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Ana Carolina Sulzbach

EFEITO DO ENRIQUECIMENTO AMBIENTAL SOBRE AS CRISES EPILÉPTICAS EM MODELO ANIMAL DE *KINDLING* INDUZIDO POR PENTILENOTETRAZOL

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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharela em Biomedicina.

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Como eu sempre disse, centenas de vezes todos os dias
Não se preocupe comigo
Eu posso sentir o gosto da falha e frustração e abaixar a cabeça
Ainda somos jovens e imaturos, nem se preocupe com isso
Porque musgo com certeza cresce
Em uma pedra que não rola
Se você não puder voltar atrás, vá embora direto
Espero que você esqueça todos os seus erros
Deixe para lá
Não é fácil, mas grave isso no seu coração
Se você sentir que irá bater, então acelere mais, seu idiota
Nevermind - BTS

RESUMO

A epilepsia é uma doença neurológica crônica que afeta milhões de pessoas em todo o mundo. Embora a terapia farmacológica seja o tratamento de primeira linha para a patologia, estima-se que cerca de um terço dos pacientes sejam refratários. Estudos anteriores sobre enriquecimento ambiental em modelos animais (EA) demonstraram que este procedimento melhora não só o bem-estar animal, mas também processos cognitivos e de aprendizagem. Além disso, também promove redução do stress e aumento da neuroplasticidade. Desta forma, o EA pode ser uma alternativa terapêutica não farmacológica promissora para a epilepsia. Neste trabalho, avaliou-se o EA em um modelo animal de crises epilépticas induzidas por pentilenotetrazol (PTZ) em diferentes períodos de exposição. Um grupo foi exposto ao EA por 30 dias antes da indução das crises, enquanto outro, durante a indução das crises, por 15 dias, com o objetivo de comparar ambas as exposições quanto à severidade e latência das crises epilépticas. Os resultados demonstraram um aumento na latência para as crises no grupo de animais que foi exposto ao EA durante a indução das crises. Além disso, este mesmo grupo apresentou crises menos severas, demonstrando o efeito protetor do EA e levantando uma possibilidade terapêutica para o mesmo na epilepsia.

Palavras-chave: Epilepsia; Crise epiléptica; Enriquecimento ambiental; Kindling; Modelo animal.

ABSTRACT

Epilepsy is a chronic neurological condition that affects millions of people worldwide. Although pharmacological therapy is the first line treatment for the condition, it is estimated that about a third of the patients are refractory. Previous studies on animal models of environmental enrichment (EE) have shown that it improves not only the well-being of the animals but also memory and learning processes level. Additionally, it provides stress reduction and increases neuroplasticity. Therefore, EE is a promising non-pharmacological therapy alternative to epilepsy. In this study, EE was tested in a pentylenetetrazole-induced epileptic seizure animal model in different exposure periods. One group was exposed to EE for 30 days before seizure induction, meanwhile the other group was exposed to EE during seizure induction for 15 days, with a goal to compare both exposures regarding the severity and latency of the seizures. Results showed an increase in latency of the animal group that was exposed to EE during the induction of the seizures. The same group also presented less severe seizures, enhancing a protective effect of EE and raising a therapeutic possibility for it regarding epilepsy.

Keywords: Epilepsy; Seizure; Environmental enrichment; Kindling; Animal model.

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1 INTRODUÇÃO

1.1 EPILEPSIA

A epilepsia é uma doença neurológica crônica, caracterizada pela presença de crises epilépticas espontâneas e recorrentes. Segundo dados de um relatório publicado pela Organização Mundial da Saúde (OMS ou, do inglês, *World Health Organization* (WHO)) em 2019 (*Epilepsy: a public health imperative*), a epilepsia afeta cerca de 50 milhões de pessoas pelo mundo, sendo uma das doenças neurológicas mais comuns. A taxa de incidência mundial da doença é estimada em cerca de 61 a cada 100.000 pessoas por ano, enquanto sua prevalência é estimada em 6,38 pessoas com epilepsia ativa a cada 1.000 pessoas (Fiest et al., 2017). De acordo com o estudo Carga Global de Doenças (*Global Burden of Disease* - GBD), realizado em 2016, que considera a mortalidade e incapacitação dos pacientes acerca de determinadas condições, a epilepsia impacta 45,9 milhões de pessoas pelo mundo. Sendo assim, a distribuição mundial da epilepsia é ampla, afetando pessoas de todas as etnias, idades, gêneros e condições de vida e, ainda que afete pessoas de todas as faixas etárias, é mais comum que ocorra em crianças e idosos (Beghi, 2020).

A definição exata do termo “epilepsia” ainda é amplamente discutida (Manford, 2017), entretanto a descrição oficial mais recente cunhada pela Liga Internacional Contra a Epilepsia (do inglês, *International League Against Epilepsy* (ILAE)), considera a Epilepsia como sendo uma doença cerebral que pode ser definida por pelo menos duas crises epilépticas espontâneas ou a partir diagnóstico apropriado (Fisher et al., 2014). A definição de “síndrome epiléptica” veio anos depois, sendo descrita pela ILAE como “um conjunto de características clínicas e de eletroencefalogramas (EEG), frequentemente corroboradas por outras características etiológicas específicas (estruturais, genéticas, metabólicas, imunes e infecciosas)” (Wirrell et al., 2022).

A epilepsia possui diversas etiologias, sendo muito heterogênea (Ali, 2018) mas a maior parte dos casos apresenta causa desconhecida (Beghi, 2020). Além da etiologia desconhecida, as causas da epilepsia podem ser divididas em outros grupos (Thijs et al., 2019). Meningite bacteriana, tuberculose do sistema nervoso central (SNC) ou outras infecções cerebrais são etiologias infecciosas para crises epilépticas, enquanto traumatismos cranianos são uma das causas estruturais mais comuns do processo epileptogênico (Ali, 2018). Distúrbios metabólicos, como hipo ou hiperglicemia, também fazem parte das causas de crises (Shneker and Fountain, 2003), bem como existe uma conexão bem estabelecida entre pacientes com doenças

autoimunes e um elevado risco de desenvolvimento de epilepsia (Steriade et al., 2021). Além disso, estudos demonstram que há genes e mutações relacionadas com a condição (Thijs et al., 2019).

A epilepsia pode ser tratada com uma vasta gama de alternativas terapêuticas e os prognósticos podem variar, dependendo de uma definição importante: o tipo de crise epiléptica em questão (Beghi et al., 2015).

1.1.1 TIPOS DE CRISES EPILÉPTICAS

Em 2017, a ILAE publicou seu relatório oficial a respeito das classificações das epilepsias e descreveu que as categoriza primeiramente entre os tipos de crises epilépticas; a partir destas, define os tipos de epilepsias e, dentro destes, temos os sintomas específicos de cada epilepsia - que também foram posteriormente descritos e classificados pela organização (Fisher et al., 2014).

Primeiramente, a ILAE define uma crise epiléptica como sendo “uma ocorrência transitória de sinais e/ou sintomas devido à atividade neuronal anormal, excessiva ou síncrona no cérebro”. Então, parte para a classificação do início da crise: focal, ou seja, em apenas um hemisfério do cérebro, ou generalizado, em ambos os hemisférios. Ainda, existe o início “desconhecido”, quando não se é possível determinar onde as crises tiveram seu início. No início focal, é possível que, durante a crise, a consciência do paciente seja afetada, e essas crises podem ter sintomas motores ou não motores por sua duração. Por fim, um tipo “especial” de crise é descrito, a “focal para tônico-clônica bilateral” que é uma crise que tem seu início focal, mas acaba por se propagar e evoluir para uma tônico-clônica. Já as crises de início generalizado podem ser divididas por seus sintomas: motores ou não motores. As de início desconhecido são classificadas de maneira similar, entre sintomas motores ou não motores, com a adição das “não classificadas”.

Dentro dos sintomas não motores, temos o que conhecemos como crises de ausência, muito comum em crianças, onde há a perda de consciência e cessação da atividade motora, como se se estivesse em um tipo de “transe”, e costuma durar cerca de 10 segundos. Nos sintomas motores, existe a chamada crise mioclônica, que é breve e envolve contrações involuntárias únicas ou múltiplas de músculos. Já a tônico-clônica envolve a perda da consciência e o simultâneo enrijecimento e estremecimento – o que se conhece popularmente

como uma “típica crise convulsiva”. Por fim, uma crise atônica gera perda do tônus muscular e gera uma queda do indivíduo (Katzung and Vanderah, 2022).

Dentro dessa classificação, além dos tipos de crises, há o que é conhecido como “estado de mal epiléptico” ou “*status epilepticus*”. É definido como: “uma condição que resulta, ou de uma falha nos mecanismos responsáveis pelo fim da crise ou da iniciação dos mecanismos, o que leva a crises anormalmente longas. É uma condição que pode ter consequências a longo prazo, incluindo morte neuronal, dano neuronal e alterações das redes neuronais, dependendo do tipo e da duração das crises” (Trinka et al., 2015).

