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Investigação dos efeitos neuroprotetores da N-acetilcisteína e acetil-L-carnitina em um modelo de crise epiléptica induzida por pentilenotetrazol em peixes-zebra

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Tese apresentada ao Programa de Pós-Graduação em Neurociências do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de doutor em Neurociências.

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RESUMO

A epilepsia é uma doença neurológica que afeta cerca de 1 a 2% da população. Caracteriza-se pela ocorrência de crises epilépticas que refletem os mecanismos neurais subjacentes da doença. Aproximadamente 30% dos pacientes não respondem aos tratamentos farmacológicos disponíveis. Portanto, é essencial explorar opções terapêuticas para o manejo dessa condição. Recentemente, o uso do peixe-zebra como organismo modelo tem sido destacado no estudo da epilepsia, pois pode contribuir para a compreensão dos mecanismos envolvidos nas crises epilépticas e para a pesquisa e desenvolvimento de novos fármacos. O objetivo desta tese foi investigar o efeito da N-acetilcisteína (NAC) e da acetil-L-carnitina (ALC) nas crises epilépticas agudas induzidas pelo pentilenotetrazol (PTZ) em peixes-zebra. Inicialmente, foi realizada uma revisão sistemática, integrando estudos que abordaram os efeitos comportamentais, eletrofisiológicos e neuroquímicos de diferentes indutores químicos de crises epilépticas nesse organismo modelo. Foram identificados 201 artigos que preenchiam os critérios de inclusão nas bases de dados *Pubmed*, *Scopus* e *Web of Science*. O pentilenotetrazol (PTZ 10, 15 e 20 mM) foi o indutor químico mais utilizado, sendo que dentre os parâmetros comportamentais mais avaliados estão os estágios de crises epilépticas e a latência para cada estágio. Foi realizada uma análise de risco de viés e qualidade de relato em uma amostra de 100 artigos, revelando que a randomização dos procedimentos e a apresentação dos dados incompletos foram pouco claras em 81% e 68% dos estudos, respectivamente. Nenhum dos estudos relatou o cálculo do tamanho da amostra. Após a revisão sistemática, foram conduzidos experimentos para avaliar os efeitos de diferentes concentrações (0,1, 1,0 e 10 mg/L) de NAC e ALC nas crises epilépticas agudas induzidas pelo PTZ em peixes-zebra em diferentes estágios de desenvolvimento. Nossos resultados indicam que, em qualquer uma das concentrações testadas, ambas as drogas não foram capazes de reduzir as crises epilépticas induzidas pelo PTZ. No entanto, a administração de diazepam demonstrou uma redução notável na intensidade das crises e um aumento nas latências para os estágios mais avançados das crises epilépticas. Nesse sentido, mais estudos são necessários para avaliar o potencial da NAC e ALC em diferentes concentrações, tempo de tratamento e protocolos no peixe-zebra.

ABSTRACT

Epilepsy is a neurological disease that affects approximately 1 to 2% of the population. It is characterized by the occurrence of epileptic seizures that reflect the underlying neural mechanisms of the disease. Approximately 30% of patients do not respond to available pharmacological treatments. Therefore, it is essential to explore alternative therapeutic options for managing this condition. Recently, the use of the zebrafish as a model organism has been highlighted in the study of epilepsy, as it can contribute to understanding the mechanisms involved in epileptic seizures and the research and development of new drugs. The aim of this thesis was to investigate the effect of N-acetylcysteine (NAC) and acetyl-L-carnitine (ALC) on acute epileptic seizures induced by pentylenetetrazole (PTZ) in zebrafish. Initially, a systematic review was conducted, integrating studies that addressed the behavioral, electrophysiological, and neurochemical effects of different chemical inducers of epileptic seizures in this model organism. A total of 201 articles meeting the inclusion criteria were identified from *PubMed*, *Scopus*, and *Web of Science* databases. pentylenetetrazole (PTZ 10, 15, and 20 mM) was the most commonly used chemical inducer, and among the most evaluated behavioral parameters were the stages of epileptic seizures and the latency for each stage. A risk of bias and quality of reporting analysis was performed on a sample of 100 articles, revealing that randomization of procedures and reporting of incomplete data were unclear in 81% and 68% of the studies, respectively. None of the studies reported sample size calculation. After the systematic review, experiments were conducted to evaluate the effects of different concentrations (0.1, 1.0, and 10 mg/L) of NAC and ALC on acute epileptic seizures induced by PTZ in different developmental stages. Assessment of behavioral parameters, such as seizure intensity and latency to seizure occurrence, provided data on the efficacy of these substances. Our results indicate that, at any of the tested concentrations, both drugs were not able to reduce PTZ-induced epileptic seizures. However, diazepam administration showed a remarkable reduction in seizure intensity and an increase in latencies to the more advanced stages of epileptic seizures. In this regard, further studies are needed to evaluate the potential of NAC and ALC at different concentrations, treatment durations, and protocols in zebrafish.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

AE	Antiepilépticos
ALC	Acetil-L-carnitina
AMPA	α-amino-3-hidroxi-5-metil-4-isoxazole propiônico
EEG	Eletroencefalograma
GABA	Ácido gama-aminobutírico
GABA-T	GABA transaminase
GAT1 ou SLC6A1	Transportador do ácido γ-aminobutírico
GSH	Glutationa
ILAE	*International League Against Epilepsy*
MDA	Malondialdeído
mGluRs	Receptores metabotrópicos extra sinápticos
mGlu2	Receptores de glutamato metabotrópico tipo 2
NAC	N-acetilcisteína
NMDA	N-metil-d-aspartato
PTZ	Pentilenotetrazol
ROS	Espécies reativas de oxigênio
SV2A	Glicoproteína 2A de vesículas sinápticas

SUMÁRIO

1. INTRODUÇÃO

1.1 Epilepsia

Epilepsia é uma doença neurológica cuja característica fundamental é a ocorrência de crises epilépticas espontâneas e recorrentes ou pelo menos uma crise epiléptica espontânea com predisposição a crises subsequentes (Fisher et al., 2014). Encontra-se entre as principais doenças neurológicas crônicas, afetando em torno de 70 milhões de pessoas no mundo (Singh & Trevick, 2016) de todas as idades independentemente de gênero ou status socioeconômico (Bialer & White, 2010).

Uma crise epiléptica é caracterizada por um breve episódio de sinais e sintomas devidos à atividade neuronal síncrona anormal ou excessiva no cérebro. As manifestações clínicas variam significativamente, dependendo da área afetada no cérebro. Os tipos de crises epilépticas foram recentemente atualizados (Fisher et al., 2017). A ILAE (*International League Against Epilepsy*, 2017) classifica as crises epilépticas de acordo com o local de início podendo ser focal, generalizado ou desconhecido. Quando possível, as crises epilépticas focais são divididas em crise epiléptica com consciência preservada ou com comprometimento da consciência. Crises epilépticas focais cientes foram previamente referidas como crises parciais simples e crises focais de consciência prejudicada foram previamente referidas como crises parciais complexas. As crises de início focal, de início generalizado e de início desconhecido podem incluir formas motoras e não motoras. Crises epilépticas focais incluem crises que progridem para tônico-clônicas bilaterais (anteriormente referidas como crises tônico-clônicas secundariamente generalizadas). Essa classificação também distingue entre crises epilépticas bilaterais (que se propagam para ambos os hemisférios) e crises epilépticas generalizadas (que se originam simultaneamente em ambos os hemisférios) (Devinsky et al., 2018). Na tabela 1 podemos ver a classificação básica para os tipos de crise epiléptica segundo a ILAE (Fisher et al., 2017).

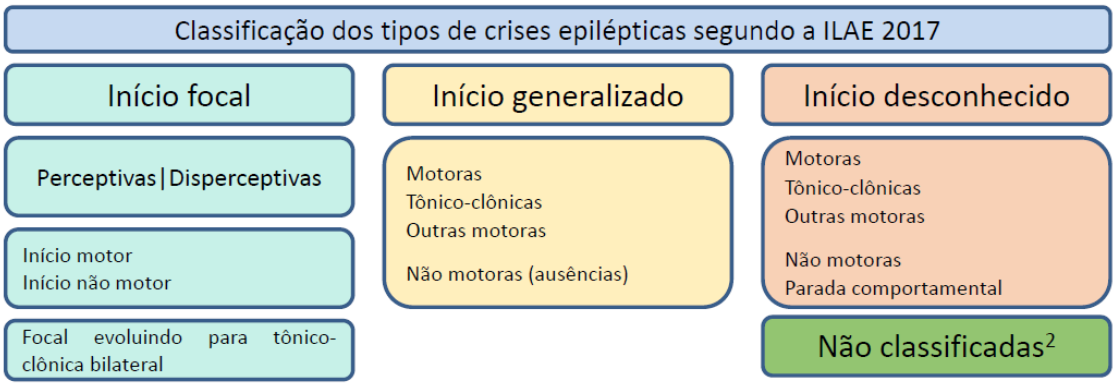

Figura 1. Classificação operacional básica da ILAE 2017 para os tipos de crises epilépticas. [2] Por informação inadequada ou impossibilidade de inserir nas outras categorias (Fisher et al., 2017).

Além disso, o estado epiléptico ou *status epilepticus* é definido por crise prolongada ou crises repetitivas sem a recuperação completa da consciência, com duração de 30 minutos ou mais, sendo considerado uma emergência médica. Embora normalmente o cérebro se recomponha entre uma crise e outra, a ocorrência dessas crises gera uma enorme sobrecarga metabólica, podendo ocorrer hipotensão, hipóxia, hipoglicemia e acidemia, levando o indivíduo a danos cerebrais permanentes, complicações sistêmicas e morte (Kandel et al., 2014).

A causa da epilepsia em muitos pacientes é desconhecida e as crises podem ser o resultado de qualquer insulto que perturbe a função cerebral, incluindo causas adquiridas, como por exemplo, acidente vascular cerebral ou traumatismo cranioencefálico, doenças infecciosas, doenças autoimunes e mutações genéticas. Até o momento, mais de 500 genes associados à epilepsia foram identificados (Devinsky et al., 2018). Há uma cascata de eventos celulares que ocorre durante as crises epilépticas, como ativação de receptores de glutamato e citocinas pró-inflamatórias, mudanças na expressão de receptores de glutamato e ácido gama-aminobutírico (GABA) e modificações na plasticidade neuronal (Chuang, 2010). Mecanismos propostos para explicar como as crises epilépticas ocorrem, incorporam múltiplos níveis de análise que variam de comportamental (estágios de crise definidos) a eventos eletrofisiológicos (por exemplo, eventos elétricos que contribuem para um episódio de crise epiléptica) e moleculares como, por exemplo, alterações na expressão gênica (Baraban et al., 2005).

1.2 Tratamentos farmacológicos e não-farmacológicos

Desde quando se recebe o diagnóstico de epilepsia, o desejo dos indivíduos que vivem com epilepsia é ficar livre das crises epilépticas. A história pela busca de tratamentos para a epilepsia ganhou força durante o século XX, onde a invenção do eletroencefalograma (EEG), o avanço da neurocirurgia, a descoberta de fármacos antiepilépticos (AEs) e o delineamento dos mecanismos fisiopatológicos subjacentes foram os avanços mais significativos no campo da pesquisa em epilepsia (Magiorkinis et al., 2014). A descoberta de antiepilépticos começou no final do século XVIII e início do século XIX onde bromidas eram usadas no tratamento de epilepsia e a descoberta do fenobarbital, em 1912 (Shorvon, 2009). Após isso, a descoberta de novos antiepilépticos pode ser dividida em 3 períodos: o primeiro período (1938-1969) viu a introdução de mais de 20 novos fármacos para epilepsia, muitos dos quais foram retirados do mercado. Foi nesse período o surgimento da carbamazepina, a fenitoína, o valproato, a etossuximida e os benzodiazepínicos. O período intermediário (1970-1988) não viu a introdução de nenhuma nova medicação importante, mas importantes avanços terapêuticos ocorreram devido à melhor compreensão das propriedades das drogas disponíveis. O último período (1989-2019) foi dominado pela introdução de fármacos de segunda geração (por exemplo: felbamato, topiramato, lacosamida, retigabina e canabidiol) e maior evolução no desenho de ensaios de monoterapia e terapia adjuvante (Perucca, 2019).

Atualmente, a epilepsia é tratada principalmente com AEs, tendo como objetivo diminuir ou impedir a ocorrência de crises. Esses fármacos atuam inibindo os disparos neuronais repetitivos, através da modulação de canais iônicos dependentes de voltagem, potencialização da neurotransmissão GABAérgica e/ou diminuição da neurotransmissão glutamatérgica (Bialer & White, 2010). Atualmente, acredita-se que os AEs disponíveis tenham como alvo moléculas na sinapse excitatória. Esses incluem canais de Na^{+} dependentes de voltagem, glicoproteína 2A de vesículas sinápticas (SV2A), a subunidade α2δ do canal de Ca^{+2} regulado por voltagem, receptores AMPA (α-amino-3-hidroxi-5-metil-4-isoxazole propiônico) e receptores NMDA (N-metil-d-aspartato). Alvos de AEs em sinapses inibitórias também foram propostos. Esses incluem o transportador do ácido γ-aminobutírico (GABA) GAT1 (também conhecido como SLC6A1), que é inibido pela tiagabina, levando a uma diminuição na captação de GABA nos terminais pré-sinápticos e na glia circundante; e GABA transaminase (GABA-T), que é inibida irreversivelmente

pela vigabatrina. Isso diminui o metabolismo do GABA em terminais pré-sinápticos e células gliais. Verificou-se que os benzodiazepínicos, barbitúricos, topiramato e felbamato aumentam a neurotransmissão inibitória por meio da modulação alostérica das correntes de cloreto mediadas pelo receptor $GABA_A$. No entanto, a ação de cada um desses fármacos é diferente e depende da conformação da subunidade do complexo receptor do $GABA_A$ (Bialer & White, 2010). O mecanismo de ação dos fármacos antiepilépticos está representado na figura abaixo (Figura 1).

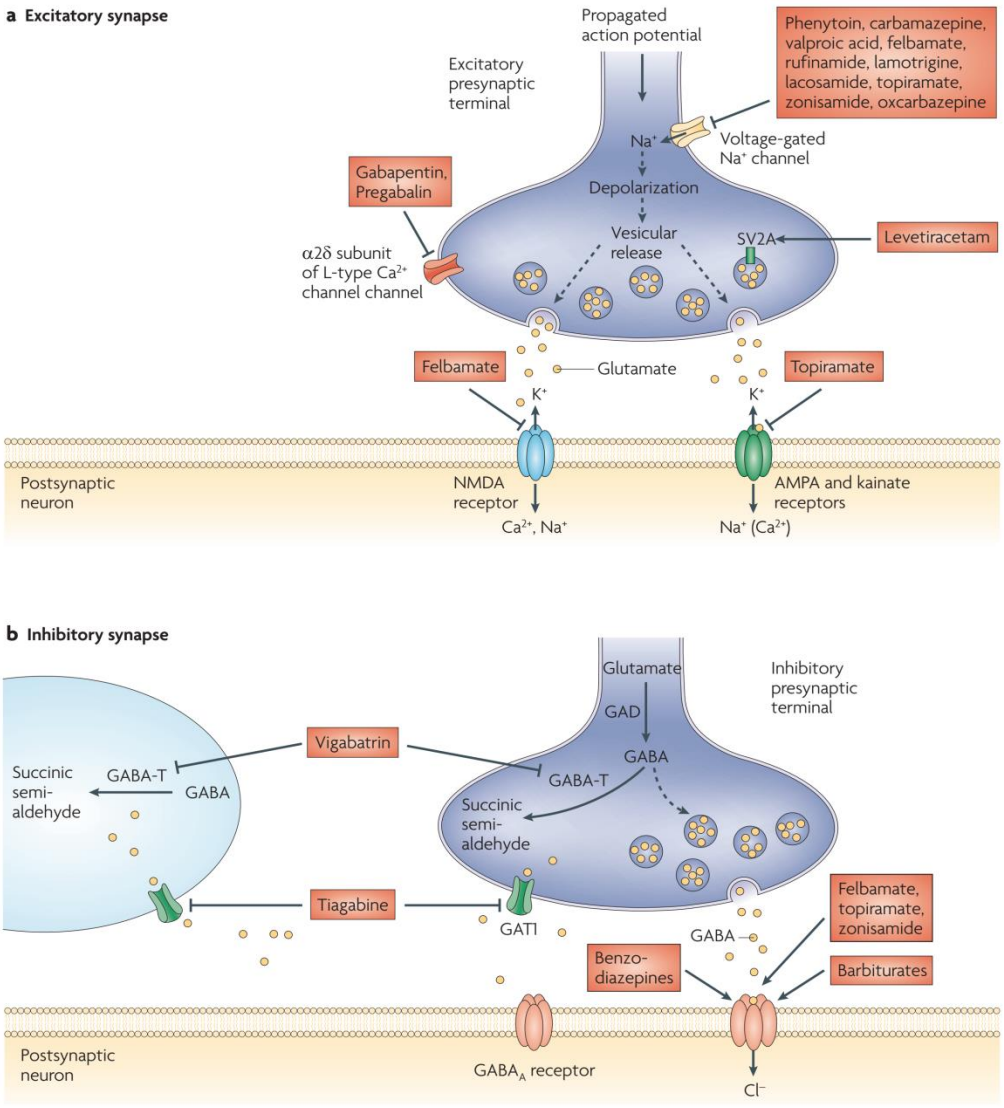

Figura 2. Mecanismos de ação propostos dos antiepilépticos nas sinapses a) excitatórias e b) inibitórias. Fonte: (Bialer & White, 2010). Reprodução autorizada.

Após o início do tratamento com antiepilépticos, 80% das pessoas que vivem com epilepsia apresentam reações adversas (como depressão, ansiedade, irritabilidade, dificuldade de concentração, alterações de humor, hiperatividade e, em casos raros,

psicose), sendo que 30% a 40% relatam prejuízo na qualidade de vida culminando em muitos casos com a interrupção ou não adesão correta ao tratamento. Os efeitos adversos e a eficácia desses fármacos são altamente variáveis entre os indivíduos com epilepsia (Devinsky et al., 2018). Nesse sentido, apesar dos mais de 20 AEs disponíveis no mercado, aproximadamente 30 a 40% da população que vive com epilepsia possuem epilepsia refratária, ou seja, quando as crises epilépticas não são controladas por dois ou mais antiepilépticos (Stafstrom & Carmant, 2015).

Além dos tratamentos farmacológicos, existem outras opções não-farmacológicas que podem ajudar no tratamento da epilepsia, sendo importante ressaltar que essas abordagens podem variar em eficácia e resultados de acordo com o tipo e a gravidade da epilepsia. Abordagens cirúrgicas tem a maior chance de tornar os indivíduos que vivem com epilepsia livres de crises epilépticas, embora apenas um pequeno número de pacientes seja elegível para a cirurgia (Devinsky et al., 2018). Além da cirurgia, a dieta cetogênica é uma opção principalmente para crianças e adolescentes. Trata-se de uma abordagem alimentar rica em gorduras e pobre em carboidratos que parece reduzir a adenosina quinase, já que seu aumento parece estar envolvido com as crises epilépticas e morte celular na epilepsia. Porém, nem todos respondem a esse tratamento, além de possuir vários efeitos adversos como náusea e vômitos (Devinsky et al., 2018; Wells et al., 2020). Outra abordagem não-farmacológica é a estimulação do nervo vago. A estimulação crônica e intermitente do nervo vago pode ser uma opção para muitos indivíduos que sofrem de epilepsias refratárias que não são candidatos à ressecção cirúrgica. Embora apenas uma pequena minoria de indivíduos com epilepsia fique totalmente livre de crises epilépticas, a estimulação do nervo vago, como adjuvante da terapia médica, pode resultar em melhorias significativas na qualidade de vida (Milby et al., 2009; González et al., 2019).

1.3 Neuroprotetores: N-acetilcisteína e acetil-L-carnitina

A N-acetilcisteína (NAC) é utilizada como mucolítico e como antídoto em intoxicações por paracetamol e vem emergindo como uma molécula promissora para o tratamento como adjuvante de vários distúrbios neuropsiquiátricos, como transtorno bipolar, esquizofrenia, transtorno obsessivo-compulsivo, ansiedade, depressão e dependência de drogas (Skvarc et al., 2017; Zheng et al., 2018; Bradlow et al., 2022).

Porém, a NAC mostrou resultados mistos no tratamento de muitos transtornos psiquiátricos, pois os achados iniciais significativos muitas vezes não foram replicados ou são limitados, portanto, embora estudos pré-clínicos tenham demonstrado um efeito positivo da NAC, sua eficácia clínica não está totalmente estabelecida, sendo necessários estudos pré-clínicos e clínicos adicionais (Smaga et al., 2021; Bradlow et al., 2022). A NAC ativa os transportadores antiporte cistina-glutamato presentes nos astrócitos, levando à liberação de glutamato que estimula os receptores metabotrópicos extra sinápticos (mGluRs), diminuindo a liberação sináptica do neurotransmissor (Baker et al., 2002; Moran et al., 2005). Além disso, essa molécula demonstrou possuir efeitos antioxidante, ansiolítico e anti-estresse em camundongos e peixes-zebra (Mocelin et al., 2015, 2018; Santos et al., 2017).

Outro composto com potenciais benefícios terapêuticos é a Acetil-L-carnitina (ALC), um suplemento dietético disponível em lojas de produtos naturais. A ALC demonstrou efeitos em modelos de depressão em roedores expostos a estresse crônico imprevisível. Esse efeito se dá possivelmente pela regulação epigenética dos receptores de glutamato metabotrópico tipo 2 (mGlu2) (Nasca et al., 2013). Recentemente, foi demonstrado que a ALC preveniu crises epilépticas induzidas por PTZ em ratos (em modelo de *kindling*) e diminuiu marcadores de estresse oxidativo, aumentou a glutationa (GSH) e reduziu a expressão gênica de marcadores de apoptose, como a caspase-3 (Hussein et al., 2018). Outros estudos têm demonstrado efeitos desse composto contra o estresse oxidativo causado por poluentes através de aumento na atividade de enzimas antioxidantes e diminuição de espécies reativas de oxigênio (ROS) (Sepand et al., 2016; Sun et al., 2017). Recentemente, nosso grupo mostrou os efeitos ansiolíticos, anti-estresse e antioxidante desse composto em peixes-zebra adultos (Pancotto et al., 2018).

Como a neurobiologia da epilepsia envolve o desequilíbrio entre sistemas de neurotransmissores excitatórios e inibitórios com prevalência do primeiro sobre o segundo, compostos moduladores de glutamato como a NAC e a ALC podem potencialmente ser úteis na regulação desse balanço e consequentemente ser utilizadas em pacientes com essa condição. Além disso, alguns estudos tem demonstrado o efeito desses compostos contra crises epilépticas em roedores (Uma Devi et al., 2006; Zaeri & Emamghoreishi, 2015; Essawy et al., 2022).

1.4 Peixes-zebra como organismo modelo

A utilização do peixe-zebra (*Danio rerio*, F. Hamilton 1822) na neurociência cresceu acentuadamente nas últimas décadas, por ser uma espécie de vertebrados com propriedades fisiológicas e genéticas homólogas com os seres humanos (Kalueff et al., 2014), bem como pela facilidade da manipulação genética e, apesar de algumas diferenças notáveis no tamanho de estruturas específicas do cérebro do peixe-zebra (por exemplo, hemisférios cerebrais menores), a organização geral dos principais componentes cerebrais é altamente conservada com a do cérebro humano (Tropepe & Sive, 2003). Além disso, apresenta capacidade de absorver compostos adicionados à água e padrão comportamental caracterizado e detalhado (Kalueff et al., 2014). Estudos demonstraram que 71% dos genes que codificam proteínas no genoma humano são relacionados a genes encontrados no genoma do peixe-zebra e 82% dos genes associados a doenças humanas possuem um ortólogo em peixe-zebra, mostrando-se como um ótimo modelo também para estudos bioquímicos e genéticos (Howe et al., 2013).

Os peixes-zebra têm se mostrado um eficiente modelo experimental para o estudo da epilepsia. As crises epilépticas induzidas pela exposição a indutores de crise epiléptica apresentam aspectos característicos de crises que ocorrem em humanos: descargas neuronais excessivas e alterações comportamentais progressivas (Baraban et al., 2005). Baraban e colaboradores iniciaram testes comportamentais em larvas de peixe-zebra em 2005 e caracterizaram o comportamento de crises epilépticas de larvas com 7 dpf usando pentilenotetrazol (PTZ) como agente convulsivo. Este comportamento dividiu-se em 3 estágios: estágio I - atividade de natação dramaticamente aumentada, estágio II - comportamento de natação em redemoinho e estágio III - convulsões do tipo clônus seguidas de perda de postura, quando o animal cai para um lado e permanece imóvel por 1 a 3 segundos (Baraban et al., 2005). Mais tarde, Mussulini e colaboradores (2013) caracterizaram o comportamento no peixe-zebra adulto, dividindo-os em 6 estágios: 0, natação curta, principalmente no fundo do tanque; 1, aumento da atividade natatória; 2, movimentos irregulares para esquerda e direita; 3, movimentos circulares; 4, comportamento do tipo crise clônica; 5; cair no fundo do tanque, comportamento do tipo convulsão tônica; 6, morte (Mussulini et al., 2013).

Larvas de peixe-zebra e também animais adultos desenvolveram padrões eletroencefalográficos correspondentes às fases ictal e inter-ictal da crise epiléptica quando expostos ao agente convulsivante pentilenotetrazol (PTZ) (Afrikanova et al.,

2013; Baraban et al., 2005). A resposta a tratamentos farmacológicos também é evidente nessa espécie, mostrando sua validação preditiva. Foi visto que larvas e animais adultos previamente tratadas com fármacos antiepilépticos, como Diazepam e valproato de sódio, tiveram crises epilépticas prevenidas quando expostas aos agentes indutores de crise epiléptica (Baraban et al., 2005; Berghmans et al., 2007; Siebel et al., 2015).

A grande maioria dos estudos sobre epilepsia usando peixe-zebra como organismo modelo são estudos de exposição aguda a um agente indutor de crise epiléptica para avaliar as crises e a descoberta de novos compostos que diminuem ou terminam crises epilépticas por meio de avaliação comportamental, como atividade locomotora e estágios de crises epilépticas (Baraban et al., 2005; Mussulini et al., 2013; Afrikanova et al., 2013) porém recentemente, tentativas de validar um protocolo de *kindling* no peixe-zebra foram realizados (Kundap et al., 2019; Kumari et al., 2020). *Kindling* é um modelo animal crônico de epilepsia que tem sido extensivamente estudado, principalmente em roedores, para entender o processo de epileptogênese e descobrir novos compostos antiepilépticos e trata-se de um fenômeno em que um estímulo subconvulsivo (seja químico ou elétrico), se aplicado de forma repetitiva e intermitente, acaba levando à geração de crises epilépticas completas no animal (Dhir, 2012). Apesar de nos estudos em peixe-zebra os pesquisadores terem conseguido observar crises completas após exposição ao PTZ, elas não eram crises espontâneas, fazendo com que seja necessário mais estudos e uma melhor validação deste tipo de protocolo.

Tendo em vista a problemática da epilepsia e os indivíduos que não correspondem à terapêutica disponível, esse estudo pretende avaliar os efeitos de compostos neuroprotetores frente a crises epilépticas agudas induzidas pelo PTZ em diferentes estágios de desenvolvimento de peixe-zebra no intuito de encontrar novas alternativas de tratamentos.

2. OBJETIVOS

2.1 Objetivo Geral

Investigar os efeitos da N-acetilcisteína (NAC) e da acetil-L-carnitina (ALC) sobre o comportamento em um modelo de crise epiléptica induzida por pentilenotetrazol (PTZ) em peixes-zebra (*Danio rerio*) em diferentes estágios de desenvolvimento.

2.2 Objetivos específicos

a. Realizar uma revisão sistemática da literatura sobre os efeitos de indutores químicos de crise epiléptica no peixe-zebra;
b. Avaliar os efeitos da NAC nas crises epilépticas agudas induzidas por PTZ em peixes-zebra em diferentes estágios de desenvolvimento;
c. Avaliar os efeitos da ALC nas crises epilépticas agudas induzidas por PTZ em peixes-zebra em diferentes estágios de desenvolvimento.

3. COLETÂNEA DE ARTIGOS

A tese está organizada no formato de coletânea de artigos científicos, dividida em 2 capítulos.

