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Daniela Freitas Câmara

Avaliação do efeito do composto MPMT-OX sobre neurotransmissão e comportamento convulsivo em *Caenorhabditis elegans*

Santa Maria, RS, Brasil

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Dissertação apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Biológicas: Bioquímica Toxicológica.**

Orientador: Prof. Dr. Félix Alexandre Antunes Soares

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Aprovado em 06 de Março de 2017:

_____

Dr. Félix Alexandre Antunes Soares, Ph.D. (UFSM)

(Presidente/Orientador)

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APRESENTAÇÃO

No item **INTRODUÇÃO** consta uma revisão sucinta da literatura sobre os temas trabalhados.

A metodologia realizada e os resultados obtidos estão apresentados no item **MANUSCRITO** sob a forma de um manuscrito redigido em inglês conforme as normas do periódico ao qual foi submetido. No mesmo constam as seções: Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas.

Os itens **CONCLUSÕES** e **PERSPECTIVAS**, apresentam conclusões gerais sobre os resultados do manuscrito e as perspectivas para futuros trabalhos.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem no item **INTRODUÇÃO**.

RESUMO

Avaliação do efeito do composto MPMT-OX sobre neurotransmissão e comportamento convulsivo em *Caenorhabditis elegans*

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A epilepsia é caracterizada como uma predisposição aumentada do cérebro para gerar crises convulsivas recorrentes, que representam os efeitos de descargas elétricas anormais, excessivas e hipersincrônicas de neurônios corticais. Atualmente, 65 milhões de pessoas no mundo têm epilepsia. Contudo, 33% dos pacientes são refratários aos tratamentos disponíveis, se fazendo necessária a busca por novos fármacos. Oxadiazois são compostos heterocíclicos com fórmula geral $C_2H_2ON_2$. O isômero 1,3,4-oxadiazol é um dos mais conhecidos e estudados, apresentando uma ampla gama de atividades biológicas, como antibacteriano, antiviral, antifúngico e anticonvulsivante. Neste trabalho testou-se o efeito do composto 2-fenil-5-[(4-metoxifenilseleno)metiltio]-1,3,4-oxadiazol (MPMT-OX) sobre a sinalização colinérgica e GABAérgica no nematoide *Caenorhabditis elegans*. Os nematoides foram expostos a 0, 5, 15 e 50 µM de MPMT-OX, a partir do estágio L1 até adultos jovens. Os resultados obtidos indicam que a exposição crônica ao MPMT-OX aumentou a sinalização GABAérgica devido à regulação dos genes *unc-25* e *unc-47*, responsáveis pela síntese e liberação de GABA na fenda sináptica, respectivamente. O aumento da sinalização inibitória atenuou a paralisia induzida por pentilenotetrazol (PTZ) e aldicarb, drogas que promovem a hiperexcitação na junção neuromuscular. MPMT-OX aumentou a atividade locomotora de nematoides com mutações nos genes *unc-30*, *unc-46* e *unc-49* (envolvidos na liberação e resposta a GABA). MPMT-OX também aumentou o tempo de latência até o início do comportamento convulsivo induzido por PTZ e auxiliou na recuperação da atividade locomotora destes nematoides após exposição ao PTZ. Estes dados sugerem que o MPMT-OX representa um promissor agente farmacológico no tratamento de condições onde o sistema GABAérgico poderia estar envolvido.

Palavras-chave: *Caenorhabditis elegans*, GABA, comportamento convulsivo, pentilenotetrazol, atividade locomotora

ABSTRACT

Effect of the MPMT-OX compound on neurotransmission and convulsive behavior in Caenorhabditis elegans

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Epilepsy is characterized as an increased predisposition of the brain to generate recurrent seizures, which represent the effects of abnormal, excessive and hypersynchronous electrical discharges of cortical neurons. Currently, 65 million people in the world have epilepsy. However, 33% of the patients are refractory to the available treatments, making necessary the search for new drugs. Oxadiazois are heterocyclic compounds which the general formula $C_2H_2ON_2$. The 1,3,4-oxadiazole isomer is one of the most known and studied, presenting a wide range of biological activities, such as antibacterial, antiviral, antifungal and anticonvulsant. In this work the effect of 2-[(4-methoxyphenylselenyl)methylthio]-5-phenyl-1,3,4-oxadiazole (MPMT-OX) on the cholinergic and GABAergic signaling in the nematode *Caenorhabditis elegans* was tested. The nematodes were exposed to 0, 5, 15 and 50 μM MPMT-OX from the L1 stage to young adults. The obtained results indicate that chronic exposure to MPMT-OX increased GABAergic signaling due to the regulation of *unc-25* and *unc-47* genes responsible for the synthesis and release of GABA in the synaptic cleft, respectively. The increase in inhibitory signaling attenuated the paralysis induced by pentylenetetrazol (PTZ) and aldicarb, drugs that promote hyperexcitation in neuromuscular junction. MPMT-OX increased the locomotor activity of nematodes with mutations in the *unc-30, unc-46* and *unc-49* genes (involved in the release and response to GABA). MPMT-OX also increased latency time to the onset of PTZ-induced seizure behavior and assisted in the recovery of the locomotor activity of these nematodes after exposure to PTZ. These data suggest that MPMT-OX represents a promising pharmacological agent in the treatment of conditions where the GABAergic system could be involved.

Keywords: *Caenorhabditis elegans*, GABA, seizure-like behavior, pentilenetetrazole, locomotor activity

SUMÁRIO

1. INTRODUÇÃO

Atualmente cerca de 65 milhões de pessoas no mundo têm epilepsia (Thurman *et al.*, 2011). A epilepsia é caracterizada como uma predisposição duradoura do cérebro para gerar crises convulsivas, com consequências neurobiológicas, cognitivas, psicológicas e sociais (Fisher *et al.*, 2005). As convulsões são a consequência de descargas elétricas anormais, excessivas e hipersincrônicas de neurônios corticais que levam à interrupção da função normal do cérebro (Scharfman, 2007). Vários mecanismos são propostos para a ocorrência de convulsões, contudo, um dos princípios mais discutidos é o desequilíbrio nos mecanismos que mantêm o equilíbrio entre os estímulos excitatórios e inibitórios (Bradford, 1995; Scharfman, 2007).

O fluxo normal de íons através dos canais iônicos permite que a membrana neuronal despolarize, repolarize e hiperpolarize, e assim ocorra a transmissão sináptica. Quando uma célula está em repouso o potencial de membrana é aproximadamente -75 mV, devido a diferença de gradientes iônicos entre o K^+ intracelular e o Na^+ extracelular. Caso ocorra estímulo (liberação de um neurotransmissor) a célula despolariza, isto é, a permeabilidade ao íon Na^+ aumenta, permitindo que este íon entre na célula, tornando o seu interior mais positivo. Após a abertura transitória em resposta a despolarização da membrana, os canais de Na^+ são espontaneamente inativados. A despolarização da membrana também tem o efeito de abrir os canais de K^+, hiperpolarizando a célula. A hiperpolarização tem como objetivo limitar a duração dos picos elétricos. O controle desta fase é dependente da abertura prolongada dos canais de K^+, da ativação dos receptores para o ácido γ–aminobutírico (GABA), e da libertação de outros neurotransmissores associados aos canais de Cl^-, entre outros fatores. Caso a hiperpolarização não ocorra, surge o risco de uma crise convulsiva (Dichter, 1989; Somjen, 2002; Scharfman, 2007).

Vários fatores podem contribuir para a falha nos mecanismos inibitórios, por exemplo, o desequilíbrio em aminoácidos e neurotransmissores como glicina, glutamato, aspartato, dopamina e serotonina, assim como no metabolismo de carboidratos, sistemas de segundo mensageiros e expressão gênica (Cavalheiro *et al.*, 1994; Marinho *et al.*, 1997). Dentro destes fatores é conhecido que glutamato e GABA estão presentes em uma parcela significativa das sinapses, servindo como importantes neurotransmissores excitatório e inibitório, respectivamente (Krnjevic, 1970; Fonnum, 1984; Foster and Kemp, 2006). Vários trabalhos sugerem que os mecanismos envolvidos na gênese das convulsões estão relacionados a um

desequilíbrio ocasionado pelo aumento da sinalização glutamatérgica e redução da resposta GABAérgica (Bradford, 1995; Akbar *et al.*, 1998; Naylor, 2010).

O glutamato é um importante neurotransmissor excitatório que é essencial em muitas funções integrativas do cérebro e no desenvolvimento do sistema nervoso. As ações do glutamato no sistema nervoso são mediadas por dois tipos de receptores: receptores ionotrópicos que exercem a excitação neuronal rápida e receptores metabotrópicos, que por outro lado, medeiam respostas relativamente lentas do glutamato por acoplamento à transdução de sinal através de proteínas G (Fonnum, 1984).

O mais abundante neurotransmissor inibitório no sistema nervoso central de mamíferos é o GABA, presente em 30-40% das sinapses (Docherty *et al.*, 1985). A síntese de GABA é mediada pela descarboxilase do ácido glutâmico (DAG), que catalisa a descarboxilação do glutamato. O GABA é empacotado em vesículas transportadoras (VTGA) e uma vez liberado na fenda sináptica pode interagir com receptores $GABA_A$ ou $GABA_C$ ionotrópicos, ou $GABA_B$ metabotrópico. A interação com receptores $GABA_A$ resulta na abertura de canais de Cl^-, e o aumento da concentração intracelular deste íon hiperpolariza ou estabiliza a célula pós-sináptica próximo a seu potencial de membrana em repouso, reduzindo a probabilidade de que estímulos excitatórios possam iniciar potenciais de ação (Smith and Olsen, 1995). Receptores GABAc também são canais pentaméricos de Cl^- regulados pelo ligante, cuja distribuição é restrita a retina (Golan *et al.*, 2009). A interação do receptor $GABA_B$ com proteínas G leva à inibição da adenilato ciclase, à ativação dos canais iônicos de K^+ e à inibição dos canais de Ca^{2+} regulados por voltagem. Nas sinapses GABAérgicas, os receptores $GABA_B$ são expressos em nível tanto pré-sináptico quanto pós-sináptico. Os auto-receptores pré-sinápticos modulam a liberação do neurotransmissor ao reduzir o influxo de Ca^{2+}, enquanto os receptores pós-sinápticos produzem potenciais inibitório pós-sináptico (PIPS) lentos, através da ativação dos canais de K^+ ativados por proteína G (Kuffler and Edwards, 1958; Mccormick, 1989; Owens and Kriegstein, 2002).

Embora existam vários fármacos para o tratamento das convulsões, 33% dos pacientes não respondem aos tratamentos disponíveis (Neligan and Shorvon, 2011). Alguns fármacos antiepilépticos podem causar efeitos adversos sérios, como hepatotoxicidade, anemia aplástica, depressão e distúrbios alimentares. Além destes, os efeitos cognitivos, como sedação, sonolência ou insônia estão presentes em vários destes fármacos (Ortinski and Meador, 2004). Neste sentido, modelos animais fornecem um meio para investigar os mecanismos

fundamentais relacionados às descargas elétricas anormais observadas durante as convulsões e para desenvolver novas terapias mais eficientes no tratamento desta patologia.

O *Caenorhabditis elegans* é um nematoide de aproximadamente 1 mm, que habita solos úmidos e utiliza bactérias como fonte alimentar. Em geral, após a eclosão do ovo, as larvas passam por quatro estágios larvais (L1, L2, L3 e L4) até o estágio adulto jovem e posteriormente adulto capaz de produzir ovos, ciclo que dura 3,5 a 4 dias. Sua expectativa de vida é de aproximadamente 20-25 dias em condições controladas de temperatura, 20 ± 2°C (Brenner, 1974; Riddle *et al.*, 1997), como mostra a Figura 1.

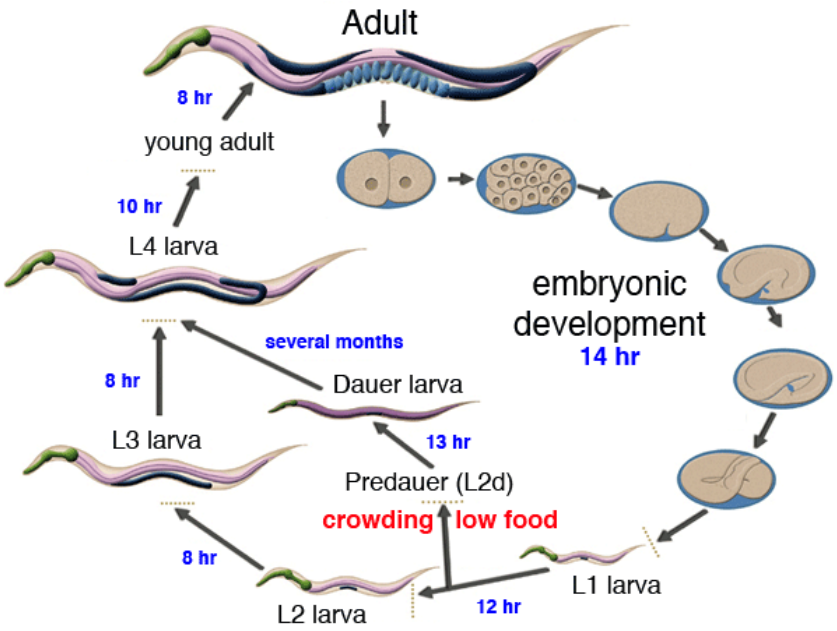


Figura 1. Ciclo de vida do *C. elegans* a 22°C, adaptada de (Murgatroyd and Spengler, 2010)

Assim como em mamíferos, no *C.elegans* a neurotransmissão GABAérgica requer um conjunto de proteínas especializadas para sintetizar, transportar e responder a GABA. O gene *unc-25* codifica a DAG, *unc-47* codifica o TVGA, *unc-46* regula a atividade e a localização do TVGA, *unc-30* é o fator que coordenadamente regula a expressão de *unc-25* e *unc-47, e unc-49* codifica o receptor $GABA_A$ *(Schuske et al., 2004)*. Os receptores GABA medeiam as ações inibitórias de GABA na junção neuromuscular (JNM), fazendo oposição à neurotransmissão excitatória, que no nematoide é mediada majoritariamente pela acetilcolina (ACh) (Richmond and Jorgensen, 1999).

Os músculos da parede do corpo do nematoide compreendem 95 células musculares individuais dispostas em quatro feixes longitudinais ancorados à cutícula (Altun and Hall, 2009). Em vermes adultos, os neurônios motores colinérgicos liberam Ach, que excita os músculos da parede do corpo do verme. Eles também excitam neurônios GABAérgicos que fazem sinapses nas paredes musculares opostas. De modo que, quando a Ach excita e contrai um conjunto de músculos, GABA é liberado sobre os músculos opostos para inibir e relaxá-los. O equilíbrio entre sinalização excitatória (E) e inibitória (I) permite que o nematoide coordene as curvaturas corporais e locomova-se (White *et al.*, 1976; Schuske *et al.*, 2004) (Figura 2).

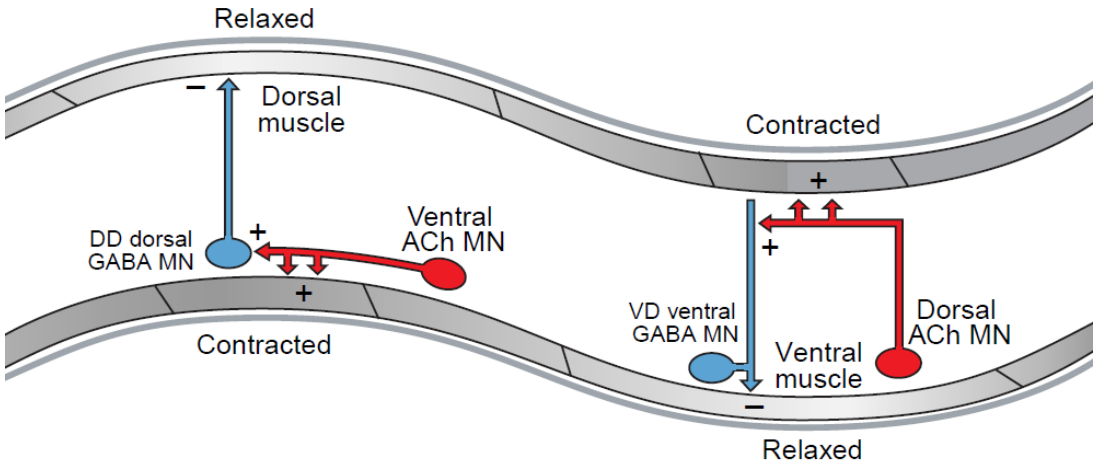


Figura 2. Conectividade entre neurônios motores Colinérgicos (vermelho) e GABAérgicos (azul), adaptada de (Han *et al.*, 2015).