1.2 TRANSMISSÃO SINÁPTICA

Uma crise epiléptica pode ser definida como um conjunto de sintomas ou manifestações clínicas referentes a uma descarga anormal, excessiva e sincronizada de atividade neuronal cerebral (Engel et al., 2008). Como já mencionado anteriormente, uma crise epiléptica isolada não representa, de imediato, um quadro de epilepsia, mas os sintomas principais dessa doença representam e, sendo assim, é necessário compreender como isso ocorre (Shneker and Fountain, 2003).

O neurônio é o tipo celular principal e mais comum do tecido cerebral (Hall, 2017). São as células que compõem o nosso sistema nervoso, funcionando através do estabelecimento de conexões entre si ao receber estímulos, resultando no que chamamos de “sinapses”. Sendo assim, uma sinapse é uma junção especializada, entre neurônios ou entre um neurônio e outra célula, que resulta em uma transmissão de informações entre estas células (Bear et al., 2017). Essa ação é desencadeada através de um processo extremamente complexo, porém extraordinário: a transmissão sináptica.

Tudo se inicia na membrana do neurônio. Quando esta membrana - excitável - se encontra em repouso, possui uma voltagem, ou um potencial de repouso, negativo (Engel et al., 2008). Essa membrana também possui, ao longo de sua extensão, diversos canais e bombas iônicas específicas, capazes de transportar moléculas com carga - os íons - para dentro ou fora da célula. As bombas iônicas, como a bomba de sódio e potássio, são essenciais e funcionam a todo momento para a manutenção do equilíbrio desses íons no ambiente intra e extracelular, bem como os canais iônicos (Brunton et al., 2018). E é fato que, para que esse equilíbrio seja mantido, é necessário que se mantenha uma concentração de K^+ maior no lado interno da membrana, enquanto tem-se uma maior concentração de Na^+ no meio extracelular (Bromfield

et al., 2006). E então, em um breve momento, a membrana torna-se mais permeável aos íons sódio, permitindo que cargas positivas passem para o interior da célula, mudando o potencial da membrana para positivo (Hall, 2017). Isso se chama despolarização.

A despolarização da membrana neuronal é o evento que ocorre nesse curto período de tempo quando temos a inversão da voltagem da membrana celular, anteriormente negativa, para uma voltagem mais positiva, gerando o que é denominado de potencial de ação (PA), um impulso elétrico desencadeado por uma alteração no fluxo de íons na membrana neuronal (Brunton et al., 2018). Sendo assim, a despolarização da membrana é essencial para que a transmissão sináptica seja efetiva, e pode ser alcançada, como citado anteriormente, através da entrada de íons Na^+ (e consequente saída de íons K^+) ou a entrada de íons Ca^{2+} por meio de canais iônicos específicos (Bear et al., 2017).

Nesse contexto, existem dois tipos de transmissão sináptica. A elétrica, induzida por correntes elétricas, ou a química, conduzida por moléculas que chamamos de mediadores químicos. No SNC, os "alvos" principais da epilepsia, as transmissões sinápticas mais comuns são as químicas que, em última análise, liberam neurotransmissores (Kandel et al., 2023). Neurotransmissores são uma parte do que chamamos de "mediadores químicos", que atuam no SNC desempenhando papéis cruciais para que a comunicação no cérebro se estabeleça (Ritter, 2022). Estes, por sua vez, irão se ligar a receptores específicos no neurónio pós-sináptico e irão exercer a função de abertura de outros canais iônicos e, dependendo de quais canais iônicos serão abertos pelos neurotransmissores, pode-se esperar diferentes efeitos na transmissão sináptica. Isso significa que neurotransmissores podem ter dois papéis em uma sinapse: exercer um potencial excitatório pós-sináptico (PEPS), ou um potencial inibitório pós-sináptico (PIPS) (Kandel et al., 2023).

Os neurotransmissores excitatórios mais comuns no cérebro são o glutamato, a acetilcolina (ACh), a norepinefrina e a histamina; já os inibitórios, são o ácido gama-aminobutírico (GABA) e a serotonina (Bromfield et al., 2006). Ainda, temos a dopamina, que pode ser tanto excitatória quanto inibitória. Sendo o neurotransmissor excitatório mais comum, o glutamato atua se ligando em dois receptores ionotrópicos principais: do tipo AMPA (amino-3-hidroxi-5-metil-4 isoxazolproprionato) e do tipo NMDA (N-metil-D-Aspartato) (Ritter, 2022). Ambos os receptores são permeáveis aos íons Na^+ e K^+, e, quando ativados, permitem a maior entrada de Na^+, o que contribui para a despolarização da membrana. O receptor tipo NMDA, ainda, possui um íon Mg^{2+} que normalmente o bloqueia, mas, com a despolarização, acaba sendo deslocado e permite também a entrada de Na^+, ação que garante a excitação dos neurônios (Kandel et al., 2023). Já o GABA, neurotransmissor inibitório mais comum, possui

uma ação diferente. Pode-se ligar em dois receptores, $GABA_A$ ou $GABA_B$. O receptor $GABA_B$ se encontra no neurônio pré-sináptico e atua aumentando o fluxo de saída de íons K^+, ou seja, atua ajudando na hiperpolarização da membrana. Isso acaba por causar uma atenuação da liberação de neurotransmissores (Ritter, 2022). O receptor $GABA_A$, por sua vez, está no neurônio pós-sináptico e é permeável para íons Cl^- e estes, bem como o K^+, possuem uma ação inibitória sobre a despolarização (Brunton et al., 2018).

A transmissão sináptica é bem estabelecida, organizada e deve ocorrer de maneira controlada. Existem diversos mecanismos guiados pelo próprio organismo para garantir o controle e equilíbrio desse processo, mas, em determinados cenários, podem ocorrer falhas. A Epilepsia, como sintoma, se estabelece quando existe uma atividade exacerbada e muito mais sincronizada de neurotransmissão do que o normal (Kandel et al., 2023). Quando se fala sobre "descarga anormal e excessiva de atividade neuronal", pode-se pensar em uma palavra que resume essa definição muito bem: hiperexcitabilidade (Engel et al., 2008). Um estado de hiperexcitabilidade é um estado de excitabilidade excessiva dos neurônios, que pode ocorrer de diversas maneiras: aumento de neurotransmissão sináptica excitatória ou a diminuição da neurotransmissão sináptica inibitória, alterações em canais iônicos dependentes de voltagem ou, por fim, através da alteração da concentração de íons - isso, a favor da despolarização da membrana (Bromfield et al., 2006).

A excitação, por natureza, tende a se propagar pela rede neural, mas normalmente é impedida pelos processos inibitórios, gerando o equilíbrio que faz o cérebro funcionar corretamente (Ritter, 2022). Sendo assim, o estado de hiperexcitabilidade pode superar esses mecanismos inibitórios, da mesma maneira que a redução destes pode levar uma excitação a se propagar mais do que deveria, gerando a crise (Kandel et al., 2023). Mutações que envolvem os canais de sódio dependentes de voltagem também podem os deixar abertos e, não ocorrendo a hiperpolarização após o PA ser gerado, também se criam mais aberturas para as crises (Brunton et al., 2018). Essa é a problemática principal na fisiopatologia da epilepsia.

Por fim, uma importante questão que ainda não foi plenamente respondida é o mecanismo por trás do fim das crises. Uma das poucas conclusões acerca do tema, é que não se trata de exaustão metabólica, mas ainda são necessárias mais investigações para que isso seja desvendado (Kandel et al., 2023).

1.3 MODELO *KINDLING* INDUZIDO POR PENTILENOTETRAZOL

Modelos animais são de extrema importância para a pesquisa acerca da epilepsia, uma vez que ajudam na compreensão da etiologia, fisiologia e neurobiologia da doença, possibilitando a pesquisa de vias e moléculas com um potencial alvo farmacológico para tratamentos, por exemplo (Dhir, 2012). Nos últimos 30 anos, diversos modelos animais para crises epilépticas foram alvo de estudos e demonstraram variados resultados; o modelo de *kindling*, no entanto, foi um dos poucos que efetivamente obtiveram utilidade clínica no tratamento e prevenção das crises (Löscher, 2017).

Kindling é o processo pelo qual, com o estímulo de qualquer agente pró-convulsivante, se obtém a indução de crises epilépticas (Samokhina and Samokhin, 2018). O primeiro *kindling* foi descrito por Graham Goddard, que utilizou correntes elétricas para estimular regiões subcorticais do cérebro de ratos e, apenas após utilizar destes estímulos repetidas vezes, conseguiu observar crises epilépticas nos animais (Goddard et al., 1969). Apesar do primeiro modelo ter se utilizado de estímulos elétricos, um estudo subsequente decidiu testar os efeitos da administração repetida do fármaco pentilenotetrazol (PTZ) e descobriu resultados extremamente similares aos de Goddard quando, ao administrar o PTZ uma vez a cada dois dias em um grupo de ratos que se tornou, progressivamente, sensível ao fármaco, obteve resultados demonstrando crises epilépticas que se tornavam mais severas com o passar do tempo (Mason and Cooper., 1972). Desta forma, mais estudos utilizando o PTZ como veículo principal tornaram esse tipo de *kindling* um modelo muito conhecido e utilizado entre os pesquisadores (Ergul Erkec, 2015).