3.1 Capítulo 1: Chemically-induced epileptic seizures in zebrafish: a systematic review

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Chemically-induced epileptic seizures in zebrafish: A systematic review

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Chemically-induced epileptic seizures in zebrafish: a systematic review

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ABSTRACT

The use of zebrafish as a model organism is gaining evidence in the field of epilepsy as it may help to understand the mechanisms underlying epileptic seizures. As zebrafish assays became popular, the heterogeneity between protocols increased, making it hard to choose a standard protocol to conduct research while also impairing the comparison of results between studies. We conducted a systematic review to comprehensively profile the chemically-induced seizure models in zebrafish. Literature searches were performed in PubMed, Scopus, and Web of Science, followed by a two-step screening process based on inclusion/exclusion criteria. Qualitative data were extracted, and a sample of 100 studies was randomly selected for risk of bias assessment. Out of the 1058 studies identified after removing duplicates, 201 met the inclusion criteria. We found that the most common chemoconvulsants used in the reviewed studies were pentylenetetrazole (n = 180), kainic acid (n = 11), and pilocarpine (n = 10), which increase seizure severity in a dose-dependent manner. The main outcomes assessed were seizure scores and locomotion. Significant variability between the protocols was observed for administration route, duration of exposure, and dose/concentration. Of the studies subjected to risk of bias assessment, most were rated as low risk of bias for selective reporting (94%), baseline characteristics of the animals (67%), and blinded outcome assessment (54%). Randomization procedures and incomplete data were rated unclear in 81% and 68% of the studies, respectively. None of the studies reported the sample size calculation. Overall, these findings underscore the need for improved methodological and reporting practices to enhance the reproducibility and reliability of zebrafish models for studying epilepsy. Our study offers a comprehensive overview of the current state of chemically-induced seizure models in zebrafish, highlighting the common chemoconvulsants used and the variability in protocol parameters. This may be particularly valuable to researchers interested in understanding the underlying mechanisms of epileptic seizures and screening potential drug candidates in zebrafish models.

Keywords: Seizure, epilepsy, chemoconvulsants, *Danio rerio*, zebrafish, animal model, behavior, systematic review.

HIGHLIGHTS

- We systematically reviewed the effects of chemically-induced seizures in zebrafish;
- PTZ is the most commonly used epileptic seizure inducer in zebrafish;
- More than 50% of studies fail to report outlier exclusion and sample size estimation;
- The results showed a need for better standardization of protocols.

INTRODUCTION

Among the main chronic neurological diseases, epilepsy is a condition characterized by recurrent unprovoked epileptic seizures that affect around 70 million people worldwide (Fisher et al., 2005; Singh and Trevick, 2016). Epilepsy is defined by the International League Against Epilepsy (ILAE) as a “disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition” (Fisher et al., 2005). An epileptic seizure is defined as a transient occurrence of signs and/or symptoms due to abnormal or excessive neuronal activity in the brain and is characterized by behavioral changes that reflect the underlying neural mechanisms of the disease (Fisher et al., 2005; Beghi, 2019). Epileptogenesis is triggered by various pathogenetic events, encompassing acquired causes (e.g., stroke or traumatic brain injury), infectious diseases, autoimmune diseases, or genetic alteration. However, the etiology remains unidentified in a significant number of patients. This process commences before and continues beyond the initial unprovoked seizure (Devinsky et al., 2018). More than 500 genes associated with epilepsy have been identified (Devinsky et al., 2018).

Patients with epilepsy are mainly treated with antiseizure drugs (ASDs), aiming to reduce or prevent the occurrence of seizures. After starting the treatment with an antiseizure medication, 80% of patients experience adverse effects impairing their quality of life or interrupting or non-adherence to treatment (Devinsky et al., 2018). Furthermore, despite the existence of over 20 antiepileptic drugs in the market, it is estimated that around 30 to 40% of patients experience refractory epilepsy, characterized by the failure of two or more

antiseizure medications to control seizures effectively (Bialer and White, 2010). There is a pressing need to develop and test novel treatment approaches and antiseizure drugs to address this issue to combat epilepsy (Bialer and White, 2010).

Most studies in the literature involving the process of epileptogenesis are carried out in rodent models (Devinsky et al., 2018). Zebrafish (*Danio rerio* Hamilton, 1822) is a model organism that has been gaining more space in the field of epileptogenesis, behavior during epileptic crises, and in search of potential antiseizure drugs and therapeutic targets (Baraban et al., 2005; Berghmans et al., 2007; Mussulini et al., 2013). Zebrafish has been widely used in neuroscience research as it is a vertebrate species with a central nervous system architecture similar to mammals and physiological and genetic properties homologous to humans (Kalueff et al., 2014). In addition, because of their external development, zebrafish present advantages in terms of genetic manipulation techniques compared to rodents (Sassen and Köster, 2015). Also, zebrafish can absorb compounds added to water and present characterized and detailed behavioral patterns (Kalueff et al., 2014). Studies have shown that 71% of the genes encoding proteins in the human genome are related to genes found in the zebrafish genome, and 84% of the genes associated with human diseases have a zebrafish homolog, proving to be a useful model organism for biochemical and genetic studies (Howe et al., 2013).

Different behavioral assays of epileptic seizures in zebrafish have been described; seizure-inducing agents, such as pentylenetetrazole, picrotoxin, and allylglycine (Baraban et al., 2005; Leclercq et al., 2015; Yang et al., 2017), among others, are employed in varying concentrations and routes of administration.

These behavioral studies have been of great importance in discovering new compounds or potential antiseizure drug candidates capable of reducing the stages of epileptic seizures that mimic the crises that happen in humans. However, the heterogeneity in methodologies described in the published literature is high, as researchers tend to adapt protocols (e.g., use a different time of exposure to a seizure inducer or a different concentration). In addition, there are differences in the behavioral characterization of the effects during exposure to epileptic seizure inducers.

We conducted a systematic review of studies using chemical inducers of epileptic seizures in zebrafish available in the indexed literature. In addition to their use in clinical research, systematic reviews are a tool used to validate models and protocols used in preclinical research externally. They contribute to improving these methods by investigating potential sources of heterogeneity and biases, also indicating the quality of the studies. We qualitatively describe the published studies, annotating the results on neurobehavioural and neurochemical parameters, detecting patterns and possible effect moderators, and evaluating the impact of bias arising from the methodological conduct and reporting quality.

METHODS

This review followed our predefined SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) protocol (de Vries et al., 2015) registered in the Open Science Framework before screening records and data collection. Preregistration is available at https://osf.io/2njhw (Chitolina et al., 2021). The

reporting of this study complies with the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (Page et al., 2021).

Search Strategy

Studies were identified through a scientific literature search in three databases: PubMed, SCOPUS, and Web of Science. Search strategies were designed to suit the characteristics of each database. Varied terms were used for the searches that describe the intervention (epileptic seizure induction protocol) combined with terms referring to the population of interest (zebrafish) to carry out a sensitive search. The complete query for each database can be found at https://osf.io/2njhw (Chitolina et al., 2021). The searches were performed without restrictions for language or year of publication on November 5th, 2021. The bibliographic data acquired were imported into Rayyan software (Ouzzani et al., 2016), where duplicates were detected and removed by one of the investigators (RC). The reference lists of the included studies were also screened in order to detect additional relevant articles.

Eligibility screening

After the removal of duplicates, the selection of eligible studies was conducted using Rayyan software. The records underwent a pre-selection based on their title and abstract. An analysis of the full text was carried out for records that did not fit the determined exclusion criteria. In both stages (title/abstract and full-text analysis), two independent reviewers analyzed each study (RC and MGL

or RB or CGR). Disagreements between reviewers' decisions were resolved by a third reviewer (MEC or APH or AP). Experimental studies using chemicals to induce seizures in zebrafish on the following outcomes were included: behavioral parameters during epileptic seizures (distance traveled, immobility, frequency, and duration of epileptic seizures, latency for stages of epileptic seizures), neurochemical outcomes (e.g., neurotransmitter levels, gene expression, protein levels, oxidative stress), and late behavioral effects (e.g., outcomes related to cognition and social behavior).

In the first screening stage (title and abstract), studies were excluded based on the following reasons: (1) design: not an original primary study (e.g., review, commentary, conference proceedings, and corrections); (2) population: studies performed in animals other than zebrafish (*Danio rerio*) or *in vitro*, *ex vivo* studies; (3) intervention: other methods of seizure induction than chemically-induced (e.g., genetically modified animals, electroshock). In the second stage (full-text screening), the remaining articles were assessed for exclusion based on the same reasons considered in the first stage plus the following additional reasons: (4) outcome: studies that did not assess behavioral or neurochemical parameters.

Data extraction

Data extraction from the included studies was conducted by two independent investigators (RC and MGL or RB or CGR or TSB), and a discussion between the two investigators resolved disagreements. Whenever available, the exact

information was extracted directly from text or table. When information was not found, it was deemed unclear.

The following characteristics were extracted: (1) study characteristics: study title, digital object identifier (DOI), first and last authors, last author's institutional affiliation, and year of publication; (2) animal model characteristics: strain, sex, developmental stage during exposure to the chemical inducer; (3) protocol characteristics: seizure inducer, administration route, frequency of exposure, duration of exposure, dose or concentration, interval between inducer exposure and test, antiepileptic treatment; (4) test characteristics: type of test, test duration, category of measured variable, measured variable and main findings of the study. To assess the relationship between researchers that publish within this field, co-authorship networks were constructed using VOSviewer software version 1.6.18 (https://www.vosviewer.com) (Van Eck, N.J. and Waltman, L., 2010, 2007).

Risk of bias and reporting quality

In order to evaluate the quality of the included studies, a sample of 100 articles was chosen at random (using random.org) for risk of bias analysis. The risk of bias assessment was conducted by two independent investigators for each paper (RC and MGL or RB or CGR or TSB), and disagreements were resolved by discussion between the two investigators. The analysis was conducted based on the SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014), with adaptations better to suit the model animal and the intervention of interest. The following items were evaluated for methodological quality: (1) description of

random allocation of animals; (2) description of baseline characteristics; (3) description of blinding methods for outcome assessment; (4) incomplete outcome data; (5) selective outcome reporting. Additionally, two other items were evaluated by the investigators to assess the overall reporting quality of the studies based on a set of reporting standards (Landis et al., 2012): (6.1) sample size estimation and (6.2) mention of inclusion/exclusion of samples criteria. For methodological quality, each item was scored with a “Yes” for a low risk of bias, “No” for a high risk of bias, or “Unclear” when it was not possible to estimate the risk of bias based on the information provided. Items regarding reporting quality were scored with only "Yes" or "No", meaning high or low reporting quality, respectively. A complete guide for assessing the risk of bias associated with each item in this review is available at https://osf.io/z698v. Risk of bias plots were created using *robvis* (McGuinness and Higgins, 2021).

RESULTS

Search results

From the search in the selected databases, 2130 records were retrieved altogether (Pubmed = 807; Scopus = 710; Web of Science = 613;). Following removing duplicates, 1058 records were screened for eligibility based on title and abstract. After the first screening phase, 283 studies remained to be assessed based on full-text (Fig. 1). In the two screening phases (title/abstract and full text), 299 did not use a chemical inducer of epileptic seizures as an intervention, 277 articles were excluded due to the study design, 268 did not meet the population criteria, and 10 did not report any of the outcomes of interest. In addition, 3 studies

were not retrieved because the full version was not available online, and the authors did not reply to our request and were therefore excluded from the final analysis. This resulted in 201 studies being included in the qualitative synthesis. Of all the studies included, only 1 was not in English, being in Mandarin. The flowchart illustrates the progressive selection of studies and the number of articles in each stage (Fig. 1).

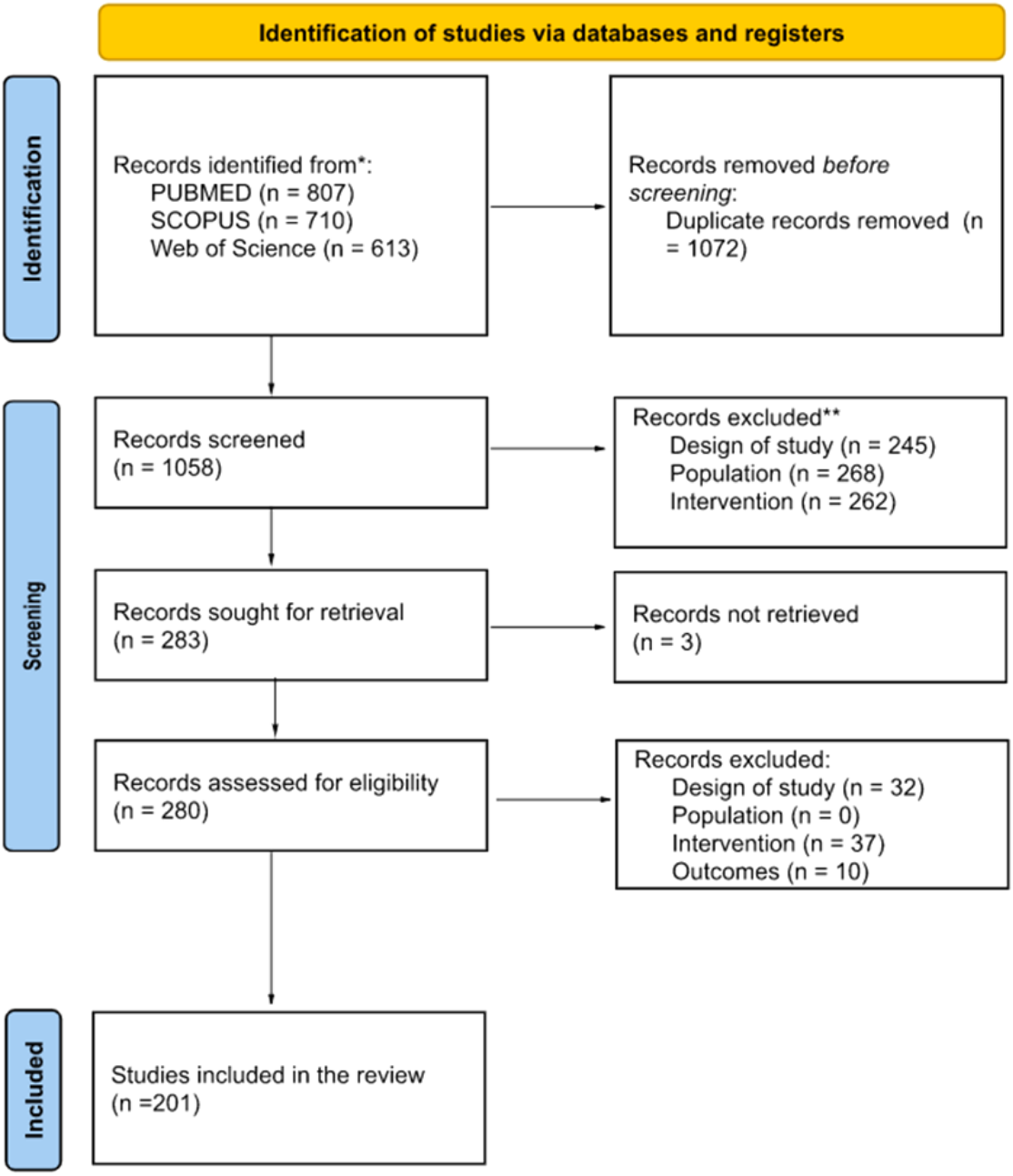

Fig. 1. Flowchart diagram of the collection of studies and selection process of the systematic review according to the PRISMA statement.

Study characteristics

In the 201 studies included in this review, most used pentylenetetrazole (PTZ) as an epileptic seizure inducer (n = 180, 89.55%); other inducers used to study epilepsy were kainic acid (n = 11, 5.47%), pilocarpine (PILO) (n = 8, 3.98%), picrotoxin (PTX) (n = 8, 3.98%), domoic acid (DA) (n = 5, 2.48%), 4-aminopyridine (4-AP) (n = 5, 2.48%), caffeine (n = 3, 1.49%) and ethyl ketopentenoate (EKP) (n = 3, 1.49%). In addition to the main inducers found, others appeared once or twice, namely strychnine, ginkgotoxin (GT), allylglycine (AG), triocresyl-phosphate (TCP), 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), N-methyl-D-aspartic acid (NMDA) and aconitine. It is important to mention that, in some studies, more than one seizure inducer was used, as described in Table 5. Figure 2 shows a pictorial representation of the studies that used larvae, juvenile, or adult zebrafish and the number of studies that used each chemoconvulsants.

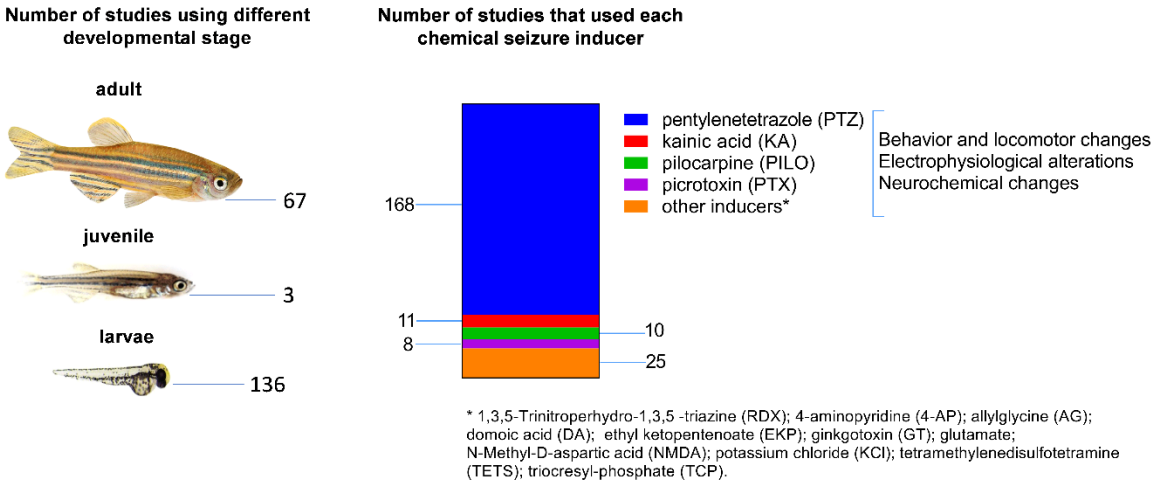

Fig. 2. Representation of the number of studies that used adults, juveniles, and larvae zebrafish and the number of studies that used each chemical seizure inducer.

The route of administration of epileptic seizure inducers in the selected articles was mostly by immersion in a solution containing the drug (n = 178, 88.56%) or intraperitoneal injection (n = 23, 11.44%). PTZ was mainly administered by immersion, with 166 studies out of 180 that used this seizure-inducer employing this route of administration, which corresponds to 92.22%. On the other hand, most exposures to kainic acid used intraperitoneal injection as the route of administration (n = 7, 63.63%), 3 used immersion, and 1 used microinjection into the pericardium. Another seizure inducer that is commonly administered by intraperitoneal (i.p.) injection is pilocarpine, where 4 studies (50%) used this method of exposure, while another 4 (50%) used immersion as the form of administration. As for picrotoxin, in all studies, immersion was used as the delivery route (n = 8, 100%). Domoic acid was administered by immersion in 3 studies, by microinjection in one, and via intraperitoneal injection in another one. For 4-aminopyridine, caffeine, and ethyl ketopentenoate, all studies used immersion as the route of administration.

As expected, the duration of exposure to seizure inducers varies between studies. In the 180 studies using PTZ, the time of exposure ranged from 2 to 390 min, with 30 min as the most frequently used exposure time (n = 37, 20.55%). Other commonly used exposure times were 10 min (n = 24, 13.33%), 15 min (n = 19, 10.55%), 60 min (n = 18, 10%), and 20 min (n = 17, 9.44%). Exposure to kainic acid via immersion ranged from 10 to 30 min in 3 studies. The duration of

exposure to pilocarpine by immersion ranged between 2 and 240 min. Picrotoxin exposure duration ranged from 20 to 240 min; in 2 studies, this information was unclear.

The data presented in Tables 1-5 shows that various doses and concentrations are used in chemically-induced seizure protocols. Notably, different research groups employ a range of inducers, resulting in variations in the experimental conditions. For PTZ, for example, we found a variation between less than 1 and more than 60 mM. The most commonly used concentration in cases where immersion was the chosen route of administration was 20 mM (n = 40, 22.22%), followed by 10 mM (n = 39, 21.66%) and 15 mM (n = 35, 19.44%). For studies that used intraperitoneal injection, the most commonly used dose was 170 mg/kg (n = 8, 4.44%). Other doses used were 220 and 225 mg/kg, in addition to 80 mg/kg administered for 10 days. In the case of kainic acid, intraperitoneal injection doses ranged from 5 or 6 mg/kg (n = 3, 27.27% for 5 mg/kg and n = 5, 45.45% for 6 mg/kg). Pilocarpine was used mainly at concentrations of 15, 30, and 60 mM when administered by immersion, with 2 studies for each concentration, and the most common intraperitoneal injection dose was 400 mg/kg. To see all the concentrations of all seizure inducers, please check Tables 1 to 5.

Most of the available studies were carried out with zebrafish in the early developmental stages, with 136 studies on embryos/larvae, corresponding to 67.66%, against 67 studies on adults (33.33%). Only 3 studies used zebrafish in the juvenile stage (1.49%). In larvae, most studies were conducted at 7 days post-fertilization (dpf) (n = 80, 58.82%), followed by 5 dpf (n = 34, 25%) and 6 dpf (n = 19, 13.97%) (Tables 1-5).

Most studies on epilepsy using zebrafish as a model organism are studies of acute exposure to a chemoconvulsant to assess epileptic seizures for discovering new compounds through behavioral assessment. 178 studies (88.55%) describe behavioral analyses, while 81 (40.29%) showed neurochemical outcomes such as gene expression and oxidative stress status, and 44 (21.89%) reported electrophysiological results. Among the behavioral outcomes most commonly assessed during epileptic seizures are distance traveled (n=89, 50%), latency to reach each seizure score (n = 60, 33.71%), and ratings of epileptic seizure scores (n = 42, 23.59%). In addition, 44 studies use electrophysiology to verify the occurrence, frequency, and duration of epileptiform discharges.

Within neurochemical analyses, changes in genes involved in epileptogenesis and neuronal activity are widely evaluated. The most evaluated gene among the studies is *c-fos* (n = 44, 54.32%), followed by brain-derived neurotrophic factor (*bdnf*) (n = 15, 18.51%). Other genes appear in fewer studies, such as neuropeptide y (*npy),* tumor necrosis factor (*tnf-α*), transcription factor cyclic-AMP response element-binding protein 1 (*creb_1*), toll like Receptor 4 (*tlr4*), nuclear factor kappa B (*nfkb*), and *caspase-3*. Additionally, glutamate and gamma-aminobutyric acid (GABA) levels are also seen in 11 and 10 studies, respectively. Oxidative stress appears in 8 studies, with analyses such as lipid peroxidation levels and activity or expression of enzymes such as superoxide dismutase (SOD) and catalase (CAT).

Co-authorship network analysis identified 76 clusters of researchers that use chemically-induced seizure protocols in their labs across the globe based on

the studies included in this review (Fig. 3). An interactive version of the co-authorship network is available at https://tinyurl.com/2g9kgyu6.

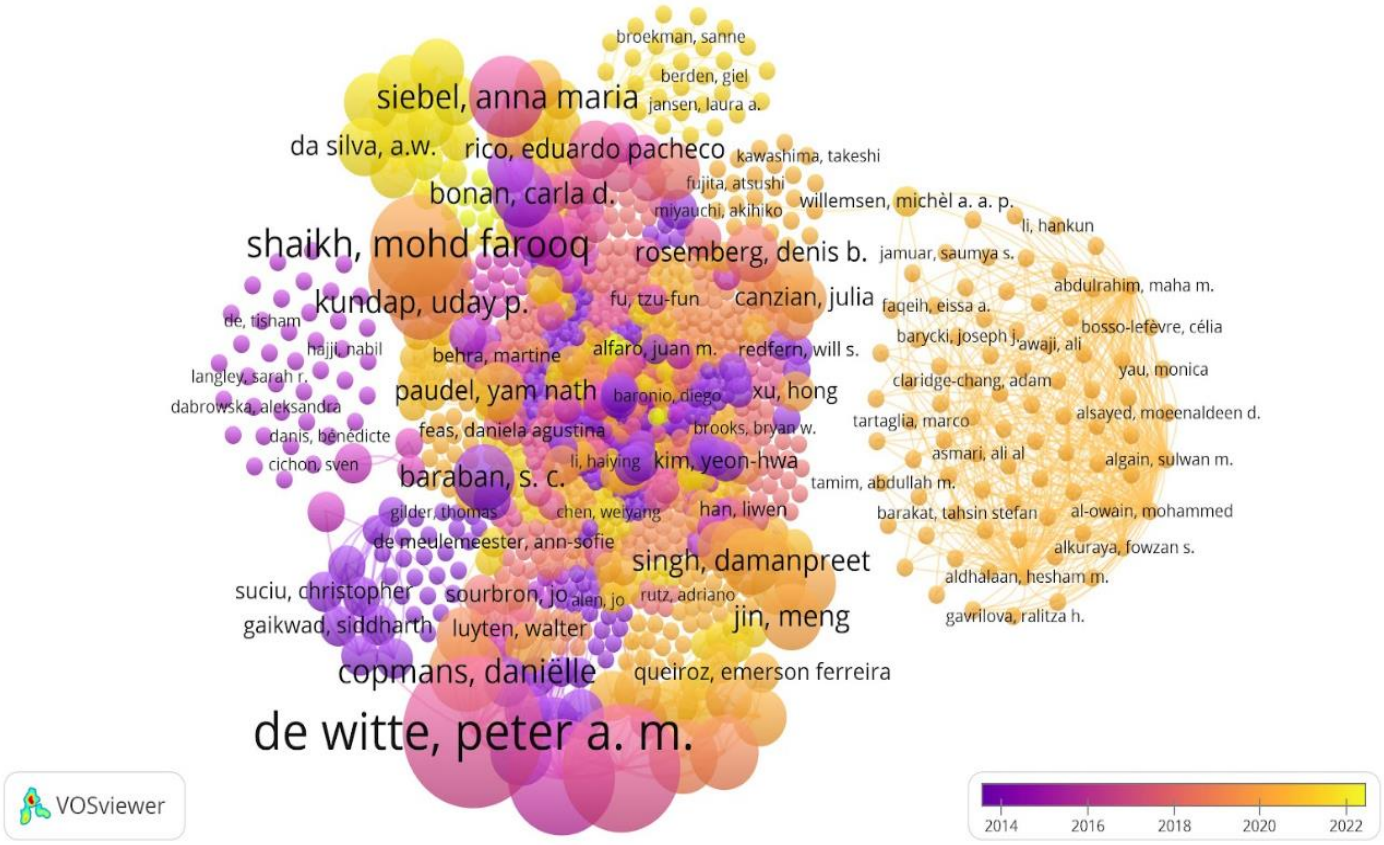

Fig. 3. Co-authorship network analysis of researchers that authored studies implementing chemically-induced seizures in zebrafish. Authors are color-coded from violet (older studies) to yellow (more recent studies), indicating the average publication year of the studies published by each researcher. The size of the circles represents the number of studies published by each author. The distance between the two circles indicates the correlations between researchers.

Table 1. Qualitative description of studies reporting chemically-induced seizure protocols by pentylenetetrazole (PTZ) in research with zebrafish.