Desequilíbrios no balaço E/I foram induzidos anteriormente para mimetizar convulsões no modelo nematoide. Williams *et al*. (2004) demonstrou que convulsões podem ser induzidas em *C. elegans* transgênicos com mutações em genes da via de sinalização GABAérgica (*unc-25, unc-46, unc-47* e *unc-49*) através da exposição destes nematoides ao Pentilenotetrazol (PTZ). O PTZ bloqueia canais de Cl^- associados aos receptores $GABA_A$, o que interrompe o influxo de Cl^-, impede a hiperpolarização da membrana celular e reduz o limiar para ocorrência de convulsões (Minoru *et al.*, 1991). O PTZ têm sido utilizado como uma das principais substâncias indutoras de convulsão na triagem pré-clínica de novos fármacos anticonvulsivantes. O desenvolvimento de benzodiazepínicos e barbitúricos para o tratamento de crises convulsivas veio através de estudos realizados com o PTZ (Löscher *et al.*, 1998; Smith *et al.*, 2007; White *et al.*, 2007).

O fenótipo convulsivo em *C. elegans* é caracterizado por movimentos repetitivos de contração da cabeça e paralisia da parte posterior do corpo. Em trabalhos anteriores, este comportamento foi relacionado a uma redução na sinalização GABAérgica e aumento na sinalização colinérgica na JNM de vermes submetidos a técnica de *patch-clamp* (Jospin *et al.*, 2009; Stawicki *et al.*, 2011). Desta forma, a sensibilidade comportamental, assim como a elevada manipulabilidade genética, são características interessantes oferecidas pelo nematoide *C. elegans* para a pesquisa de novas drogas com potencial farmacológico reprodutível em organismos mais complexos (Kaletta and Hengartner, 2006; Leung *et al.*, 2008).

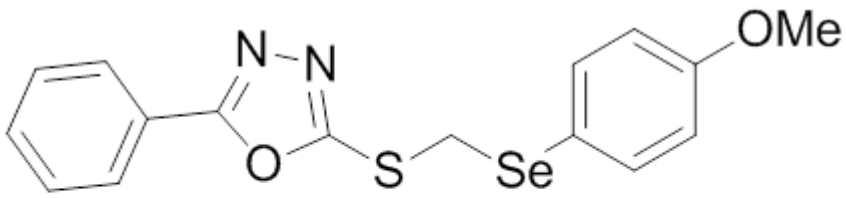
Os oxadiazois são compostos heterocíclicos que apresentam um átomo de oxigênio e dois átomos de nitrogênio em um anel de cinco membros, com fórmula geral $C_2H_2ON_2$. Moléculas com esta unidade estrutural tem demonstrado uma ampla gama de atividades biológicas, como antibacteriana (Andotra and Manhas, 1992), antimalárica (Hutt *et al.*, 1970), anti-inflamatória (Silvestrini and Pozzatti, 1961), antifúngica (Sharma and Bahel, 1983) e anticonvulsivante (Omar *et al.*, 1984), entre outras.

Dentre os possíveis isômeros oxadiazois, o 1,3,4-oxadiazol é um dos mais conhecidos e amplamente estudados pelos pesquisadores. Vários medicamentos comercializados atualmente apresentam esta unidade estrutural em sua fórmula, como o Raltegravir®, um fármaco anti-retroviral (Savarino, 2006); Nesapidil®, um fármaco antiarrítmico (Schlecker and Thieme, 1988); Furamizole®, antibacteriano (Hirao *et al.*, 1971; Ogata *et al.*, 1971); e Zibotentan®, um agente anticâncer (James and Growcott, 2009).

Diversos trabalhos têm atribuído ao 1,3,4-oxadiazol um potencial anticonvulsivante (Kashaw *et al.*, 2010; Rajak *et al.*, 2010; Jain *et al.*, 2011). Em trabalho realizado por Zarghi *et al.*,(2005), o análogo benzodiazepínico 2-amino-5-(2-fenoxibenzil)-1,3,4-oxadiazol apresentou elevada afinidade de ligação ao receptor $GABA_A$/Benzodiazepínico, baixa toxicidade, e aumentou o limiar para convulsões induzidas por PTZ e eletrochoque máximo (ECM) em ratos. Uma série de compostos derivados da ftalimida com o anel 1,3,4-oxadiazol como substituinte foi sintetizada e testada quanto a neurotoxicidade e atividade anticonvulsiva. Todos os compostos apresentaram atividade anticonvulsiva em modelo de ECM e menor neurotoxicidade que o fármaco clássico Fenitoína (Bhat *et al.*, 2010). Ali Almasirad *et al.*, (2004), sintetizou uma série de compostos com os anéis 1,3,4- oxadiazol ou 1,2,3- triazol inseridos nas estruturas. O composto 2-amino-5-[2-(2-fluorfenoxi)fenil]-1,3,4-oxadiazole

destacou-se pela considerável atividade anticonvulsivante em modelo de convulsão induzida por PTZ e ECM.

Considerando a relevância da busca por novos fármacos para o tratamento das convulsões e devido ao já reconhecido potencial anticonvulsivante de drogas que contêm a unidade estrutural 1,3,4-oxadiazol em sua fórmula, no presente trabalho investigou-se os efeitos da molécula 2-fenil-5-[(4-metoxifenilseleno)metiltio]-1,3,4-oxadiazol (Sauer *et al.*, 2016) (Figura 3), sobre a neurotransmissão em nematoides *C.elegans*,.



$C_{16}H_{14}N_2O_2SSe$

Figura 3. Estrutura química 2-fenil-5-[(4-metoxifenilseleno)metiltio]-1,3,4-oxadiazol (MPMT-OX)

2. OBJETIVOS

2.1 Objetivo Geral

Avaliar a possível atividade neuromoduladora e anticonvulsiva da molécula 2-fenil-5-[(4-metoxifenilseleno)metiltio]-1,3,4-oxadiazol em *Caenorhabditis elegan.*

2.2 Objetivos específicos

- Determinar se o MPMT-OX altera a neurotransmissão excitatória e/ou inibitória no *C. elegans,* através da avaliação da atividade locomotora;
- Investigar possíveis genes modulados pelo MPMT-OX; (mecanismo de ação)
- Determinar a atividade do MPMT-OX frente a convulsões induzidas por PTZ em *C. elegans.*

3. DESENVOLVIMENTO

O desenvolvimento está apresentado sob a forma de artigo científico. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se no próprio artigo. O artigo encontra-se na formatação para publicação da revista científica *Molecular and Cellular Neuroscience*.

3.1 Manuscript

New molecule derived from 1, 3, 4-oxadiazole modulates GABAergic system and protects against seizure-like behavior in *Caenorhabditis elegans*

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Abstract

The nervous system operates through a sensitive balance between excitatory (E) inputs and inhibitory (I) response. Changes in this balance contribute to the development of pathologies such as seizures. In *Caenorhabditis elegans*, the locomotor circuit operates via the coordinated activity of cholinergic excitatory (E) and GABAergic inhibitory (I) transmission. Changes in E/I inputs can cause uncontrolled electrical discharges, mimicking the physiology of seizures. Molecules derived from 1,3,4-oxadiazole have already been characterized by their potential against seizures due to the positive modulation of GABAergic signaling. Accordingly, we used *C. elegans* with mutations in genes related to the synthesis, release and uptake of GABA (*unc-25, unc-30, unc-46, unc-47* and *unc-49*) to evaluate the potential of a new molecule 2-[(4-methoxyphenylselenyl)methylthio]-5-phenyl-1,3,4-oxadiazole (MPMT-OX) on the synaptic GABAergic transmission. Chronic exposure to MPMT-OX attenuated the paralysis induced by pentylenetetrazole (PTZ) and aldicarb, drugs that promote hyperexcitation in the neuromuscular junction. MPMT-OX increased the locomotor activity of the *unc-30, unc-46* and *unc-49* worms and protected the *unc-46* and *unc-49* worms against PTZ-induced seizures. These data suggest that MPMT-OX enhances inhibitory signaling, probably by up-regulating the *unc-25* and *unc-47* genes. Our data point to MPMT-OX as a promising drug in the treatment of disturbs related to decreased inhibitory GABA signaling.

Keywords: *Caenorhabditis elegans*, GABA, seizure-like behavior, Pentilenetetrazole, 1,3,4-oxadiazole, neurotransmission

Abbreviations: (GABA), γ-aminobutyric acid; (WT), wild-type; (ACh), Acetylcholine; (PTZ), pentylenetetrazole; (VGAT), vesicular GABA transporter; (GAD), glutamic acid decarboxylase; (AChE), Acetylcholinesterase; (NGM), nematode growth medium; (GFP), green fluorescent protein; (NMJ), neuromuscular junction; (MPMT-OX), 2-[(4-methoxyphenylselenyl)methylthio]-5-phenyl-1,3,4-oxadiazole.

Introduction:

The nervous system works on the subtle balance of excitatory and inhibitory signals (Mccormick and Contreras, 2001). Several neurotransmitters are involved in this balance, such as γ-aminobutyric acid (GABA). GABA is the primary inhibitory neurotransmitter in the mammalian brain (Mody *et al.*, 1994). Chlorine (Cl^-) influx through $GABA_A$ receptors results in neuronal hyperpolarization and inhibition. This response depends on the maintenance of the relatively low intracellular Cl^- concentration, resulting in a Cl^- reversal potential more negative than the cell resting membrane potential (Farrant and Kaila, 2007). Acetylcholine (ACh) is a fast-acting, point-to-point neurotransmitter at the neuromuscular junction (NMJ) and in the autonomic ganglia. However, in the central nervous system, ACh acts by modulating neuronal excitability, altering presynaptic release of neurotransmitters and coordinating the firing of groups of neurons (Rice and Cragg, 2004; Kawai *et al.*, 2007; Zhang *et al.*, 2007). The imbalance between excitatory (E) and inhibitory (I) signaling pathways is essential for seizures occurrence. Seizures can be described as paroxysmal hypersynchronous transient electrical discharges in the brain that result from too much excitation or too little inhibition in the area in which the abnormal discharge starts (Scharfman, 2007; Berg *et al.*, 2010).

Despite its simple nervous system, *Caenorhabditis elegans* has important conserved features of neuronal function at the level of ion channels, axon guidance cues, receptors, transporters, synaptic components, and neurotransmitters, i.e., ACh, dopamine, GABA and serotonin (Bargmann, 1998). Previous studies have established *C. elegans* as a simple model for the study of seizure-like behavior (Williams *et al.*, 2004; Risley *et al.*, 2016). In adult worms, cholinergic motor neurons excite body wall muscles and also GABAergic neurons that synapses onto opposing body wall muscles. Thus, when ACh excites and contracts one set of muscles, GABA is released onto the opposing muscles to inhibit and relax them (White *et al.*, 1976), allowing adults to coordinate body bending (Mclntire, Jorgensen, Kaplan, *et al.*, 1993; Schuske *et al.*, 2004). Due to the sensitivity of this nematode to changes in this E/I balance, some studies have used pentylenetetrazole (PTZ) to produce seizures in *C. elegans* (Williams et al., 2004). PTZ is a selective blocker of the $GABA_A$ receptor that decreases the seizure threshold by altering the E/I input ratio to muscles (Huang *et al.*, 2001). However, detecting the effects of PTZ requires seizure-sensitive strains, such as *unc-25*, *unc-47*, *unc-49*, or *lis-1* mutants. Recently, was observed that anticonvulsant drugs (retigabine, sodium valproate and levetiracetam) promoted the recovery of *unc-25* and *unc-49* mutants after induction of seizures

(Risley *et al.*, 2016). This response confirms the suitability of the experimental model *C. elegans* for the study of new molecules with pharmacological potential in the GABAergic system and related diseases.

In this sense, oxadiazole-derived molecules have attracted attention due to their neuroprotective activity and low toxicity in mammalian models (Liu *et al.*, 2001; Jiang *et al.*, 2015). Oxadiazole is a five-membered heterocyclic ring consisting of two carbons, two nitrogens, one oxygen and two double bonds, with a general formula of $C_2H_2ON_2$. Several molecules derived from 1,2,3-oxadiazole have shown anticonvulsant activity in maximal electroshock seizure models (MES), subcutaneous pentylenetetrazole (scPTZ) and subcutaneous strychnine models (scSTY), by positively modulating GABAergic signaling in mammals (Zarghi *et al.*, 2005; Rajak *et al.*, 2010; Mashayekh *et al.*, 2014).

Considering the explained above, the aim of this study was to investigate the effects of a new molecule, 2-[(4-methoxyphenylselenyl)methylthio]-5-phenyl-1,3,4-oxadiazole (MPMT-OX), on GABAergic signaling and seizure-like behavior induced by PTZ in *C. elegans*. We tested the hypothesis that MPMT-OX protects against PTZ-induced seizures by up-regulating specific genes in the GABAergic system.

Material and methods

Chemicals and reagents

Aldicarb, dimethyl sulfoxide (DMSO), muscimol, PTZ, bovine serum albumin (BSA), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), sodium azide and acetylthiocholine iodide (ASChI) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The MPMT-OX compound was synthesized as described elsewhere (Sauer *et al.*, 2016), and chemical purity (78%) was assessed by hydrogen and carbon nuclear magnetic resonance and gas chromatography.

C. elegans strains and their maintenance

Wild-type (WT) *C. elegans* strain N2 (Bristol) and transgenic worms CB 156 *unc-25 (e156) III*, CB 307 *unc-47 (e307) III*, CB 407 *unc-49 (e407) III*, BC 277 *unc-46 (e177), dpy-11 (e224) V*, EW 49 *unc-30 (e191) IV; deIs12*, RM 2710 *snf-11(ok156) V*, and CB 193 *unc-*

29(e193) I, CZ333 *juIs1 [unc-25p::snb-1::GFP+lin-15(+)] IV* and *Escherichia coli* OP50 were obtained from the *C. elegans* Genetics Center (University of Minnesota, Minneapolis, MN, USA). All strains were grown at 20°C on nematode growth medium (NGM) plates (Brenner, 1974).

The following genes are involved in GABAergic signaling: *unc-25* encodes the biosynthetic enzyme glutamic acid decarboxylase (GAD); *unc-47* encodes the vesicular GABA transporter VGAT; *unc-46* may regulate the transport of GABA into vesicles; *unc-30* regulates the expression of *unc-25* and *unc-47* in 19 D-type neurons; *unc-49* encodes the $GABA_A$ receptor that mediates body muscle inhibition during locomotion; and *snf-11* encodes plasma membrane transporter required in vivo for GABA re-uptake from the synaptic cleft (Mclntire, Jorgensen and Horvitz, 1993; Schuske *et al.*, 2004).

Treatment and survival test

NGM agar plates for treatment were seeded with *E.coli OP50* and incubated overnight at 37°C to allow bacterial growth. MPMT-OX was added to the agar surface at final concentrations of 0, 5, 15 and 50 µM. DMSO was added to control plates and the final concentration never exceeded 0.5%.

WT or transgenic gravid adult hermaphrodite worms were lysated by bleach solution containing 1% NaCl and 0.1 M NaOH (Sulston and Hodgkin, 1988) and the eggs obtained hatched after 16 h in M9 buffer. The population of L1-larval stage worms was transferred to NGM plates with DMSO or MPMT-OX and cultured until 1-day adult stage, about 46 h. After exposure, WT and *unc-47* worms were analyzed for lethality. All other tests were performed after this period of exposure to the test compound.

PTZ-induced paralysis assay

Approximately 20 WT or *unc-30* worms were transferred from the treatment plates to new NGM plates seeded with *E. coli* and containing a final concentration of 5 mg PTZ/ml of agar, the worms were assayed for paralysis every 15 min for 120 min. Paralysis was defined as the absence of movement when the worm was prodded three times with a platinum wire on the head and tail, and when pharyngeal pumping was absent (Calahorro and Ruiz-Rubio, 2013).