O PTZ é um derivado bicíclico do tetrazol (Samokhina and Samokhin, 2018) que atua como um antagonista de receptores GABAérgicos do tipo $GABA_A$, resultando no bloqueio da atividade sináptica inibitória que acaba gerando aumento de atividade neuronal e, consequentemente, as crises epilépticas (Corda et al., 1991). Este modelo pode ser utilizado de duas formas: administrando uma alta dose de PTZ para gerar crises agudas e severas, ou administrando doses subconvulsivantes, ao longo de um período de tempo, com intervalos, para se obter um modelo crônico de crises epilépticas (Shimada and Yamagata, 2018). Em ambos os modelos, as crises induzidas normalmente são modelos de crises de ausência, mioclônicas, até tônico-clônicas (Ergul Erkec, 2015). Além disso, o *kindling* induzido pelo PTZ não gera crises epilépticas espontâneas, ao contrário de outros fármacos, como a pilocarpina (Singh et al., 2021).

1.4 TRATAMENTO

A epilepsia, além de ser uma condição anormal que deve, portanto, ser controlada, também pode causar danos físicos e sociais ao impactar na vida, convívio e interações coletivas e profissionais da pessoa afetada (Brunton et al., 2018). Com tal preocupação em mente, a pesquisa e desenvolvimento dos fármacos denominados antiepilépticos - também conhecidos como anticonvulsivantes - se iniciou. Através de modelos animais como o *kindling* elétrico ou químico, mencionados anteriormente, foi possível um progresso notável tanto em relação aos fármacos antiepilépticos (Katzung and Vanderah, 2022), quanto a alternativas não farmacológicas, que ganham força nas pesquisas.

1.4.1 TRATAMENTOS FARMACOLÓGICOS

Os fármacos antiepilépticos (FAC) começaram a ser desenvolvidos no final do século XIX, tendo o fenobarbital como um dos pioneiros na área (Brunton et al., 2018), bem como outros medicamentos que são utilizados até os dias atuais. O tratamento farmacológico é realizado de maneira crônica e é feito por via oral, tendo como objetivo a prevenção das crises epilépticas (Katzung and Vanderah, 2022). Existem 4 mecanismos de ação principais desenvolvidos para tal fim:

a) Modulação dos canais iônicos de Na^{+}, K^{+} e $Ca2^{+}$ dependentes de voltagem;

b) Potencialização da ação do GABA;

c) Inibição da ação do glutamato;

d) Modulação dos processos de liberação sináptica.

Ainda, existem alguns FAC que atuam em mecanismos diferentes dos citados, que surgiram de acordo com a evolução das pesquisas acerca das vias envolvidas no processo epileptogênico, principalmente FACs mais modernos (Ritter, 2022).

De maneira geral, o tipo de crise epiléptica em questão influencia no fármaco que será recomendado para o paciente. Existem medicamentos excelentes no controle das crises tônico-clônicas, mas ineficazes contra as crises de ausência, mesmo que o FAC ideal devesse, em teoria, ser eficaz em todos os tipos de crises (Shneker and Fountain, 2003).

1.4.2 TRATAMENTOS NÃO FARMACOLÓGICOS

Por mais que o tratamento farmacológico seja o mais comum e recomendado para os pacientes, alternativas não farmacológicas podem ser muito eficazes em, por exemplo, reduzir a recorrência de crises em pacientes com epilepsia refratária (Shneker and Fountain, 2003).

1.4.2.1 CIRURGIA E ELETROESTIMULAÇÃO

Uma alternativa efetiva, porém, delicada e complexa, é a realização de cirurgias para que se obtenha melhor controle das crises e melhor qualidade de vida dos pacientes, especialmente em casos que resistem aos medicamentos disponíveis (Shneker and Fountain, 2003). Pacientes que possuem epilepsia do lobo temporal mesial são os que possuem a maior chance de sucesso em um modelo de cirurgia ressectiva, a qual remove a parte do encéfalo responsável pelo início focal das crises (Devinsky et al., 2018).

Porém, ainda existe uma boa porcentagem de pacientes que não se encaixam nos requisitos para uma cirurgia ressectiva, então uma boa alternativa é a neuroestimulação (Devinsky et al., 2018). A neuroestimulação consiste na utilização de estímulos cerebrais para se obter o controle dos impulsos das crises, e pode ser utilizada diretamente no nervo vago - uma alternativa de mínimo risco (Manford, 2017) - ou como uma estimulação cerebral profunda, através de eletrodos intracranianos (Laxer et al., 2014).

1.4.2.2 DIETA CETOGÊNICA

Outra opção não farmacológica para tratamento de epilepsia resistente, especialmente em crianças, é a dieta cetogênica, uma dieta com foco no consumo elevado de gorduras e baixo de carboidratos (Sampaio, 2016). Já muito estudada, a dieta cetogênica atualmente é considerada um tratamento estabelecido para a redução das crises, podendo ser aplicada de diversas maneiras e com boa eficácia em crianças e adolescentes (van der Louw et al., 2016). O mecanismo exato por trás da baixa na frequência das crises ainda é incerto e está sob investigação (Sourbron et al., 2020); os relatos até então indicam que o aumento de corpos cetônicos causados pela dieta podem impactar na função mitocondrial, função neuronal ou

liberação de neurotransmissores e, assim, apresentar um efeito anticonvulsivante (Sampaio, 2016).

1.4.2.3 EXERCÍCIO FÍSICO

A epilepsia afeta a vida dos pacientes de inúmeras formas, sejam elas sociais, ou físicas. Fisicamente, o que se observa é o afastamento dessas pessoas de quaisquer formas de exercício físico, por conta de medos e estigmas, mesmo que os benefícios de uma rotina de exercícios possam oferecer aos pacientes sejam de conhecimento geral (Arida et al., 2008). Estudos já observaram um efeito positivo em relação ao exercício físico, tanto aeróbico quanto anaeróbico, na epilepsia, contribuindo para a redução da frequência das crises (Peixinho-Pena et al., 2012), atenuação de níveis de fatores associados com atividade epiléptica, como o fator de crescimento semelhante à insulina tipo 1 (IGF-1) e o fator neurotrófico derivado do cérebro (BDNF) (de Almeida et al., 2017) e inclusive redução de níveis de citocinas pró-inflamatórias, como o fator de necrose tumoral alfa (TNF-α) e a interleucina 1 beta (IL-1β) (de Lima Rosa et al., 2021).

1.5 ENRIQUECIMENTO AMBIENTAL

Enriquecimento ambiental (EA), termo relativamente recente, surgiu a partir de estudos realizados em 1947, por Donald O. Hebb. Ele descreveu que os ratos criados livremente em casa obtiveram performances melhores em testes cognitivos do que os animais que se encontravam abrigados em laboratório (Hebb et al., 1947). Não existe um consenso na definição do termo “enriquecimento ambiental”, e os protocolos são variados, mas o objetivo é o mesmo: o EA normalmente envolve melhorias nas condições de habitação dos animais, incluindo estímulos que tornem o ambiente mais complexo a níveis cognitivos, sociais, motores e sensoriais (Rosenzweig et al., 1978; Tudella and Formiga, 2021). No caso de animais domesticados, como cães, gatos e até mesmo ratos, é fácil ignorar o fato de que a domesticação não apaga comportamentos ou hábitos, mas sim, apenas altera sua frequência (Ratuski and Weary, 2022), e que o estímulo, seja ele motor ou social, melhora a qualidade de vida e o bem-estar físico e mental dos animais (Martins and Costa, 2023). Sendo assim, é de grande interesse prover o animal com materiais, brinquedos, estruturas ou desafios que realmente facilitem e

estimulem os comportamentos e características típicas da sua espécie (*Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 2010).

As técnicas de EA podem ser utilizadas em diversos contextos. Arranhadores, brinquedos de corda ou com sinos são exemplos comuns de EA para gatos domésticos (Martins and Costa, 2023), mas não são a única opção de EA existente. Animais mantidos em cativeiro como em zoológicos ou centros de reabilitação se beneficiam do EA (Hoy et al., 2010; Mason et al., 2007). A indústria alimentícia também pode utilizar do EA para melhorar a qualidade de vida dos animais que mantém, como galinhas (van Staaveren et al., 2021). E animais mantidos em laboratório também recebem benefícios provindos do EA (*Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 2010).

No caso de EA especificamente voltado para animais de laboratório, existem inúmeras possibilidades. Algumas abordagens optam em, por exemplo, aumentar a habitação em mais de uma caixa ou apenas aumentar o tamanho da caixa de habitação, podendo contar com a presença de túneis, abrigos, itens como rodinhas de correr ou materiais para ninho (Suemaru et al., 2018; Zeraati et al., 2021). Os protocolos são extremamente diversos e demonstram resultados impressionantes (Kempermann, 2019).

1.5.1 TIPOS DE ENRIQUECIMENTO AMBIENTAL

Dentro desse contexto, o EA pode ser classificado em cinco categorias: físico, social, cognitivo/ocupacional, nutricional/alimentar e sensorial (Bloomsmith et al., 1991). É importante citar, no entanto, que os melhores resultados acerca do EA são obtidos quando se utilizam de todas as ferramentas possíveis dentro do que seu contexto permite. Sendo assim, pode-se dizer que um protocolo cobrindo todos os tipos de EA terá resultados positivos e distintos dos resultados de um protocolo de, por exemplo, apenas exercício físico (Kempermann, 2019).