Reference	Seizure inducer	Duration of exposure (min)	Administration route	Interval inducer exposure and test	Developmental stage during outcome assessment	Sex	Duration test (min)	Main findings

(Baraban et al., 2005)	PTZ 2.5, 5, and 15 mM	10	immersion	-	Larvae (7 dpf)	N.A.	10	**Seizure Behavior** ↑ Seizure score in a concentration-dependent manner ↓ Latency to seizures in a concentration-dependent manner ↑ Distance traveled
	PTZ 15 mM	15, 30, 60					unclear	**Electrophysiology** ↑ Epileptiform discharges ↑ Interictal-like bursts **Neurochemical outcomes** ↑ *c-fos* expression
(Baraban et al., 2007)	PTZ 15 mM	40	Immersion	-	Larvae (3 and 7 dpf)	N.A.	unclear	**Seizure Behavior** ↑ % of movements **Electrophysiology** ↑ Epileptiform discharges ↑ ictal-like discharges
(Berghmans et al., 2007)	PTZ 5, 10, 15, 20, 25 and 30 mM	60	Immersion	-	Larvae (7 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled in all concentrations
(Goldsmith et al., 2007)	PTZ 15 mmol/L	390	Immersion	17 h	Larvae (7 dpf)	N.A.	unclear	**Seizure Behavior** Animals entered *status epilepticus*. The frequency of seizures decreased over the next few hours until the majority of the fish were immobile and unresponsive to a tap on the dish ↑ Distance traveled in 60 min
(Winter et al., 2008)	Various drugs tested as convulsive	17	Immersion	-	Larvae (7 dpf)	N.A.	17	**Seizure Behavior** The tested drugs were classified for positive or negative convulsive activity pentylenetetrazole showed convulsive activity
(Kim et al., 2009)	PTZ 10 mM	10	Immersion	unclear	Adult	unclear	10	**Seizure Behavior** ↑ Velocity ↑ Turn angle in stage III ↓ Avoidance response (passive avoidance test) **Neurochemical outcomes** ↑ *hsp 70* expression = *β-actin* expression = *fabp-7* expression = *ngb* expression
(Hortopan et al., 2010)	PTZ 15 mM	30	Immersion	-	Larvae (3 dpf)	N.A.	30	**Electrophysiology** ↑ typical long-duration high-amplitude burst with multispike activity
(Kim et al., 2010)	PTZ 10 mM	10	Immersion	-	Larvae (5 and 15 dpf)	N.A.	-	**Electrophysiology** ↑ Epileptiform discharges **Neurochemical outcomes** ↓ Number of BrdU labeled cells at 5 dpf in different brain areas = Number of BrdU labeled cells at 15 dpf in different brain areas
(Lee et al., 2010)	PTZ 10 mM	2	Immersion	-	Adult	unclear	2	**Seizure Behavior** ↑ Distance traveled ↑ Angular velocity
		10		360 min			5	**Learning Behavior** ↑ Crossing time **Neurochemical outcomes** ↑ *hsp70* expression

(Wong et al., 2010a)	PTZ 900 mg/L	10 (2 weeks)	Immersion	-	Adult	M:F	6	**Locomotor Behavior** ↑ Erratic movements = Time in top = Transitions to top
(Wong et al., 2010b)	PTZ 1.5 g/L	20	Immersion	-	Adult	M:F	6	**Seizure Behavior** ↓ Latency to the upper half ↑ Transitions to the upper half ↑ Time in the upper half ↑ Freezing bouts ↓ Freezing duration = Erratic movements = Distance traveled ↓ Average velocity = Meandering = Turn angle ↑ Bursts of hyperactivity ↑ Spasms ↑ Corkscrew swimming ↑ Circular swimming **Neurochemical outcomes** ↑ Cortisol levels
(Pineda et al., 2011)	PTZ 3.75, 7.5 and 15 mM PTZ 15 mM	60	Immersion	- 3 min	Adult	unclear	60	**Seizure Behavior** PTZ caused seizures at different times than 15 mM was chosen for the other experiment **Electrophysiology** ↑ interictal epileptiform discharges
(Siebel et al., 2011)	PTZ 2.5, 5 and 15 mM	20	Immersion	-	Adult	M:F	-	**Neurochemical outcomes** = ADP, ATP, and AMP hydrolysis ↓ Adenosine deaminase (ADA) activity (5 and 15 mM) = *ada1* expression = *ada2.1* expression = *ada2.1* expression = *ada2.2* expression = *adal* expression
(Teng et al., 2011)	PTZ 2.5 mM	120	Immersion	-	Embryo (3 and 72 hpf)	N.A.	120	**Seizure Behavior** ↑ Activity **Neurochemical outcomes** ↑ *c-fos* expression
(Vermoesen et al., 2011)	PTZ 20 mM	2	Immersion	-	Larvae (7 dpf)	N.A.	2	**Seizure Behavior** ↑ Total movement
(Baxendale et al., 2012)	PTZ 1.25, 2.5, 5, 10, 20, 40 and 80 mM PTZ 20 mM	10 unclear	Immersion Immersion	- -	Larvae (4 dpf) Embryo (48 hpf) Embryo (50 hpf)	N.A. N.A. N.A.	10 10 90	**Seizure Behavior** ↑ Distance traveled in a concentration-dependent manner ↑ Traces of locomotor activity **Neurochemical outcomes** ↑ *fos* expression ↑ gabra1 expression ↑ gabrg2 expression ↑ *npas4* expression ↑ *bdnf* expression ↑ *c-fos* expression ↑ *pyya* expression
(Ellis et al., 2012)	PTZ 1, 2.5, 5 and 10 mM	10	Immersion	30 min	Larvae (100 – 106 hpf)	N.A.	80	**Light/Dark** ↓ Center/total distance in light

								↑ Fast/center distance in a concentration-dependent manner **Neurochemical outcomes** ↑ *c-fos* in a concentration-dependent manner
(Braida et al., 2012)	PTZ 1,5g/L	20	Immersion	-	Adult	M:F	20	**Seizure Behavior** ↑ Seizure score ↓ Latency to scores
(Orellana-Paucar et al., 2012)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Total movement **Electrophysiology** ↑ Number of interictal-like spikes ↑ Average duration of interictal-like spikes ↑ Number of ictal-like discharges ↑ Average duration of ictal-like discharges ↑ Cumulative duration of epileptiform activity
(Pineda et al., 2013)	PTZ 15 mM	Until time to reach stage III	Immersion	-	Adult	F	Until the time to reach stage III	**Seizure Behavior** Latency to Stage III was measured in fish with stimulator wire insertion and no stimulation. Hindbrain stimulation increases seizure onset latency.
	PTZ 7.5 and 15 mM	30					30	**Electrophysiology** ↑ Cerebral field potential ↓ Scalar entropy
(Afrikanova et al., 2013)	PTZ 20 mM	65	Immersion	5	Larvae (7 dpf)	N.A.	60	**Seizure Behavior** ↑ Average movement ↑ Movement relative to PTZ (%)
							10	**Electrophysiology** ↑ Interictal-like activity = Duration of interictal-like activity
(Banote et al., 2013)	PTZ 220 mg/kg	-	Intraperitoneal injection	2 min	Adult	M	8	**Seizure Behavior** ↑ Seizure scores ↑ Distance traveled ↑ Mean velocity ↑ Mobility (duration and frequency) ↑ Rotation
							240	**Electrophysiology** ↑ Sharp rises in peak amplitude
(Buenafe et al., 2013)	PTZ 20 mM	35	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** PTZ causes seizure behavior; the induced activity was used to compare with other groups **Neurochemical outcomes** ↑ *c-fos* expression
(Fleisch et al., 2013)	PTZ 2.5 mM	20	Immersion	-	Larvae (3 and 5 dpf)	N.A.	40	**Seizure Behavior** ↑ Velocity
(Mei et al., 2013)	PTZ 1, 2.5, 5, 10 and 15 mM	30	Immersion	-	Larvae (3 dpf)	N.A.	30	**Seizure Behavior** ↑ total activity (cm) in a concentration-dependent manner
(Mussulini et al., 2013)	PTZ 5, 7.5, 10 and 15 mM	20	Immersion	-	Adult	M:F	20	**Seizure Behavior** ↑ Seizure score in a concentration-dependent manner

								↓ Latency to seizures in a concentration-dependent manner PTZ 15 mM ↑ Latency to return to score 0 than the other concentrations
(Orellana-Paucar et al., 2013)	PTZ 20 mM	unclear	Immersion	-	Larvae (7 dpf)	N.A.	-	**Neurochemical outcomes** ↑ *c-fos* expression ↑ *bdnf* expression = *gabra* expression = *il10* expression
(Pagnussat et al., 2013)	PTZ 10mM	Until time to reach stage III	Immersion	-	Adult	M	Until the time to reach stage III	**Seizure Behavior** The study exposed single and triplets animals to compare. The mean latency to reach stage III in single animals was lower than in triplets.
(Siebel et al., 2013)	PTZ 7.5 mM	unclear	Immersion	-	Adult	M:F	Until animals reach score III	**Seizure Behavior** PTZ causes seizures, and the latency to reach each seizure score was used to compare with other groups **Neurochemical outcomes** ↑ Ectonucleotidases = *ada1* expression = *ada2.1* expression = *ada2.2* expression
(Challal et al., 2014)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** PTZ showed seizure behavior ↑ Duration of movements
(Gupta et al., 2014)	PTZ 2, 4, 6 and 8 mM	Until reach seizure	Immersion	-	Adult	M:F	Until reach seizure	**Seizure Behavior** ↓ Latency to seizure scores I, II, and III in a concentration-dependent manner
(Koseki et al., 2014)	PTZ 62.5, 125, 250, 500, 1000 µM	60	Immersion	-	Larvae (7 dpf)	N.A.	60	**Locomotor Behavior** ↑ Movement frequency (1000 µM) ↑ Movement duration (1000 µM) ↑ Distance at high-speed movement (1000 µM)
(Long et al., 2014)	PTZ 2.5, 5, 10, 20 and 40 mM	unclear	Immersion	-	Larvae (5 dpf)	N.A.	90	**Locomotor Behavior** ↑ Distance traveled (5 and 10 mM) **Neurochemical outcomes** ↑ *c-fos* expression
(Rahn et al., 2014)	PTZ 15 mM	22	Immersion	7 min	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** ↑ Distance traveled **Neurochemical outcomes** ↑ *c-fos* expression
(Rosa-Falero et al., 2014)	PTZ 3 mg/mL	unclear	Immersion	-	Adult	M:F	unclear	**Seizure Behavior** PTZ causes seizure behavior, and the latency to seizure onset was used to compare with other groups
(Dubey et al., 2015)	PTZ 7.5 mM	unclear	Immersion	-	Adult	unclear	5	**Seizure Behavior** PTZ causes seizure behavior, the latency to reach each seizure score was used to compare with other groups
(Jain et al., 2015)	PTZ 225 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	10	**Seizure Behavior** PTZ causes seizure behavior, and the latency to

								seizure onset was used to compare with other groups
(Johnson et al., 2015)	PTZ 20 mM	60	Immersion	-	Larvae (3 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled **Neurochemical outcomes** ↑ *c-fos* expression
(J.-L. Li et al., 2015)	PTZ 10 mM	60	Immersion	10 min	Larvae (6 dpf)	N.A.	60	**Seizure Behavior** The increase in high-speed moved distance was used as a parameter to compare with other groups
(X. Li et al., 2015)	PTZ 7.5 mM	10	Immersion	-	Larvae (7 dpf)	N.A.	10	**Seizure Behavior** ↑ Distance traveled
(Siebel et al., 2015a)	PTZ 7.5 mM	unclear	Immersion	-	Adult	unclear	unclear	**Seizure Behavior** Animals showed progressive alterations until they reached seizure stage III **Neurochemical outcomes** ↑ *c-fos* expression
(Siebel et al., 2015b)	PTZ 7.5 mM	unclear	Immersion	-	Larvae (7 dpf) Juvenile (45 dpf) and adult	N.A. unclear	30	**Seizure Behavior** PTZ causes seizure behavior, the latency to reach each seizure score, and distance traveled and were used to compare with other groups
(Torres-Hernández et al., 2015)	PTZ 3 mg/mL	30	Immersion	-	Adult	M:F	30	**Seizure Behavior** PTZ was used as a parameter to compare the latency between treated groups
(Barbalho et al., 2016a)	PTZ 15 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Velocity **Neurochemical outcomes** ↑ *c-fos* expression ↑ *cox1* expression
(Barbalho et al., 2016b)	PTZ 15 mM	20	Immersion	- 0.05, 1, 6 h 0.05, 1, 6, 12, 24 and 48 h	Larvae (7 dpf)	N.A.	20	**Seizure Behavior** PTZ causes seizures, the seizure onset latency and number of seizures were used to compare with other groups **Neurochemical outcomes** = *cox2a* expression ↑ *cox2b* expression (0.05 and 1 h after exposure) ↑ *c-fos* expression (0.05h) ↑ *il1b* expression (0.05 and 1 h after exposure)
(Hoffman et al., 2016)	PTZ 10 mM	60	Immersion	-	Larvae (4-7 dpf)	N.A.	60	**Seizure Behavior** ↑ Average activity
(Hong et al., 2016)	PTZ 10 mM	70	Immersion	-	Larvae (3-7 dpf)	N.A.	70	**Electrophysiology** ↑ Field potential ↑ Frequency (Hz) signals ↑ Ictal and interictal-like bursts
(Lopes et al., 2016)	PTZ 2.5 and 15 mM	unclear	Immersion	-	Larvae (3 and 7 dpf)	N.A.	18	**Seizure Behavior** ↑ Cumulative bouts in a concentration-dependent manner

								↓ Latency to the first bout in a concentration-dependent manner
	PTZ 15 mM	60		60 min				**Neurochemical outcomes** ↑ *creb1a* expression ↑ *bdnf* expression ↑ *c-fos* expression = *cpe* expression. = *cpa6* expression ↑ *npy* expression. = *pomca* expression ↑ *chga* expression ↑ *pcsk1nl* expression ↑ *tac1* expression ↑ *nts* expression ↑ *edn1* expression = *gria1a* expression = *gria2a* expression = *grin1a* expression = *grin2b* expression = *slc1a2b* expression
				300 min				= *creb1a* expression ↑ *bdnf* expression ↑ *c-fos* expression = *cpe* expression = *cpa6* expression ↑ *npy* expression = *pomca* expression ↑ *chga* expression ↑ *pcsk1nl* expression ↑ *tac1* expression ↑ *nts* expression = *edn1* expression = *gria1a* expression = *gria2a* expression = *grin1a* expression = *grin2b* expression = *slc1a2b* expression
				1380 min				= *creb1a* expression ↑ *bdnf* expression = *c-fos* expression = *cpe* expression = *cpa6* expression = *npy* expression = *pomca* expression = *chga* expression ↑ *pcsk1nl* expression = *tac1* expression = *nts* expression = *edn1* expression = *gria1a* expression = *gria2a* expression = *grin1a* expression = *grin2b* expression = *slc1a2b* expression
(Meyer et al., 2016)	PTZ 15 mM	30	Immersion (solution in artificial cerebrospinal fluid (aCSF)	-	Larvae (4-5 dpf)	N.A.	60	**Electrophysiology** ↑ Burst rate ↑ Spike rate ↑ Synchrony of neural firing across channels
(Peng et al., 2016)	PTZ 1, 2, 4, 8 and 16 mM	95	Immersion	10	Larvae (5 and 7 dpf)	N.A.	85	**Seizure Behavior** ↑ Average distance moved per minute (8 and 16 mM) at 5 dpf ↑ Average distance moved per minute (4, 8, and 16 mM) at 7 dpf

								Light/Dark test ↑ Average distance moved per minute (8 and 16 mM) in light at 5 dpf ↓ Average distance moved per minute (4 mM) in the dark at 5 dpf ↑ Average distance moved per minute (4 and 8 mM) in light at 7 dpf ↓ Average distance moved per minute (16 mM) in the dark at 7 dpf **Thigmotaxis** ↑ Outer ring / total distance (4 and 16 mM) at 5 dpf ↑ Time spent in the outer ring (2, 4, 8 and 16 mM) at 5 dpf Thigmotaxis parameters are the same at 7 dpf
(Sheng et al., 2016)	PTZ 10 mM	30	Immersion	-	Larvae (6 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled **Neurochemical outcomes** ↑ *c-fos* expression ↑ *BAX/Bcl2* ratio ↓ *Bcl-2* expression
(Torres-Hernández et al., 2016)	PTZ 7.5 mM	80	Immersion	-	Larvae (5-6 dpf)	N.A.	80	**Light/Dark test** ↑ Distance traveled in light ↓ Distance traveled in the dark **Neurochemical outcomes** ↑ *c-fos* expression ↑ *npas4a* expression ↑ *bdnf* expression
(Zheng et al., 2016)	PTZ 2, 4, 10 and 15 mmol	60	Immersion	-	Larvae (7 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled in a concentration-dependent manner ↑ Mean velocity in a concentration-dependent manner **Neurochemical outcomes** ↑ *c-fos* expression in a concentration-dependent manner ↓ *lgi1* expression in a concentration-dependent manner
(Cho et al., 2017)	PTZ 15 mM	-	Gavage	1,5 min	Adult	M+F	60	**Electrophysiology** ↑ Seizures Males had more seizures than Females 50% of males developed SE-like events, while only 25% of females developed SE-like events
(Duy et al., 2017)	PTZ 15 mM	unclear (5 days)	Immersion	1, 5, 14 and 21 days	Adult	unclear	-	**Neurochemical outcomes** ↑ Blood vessel diameter (day 1 interval) ↑ Leukocytes in the brain (day 1 interval) ↑ Total number of leukocytes ↑ Proliferation ↑ Neurogenesis ↑ Proliferating glia

								↑ Reactive gliosis
(Hunyadi et al., 2017)	PTZ 20 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	10	**Electrophysiology** ↑ Frequency of epileptiform discharges ↑ Cumulative duration ↑ % seizing larvae
(Kundap et al., 2017)	PTZ 170 mg/kg	-	Intraperitoneal injection	- 180 min 24 h	Adult	M	10	**Seizure Behavior** PTZ showed seizure scores behavior and low latency to stage 4 **Locomotor activity** = Total distance ↑ Time spent in the lower half of the tank ↓ Time spent in the upper half of the tank **T-maze test** ↑ Total distance traveled ↑ Time spent in the wrong arm ↓ Inflexion ratio ↓ Inflexion ratio **Neurochemical outcomes** ↓ GABA concentration ↑ Glutamate concentration ↓ Acetylcholine concentration ↓ *bdnf* expression = *creb_1* expression ↓ *npy* expression
(Menezes and Da Silva, 2017)	PTZ 10 mM	10	Immersion	-	Adult	M:F	10	**Seizure Behavior** ↓ Latency to stages III, IV, and V with increasing water temperature (22-30 °C)
(Moradi-Afrapoli et al., 2017a)	PTZ 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 mM PTZ 10 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled in all concentrations, but high concentrations have declined in distance in the last minutes ↑ Distance traveled
(Moradi-Afrapoli et al., 2017b)	PTZ 6 mM	3	Immersion	-	Larvae (6 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
(Pisera-Fuster et al., 2017)	PTZ 6 mM	15	Immersion	-	Adult	unclear	15	**Seizure Behavior** ↑ Burst of hyperactivity ↑ Erratic movement ↑ Spasms ↓ Latency to seizures ↓ Latency to spasm
(Turrini et al., 2017)	PTZ 1 and 15 mM	80	Immersion	-	Larvae (4 dpf)	N.A.	80	**Seizure Behavior** ↑ Swimming velocity ↑ Tail movements **Electrophysiology** ↑ Amplitude ($\Delta F/F_0$) (15 mM) ↑ Frequency (peaks/min)
(Winter et al., 2017)	PTZ 5 mM	55	Immersion	-	Larvae (4 dpf)	N.A.	70	↓ Frequency of neuronal network events (Hz) = Power of neuronal network events (mV^2)
(Yang et al., 2017)	PTZ 1, 2, 4, 8 and 16 mM	35	Immersion	-	Larvae (5 dpf)	N.A.	10	**Locomotor Behavior** ↑ Average distance moved (4, 8, and 16 mM) ↓ Distance moved in the center area (4, 8, and 16 mM)

								↓ Time spent in the center (2, 4, 8 and 16 mM)
(Zhang et al., 2017)	PTZ 20 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	120	**Neurochemical outcomes** ↑ *c-fos* expression
(Bertoncello et al., 2018)	PTZ 3 mM	10	Immersion	-	Larvae (7 dpf) and Adults	M:F	10	**Seizure Behavior** PTZ causes seizure behavior, and the seizure scores and the latency to seizure onset were used to compare with other groups
(Brillatz et al., 2018)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Average movement
(Choo et al., 2018)	PTZ 170 mg/kg	-	Intraperitoneal injection	-	Adult	unclear	10	**Seizure Behavior** ↑ Mean seizure score ↓ Latency of seizure onset (stage IV) **Neurochemical outcomes** = *bdnf* expression ↑ *nfkb* expression ↑ *npy* expression ↑ *c-fos* expression ↑ *tnf-α* expression ↑ *il-1* expression
(Copmans et al., 2018a)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Mean actinteg **Electrophysiology** ↑ % of larvae with epileptiform activity ↑ Number of events ↑ Cumulative duration of events
(Copmans et al., 2018b)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Mean actinteg PTZ-induced activity was used to compare with other groups **Electrophysiology** ↑ % of larvae with epileptiform activity ↑ Number of events ↑ Cumulative duration of events
(Fuller et al., 2018)	PTZ 5 and 20 mM	30	Immersion	-	Larvae (3 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled
(Grünspan et al., 2018)	PTZ 10 mM	20	Immersion	-	Adult	unclear	20	**Seizure Behavior** PTZ causes seizures, seizure intensity, % of animals in each score, and latency to score 4 were used to compare with other groups
(Huang et al., 2018)	PTZ 2 and 4 mM	20	Immersion	-	Larvae (7 dpf)	N.A.	20	**Seizure Behavior** ↑ High-speed (greater than 20 mm/s) ↑ Distance traveled
(Ibhazehiebo et al., 2018)	PTZ 10 mM	30	Immersion	-	Larvae (5 dpf)	N.A.	30	**Seizure Behavior** ↑ Total swim activity **Metabolic measurement** ↑ Basal respiration = Maximum respiratory capacity = Non-mitochondrial respiration ↑ Total mitochondrial respiration ↑ Proton leak ↑ ATP-linked respiration

(Jin et al., 2018a)	PTZ 15 mM	20	Immersion	-	Larvae (7 dpf)	N.A.	20	**Seizure Behavior** ↑ Distance traveled The latency to seizure was used to compare to other groups **Neurochemical outcomes** ↑ ROS production ↓ SOD activity ↓ CAT activity ↓ GPx activity ↑ MDA levels ↑ *c-fos* expression ↓ *Mn-sod* expression ↓ *Cu/Zn-sod* expression ↓ *cat* expression ↓ *gpx1a* expression
(Jin et al., 2018b)	PTZ 15 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Speed ↓ Seizure latency **Neurochemical outcomes** ↑ *bdnf* expression ↑ *trkB* expression ↑ *c-fos* expression ↑ *il1β* expression ↑ *nfxb* expression ↑ *hsp70.3* expression ↑ Neutrophil numbers ↑ Macrophage numbers
(Li et al., 2018)	PTZ 1, 2, 4, 8 and 16 mM	unclear	Immersion	-	Larvae (7 dpf)	N.A.	15	**Locomotor Behavior (light/dark)** ↑ Average distance moved in the light (4 and 8 mM) ↓ Average distance moved in the dark (16 mM) ↓ Lighting motor index (fold change) (4, 8 and 16 mM)
(Lin et al., 2018)	PTZ 5 mM	15	Immersion	-	Larvae (5 dpf)	N.A.	15	**Seizure Behavior** ↑ Distance traveled
(Marin-Valencia et al., 2018)	PTZ 5 mM	15	Immersion	-	Larvae (5 dpf)	N.A.	15	**Electrophysiology** ↑ Normalized intensity (Z-scores) ↑ Duration of events ↑ Peak intensity
(Martinez et al., 2018)	PTZ 2.5 mM	10	Immersion	-	Larvae (5 dpf)	N.A.	15	**Seizure Behavior** ↑ Spontaneous movements
(Menezes et al., 2018)	PTZ 10 mM	10	Immersion	-	Larvae (7 dpf) and Adult	unclear	10	**Seizure Behavior** PTZ causes seizure behavior, the latency to reach each seizure score was used to compare with other groups
(Podlasz et al., 2018)	PTZ 10 mM	10	Immersion	-	Larvae (5 and 7 dpf)	N.A.	10	**Seizure Behavior** ↑ Mean velocity ↑ Active velocity = Time moving = Rest duration ↑ Active duration **Neurochemical outcomes** ↑ *c-fos* expression = *galn* expression
(Rishitha and Muthuraman, 2018)	PTZ 7.5 mM	3-4	Immersion	-	Adult	M	unclear	**Cognitive Behavior (light/dark test)** ↓ Time spent in the light chamber

								↑ Number of entry to the dark chamber **Memory Behavior (Partition preference test)** ↓ Time spent in the target chamber ↓ Entry to the target chamber **Three horizontal compartment test** ↓ Time spent in the upper segment ↑ Time spent in the lower segment **Neurochemical outcomes** ↓ GSH levels ↑ AChE levels ↑ TBARS
(Rosch et al., 2018)	PTZ 20 mM	150	Immersion	-	Larvae (5 dpf)	N.A.	150	**Electrophysiology** ↑ Largery in low frequencies (<2Hz) ↑ Broadband activity
(Steele et al., 2018)	PTZ 2.65, 26.5, 132.25, 264.49, 1057.97 mg/L	50	Immersion	-	Larvae	N.A.	50	**Locomotor Behavior (light/dark test)** ↓ Total distance in the dark (132.25 and 264.49 mg/L) ↓ Total counts in the dark (132.25 and 264.49 mg/L) ↑ Bursting Distance in the dark (264.49 mg/L) ↓ Duration bursting in the dark (1057.97 mg/L) ↓ Cruising distance in the dark (132.25 and 264.49 mg/L) ↓ Cruising duration in the dark (132.25 and 264.49 mg/L) ↓ Freezing distance in the dark (132.25, 264.49, 1057.97 mg/L) ↓ Freezing duration in the dark (132.25, 264.49, 1057.97 mg/L) ↑ Total distance in the light (1057.97 mg/L) ↑ Total counts in the light (1057.97 mg/L) ↑ Bursting Distance in the light (264.49 and 1057.97 mg/L) ↑ Duration bursting in the light (132.25, 264.49 and 1057.97 mg/L) = Cruising distance in the light (132.25 and 264.49 mg/L) = Cruising duration in the light (132.25 and 264.49 mg/L) ↑ Freezing distance in the light (1057.97 mg/L) ↑ Freezing duration in the light (264.49 and 1057.97 mg/L)
(Wang et al., 2018)	PTZ 2.5, 5 and 15 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior**

								↑ % fish that reach each seizure stage in a concentration-dependent manner ↑ Distance traveled in a concentration-dependent manner ↑ Duration of time spent in a mobile state in a concentration-dependent manner ↑ Duration of time spent in a highly mobile state in a concentration-dependent manner ↓ Latency to reach each seizure stage in a concentration-dependent manner
(Zheng et al., 2018)	PTZ 2, 4 and 6 mM	60	Immersion	-	Larvae (7 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled (4 and 6 mM) ↑ Mean velocity (4 and 6 mM) **Neurochemical outcomes** ↓ *stx1b* expression
(Fu et al., 2019)	PTZ 10mM	15	Immersion	-	Larvae (6 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
(Aourz et al., 2019)	PTZ 20 mM	65	Immersion	5 min	Larvae (7 dpf)	N.A.	60	**Seizure Behavior** ↑ Total movement **Electrophysiology** ↑ Interictal-like spikes ↑ Ictal spikes ↑ Total cumulative duration of epileptiform activity
(Acevedo-Canabal et al., 2019)	PTZ 7.5 mM	50	Immersion	-	Larvae (5 dpf)	N.A.	20	**Light/Dark test** ↑ Distance traveled in light = center occupancy (%)
(Brueggeman et al., 2019)	PTZ 2µmol/L	30	Immersion	-	Larvae (5 and 7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
(Canzian et al., 2019a)	PTZ 10 mM	20	Immersion	-	Adult	M:F	20	**Seizure Behavior** ↑ Seizure scores in 20 min
				60 min			6	**Aggressive Behavior** ↑ Number of aggressive episodes ↑ Duration of aggressive episodes
							6	**Exploratory activity** ↑ Transitions to close area
				180 min			6	**Aggressive Behavior** ↑ Number of aggressive episodes ↑ Duration of aggressive episodes
								Exploratory activity ↑ Transitions to close area
				360 min			6	**Aggressive Behavior** ↑ Number of aggressive episodes ↑ Duration of aggressive episodes
							6	**Exploratory activity** ↑ Transitions to close area
				24 h			6	**Aggressive Behavior** ↑ Number of aggressive episodes

								↑ Duration of aggressive episodes
							6	**Exploratory activity** ↑ Transitions to close area
				48 h			6	**Aggressive Behavior** ↑ Number of aggressive episodes ↑ Duration of aggressive episodes
							6	**Exploratory activity** ↑ Transitions to close area
				57 h			6	**Aggressive Behavior** = Number of aggressive episodes = Duration of aggressive episodes
							6	**Exploratory activity** **=** Transitions to close area
(Canzian et al., 2019b)	PTZ 10 mM	20	Immersion	24h	Adult	M:F	6	**Locomotor Activity** = Distance traveled = Absolut turn angle **Anxiety Behavior (novel tank)** ↑ Transitions to the top area ↑ Time spent in the top area = Average duration per entry in the top area ↓ Latency to enter top área **Anxiety Behavior (light/dark)** ↓ Time in the lit area = Number of crossings = Latency to enter the dark area ↓ Episodes of risk assessment
							5	**Shoaling Behavior** ↑ Inter-fish distance ↑ Shoal area ↓ Social interaction **Memory (inhibitory avoidance)** ↓ Memory retention index **Biochemical outcome** = Cortisol levels (whole body)
(Choo et al., 2019)	PTZ 170 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	10	**Seizure Behavior** ↑ Mean seizure score ↓ Mean seizure onset time ↑ Distance traveled = Time spent in the upper zone = Time spent in the lower zone
				180 min 24 h			5	**T-maze test** = 3 hour inflexion ratio ↓ 24 hour inflexion ratio Neurochemical outcomes ↓ GABA levels ↓ Glutamate levels ↓ Acetylcholine levels = *bdnf* expression = *npy* expression = *creb-1* expression
(Copmans et al., 2019)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Movement (actinteg/5 min) **Electrophysiology**