Aldicarb-induced paralysis assay

Resistance of WT worms to aldicarb was performed as described elsewhere (Nurrish *et al.*, 1999) with minor modifications. Approximately 20 worms were transferred to NGM plates seeded with *E. coli* and containing 1 mM aldicarb and assayed for paralysis every 30 min for 2h and 30 min. Paralysis was defined by the absence of movement when prodded three times with a platinum wire on the head and tail.

Acetylcholinesterase (AChE) activity assay

AChE activity was measured in WT and transgenic worms, using the colorimetric method (Ellman *et al.*, 1961) with adaptations (Cole *et al.*, 2004). After exposure to treatments, 8,000-10,000 adult worms were washed three times in M9 buffer and transferred to Eppendorf tubes. The samples were frozen and thawed three times using dry ice and sonicated 5×15 s at 30% amplitude with 10 s breaks on ice and centrifuged for 30 min at 15,000 × g at 4°C. The resulting supernatant was used to measure AChE activity. The proportions of reagents were adjusted for ELISA plates, where 40 µL of sample were mixed with 200 µL of 0.25 mM DTNB, and 10 µL of 156 mM ASChI, and the plates incubated at 30°C for 5 min. The rate of change in absorbance was measured at 405 nm at 60 s intervals for 5 min in a spectrophotometer. Protein content was determined as described previously (Bradford, 1976).

Muscimol assay

Muscimol assays were performed as described previously (Dabbish and Raizen, 2011). Twenty worms were transferred to NGM plates seeded with *E. coli* containing 1 mM muscimol. After 1 h of exposure, worms were scored for the following categories: category 0, the worm moved away rapidly from the mechanical stimulus; category 1, the worm briefly contracted and relaxed and then moved away from the stimulus; category 2, the worm contracted and then relaxed while at the same time moving slightly away from the stimulus; category 3, the worm contracted and then relaxed but showed no moving away; category 4, the worm contracted incompletely and then relaxed and showed no moving away; and category 5, the worm showed flaccid paralysis and did not respond at all to the mechanical stimulus.

Seizure-like behavior assay

Seizure-like behavior assays were performed as previously described (Williams *et al.*, 2004) with minor modification. About 20-30 transgenic worms (*unc-25, unc-46, unc-47* and *unc-49*) were transferred from the treatment plates to new NGM plates with *E.coli* OP50 and 5 mg PTZ/ml of agar, and observed every 10 min for 1 h. Worms were scored positive for seizure-like behavior if they demonstrated contractions in the whole body or head-bobbing, posterior immobilization and lack of pharyngeal pumping, an observation indicative of disrupted GABA neurotransmission (Williams *et al.*, 2004).

Locomotion behavior assay

WT or transgenic worms were transferred from the treatment plates to new food-free NGM plates and allowed to move freely. After 1 min. of adaptation, worms were scored for the number of body bends during 1 min. A body bend was defined as a change in the direction of propagation of the part of the worm corresponding to the posterior bulb of the pharynx along the *y* axis, assuming the worm was traveling along the *x* axis (Tsalik and Hobert, 2003). The locomotor capacity of worms was evaluated in two situations: after exposure to MPMT-OX or DMSO and 1 h after the end of induction of seizure-like behavior with PTZ. After exposure to PTZ worms were transferred to new NGM plates containing only *E.coli* and recovered for 1 h. This time period was stipulated for the re-establishment of basic behaviors: body bends, pharyngeal pumping and defecation.

Distribution of synaptic vesicles at the pre-synaptic terminals in GABAergic neurons

Neuronal trafficking of GABA transporter vesicles (VGAT) was measured in *juIs1 [unc-25p::snb-1::GFP+ lin-15(+)] IV* worms. SNB-1::GFP worms were analyzed after exposure to MPMT-OX or DMSO, and after 1 h recovery of exposure to 5 mg PTZ/mL. Nematodes were washed from exposure plates with M9 and placed on slides containing 3 mM sodium azide. The images were obtained under a confocal microscope (Olympus® FLUOVIEW FV101) at 60x magnification. Green fluorescent protein (GFP) is expressed in presynaptic terminals of GABAergic DD and VD motor neurons as discrete fluorescent punctas. Gaps were defined as GFP failures of GABAergic motor neurons.

Digital videos of convulsions

Worms were exposed to 5 mg PTZ/ml of agar for 15 min. and then examined under a stereomicroscope (OLYMPUS® CX21). We chose this time because approximately 50% of the worms presented convulsion-like behavior, as shown in Fig 5. Seizures were recorded for 20 s using a digital video camera at 25 frames/s. The captured videos were saved using the ISCapture program.

Statistical analysis

Statistical analysis was performed using GraphPad (Version 6.0 for Macintosh OSX, GraphPad Software, San Diego, CA, USA). Significance was assessed by Student's t-test (for two groups). For more than two groups, we used one-way analysis of variance (ANOVA) or two-way ANOVA, followed by a suitable post hoc test. All assays were repeated at least three times, and $p < 0.05$ was considered statistically significant. We measured the mean fluorescence of the punctas of each animal through the ImageJ program and transformed the values in percentage of control, and submitted to the statistical tests in the GraphPad.

Results

MPMT-OX changes locomotor activity and reduces paralysis

We first evaluated whether MPMT-OX caused toxicity in WT or transgenic worms (*unc-47*). In this assay, both strans showed similar tolerance to 5, 15 and 50 µM MPMT-OX during exposure of 46 h, without increasing mortality in relation to the DMSO control (data not show). Figure 1 shows the effect of MPMT-OX on locomotor activity in *C. elegans*. WT worms exposed to MPMT-OX showed a 23.2 and 20.8% decrease in the number of body bends at 15 and 50 µM, respectively, compared to the control group (Fig. 1A).

A decrease in locomotor activity have been related to a decrease in excitatory signaling (cholinergic) or to an increase in inhibitory signaling (GABAergic) (Dabbish and Raizen, 2011; Stawicki *et al.*, 2011). To determine if treatment with MPMT-OX altered the E/I ratio at the NMJ, we exposed the WT worms to PTZ. Fig. 1B shows that treatment with 15 µM MPMT-

OX reduced the percentage of PTZ-paralyzed worms by 20% starting at 30 min and that 50 µM reduced the number of PTZ-paralyzed worms by approximately 20.5% only at 45 and 60 min of exposure.

MPMT-OX slows aldicarb-induced paralysis without acting on the cholinergic system

To investigate possible alterations in cholinergic signaling, worms treated with MPMT-OX were tested for resistance to paralysis induced by aldicarb. The rate at which worms become paralyzed reflects the balance of excitatory cholinergic and inhibitory GABAergic inputs onto the muscle (Vashlishan *et al.*, 2008). Treatment with 5 and 15 µM MPMT-OX reduced the number of paralyzed WT worms by 20%, from 60 to 90 min of exposure to aldicarb respectively, compared to the control (Fig. 2A). Resistance to aldicarb may be associated with a reduction in ACh levels at the neuromuscular synapse (Locke *et al.* 2006; Mahoney *et al.* 2006). To investigate this possibility, we measured AChE activity in WT and GABA mutants *unc-25, unc-47, unc-49, unc-46* and *unc-30*. The concentration of 15 µM was chosen for further analysis. WT and transgenic worms showed no significant differences in AChE activity in response to treatment with 15 µM MPMT-OX compared to control (Fig. 2B). To determine the participation of the Ach receptor in the effect of MPMT-OX, we evaluated the locomotor activity of *unc-29* worms. Fig. 2C shows that exposure to MPMT-OX significantly reduced the number of body bends of *unc-29* worms per minute compared to the control.

MPMT-OX acts on inhibitory signaling and alters the degree of relaxation of body muscle

We exposed WT worms to muscimol, which acts as a $GABA_A$-receptor agonist. *C. elegans* placed on agar containing GABA agonists shows a variety of responses that reflect the degree of relaxation of body muscle (De La Cruz *et al.*, 2003). Fig. 3A shows that MPMT-OX exposure reduced the number of worms with normal response to the stimulus to 12.4%, (category 0, the worm moved away rapidly from the mechanical stimulus) compared to control worms. In contrast, the treatment caused a 17% increase in the number of worms in category 4 (the worm contracted incompletely and then relaxed and showed no moving away) and 12% increase in category 5 (the worm showed flaccid paralysis and did not respond to the mechanical stimulus). To determine the effect of MPMT-OX on *C. elegans* with high levels of GABA in the neuromuscular synapse, we evaluated the locomotor activity of *snf-11* worms (Mullen *et*

al., 2006). Treatment with MPMT-OX reduced the number of body bends per minute of *snf-11* worms by 15% compared to control (Fig. 3B).

MPMT-OX modulates locomotor activity of worms with deficiency in GABAergic signaling

C. elegans unc-25, unc-30, unc-46, unc-47 and *unc-49* have reduced GABA inhibitory activity, which compromises the locomotor capacity of these animals, since body wall muscles operate on a greater proportion of excitatory inputs (Schuske *et al.*, 2004; Schuske *et al.*, 2007). We found that the *C. elegans* GABA mutants *unc-25, unc-30, unc-46, unc-47 and unc-49* respectively showed decreased locomotion by 23, 19, 31, 32 and 45% compared to WT worms (Fig. 4A). This indicated that a reduction in inhibitory signaling contributes to a reduction in locomotion rate, because the balance of E/I is critical for the normal nematode locomotion rate. Treatment with the MPMT-OX increased the number of body bends in *unc-30* (10.46%), *unc-46* (16.75%) and *unc-49* (15.6%) worms compared to controls of each strain. *unc-25* and *unc-47* worms showed no significant change in body bends rate compared to controls (Fig. 4B).

MPMT-OX increases GABAergic signaling and protects against seizure-like behavior

We tested whether MPMT-OX was able to alter susceptibility to PTZ-induced seizures in *C. elegans* GABAergic mutants (*unc-25, unc-46, unc-47* and *unc-49*). Using digital video imaging we captured the phenotypic effects of chemically induced seizures in *C. elegans* genetic backgrounds (supplementary material: video 1A, N_2 control (normal behavior); convulsion-like behavior videos: 1B. *unc-25*; 1C. *unc-46*; 1D. *unc-47* and 1E. *unc-49*). Only animals with posterior paralysis, absence of pharyngeal pumping and defecation cycle, and head-bobbing movements or spasms all over the body were scored positive for seizure.

Treatment with MPMT-OX did not affect the susceptibility of *unc-25* (Fig. 5A) and *unc-47* worms (Fig. 5C) to PTZ-induced seizures. The number of *unc-46* worms treated with MPMT-OX that displayed seizure-like behavior was 25% lower in comparison to the control, from 30 min of PTZ exposure (Fig. 5B). The number of treated *unc-49* worms that showed convulsion-like behavior was approximately 20% lower than that of control worms, from 20 min of exposure to PTZ (Fig. 5D). *unc-30* worms did not convulse in the presence of PTZ, and therefore, we tested whether treatment with MPMT-OX could affect the susceptibility of these

worms to PTZ-induced paralysis. Fig. 5E shows that the treatment reduced the number of paralyzed *unc-30* worms by 20-30% after 45 min of PTZ exposure, compared to control.

MPMT-OX facilitates recovery of GABAergic signaling

Previous work has shown a reduction in GABA levels after seizures in mammals (Bradford, 1995; Treiman, 2001). In the present study, we observed that after one hour of PTZ exposure, worms were not able to coordinate body bends due to the drastic imbalance in E/I signaling.

The recovery of locomotor activity is indicative of reestablishment of inhibitory signaling. Therefore, after one hour of recovery, we evaluated the locomotion rate of these animals (Fig. 6A). All control worms of strains *unc-25, unc-30, unc-46, unc-47* and *unc-49* showed significant reduction in the number of body bends after exposure to PTZ. The unc-30, *unc-46* and *unc-49* worms treated with MPMT-OX showed a significant increase (mean of 6%) in the number of body bends after the recovery interval. Again, the *unc-25* and *unc-47* transgenic strains did not respond to treatment with MSMTP-OX.

MPMT-OX acts pre-synaptically altering traffic of GABAergic vesicles

Figure 7 shows the density of synapses in the GABAergic nerve cords in SNB-1::GFP worms treated with MPMT-OX, before and after exposure to PTZ. SNB-1::GFP worms have a WT phenotype, and exposure to PTZ caused a paralysis rate similar to that of WT worms (data not shown).

Control worms showed a small number of gaps in the GABAergic motor neurons (12.5%) (Fig. 7A (I) and Fig. 7B), approximated values have been previously reported (Williams *et al.*, 2004). Worms treated with MPMT-OX showed similar numbers of gaps compared to control (Fig. 7A (II) and Fig. 7B), however, fluorescence of SNB-1::GFP punctas increased significantly compared to the control (Fig. 7A (II) and Fig. 7C). Exposure only to PTZ showed a 16% increase in number of gaps (Fig. 7A (III) and Fig. 7B) and a reduction in fluorescence of GFP punctas compared to control (Fig. 7A (III) and Fig. 7C). Worms that were treated with MPMT-OX before PTZ exposure showed higher fluorescence of GFP punctas (Fig.

7A (IV) and Fig. 7C), and reduction in number of gaps compared to the control exposed to PTZ (Fig. 7A (IV) and Fig. 7B).

Discussion

In the present study, we investigated the effect of a new molecule derived from 1,3,4-oxadiazole on neurotransmission in *C. elegans*. The data presented here are in line with previous studies demonstrating the anticonvulsant activity of other 1,3,4-oxadiazole derivatives (Zarghi *et al.*, 2008; Rajak *et al.*, 2010; Siddiqui *et al.*, 2014). We also observed that MPMT-OX showed no toxicity to the worms during the exposure time. This corroborates recent work with the same compound, where the EC_{50} was found to be greater than 500 µg/mL in rodents (Sauer *et al.*, 2016).

Genetic studies have shown that neurotransmitters and ion channels that mediate synaptic transmission in mammals are highly conserved in *C. elegans* (Bargmann and Kaplan, 1998; Leung *et al.*, 2008). In this regard, the NMJ of this nematode is a genetic model system appealing to the more complex and often inaccessible polynerved synapses of vertebrate central neurons.

C.elegans locomotor activity is directly related to ACh and GABA neurotransmitters, where changes in this balance may reflect alterations in locomotor ability of the worm (Jospin *et al.*, 2009). WT worms treated with MPMT-OX showed decreased locomotor activity. To determine if this behavioral change was related to imbalances in E/I signaling, we exposed MPMT-OX-treated worms to PTZ. Induction of paralysis with PTZ in WT worms is controversial, where previous work have reported that WT worms show normal behavior when exposed to up to 20 mg/mL PTZ (Williams *et al.*, 2004). However, other studies have reported reduced locomotor activity and paralysis in WT worms (Calahorro and Ruiz-Rubio, 2013). We found that 5 mg/mL PTZ induced a high paralysis rate in WT worms, and that MPMT-OX at concentrations of 15 and 50 µM MPMT-OX reduced these rates. These data suggest that MPMT-OX increases inhibitory signaling since it decreases the number of body bends of worms with normal levels of Ach/GABA (Fig. 1A) and increases resistance to paralysis in worms with inhibition of GABA response (Fig.1B).

The aldicarb assay has been previously used for assessing alterations in cholinergic synaptic transmission at the NMJ (Mahoney *et al.*, 2006). Aldicarb inhibits AChE, causing the

accumulation of Ach in the synaptic cleft. This leads to increased excitatory input and eventually to hypercontractive paralysis. Worms treated with MPMT-OX were more resistant to aldicarb-induced paralysis (Fig. 2A). This can be attributed to changes in Ach levels at the neuromuscular synapse. We measured AChE activity in worms exposed to MPMT-OX, since the ability of some 1,3,4-oxadiazole derivatives to inhibit AChE activity has been previously described (Khan *et al.*, 2013; Kamal *et al.*, 2014). Our results indicated that MPMT-OX did not affect AChE activity in WT worms or GABA mutants (Fig. 2B).