1.5.1.1 ENRIQUECIMENTO AMBIENTAL FÍSICO

O enriquecimento ambiental físico consiste na melhoria estrutural do ambiente do animal. Alterações ambientais podem ser cordas para balanço, rampas ou estruturas de madeira para escalada, lagos artificiais, entre outros; desde que tornem o ambiente rico, interativo e

estimulador para o animal (Hosey et al., 2013). Assim como o EA é específico para cada espécie, o enriquecimento físico também será: animais como macacos, se beneficiam de estruturas altas e variadas para escaladas (Hosey et al., 2013), já animais menores e presas naturais, como coelhos e ratos, preferem lugares fechados onde possam se esconder (Hansen and Berthelsen, 2000). De maneira geral, um ambiente enriquecido que fornece escolhas e interações aos animais é essencial e pode ajudá-los com fatores de estresse e melhorar sua qualidade de vida (*Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 2010).

1.5.1.2 ENRIQUECIMENTO AMBIENTAL SOCIAL

A interação social é uma necessidade que surge não apenas do ambiente humano. A presença de outros animais, seja da mesma ou de diferentes espécies, proporciona experiências imprevisíveis e interações dinâmicas que nenhum brinquedo ou estrutura física podem fornecer (Hosey et al., 2013). Ratos, por exemplo, são animais naturalmente sociais, normalmente se mantendo em grupos ou pares, provando que para diversas espécies - inclusive as mais solitárias - interações sociais são importantes (Crofton et al., 2015). Os estudos acerca dos benefícios do enriquecimento social se concentram mais no âmbito laboratorial, uma vez que já se observaram diferenças significativas na presença ou não de mais de um animal por gaiola/caixa; também foram verificados benefícios em animais de zoológico mantidos em grupos ou pares, em função da mais vasta gama de comportamentos exibidos por animais com interações sociais (Hosey et al., 2013).

1.5.1.3 ENRIQUECIMENTO AMBIENTAL COGNITIVO/OCUPACIONAL

Com o objetivo de estímulo mental e racional, o enriquecimento cognitivo pode vir em formas diferentes, como um quebra-cabeças ou uma situação em que o animal se depara com um problema que precisa solucionar para ganhar algo - geralmente, comida. (Hosey et al., 2013). Ainda, metodologias que encorajem o exercício físico também se encaixam nesse enriquecimento, sendo muito importantes quando consideramos que atividades motoras são extremamente benéficas no aspecto cognitivo (*Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 2010).

1.5.1.4 ENRIQUECIMENTO AMBIENTAL ALIMENTAR

O enriquecimento alimentar visa o estímulo de comportamentos espécie-específicos tendo como foco a alimentação. Consiste não apenas em variedade alimentar, mas objetiva o estímulo, por exemplo, à caça que animais predadores fariam em um ambiente selvagem (Hosey et al., 2013). Estudos já observaram que a previsibilidade em relação à alimentação, como em zoológicos, onde os animais possuem um horário fixo para comer, acabaram por gerar comportamentos agressivos, além de indícios de estresse (Bassett and Buchanan-Smith, 2007). Sendo assim, horários flexíveis e imprevisíveis promovem benefícios; bem como esconder a comida dentro do ambiente onde o animal se encontra, que é outro ótimo exemplo de enriquecimento alimentar, que incita a busca por alimento e reduz comportamentos indesejados (Gilbert-Norton et al., 2009).

1.5.1.5 ENRIQUECIMENTO AMBIENTAL SENSORIAL

Animais se comunicam e experienciam o mundo com muito mais do que apenas os olhos ou a "fala". Sendo assim, um enriquecimento ambiental focado na parte sensorial do ambiente é benéfico e, com um pouco de criatividade, é possível implementar sons ambientes, músicas, cheiros ou até mesmo modificar temperaturas, dependendo da espécie animal que se trata (Hosey et al., 2013). Já existem estudos que evidenciaram efeitos de maior produção e relaxamento em animais de fazenda quando expostos a música *country* ou clássica, bem como o odor de lavanda difuso no ar de um canil proveu comportamentos menos agitados e mobilidade reduzida, também sinais de relaxamento (Wells, 2009).

1.5.2 BENEFÍCIOS DO ENRIQUECIMENTO AMBIENTAL

A partir dos primeiros resultados obtidos com EA, surgiram, então, outros estudos que exploraram os seus efeitos em diversas áreas.

Ao nível do sistema nervoso, o EA se mostrou efetivo, por exemplo, além dos estudos já citados anteriormente, em diminuir efeitos de estresse em relação a aprendizado e memória

em roedores (Macartney et al., 2022). Diversas pesquisas apontaram uma ação do EA direcionada ao hipocampo, estrutura conectada com ambos os processos (Kempermann, 2019). Foram encontradas evidências de aumento da transmissão sináptica e plasticidade celular além de um aumento na excitabilidade celular tanto na área CA1 quanto no giro denteado hipocampal (Ohline and Abraham, 2019; Smail et al., 2020). Acredita-se que tais efeitos sejam possíveis devido a diversas alterações e modulações induzidas pelo EA, como o aumento da atividade neurotrófica induzido no hipocampo. Quando falamos, por exemplo, do fator neurotrófico derivado do cérebro (em inglês, BDNF) ou do fator de crescimento nervoso (em inglês, NGF), o EA proporciona elevação dos seus níveis, prevenção da sua queda provocada pelo estresse e aumento dos níveis dos seus respectivos receptores (Angelucci et al., 2009; Cao et al., 2014; Ickes et al., 2000). As funções neurotróficas de ambos fatores são extremamente importantes para o crescimento, desenvolvimento e plasticidade das células neuronais (Smail et al., 2020).

O EA também foi objeto de estudo em relação a drogas e substâncias de abuso. Utilizando protocolos com processos de "autoadministração de drogas", no qual os animais realizam uma tarefa para serem recompensados com uma dose de alguma droga, de maneira intravenosa (Panlilio and Goldberg, 2007), os pesquisadores foram capazes de observar redução na autoadministração de substâncias como cocaína, anfetamina ou metilfenidato (Alvers et al., 2012; Bardo et al., 2001; Gipson et al., 2011).

Outra área de destaque foi a investigação do EA em relação ao seu potencial de modulação do sistema imune (Smail et al., 2020). Os efeitos de protocolos de EA no sistema imune foram amplamente observados e documentados, demonstrando seus resultados positivos e neuroprotetores, principalmente na fisiologia e em hábitos comportamentais relacionados ao estresse (Dandi et al., 2023). Além disso, estudos também relataram a diminuição de citocinas pró-inflamatórias como a IL-6, IL-1β e TNF-α (de Sousa Fernandes et al., 2022) e aumento de fatores anti-inflamatórios como a interleucina 10 (IL-10) (Smail et al., 2020) em animais submetidos ao EA. Os benefícios da exposição ao EA tanto antes, como após cenários de estresse também foram descritos (Crofton et al., 2015).

No contexto epileptogênico, já existem alguns estudos acerca dos efeitos do EA em modelos de crises epilépticas (Auvergne et al., 2002; Young et al., 1999). No entanto, estes estudos ainda são escassos. Dessa forma, torna-se evidente a importância de mais investigações sobre o efeito do EA em um modelo animal de *kindling*. Nesse contexto, nossa hipótese é de que o EA interfere positivamente no comportamento de animais submetidos ao modelo de *kindling*, atuando de maneira a diminuir a latência das crises epilépticas e sua severidade.

2 JUSTIFICATIVA

Dados já mencionados acerca da incidência e prevalência da epilepsia no mundo demonstram a magnitude da doença e os diversos estigmas e desinformações a respeito desse tópico (Ali, 2018). É uma condição que influencia em todos os aspectos da vida de quem é afetado, trazendo consequências neurológicas, cognitivas e sociais (*Epilepsy: a public health imperative* - WHO, 2019). Condições psiquiátricas como depressão e ansiedade também já foram correlacionadas com a epilepsia (Thijs et al., 2019). Além disso, o aspecto imprevisível relacionado ao início das crises é extremamente debilitante no dia a dia do paciente (Kandel et al., 2023).

Uma questão ainda pendente é: cerca de um terço dos pacientes com epilepsia apresentam crises resistentes ao tratamento farmacológico (Janson and Bainbridge, 2021). Além disso, a doença é mais comum em países em desenvolvimento. Demonstra-se que 80 a 90% das pessoas com epilepsia nestes locais podem não ter acesso a tratamento, tanto por estigmas quanto por baixas condições financeiras (Ali, 2018).

Dentro deste contexto, são necessárias mais investigações sobre novos tratamentos e alternativas não farmacológicas para pacientes afetados pela epilepsia. Desta forma, o presente estudo investigou o efeito do EA em um modelo animal de crises epilépticas. O EA já demonstrou efeitos protetores em relação ao estresse e agressividade, além de um papel positivo na modulação do sistema imune, que também se correlaciona com a epilepsia. Além disso, estudos estão avançando em relação a efeitos neuroprotetores do EA sob condições neurodegenerativas como a doença de Alzheimer (Kempermann, 2019).