								↑ % of larvae with epileptiform activity ↑ Number of events ↑ Cumulative duration of events
(Fontana et al., 2019)	PTZ 10 mM	20	Immersion	-	Adult	M:F	20	**Seizure Behavior** PTZ causes seizures, the seizure intensity (area under the curve), score IV events, and latency to reach seizure score IV were used to compare with other groups **Neurochemical outcomes** = SOD activity ↑ CAT activity = GST activity ↓ NPSH levels ↑ TBARS levels ↑ Carbonylated proteins levels = Cell viability (MTT assay) = LDH activity
(Kozioł et al., 2019)	PTZ 20 mM	unclear	Immersion	-	Larvae (6-7 dpf)	N.A.	unclear	**Seizure Behavior** ↑ Average movement
(Kumari et al., 2019)	PTZ 8 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** PTZ causes seizures, and the latency to clonus-like seizures was used to compare with other groups ↑ Distance traveled ↑ Mean speed **Neurochemical outcomes** ↑ *c-fos* expression
(Kundap et al., 2019b)	PTZ 80 mg/kg	10 days	Intraperitoneal injection	unclear	Adult	unclear	10	**Seizure Behavior** ↑ Seizure score from day 4 to day 10 **Three-axis maze test** ↑ Latency to reach the feeding chamber ↓ Inflexion ratio **Neurochemical outcomes** ↓ GABA concentration ↓ Glutamate concentration ↓ Acetylcholine concentration ↓ GABA/glutamate ratio = *ccl2* expression ↑ *tlr4* expression ↑ *ifn- γ* expression = *tnf-α* expression ↑ *il-1* expression
(Kundap et al., 2019b)	PTZ 170 mg/kg	-	Intraperitoneal injection	- 180 min	Adult	M:F	10	**Seizure Behavior** PTZ showed seizure scores behavior and low latency to stage 4 **Locomotor activity** = Total distance = Distance in the upper half ↑ Time spent in the lower half of the tank ↓ Time spent in the upper half of the tank **T-maze test** ↑ Total distance traveled ↑ Time spent in the wrong arm ↓ Inflexion ratio

				24 h				↓ Inflexion ratio **Neurochemical outcomes** ↓ GABA concentration ↑ Glutamate concentration = Acetylcholine concentration ↓ *bdnf* expression ↑ *creb_1* expression ↓ *npy* expression ↓ BrdU labeled cells (proliferation) ↓ BrdU labeled cells (cerebellum)
(Liao et al., 2019)	PTZ 10 mM	60	Immersion	-	Larvae (6 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled **Neurochemical outcomes** ↑ *c-fos* expression ↑ *npas4a* expression
(M. Liu et al., 2019)	PTZ 40 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Movement (actinteg/5 min)
(Liu and Baraban, 2019)	PTZ 10 mM	90	Immersion	40 min	Larvae (5 and 6 dpf)	N.A.	unclear	**Electrophysiology** ↑ Epileptiform events with ictal-like seizures
(Mazumder et al., 2019)	PTZ 8 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** ↑ Distance traveled ↑ Average speed The latency to seizure was used to compare with other groups **Neurochemical outcomes** ↑ *c-fos* expression
	PTZ 6 mM	30	Immersion	-	Adult	unclear	30	**Seizure Behavior** PTZ causes seizure behavior, the seizure score, latency to clonic-like seizure, and occurrence of tonic-like seizure were used to compare with other groups **Neurochemical outcomes** ↑ *pik3ca* expression ↑ *pik3r1* expression ↑ *akt1* expression ↑ *mtor* expression ↑ *rps6* expression = *rps6kb1* expression
(Nieoczym et al., 2019)	PTZ 20 mM	30	immersion	5 min	Larvae (117 hpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
		5					20	**Electrophysiology** ↑ Epileptiform events
(Rajesh et al., 2019)	PTZ 20 mM	unclear	Immersion	-	Larvae (6 dpf)	N.A.	60	**Seizure Behavior** ↑ Number of twitches/s ↑ Distance traveled
(Ren et al., 2019a)	PTZ 5 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** PTZ causes seizure behavior, the seizure frequency was used to compare with other groups ↑ Movement
(Ren et al., 2019b)	PTZ 5 mmol/L	30	Immersion	10 min	Larvae (7 dpf)	N.A.	20	**Seizure Behavior** ↑ Distance traveled
(Samarut et al., 2019)	PTZ 2.5 and 5 mM	30	Immersion	-	Larvae (5 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Fast activity
(Zhang et al., 2019)	PTZ 20 mmol	10	Immersion	-	Larvae (4 df)	N.A.	10	**Seizure Behavior** ↑ Movement times

								↑ Distance traveled **Neurochemical outcomes** ↑ *c-fos* expression
(Sourbron et al., 2019)	PTZ 20 mmol	10	Immersion	-	Larvae (7 dpf)	N.A.	10	**Seizure Behavior** ↑ Locomotor activity **Electrophysiology** ↑ epileptiform events
(Tanwar et al., 2019)	PTZ 8 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** PTZ causes seizure behavior, and the latency to clonic seizure was used to compare with other groups **Neurochemical outcomes** ↑ *c-fos* expression ↑ *pik3ca* expression ↑ *pik3r1* expression ↑ *akt* expression ↑ *mtor* expression ↑ *rps6* expression ↑ *rps6kb1* expression
(Diaz Verdugo et al., 2019)	PTZ 20 mM	90	Immersion	-	Larvae (5-7 dpf)	N.A.	90	**Electrophysiology** ↑ Overall ratio of active neurons
(Xu et al., 2019)	PTZ 1.25, 2.5, 5, 10, 20 and 30 mM	70	Immersion	10 min	Larvae (5 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled ↑ High-speed moved distance **Neurochemical outcomes** ↑ *c-fos* expression
(Bandara et al., 2020)	PTZ 0, 0.1, 0.4, 1, 4, 10, 40 and 100 mM	20	Immersion	-	Larvae (5 dpf)	N.A.	20	**Seizure Behavior** ↑ Seizure score in a concentration-dependent manner ↓ Latency to seizures in a concentration-dependent manner ↑ Distance traveled **Electrophysiology** ↑ Epileptiform-like discharges ↑ Burst frequency
(Brillatz et al., 2020a)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Average movement
(Brillatz et al., 2020b)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Average movement
(Brillatz et al., 2020c)	PTZ 20 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Average movement (%)
(Brunal et al., 2020)	PTZ 2, 5, 10 and 20 mM	15, 30 and 60	Immersion	-	Larvae (6 dpf)	N.A.	-	**Neurochemical outcomes** ↓ cx36 protein levels (30 min exposure) = *caspase-3* positive cells
(Campos-Rodriguez et al., 2020)	PTZ 20 mM	45	Immersion	-	Larvae (7 – 10 dpf)	N.A.	45	**Seizure Behavior** ↑ Distance traveled ↑ Cumulative jump duration
(Y.-C. Chen et al., 2020)	PTZ 10 mM	5 (3 days)	Immersion	-	Adult	M	5	**Seizure Behavior** Causes seizure behavior ↑ latency to score 4 throughout the days of exposure **Neurochemical outcomes** = *cdnf* expression = *manf* expression = *slc17a6a/ vglut2* expression = *slc32a1/vgat* expression = *gad2a/gad65* expression

								= *gad1b/gad67* expression = *gfap* expression
(P.-Y. Chen et al., 2020)	PTZ 10 mM	120	Immersion	-	Larvae (5 dpf)	N.A.	300	**Seizure Behavior** PTZ causes seizure behavior and was used to compare whether pyridoxine had any effect on the survival of animals exposed to inducers **Neurochemical outcomes** ↑ *leptin-b* expression
(Chung et al., 2020)	PTZ 170 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	10	**Seizure Behavior** ↑ Seizure score ↓ Mean seizure onset time ↑ Total distance ↓ Time spent in the upper zone
(Decui et al., 2020)	PTZ 3 mM	10	Immersion	-	Larvae (7 dpf)	N.A.	10	**Seizure Behavior** PTZ causes seizure behavior, and the seizure scores and the latency to seizure onset were used to compare with other groups **Exploratory/Locomotor Behavior** ↓ Number of crossings
(Gawel et al., 2020)	PTZ 20 mM	30	Immersion	5 min	Larvae (7 dpf)	N.A.	30 20	**Seizure Behavior** ↑ Distance traveled **Electrophysiology** ↑ Epileptiform events ↑ Mean duration of events **Neurochemical outcomes** ↑ *c-fos* expression ↑ *bdnf* expression
(Gong et al., 2020)	PTZ 10 mM	35	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Velocity ↑ Seizure activity ↑ Seizure duration ↑ Number of bursts **Neurochemical outcomes** ↑ *npas4a* expression ↑ *c-fos* expression ↑ *pyya* expression ↑ *bdnf* expression = *pvalb5* expression = *calb2* expression = *prkacab* expression ↑ *gabra1* expression ↑ *gad1* expression ↑ *glsa* expression ↑ *glula* expression = *glud1a* expression = *abat* expression = *gat1* expression
(Gupta et al., 2020)	PTZ 15 mM	30	Immersion	-	Larvae (5 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
(Hamanaka et al., 2020)	PTZ 2.5, 5 and 15 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** ↑ Swimming distance in a concentration-dependent manner The % of fish that reach each seizure stage was used to compare with other groups
(Hengel et al., 2020)	PTZ 15 mM	10	Immersion	-	Larvae (7 dpf)	N.A.	10	**Seizure Behavior** ↑ Time spent moving

		45						↑ Time spent at high speed ↑ Distance traveled **Neurochemical outcomes** ↑ *c-fos* expression
(Hwang et al., 2020)	PTZ ([ ] unclear)	30	Immersion	-	Larvae (5 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Moving duration ↑ Frequency of movements
	PTZ 220 mg/kg	N.A.	Gavage	-	Adult	unclear	25	**Electrophysiology** ↑ Relative power signal (%) ↑ Average numbers of seizure-like events ↑ Total duration of seizure-like events
(Jaiswal et al., 2020)	PTZ 170 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	10	**Seizure Behavior** ↑ Mean seizure score ↓ Mean seizure score onset for stage 4 **Neurochemical outcomes** = *bdnf* expression = *nfkb* expression = *c-fos* expression
(Jin et al., 2020)	PTZ 15 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	5 30	**Seizure Behavior** ↑ Seizure scores **Locomotor Behavior** ↑ Distance traveled ↑ Speed **Electrophysiology** ↑ Abnormal discharges frequency **Neurochemical outcomes** ↑ *c-fos* expression ↑ *ppar α* expression ↑ *ppar γ* expression
(Jones et al., 2020)	PTZ 20 mM	60	Immersion	-	Larvae (3 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled **Neurochemical outcomes** ↑ *c-fos* expression
(Kamiński et al., 2020)	PTZ 20 mM	5	Immersion	-	Larvae (6 dpf)	N.A.	20	**Electrophysiology** ↑ Number of epileptiform events ↑ Cumulative duration of epileptiform events ↑ Mean duration of epileptiform events
(Kanyo et al., 2020)	PTZ 10 mM	60	Immersion	-	Larvae (7 dpf)	N.A.	120	**Seizure Behavior** ↑ Activity (stage I and II)
(Kumari et al., 2020)	PTZ 1,25 mM	30 (22 days)	Immersion	-	Adult	M	30	**Seizure Behavior** ↑ Seizure score over 22 days **Neurochemical outcomes** ↑ *c-fos* expression ↑ *crebbpa* expression ↑ *crebbpb* expression ↑ Glutamate/GABA ratio
(Lee et al., 2020a)	PTZ 15 mM	10	Immersion	-	Adult	M:F	10	**Electrophysiology** ↑ Ictal events ↑ Inter-ictal events ↑ Amplitude (a.u.) ↑ Delta amplitude ↑ Theta amplitude ↑ Alpha amplitude ↑ Beta amplitude
(Lee et al., 2020b)	PTZ 15 mM	60	Immersion	-	Larvae (5 – 7 dpf)	N.A.	60	**Electrophysiology** ↑ Ictal and interictal events
(J. Li et al., 2020)	PTZ 40 mM	30	Immersion	-	Larvae (5 dpf)	N.A.	30	**Locomotor Activity**

	PTZ 40 mM	15			Larvae (7 dpf)	N.A.	unclear	↑ Average actinteg (hyperlocomotion) **Electrophysiology** ↑ Epileptiform discharges ↑ Power spectral density
(X. Li et al., 2020)	PTZ 15 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** ↑ Distance traveled
(Sharma et al., 2020)	PTZ 8 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** ↑ Distance traveled ↑ Velocity Latency to stage III was used to compare with other groups **Neurochemical outcomes** ↑ *c-fos* expression ↑ *bdnf* expression = *il10* expression
(Thornton et al., 2020)	PTZ 5 mM	20	immersion	-	Larvae (5 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled Hyperlocomotion
(Wasilewska et al., 2020)	PTZ 1.5 and 15 mM	30	Immersion	-	Larvae (4 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Low activity phase light ON (15 mM) ↑ High activity phase light OFF (15 mM) ↑ Velocity
(Weuring et al., 2020)	PTZ 5 mM	unclear	Immersion	-	Larvae	N.A.	unclear	**Electrophysiology** ↑ Burst movements
(Zhang et al., 2020)	PTZ 5 mM	30 3 30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Speed Latency to reach seizure stages I-III was used to compare with other groups **Neurochemical outcomes** ↑ *c-fos* expression ↑ c-fos protein ↑ *il-6* expression ↑ *il-1β* expression ↑ tnf-α protein ↑ il-1β protein ↑ Neutrophils number ↑ Macrophages number ↑ *c-fos* expression ↑ *il-6* expression ↑ *il-1β* expression ↑ *tnf-α* expression ↑ Neutrophils number ↑ Macrophages number **Electrophysiology** PTZ presents abnormal discharge frequency
(Almeida et al., 2021)	PTZ 5 mM	10	Immersion	-	Adult	M:F	10	**Seizure Behavior** PTZ causes seizure behavior, and the seizure scores and the latency to seizure onset were used to compare with other groups **Neurochemical outcomes** ↑ *c-fos* expression = *caspase-3* expression = *interleukin 1β* expression
(Brillatz et al., 2021)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Average movement
(Canzian et al., 2021)	PTZ 7.5 mM	10	Immersion	-	Adult	M:F	10	**Seizure Behavior**

								PTZ causes seizures, the seizure scores, seizure intensity, % of animals in each score, latency to score 4, and number of score 4 events were used to compare with other groups
(Chipiti et al., 2021)	PTZ 20 mM	35	Immersion	-	Larvae (6 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
(Choo et al., 2021)	PTZ 12 mM	30	Immersion	-	Larvae (5 dpf)	N.A.	150	**Seizure Behavior** ↑ Distance traveled ↑ Maximum acceleration
(Dang et al., 2021)	PTZ 15 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Seizure scores ↓ Latency to seizure ↑ Distance traveled ↑ Swimming speed **Neurochemical outcomes** ↑ *c-fos* expression ↑ *il-6* expression ↑ *il-1β* expression ↑ *nfkb* expression ↑ *cat* expression ↓ *gpx1a* expression ↓ *Mn-sod* expression ↓ CAT activity ↓ GPx activity ↓ SOD activity ↑ Number of apoptotic cells ↑ Immunocytes recruitment
(Engelke et al., 2021)	PTZ 15 mM	20	Immersion	15 min	Larvae (5 dpf)	N.A.	5	**Light/dark test** PTZ was used as a positive-control compound to induce seizure-like behavior in zebrafish. Distance, high-speed movements, movement frequency and distance on light on, movement frequency and distance in cycles were used as parameters to compare with other groups
(Ferreira et al., 2021)	PTZ 7.5 mM	unclear	Immersion	-	Adult	M:F	unclear	**Seizure Behavior** PTZ causes seizure behavior, the latency to reach each seizure score was used to compare with other groups
(Garbinato et al., 2021)	PTZ 5 mM	10	Immersion	-	Adult	M:F	10	**Seizure Behavior** PTZ causes seizure behavior. The occurrence (%) for each stage and latency to reach each stage of seizure were used to compare with other groups **Neurochemical outcomes** = *p70-s6k* expression = *il-1β* expression = *caspase-3* expression
(Gawel et al., 2021)	PTZ 20 mM	35	Immersion	5 min	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled **Electrophysiology** ↑ Number of events ↑ Mean duration of events **Neurochemical outcomes** ↓ GABA levels = Glutamate levels

								↑ GABA/glutamate levels ↓ *gabra1a* expression ↑ *grin1a* expression ↑ *grin2b* expression ↑ *gria1a* expression = *gria2a* expression ↓ *gria3b* expression
(Goi et al., 2021)	PTZ 10 mM	10	Immersion	-	Adult	M:F	10	**Seizure Behavior** PTZ causes seizures, and the latency to reach seizure scores I, II, and III was used to compare with other groups
(Griffin et al., 2021)	PTZ 1, 2.5, 5 and 15 mM	10, 30 and 60	Immersion	-	Larvae	N.A.	10, 30 and 60	**Seizure Behavior** ↑ Seizure-like events in a concentration-dependent manner. PTZ 15 mM had a lower number of events in 60 min because of increased larvae mortality
(Hacke et al., 2021)	PTZ 7.5 mM	20	Immersion	-	Adult	unclear	20	**Seizure Behavior** PTZ causes seizure behavior, the latency to seizure score IV, % animals that reach each stage were used to compare with other groups **Neurochemical outcomes** ↑ MDA levels ↓ GSH levels ↑ NO levels ↓ CAT activity
(Jain et al., 2021)	PTZ 20 mM	unclear	Immersion	-	Larvae (6 dpf)	N.A.	unclear	**Seizure Behavior** ↑ Total distance traveled
(Kim et al., 2021)	PTZ 1 and 5 mM	60	Immersion	-	Larvae (5 dpf) and Adult	N.A.	-	**Neurochemical outcomes** ↓ 5-HT concentration ↑ Normetanephrine concentration = Glutamine concentration ↑ GABA concentration ↓ Excitatory/Inhibitory ratio ↓ Neurosteroid E2 concentration = Dihydrotestosterone concentration ↑ Cortisol concentration ↓ Progesterone concentration ↓ 5α-dihydroProgesterone concentration ↓ Allo-Pregnanolone concentration ↓ 5α-reductase ratio ↓ Aromatase ratio ↑ ROS levels
(Kozioł et al., 2021)	PTZ 20 mM	15	Immersion	-	Larvae (7 dpf)	N.A.	15	**Seizure Behavior** ↑ Distance traveled **Electrophysiology** ↑ Epileptiform events
(Mante et al., 2021)	PTZ 170 mg/kg	N.A.	Intraperitoneal injection	unclear	Adult	M	10	**Seizure Behavior** ↑ Seizure scores ↓ Latency to seizure ↑ Distance traveled **Anxiety Behavior** ↑ Time spent in the lower zone

(Partoens et al., 2021)	PTZ 6 mM	30 25	Immersion	-	Larvae (5 and 8 dpf)	N.A.	30 10	**Seizure Behavior** ↑ Epileptiform activity in kif2a-/- larvae after PTZ incubation **Electrophysiology** Enlargement of a polyspiking event in kif2a-/- larvae after PTZ incubation
(Matias Pereira et al., 2021)	PTZ 170 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	10	**Seizure Behavior** ↑ Seizure duration ↑ hyperlocomotion ↑ % Seizure = Freezing ↑ Loss of posture ↑ Immobility ↑ Corkscrew swimming **Locomotor Behavior** ↓ Distance traveled = Average acceleration = Average velocity
(Pieróg et al., 2021a)	PTZ 10 mM	unclear	Immersion	-	Adult	M:F	unclear	**Seizure Behavior** PTZ causes seizure behavior, the latency to reach each seizure score was used to compare with other groups
(Pieróg et al., 2021b)	PTZ 10 mM	unclear	Immersion	-	Adult	M:F	unclear	**Seizure Behavior** PTZ causes seizures, and the latency to reach each seizure score was used to compare with other groups
(Ren et al., 2021)	PTZ 15 mM	30	Immersion	-	Larvae (6 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled ↑ Velocity Latency to reach seizure scores I, II, and III was used to compare with other groups **Electrophysiology** ↑ Abnormal discharge frequency **Neurochemical outcomes** ↑ *c-fos* expression ↓ *cat* expression ↑ *gpx* expression ↑ *gpx4a* expression ↓ *sod1* expression = *sod2* expression = *gsr* expression ↑ *gss* expression ↑ *gsto2* expression ↑ *gstp1* expression ↑ *gclm* expression
(da Silva et al., 2021)	PTZ 7.5 mM	unclear	Immersion	-	Adult	M:F	unclear	**Seizure Behavior** PTZ causes seizure behavior, and the latency to seizure onset was used to compare with other groups
(Sturgeon et al., 2021)	PTZ 5 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
(Suo et al., 2021)	PTZ 10 mM	30	Immersion	-	Larvae (5 dpf)	N.A.	30	**Seizure Behavior** PTZ causes seizures, total distance traveled, average swimming velocity, movement/no movement time (s), and mobility were

								used to compare with other groups **Neurochemical outcomes** ↑ *c-fos* expression.
(Xavier et al., 2021)	PTZ 7.5 mM	unclear	Immersion	-	Adult	unclear	unclear	**Seizure Behavior** PTZ causes seizures, and the latency to reach seizure scores I, II, and III was used to compare with other groups
(Wang et al., 2021)	PTZ 3 mmol/L	10	Immersion	-	Larvae (4 dpf)	N.A.	10	**Seizure Behavior** ↑ Movement velocity **Neurochemical outcomes** ↑ *c-fos* expression
(Gwedela et al., 2022)	PTZ 14 mM	40, 15, 30, 60 and 70	Immersion	-	Larvae (7 dpf)	N.A.	40 15 70 30 and 60	**Seizure Behavior** ↑ Distance traveled Latency to stage III and frequency of stage III were used to compare with other groups **Electrophysiology** ↑ Frequency of seizure-like activity (Hz) **Neurochemical outcomes** ↑ *c-fos* expression ↑ *npas4a* expression
(Lima et al., 2022)	PTZ 7.5 mM	unclear	unclear	-	Juvenile	M:F	60	**Seizure Behavior** PTZ causes seizure behavior, and the latency to seizure scores onset was used to compare with other groups
(Sharma et al., 2022)	PTZ 6 mM	30	Immersion	-	Adult	M	30	**Seizure Behavior** PTZ causes seizures, the seizure latency, maximum seizure severity score, and seizure incidence were used to compare with other groups
	PTZ 1.25 mM	30 (25 days)	Immersion	24 h	Adult	M	30	↑ Seizure severity on 25th day as compared to 5th, 10th, 15th and 20th days
(da Silva et al., 2022)	PTZ 7.5 mM	unclear	Immersion	-	Adult	M:F	unclear	**Seizure Behavior** PTZ causes seizures, and the latency to reach seizure scores I, II, and III was used to compare with other groups

The sex of the animals used was computed as: M, for males; F, for females; M:F, when male and female were included but tested and analyzed as a mixed group; not applicable (N.A.) for larvae and unclear when the sex of the animals was not reported; -, means no interval between exposure and test. Unclear was used to address missing information in the articles. The main findings were described as: ↑, higher than the control group; ↓, lower than the control group; =, no difference when compared to the control group; Abbreviations: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT); 5-hydroxytryptamine (5-HT)*;* acetylcholinesterase (AchE); adenosine diphosphate (ADP)*;* adenosine monophosphate (AMP)*;* adenosine triphosphate (ATP); bromodeoxyuridine (BrdU)*;* catalase (CAT)*;* days post-fertilization (dpf); gamma-aminobutyric acid (GABA); glutathione (GSH)*;* glutathione peroxidase (GPx)*;* glutathione s-transferase (GST)*;* hours post-fertilization (hpf); lactate dehydrogenase (LDH)*;* malondialdehyde (MDA); nitric oxide (NO)*;* non-protein sulfhydryl groups (NPSH); reactive oxygen species (ROS)*;* superoxide dismutase (SOD); thiobarbituric acid reactive species (TBARS)*;*

Table 2. Qualitative description of studies reporting chemically-induced seizure protocols by kainic acid (KA) in research with zebrafish.

Reference	Seizure inducer	Duration of	Administration route	Interval inducer exposur	Developmental stage during	Sex	Duration test (min)	Main findings

		exposure (min)		e and test	outcome assessment			
(Kim et al., 2010)	KA 50, 100 and 200 µM	10	Immersion	-	Larvae (5 and 15 dpf)	N.A.	-	**Electrophysiology** ↑ Epileptiform discharges **Neurochemical outcomes** ↓ Number of BrdU labeled cells at 5 dpf (KA 200 µM) in different brain areas
(Alfaro et al., 2011)	KA 1, 2, 4, 6 and 8 mg/kg	-	Intraperitoneal injection	0	Adult	M:F	60	**Seizure Behavior** ↑ Seizure scores in a dose-dependent manner 8 mg/kg showed status epilepticus ↓ Latency to seizures in a dose-dependent manner 4 mg/kg above evoked levels of mortality of around 20%
(Sierra et al., 2012)	KA 6 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	60	**Seizure Behavior** ↑ Seizure scores in 60 min ↓ Latency to seizures KA causes *status epilepticus* in some animals KA causes some deaths
(Menezes et al., 2014)	KA 100, 300 and 500 µM	31	Immersion	-	Larvae (7, 15 and 30 dpf)	N.A.	30	**Seizure Behavior** ↓ Distance traveled on 7 dpf ↑ Distance traveled on 15 dpf (500 µM) = Distance traveled on 30 dpf
	KA 500 µM at 24 hpf, 7 dpf and 15 dpf + 6 mg/kg at 2 mpf	30	Intraperitoneal injection	2 months	Juvenile (2 mpf)	N.A.	30	**Seizure Behavior** ↑ Seizure scores
(Feas et al., 2017)	KA 1.25, 2.5, 5 and 10 mM	25	Immersion	10 min	Larvae (5 dpf)	N.A.	15	**Locomotor Behavior** ↑ Spontaneous movements
(Mussulini et al., 2018)	KA 4, 5 and 6 mg/kg	-	Intraperitoneal injection	-	Adult	M	60	**Seizure Behavior** ↑ Seizure scores in 60 min ↓ Latency to score V in a dose-dependent manner ↑ Seizure intensity in first 20 min in a dose-dependent manner ↑ Seizure intensity in 60 min in a dose-dependent manner ↑ % of animals in status epilepticus (5 and 6 mg/kg) ↑ Mortality in a dose-dependent manner **Neurochemical outcomes** ↓ Glial fibrillary acidic protein (GFAP) brain (12 h after KA exposure) ↓ Intracellular brain S100B levels (12 and 72h after KA exposure) ↓ Glutamate uptake (3 and 12 h after KA exposure) in the forebrain
	KA 5 mg/kg			24 h				**Locomotor Behavior** ↓ Distance traveled ↓ Speed ↑ Immobility
				72 h				↓ Distance traveled

				96 h 168 h				↓ Speed ↑ Immobility ↓ Distance traveled = Speed ↑ Immobility ↓ Distance traveled = Speed ↑ Immobility
(Kundap et al., 2019b)	KA 3 mg/kg	1 day	Intraperitoneal injection	unclear	Adult	unclear	10	**Seizure Behavior** ↑ Seizure score at day 1 but decreases with time **Three-axis maze test** ↑ Latency to reach the feeding chamber
(Heylen et al., 2021)	KA 2.5, 5, 10 and 20 mg/kg	-	Microinjection into pericardium	1 day post injection (dpi) until 4 days post injection (dpi)	Larvae (7 dpf)	N.A.	150	**Seizure Behavior** ↓ Movement at 3dpi ↑ Seizure activity **Electrophysiology** ↑ Epileptiform discharges starting from 2dpi **Neurochemical outcomes** ↑ *bax* expression (1 dpi) ↑ *casp9* expression (3 dpi) ↑ *il1b* expression (1 and 3 dpi) ↑ *il8* expression (1 dpi) = *tnf-α* expression ↑ *faz* expression (2 dpi) ↑ *irf1b* expression ↑ *c4* expression (2 dpi) ↑ *csf1ra* expression (2 dpi) = *nrros* expression
(Moro et al., 2021)	KA 6 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	-	**Neurochemical outcomes** = Oxygen consumption rate representation = Routine respiration = ATP-linked respiration = Proton leak = Maximal respiration; ↑ Spare respiratory capacity = Non-mitochondrial respiration
(de Farias et al., 2022)	KA 5 mg/kg	-	Intraperitoneal injection	-	Adult	M:F	60	**Seizure Behavior** KA causes seizures, the seizure scores, the area under the curve, latency to score 5, and time of SE were used to compare with other groups **Neurochemical outcomes** ↓ Glutamate uptake ↑ DCFH oxidation ↑ TBARS levels = SOD activity = CAT activity ↑ SOD/CAT activities = GR activity = Gpx activity

The sex of the animals used was computed as: M, for males; F, for females; M:F, when male and female were included but tested and analyzed as a mixed group; not applicable (N.A.) for larvae and unclear when the sex of the animals was not reported; -, means no interval between exposure and test. Unclear was used to address missing information in the articles. The main findings were described as: ↑, higher than the control group; ↓, lower than the control group; =, no difference when compared to the control group; Abbreviations: adenosine triphosphate (ATP); bromodeoxyuridine (BrdU)*;* catalase (CAT)*;* days post-fertilization (dpf); diacetyldichlorofluorescein (DCFH)*;* glutathione peroxidase (GPx)*;* glutathione reductase (GR)*;* hours post-fertilization (hpf); superoxide dismutase (SOD)*;* thiobarbituric acid reactive species (TBARS).