Until now, our data suggest that MPMT-OX does not act at any level on cholinergic signaling. This is more evident in the reduction of locomotor activity *of unc-29* worms after MPMT-OX treatment (Fig. 2C). *unc-29* gene encodes a non-alpha subunit of the nicotinic ACh receptor (nAChR), which mediates fast actions of ACh at NMJ and in the nervous system (Fleming *et al*., 1997). Worms with deletion of this subunit have a deficiency in cholinergic signaling, which is reflected in low locomotor activity compared to WT worms (compare Fig. 1A and 2C, reduction of 38%). The additional reduction in the locomotor activity of treated *unc-29* worms indicated a greater imbalance between (E) and (I), which tends to increase inhibitory signaling.

To investigate whether MPMT-OX may up-regulate GABA, we exposed the WT worms to muscimol, a restricted conformational analog of GABA. Muscimol produced an inward current in the body wall muscle with a reversal potential of +20 mV, similar to the GABA response. The "elastic" phenotype in which the animals contract and relax without displacement, is the more severe response to the tactile stimulus, indicating the loss of muscle tonus due to excessive inhibitory signaling in NMJ (Richmond and Jorgensen, 1999). The increased severity of response observed in treated worms is an indication of the greater release of GABA in the synaptic cleft (Fig. 3A).

snf-11 worms treated with the MPMT-OX had a reduction in locomotor activity (Fig. 3B). The *snf-11* gene encodes an electrogenic [+]/Cl[-]-coupled, high-affinity GABA transporter that is required *in vivo* for GABA uptake. Worms with *snf- 11* gene knockout have increased basal levels of GABA in the cleft (Mullen *et al.*, 2006). Therefore, the reduction in the locomotor activity of *snf-11* worms treated with MPMT-OX can be explained by the increase in GABA release in the synaptic cleft, in addition to the high levels of pre-existing GABA, leading to a reduction in the locomotor capacity of these animals.

Our data demonstrated that MPMT-OX acts by modulating GABAergic signaling, so we tested several mutants with defects in biosynthetic enzymes, transporters and receptors.

Defects in these proteins can lead to a specific imbalance of GABA neurotransmission and to diseases, such as epilepsy (Baulac *et al.*, 2001).

The *unc-30* gene encodes a homeodomain transcription factor that regulates the expression of *unc-25*-GAD and *unc-47*-VGAT in 19 D-type neurons (Eastman *et al.*, 1999). *unc-30* knockout worms showed a 10-fold reduction in *unc-25* mRNA in D-type neurons and normal levels of GAD and VGAT in RME, AVL, DVB, RIS GABAergic neurons (Eastman *et al.*, 1999). Therefore, we suggest that MPMT-OX treatment increased the levels of GAD and VGAT in RME, AVL, DVB, RIS neurons, which was reflected in the increase in locomotor activity (Fig. 4B), greater resistance to PTZ-induced paralysis (Fig. 5E) and increased locomotion rate (Fig. 6) after exposure to PTZ compared to controls. We did not observe seizures in these animals exposed to PTZ, which corroborates previously reported data (Locke *et al.*, 2009). We believe this occurs because these animals express WT levels of GABA in RME, AVL, DVB, RIS neurons (Jin *et al.*, 1994). Although these worms did not convulse, they did become paralyzed in the presence of PTZ at a higher rate than did WT worms (compare Fig. 1B and 5E), which corroborates the lower levels of GABA described in these animals

The *unc-46* gene is required primarily at the synapse to localize VGAT to synaptic vesicles (Schuske *et al.*, 2007). Previous work has demonstrated that the overexpression of the *unc-47*(VGAT) in an *unc-46* mutant background, partially rescues the defects in these animals (Schuske *et al.*, 2007). Therefore, the increase in locomotor activity of treated *unc-46* animals (Fig. 4B), as well as reduction of the number of worms with convulsion-like behavior in the presence of PTZ (Fig. 5B) and the improvement locomotor capacity after seizure (Fig. 6), can be related to increased release VGAT in neurons.

MPMT-OX-treated *unc-49* worms showed an increase in locomotor activity before and after induction of seizure behavior (Fig. 4B and Fig. 6) and reduction in the number of worms with seizure-like behavior (Fig 5D). *C. elegans unc-49* do not have $GABA_A$ receptors, but our data demonstrate that these animals respond to the possible increase in release of the GABA in the synaptic cleft, caused by MPMT-OX treatment. We believe that it is possible, because *C. elegans* possess $GABA_B$ receptors, expressed by cholinergic motor neurons, which inhibit cholinergic neurons by feedback in response to GABA spillover (Schultheis *et al.*, 2011). Thus, even in the absence of $GABA_A$, $GABA_B$ receptors may respond to increased release of GABA in the synaptic cleft, inhibiting cholinergic motor neurons.

As in other works, we demonstrated that *unc-49* worms convulsed in the presence of PTZ (Williams *et al.*, 2004; Dabbish and Raizen, 2011). How can a null mutant for the $GABA_A$ receptor respond to a chemical antagonist? PTZ is known to induce convulsions through the suppression of GABAergic signaling, however, it also alters other neurotransmission systems in mammals, such as the adenosinergic system (Pagonopoulou and Angelatou, 1998) and glutamate/glutamine homeostasis (Eloqayli *et al.*, 2003). Thus, we believe that imbalance in several neurotransmission pathways may contribute to the convulsion effect of PTZ in *unc-49*/$GABA_A$ worms.

unc-25 and *unc-47* worms treated with MPMT-OX showed no changes in locomotor activity (Fig. 5B) in the latency time up to the beginning of the convulsion-like behavior (Fig. 5A and C), and they did not recover locomotor activity after seizure (Fig. 6A). Therefore, we hypothesized that this compound is able to act on GABA synthesis and on release of the transporter vesicles, since these processes are interconnected. To verify this hypothesis, we used a method to observe the GABAergic synapses in vivo.

snb-1 encodes a neuronally expressed *C. elegans* homolog of the synaptic vesicle-associated membrane protein synaptobrevin (SNB-1), involved in vesicle docking and exocytosis (Nonet *et al.*, 1998). The *unc-25* gene serves as a ubiquitous marker for the GABAergic system (Nonet, 1999). A transgenic strain carrying a fusion of GFP to the protein SNB-1 driven by the *unc-25* promoter allows the specific visualization of the GABAergic vesicle system.

Previous work has shown that differences in the fluorescence intensity of SNB-1::GFP punctas are correlates with changes in the number of synaptic vesicles that reach the synaptic terminals (Dittman and Kaplan, 2006; Bessa *et al.*, 2013). In *C.elegans* synapses occur *en passant*, that is, synaptic boutons are formed along the axon shaft (Jin, 2005). Therefore, our data indicate that the increase of the fluorescence of SNB-1::GFP punctas, as well as, the reduction of the number of gaps in the GABAergic motor neurons of worms treated with MPMT-OX and post-exposed to PTZ, is related to the greater recruitment of SNB-1 due to increased docking and release of GABAergic vesicles. This data, in addition with ours behavioral results make plausible describe a mechanism to the effects observed in *C. elegans* treated with MPMT-OX.

Considering our data, we believe MPMT-OX could leads to release of GABA in the synaptic cleft due to up-regulation of *unc-25* and *unc-47* genes. In this way, the increase in inhibitory input signals in NMJ reduces the locomotor activity of WT worms. This increase in

excitation threshold protects animals against PTZ-induced paralysis and seizure, and could accelerates the recovery of locomotion after convulsion by facilitating the restoration of E/I balance.

Conclusions

The effects of MPMT-OX on neurotransmission were tested in *C. elegans*. Pre-treatment with it reduced the number of paralyzed worms, after exposure to drugs that alter the balance between E/I inputs in body wall muscles, as PTZ. MPMT-OX act by modulating *unc-25* and *unc-47*, genes responsible for the synthesis and transport of GABA, respectively. This mechanism is clearly reflected in the locomotor aspects of worms deficient in GABAergic signaling, as well as in protection against PTZ-induced seizure. Our data indicate that MPMT-OX has pharmacological potential in the treatment of conditions such as seizures condition in which the GABAergic system could be involved.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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References

Abraham, C., Bai, L., Leube, R.E., 2011. Synaptogyrin-dependent modulation of synaptic neurotransmission in Caenorhabditis elegans. Neuroscience 190, 75-88.

Bargmann, C.I., 1998. Neurobiology of the Caenorhabditis elegans genome. Science 282, 2028-2033.

Bargmann, C.I., Kaplan, J.M., 1998. Signal transduction in the Caenorhabditis elegans nervous system. Annual review of neuroscience 21, 279-308.

Baulac, S., Huberfeld, G., Gourfinkel-An, I., Mitropoulou, G., Beranger, A., Prud'homme, J.-F., Baulac, M., Brice, A., Bruzzone, R., LeGuern, E., 2001. First genetic evidence of GABAA receptor dysfunction in epilepsy: a mutation in the γ2-subunit gene. Nature genetics 28, 46-48.

Bradford, H., 1995. Glutamate, GABA and epilepsy. Progress in neurobiology 47, 477-511.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72, 248-254.

Brenner, S., 1974. The genetics of Caenorhabditis elegans. Genetics 77, 71-94.

Calahorro, F., Ruiz-Rubio, M., 2013. Human alpha-and beta-NRXN1 isoforms rescue behavioral impairments of Caenorhabditis elegans neurexin-deficient mutants. Genes, Brain and Behavior 12, 453-464.

Cole, R.D., Anderson, G.L., Williams, P.L., 2004. The nematode Caenorhabditis elegans as a model of organophosphate-induced mammalian neurotoxicity. Toxicology and Applied Pharmacology 194, 248-256.

Dabbish, N.S., Raizen, D.M., 2011. GABAergic synaptic plasticity during a developmentally regulated sleep-like state in C. elegans. The Journal of Neuroscience 31, 15932-15943.

de la Cruz, I.P., Levin, J.Z., Cummins, C., Anderson, P., Horvitz, H.R., 2003. sup-9, sup-10, and unc-93 may encode components of a two-pore K+ channel that coordinates muscle contraction in Caenorhabditis elegans. The Journal of neuroscience 23, 9133-9145.

Eastman, C., Horvitz, H.R., Jin, Y., 1999. Coordinated Transcriptional Regulation of the unc-25Glutamic Acid Decarboxylase and the unc-47 GABA Vesicular Transporter by the Caenorhabditis elegans UNC-30 Homeodomain Protein. The Journal of neuroscience 19, 6225-6234.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. pp. 88-95.

Eloqayli, H., Dahl, C.B., Götestam, K.G., Unsgård, G., Hadidi, H., Sonnewald, U., 2003. Pentylenetetrazole decreases metabolic glutamate turnover in rat brain. Journal of neurochemistry 85, 1200-1207.

Farrant, M., Kaila, K., 2007. The cellular, molecular and ionic basis of GABA A receptor signalling. Progress in brain research 160, 59-87.

Huang, R.-Q., Bell-Horner, C.L., Dibas, M.I., Covey, D.F., Drewe, J.A., Dillon, G.H., 2001. Pentylenetetrazole-induced inhibition of recombinant γ-aminobutyric acid type A (GABAA) receptors: mechanism and site of action. Journal of Pharmacology and Experimental Therapeutics 298, 986-995.

Jiang, C.-S., Fu, Y., Zhang, L., Gong, J.-X., Wang, Z.-Z., Xiao, W., Zhang, H.-Y., Guo, Y.-W., 2015. Synthesis and biological evaluation of novel marine-derived indole-based 1, 2, 4-oxadiazoles derivatives as multifunctional neuroprotective agents. Bioorganic & medicinal chemistry letters 25, 216-220.

Jin, Y., Hoskins, R., Horvitz, H.R., 1994. Control of type-D GABAergic neuron differentiation by C. elegans UNC-30 homeodomain protein.

Jospin, M., Qi, Y.B., Stawicki, T.M., Boulin, T., Schuske, K.R., Horvitz, H.R., Bessereau, J.-L., Jorgensen, E.M., Jin, Y., 2009. A neuronal acetylcholine receptor regulates the balance of muscle excitation and inhibition in Caenorhabditis elegans. PLoS Biol 7, e1000265.
Kamal, A., Shaik, A.B., Reddy, G.N., Kumar, C.G., Joseph, J., Kumar, G.B., Purushotham, U., Sastry, G.N., 2014. Synthesis, biological evaluation, and molecular modeling of (E)-2-aryl-5-styryl-1, 3, 4-oxadiazole derivatives as acetylcholine esterase inhibitors. Medicinal Chemistry Research 23, 2080-2092.
Kawai, H., Lazar, R., Metherate, R., 2007. Nicotinic control of axon excitability regulates thalamocortical transmission. Nature neuroscience 10, 1168-1175.
Khan, K.M., Ashraf, M., Ahmad, I., Ejaz, S.A., 2013. Synthesis, characterization and biological screening of 5-substituted-1, 3, 4-oxadiazole-2yl-N-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide. Pak. J. Pharm. Sci 26, 345-352.
Leung, M.C., Williams, P.L., Benedetto, A., Au, C., Helmcke, K.J., Aschner, M., Meyer, J.N., 2008. Caenorhabditis elegans: an emerging model in biomedical and environmental toxicology. Toxicological Sciences 106, 5-28.
Liu, Z., Yang, G., Qin, X., 2001. Syntheses and biological activities of novel diheterocyclic compounds containing 1, 2, 4-triazolo [1, 5-a] pyrimidine and 1, 3, 4-oxadiazole. Journal of Chemical Technology and Biotechnology 76, 1154-1158.
Locke, C.J., Kautu, B.B., Berry, K.P., Lee, S.K., Caldwell, K.A., Caldwell, G.A., 2009. Pharmacogenetic analysis reveals a post-developmental role for Rac GTPases in Caenorhabditis elegans GABAergic neurotransmission. Genetics 183, 1357-1372.
Locke, C.J., Williams, S.N., Schwarz, E.M., Caldwell, G.A., Caldwell, K.A., 2006. Genetic interactions among cortical malformation genes that influence susceptibility to convulsions in C. elegans. Brain research 1120, 23-34.
Mahoney, T.R., Luo, S., Nonet, M.L., 2006. Analysis of synaptic transmission in Caenorhabditis elegans using an aldicarb-sensitivity assay. Nature protocols 1, 1772-1777.
Mashayekh, S., Rahmanipour, N., Mahmoodi, B., Ahmadi, F., Motaharian, D., Shahhosseini, S., Shafaroodi, H., Banafshe, H.R., Shafiee, A., Navidpour, L., 2014. Synthesis, receptor affinity and effect on pentylenetetrazole-induced seizure threshold of novel benzodiazepine analogues: 3-Substituted 5-(2-phenoxybenzyl)-4H-1, 2, 4-triazoles and 2-amino-5-(phenoxybenzyl)-1, 3, 4-oxadiazoles. Bioorganic & medicinal chemistry 22, 1929-1937.
McCormick, D.A., Contreras, D., 2001. On the cellular and network bases of epileptic seizures. Annual review of physiology 63, 815-846.
Mclntire, S.L., Jorgensen, E., Horvitz, H.R., 1993a. Genes required for GABA function in Caenorhabditis elegans.
Mclntire, S.L., Jorgensen, E., Kaplan, J., Horvitz, H.R., 1993b. The GABAergic nervous system of Caenorhabditis elegans.
Mody, I., De Koninck, Y., Otis, T.S., Soltesz, I., 1994. Bridging the cleft at GABA synapses in the brain. Trends in neurosciences 17, 517-525.
Mullen, G.P., Mathews, E.A., Saxena, P., Fields, S.D., McManus, J.R., Moulder, G., Barstead, R.J., Quick, M.W., Rand, J.B., 2006. The Caenorhabditis elegans snf-11 gene encodes a sodium-dependent GABA transporter required for clearance of synaptic GABA. Molecular biology of the cell 17, 3021-3030.
Nonet, M.L., 1999. Visualization of synaptic specializations in live C. elegans with synaptic vesicle protein-GFP fusions. Journal of neuroscience methods 89, 33-40.
Nonet, M.L., Saifee, O., Zhao, H., Rand, J.B., Wei, L., 1998. Synaptic transmission deficits in Caenorhabditis eleganssynaptobrevin mutants. The Journal of neuroscience 18, 70-80.
Nurrish, S., Ségalat, L., Kaplan, J.M., 1999. Serotonin inhibition of synaptic transmission: Gαo decreases the abundance of UNC-13 at release sites. Neuron 24, 231-242.