O emprego do EA, além de promissor na epilepsia, ainda pode vir a ser uma ótima alternativa, mais simples, e barata, auxiliando nos casos de refratariedade. Assim, o presente estudo investigou protocolos de diferentes períodos de tempo de exposição ao EA em relação à indução de crises epilépticas - antes e durante a indução das crises. Espera-se que os resultados contribuam para o emprego de protocolos de EA no auxílio ao tratamento da epilepsia.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Investigar o efeito do enriquecimento ambiental sobre as crises epilépticas no modelo animal de *kindling* induzido pelo pentilenotetrazol (PTZ).

3.1.1 OBJETIVOS ESPECÍFICOS

a) Investigar o efeito do enriquecimento ambiental aplicado anteriormente à indução das crises no modelo animal de *kindling*;

b) Investigar o efeito do enriquecimento ambiental aplicado concomitantemente à indução das crises no modelo animal de *kindling*.

4 ARTIGO CIENTÍFICO

Artigo a ser submetido na revista Neurobiology of Disease.

Environmental Enrichment reduces severity and increases epileptic seizure latency in an animal model of epileptic seizures.

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ABSTRACT

Epilepsy is a chronic neurological condition that affects millions of people worldwide. Although pharmacological therapy is the first line treatment for the condition, it is estimated that about a third of the patients are refractory. Previous studies on animal models of environmental enrichment (EE) have shown that it improves not only the well-being of the animals but also memory and learning processes level. Additionally, it provides stress reduction and increases neuroplasticity. Therefore, EE is a promising non-pharmacological therapy alternative to epilepsy. In this study, EE was tested in a pentylenetetrazole-induced epileptic seizure animal model in different exposure periods. One group was exposed to EE for 30 days before seizure induction, meanwhile the other group was exposed to EE during seizure induction for 15 days, with a goal to compare both exposures regarding the severity and latency of the seizures. Results showed an increase in latency of the animal group that was exposed to EE during the induction of the seizures. The same group also presented less severe seizures, enhancing a protective effect of EE and raising a therapeutic possibility for it regarding epilepsy.

Keywords: Epilepsy, Seizures, Environmental enrichment, Kindling, Animal model

Highlights:

- EE reduced the severity of seizures in the group enriched during kindling.
- Latency time for seizures was increased in the animals enriched during kindling.
- There was no effect in the group that was previously submitted to EE.

1. INTRODUCTION

Epilepsy is a chronic neurological condition characterized by spontaneous and recurrent seizures. According to the World Health Organization (WHO), it affects 50 million people worldwide being more prevalent in children and the elderly (Beghi, 2020). It is a debilitating condition and impacts every aspect of someone's life, including physically, socially, and even professionally (Ali, 2018).

Being one of the most common neurological conditions, epilepsy has an established treatment that starts with medication therapy (Devinsky et al., 2018). Antiepileptic drugs (AED) or anti-seizure drugs (ASD) are the basis of epilepsy management, but although effective in most cases, studies revealed that around 30% of epilepsy patients have refractory episodes, not benefit from the AEDs (Laxer et al., 2014; Shneker and Fountain, 2003). For that, new alternatives, especially non-pharmacological ones, need to be studied and discovered.

Environmental Enrichment (EE) is a relatively recent term that has gained strength over the past years. First mentioned by Donald O. Hebb, who detailed in his studies that rats who were raised as pets at home would perform better in cognitive tests rather than the ones kept in laboratory facilities (Brown and Milner, 2003). EE is a practice whose objective revolves around improving animal housing conditions, including stimuli that make the environment more complex at cognitive, social, motor, and sensory levels (Rosenzweig et al., 1978). EE can be used in a variety of ways, and the protocols are always diverse and dependent on animal species and necessities (Kempermann, 2019), but for laboratory animals, such as rats, it involves bigger or multiple cages, collective housing of animals on larger numbers, and the presence of stimuli such as tunnels, chewing toys, sheltering options or running wheels (Suemaru et al., 2018; Zeraati et al., 2021). It is of great interest to provide the animal with

materials, toys, structures, or challenges that facilitate and stimulate the behaviors and characteristics typical of its species (Katuski and Weary, 2022).

Since Hebb's studies, EE has collected a wide range of results demonstrating positive effects in various situations. It has already been demonstrated that EE can attenuate stress and anxiety symptoms and benefit animals cognitively, on learning and memory processes, also being capable of reducing aggressive and stress-related behaviors (Akyuz and Eroglu, 2021; Mccartney et al., 2022; Simpson and Kelly, 2011). There are some investigations on the effects of EE in epilepsy (Akyuz and Eroglu, 2021) but, especially in a PTZ-induced epileptic seizure model (Dolu et al., 2013; Keloglan et al., 2016), these studies are scarce. Therefore, new studies need to be done. On this front, we investigated the effects of EE before and during an epileptic seizure animal model, with the hypothesis of EE having protective effects on the seizures.

2. MATERIALS AND METHODS

2.1 ANIMALS

Male Wistar rats, 30 days old, adolescents, weighing approximately 100 g, from the Center for Reproduction and Experimentation of Laboratory Animals (CREAL) at Universidade Federal do Rio Grande do Sul (UFRGS) were used in the study. The animals had free access to water and food and were kept in polypropylene boxes measuring 41x34x16 cm, with a maximum of 4 animals per box, in an environment with a 12-hour light-dark cycle (07:00 AM - 07:00 PM) at a temperature of 23 ± 1°C. The animals were kept at CREAL. The experiments were approved by the ethical committee of the Universidade Federal do Rio Grande do Sul and the Ethical Committee of Animal Usage (CEUA), under project number 44316, and followed the ARRIVE guidelines.

2.2 EXPERIMENTAL GROUPS

The animals were randomly divided into three groups (n = 10–12): one group that was exposed to EE for 30 days exclusively before applying the kindling model (EEB); one group that was exposed to EE only during the 14 days of the kindling model (EED) and, lastly, a

control group (C) that remained without any type of enrichment, both before and during the application of the kindling model.

2.3 ENVIRONMENTAL ENRICHMENT

The groups of enriched animals were housed in boxes with high bars, which increased the proportions of the boxes to 41 cm x 34 cm x 24 cm. Furthermore, the boxes contained a hanging tricoline hammock measuring 25 cm x 25 cm and various wooden and calcium toys, as well as materials such as toilet paper rolls and pieces of paper, which were used as improvised toys (tunnels and food-based toys) (Figure 1). The knitting nets were present in all the boxes and were maintained every day and the improvised toys were remade every day, also always being available. The various wooden and calcium toys were rotated between the boxes of enriched animals at intervals of 4 to 5 days (Figure 2). Lastly, the control group was housed in common boxes without any enrichment.

2.4 BEHAVIOURAL ACTIVITY AND KINDLING

From the thirty-second day of experimentation, on alternate days over a period of 14 days, it was used the kindling model of seizures. In this protocol, the animals received sub-convulsant doses (30 mg/kg) intraperitoneally of the pro-convulsant drug pentylenetetrazole (PTZ) on alternate days, totaling seven administrations (Figures 2, 4, and 5). After PTZ administration, the animals underwent 20 minutes of observation in which the severity of the seizures was graded according to the Racine scale (Racine, 1972). The latency time to the onset of the first epileptic seizure was also registered.

2.5 STATISTICAL ANALYSIS

After defining the groups and variables, the Shapiro-Wilk Normality test was performed. Then, the Kruskal-Wallis test was performed, followed by the Dunn-Bonferroni post hoc test. Results with a value of $p < 0.05$ were considered with statistical significance. All

statistical analyses were conducted using a database set based on the SPSS statistical software, version 18.0.

3. RESULTS

The results obtained revealed that, on the 4th of PTZ administration, the latency for the onset of seizures (Figure 6) was greater in the EED group compared to the EEB group ($p = 0.017$, Kruskal-Wallis and Dunn-Bonferroni post hoc). Also, on 6th day of PTZ administration, latency was longer in the EED group compared to both the EEB group ($p < 0.01$, Kruskal-Wallis and Dunn-Bonferroni post hoc) and C group ($p = 0.044$, Kruskal-Wallis and Dunn-Bonferroni post hoc). There were no differences between other groups or in other moments.

Regarding the severity of the seizures (Figure 7), a protective effect from EE was observed in the last two days of PTZ administration. On the 6th ($p = 0.026$, Kruskal-Wallis and Dunn-Bonferroni post hoc) and 7th days ($p = 0.018$, Kruskal-Wallis and Dunn-Bonferroni post hoc), the EED group had less severe crises than the C group.

4. DISCUSSION

Epilepsy is a very debilitating condition, and despite all the pharmacological treatments, up to a third of the patients will still experience refractory episodes. The existing non-pharmacological alternatives, such as surgery, are expensive and, besides being considered only for a few cases, are also commonly not available to a lot of the patients who live in developing countries - where epilepsy is more predominant (Fiest et al., 2017). This shows the need to look further into other easy-applied and possibly cheaper treatment alternatives, such as EE.

Evidence suggests that EE is highly effective in mitigating stress levels, enhancing cognitive functions such as memory and learning, and protecting against neurodegenerative conditions. Each of these outcomes has been seen along epilepsy researches as positive effects of EE demonstrated a significant correlation with epilepsy in various studies (Dandi et al., 2023; Macartney et al., 2022; Smail et al., 2020). The results obtained in this investigation supported the benefits already observed by these other investigations which were able to find a protective effect that is very likely originated from EE exposure. Previous studies could observe that EE

does affect the brain in epilepsy-related pathways, such as hippocampal neurotrophic functions, which are increased by EE, benefiting neuronal cells on growth, development and plasticity wise (Angelucci et al., 2009; Cao et al., 2014; Ickes et al., 2000).