Table 3. Qualitative description of studies reporting chemically-induced seizure protocols by pilocarpine (PILO) in research with zebrafish.

Reference	Seizure inducer	Duration of exposure (min)	Administration route	Interval inducer exposure and test	Developmental stage during outcome assessment	Sex	Duration test (min)	Main findings
(Vermoesen et al., 2011)	PILO 30 mM	2	Immersion	-	Larvae (7 dpf)	N.A.	2	**Seizure Behavior** ↑ Total movement
(Lopes et al., 2016)	PILO 15, 30, and 60 mM	unclear	Immersion	-	Larvae (3 and 7 dpf)	N.A.	18	**Locomotor activity** ↑ Number of crossings in a concentration-dependent manner
	PILO 60 mM	60		60 min				**Neurochemical outcomes** = *creb1a* expression = *bdnf* expression ↑ *c-fos* expression = *cpe* expression = *cpa6* expression = *npy* expression = *pomca* expression = *chga* expression = *pcsk1nl* expression = *tac1* expression ↑ *nts* expression ↑ *edn1* expression = *gria1a* expression = *gria2a* expression = *grin1a* expression = *grin2b* expression = *slc1a2b* expression
				300 min				= *creb1a* expression = *bdnf* expression ↑ *c-fos* expression = *cpe* expression = *cpa6* expression = *npy* expression = *pomca* expression = *chga* expression = *pcsk1nl* expression = *tac1* expression = *nts* expression = *edn1* expression = *gria1a* expression = *gria2a* expression = *grin1a* expression = *grin2b* expression = *slc1a2b* expression
				1380 min				= *creb1a* expression = *bdnf* expression ↑ *c-fos* expression = *cpe* expression = *cpa6* expression = *npy* expression = *pomca* expression = *chga* expression = *pcsk1nl* expression = *tac1* expression = *nts* expression = *edn1* expression = *gria1a* expression = *gria2a* expression = *grin1a* expression = *grin2b* expression ↓ *slc1a2b* expression

(Winter et al., 2017)	PILO 1 mM	55	Immersion	-	Larvae (4 dpf)	N.A.	70	**Electrophysiology** = Frequency of neuronal network events (Hz) ↑ Power of neuronal network events (mV^2)
(Diaz Verdugo et al., 2019)	PILO 60 mM	120-240	Immersion	-	Larvae (5-7 dpf)	N.A.	90	**Electrophysiology** ↑ Overall ratio of active neurons
(Paudel et al., 2020a)	PILO 400 mg/kg (single dose and every other day for 10 days)	-	Intraperitoneal injection	-	Adult	M:F	15	**Seizure Behavior** ↑ Seizure scores ↓ Distance traveled (injection days 1, 3, 5, 7, 9) ↑ Time in the upper half (injection days 1, 3, 5, 7, 9) ↓ Time in the lower half (injection days 1, 3, 5, 7, 9) **Neurochemical outcomes** ↑ *hmgb1* expression ↑ *tlr4* expression ↓ *nf-κb* expression = *tnf-α* expression ↑ *il-1* expression = *bdnf* expression = *creb-1* expression = *npy* expression ↓ GABA levels = Glutamate levels = Acetylcholine levels
(Budaszewski Pinto et al., 2021)	PILO 350 mg/kg	-	Intraperitoneal injection	60 min	Adult	M:F	unclear	**Seizure Behavior** PILO-induced seizure-like behavior 13% of animals showed lower scores (I–III) 87% reached scores IV-V Only fish presenting high scores (IV-V) were used in the PILO group to evaluate behavioral and biochemical parameters.
				24 h			6	**Light/dark test** = Distance traveled = Time spent in the light zone **Aggressive Behavior** ↑ Number of aggressive events ↑ Time spent in proximal zone (close to the mirror)
							10	**Anxiety** = Time in upper = Distance in the upper zone
				30 days			6	**Light/dark test** ↓ Distance traveled ↓ Time spent in the light zone **Aggressive Behavior** ↑ Number of aggressive events ↑ Time spent in the proximal zone (close to the mirror)
							10	**Anxiety** = Time in upper = Distance in the upper zone **Neurochemical outcomes**

								= PSD95 levels ↓ SNAP25 levels = GAD 67 levels = gephyrin 93 levels = gephyrin 75 levels = GFAP levels = iba-1 levels
					24 h			↑ Levels of cleaved parp1 ↑ Levels of cleaved caspase 3
					2 days			= Levels of cleaved parp1 = Levels of cleaved caspase 3 = BrdU + cells ↓ gephyrin 93 levels = gephyrin 75 levels
					3 days			↑ BrdU + cells ↓ PSD95 levels ↓ SNAP25 levels ↓ GAD 67 levels = gephyrin 93 levels = gephyrin 75 levels = GFAP levels ↓ iba-1 levels
					5 days 10 days			= BrdU + cells = PSD95 levels = SNAP25 levels = GAD 67 levels = gephyrin 93 levels ↑ gephyrin 75 levels ↑ GFAP levels = iba-1 levels

The sex of the animals used was computed as: M, for males; F, for females; M:F, when male and female were included but tested and analyzed as a mixed group; not applicable (N.A.) for larvae and unclear when the sex of the animals was not reported; -, means no interval between exposure and test. Unclear was used to address missing information in the articles. The main findings were described as: ↑, higher than the control group; ↓, lower than the control group; =, no difference when compared to the control group. Abbreviations: bromodeoxyuridine (BrdU)*;* days post-fertilization (dpf); gamma-aminobutyric acid (GABA)*;* glial fibrillary acidic protein (GFAP); glutamic acid decarboxylase 67 (GAD 67); hours post-fertilization (hpf); ionized calcium-binding adapter *protein* 1 (iba-1); Poly [ADP-ribose] polymerase 1 (parp1); Postsynaptic density *protein*-95 (PSD95), Synaptosomal-Associated *Protein*, 25kDa (SNAP25)

Table 4. Qualitative description of studies reporting chemically-induced seizure protocols by picrotoxin (PTX) in research with zebrafish.

Reference	Seizure inducer	Duration of exposure (min)	Administration route	Interval inducer exposure and test	Developmental stage during outcome assessment	Sex	Duration test (min)	Main findings
(Winter et al., 2008)	Various drugs tested as convulsive	17	Immersion	-	Larvae (7 dpf)	N.A.	17	**Seizure Behavior** The tested drugs were classified for positive or negative convulsive activity. Picrotoxin showed convulsive activity
(Wong et al., 2010b)	PTX 100 mg/L	20	Immersion	-	Adult	M:F	6	**Seizure Behavior** ↓ Latency to the upper half ↑ Transitions to the upper half ↑ Time in the upper half ↑ Freezing bouts ↓ Freezing duration = Erratic movements = Distance traveled ↓ Average velocity = Meandering = Turn angle

								↑ Bursts of hyperactivity ↑ Spasms ↑ Corkscrew swimming ↑ Circular swimming **Neurochemical outcomes** ↑ Cortisol levels
(Baxendale et al., 2012)	PTX 300 µM	unclear	Immersion	-	Embryo (50 hpf)	N.A.	90	↑ *fos* expression
(Cassar et al., 2017)	Various drugs tested as convulsive at 0.01, 0.03, 0.1, 0.3, 1 and 3 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** The tested drugs were classified as "high", "moderate," and "none" for convulsive activity. Picrotoxin was classified as high.
(Yang et al., 2017)	PTX 1, 5, 25, 125 and 625 µM	35	Immersion	-	Larvae (5 dpf)	N.A.	10	↑ Average distance moved (25, 125, and 625 µM) ↓ Distance moved in the center area (125 and 625 µM) ↓ Time spent in the center (125 and 625 µM)
(Shao et al., 2018)	PTX 12.5, 25 and 50 µM	unclear	Immersion	-	Larvae (5 dpf)	N.A.	60	**Locomotor activity** ↑ Mean speed (50 µM) ↓ Time moving (12.5 and 25 µM) ↑ Active swimming speed in a concentration-dependent manner
(Bandara et al., 2020)	PTX at 0, 0.01, 0.04, 0.1, 0.4, 1, 4 mM	20	Immersion	-	Larvae (5 dpf)	N.A.	20	**Seizure Behavior** ↑ Seizure score in a concentration-dependent manner ↓ Latency to seizures in a concentration-dependent manner ↑ Distance traveled **Electrophysiology** ↑ Epileptiform-like discharges ↑ Burst frequency
(Knoll-Gellida et al., 2021)	PTX 400 µM	240	Immersion	-	Larvae (4 e 7 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled

The sex of the animals used was computed as: M, for males; F, for females; M:F, when male and female were included but tested and analyzed as a mixed group; not applicable (N.A.) for larvae and unclear when the sex of the animals was not reported; -, means no interval between exposure and test. Unclear was used to address missing information in the articles. The main findings were described as: ↑, higher than the control group; ↓, lower than the control group; =, no difference when compared to the control group; Abbreviations: days post-fertilization (dpf); hours post-fertilization (hpf);

Table 5. Qualitative description of studies reporting chemically-induced seizure protocols by other inducers or a combination of two or more drugs in research with zebrafish.

Reference	Seizure inducer	Duration of exposure (min)	Administration route	Interval inducer exposure and test	Developmental stage during outcome assessment	Sex	Duration test (min)	Main findings
(Tiedeken and Ramsdell, 2007)	DA 0.22, 0.4, 0.7 and 1.26 ng/mg + PTZ 1, 1.25, 2.5, 5, 10, 15 and 25 mM	15	Microinjection (DA) + Immersion (PTZ)	-	Embryo (3 hpf – 7 dpf)	N.A.	15	**Seizure Behavior** The microinjection of DA increased the susceptibility to PTZ-induced crisis

(Winter et al., 2008)	Various drugs tested as convulsive	17	Immersion	-	Larvae (7 dpf)	N.A.	17	**Seizure Behavior** The tested drugs were classified for positive or negative convulsive activity maprotiline, bemegride, bicuculine, strychnine, physostigmine, amoxapine, enoxacin, semicarbazine, aminophylline, 4-AP, acetylsalicylic acid, verapamil, naproxen, and tobramycin showed convulsive activity
(Lefebvre et al., 2009)	DA 0.47 µg/g and 1.2 µg/g total body weight	-	Intraperitoneal injection	-	Adult	unclear	360	**Behavior** ↑ Abnormal behavior **Mortality** ↑ Rate of deaths (1.2 µg/g)
(Tiedeken and Ramsdell, 2009)	*o,p´-DDT* + PTZ 5 mM or DA 0.36 mM *p,p´- DDE* contaminat + PTZ 5 mM or DA 0.36 mM	15	Immersion	-	Larvae (6 hpf – 7 dpf)	N.A.	15	**Seizure Behavior** The pre-exposure of *o,p´-DDT,* and *p,p´- DDE* increased the susceptibility for PTZ and DA-induced crisis
(Tiedeken and Ramsdell, 2010)	*p,p´- DDE* contaminat + PTZ 5 mM or DA 0.36 mM	20	Immersion	-	Larvae (30 hpf -7 dpf)	N.A.	20	**Seizure Behavior** The pre-exposure of *p,p´- DDE* increased the susceptibility for PTZ and DA-induced crisis
(Wong et al., 2010b)	Caffeine 250 mg/L	20	Immersion	-	Adult	M:F	6	**Seizure Behavior** = Latency to the upper half = Transitions to the upper half = Time in the upper half ↑ Freezing bouts ↑ Freezing duration = Erratic movements ↓ Distance traveled ↓ Average velocity = Meandering = Turn angle ↑ Bursts of hyperactivity ↑ Spasms = Corkscrew swimming = Circular swimming **Neurochemical outcomes** ↑ Cortisol levels
(Wong et al., 2010a)	Caffeine 100 mg/L	15 (2 weeks)	Immersion	-	Adult	M:F	6	↑ Erratic movements = Time in top = Transitions to top
(Ellis et al., 2012)	Aconitine 1, 2, 5, 10 and 20 µM	10	Immersion	30 min	Larvae (100 – 106 hpf)	N.A.	80	**Light/Dark** ↑ Center/total distance in dark (5 and 10 µM) ↑ Fast/center distance (5, 10 and 20 µM) **Neurochemical outcomes** ↑ *c-fos* in a concentration-dependent manner
	4-AP 0.6, 0.8, 1 and 2.5 mM	10	Immersion	30 min	Larvae (100 – 106 hpf)	N.A.	80	**Light/Dark** ↓ Center/total distance in light and dark (0.8, 1 and 2.5 mM) ↑ Fast/center distance in light and dark **Neurochemical outcomes** ↑ *c-fos* in a concentration-dependent manner

(Lee et al., 2012)	GT 0.2, 0.5 and 1 mM	60 and 120	Immersion	-	Larvae (5 dpf)	N.A.	1.5	**Seizure Behavior** ↑ Seizure score ↑ Distance traveled
(Williams et al., 2012)	RDX 0.1 mM	20	Immersion	-	Adult	M:F	6	**Locomotor Behavior** ↓ Latency to top (0.1 mM) ↑ Transitions to top ↑ Time in top = Freezing frequency ↓ Freezing duration = Distance traveled ↑ Velocity = Angular velocity = Meandering = Turn angle = Latency to white = Transitions to White = Total time in White = Avg entry duration
	1 mM							↑ Average seizure score = Latency to top ↓ Transitions to top ↓ Time in top = Erratic movements = Freezing frequency = Freezing bouts ↓ Distance traveled ↓ Velocity = Angular velocity = Meandering = Turn angle ↑ Bursts of hyperactivity ↑ Spasms ↑ Corkscrew swimming **Neurochemical outcomes** ↑ Cortisol levels
(Leclercq et al., 2015)	AG 30, 50, 100, 200 and 300 mM	480	Immersion	5 min	Larvae (7 dpf)	N.A.	480	**Seizure Behavior** ↑ Swimming activity ↑ Whole body seizure ↑ Motility (50 and 100 mM)
	AG 300 mM	120		5 min			10	**Electrophysiology** ↑ Epileptiform discharges
							120	**Neurochemical outcomes** ↓ GABA in the head homogenates = glutamate in the head homogenates ↓ GABA/glutamate ratio
(Meyer et al., 2016)	KCL 15 mM	30	Immersion (solution in artificial cerebrospinal fluid (aCSF)	-	Larvae (4-5 dpf)	N.A.	60	**Electrophysiology** ↑ Burst rate ↑ Spike rate ↑ Synchrony of neural firing across channels
(Cassar et al., 2017)	Various drugs tested as convulsive at 0.01, 0.03, 0.1, 0.3, 1 and 3 mM	30	Immersion	-	Larvae (7 dpf)	N.A.	30	**Seizure Behavior** The tested drugs were classified as “high”, “moderate,” and “none” for convulsive activity. 4-AP, bicuculline, methotrexate, pentetrazol, quinolinic acid, donepezil, naproxen, scopolamine, vitamin b6, and buspirone were classified as high gabazine, clozapine, and naloxone was classified as moderate

								other drugs showed no effect on convulsive activity
(Vada et al., 2017)	Caffeine 250, 350 and 500 mg/L	20	Immersion	-	Adult	M:F	20	**Seizure Behavior** Higher concentrations of caffeine induced intense behavioral activity similar to tonic seizures. Lower concentrations resulted in less intense behavior but sustained for longer periods
(Winter et al., 2017)	4-aminopyridine (4AP) 1 mM	55	Immersion	-	Larvae (4 dpf)	N.A.	70	**Electrophysiology** ↑ Frequency of neuronal network events (Hz) = Power of neuronal network events (mV^2)
	Strychnine 200 µM	55					70	= Frequency of neuronal network events (Hz) = Power of neuronal network events (mV^2)
(Zhang et al., 2017)	EKP 200, 300, 400, 500, 600, 700 and 800 µM	120	Immersion	-	Larvae (7 dpf)	N.A.	120	**Seizure Behavior** ↑ Total amount of movement in a concentration-dependent manner ↑ Number of larvae with stage 2 behavior in a concentration-dependent manner **Electrophysiology** ↑ epileptiform discharges **Neurochemical outcomes** ↑ *c-fos* expression. ↑ Neuroluminescence
(Liu and Baraban, 2019)	4-AP 4 mM	90	Immersion	40 min	Larvae (5 and 6 dpf)	N.A.	unclear	**Electrophysiology** ↑ Epileptiform events
(Sourbron et al., 2019)	EKP 400 µmol	10	Immersion	-	Larvae (7 dpf)	N.A.	10	**Seizure Behavior** ↑ Locomotor activity **Electrophysiology** ↑ Epileptiform events
(Bandara et al., 2020)	TETS 0, 0.1, 0.4, 1, 4, 10, 40 and 100 mM	20	Immersion	-	Larvae (5 dpf)	N.A.	20	**Seizure Behavior** ↑ Seizure score in a concentration-dependent manner ↓ Latency to seizures in a concentration-dependent manner ↑ Distance traveled **Electrophysiology** ↑ Epileptiform-like discharges ↑ Burst frequency
(P.-Y. Chen et al., 2020)	GT 0.5 mM	120	Immersion	-	Larvae (5 dpf)	N.A.	300	**Seizure Behavior** GT causes seizure behavior and was used to compare whether pyridoxine had any effect on the survival of animals exposed to inducers **Neurochemical outcomes** ↑ *leptin-b* expression
(Gupta et al., 2020)	Glutamate 600 µM	30	Immersion	-	Larvae (5 dpf)	N.A.	30	**Seizure Behavior** ↑ Distance traveled
(Kanyo et al., 2020)	4-AP 400 µM	unclear	Immersion	-	Larvae (4 dpf)	N.A.	unclear	**Neurochemical outcomes** ↑ Hyperexcitability in the hindbrain or lateral optic tectum

(J. Li et al., 2020)	EKP 1mM	20 8	Immersion	-	Larvae (5 dpf)	N.A.	20 unclear	↑ Average actinteg (hyperlocomotion) **Electrophysiology** ↑ Epileptiform discharges ↑ Power spectral density
(Long et al., 2020)	NMDA 300 and 500 µmol	60	Immersion	-	Adult	unclear	60	**Seizure Behavior** ↑ Seizure score in a concentration-dependent manner ↓ Latency to seizure onset in a concentration-dependent manner
	NMDA 8 and 16 mg/kg	-	Intraperitoneal injection	-			60	↑ Seizure score in a concentration-dependent manner ↓ Latency to seizure onset in a concentration-dependent manner
	NMDA 0.1 and 0.5 mol	-	Intravitreal injection	-			60	↑ Seizure score in a concentration-dependent manner ↓ Latency to seizure onset in a concentration-dependent manner
(Paudel et al., 2020b)	PILO 400 mg/kg (day 1) + PTZ 80 mg/kg (day 10)	-	Intraperitoneal injection	- 180 min 24 h	Adult	unclear	15 (day 1 and 10)	**Seizure Behavior** ↑ Seizure score (stage IV) ↓ Distance traveled ↑ Time spent in the upper half ↓ Time spent in the lower half **T-Maze test** ↓ Inflection ratio ↓ Inflection ratio **Neurochemical outcomes** ↓ GABA concentration = Glutamate concentration ↓ GABA/Glutamate ratio ↑ *hmgb1* expression ↑ *tlr4* expression ↑ *nfkb* expression ↑ *tnf-α* expression ↑ *creb-1* expression ↓ *npy* expression
(Knoll-Gellida et al., 2021)	Triocresyl-fosfate (TCP) 10, 20, 50, 75 µM	240	Immersion	-	Larvae (4 e 7 dpf)	N.A.	60	**Seizure Behavior** ↑ Distance traveled
(Mundy et al., 2021)	TETS 4 µM	20	Immersion	5 min	Larvae (5 dpf)	N.A.	20	**Seizure Behavior** TETS causes seizure-like behavior ↑ Distance traveled
(Paudel et al., 2021)	PILO 400 mg/kg (day 1) + PTZ 80 mg/kg (day 10)	-	Intraperitoneal injection	-	Adult	unclear	15 (day 1 and 10)	**Seizure Behavior** ↑ Mean seizure score ↓ Latency to score IV **Locomotor Behavior** ↓ Total distance traveled ↑ Mean time spent in the upper half ↓ Mean time spent in the lower half **Neurochemical outcomes** = GABA concentration = Glutamate concentration = GABA/glutamate ratio ↑ *c-fos* expression ↑ *hmgb1* expression

		↑ *tlr4* expression ↑ *nf-kb* expression ↑ *tnf-α* expression ↑ *bdnf* expression ↑ *creb1* expression ↓ *npy* expression
		T-maze test
	3 h	= inflexion ratio
	24 h	= inflexion ratio

The sex of the animals used was computed as: M, for males; F, for females; M:F, when male and female were included but tested and analyzed as a mixed group; not applicable (N.A.) for larvae and unclear when the sex of the animals was not reported; -, means no interval between exposure and test. Unclear was used to address missing information in the articles. The main findings were described as: ↑, higher than the control group; ↓, lower than the control group; =, no difference when compared to the control group; Abbreviations: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (*p,p´- DDE);* 1,3,5-Trinitroperhydro-1,3,5 -triazine (RDX); 4-aminopyridine (4-AP); allylglycine (AG); days post-fertilization (dpf); dichlorodiphenyltrichloroethane (*o,p´-DDT);* domoic acid (DA); ethyl ketopentenoate (EKP); gamma-aminobutyric acid (GABA)*;* ginkgotoxin (GT); glutathione (GSH)*;* glutathione peroxidase (GPx)*;* glutathione reductase (GR)*;* glutathione s-transferase (GST)*;* hours post-fertilization (hpf); N-Methyl-D-aspartic acid (NMDA); pentylenetetrazole (PTZ); picrotoxin (PTX); pilocarpine (PILO); tetramethylenedisulfotetramine (TETS); triocresyl-phosphate (TCP).

Risk of bias and reporting quality

A sample of 100 studies (49.75%) was randomly chosen for risk of bias and reporting quality assessment (Figure 4). About 94% of the studies were rated as presenting a low risk of bias for selective reporting, 67% for baseline characteristics of the animals, and 54% for blinding. Randomization procedures and incomplete data were rated unclear in 81% and 68% of the studies, respectively.

As for the reporting quality, more than 50% of the studies failed to report any information on the items assessed. Reporting quality was considered unsatisfactory when evaluating the inclusion/exclusion criteria report since there were no reports for this item in 86 (86%) of the studies. Sample size calculation was not reported in any of the studies. Individualized scores for each included study are available at https://osf.io/bgcdv.

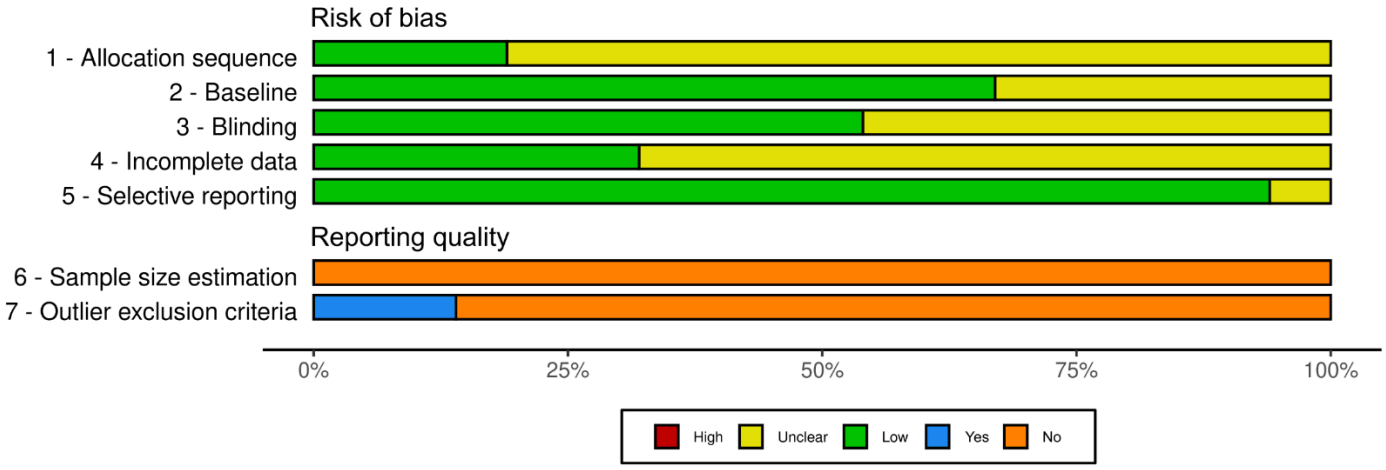

Fig. 4. Risk of bias assessment of included studies. The risk of bias assessment was performed by two independent investigators based on the SYRCLE's risk of bias assessment tool. Items 1 to 5 (Allocation sequence, Baseline, Blinding, Incomplete data, and Selective reporting) account for methodological quality and were scored as presenting a high, unclear, or low risk of bias. Sample size estimation and outlier exclusion criteria evaluated the reporting quality of the studies and were scored as "Yes" or "No", meaning satisfactory or unsatisfactory reporting quality, respectively. Classification is given as the percentage of assessed studies (n = 100) presenting each score.

DISCUSSION

For the behavioral characterization of epileptic seizures and the discovery of new therapies to control epileptic seizures, different model organisms have been employed, including rodents, flies, worms, and fish (Baraban et al., 2007). Zebrafish has proved to be an efficient experimental model for chemically-induced epileptic seizures. The features of seizures are similar in humans: excessive neuronal discharges and progressive behavioral changes (Baraban et

al., 2005). The response to pharmacological treatments is also evident in zebrafish. Larvae and adult animals that were pretreated with ASDs had seizures prevented when exposed to seizure inducers (Baraban et al., 2005; Berghmans et al., 2007; Siebel et al., 2015b; Pieróg et al., 2021a). Berghmans et al. (2007) extended the studies by Baraban et al. (2005) by testing 13 known ASDs against exposure to pentylenetetrazole (PTZ), validating the use of zebrafish in the search for new antiepileptic drugs and serving as a primary study, before being tested in other animal models, such as mammals (Berghmans et al., 2007). The use of zebrafish in epilepsy research has grown exponentially in recent years, as this organism model offers many advantages: zebrafish have known homologs for 85% of the recognized epilepsy genes found in humans, have easy genetic manipulation, and can be maintained in large quantities, in addition to being used at different stages of development (Hortopan et al., 2010). It is important to say that studies involving this species generally require analysis and approval by an institutional ethics committee to assess the protocol and the risks to the animal during the experiments.

Here, we conducted a systematic review of studies that used chemical inducers to provoke seizures in zebrafish. As shown in Figure 3, there are different circuits between authors in the studies included in this review. Furthermore, the first study included was carried out in 2005 by Baraban and collaborators, with an increase in the number of publications using zebrafish as an animal model of chemically induced epileptic seizures over the years, with the largest number of publications being given between 2014-2021. We can see well-established connections and authors who participated in only one study. In addition, De Witte, Peter A.M. is the author with the most participation in the

studies, with 16 appearances. For instance, most of the studies included in this review determined the behavioral responses and expression of genes involved in epileptogenesis to assess the mechanisms involved in epilepsy and discover new antiepileptic drugs, in addition to biomarkers of oxidative stress, neural activity, and inflammation markers. The following subsections describe the effects of different chemical inducers of seizure and their capacity to reproduce behavioral phenotypes in zebrafish at different developmental stages.

Pentylenetetrazole (PTZ)

In 180 studies that are included in this review, the seizure agent used is pentylenetetrazole (PTZ), a γ-Aminobutyric acid type A ($GABA_A$) receptor antagonist, that demonstrates characteristic and well-defined seizure behavior in zebrafish, both in larvae and in adults (Baraban et al., 2005; Mussulini et al., 2013).

Baraban et al. 2005 characterized the behavior of epileptic seizures of larvae with 7 dpf using PTZ as a convulsant agent for 10 minutes and divided it into 3 stages: stage I — dramatically increased swimming activity, stage II — whirlpool swimming behavior, and stage III — clonus-like seizures followed by loss of posture, when the animal falls to one side and remains immobile for 1–3 s. Also, in this study, the latency for stage I, II, or III seizure onset depended on PTZ concentration: at higher PTZ concentrations, the latency to a given seizure stage was shorter than that measured at lower concentrations. Another finding was that lower concentrations of PTZ (2.5 and 5.0 mM) evoked only Stages I and II of seizure behavior. Still, nearly 75% of all fish exhibited at least one clonus-

like convulsion (Stage III) in the presence of 15 mM PTZ. In addition, the distance traveled also increased at the concentrations tested. This study serves as the basis for the following studies using behavioral characterization to study new drugs with antiepileptic activity (Goldsmith et al., 2007; Kim et al., 2010; Copmans et al., 2019).