Pagonopoulou, O., Angelatou, F., 1998. Time development and regional distribution of [3H] nitrobenzylthioinosine adenosine uptake site binding in the mouse brain after acute pentylenetetrazol-induced seizures. Journal of neuroscience research 53, 433-442.
Rajak, H., Deshmukh, R., Veerasamy, R., Sharma, A.K., Mishra, P., Kharya, M.D., 2010. Novel semicarbazones based 2, 5-disubstituted-1, 3, 4-oxadiazoles: One more step towards establishing four binding site pharmacophoric model hypothesis for anticonvulsant activity. Bioorganic & medicinal chemistry letters 20, 4168-4172.
Rice, M.E., Cragg, S.J., 2004. Nicotine amplifies reward-related dopamine signals in striatum. Nature neuroscience 7, 583-584.
Richmond, J.E., Jorgensen, E.M., 1999. One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction. Nature neuroscience 2, 791-797.
Risley, M.G., Kelly, S.P., Jia, K., Grill, B., Dawson-Scully, K., 2016. Modulating Behavior in C. elegans Using Electroshock and Antiepileptic Drugs. PloS one 11, e0163786.
Sauer, A.C., Leal, J.G., Stefanello, S.T., Leite, M.T., Souza, M.B., Soares, F.A., Rodrigues, O.E., Dornelles, L., 2016. Synthesis and antioxidant properties of organosulfur and organoselenium compounds derived from 5-substituted-1, 3, 4-oxadiazole/thiadiazole-2-thiols. Tetrahedron Letters.
Schultheis, C., Brauner, M., Liewald, J.F., Gottschalk, A., 2011. Optogenetic analysis of GABAB receptor signalling in Caenorhabditis elegans motor neurons. Journal of neurophysiology, jn. 00578.02010.
Schuske, K., Beg, A.A., Jorgensen, E.M., 2004. The GABA nervous system in C. elegans. Trends in neurosciences 27, 407-414.
Schuske, K., Palfreyman, M.T., Watanabe, S., Jorgensen, E.M., 2007. UNC-46 is required for trafficking of the vesicular GABA transporter. Nature neuroscience 10, 846-853.
Schwartz, R.D., 1988. The GABAa receptor-gated ion channel: Biochemical and pharmacological studies of structure and function. Biochemical Pharmacology 37, 3369-3375.
Siddiqui, N., Akhtar, M.J., Yar, M.S., Ahuja, P., Ahsan, W., Ahmed, S., 2014. Substituted phenyl containing 1, 3, 4-oxadiazole-2-yl-but-2-enamides: synthesis and preliminary evaluation as promising anticonvulsants. Medicinal Chemistry Research 23, 4915-4925.
Sulston, J., Hodgkin, J., 1988. The nematode C. elegans. Wood WB and the Community of C. elegans Researchers. New York: Cold Spring Harbor Laboratory Press.
Treiman, D.M., 2001. GABAergic mechanisms in epilepsy. Epilepsia 42, 8-12.
Tsalik, E.L., Hobert, O., 2003. Functional mapping of neurons that control locomotory behavior in Caenorhabditis elegans. Journal of neurobiology 56, 178-197.
Vashlishan, A.B., Madison, J.M., Dybbs, M., Bai, J., Sieburth, D., Ch'ng, Q., Tavazoie, M., Kaplan, J.M., 2008. An RNAi screen identifies genes that regulate GABA synapses. Neuron 58, 346-361.
Wagner, O.I., Esposito, A., Köhler, B., Chen, C.-W., Shen, C.-P., Wu, G.-H., Butkevich, E., Mandalapu, S., Wenzel, D., Wouters, F.S., 2009. Synaptic scaffolding protein SYD-2 clusters and activates kinesin-3 UNC-104 in C. elegans. Proceedings of the National Academy of Sciences 106, 19605-19610.
White, J.G., Southgate, E., Thomson, J.N., Brenner, S., 1976. The structure of the ventral nerve cord of Caenorhabditis elegans. Philosophical Transactions of the Royal Society B: Biological Sciences 275, 327-348.
Williams, S.N., Locke, C.J., Braden, A.L., Caldwell, K.A., Caldwell, G.A., 2004. Epileptic-like convulsions associated with LIS-1 in the cytoskeletal control of neurotransmitter signaling in Caenorhabditis elegans. Human molecular genetics 13, 2043-2059.
Wonnacott, S., 1997. Presynaptic nicotinic ACh receptors. Trends in neurosciences 20, 92-98.

Zarghi, A., Hamedi, S., Tootooni, F., Amini, B., Sharifi, B., Faizi, M., Tabatabai, S.A., Shafiee, A., 2008. Synthesis and pharmacological evaluation of new 2-substituted-5-{2-[(2-halobenzyl) thio) phenyl}-1, 3, 4-oxadiazoles as anticonvulsant agents. Scientia pharmaceutica 76, 185.
Zarghi, A., Tabatabai, S.A., Faizi, M., Ahadian, A., Navabi, P., Zanganeh, V., Shafiee, A., 2005. Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1, 3, 4-oxadiazoles. Bioorganic & medicinal chemistry letters 15, 1863-1865.
Zhang, H., Darwanto, A., Linkhart, T.A., Sowers, L.C., Zhang, L., 2007. Maternal cocaine administration causes an epigenetic modification of protein kinase Cepsilon gene expression in fetal rat heart. Mol Pharmacol 71, 1319-1328.

Figures and Captions:

Fig. 1

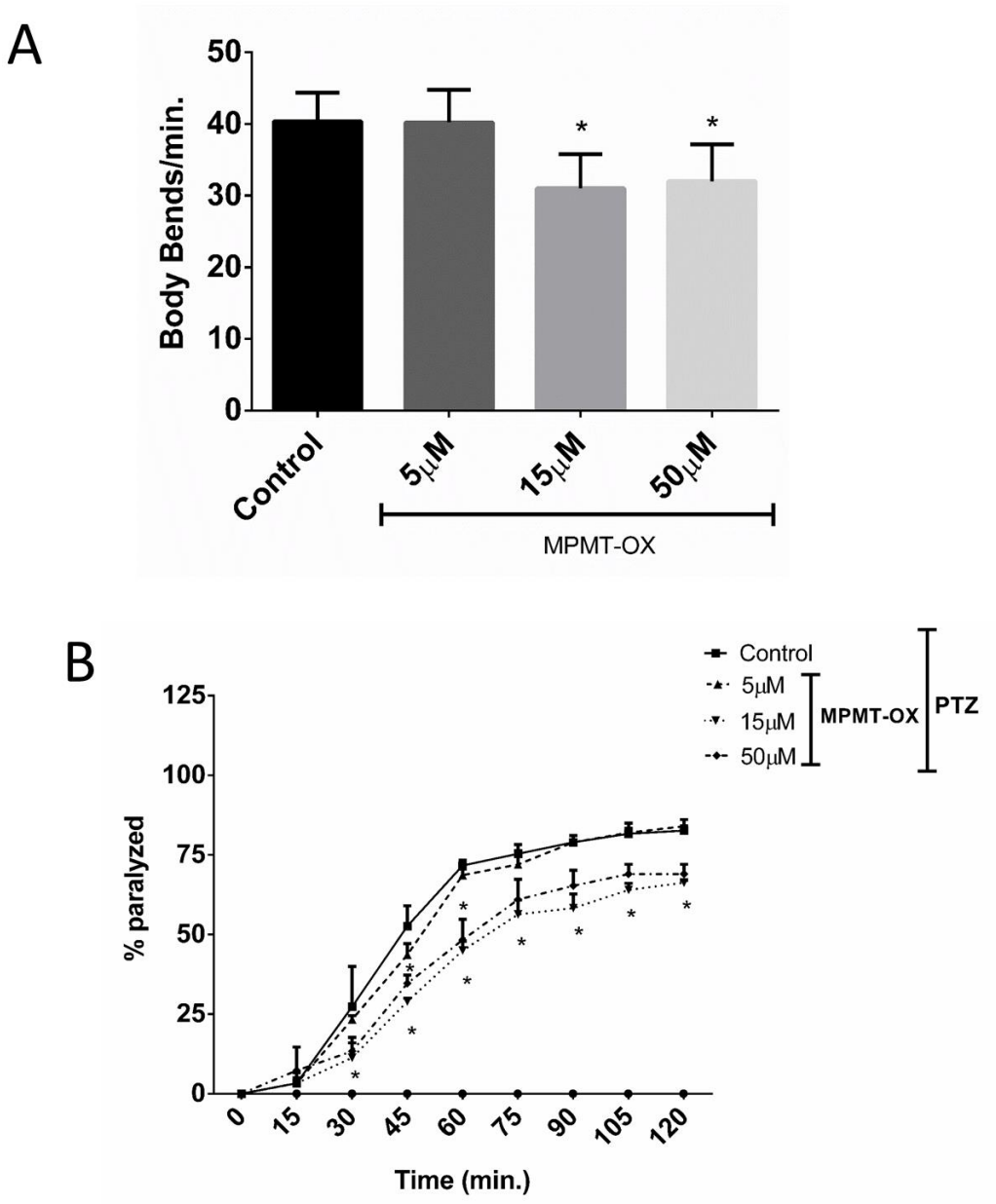


Figure 1: Effect of MPMT-OX on locomotor activity in WT *C. elegans*. **(A)** Data are expressed as number of body bends from three independent assays with 10 worms in each group (n=30). * indicates $p < 0.05$ compared to the control group by one-way ANOVA followed by the Tukey multiple comparison test. **(B)** PTZ-induced paralysis. Worms were placed on plates containing 5 mg/mL PTZ and assayed for body bends every 15 min for 2 h. Data represent the percentage of paralyzed animals from three independent assays with 20 worms in each group (n=60). * indicates $p < 0.05$, 15 and 50 µM MPMT-OX versus control, by two-way ANOVA followed by the Tukey multiple comparison test. Data are represented as means ± SEM.

Fig. 2

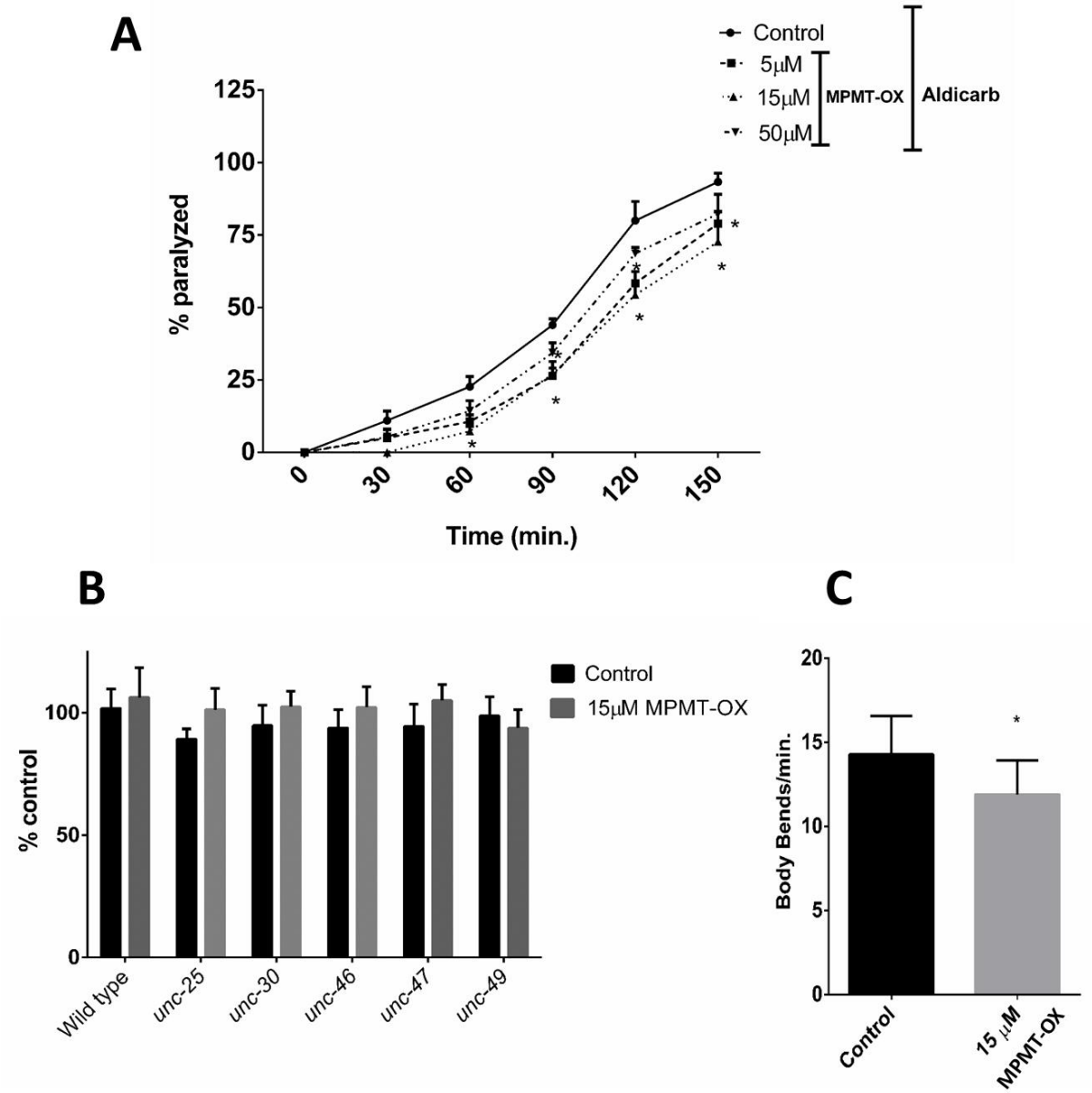


Figure 2: Effect of MPMT-OX on cholinergic signaling. **(A)** Paralysis was induced with 1 mM aldicarb in WT. Locomotion was evaluated every 30 min. for 150 min. Data represent the percentage of paralyzed animals from three independent assays with 20 worms in each group (n=60). * indicates $p < 0.05$, 15 and 50 µM MPMT-OX versus control, with two-way ANOVA followed by the Tukey multiple comparison test. **(B)** AChE activity in WT and transgenic worms. Data are expressed as the percentage of control of each strain, from four independent assays, n = 4. **(C)** Effect of MPMT-OX on locomotor activity of *C. elegans unc-29* knockout. Data are expressed as number of body bends performed by the worms from four independent assays with 10 worms in each group (n=40). * indicates $p < 0.05$ by Student's t-test. Data are represented as means ± SEM.