Additionally, while researchers were able to find similar results regarding a protective effect promoted by EE, there are not many observations relating to EE exposure in animal models of PTZ-induced epileptic seizures. In this study, we verified the effects of EE done both before (EEB group) and during (EED group) the application of the PTZ-induced kindling model. Therefore, the animals were exposed to EE at different moments, for different periods of time. We could notice that EE does not seem to have a preventive effect, given that the group that was enriched before the application of the kindling model did not present any significant results compared to the group that was enriched during the induction of the epileptic seizures. This evidence supports considering EE as a therapeutic adjuvant rather than a preventive measure. This could be related to, for example, inflammatory pathways, which have been found to be connected to epilepsy (de Lima Rosa, 2021; Vezzani, 2014). Epilepsy causes brain inflammation (Rana and Musto, 2018), so exposure to EE during seizure induction could ameliorate the inflammatory response caused by the seizures; that, though, would not happen with the animals being enriched before the seizure induction, since the increased inflammatory cascade has not yet been established.

The study from Dolu and colleagues which used the PTZ kindling model found that the enriched animals had lower stress levels and sympathetic activity. Another research that utilized the PTZ kindling model also showed that EE reversed learning and memory impairments caused by the seizure induction (Keloglan et al., 2016). Both studies showed benefits and protective effects regarding EE, which are consistent with our results. In this study though, we used a 14-day protocol for the kindling model, meanwhile the previously mentioned investigations used protocols with more than 30 days.

Aside from the PTZ-induced kindling model, other epilepsy models observed benefit from EE on the condition. Investigations on a *status epilepticus* model using lithium and pilocarpine found that enriched animals performed better on memory and learning tests rather than the animals in standard housing (Faverjon et al., 2002; Rutten et al., 2002; Wang et al., 2007). Also, a temporal lobe epilepsy kainate model submitted animals to an early EE exposure - right after kainic acid lesion - and late EE exposure - 60 days after kainic acid lesion - and found that both early and late exposures to EE had the animals performing better on behavioral tests (Gorantla et al., 2019).

Despite the fact that the previously mentioned results do support the results in this study, it is complex to actually compare them due to the lack of investigations with this protocol. As mentioned, there are a few studies that used the PTZ-induced epileptic seizures model, but both had a larger timeline on PTZ administration (Dolu et al., 2013; Keloglan et al., 2016). Thus, further studies will improve knowledge and make it possible to obtain better comparisons with the obtained results.

Our study should be interpreted in light of their limitations. As already mentioned, the protocols for EE are very variable (Kempermann, 2019), which could be a complication in the standardization and comparison of the results. It is one of the topics that are very much brought up by lots of authors, yet still to be resolved. The type of housing, quantity and size of toys used in our study were limited by the available structure, not allowing us to reproduce a more enriched environment as shown as other studies protocols that use bigger cages or connected two or more of them (Suemaru et al., 2018; Zeraati et al., 2021). Therefore, new studies are needed to understand the effect of EE on epilepsy.

5. CONCLUSION

In this study it was observed that animals exposed to EE during a PTZ-induced epileptic seizures protocol had less severe seizures and higher latency to their onset, being consistent with other epilepsy and epileptic seizures models findings.

Artwork



Figure 1: Demonstration of how EE was arranged in the cages.

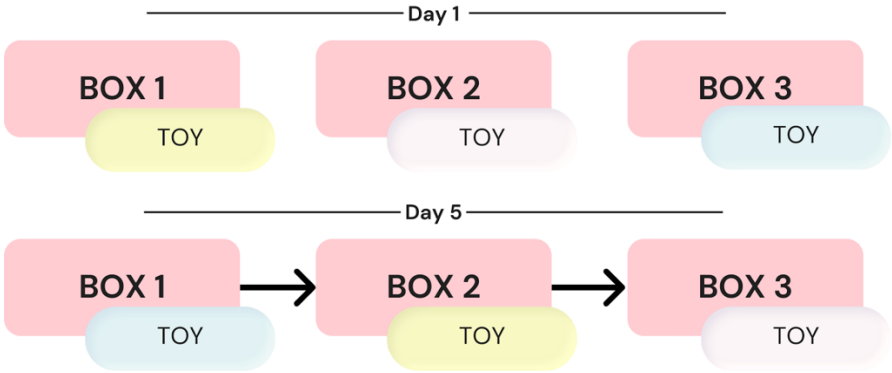


Figure 2: Demonstration of the wooden and calcium toys distribution and alternation. The toys were alternated in 4 to 5-day intervals, following a pattern for each box to experience every kind of toy at least once.

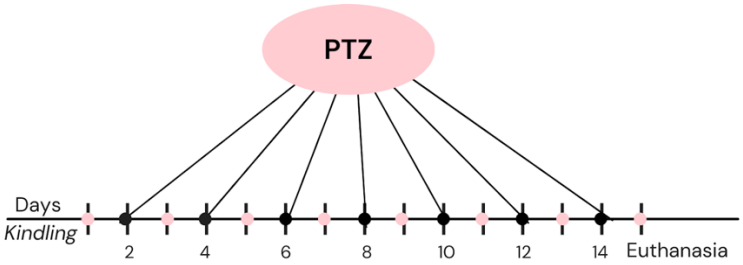


Figure 3: Demonstrative figure of the PTZ administration pattern. PTZ was administered on alternate days under 14 days, 7 administrations total. On the 15th day, the euthanasia was conducted.

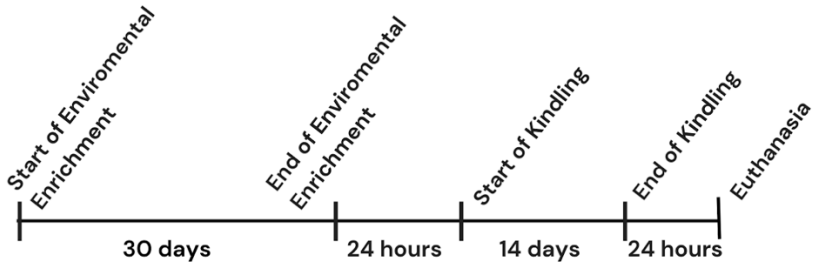


Figure 4: Experiment timeline on the animal group enriched before kindling.

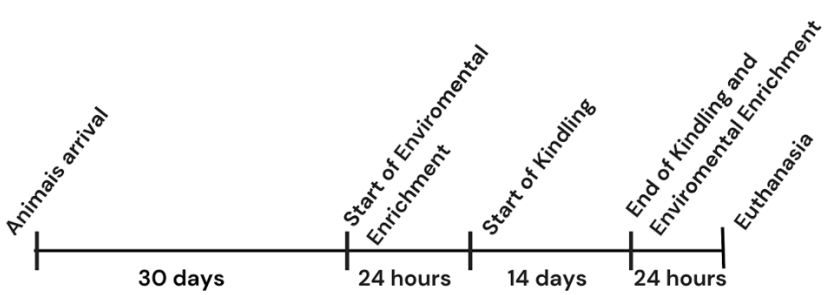


Figure 5: Experiment timeline on the animal group enriched during kindling.

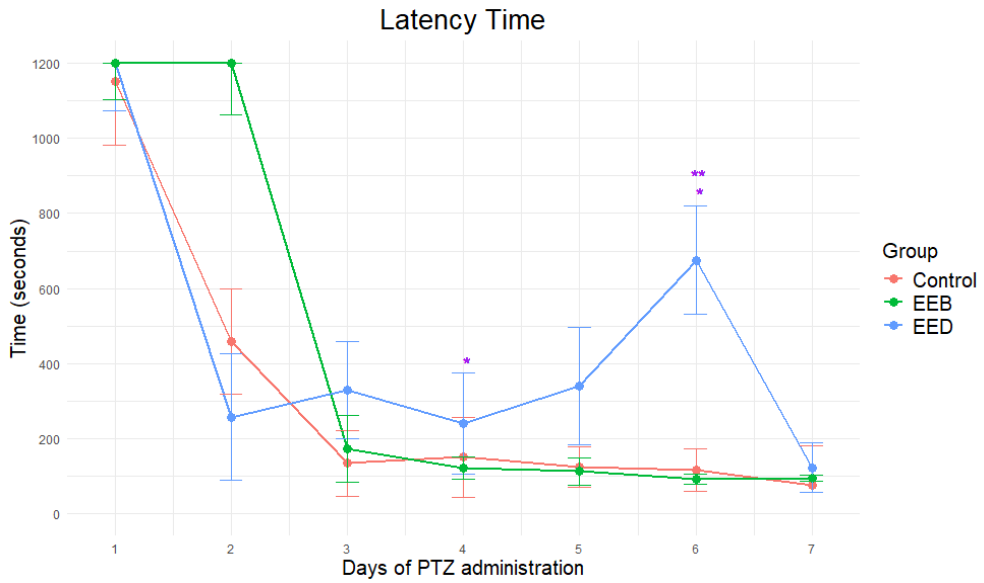


Figure 6: Effect of environmental enrichment on the latency of PTZ-induced seizures. Results are expressed as median. ** $p < 0.01$. * $p < 0.05$. Kruskal-Wallis test followed by Dunn post hoc test. The x-axis represents the days of the PTZ-induced protocol, as shown in Figure 2. Results expressed in median ± SE. n = 10-12 animals per group. EEB: Environmental enrichment before kindling model. EED: Environmental enrichment during kindling model.