In a study conducted by Afrikanova et al. (2013), an alternative protocol was employed to investigate the effects of acute exposure in zebrafish larvae. Larvae of 6 dpf were placed in 96-well plates with ASD or vehicle for 18 hours of pre-treatment. After pre-treatment, 20 mM PTZ was added. Then, animals were habituated for 5 min, and locomotor activity was assessed for 30 min. The epileptic seizure stages observed were the same as in the study by Baraban et al. (2005), but the difference between the two studies is the concentration of PTZ (15 mM PTZ, Baraban; 20 mM PTZ, Afrikanova) and the sequence of application of the drugs used (PTZ added before ASD, Baraban; ASD added before PTZ, Afrikanova). Other researchers began to use the protocol by Afrikanova et al. (2013) in their epilepsy experiments using zebrafish larvae (Barbalho et al., 2016b; Brillatz et al., 2020a).

In adult zebrafish, the protocols followed the 3 stages characterized by Baraban et al. 2005 until the study by Mussulini et al. (2013), which characterized 6 stages of epileptic seizures induced by PTZ where different concentrations of the inducer were evaluated. The stages were defined as: (0) short swim, (1) increased swimming activity and high frequency of opercular movement, (2) erratic movements, (3) circular movements, (4) clonic seizure-like behavior, (5) fall to the bottom of the tank and tonic seizure-like behavior, (6) death. The seizure stages were evaluated for 20 min, and they found that 10 mM was the

ideal concentration to induce seizures, with animals reaching stage 5 in 240 s, approximately.

As we can see in Tables 1 and 5, PTZ was used in 125 studies with zebrafish at larval stages. In larvae, the most common concentration was 20 mM for 30 min, where an increase in epileptic seizure stages and distance traveled was observed. At this concentration, PTZ increases gene expression of *c-fos* and *bdnf* (Baxendale et al., 2012; Buenafe et al., 2013; Gawel et al., 2020). The same was seen in other widely used concentrations, such as 10 mM and 15 mM (Jin et al., 2018b; Podlasz et al., 2018; Gong et al., 2020). The study by Lopes et al. (2016) showed that approximately 24 hours after acute exposure to 15 mM of PTZ for 60 min, *c-fos* gene expression was not statistically different when compared to the control group, indicating that this transcript factor, although altered soon after an acute induction, returns to its baseline levels after 1 day. In addition, changes in oxidative stress were observed, such as increased production of reactive oxygen species (ROS) and decreased activity and gene expression of antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (GPx), which may lead to neuronal death, at a concentration of 15 mM for 20 min in the study by Jin, et al. 2018 (Jin et al., 2018a). The expression of genes involved in neuroinflammation, such as interleukin-1B (il-1B) and interleukin-6 (il-6), appeared already increased at 5 mM PTZ exposure for 3 and 30 min (Zhang et al., 2019).

In adults, the most common PTZ exposures were at concentrations of 7.5 and 10 mM for 10 to 20 minutes, where it was found that zebrafish showed increased distance traveled, speed, as well as increased gene expression of *c-fos* also seen in the larval stages (Lee et al., 2010; Siebel et al., 2015a). Few

studies evaluated *bdnf* expression, which is involved in neuroplasticity, in adults, and the majority used 170 mg/kg via ip. injection to induce seizures and some did not find any alteration (Choo et al., 2018, 2019; Jaiswal et al., 2020), while some observed a decrease in this gene expression (Kundap et al., 2019a, 2017). Little is known about oxidative stress in adult zebrafish exposed to PTZ. Fontana et al. (2019) found that 10 mM PTZ for 20 min causes an increase in thiobarbituric acid reactive species (TBARS) and carbonyl proteins, indicating lipid peroxidation, in addition to a decrease in non-protein thiol levels (NPSH) (Fontana et al., 2019).

PTZ has recently been utilized in chronic experiments to establish a kindling model in zebrafish, as highlighted in studies by Kumari et al. (2020) and Kundap, Paudel et al. (2019) (Kundap et al., 2019b; Kumari et al., 2020). However, it is worth noting that in these studies, the assessment of seizures occurred shortly after PTZ induction, possibly confounding the acute effects of PTZ administration. In rodent models, it has been observed that PTZ concentrations in the brain significantly decline approximately 6 hours after intraperitoneal application, as determined through high-performance liquid chromatography (HPLC) methods (Sierra-Paredes et al., 1989). Consequently, after this time point, the acute effects of PTZ diminish significantly. Therefore, when evaluating the PTZ kindling model, a comprehensive assessment should consider the time elapsed since the last PTZ injection to mitigate any acute effects.

Kindling in rodents is a well-established chronic model that involves repeated electrical stimuli of sub-effective intensity or repeated low-dose administration of a seizure-inducer until the induction of complete tonic-clonic seizures (Mason and Cooper., 1972; Dhir, 2012; Davoudi et al., 2013). While

spontaneous seizures remain challenging to observe in this model, some studies have reported such occurrences (Brandt et al., 2004; Tian et al., 2009; Song et al., 2018; H. Liu et al., 2019). Research in which spontaneous seizures are observed in animals bears greater relevance to human epilepsy and could be a good epilepsy model. In experiments with zebrafish, Kundap et al. (2019b) used an intraperitoneal dose of 170 mg/kg PTZ for 10 days, while Kumari et al. (2020) employed an immersion method with 1.25 mM PTZ for 22 days to induce kindling. Both studies noted an increase in seizure scores over time, albeit without spontaneous crises or an electrophysiological approach. This gap underscores the need for further investigation in zebrafish models. Despite this, existing studies bring new approaches for testing new compounds in a chronic model of epileptic seizures in zebrafish, but to better develop a robust protocol for inducing kindling in zebrafish and comprehensively understand the epileptogenesis process, it is imperative to conduct additional studies. Such efforts can contribute to the discovery of novel treatments for epilepsy and enhance our understanding of this neurological disorder.

Most electrophysiological studies in zebrafish exposed to PTZ to record local field potential were performed in the larval phase, showing interictal and ictal-like epileptiform discharges with different concentrations of PTZ (Rosch et al., 2018; Aourz et al., 2019; Liu and Baraban, 2019; Copmans et al., 2019). These results corroborate other studies carried out in mammals, where behavioral changes, alterations in the gene expression of *c-fos, bdnf*, and changes in oxidative stress are also observed (Méndez-Armenta et al., 2014; Barros et al., 2015; Chmielewska et al., 2020). Through this, PTZ in different concentrations seems to be an adequate chemical inducer of epileptic seizures

in zebrafish used in most studies in this review. However, there are discrepancies in some results, making it necessary to conduct more studies on these parameters, and the dose/concentration should be decided according to the objectives of the study.

Kainic acid (KA)

The second major chemical inducer of seizures found in this systematic review is KA, a potent excitatory neurotransmitter commonly used to induce seizures in animal studies (Lothman and Collins, 1981; Rojas et al., 2014). The mechanism of action of KA in inducing seizures is through activation of the ionotropic glutamate receptor, specifically the kainate receptor subtype. Activation of these receptors leads to the influx of sodium ions into the neurons, which triggers a cascade of events leading to neuronal hyperexcitability and, ultimately, seizure activity (Stafstrom et al., 1992). The first study in this review that used kainic acid to induce seizures in zebrafish was conducted by Kim et al. (2010), to determine whether PTZ- or KA-induced seizures influence cell proliferation in zebrafish larvae using bromodeoxyuridine (BrdU) to label dividing cells. They found that exposure to 200 µM KA for 10 min decreased the number of BrdU labeled cells in 5 dpf larvae in different brain areas, and the same was seen after exposure to 10 mM PTZ for 10 min, which indicated that seizures result in a massive reduction in cell proliferation in wide-ranging areas of the developing brain. In electrophysiology, KA at 50 µM increased epileptiform discharges characterized by short-duration interictal events (100−200 ms) and long-lasting bursting discharges (4−5 s) occurring 8 times per minute on average. Menezes

et al. (2014) have shown that exposure of larvae to 100–500 μM KA decreased locomotor activity at 7 dpf and increased locomotion at 15 dpf. In addition, pre-exposure to KA at 24 hpf reduces the susceptibility of juvenile fish to generate seizures when later exposed to KA. Still in larvae, (Feas et al., 2017) tested concentrations between 1.25 to 10 mM of KA for 25 min, and an increase in spontaneous movements in 5 dpf larvae was seen.

Alfaro et al. (2011) conducted an experiment using kainic acid (KA) to assess the behavior of adult zebrafish following intraperitoneal injections of varying doses (1, 2, 4, 6, and 8 mg/kg) of KA. The seizure stages used to characterize the seizures were similar to those observed with PTZ: stage I involved immobility and hyperventilation of the animal, stage II displayed whirlpool-like swimming behavior, stage III showed rapid movements from right to left, stage IV exhibited abnormal and spasmodic muscular contractions, stage V displayed rapid whole-body clonus-like convulsions, stage VI involved sinking to the bottom of the tank and spasms for several minutes, and stage VII resulted in death. They observed a dose-dependent increase in seizure scores. Moreover, higher doses (above 6 mg/kg) significantly reduced the latency to the first stage V seizure compared to lower doses. Animals administered with 8 mg/kg of KA exhibited *status epilepticus,* defined as one continuous unremitting seizure lasting longer than 30 minutes. Sierra et al. (2012) also used 6 mg/kg of KA to induce seizures in adult zebrafish by intraperitoneal injection, and an increase in seizure scores was seen in 60 min, with some animals reaching *status epilepticus*.

The study by Mussulini et al. (2018) aimed to investigate the effects of kainic acid-induced *status epilepticus* on glutamate uptake and behavioral

parameters in adult zebrafish. The authors found that kainic acid significantly decreased glutamate uptake in zebrafish forebrain, suggesting that glutamate neurotransmission is altered during *status epilepticus*. Additionally, the study showed that zebrafish exhibited several behavioral changes following kainic acid administration, including hyperactivity, freezing, and erratic swimming patterns. The authors suggest that alterations in glutamate uptake and release in the forebrain may underlie these behavioral changes. Kundap et al. (2019b) compared a single dose of KA 3 mg/kg with 10 days of PTZ 80 mg/kg, and the results showed that KA increased the seizure score on day 1 but decreased with time, while PTZ increased the seizure scores during the 10 days.

More recently, a study by Heylen et al. (2021) investigated the use of pericardial injection of kainic acid to induce a chronic epileptic state in larval zebrafish. The authors found that this method sustained increased seizure frequency and duration for several days, indicating the development of a chronic epileptic state. The study also identified changes in gene expression in the brain of zebrafish following KA injection, including upregulation of genes involved in inflammation and neurodegeneration at different time points after the microinjection. These results are accompanied by increased epileptiform discharges starting 2 days post-injection. In oxidative stress, de Farias et al. (2022) showed that 5 mg/kg increased the levels of reactive oxygen species (ROS) and lipid peroxidation, but did not alter the activity of antioxidant enzymes like SOD, CAT, glutathione reductase (GR) and GPx, just an increase in the SOD/CAT activity ratio. Overall, the studies reviewed highlight the usefulness of KA as a reliable chemical inducer of seizures in zebrafish models and provide

insights into the underlying mechanisms and behavioral outcomes of KA-induced seizures.

Pilocarpine (PILO)

Pilocarpine, a muscarinic cholinergic receptor agonist, is another agent used to induce epileptic seizures that progress to status epilepticus in rodents, and behavioral and biochemical changes are also observed when administered to adult zebrafish (Lin et al., 2018; Paudel et al., 2020a).

Ten studies used pilocarpine as a seizure inducer in zebrafish. Vermoesen et al. (2011) tested PTZ and pilocarpine in *Tg(fli1a:EGFP)y1* zebrafish larvae (7dpf) and found an increase of total movement when 20 mM pilocarpine was administered by immersion for 10 minutes. Antiseizure effects of three antidepressants (citalopram, reboxetine, bupropion) against pilocarpine were tested, but only citalopram minimized the effect of pilocarpine. Lopes et al. (2016) found that 60 mM pilocarpine administered for 60 min to larvae resulted in increased seizure activity, as evidenced by rapid, rhythmic, and repetitive movements. The knockdown of carboxypeptidase A6 (*Cpa6*) using morpholino antisense oligonucleotides reduced the response to seizure-inducing drugs and caused changes in the expression levels of mRNAs encoding signaling molecules. Specifically, the expression of genes associated with neurotransmitter release, ion channel activity, and G protein-coupled receptor signaling was altered. Winter et al. (2017) showed electrophysiological outcomes in *elavl3:GCaMP6s* zebrafish larvae (4 dpf) exposed to 1 mM of pilocarpine for 55 minutes. This resulted in increased event power of neuronal network events

(mV^2) but not in frequency (Hz). The electrophysiology and the imaging data for pilocarpine suggested a relatively widespread reduction in activity; however, this was predominantly observed in the rhombencephalon. This was the only study that performed electrophysiological recording to evaluate the effect of pilocarpine in zebrafish.

Recently, the study by Paudel et al. (2020) investigated the behavioral and biochemical changes induced by pilocarpine in adult zebrafish in a chronic seizure-like condition. The researchers administered pilocarpine (400 mg/kg) to the zebrafish through intraperitoneal injection and observed the development of seizure-like behaviors over time through the scores: 0 - Normal swimming, 1 - Jittery movement at the top of the tank, 2 - Ataxia or hyperactivity, 3 - Circular movement, circling around a small area, 4 - Erratic burst movement with loss of posture/corkscrew swimming. The results showed that pilocarpine induced seizure-like behaviors in adult zebrafish, characterized by hyperactivity, seizures, and loss of posture. The chronic administration of pilocarpine also led to biochemical alterations, such as significant increases in mRNA expression of high-mobility group protein 1 (*hmgb1), tlr4,* interleukin-1 (*il-1*); and decreased expression of factor nuclear kappa B (*nfκb*). In addition, the group exposed to pilocarpine decreased GABA levels. The seizure behavior was also observed by Budaszewski Pinto et al. (2021), where adult zebrafish were injected with pilocarpine (350 mg/Kg, i.p.), and 13% of animals showed lower scores (I–III) and 87% reached scores IV-V. Only fish presenting high scores (IV-V) were used in the PILO group to evaluate behavior on the light/dark test, open tank, aggressiveness test, and biochemical parameters at different times. The study identified increased aggressive behavior and cell death in the PILO group, with

increased levels of cleaved proteins caspase 3 and parp1 24 hours after seizure-like behavior induction. In addition, there were decreased protein levels of PSD95 and SNAP25 and increased BrdU-positive cells 3 days after induction. Persistent aggressive and anxiolytic-like behaviors were still detected 30 days after the induction, although most synaptic and cell death marker levels seemed normal.

Most recent studies with pilocarpine in this review (Paudel et al., 2020b, 2021) showed that a second hit with 80 mg/kg PTZ after 10 days of 400 mg/kg pilocarpine exposure increased the time spent in the upper half of the observation tank, and changed the expression of different genes, like increase in *hmgb1*, *tlr4*, *nfkb*, *tnf-α*, *bdnf*, *creb1* and a decrease in *npy* expression. Paudel et al. (2021) also observed increased *c-fos* expression. The use of pilocarpine as an epileptic seizure inducer in zebrafish models has provided important insights into the underlying neurochemical changes that occur during epilepsy and has implications for developing new antiepileptic treatments.

Picrotoxin (PTX)

The mechanism of action of picrotoxin (PTX) is similar to that of PTZ. PTX is a non-competitive GABA-A receptor antagonist that induces tonic and/or clonic seizures in different species, including rats and mice (Hamani and Mello, 1997; Kamal, 2012; Pinar, 2021).

The first study to use picrotoxin in zebrafish was by Wong et al. (2010b), where they tested PTZ, caffeine, and PTX on behavioral and cortisol levels. The results showed that adult zebrafish exposed to 100 mg/L PTX for 20 minutes had an increase in bursts of hyperactivity, spasms, corkscrew swimming, circular

swimming, and time spent in the upper half of the tank; cortisol levels were increased. Baxendale et al. (2012) found an increase in *fos* expression in zebrafish embryos exposed to 300 μM PTX, and the same was seen with 20 mM of PTZ.

The studies using zebrafish with PTX as a seizure-inducer basically evaluated behavioral outcomes and found alterations in distance traveled, average of movements, and speed (Yang et al., 2017; Shao et al., 2018; Bandara et al., 2020; Knoll-Gellida et al., 2021). There are discrepancies in locomotor parameters, such as distance traveled, and velocity between the studies, and it could be due to the use of different doses or concentrations tested and the time of exposure. In electrophysiology, just one study by Bandara et al. (2020) showed increased epileptiform-like discharges and burst frequencies. In short, further studies are needed to elucidate and characterize epileptic seizures and molecular alterations caused by picrotoxin in zebrafish.

Other chemical seizure-inducers

Other drugs are used to induce seizures in zebrafish, but they are not as usual, including caffeine, domoic acid, allylglycine, ethyl ketopentenoate, ginkgotoxin (GT), strychnine, 4-aminopyridine (4-AP), and 1,3,5-Trinitroperhydro-1,3,5-triazine (RDX), among others.

Domoic acid is a neurotoxin produced by certain species of algae that can cause seizures and other neurological symptoms in animals that consume contaminated shellfish (Lefebvre et al., 2002). Studies included in this review showed that domoic acid increases the susceptibility to seizures when

administered in larvae with other drugs like PTZ and contaminants (Tiedeken and Ramsdell, 2007, 2009, 2010). Lefebvre, et al. (2009) showed that intraperitoneal injection of 0.47 µg/g and 1.2 µg/g of domoic acid in adult zebrafish increased abnormal behavior and mortality.

Another drug used to study epileptic seizures in zebrafish is 4-aminopyridine (4-AP), a potent voltage-gated potassium channel blocker (Riazanski et al., 2001). 4-AP seems to cause seizure behavior in zebrafish (Winter et al., 2008; Cassar et al., 2017), increase epileptiform events (4-AP 4mM) (Liu and Baraban, 2019), and hyperexcitability in the hindbrain or lateral optic tectum (Kanyo et al., 2020). Also, Ellis et al. (2012) showed an increase in c-fos expression in a concentration-dependent manner at concentrations between 0.6 to 2.5 mM for 10 minutes.

Caffeine is a central nervous system stimulant that induces seizures in zebrafish at high concentrations (Vada et al., 2017). Also, Wong et al. (2010b) noticed that 250 mg/L caffeine for 20 minutes alters zebrafish locomotor behavior, increasing spasms and bursts of hyperactivity but decreasing the distance traveled. Another study by Wong et al. (2010a) showed that 15 minutes of exposure to caffeine 100 mg/L for 2 weeks increased erratic movements in the animal.

Leclercq et al. (2015) tested concentrations of allylglycine (AG) (30 to 300mM) for 480 minutes in 7 dpf zebrafish larvae and found an increase in swimming activity and a whole-body seizure. Also, 300 mM AG for 120 minutes presents epileptiform discharges in electrophysiological analyses and a decrease of GABA in brain homogenates. Ethyl ketopentanoate (EKP) is a derivative of AG, and both are glutamate decarboxylase (GAD) inhibitors. Zhang et al. (2017)

tested different concentrations of EKP (200-800 µM), and the results showed that EKP evoked robust convulsive locomotor activities, excessive epileptiform discharges, and upregulated *c-fos* expression in zebrafish. Li et al. (2020) tested the concentration of 1 mM, and the results also showed hyperlocomotion and epileptiform discharges. Alterations in locomotor behavior and epileptiform activity also were seen by (Sourbron et al. (2019).

The other drugs that appear in Table 5 also showed capacity to alter the zebrafish behavior, like 0.2 to 1 mM of ginkgotoxin (GT) that increases seizure score and distance traveled (Lee et al., 2012) , 1 mM RDX and NMDA (different concentrations), that increase average seizure score (Williams et al., 2012; Long et al., 2020), and 4 µM tetramethylenedisulfotetramine (TETS) that increases distance traveled and causes seizure-like behavior (Mundy et al., 2021). Despite these results, further studies are needed to characterize better epileptic seizures in zebrafish and their molecular and electrophysiological changes.

Risk of bias and reporting quality

To assess the risk of bias and reporting quality, we randomly chose a sample of 100 articles included in this review. Most studies were rated as having a low risk of bias for selective reporting, suggesting that most studies included in the review reported all mentioned outcomes. This, however, should be interpreted with caution as protocol preregistration is not standard in preclinical literature, and we thus cannot rule out that other outcomes were collected and not reported. In addition, the results indicate that more than half of the studies have a low risk of bias for baseline characteristics of animals and blinding, but

these points could still be potential sources of bias for some studies. The fact that randomization procedures and incomplete data were frequently rated as unclear is also a concern, as this suggests that some studies have not adequately reported on these important aspects of study design. The lack of information might compromise the credibility and reproducibility of data. This information is necessary to assess the methodological rigor of the studies and for other researchers to repeat experimental procedures (Macleod et al., 2015; Percie du Sert et al., 2020).

As for reporting quality, the fact that more than 50% of studies failed to report information on the items is concerning. The lack of reporting on inclusion/exclusion criteria in most studies is particularly concerning, making it difficult to assess the validity of study results. Additionally, the fact that sample size calculation was not reported in any of the studies is another important limitation, as it makes it difficult to assess the statistical power of the studies. Statistical testing is crucial for evaluating whether observed data align with the expected values under a given hypothesis (Krzywinski and Altman, 2013). While specific guidelines for sample size determination in zebrafish research may be lacking, several factors should be considered when making this critical decision, as articulated by Lakens (2022): the possibility of collecting data from nearly the entire population, constraints imposed by available resources, desired statistical power, and the level of precision required. Furthermore, Lakens (2013) has outlined methods for calculating sample sizes and effect sizes, providing researchers with valuable tools to enhance the robustness of their studies. In addition to these quantitative approaches, guidelines and checklists, such as

ARRIVE 2.0 (Percie du Sert et al., 2020), are readily accessible to facilitate the production of high-quality research reports.

Conclusions

This systematic review carried out a detailed literature search, looking for studies on the effects of chemical seizure inducers in zebrafish. We summarized the information to provide a tool for the researcher to select the best protocol to chemically induce seizures in zebrafish larvae and adults to study epileptic crisis or assess the antiseizure effects of different compounds. Choosing the best model needs to consider specific objectives to address the research question and outcomes of interest. Although there are several available inducers for epileptic seizures, studies using PTZ are by far the most common and extensively characterized. However, it is crucial to consider the risk of bias and the quality of reports observed in the studies to improve the protocol to be used.

It is clear that seizure models are continually being improved; however, better standardization and better models, such as kindling, are still needed to advance knowledge in the field of epilepsy.

AUTHOR CONTRIBUTION

Rafael Chitolina: conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization and writing - original draft; **Matheus Gallas-Lopes**: conceptualization, data curation, investigation, methodology, visualization and writing - review & editing; **Carlos Guilherme**

Rosa Reis: conceptualization, investigation, methodology, visualization and writing - review & editing; **Radharani Benvenutti**: conceptualization, investigation, methodology, visualization and writing – review & editing; **Thailana Stahlhofer-Buss**: investigation, visualization and writing – review & editing; **Maria Elisa Calcagnotto**: conceptualization, investigation, methodology, project administration, supervision, visualization and writing – review & editing; **Ana P. Herrmann**: conceptualization, investigation, methodology, project administration, supervision, visualization and writing – review & editing; **Angelo Piato:** conceptualization, investigation, methodology, project administration, supervision, visualization and writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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3.2 Capítulo 2: Effects of N-acetylcysteine and acetyl-L-carnitine on acute PTZ induced seizures in larval and adult zebrafish

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ARTICLE

Effects of *N*-acetylcysteine and acetyl-L-carnitine on acute PTZ-induced seizures in larval and adult zebrafish

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Abstract

Background Epilepsy is a prevalent neurological disease, affecting approximately 1 to 2% of the global population. The hallmark of epilepsy is the occurrence of epileptic seizures, which are characterized by predictable behavioral changes reflecting the underlying neural mechanisms of the disease. Unfortunately, around 30% of patients do not respond to current pharmacological treatments. Consequently, exploring alternative therapeutic options for managing this condition is crucial. Two potential candidates for attenuating seizures are N-acetylcysteine (NAC) and Acetyl-L-carnitine (ALC), as they have shown promising neuroprotective effects through the modulation of glutamatergic neurotransmission.

Methods This study aimed to assess the effects of varying concentrations (0.1, 1.0, and 10 mg/L) of NAC and ALC on acute PTZ-induced seizures in zebrafish in both adult and larval stages. The evaluation of behavioral parameters such as seizure intensity and latency to the crisis can provide insights into the efficacy of these substances.

Results Our results indicate that both drugs at any of the tested concentrations were not able to reduce PTZ-induced epileptic seizures. On the other hand, the administration of diazepam demonstrated a notable reduction in seizure intensity and increased latencies to higher scores of epileptic seizures. **Conclusion** Consequently, we conclude that, under the conditions employed in this study, NAC and ALC do not exhibit any significant effects on acute seizures in zebrafish.

Keywords: *Danio rerio*, seizure, epilepsy, pentylenetetrazole, N-acetylcysteine, acetyl-L-carnitine

Abbreviations

ASD antiseizure drugs

AUC area under the curve

BOD biochemical oxygen demand incubator

CEUA Ethics Committee for Animal Use

CONCEA Conselho Nacional de Controle de Experimentação Animal

dpf days post-fertilization

DZP Diazepam

GABA gamma-aminobutyric acid

$GABA_A$ Gamma-aminobutyric acid type A receptor

Grm2 glutamate metabotropic receptor 2 gene

GSH glutathione

ALC acetyl-L-carnitine

MDA malondialdehyde

mGlu2 metabotropic glutamate type 2 receptors

mGluRs extra-synaptic metabotropic receptors

NAC N-acetylcysteine

PTZ pentylenetetrazole

UFRGS Universidade Federal do Rio Grande do Sul

Introduction

Epilepsy is a prevalent neurological disease that affects approximately 1 to 2% of the global population [1]. It is characterized by two or more unprovoked or reflex seizures, with a minimum interval of 24 hours between them. Alternatively, it can be defined as a single unprovoked or reflex seizure in an individual with a greater than 60% risk of experiencing another seizure within the next 10 years or as an epilepsy syndrome [2]. The primary manifestation of epilepsy is the occurrence of epileptic seizures, which are transient events characterized by abnormal neuronal activity in the brain. These seizures are often accompanied by stereotyped behavioral changes that reflect the underlying neural mechanisms of the disease [3,4].

The primary treatment for epilepsy involves pharmacological interventions aimed to reduce or prevent seizure occurrence. However, approximately 80% of patients experience adverse effects or side effects from antiseizure medications, which can significantly impact their quality of life and lead to treatment interruption or non-adherence [5]. Moreover, despite more than 20 antiseizure drugs, around 30% of individuals with epilepsy do not achieve seizure control with these medications [6]. Recent systematic reviews with meta-analyses have reported a cumulative incidence of drug-resistant epilepsy of 25% in studies involving children and 14.6% in studies involving adults or mixed-age populations [7]. Consequently, exploring new treatment approaches and developing novel antiseizure drugs is crucial to managing epilepsy effectively [8].

N-acetylcysteine (NAC) has emerged as a promising adjunctive treatment for various neuropsychiatric disorders, including bipolar disorder, schizophrenia,

obsessive-compulsive disorder, anxiety, depression, and drug addiction [9,10]. Additionally, NAC exhibits antioxidant, anxiolytic, and anti-stress effects in animal models, such as rodents and zebrafish [11–13]. A recent study by Bilister Egilmez et al. [14] demonstrated a dose-dependent reduction in seizure severity and prolonged onset time of the first myoclonic jerk in rats pretreated with NAC.

Another compound with potential therapeutic benefits is acetyl-L-carnitine (ALC), a dietary supplement available in health food stores. ALC has shown positive effects in mice exposed to unpredictable chronic stress, an environmentally induced depression, enhancing the transcription of glutamate metabotropic receptor 2 (*Grm2*) gene encoding for the metabotropic glutamate type 2 receptor (mGlu2) in the hippocampus and prefrontal cortex in addition to reducing the immobility time in the forced swim test and increased sucrose preference as early as 3 days of treatment [15]. Furthermore, ALC has been found to prevent PTZ-induced epileptic seizures in rats, decrease oxidative stress markers, increase glutathione (GSH) levels, and reduce the expression of apoptosis markers such as caspase-3 [16].

The increase of glutamatergic neurotransmission is associated with epileptic crisis [5]. NAC and ALC have demonstrated neuroprotective effects by modulating glutamate levels [15,17,18]. These compounds hold promise as potential treatments for epilepsy, as they could potentially regulate the excitatory/inhibitory imbalance associated with the disease.

Zebrafish (*Danio rerio,* Hamilton 1822) is a small freshwater teleost species commonly used as a model organism to study epileptic seizure occurrence, the action of antiseizure drugs, and alternative therapies for seizure control in epilepsy [19–21]. In this model organism, the chemoconvulsant

pentylenetetrazole (PTZ), a gamma-aminobutyric acid GABAA antagonist, can induce acute seizures. PTZ reliably triggers characteristic and well-defined seizure behaviors in zebrafish, both in larvae and adult stages [19,22]. Additionally, zebrafish treated with antiseizure drugs are protected against epileptic seizures when exposed to convulsive agents [19,23].