Fig. 3

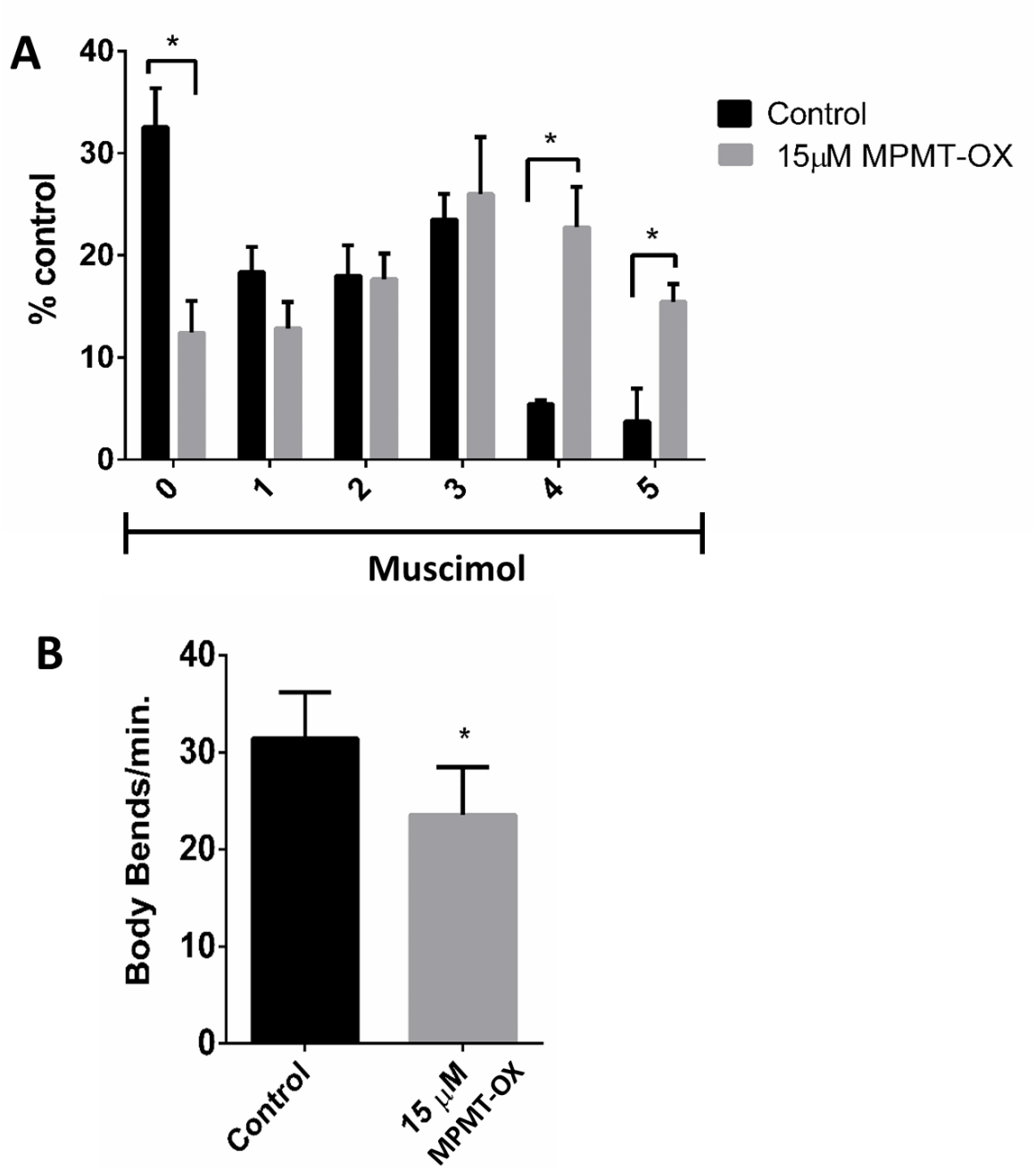


Figure 3: Effect of MPMT-OX on GABaergic signaling. **(A)** Treatment increases sensitivity of WT worms to GABA$_A$ receptor agonists. Data are expressed as the fraction of worms that displayed each of five phenotypes after a 1-h exposure to 0.5 mM of muscimol, from three independent assays with 20 worms in each group (n=60). (See Materials and Methods for full description of phenotypes).* indicates $p < 0.05$ by Student's t-test. **(B)** Data are expressed as number of body bends performed by the *snf-11* worms, from three independent assays with 10 worms in each group (n=30).* indicates $p < 0.05$ by Student's t-test. Data are represented as means ± SEM

Fig. 4

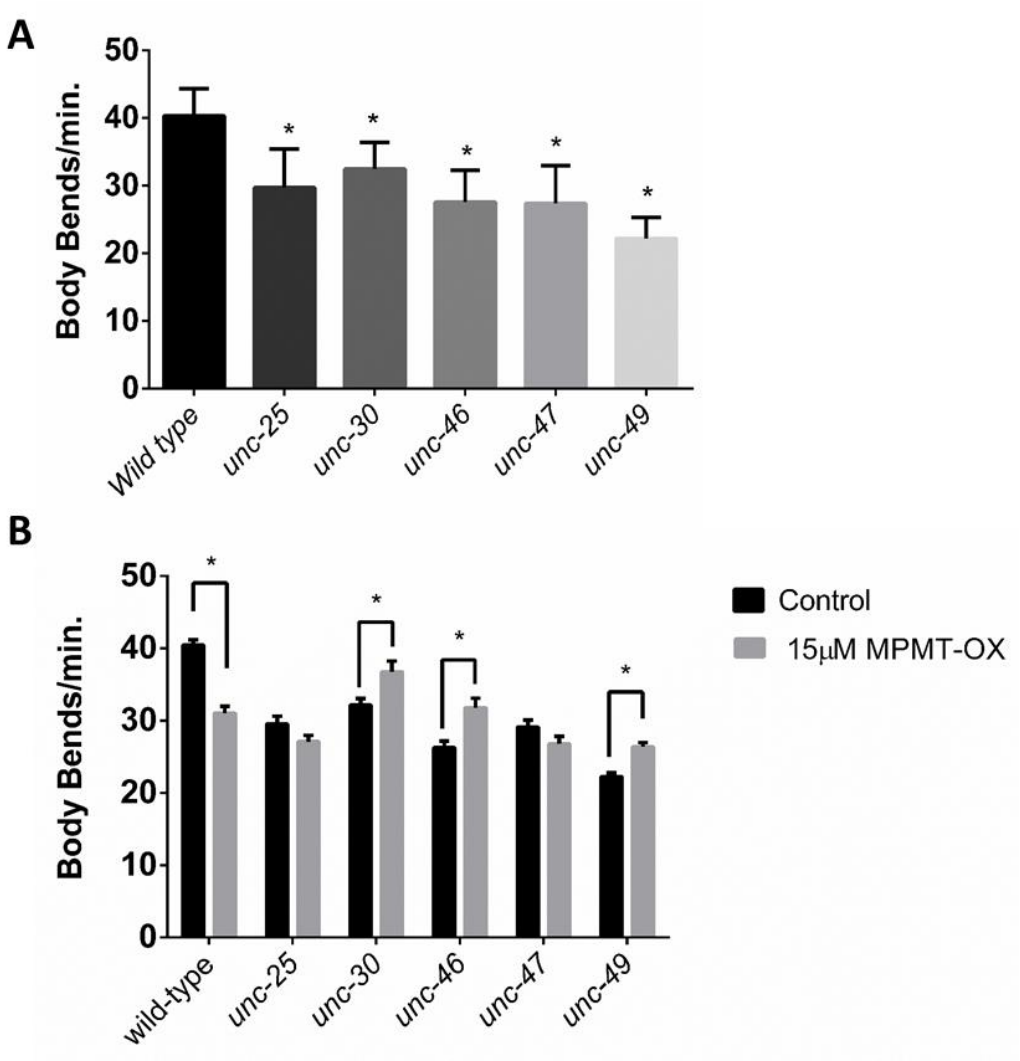


Figure 4: MPMT-OX modulates genes of GABAergic pathway and alters locomotion rate: **(A)** All transgenic worms (*unc-25*, *unc-30*, *unc-46*, *unc-47* and *unc-49*) showed a reduction in locomotor activity when compared to the wild-type strain. * indicates $p < 0.05$ with one-way ANOVA followed by the Tukey multiple comparison test. **(B)** Number of body bends of the WT and transgenic worms after exposure to MPMT-OX.* indicates $p < 0.05$ by Student's t-test. (**A-B)**. Data are expressed as mean body bends/min. performed by the worms, from three independent assays with 10-15 worms in each group (n=30-45). Data are represented as means ± SEM.

Fig. 5

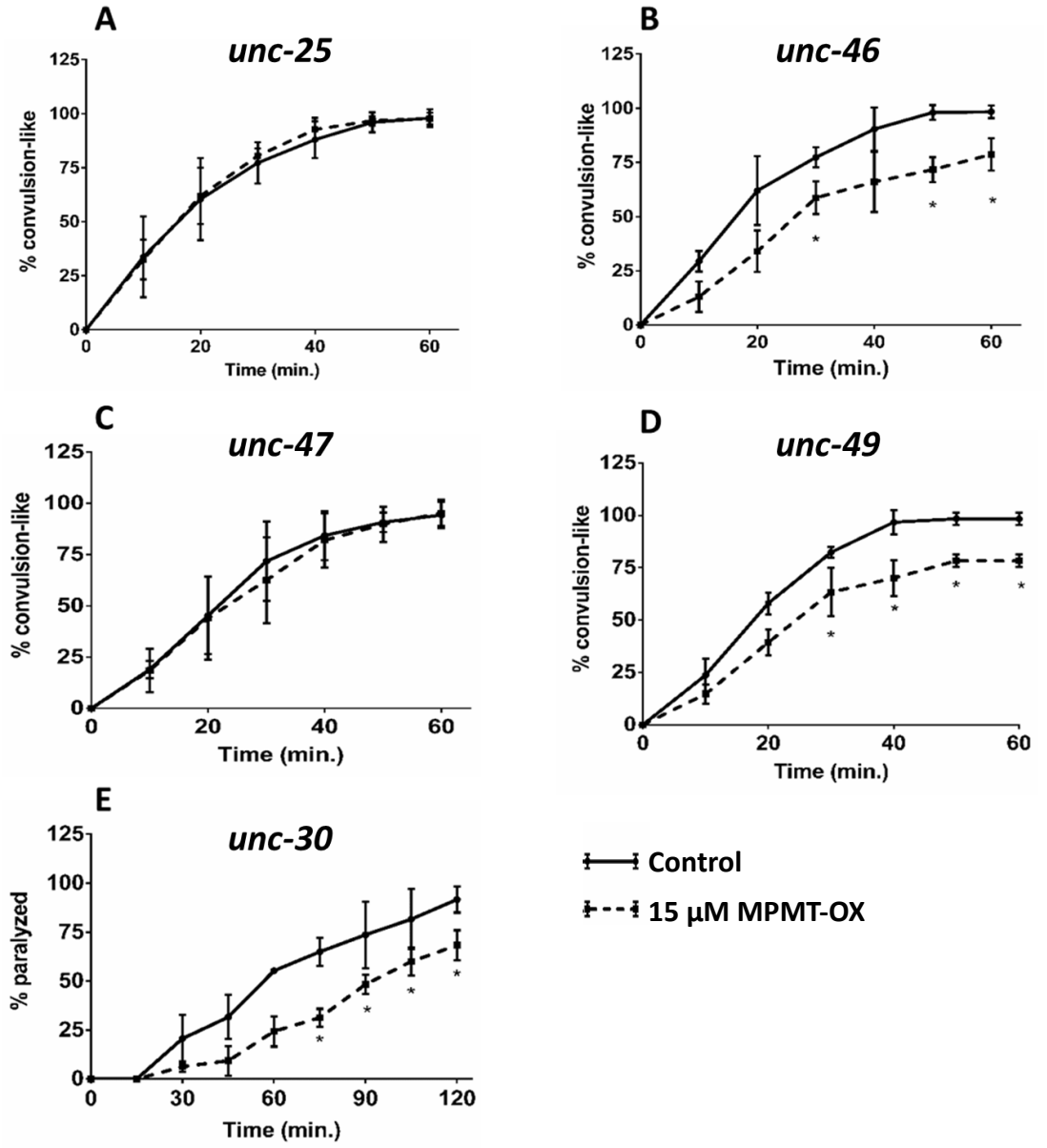


Figure 5: MPMT-OX increases threshold for seizure-like behavior. (A, B, C and D) Data are expressed as the percentage of worms with seizure-like behavior exposed to 5 mg/mL PTZ for 1 h. **(A)** *unc-25*, four independent assays with 24 worms in each group (n=96). **(B)** *unc-46*, three independent assays with 30 worms in each group (n=93), *p < 0.05 with two-way ANOVA followed by Bonferroni's multiple comparison test. **(C)** *unc-47,* four independent assays with 28 worms in each group (n=112). **(D)** *unc-49,* three independent assays with 20 worms in each group (n=60), *p < 0.05 with two-way ANOVA followed by Bonferroni's multiple comparison test. **(E)** *unc-30***,** paralysis was induced with 5 mg/mL PTZ. Mobility was evaluated every 15 min. for 2 h. Data represent the mean percentage of paralyzed *unc-30* worms, from three independent assays with 23 worms in each group (n=70). *p < 0.05 with two-way ANOVA followed by Bonferroni's multiple comparison test. Data are represented as means ± SEM.

Fig. 6

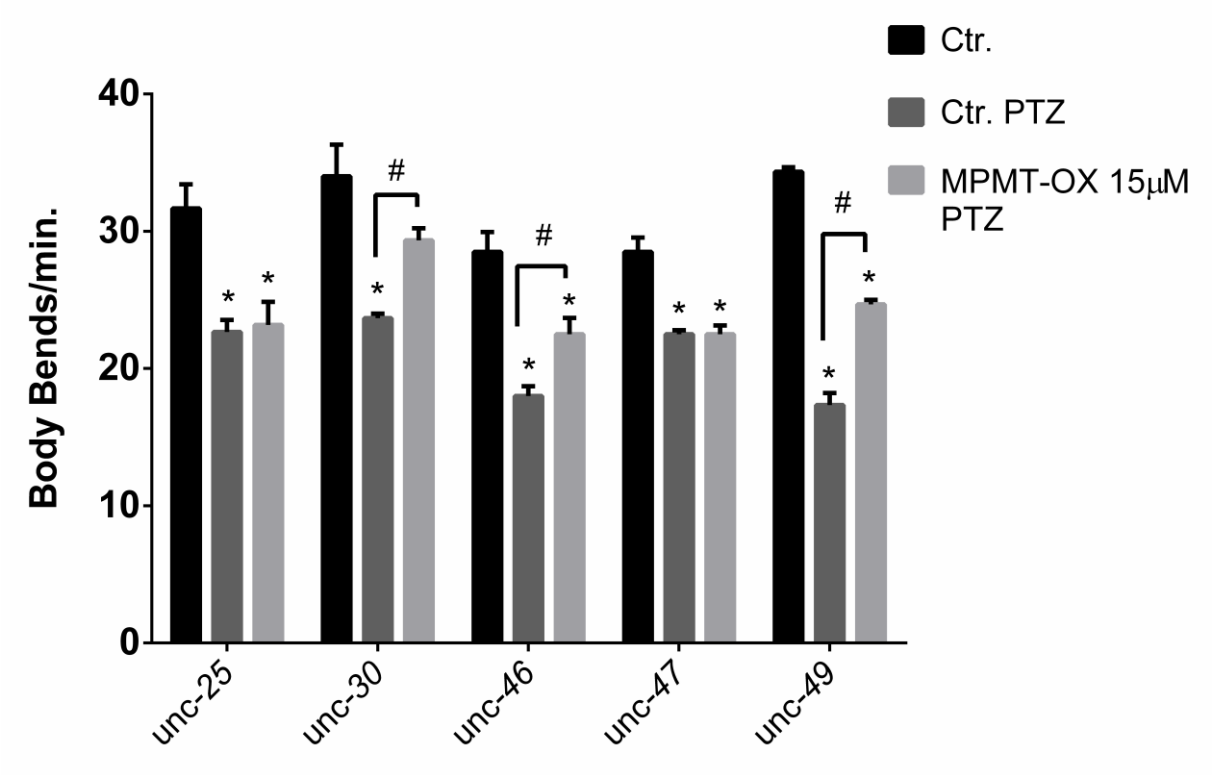
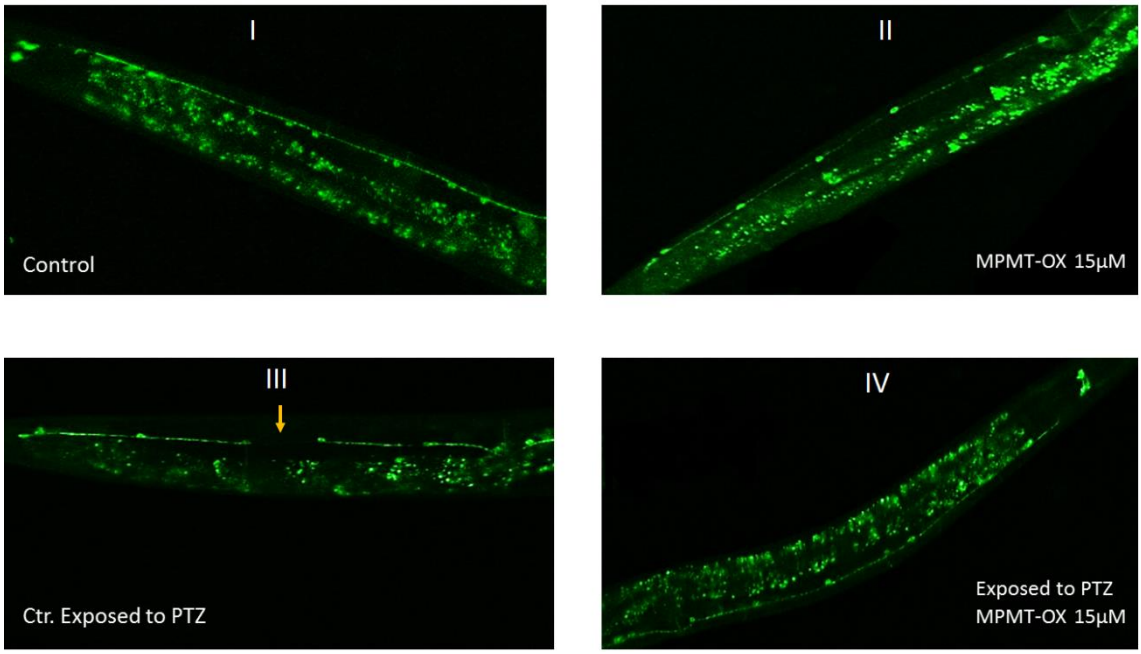


Figure 6: MPMT-OX assists in locomotor recovery after seizure-like behavior. Data are expressed as number of body-bends/min of Ctr. (control worms before seizure), Ctr. PTZ (control after seizure) and MPMT-OX 15 μM PTZ (worms treated before seizure). The worms recovered for one hour after seizure induced by 5 mg/mL PTZ. Locomotion was evaluated in three independent assays with 10-15 worms in each group (n = 30-45). * indicates $p < 0.05$ compared to Ctr., # $p < 0.05$ versus Ctr. PTZ. Two-way ANOVA followed by Bonferroni's multiple comparison test. Data are represented as means ± SEM.