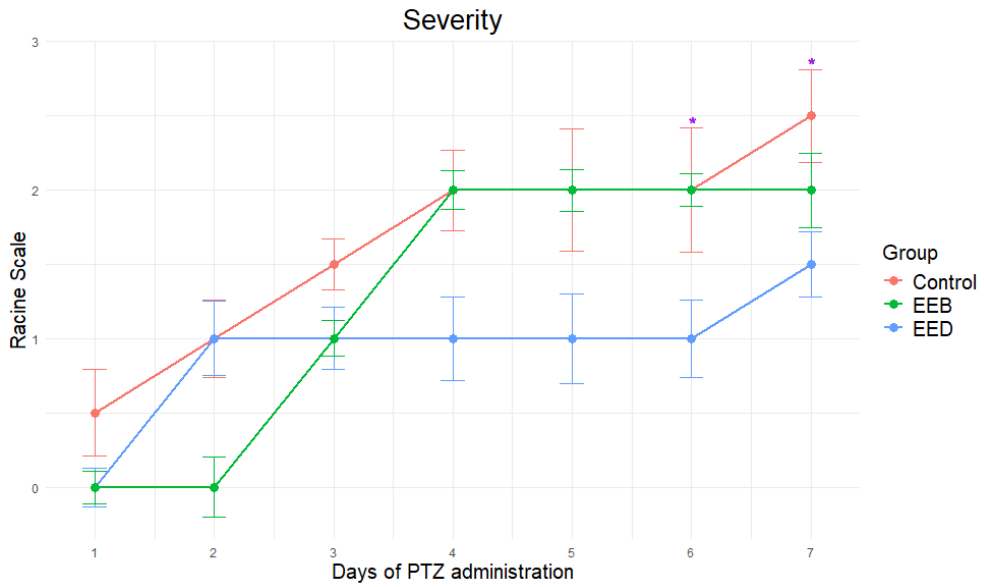


Figure 7: Effect of environmental enrichment on the severity of PTZ-induced seizures. Results are expressed as median. * $p < 0.05$. Kruskal-Wallis test followed by Dunn post hoc test. The x-axis represents the days of the PTZ-induced protocol, as shown in Figure 2. Results expressed in median ± SE. n = 10-12 animals per group. EEB: Environmental enrichment before kindling model. EED: Environmental enrichment during kindling model.

Abbreviations

EE: Environmental Enrichment

EEB: Environmental Enrichment Before

EED: Environmental Enrichment During

AED: Anti-epileptic drugs

ASD: Anti-seizure drugs

PTZ: Pentylenetetrazole

Author contributions

Ana Carolina Sulzbach: Conceptualization, Formal Analysis, Investigation, Methodology, Writing - Original Draft, Laura Gentilini: Investigation, Rafael Bremm Padilha: Investigation, Amanda Muliterno Domingues: Investigation, Edson Fernando Muller Guzzo: Investigation, Brayan Braz Barbosa: Investigation, Gabriel de Lima Rosa: Formal Analysis, Investigation, Methodology, Writing - Review & Editing, Project administration, Supervision, Funding acquisition, Adriana Simon Coitinho: Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Competing of Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Data availability

The anonymized database utilized in this study can be obtained from the corresponding author upon reasonable request.

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5 CONCLUSÕES E PERSPECTIVAS

O presente estudo evidenciou um efeito protetor do EA em relação, tanto a latência, quanto a severidade das crises epilépticas apresentadas pelos animais, corroborando os diversos estudos que demonstraram benefícios acerca do EA, tanto em modelos de crises epilépticas, quanto em outras condições e cenários.

Uma vez que apenas consideraram-se a latência e a severidade das crises epilépticas, uma perspectiva seria uma análise mais completa, investigando possíveis mecanismos envolvidos nos efeitos observados através de marcadores inflamatórios e de estresse oxidativo.

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ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA NEUROBIOLOGY OF DISEASE

Revista Neurobiology of Disease: Guide for authors

ABOUT THE JOURNAL

Aims and scope

Neurobiology of Disease is a major international open access journal at the interface between basic and clinical neuroscience. The journal provides a forum for the publication of top quality research papers on: molecular and cellular definitions of disease mechanisms, the neural systems and underpinning behavioral disorders, the genetics of inherited neurological and psychiatric diseases, nervous system aging, and findings relevant to the development of new therapies whether those are informative about the neurobiology of the considered condition. *Neurobiology of Disease* also welcomes manuscripts reporting negative results. Negative data and refutations are important to foster critical mind and scientific debate around important neurobiological questions. Authors must provide solid arguments to the Editors of the paramount importance of their observations. Validation of putative therapeutics without gaining significant new knowledge is not appropriate for *Neurobiology of Disease*.

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This journal follows a single anonymized review process. Your submission will initially be assessed by our editors to determine suitability for publication in this journal. If your submission is deemed suitable, it will typically be sent to a minimum of two reviewers for an independent expert assessment of the scientific quality. The decision as to whether your article is accepted or rejected will be taken by our editors. Authors who wish to appeal the editorial decision for their manuscript may submit a formal appeal request in accordance with the procedure outlined in Elsevier's Appeal Policy. Only one appeal per submission will be considered and the appeal decision will be final.

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Any such submissions will be subject to the journal's usual procedures and peer review will be handled independently of the editor involved and their research group. Read more about editor duties.

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- the article's publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out.
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All authors should have made substantial contributions to all of the following:

1. The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
2. Drafting the article or revising it critically for important intellectual content.
3. Final approval of the version to be submitted.

All authors should agree to be accountable for all aspects of the work to ensure that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The editors of this journal generally will not consider changes to authorship once a manuscript has been submitted. It is important that authors carefully consider the authorship list and order of authors and provide a definitive author list at original submission.

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- Consultancies
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The Declaration of Interests tool should always be completed.

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The resulting Word document containing your declaration should be uploaded at the "attach/upload files" step in the submission process. It is important that the Word document is saved in the .doc/.docx file format. Author signatures are not required.

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Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants, scholarships and awards. When funding is from a block grant or other resources available to a

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The use of generative AI and AI-assisted technologies in scientific writing must be declared by adding a statement at the end of the manuscript when the paper is first submitted. The statement will appear in the published work and should be placed in a new section before the references list. An example:

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- gender
- race
- ethnicity
- culture
- sexual orientation
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No assumptions should be made about the beliefs of readers and writing should be free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions.

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Authors must follow ethical guidelines for studies carried out in humans and animals.

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You are required to provide a concise and factual abstract. The abstract should briefly state the purpose of your research, principal results and major conclusions. Some guidelines:

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- Provide files in one of our recommended file formats. Files should be within our preferred maximum file size of 150 MB per file, 1 GB in total.
- Provide "stills" for each of your files. These will be used as standard icons to personalize the link to your video data. You can choose any frame from your video or animation or make a separate image.
- Provide text (for both the electronic and the print version) to be placed in the portions of your article that refer to the video content. This is essential text, as video and animation files cannot be embedded in the print version of the journal.

We publish all video and animation files supplied in the electronic version of your article.

For more detailed instructions, we recommend that you read our guidelines on submitting video content to be included in the body of an article.

Research data
We are committed to supporting the storage of, access to and discovery of research data, and our research data policy sets out the principles guiding how we work with the research community to support a more efficient and transparent research process.

Research data refers to the results of observations or experimentation that validate research findings, which may also include software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Please read our guidelines on sharing research data for more information on depositing, sharing and using research data and other relevant research materials.

For this journal, the following instructions from our research data guidelines apply.

Option C: Research data deposit, citation and linking

You are required to:

- Deposit your research data in a relevant data repository.
- Cite and link to this dataset in your article.
- If this is not possible, make a statement explaining why research data cannot be shared.

Data statement
To foster transparency, you are required to state the availability of any data at submission.

Ensuring data is available may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you can state the reason why (e.g., your research data includes sensitive or confidential information such as patient data) during the submission process. This statement will appear with your published article on ScienceDirect.

Read more about the importance and benefits of providing a data statement.

Data linking
Linking to the data underlying your work increases your exposure and may lead to new collaborations. It also provides readers with a better understanding of the described research.

If your research data has been made available in a data repository there are a number of ways your article can be linked directly to the dataset:

- Provide a link to your dataset when prompted during the online submission process.
- For some data repositories, a repository banner will automatically appear next to your published article on ScienceDirect.
- You can also link relevant data or entities within the text of your article through the use of identifiers. Use the following format: Database: 12345 (e.g. TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Learn more about linking research data and research articles in ScienceDirect.

Research Elements

This journal enables the publication of research objects (e.g. data, methods, protocols, software and hardware) related to original research in Elsevier's Research Elements journals.