Considering the challenges posed by epilepsy and the limitations of current therapies, this study aims to evaluate the effects of neuroprotective compounds (NAC and ALC) on acute epileptic seizures induced by PTZ in different stages of zebrafish development. The objective is to identify new alternatives for effective treatments with fewer adverse effects.

MATERIALS AND METHODS

Chemicals

N-acetylcysteine (NAC), acetyl-L-carnitine (ALC), and pentylenetetrazole (PTZ) compounds were obtained from Sigma-Aldrich® (CAS number 616-91-1, 204259-54-1, 54-95-5, respectively). Diazepam (DZP) (CAS number: 439-14-5) was chosen as a positive control for preventing epileptic seizures.

Animals

Adult short-fin wild-type zebrafish of both sexes were obtained from a local pet shop and acclimated for at least 2 weeks in an aquarium system with automated control of physical-chemical parameters and water recirculation

(Altamar, Santos, SP) before the experiments were conducted. The following parameters were noted: temperature 28 ± 1 °C, pH 7.5 ± 0.5, conductivity, 500 uS/cm and 14/10 h (light/dark). The animals were housed in 14 L aquariums with a maximum density of 5 animals/L. The fish were fed twice daily with commercial feed (Poytara®, Brazil) and *Artemia* sp.

Zebrafish embryos were obtained from the natural mating of adult zebrafish maintained in static aquariums in our laboratory. For breeding, 2 male and 1 female adult zebrafish were randomly selected from home aquariums and placed in breeding tanks (approximately 5:00 p.m.) with a grid designed to prevent egg predation by separating the adults from the embryos. Females and males were separated overnight by a transparent barrier, which was removed after the lights went on the following morning at approximately 9:00 a.m. Then, at approximately 10:00 a.m., the adult animals were removed, and the embryos were collected with a Pasteur pipette. Fertilized eggs were collected, washed with system water, and transferred to Petri dishes (30 larvae per dish). Embryos were maintained in a biochemical oxygen demand incubator (BOD) at 28 °C and monitored daily until 6 days post-fertilization (dpf). Since zebrafish larvae can absorb all the necessary nutrients through the yolk sac up to 7 dpf [24,25], feeding the animals during the experiment was not necessary. The dead or abnormal embryos (i.e., asymmetrical, showing coagulation, formation of vesicles, or damaged chorions) were discarded.

Euthanasia of animals was performed after the tests by immersion in cold water (0 to 4 °C) until the cessation of the movements to ensure death by hypoxia according to the AVMA Guidelines for the Euthanasia of Animals [26]. The protocol was approved by the Ethics Committee for Animal Use (CEUA) of

Universidade Federal do Rio Grande do Sul (UFRGS) (CEUA/UFRGS n° 35823) and followed the guidelines of Conselho Nacional de Controle de Experimentação Animal (CONCEA) and experiments are reported in compliance with the ARRIVE guidelines 2.0 [27].

Treatments

For the tests conducted in adult animals, 160 adult male and female zebrafish (50:50 ratio) were randomly selected from their home aquariums using a random number generator (random.org) to fulfill the experimental groups. Different experiments with negative and positive controls were carried out for each drug tested, resulting in two independent experiments with 5 experimental groups each (n=16 animals per group). The concentrations of NAC and ALC chosen for the experiment were based on previous studies [13,28,29]. The following concentrations were used: 0.1, 1.0, and 10 mg/L. DZP was used as a positive control at a concentration of 50 µM (or 14,23 mg/L). The control group received only system water. Zebrafish were individually treated for 40 min in a beaker containing the respective concentrations of NAC, ALC, or DZP (400 mL of solution), following the protocol described by Mussulini et al. [22] and Fontana et al. [30].

For larvae experiments, 200 individuals were used in two independent experiments for each tested drug with 5 experimental groups (n=20 animals per group). After breeding and daily monitoring of the embryos, until they reached 6 days post-fertilization (dpf), the larvae were randomized within the plate with a Random number generator (random.org) to spread the experimental groups

across the plate. Then, larvae were placed individually in a 24-well plate (1 per well), with each well containing 2 mL of the respective treatments: NAC and ALC at concentrations of 0.1, 1.0, and 10 mg/L, and DZP at a concentration of 18 µM (or 5,12 mg/L). The control group received only system water. The zebrafish larvae were arranged in a 6 by 6 pattern on the plate, with a 15-minute interval, to facilitate video analysis after treatment. The filming captured groups of 6 larvae at a time. The duration of treatment was 18 hours, as per the protocol established by Afrikanova et al. (2013) [31].

PTZ-induced seizures

After pretreatments, adult zebrafish were individually exposed to a 10 mM PTZ solution in an observation tank (13 cm high x 15 cm long x 10 cm wide) with 1L of solution, and their behavior was recorded on video for 20 min. The videos were recorded by a Logitech c920 webcam camera placed in front of the test aquarium. The behavioral phenotype of PTZ-induced seizures was manually quantified from the recorded video analysis using a standardized staged analysis model, as shown in Table 1 [22]. The highest epileptic seizure stage that each animal reached in 30-second intervals over the total analysis time (20 min) was recorded. The median of the epileptic seizure scores seen during the analysis were used to measure the area under the curve and verify the intensity of the seizure during the analyzed time. Also, the latency to reach stage 4 and stage 5 of the seizure was also considered to assess the seizure onset.

Table 1. Scores and behavior phenotypes used to characterize PTZ seizure model in adult zebrafish (Mussulini, et al. 2013).

SCORES	Behavior phenotype
0	Short swim mainly in the bottom of the tank.
1	Increased swimming activity and high frequency of opercular movement.
2	Burst swimming, left and right movements, and erratic movements.
3	Circular movements.
4	Clonic seizure-like behavior (abnormal whole-body rhythmic muscular contraction).
5	Fall to the bottom of the tank, tonic seizure-like behavior (sinking to the bottom of the tank, loss of body posture, and principally by rigid extension of the body).
6	Death.

For larvae, after treatment for 18 hours, the animals (7 dpf) were individually exposed to a 10 mM PTZ solution for 10 min in a 24-well plate with 2 mL of the solution. The seizure scores were quantified using a previously standardized behavior according to Table 2 [19] at intervals of 30 seconds, as done in adult animals. In addition, latencies for scores 2 and 3 were assessed to assess the seizure onset.

Table 2. Scores and behavior phenotypes used to characterize PTZ seizure model in zebrafish larvae (Baraban, et al. 2005).

SCORES	Behavior phenotype
0	Normal swim.
1	Increased swimming activity.

2	Whirlpool swimming behavior.
3	Clonus-like seizures followed by loss of posture.

All PTZ exposures were recorded, and researchers analyzed the behavior blinded to treatments in the software BORIS® [32]. All tests were done in the morning.

Statistical analysis

The sample size was calculated using G*Power 3.1.9.7 for Windows to detect an effect size of 0.4 with a power of 0.8 for adults and 0.9 for larvae and an alpha of 0.05, considering 5 experimental groups. The seizure latency was defined as the primary outcome. The total sample size was 80 for adults (n=16) and 100 for larvae (n=20) for each experiment.

The normality and homogeneity of variances were confirmed for all data sets using D'Agostino-Pearson and Levene tests, respectively. Non-parametric data of seizure scores were expressed as median ± interquartile range and analyzed by Kruskal-Wallis, followed by Dunn's test. The area under the curve (AUC) is presented as mean ± SD and analyzed by the one-way ANOVA followed by the Dunnet post-hoc test. The significance level was set at $p<0.05$.

Results

Effects of NAC pretreatment on zebrafish acute PTZ-induced seizure behavior

Progressive behavioral alterations were observed in adult zebrafish exposed to PTZ, leading to the development of severe seizures with scores of 4-5, which corresponded to tonic-clonic seizures (Fig. 1A). As expected, the administration of DZP at a concentration of 50 µM resulted in a notable response, reducing seizure intensity as indicated by the area under the curve (Fig. 1B; $F_{4,74}$ = 8.73; p = 0.0044). Additionally, a significant difference was observed for latency to score 4 (Fig. 2A; H = 13.09, p = 0.0109); DZP pretreatment increased this parameter as compared to the PTZ group (p = 0.0387), confirming its effectiveness as a classic antiseizure drug (ASD). No significant difference was observed for latency to score 5 (Fig. 2B; H = 7.23, p = 0.1241).

On the other hand, pretreatments with NAC at concentrations of 0.1, 1.0, and 10 mg/L did not prevent or attenuate acute PTZ-induced seizures in adult zebrafish, as demonstrated by the analysis of seizure intensity (Fig. 1B) and latencies to scores 4 and 5 (Fig. 2A and Fig. 2B).

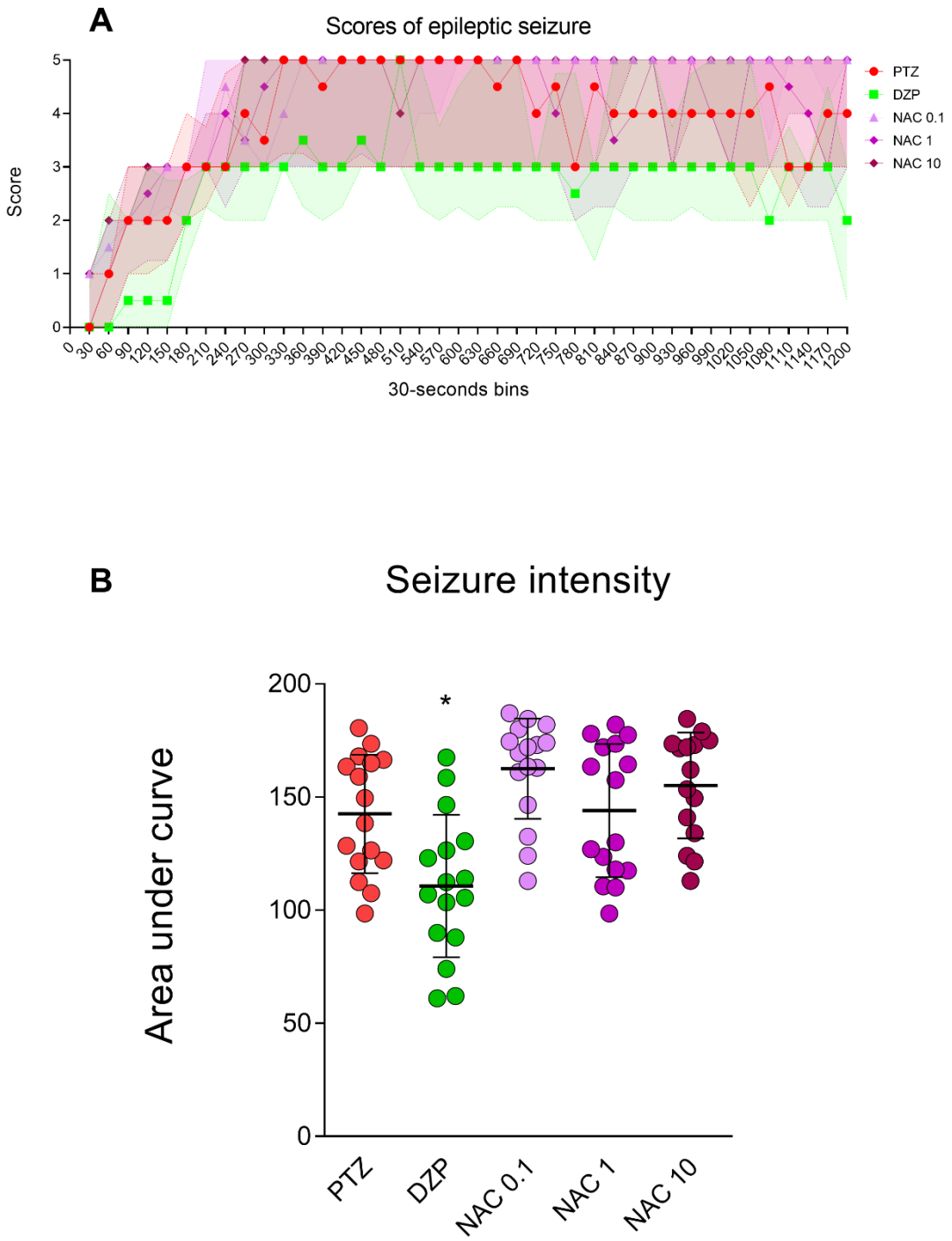

Fig. 1. Effect of N-acetylcysteine (NAC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 50 µM) on (A) scores of epileptic seizures during 20 min (only the highest score reached was considered in each interval) and (B) seizure intensity evaluated by area under the curve (AUC) in adult zebrafish. Data are presented as medians ± interquartile ranges for scores of epileptic seizures (A) and mean ±

SD for seizure intensity (B). The AUC was analyzed by one-way ANOVA followed by a Dunnet's post-hoc test. * indicates a significant difference as compared to the control group ($p<0.05$). n=16, except for NAC 10 mg/L n=15.

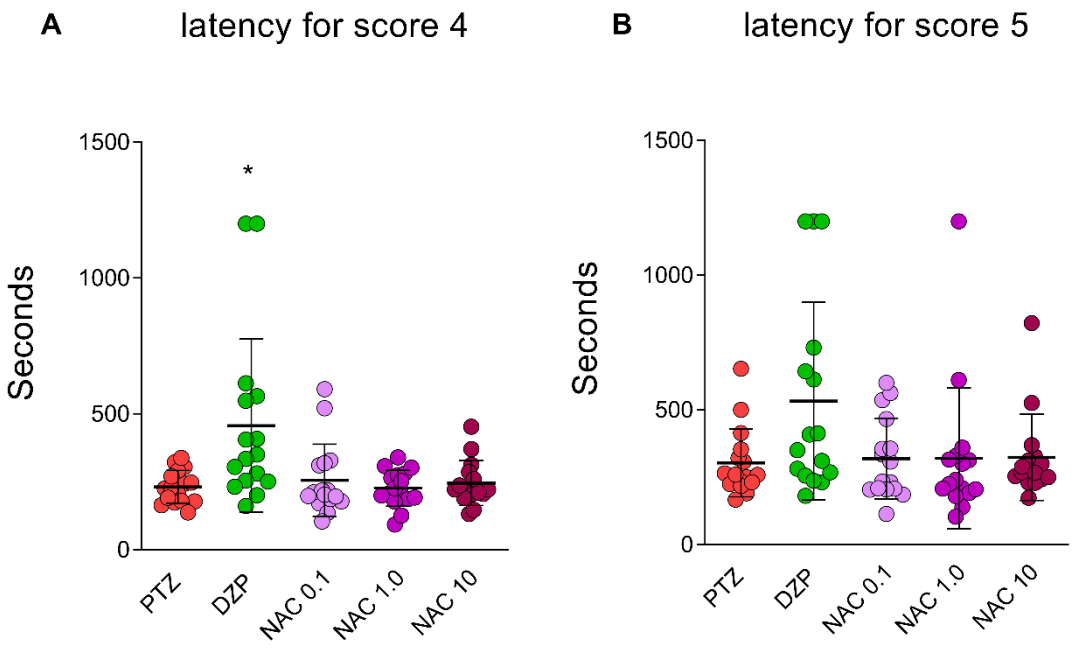

Fig. 2. Effect of N-acetylcysteine (NAC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 50 µM) on (A) latency for score 4 onset and (B) latency for score 5 onset in adult zebrafish. Data are presented as medians ± interquartile ranges and analyzed by Kruskal-Wallis followed by Dunn's test. * indicates a significant difference as compared to the control group ($p<0.05$). n=16, except for NAC 10 mg/L n=15.

In larvae, we observed results similar to those observed in adults. PTZ-treated larvae showed progressive seizure behavior until they reached the most severe seizure scores (Fig. 3A). Pretreatment with DZP at a concentration of 18 µM reduced the severity of acute PTZ-induced seizures in larvae. This was

evident from the decrease in seizure intensity across the area under the curve (Fig. 3 B, $F_{4,93} = 13.68$; $p < 0.0001$). Significant differences between groups were also observed for latency to scores 2 and 3 of epileptic seizures (Fig. 4A, $H = 23.29$, $p = 0.0001$, and Fig. 4B, $H = 28.37$, $p < 0.0001$, respectively); diazepam increased the latency to score 2 ($p = 0.0007$) and 3 ($p = 0.0001$) as compared to the PTZ group. Unfortunately, despite testing three different concentrations, NAC was unable to prevent the increase in seizure scores (Fig. 3A and Fig. 3B) or reduce the latency for scores 2 and 3 in the larvae compared to the PTZ group (Fig. 4A and Fig. 4B).

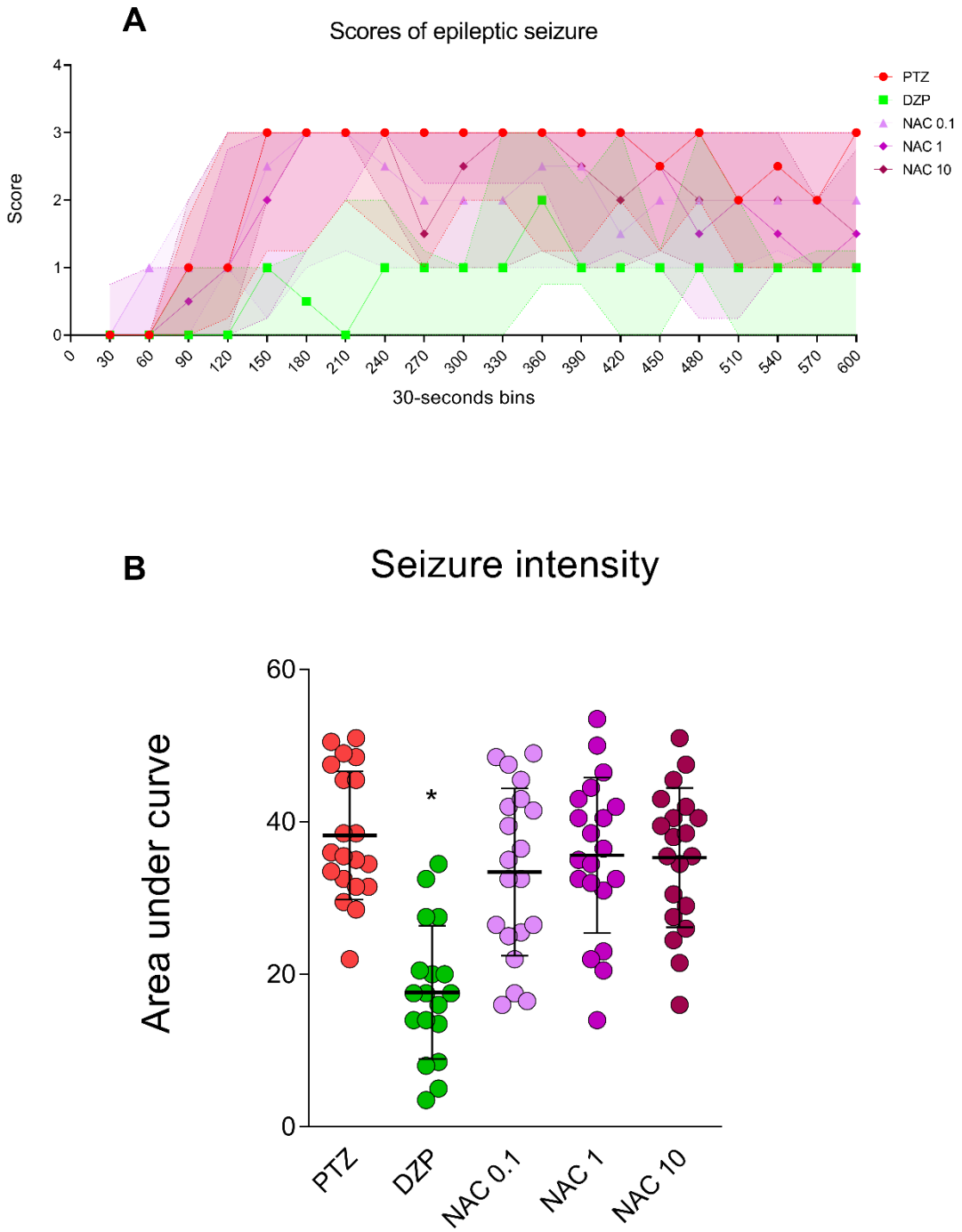

Fig. 3. Effect of N-acetylcysteine (NAC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 18 µM) on (A) scores of epileptic seizures during 10 min (only the highest score reached was considered in each interval) and (B) seizure intensity evaluated by area under the curve (AUC) in zebrafish larvae. Data are presented as medians ± interquartile ranges for scores of epileptic seizures (A) and mean ±

SD for seizure intensity (B). The AUC was analyzed by one-way ANOVA followed by Dunnet's post-hoc test. * indicates a significant difference as compared to the control group ($p<0.05$). n=20, except for DZP n=18.

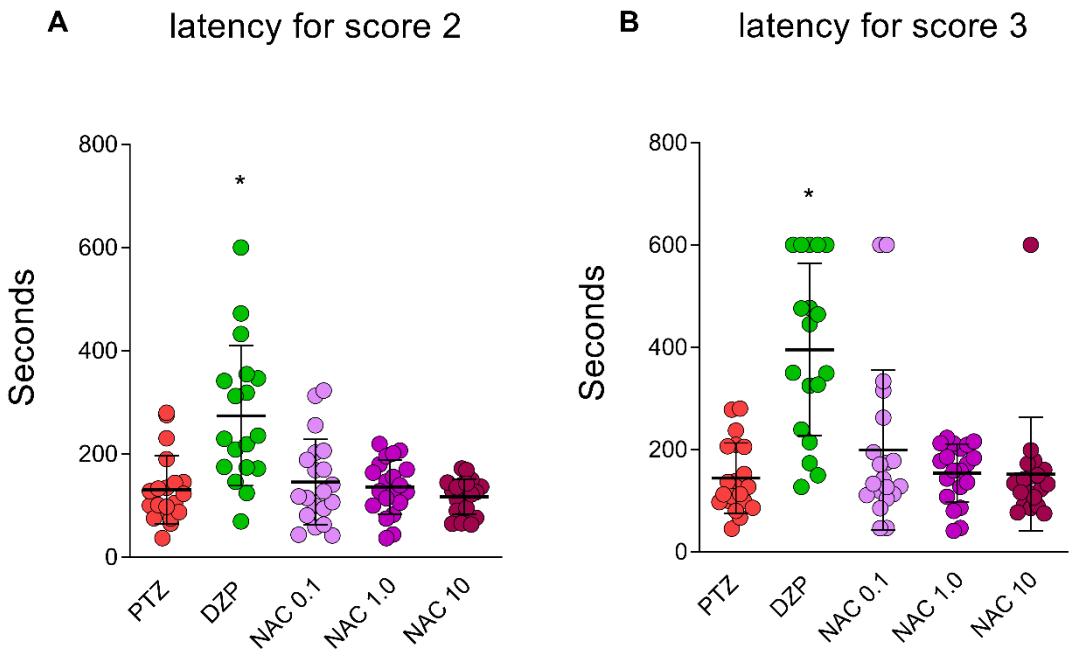

Fig. 4. Effect of N-acetylcysteine (NAC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 18 µM) on (A) latency for score 2 onset and (B) latency for score 3 onset in zebrafish larvae. Data are presented as medians ± interquartile ranges and analyzed by Kruskal-Wallis followed by Dunn's test. * indicates a significant difference as compared to the control group ($p<0.05$). n=20, except for DZP n=18.

Effects of ALC pretreatment on zebrafish acute PTZ-induced seizure behavior

In the experiments with ALC, the impact of PTZ on the animals mirrored that of the experiments with NAC. DZP demonstrated a reduction in the effect of

PTZ (Fig. 5A and Fig. 5B, $F_{4,75}$ = 6.034, p = 0.0063). However, ALC was devoid of effects at concentrations of 0.1, 1.0, and 10 mg/L on seizures caused by PTZ, as there was no observed decrease in seizure intensity (Fig. 5A and Fig. 5B).

A significant difference was observed for latency to score 4 (Fig. 6A; H = 23.14, p = 0.0001); DZP pretreatment increased this parameter as compared to the PTZ group (p = 0.0159). Regarding latency to score 5 (Fig. 6B; H = 16.61, p = 0.0023), no statistically significant differences were observed for the relevant post-hoc comparisons. ALC did not show any significant differences compared to the PTZ group at any of the tested concentrations.

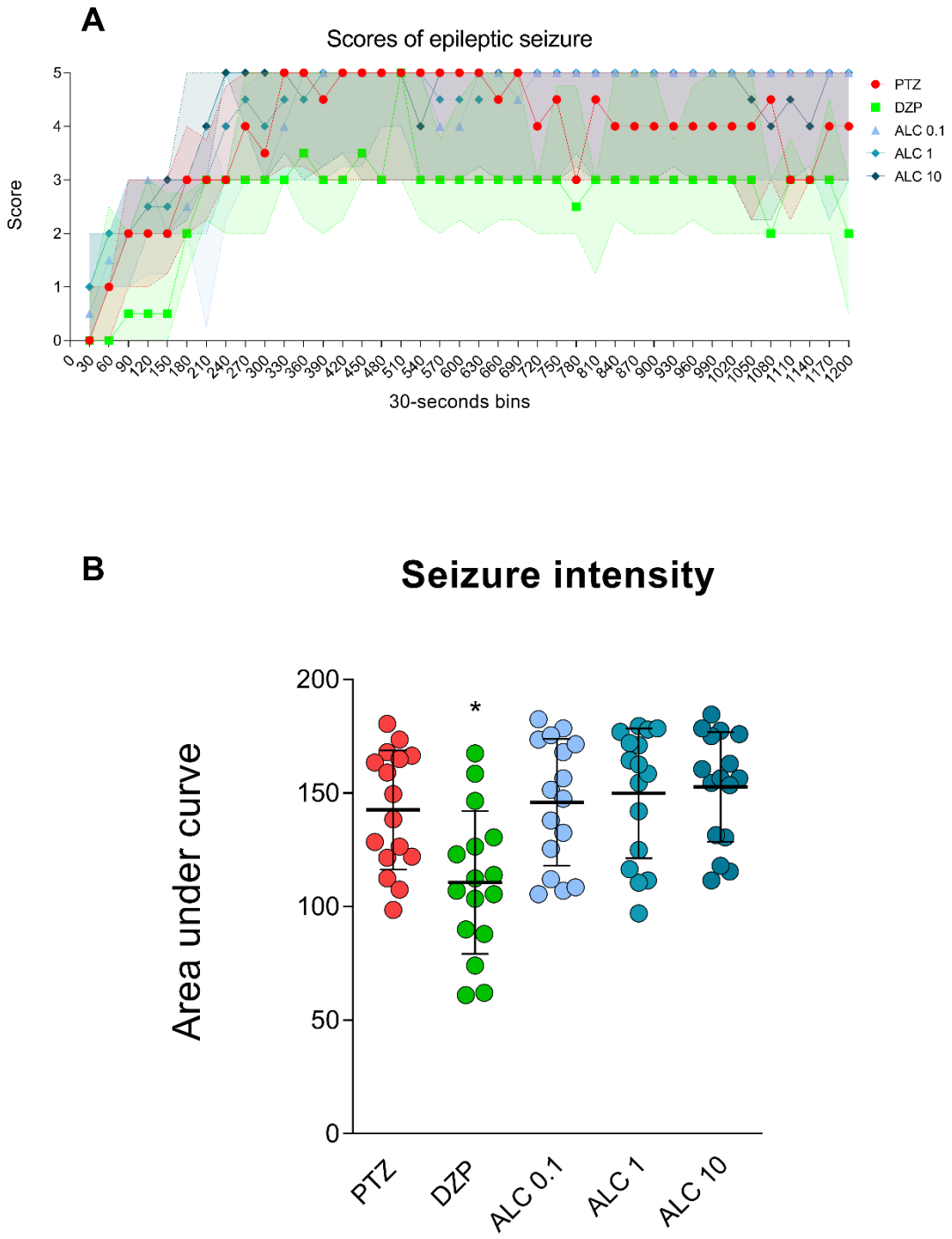

Fig. 5. Effect of acetyl-L-carnitine (ALC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 50 µM) on (A) Scores of epileptic seizures during 20 min (only the highest score reached was considered in each interval) and (B) seizure intensity evaluated by area under the curve (AUC) in adult zebrafish. Data are presented as medians ± interquartile ranges for scores of epileptic seizures (A) and mean ±

SD for seizure intensity (B). The AUC was analyzed by one-way ANOVA followed by Dunnet's test as post-hoc. * indicates a significant difference as compared to the control group ($p<0.05$). n=16.