Fig. 7

A



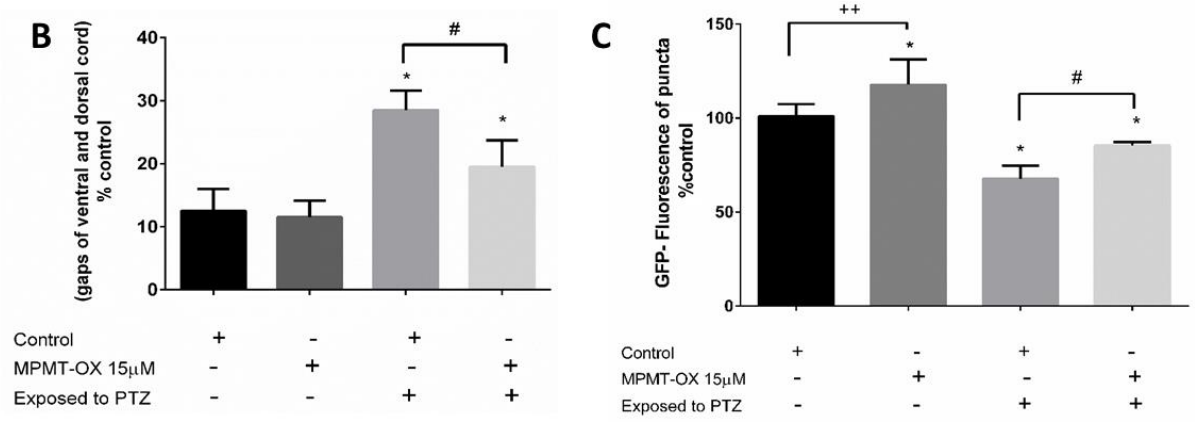


Figure 7: Traffic of GABAergic vesicles before and after exposure to PTZ: (A) GABAergic presynaptic terminals are visualized in live adult animals with P*unc-25*-SNB-1::GFP (juIs1), and all images were obtained from ventral and dorsal cords before and after PTZ exposure. Arrow indicates gaps in nerve cord. **(B)** Quantification of gaps in GFP of the GABAergic nerve cords. **(C)** Quantification of SNB-1::GFP fluorescent puncta from images obtained from three independent assays with 10 animals in each group (n = 30).* indicates $p < 0.05$ compared to the Control group, # $p< 0.05$, MPMT-OX 15µM-Exposed to PTZ group versus Control-Exposed to PTZ group, ++ $p < 0.05$, MPMT-OX 15µM group versus Control group. Two-way ANOVA followed by the Tukey multiple comparison test. Data are represented as means ± SEM.

4. CONCLUSÕES

De acordo com os resultados apresentados neste trabalho pode-se concluir que o tratamento crônico com o composto MPMT-OX, no nematoide *C. elegans*:

- Modula positivamente os genes *unc-25* e *unc-47*, responsáveis pela síntese e liberação de GABA na fenda sináptica, o que provavelmente resulta na maior ligação de GABA aos receptores GABAérgicos, causando:

- Redução da atividade locomotora de nematoides com níveis normais de neurotransmissão E/I, e aumento na atividade locomotora de vermes com deficiências na via de sinalização GABAérgica (*unc-30, unc-46* e *unc-49*);

- Proteção parcialmente contra a paralisia induzida por hiper-excitação na JNM induzida por Aldicarb e PTZ;

- Maior resistência de vermes transgênicos *unc-46* e *unc-47*, frente ao comportamento convulsivo induzido pela exposição ao PTZ, devido ao aumento do limiar de excitação necessário para ocorrência das convulsões;

- Melhora na recuperação da atividade locomotora após indução de convulsões, pois a maior liberação de GABA auxilia no reestabelecimento do equilíbrio entre E/I, permitindo a coordenação das curvaturas corporais;

- Não apresenta efeito sobre atividade da AChE, ou receptor nicotínico de Ach, sugerindo que o MPMT-OX atua prioritariamente sobre a sinalização GABAérgica.

5. PERSPECTIVAS

Este trabalho tinha como objetivo determinar o mecanismo de ação do composto MPMT-OX em modelo nematoide. Os próximos estudos devem se direcionar a elucidar os efeitos deste composto sobre parâmetros básicos relacionados à qualidade de vida do *C. elegans*, como reprodução, expectativa de vida, memória associativa e aprendizagem, e atividade de genes relacionados ao estresse oxidativo e detoxificação. Além disso, pode-se pensar no uso de MPMT-OX em modelos de convulsão com PTZ em roedores bem como estudos toxicológicos mais aprimorados.

6. REFERÊNCIAS BIBLIOGRÁFICAS

ALMASIRAD, A. et al. Synthesis and anticonvulsant activity of new 2-substituted-5-[2-(2-fluorophenoxy) phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles. **Bioorg. Med. Chem. Lett**, v.14, p. 6057–6059, 2004.

AKBAR, M. et al. Reduction of GABA and glutamate transporter messenger RNAs in the severe-seizure genetically epilepsy-prone rat. **Neuroscience,** v. 85, n. 4, p. 1235-1251, 1998. ISSN 0306-4522.

ALTUN, Z.; HALL, D. Muscle System, somatic muscle [WWW Document]. **WormAtlas. URL www. wormatlas. org (accessed 9.2. 15)**, 2009.

ANDOTRA, C.; MANHAS, B. Synthesis and antimicrobial activity of some 2, 5-disubstituted 1, 3, 4-oxadiazoles. **Acta Ciencia Indica, Series Chemistry,** v. 18, p. 99-108, 1992.

BARGMANN, C. I. Neurobiology of the Caenorhabditis elegans genome. **Science,** v. 282, n. 5396, p. 2028-2033, 1998. ISSN 0036-8075.

BARGMANN, C. I.; KAPLAN, J. M. Signal transduction in the Caenorhabditis elegans nervous system. **Annual review of neuroscience,** v. 21, n. 1, p. 279-308, 1998. ISSN 0147-006X.

BAULAC, S. et al. First genetic evidence of GABAA receptor dysfunction in epilepsy: a mutation in the γ2-subunit gene. **Nature genetics,** v. 28, n. 1, p. 46-48, 2001.

BERG, A. T. et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. **Epilepsia,** v. 51, n. 4, p. 676-685, 2010. ISSN 1528-1167.

BESSA, C.; MACIEL, P.; RODRIGUES, A. J. Using C. elegans to decipher the cellular and molecular mechanisms underlying neurodevelopmental disorders. **Molecular neurobiology,** v. 48, n. 3, p. 465-489, 2013. ISSN 0893-7648.

BHAT, M. A.; AL-OMAR, M. A.; SIDDIQUI, N. Synthesis, anticonvulsant and neurotoxicity of some novel 1, 3, 4-oxadiazole derivatives of phthalimide. **Der Pharma Chemica,** v. 2, n. 2, p. 1-10, 2010.

BRADFORD, H. Glutamate, GABA and epilepsy. **Progress in neurobiology,** v. 47, n. 6, p. 477-511, 1995. ISSN 0301-0082.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical biochemistry,** v. 72, n. 1, p. 248-254, 1976. ISSN 0003-2697.

BRENNER, S. The genetics of Caenorhabditis elegans. **Genetics,** v. 77, n. 1, p. 71-94, 1974. ISSN 0016-6731.

CALAHORRO, F.; RUIZ-RUBIO, M. Human alpha-and beta-NRXN1 isoforms rescue behavioral impairments of Caenorhabditis elegans neurexin-deficient mutants. **Genes, Brain and Behavior,** v. 12, n. 4, p. 453-464, 2013. ISSN 1601-183X.

CAVALHEIRO, E. et al. Spontaneous recurrent seizures in rats: amino acid and monoamine determination in the hippocampus. **Epilepsia,** v. 35, n. 1, p. 1-11, 1994. ISSN 1528-1167.

COLE, R. D.; ANDERSON, G. L.; WILLIAMS, P. L. The nematode Caenorhabditis elegans as a model of organophosphate-induced mammalian neurotoxicity. **Toxicology and Applied Pharmacology,** v. 194, n. 3, p. 248-256, 2004. ISSN 1706542747.

DABBISH, N. S.; RAIZEN, D. M. GABAergic synaptic plasticity during a developmentally regulated sleep-like state in C. elegans. **The Journal of Neuroscience,** v. 31, n. 44, p. 15932-15943, 2011. ISSN 0270-6474.

DE LA CRUZ, I. P. et al. sup-9, sup-10, and unc-93 may encode components of a two-pore K+ channel that coordinates muscle contraction in Caenorhabditis elegans. **The Journal of neuroscience,** v. 23, n. 27, p. 9133-9145, 2003. ISSN 0270-6474.

DICHTER, M. A. Cellular mechanisms of epilepsy and potential new treatment strategies. **Epilepsia,** v. 30, n. s1, p. S3-S12, 1989. ISSN 1528-1167.

DITTMAN, J. S.; KAPLAN, J. M. Factors regulating the abundance and localization of synaptobrevin in the plasma membrane. **Proceedings of the National Academy of Sciences,** v. 103, n. 30, p. 11399-11404, 2006. ISSN 0027-8424.

DOCHERTY, M. et al. Evidence for specific immunolysis of nerve terminals using antisera against choline acetyltransferase, glutamate decarboxylase and tyrosine hydroxylase. **Brain research,** v. 339, n. 1, p. 105-113, 1985. ISSN 0006-8993.

EASTMAN, C.; HORVITZ, H. R.; JIN, Y. Coordinated Transcriptional Regulation of the unc-25Glutamic Acid Decarboxylase and the unc-47 GABA Vesicular Transporter by the Caenorhabditis elegans UNC-30 Homeodomain Protein. **The Journal of neuroscience,** v. 19, n. 15, p. 6225-6234, 1999. ISSN 0270-6474.

ELLMAN, G. L. et al. **A new and rapid colorimetric determination of acetylcholinesterase activity**. 7**:** 88-95 p. 1961.

ELOQAYLI, H. et al. Pentylenetetrazole decreases metabolic glutamate turnover in rat brain. **Journal of neurochemistry,** v. 85, n. 5, p. 1200-1207, 2003. ISSN 1471-4159.

FARRANT, M.; KAILA, K. The cellular, molecular and ionic basis of GABA A receptor signalling. **Progress in brain research,** v. 160, p. 59-87, 2007. ISSN 0079-6123.

FISHER, R. S. et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). **Epilepsia,** v. 46, n. 4, p. 470-472, 2005. ISSN 1528-1167.

FONNUM, F. Glutamate: a neurotransmitter in mammalian brain. **Journal of neurochemistry,** v. 42, n. 1, p. 1-11, 1984. ISSN 1471-4159.

FOSTER, A. C.; KEMP, J. A. Glutamate-and GABA-based CNS therapeutics. **Current opinion in pharmacology,** v. 6, n. 1, p. 7-17, 2006. ISSN 1471-4892.

GOLAN, D. et al. **Princípios de farmacologia: a base fisiopatológica da farmacoterapia**. Guanabara Koogan, 2009. ISBN 8527715201.

HAN, B.; BELLEMER, A.; KOELLE, M. R. An evolutionarily conserved switch in response to GABA affects development and behavior of the locomotor circuit of Caenorhabditis elegans. **Genetics,** v. 199, n. 4, p. 1159-1172, 2015. ISSN 0016-6731.

HIRAO, I.; KATO, Y.; HIROTA, T. Studies of the Synthesis of Furan Compounds. XXIV. The Synthesis of 5-[2-(5-Nitro-2-furyl)-1-(4-nitrophenyl) vinyl]-1, 3, 4-oxadiazole and Its Related Compounds. **Bulletin of the Chemical Society of Japan,** v. 44, n. 7, p. 1923-1927, 1971. ISSN 1348-0634.

HUANG, R.-Q. et al. Pentylenetetrazole-induced inhibition of recombinant γ-aminobutyric acid type A (GABAA) receptors: mechanism and site of action. **Journal of Pharmacology and Experimental Therapeutics,** v. 298, n. 3, p. 986-995, 2001. ISSN 1521-0103.

HUTT, M. P.; ELSLAGER, E. F.; WERBEL, L. M. 2-Phenyl-5-(trichloromethyl)-1, 3, 4-oxadiazoles, A new class of antimalarial substances. **Journal of Heterocyclic Chemistry,** v. 7, n. 3, p. 511-518, 1970. ISSN 1943-5193.

JAIN, N. et al. Synthesis, anticonvulsant and neurotoxic activity of some new 2, 5-disubstituted-1, 3, 4-oxadiazoles. **Medicinal Chemistry Research,** v. 20, n. 9, p. 1696-1703, 2011. ISSN 1054-2523.

JAMES, N. D.; GROWCOTT, J. W. ZIBOTENTAN Endothelin ETA Receptor Antagonist Oncolytic. **Drugs of the Future,** v. 34, n. 8, p. 624-633, 2009. ISSN 0377-8282.

JIANG, C.-S. et al. Synthesis and biological evaluation of novel marine-derived indole-based 1, 2, 4-oxadiazoles derivatives as multifunctional neuroprotective agents. **Bioorganic & medicinal chemistry letters,** v. 25, n. 2, p. 216-220, 2015. ISSN 0960-894X.

JIN, Y. Synaptogenesis. 2005.

JIN, Y.; HOSKINS, R.; HORVITZ, H. R. Control of type-D GABAergic neuron differentiation by C. elegans UNC-30 homeodomain protein. 1994.

JOSPIN, M. et al. A neuronal acetylcholine receptor regulates the balance of muscle excitation and inhibition in Caenorhabditis elegans. **PLoS Biol,** v. 7, n. 12, p. e1000265, 2009. ISSN 1545-7885.

KALETTA, T.; HENGARTNER, M. O. Finding function in novel targets: C. elegans as a model organism. **Nature reviews Drug discovery,** v. 5, n. 5, p. 387-399, 2006. ISSN 1474-1776.

KAMAL, A. et al. Synthesis, biological evaluation, and molecular modeling of (E)-2-aryl-5-styryl-1, 3, 4-oxadiazole derivatives as acetylcholine esterase inhibitors. **Medicinal Chemistry Research,** v. 23, n. 4, p. 2080-2092, 2014. ISSN 1054-2523.

KASHAW, S. K. et al. Anticonvulsant and sedative-hypnotic activity of some novel 3-[5-(4-substituted) phenyl-1, 3, 4-oxadiazole-2yl]-2-styrylquinazoline-4 (3H)-ones. **Medicinal chemistry research,** v. 19, n. 3, p. 250-261, 2010. ISSN 1054-2523.