Research Elements are peer-reviewed, open access journals which make research objects findable, accessible and reusable. By providing detailed descriptions of objects and their application with links to the original research article, your research objects can be placed into context within your article.

You will be alerted during submission to the opportunity to submit a manuscript to one of the Research Elements journals. Your Research Elements article can be prepared by you, or by one of your collaborators.

ARTICLE STRUCTURE

Article sections

Divide your manuscript into clearly defined sections covering all essential elements. For example:

- Abstract
- Keywords
- Introduction
- Materials and methods
- Results
- Conclusions
- Artwork
- Tables with captions

Introduction

The introduction should clearly state the objectives of your work. We recommend that you provide an adequate background to your work but avoid writing a detailed literature overview or summary of your results.

Material and methods

The materials and methods section should provide sufficient details about your materials and methods to allow your work to be reproduced by an independent researcher. Some guidelines:

- If the method you used has already been published, provide a summary and reference the originally published method.
- If you are quoting directly from a previously published method, use quotation marks and cite the source.
- Describe any modifications that you have made to existing methods.

Results

Results should be clear and concise. We advise you to read the sections in this guide on supplying tables, artwork, supplementary material and sharing research data.

Discussion

The discussion section should explore the significance of your results but not repeat them. You may combine your results and discussion sections into one section, if appropriate. We

recommend that you avoid the use of extensive citations and discussion of published literature in the discussion section.

Conclusion
The conclusion section should present the main conclusions of your study. You may have a stand-alone conclusions section or include your conclusions in a subsection of your discussion or results and discussion section.

Glossary
Please provide definitions of field-specific terms used in your article, in a separate list.

Abbreviations
Abbreviations which are not standard in the field should be defined in a footnote on the first page of your article.

Abbreviations which are essential to include in your abstract should be defined at first mention in your abstract, as well as in a footnote on the first page of your article.

Before submission we recommend that you review your use of abbreviations throughout your article to ensure that it is consistent.

Acknowledgements
Include any individuals who provided you with help during your research, such as help with language, writing or proof reading, in the acknowledgements section. Acknowledgements should be placed in a separate section which appears directly before the reference list. Do not include acknowledgements on your title page, as a footnote to your title, or anywhere else in your article other than in the separate acknowledgements section.

Author contributions: CRediT
Corresponding authors are required to acknowledge co-author contributions using CRediT (Contributor Roles Taxonomy) roles:

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- Software
- Supervision
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- Writing – original draft
- Writing – review and editing

Not all CRediT roles will apply to every manuscript and some authors may contribute through multiple roles.

We advise you to read more about CRediT and view an example of a CRediT author statement.

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Authors must disclose any funding sources who provided financial support for the conduct of the research and/or preparation of the article. The role of sponsors, if any, should be declared in relation to the study design, collection, analysis and interpretation of data, writing of the report and decision to submit the article for publication. If funding sources had no such involvement this should be stated in your submission.

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants, scholarships and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendices
We ask you to use the following format for appendices:

- Identify individual appendices within your article using the format: A, B, etc.
- Give separate numbering to formulae and equations within appendices using formats such as Eq. (A.1), Eq. (A.2), etc. and in subsequent appendices, Eq. (B.1), Eq. (B. 2) etc. In a similar way, give separate numbering to tables and figures using formats such as Table A.1; Fig. A.1, etc.

REFERENCES

References within text
Any references cited within your article should also be present in your reference list and vice versa. Some guidelines:

- References cited in your abstract must be given in full.
- We recommend that you do not include unpublished results and personal communications in your reference list, though you may mention them in the text of your article.
- Any unpublished results and personal communications included in your reference list must follow the standard reference style of the journal. In substitution of the publication date add "unpublished results" or "personal communication."
- References cited as "in press" imply that the item has been accepted for publication.

Linking to cited sources will increase the discoverability of your research.

Before submission, check that all data provided in your reference list are correct, including any references which have been copied. Providing correct reference data allows us to link to abstracting and indexing services such as Scopus, Crossref and PubMed. Any incorrect surnames, journal or book titles, publication years or pagination within your references may prevent link creation.

We encourage the use of Digital Object Identifiers (DOIs) as reference links as they provide a permanent link to the electronic article referenced. See the example below, though be aware that the format of such citations should be adapted to follow the style of other references in your paper.

DOI link example (for an article not yet in an issue):
VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884.

Reference format
This journal does not set strict requirements on reference formatting at submission. Some guidelines:

- References can be in any style or format as long as the style is consistent.
- Author names, journal or book titles, chapter or article titles, year of publication, volume numbers, article numbers or pagination must be included, where applicable.
- Use of DOIs is recommended.

Our journal reference style will be applied to your article after acceptance, at proof stage. If required, at this stage we will ask you to correct or supply any missing reference data.

Reference style
All citations in the text should refer to:

- Single author: the author's name (without initials, unless there is ambiguity) and the year of publication.
- Two authors: both authors' names and the year of publication.
- Three or more authors: first author's name followed by 'et al.' and the year of publication.

Citations can be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa. Examples: "as demonstrated (Allan, 2020a, 2020b; Allan and Jones, 2019)" or "as demonstrated (Jones, 2019; Allan, 2020). Kramer et al. (2023) have recently shown".

The list of references should be arranged alphabetically and then chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Van der Geer, J., Handgraaf, T., Lupton, R.A., 2020. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.sc.2020.00372.

Reference to a journal publication with an article number:

Van der Geer, J., Handgraaf, T., Lupton, R.A., 2022. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2022.e00205.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in a book:

Mettam, G.R., Adams, L.B., 2023. How to prepare an electronic version of your article, in: Jones, B.S., Smith, R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 2023. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2023).

Reference to a dataset:

Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions [dataset]. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Reference to software:

Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88) [software]. Zenodo. https://doi.org/10.5281/zenodo.3727209.

Journal abbreviations
We ask you to abbreviate journal names according to the List of Title Word Abbreviations (LTWA).

Web references
When listing web references, as a minimum you should provide the full URL and the date when the reference was last accessed. Additional information (e.g. DOI, author names, dates or reference to a source publication) should also be provided, if known.

You can list web references separately under a new heading directly after your reference list or include them in your reference list.

Data references

We encourage you to cite underlying or relevant datasets within article text and to list data references in the reference list.

When citing data references, you should include:

- author name(s)
- dataset title
- data repository
- version (where available)
- year
- global persistent identifier

Add [dataset] immediately before your reference. This will help us to properly identify the dataset. The [dataset] identifier will not appear in your published article.

Preprint references
We ask you to mark preprints clearly. You should include the word "preprint" or the name of the preprint server as part of your reference and provide the preprint DOI.

Where a preprint has subsequently become available as a peer-reviewed publication, use the formal publication as your reference.

If there are preprints that are central to your work or that cover crucial developments in the topic, but they are not yet formally published, you may reference the preprint.

Reference management software
Most Elsevier journals have their reference template available in popular reference management software products. These include products that support Citation Style Language (CSL) such as Mendeley Reference Manager.

If you use a citation plug-in from these products, select the relevant journal template and all your citations and bibliographies will automatically be formatted in the journal style. We advise you to remove all field codes before submitting your manuscript to any reference management software product.

If a template is not available for this journal, follow the format given in examples in the reference style section of this Guide for Authors.

Additional information

Electrophoretic gels and blots
All Western blot and other electrophoresis data should be supported by the underlying raw images. The image of the full gel and blot, uncropped and unprocessed, must be provided in the supplementary material on submission.

For publication at NBD, authors must include at least one molecular weight marker in electrophoretic gels (though markers above and below the band(s) of interest are preferred).

Authors must also include appropriate rulers or scale bars in any microscopy images and in any photographic images of tissues or animals.

Avoid assembling figures of blots by splicing lanes from different sections of a gel. If blots must be spliced, borders must be clearly marked and explained in the figure legend. Splicing between different blots or gels is not allowed.

Please review this information carefully to avoid publication delays stemming from the use of poor quality images or inappropriately spliced gels.

Scatter plots
Simple bar graphs to report mean±SEM values are not generally permitted: authors should super-impose a scatter plot to report the reproducibility of independent biological replicates within such data sets, and report mean±S.D. values to make the distribution and variation transparent.

ANEXO B – CARTA DE APROVAÇÃO DO 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: 44316
Título: INVESTIGACAO DO EFEITO DE ANTI-INFLAMATORIOS SOBRE PARAMETROS COMPORTAMENTAIS, IMUNOLOGICOS E MICROBIOLOGICOS EM MODELO ANIMAL DE CRISES EPILETICAS INDUZIDAS PELO PENTILENOTETRAZOL

Vigência: 01/08/2023 à 01/12/2027

Pesquisadores:

Equipe UFRGS:

ADRIANA SIMON COITINHO - coordenador desde 01/08/2023
RAFAEL BREMM PADILHA - desde 01/08/2023
GABRIEL DE LIMA ROSA - desde 01/08/2023
EDSON FERNANDO MÜLLER GUZZO - desde 01/08/2023

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 04/12/2023 - Mconf, em seus aspectos éticos e metodológicos, para a utilização de 320 ratos Wistar machos, com 2 meses de idade, provenientes do biotério central 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, Quarta-Feira, 24 de Janeiro de 2024

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MAITE DE MORAES VIEIRA
Coordenador da comissão de ética