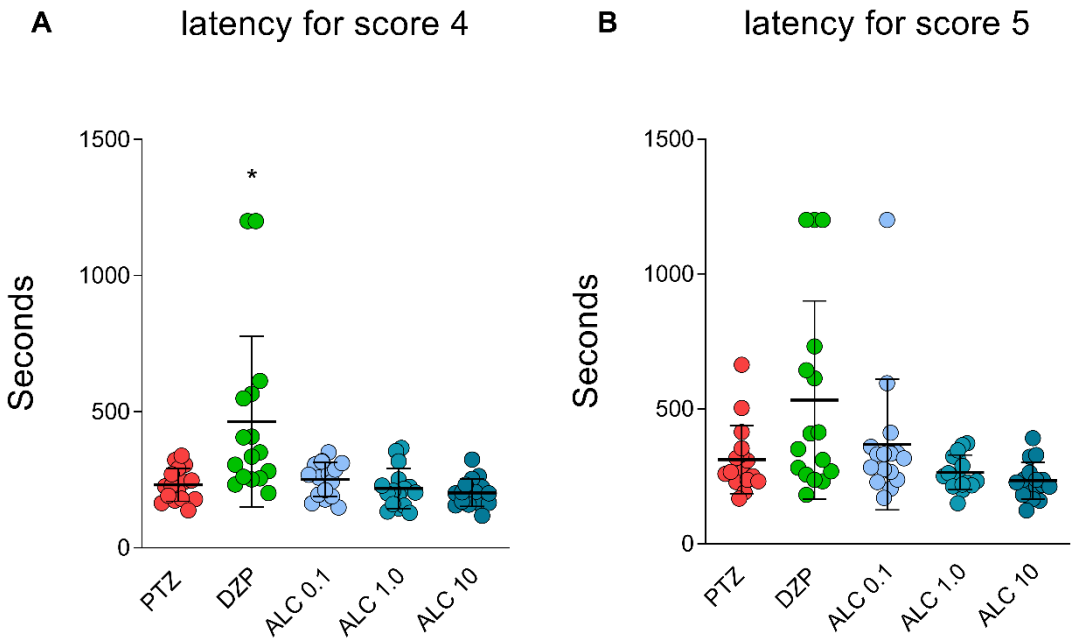

Fig. 6. Effect of acetyl-L-carnitine (ALC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 50 µM) on (A) latency for score 4 onset and (B) latency for score 5 onset in adult zebrafish. Data are presented as medians ± interquartile ranges and analyzed by Kruskal-Wallis followed by Dunn's test. * indicates a significant difference as compared to the control group ($p<0.05$). n=16.

In zebrafish larvae, the positive control diazepam at 18 µM reduced the severity of the seizures (Fig. 7A and Fig. 7B, $F_{4,91} = 19.38$, $p < 0.0001$). The latencies for scores 2 and 3 increased when treated with DZP at 18 µM compared to the PTZ group. However, ALC at concentrations of 0.1, 1.0, and 10 mg/L failed to prevent the effects of PTZ on epileptic seizures.

Significant differences between groups were also observed for latency to scores 2 and 3 of epileptic seizures (Fig. 8A, $H = 26.69$, $p < 0.0001$, and Fig. 8B, $H = 23.29$, $p < 0.0001$, respectively); diazepam increased the latency to score 2 ($p < 0.0001$) and 3 ($p < 0.0001$) as compared to the PTZ group. Larvae treated with ALC showed no significant differences compared to the PTZ group at any tested concentrations in the latencies to scores 2 and 3.

It is important to mention that in the experiments with adults, one animal of the experimental group NAC 10 mg/L jumped from the tank during the analysis of seizure scores and was excluded from the results. In the experiments with NAC in larvae, two larvae from the DZP group were excluded because they died during treatment. In the experiment with ALC in larvae, three larvae from DZP and one from the ALC 10 mg/L group were excluded because they were injured during manipulation.

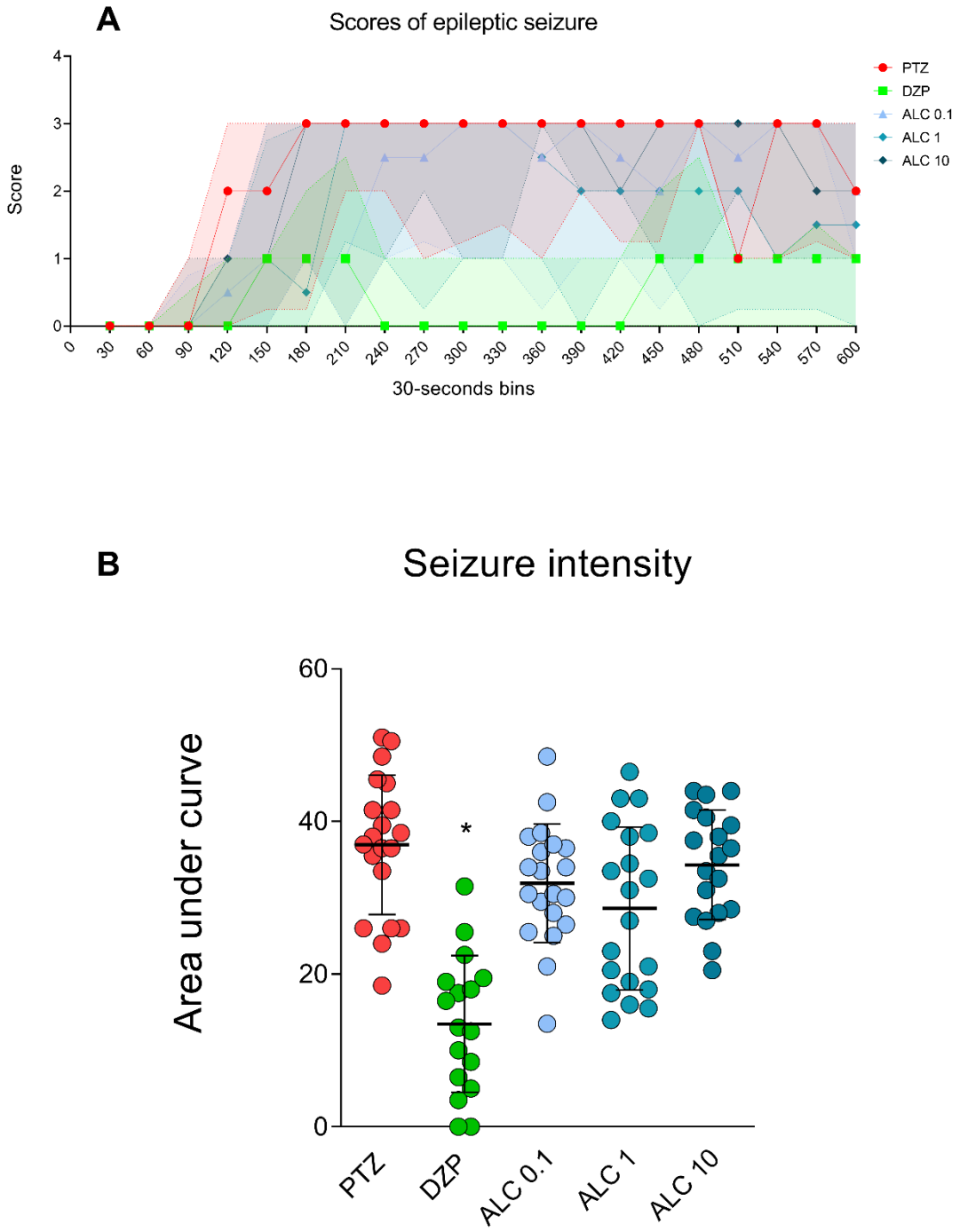

Fig. 7. Effect of acetyl-L-carnitine (ALC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 18 µM) on (A) scores of epileptic seizures during 10 min (only the highest score reached was considered in each interval) and (B) seizure intensity evaluated by area under the curve (AUC) in zebrafish larvae. Data are presented as medians ± interquartile ranges for scores of epileptic seizures (A) and mean ±

SD for seizure intensity (B). The AUC was analyzed by one-way ANOVA followed by Dunnet's test as post-hoc. * indicates a significant difference as compared to the control group ($p<0.05$). n=20, except for DZP group n=17, ALC 1.0 mg/L n=19 and ALC 10 mg/L n=19.

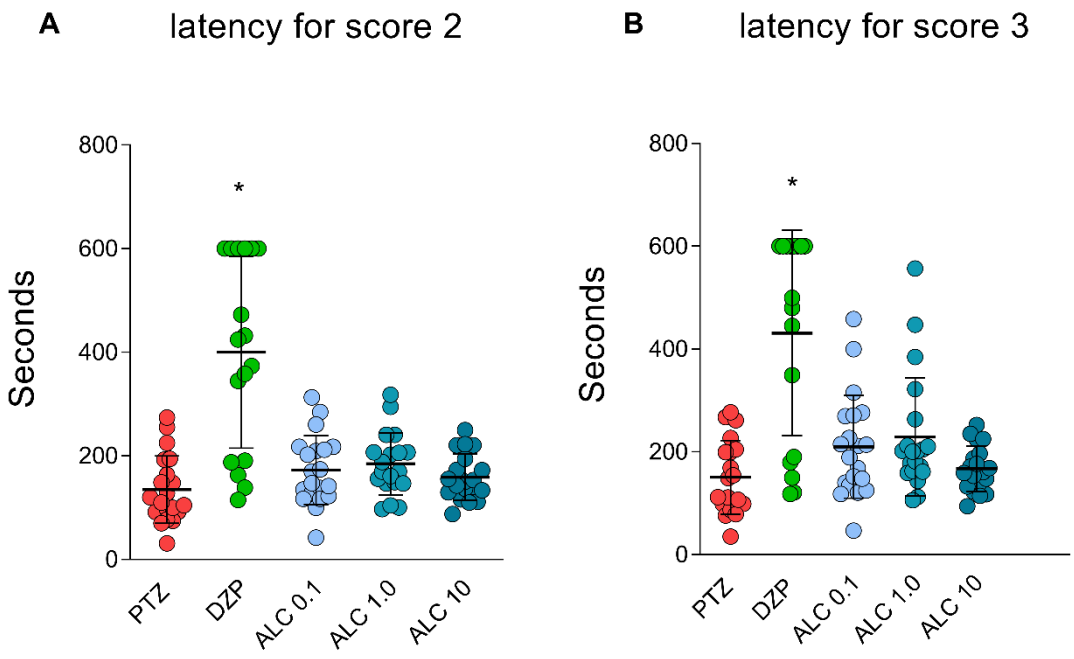

Fig. 8. Effect of acetyl-L-carnitine (ALC 0.1, 1.0, and 10 mg/L) and diazepam (DZP 18 µM) on (A) latency for score 2 onset and (B) latency for score 3 onset in zebrafish larvae. Data are presented as medians ± interquartile ranges and analyzed by Kruskal-Wallis followed by Dunn's test. * indicates a significant difference as compared to the control group ($p<0.05$). n = 20, except DZP group n=17, ALC 1.0 mg/L n=19 and ALC 10 mg/L n=19.

Discussion

In this study, we examined the effects of NAC and ALC on acute PTZ-induced seizures in adult and larval zebrafish. Our findings indicate that both

drugs at the tested concentrations were not able to reduce PTZ-induced epileptic seizures in zebrafish. These molecules failed to reduce seizure intensity or increase latency for the highest seizure scores. In contrast, diazepam effectively reduced seizure intensity and increased latency for the highest scores, validating the test.

We used a 10 mM concentration of PTZ to induce acute seizures in zebrafish. A 20-minute exposure to this concentration resulted in all seizure-like behavioral phenotypes and demonstrated lower mortality compared to a 15 mM concentration in adult zebrafish [22], indicating that 10 mM is a suitable concentration for studying new potential antiseizure molecules [30,33]. Additionally, PTZ at 10 mM exhibited similar effects on larvae, accompanied by changes in electrophysiology, including increased epileptiform discharges and ictal and interictal bursts [34–36]. Our study using 10 mM PTZ corroborated the findings of Baraban et al. [19], showing an increase in seizure scores over time in larvae zebrafish.

In epileptogenesis, it is known that activated astrocytes undergo molecular alterations that can contribute to neuronal hyperexcitability, such as the downregulation of gap junction connexins, glutamate transporters, and potassium channels [5]. Impaired astrocyte function can result in decreased elimination of extracellular glutamate, leading to a reduced seizure threshold and an imbalance between neuronal excitation and inhibition [8]. Drugs that modulate glutamate, such as topiramate, perampanel, levetiracetam, and felbamate, have been used to treat epileptic seizures [37]. Some of these molecules have shown effects in increasing latency to seizures induced by acute PTZ administration [38]. NAC and ALC have been reported to have glutamate-modulating effects. NAC

activates the cystine-glutamate antiporter present in astrocytes, leading to the release of glutamate that stimulates extra-synaptic metabotropic receptors (mGluRs), thereby reducing the synaptic release of the neurotransmitter [17,18]. On the other hand, ALC has demonstrated effects in models of depression in mice exposed to unpredictable chronic stress through a possible epigenetic regulation of mGlu2 receptors. This was observed after 3 days of treatment [15].

Several studies have demonstrated the antiseizure effects of NAC in mice. Uma Devi et al. [39] studied the effect of NAC (50 and 100 mg/kg for 8 days) on PTZ-induced seizures in mice (60 mg/kg via intraperitoneal injection). NAC significantly prolonged the latencies to jerks and clonic generalized seizures during the 30-minute analysis, with this effect not being dose-dependent. Coadministration of NAC with sodium valproate resulted in a significant enhancement of the anticonvulsant effect. Zaeri and Emamghoreishi [40] also demonstrated the antiseizure effect of NAC (50-150 mg/kg) in an acute and chronic PTZ seizure model (90 mg/kg via intraperitoneal injection). Our study did not observe an antiseizure effect of NAC at concentrations of 0.1, 1.0, and 10 mg/L when administered for 40 minutes (adults) or 18 hours (larvae) in the PTZ seizure model in zebrafish. This lack of effect may be attributed to the low concentrations used in our study and the duration of treatment (acute vs. chronic in other studies). It is important to note that a study by Tallarico et al. [41] demonstrated that NAC treatment significantly increased the number and duration of spike-wave discharges in WAG/Rij rats (rat model of absence epilepsy), exacerbating absence epilepsy while ameliorating neuropsychiatric comorbidities. Therefore, the effect of NAC on seizures may depend on the seizure type.

ALC also did not demonstrate an antiseizure effect on acute PTZ-induced seizures in zebrafish, despite other researchers having demonstrated the antiseizure effect in rodents using the kindling model. The kindling model involves repeated electrical stimuli of sub-effective intensity or repeated administration of a low dose of a chemoconvulsant until complete tonic-clonic seizures are induced [42–44]. Hussein et al. [16] exposed rats to PTZ (40 mg/kg *ip*, 3 times/week, for 3 weeks) and divided them into two groups. The first group received oral ALC (100 mg/kg/day for 4 weeks), while the second group received saline. ALC treatment decreased seizure scores and duration, increased latency to the first seizure, attenuated PTZ-induced increase in the oxidative stress marker malondialdehyde (MDA) level, and increased antioxidant GSH activity. More recently, Essawy et al. [45] also demonstrated the antiseizure effect of ALC (300 mg/kg) in a rodent kindling model using male albino rats. Pre- and post-treatment with ALC suppressed the kindling acquisition process and remarkably alleviated the PTZ-induced effects. Additionally, a study showed the antiseizure effect of ALC (100 mg/kg) in the kainate murine model of temporal lobe epilepsy by attenuating status epilepticus and reducing oxidative stress and neuroinflammation [46]. Also, the differences between the published studies and our findings may be due to the low concentrations used in our study and the duration of treatment, in addition to the different protocols (acute seizures x kindling model).

Diazepam, a benzodiazepine medication, is commonly used to treat acute recurrent seizures by enhancing the activity of gamma-aminobutyric acid (GABA) [47]. Our study showed that diazepam effectively increased seizure latency and reduced seizure intensity at 50 µM (adults) and 18 µM (larvae) concentrations in

zebrafish. These results support other studies where diazepam was used as a positive control to reduce seizures induced by PTZ in zebrafish [48,49].

As prospects, further studies can be conducted to evaluate the effect of acute treatment with NAC and ALC in higher concentrations on PTZ-induced seizures. Furthermore, NAC and ALC could be tested as adjuncts to other antiseizure treatments to determine if they enhance the overall treatment response. Additionally, the kindling model is not well standardized in zebrafish, as the existing studies only exposed the animals to chronic low doses of PTZ and evaluated the seizure behavior just after the exposure. Spontaneous seizures do not occur in existing protocols [50,51], therefore, NAC and ALC could be evaluated in a new kindling model in zebrafish to assess their antiseizure effects.

Conclusion

This study aimed to investigate the potential antiseizure effects of NAC and ALC by utilizing an acute PTZ-induced seizure model in adult and larvae zebrafish. Our findings indicate that acute treatment with both drugs did not exhibit significant antiseizure effects. The external validity of previous findings from rodent experiments is thus limited. Nevertheless, it is important to note that further investigations are warranted to fully assess the effects of NAC and ALC on PTZ-induced epileptic seizures. Potential future research approaches include exploring higher concentrations of NAC and ALC, extended treatment durations, and incorporating electrophysiological evaluation into the experimental design. By conducting these additional studies, it will be possible to more

comprehensively understand the potential antiseizure properties of NAC and ALC in the context of PTZ-induced seizures.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available in the Open Science Framework at https://osf.io/uezfm/.

AUTHOR CONTRIBUTION

Conceptualization, RC, and AP; methodology, RC, CGR, RB, TSB, AL, MM, AP; investigation, RC, CGR, RB, TSB, AL, MM; formal analysis, RC, CGR, RB, TSB, AL, MM, AP; resources, AP; writing—original draft, RC; writing—review and editing, RC, CGR, RB, TSB, AL, MM, APH, AP; supervision, AP; funding acquisition, AP.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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4. DISCUSSÃO GERAL

A hipótese dessa tese foi de que a NAC e ALC seriam capazes de proteger os peixes-zebra de crises epilépticas agudas induzidas pelo PTZ através de parâmetros comportamentais, como estágios de crise epiléptica e a latência para atingir os estágios mais severos da crise. Essa hipótese não foi confirmada, já que os dois compostos, nas condições testadas, não diminuíram as crises epilépticas (através da análise dos escores de crise epiléptica) e nem aumentaram a latência para os maiores estágios da crise que correspondem a crise tônico-clônica.

Como primeiro objetivo específico, uma revisão sistemática da literatura foi realizada, a fim de mapear os protocolos existentes e responder à seguinte pergunta: quais são os efeitos dos indutores químicos usados para induzir crise epiléptica no peixe-zebra? Através dessa revisão foi possível integrar os principais achados relatados em peixes-zebra sobre os efeitos de diferentes indutores químicos de crise epiléptica com relevância no estudo da epilepsia. Além disso, os resultados obtidos nessa revisão possibilitaram discutir as metodologias e os protocolos experimentais utilizados nessa espécie. Outra análise realizada, de risco de viés e qualidade de relato, possibilitou discutir os principais problemas nesses estudos que podem levar a problemas de reprodutibilidade.

Através de uma busca nas bases de dados MEDLINE (*PubMed*), *SCOPUS* e *Web of Science* foram identificados 2130 estudos. Após exclusão de duplicatas, 1072 artigos passaram por etapas de inclusão/exclusão através de critérios pré-definidos e ao final 201 artigos foram selecionados para compor o artigo de revisão (capítulo I). Desses 201 artigos, o PTZ foi a droga utilizada com maior frequência, sendo utilizado em 180 estudos. Já a concentração de PTZ mais utilizada é a de 20 mM (40 estudos), seguida pela concentração de 10 mM (39 estudos) e 15 mM (35 estudos). O tempo de exposição também variou entre os estudos, sendo 30 minutos o tempo mais utilizado (37 estudos), seguido por 10 e 15 minutos (24 e 19 estudos, respectivamente). Dentre os desfechos comportamentais mais avaliados estão a distância percorrida, a latência para atingir estágios de crise epiléptica e a variação dos estágios de crise epiléptica.

O segundo objetivo foi avaliar os efeitos da N-acetilcisteína e da acetil-L-carnitina frente à crise epiléptica induzida por PTZ nos peixes-zebra nas fases larval e adulta (Capítulo II). Em peixes-zebra adultos, os animais foram expostos ao tratamento por 40 minutos, e após filmados por 20 minutos numa solução de PTZ 10 mM conforme Mussulini et al. (2013). Nas larvas, os tratamentos foram realizados no 6º dia pós-

fertilização por 18 horas conforme Afrikanova, et al. (2013), e após o tratamento, no 7º dia pós-fertilização, foram colocadas em solução de PTZ 10 mM por 10 minutos em uma placa de 24 poços contendo 2 mL da solução. O diazepam foi escolhido como controle positivo na concentração de 50 µM para adultos e 18 µM para larvas. Foram avaliados os estágios de crise epiléptica em intervalos de 30 segundos e a latência para atingir os estágios mais severos da crise (estágios 4 e 5 para adultos e estágios 2 e 3 para larvas). Nenhuma das drogas testadas (NAC e ALC nas concentrações de 0,1, 1,0 e 10 mg/L) conseguiu prevenir ou atenuar a crise epiléptica causada pelo PTZ. Por outro lado, o diazepam diminuiu a intensidade da crise e também aumentou a latência para o início da crise epiléptica clônica, considerando os estágios mais severos tanto em adultos quanto em larvas. Esses resultados corroboram outros estudos em que o diazepam foi usado como controle positivo para reduzir as convulsões induzidas por PTZ em peixes-zebra (Choo et al., 2018; da Silva et al., 2021).

Apesar de na revisão sistemática a concentração de PTZ mais utilizada ter sido de 20 mM e o tempo de exposição ser de 30 minutos, utilizamos a concentração de 10 mM por 20 minutos em adultos e 10 minutos em larvas já que estudos demonstram que essa concentração já é capaz de desencadear uma crise epiléptica completa no animal visto através dos estágios de crise epiléptica. Além disso, essa concentração demonstrou menor mortalidade em comparação com 15 mM em peixe-zebra adulto (Mussulini et al., 2013), indicando que 10 mM é uma concentração adequada para estudar novos potenciais moléculas antiepilépticas (Canzian et al., 2019; Fontana et al., 2019). Nas larvas, o PTZ a 10 mM exibiu efeitos semelhantes, acompanhados por alterações na eletrofisiologia, incluindo aumento das descargas epileptiformes e explosões ictais e interictais (Hong et al., 2016; Y. Kim et al., 2009; Y.-H. Kim et al., 2010).

Como já discutido no capítulo 2, sabe-se que na epileptogênese os astrócitos ativados sofrem alterações moleculares que contribuem para a hiperexcitabilidade neuronal, como a regulação negativa de conexinas de junções comunicantes, transportadores de glutamato e canais de potássio (Devinsky et al., 2018). O comprometimento da função dos astrócitos pode resultar na diminuição da eliminação do glutamato extracelular, levando a um limiar convulsivo reduzido e a um desequilíbrio entre a excitação e a inibição neuronal (Bialer & White, 2010). Fármacos que modulam a transmissão glutamatérgica, como topiramato, perampanel, levetiracetam e felbamato, têm sido usadas para o tratamento de crises epilépticas e também mostraram atenuar os efeitos causados pela administração aguda de PTZ no peixe-zebra (Pieróg et al., 2021;

Sills & Rogawski, 2020). Foi relatado que NAC e ALC têm efeitos moduladores do glutamato. A NAC ativa o antiportador cistina-glutamato presente nos astrócitos, levando à liberação de glutamato que estimula os receptores metabotrópicos extra-sinápticos (mGluRs), reduzindo assim a liberação sináptica do neurotransmissor (Baker et al., 2002; Moran et al., 2005). Já a ALC demonstrou efeito modulador de glutamato em camundongos expostos a um protocolo de estresse crônico imprevisível possivelmente através de uma regulação epigenética de receptores metabotrópicos de glutamato tipo 2 (mGlu2) (Nasca et al., 2013). Esses mecanismos poderiam explicar algum efeito na diminuição de crises epilépticas e por isso fazem esses compostos serem pensados como candidatos para combater as crises.

Em nosso estudo, a NAC e ALC não demonstraram ter efeitos contra a crise epiléptica induzida por PTZ, ao contrário de outros estudos em roedores, como o estudo de Uma Devi et al. (2006) que avaliou o efeito de NAC (50 e 100 mg/kg por 8 dias) em crises epilépticas induzidas por PTZ em camundongos (60 mg/kg via injeção intraperitoneal). Nesses animais, a NAC prolongou significativamente as latências para crises generalizadas clônicas durante a análise de 30 minutos, sendo esse efeito não dependente da dose. Além disso, a associação de NAC com valproato de sódio resultou em aumento significativo do efeito antiepiléptico. Zaeri & Emamghoreishi (2015) também demonstraram o efeito antiepiléptico de NAC (50-150 mg/kg) em um modelo de crise PTZ aguda e crônica (90 mg/kg via injeção intraperitoneal).

Já outros estudos demonstraram os efeitos da ALC em modelo de *kindling*. O modelo *kindling* envolve estímulos elétricos repetidos de intensidade subefetiva ou administração repetida de uma dose baixa de um indutor químico até que sejam induzidas crises tônico-clônicas completas (Mason & Cooper., 1972; Dhir, 2012; Davoudi et al., 2013). Hussein et al. (2018) expuseram animais a PTZ (40 mg/kg i.p., 3 vezes/semana, durante 3 semanas) e observaram que o tratamento com ALC diminuiu os escores e a duração das crises epilépticas, aumentou a latência para a primeira crise, atenuou o aumento induzido por PTZ no nível do marcador de estresse oxidativo malondialdeído (MDA) e aumentou a atividade antioxidante da glutationa (GSH). Mais recentemente, Essawy et al. (2022) também demonstraram o efeito antiepiléptico de ALC (300 mg/kg) em um modelo de *kindling* de roedores. O pré e pós-tratamento com ALC suprimiu o processo de aquisição de *kindling* e aliviou notavelmente os efeitos induzidos por PTZ.

Em nosso estudo, a falta de efeito observado pode ser atribuída às baixas concentrações utilizadas e/ou à duração do tratamento (agudo x crônico em outros

estudos). Novos estudos podem ser conduzidos para avaliar o efeito do tratamento agudo com NAC e ALC em concentrações mais altas nas crises induzidas por PTZ. Além disso, NAC e ALC podem ser testados como adjuvantes de outros tratamentos anticrise para determinar se eles melhoram a resposta geral ao tratamento. Além disso, o modelo de *kindling* não está bem estabelecido em peixes-zebra, pois os estudos existentes apenas expuseram os animais a baixas concentrações de forma crônicas ao PTZ e avaliaram o comportamento logo após a exposição. As convulsões espontâneas não ocorrem nos protocolos existentes e também não há análises eletrofisiológicas (Kundap et al., 2017; Kumari et al., 2020), portanto, NAC e ALC podem ser avaliados em um novo modelo de *kindling* nessa espécie para avaliar seus potenciais efeitos antiepilépticos. Além disso, uma padronização de protocolo se faz necessária, visto a heterogeneidade dos protocolos que fizeram parte da revisão sistemática, levando em consideração os objetivos de estudo e os resultados de interesse.

5. CONCLUSÕES

Nossa revisão sistemática trouxe informações para fornecer uma ferramenta para o pesquisador selecionar o melhor protocolo para induzir quimicamente crises epilépticas nos peixes-zebra e/ou avaliar os efeitos antiepilépticos de diferentes compostos. Embora existam vários indutores químicos disponíveis para crises epilépticas, os estudos usando PTZ são de longe os mais comuns e amplamente caracterizados. No entanto, é fundamental considerar o risco de viés e a qualidade dos relatos observados nos estudos para aprimorar o protocolo a ser utilizado. Além disso, conclui-se que os compostos estudados NAC e ALC não foram capazes de atenuar as crises epilépticas agudas induzidas pelo PTZ nas condições testadas nessa tese, sendo nossa hipótese inicial não confirmada, porém mais estudos se fazem necessários para elucidar os efeitos do tratamento agudo e crônico destes compostos, como no protocolo de *kindling*, por exemplo.

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ANEXOS

Anexo 1. Carta de aprovação da CEUA

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 35823
Título: Investigação dos efeitos de neuroprotetores sobre o comportamento e mecanismos moleculares subjacentes em um modelo de crise epiléptica induzida por pentilenotetrazol (PTZ) em peixes-zebra (Danio reri

Vigência: 15/08/2018 à 15/08/2022

Pesquisadores:

Equipe UFRGS:

ÂNGELO LUIS STAPASSOLI PIATO - coordenador desde 15/08/2018
Ricieri Naue Mocelin - Aluno de Doutorado desde 15/08/2018
Matheus Felipe Marcon - Aluno de Doutorado desde 15/08/2018
Adrieli Sachett - Aluno de Doutorado desde 15/08/2018
Rafael Chitolina - Aluno de Doutorado desde 15/08/2018

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 01/10/2018 - Sala 330 do Anexo I do Prédio da Reitoria - Campus Centro - Av. Paulo Gama, 100. Porto Alegre- RS, em seus aspectos éticos e metodológicos, para a utilização de 1920 larvas de peixes-zebra e 760 peixes-zebra adultos, provenientes do Depto. de Bioquímica da UFRGS; 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, Sexta-Feira, 5 de Outubro de 2018

MARCELO MELLER ALIEVI
Coordenador da comissão de ética