KAWAI, H.; LAZAR, R.; METHERATE, R. Nicotinic control of axon excitability regulates thalamocortical transmission. **Nature neuroscience,** v. 10, n. 9, p. 1168-1175, 2007. ISSN 1097-6256.

KHAN, K. M. et al. Synthesis, characterization and biological screening of 5-substituted-1, 3, 4-oxadiazole-2yl-N-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide. **Pak. J. Pharm. Sci,** v. 26, n. 2, p. 345-352, 2013.

KRNJEVIC, K. Glutamate and gamma-aminobutyric acid in brain. **Nature,** v. 228, p. 119-124, 1970. ISSN 0028-0836.

KUFFLER, S. W.; EDWARDS, C. Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. **Journal of neurophysiology,** v. 21, n. 6, p. 589-610, 1958. ISSN 0022-3077.

LEUNG, M. C. et al. Caenorhabditis elegans: an emerging model in biomedical and environmental toxicology. **Toxicological Sciences,** v. 106, n. 1, p. 5-28, 2008. ISSN 1096-6080.

LIU, Z.; YANG, G.; QIN, X. Syntheses and biological activities of novel diheterocyclic compounds containing 1, 2, 4-triazolo [1, 5-a] pyrimidine and 1, 3, 4-oxadiazole. **Journal of Chemical Technology and Biotechnology,** v. 76, n. 11, p. 1154-1158, 2001. ISSN 1097-4660.

LOCKE, C. J. et al. Pharmacogenetic analysis reveals a post-developmental role for Rac GTPases in Caenorhabditis elegans GABAergic neurotransmission. **Genetics,** v. 183, n. 4, p. 1357-1372, 2009. ISSN 0016-6731.

LÖSCHER, W.; HÖNACK, D.; RUNDFELDT, C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. **Journal of Pharmacology and Experimental Therapeutics,** v. 284, n. 2, p. 474-479, 1998. ISSN 0022-3565.

MAHONEY, T. R.; LUO, S.; NONET, M. L. Analysis of synaptic transmission in Caenorhabditis elegans using an aldicarb-sensitivity assay. **Nature protocols,** v. 1, n. 4, p. 1772-1777, 2006. ISSN 1754-2189.

MARINHO, M. et al. Inhibitory action of a calcium channel blocker (nimodipine) on seizures and brain damage induced by pilocarpine and lithium-pilocarpine in rats. **Neuroscience letters,** v. 235, n. 1, p. 13-16, 1997. ISSN 0304-3940.

MASHAYEKH, S. et al. Synthesis, receptor affinity and effect on pentylenetetrazole-induced seizure threshold of novel benzodiazepine analogues: 3-Substituted 5-(2-phenoxybenzyl)-4H-1, 2, 4-triazoles and 2-amino-5-(phenoxybenzyl)-1, 3, 4-oxadiazoles. **Bioorganic & medicinal chemistry,** v. 22, n. 6, p. 1929-1937, 2014. ISSN 0968-0896.

MCCORMICK, D. A. GABA as an inhibitory neurotransmitter in human cerebral cortex. **Journal of Neurophysiology,** v. 62, n. 5, p. 1018-1027, 1989. ISSN 0022-3077.

MCCORMICK, D. A.; CONTRERAS, D. On the cellular and network bases of epileptic seizures. **Annual review of physiology,** v. 63, n. 1, p. 815-846, 2001. ISSN 0066-4278.

MCLNTIRE, S. L.; JORGENSEN, E.; HORVITZ, H. R. Genes required for GABA function in Caenorhabditis elegans. 1993.

MCLNTIRE, S. L. et al. The GABAergic nervous system of Caenorhabditis elegans. 1993.

MINORU, O.; KIN-YA, K.; SATORU, O. The molecular mechanism underlying pentylenetetrazole-induced bursting activity in Euhadra neurons: involvement of protein phosphorylation. **Comparative Biochemistry and Physiology Part C: Comparative Pharmacology,** v. 100, n. 3, p. 423-432, 1991. ISSN 0306-4492.

MODY, I. et al. Bridging the cleft at GABA synapses in the brain. **Trends in neurosciences,** v. 17, n. 12, p. 517-525, 1994. ISSN 0166-2236.

MULLEN, G. P. et al. The Caenorhabditis elegans snf-11 gene encodes a sodium-dependent GABA transporter required for clearance of synaptic GABA. **Molecular biology of the cell,** v. 17, n. 7, p. 3021-3030, 2006. ISSN 1059-1524.

MURGATROYD, C.; SPENGLER, D. Histone tales: echoes from the past, prospects for the future. **Genome biology,** v. 11, n. 2, p. 105, 2010. ISSN 1474-760X.

NAYLOR, D. E. Glutamate and GABA in the balance: convergent pathways sustain seizures during status epilepticus. **Epilepsia,** v. 51, n. s3, p. 106-109, 2010. ISSN 1528-1167.

NELIGAN, A.; SHORVON, S. Prognostic factors, morbidity and mortality in tonic–clonic status epilepticus: a review. **Epilepsy research,** v. 93, n. 1, p. 1-10, 2011. ISSN 0920-1211.

NONET, M. L. Visualization of synaptic specializations in live C. elegans with synaptic vesicle protein-GFP fusions. **Journal of neuroscience methods,** v. 89, n. 1, p. 33-40, 1999. ISSN 0165-0270.

NONET, M. L. et al. Synaptic transmission deficits in Caenorhabditis eleganssynaptobrevin mutants. **The Journal of neuroscience,** v. 18, n. 1, p. 70-80, 1998. ISSN 0270-6474.

NURRISH, S.; SÉGALAT, L.; KAPLAN, J. M. Serotonin inhibition of synaptic transmission: Gαo decreases the abundance of UNC-13 at release sites. **Neuron,** v. 24, n. 1, p. 231-242, 1999. ISSN 0896-6273.

OGATA, M. et al. In vitro sensitivity of mycoplasmas isolated from various animals and sewage to antibiotics and nitrofurans. **The Journal of antibiotics,** v. 24, n. 7, p. 443-451, 1971. ISSN 0021-8820.

OMAR, A.; MOHSEN, M.; ABOULWAFA, O. M. Synthesis and anticonvulsant properties of a novel series of 2-substituted amino-5-aryl-1, 3, 4-oxadiazole derivatives. **Journal of heterocyclic chemistry,** v. 21, n. 5, p. 1415-1418, 1984. ISSN 1943-5193.

ORTINSKI, P.; MEADOR, K. J. Cognitive side effects of antiepileptic drugs. **Epilepsy & Behavior,** v. 5, p. 60-65, 2004. ISSN 1525-5050.

OWENS, D. F.; KRIEGSTEIN, A. R. Is there more to GABA than synaptic inhibition? **Nature Reviews Neuroscience,** v. 3, n. 9, p. 715-727, 2002. ISSN 1471-003X.

PAGONOPOULOU, O.; ANGELATOU, F. Time development and regional distribution of [3H] nitrobenzylthioinosine adenosine uptake site binding in the mouse brain after acute pentylenetetrazol-induced seizures. **Journal of neuroscience research,** v. 53, n. 4, p. 433-442, 1998. ISSN 1097-4547.

RAJAK, H. et al. Novel semicarbazones based 2, 5-disubstituted-1, 3, 4-oxadiazoles: One more step towards establishing four binding site pharmacophoric model hypothesis for anticonvulsant activity. **Bioorganic & medicinal chemistry letters,** v. 20, n. 14, p. 4168-4172, 2010. ISSN 0960-894X.

RICE, M. E.; CRAGG, S. J. Nicotine amplifies reward-related dopamine signals in striatum. **Nature neuroscience,** v. 7, n. 6, p. 583-584, 2004.

RICHMOND, J. E.; JORGENSEN, E. M. One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction. **Nature neuroscience,** v. 2, n. 9, p. 791-797, 1999.

RIDDLE, D. L. et al. Introduction to C. elegans. In: RIDDLE, D. L.;BLUMENTHAL, T*., et al* (Ed.). **C. elegans II**. 2nd. Cold Spring Harbor (NY), 1997. ISBN 0879695323.

RISLEY, M. G. et al. Modulating Behavior in C. elegans Using Electroshock and Antiepileptic Drugs. **PloS one,** v. 11, n. 9, p. e0163786, 2016. ISSN 1932-6203.

SAUER, A. C. et al. Synthesis and antioxidant properties of organosulfur and organoselenium compounds derived from 5-substituted-1, 3, 4-oxadiazole/thiadiazole-2-thiols. **Tetrahedron Letters**, 2016. ISSN 0040-4039.

SAVARINO, A. A historical sketch of the discovery and development of HIV-1 integrase inhibitors. **Expert opinion on investigational drugs,** v. 15, n. 12, p. 1507-1522, 2006. ISSN 1354-3784.

SCHARFMAN, H. E. The neurobiology of epilepsy. **Current neurology and neuroscience reports,** v. 7, n. 4, p. 348-354, 2007. ISSN 1528-4042.

SCHLECKER, R.; THIEME, P. C. The synthesis of antihypertensive 3-(1, 3, 4-oxadiazol-2-yl) phenoxypropanolahines. **Tetrahedron,** v. 44, n. 11, p. 3289-3294, 1988. ISSN 0040-4020.

SCHULTHEIS, C. et al. Optogenetic analysis of GABAB receptor signalling in Caenorhabditis elegans motor neurons. **Journal of neurophysiology**, p. jn. 00578.2010, 2011. ISSN 0022-3077.

SCHUSKE, K.; BEG, A. A.; JORGENSEN, E. M. The GABA nervous system in C. elegans. **Trends in neurosciences,** v. 27, n. 7, p. 407-414, 2004. ISSN 0166-2236.

SCHUSKE, K. et al. UNC-46 is required for trafficking of the vesicular GABA transporter. **Nature neuroscience,** v. 10, n. 7, p. 846-853, 2007. ISSN 1097-6256.

SHARMA, R.; BAHEL, S. SYNTHESIS OF ARYLOXY/ARYL ACETYL THIOSEMICARBAZIDES, SUBSTITUTED 1, 3, 4-OXADIAZOLES, 1, 3, 4-THIADIAZOLES, 1, 2, 4-TRIAZOLES AND RELATED COMPOUNDS AS POTENTIAL FUNGICIDES. **Chemischer Informationsdienst,** v. 14, n. 5, 1983. ISSN 2199-2924.

SIDDIQUI, N. et al. Substituted phenyl containing 1, 3, 4-oxadiazole-2-yl-but-2-enamides: synthesis and preliminary evaluation as promising anticonvulsants. **Medicinal Chemistry Research,** v. 23, n. 11, p. 4915-4925, 2014. ISSN 1054-2523.

SILVESTRINI, B.; POZZATTI, C. PHARMACOLOGICAL PROPERTIES OF 3-PHENYL-5 β DIETHYLAMINOETHYL-1, 2, 4-OXADIAZOLE. **British journal of pharmacology and chemotherapy,** v. 16, n. 3, p. 209-217, 1961. ISSN 1476-5381.

SMITH, G. B.; OLSEN, R. W. Functional domains of GABA A receptors. **Trends in Pharmacological Sciences,** v. 16, n. 5, p. 162-168, 1995. ISSN 0165-6147.

SMITH, M.; WILCOX, K. S.; WHITE, H. S. Discovery of antiepileptic drugs. **Neurotherapeutics,** v. 4, n. 1, p. 12-17, 2007. ISSN 1933-7213.

SOMJEN, G. G. Ion regulation in the brain: implications for pathophysiology. **The Neuroscientist,** v. 8, n. 3, p. 254-267, 2002. ISSN 1073-8584.

STAWICKI, T. M. et al. TRPM channels modulate epileptic-like convulsions via systemic ion homeostasis. **Current Biology,** v. 21, n. 10, p. 883-888, 2011. ISSN 0960-9822.

SULSTON, J.; HODGKIN, J. **The nematode C. elegans**: Wood WB and the Community of C. elegans Researchers. New York: Cold Spring Harbor Laboratory Press 1988.

THURMAN, D. J. et al. Standards for epidemiologic studies and surveillance of epilepsy. **Epilepsia,** v. 52, n. s7, p. 2-26, 2011. ISSN 1528-1167.

TREIMAN, D. M. GABAergic mechanisms in epilepsy. **Epilepsia,** v. 42, n. s3, p. 8-12, 2001. ISSN 1528-1167.

TSALIK, E. L.; HOBERT, O. Functional mapping of neurons that control locomotory behavior in Caenorhabditis elegans. **Journal of neurobiology,** v. 56, n. 2, p. 178-197, 2003. ISSN 1097-4695.

VASHLISHAN, A. B. et al. An RNAi screen identifies genes that regulate GABA synapses. **Neuron,** v. 58, n. 3, p. 346-361, 2008. ISSN 0896-6273.

WHITE, H. S.; SMITH, M. D.; WILCOX, K. S. Mechanisms of action of antiepileptic drugs. **International review of neurobiology,** v. 81, p. 85-110, 2007. ISSN 0074-7742.

WHITE, J. G. et al. The structure of the ventral nerve cord of Caenorhabditis elegans. **Philosophical Transactions of the Royal Society B: Biological Sciences,** v. 275, n. 938, p. 327-348, 1976. ISSN 0962-8436.

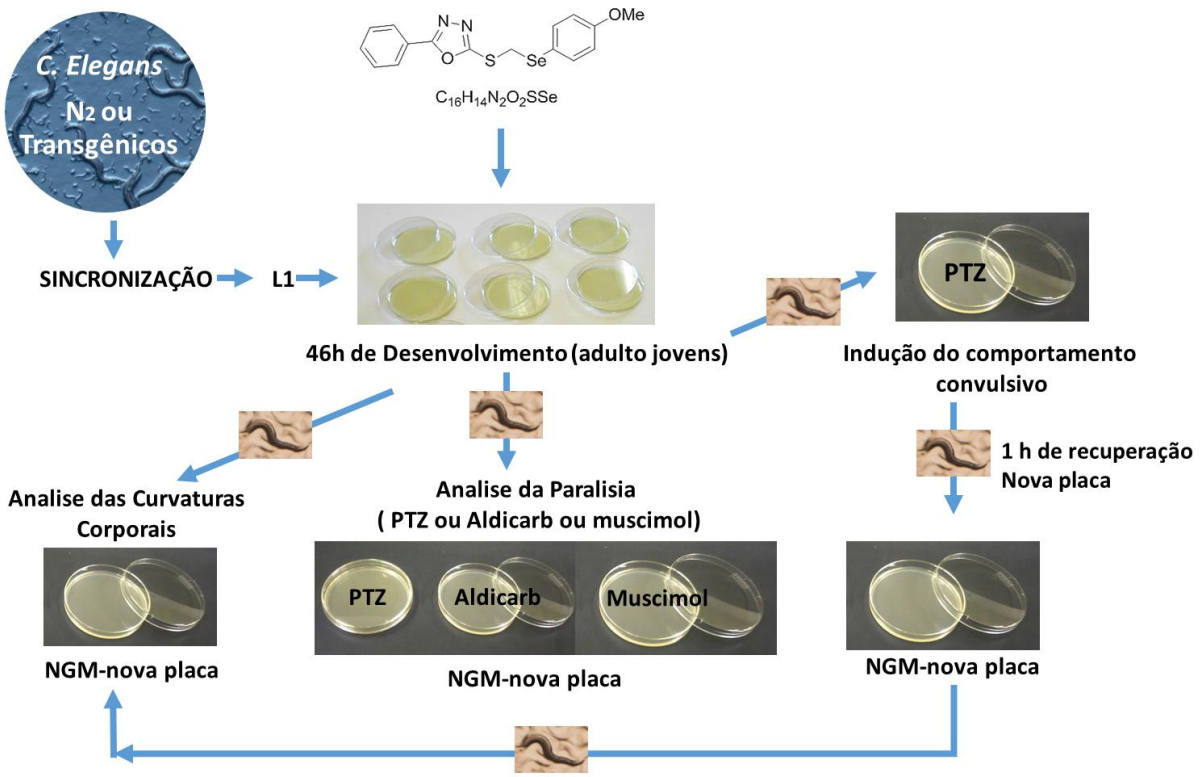
WILLIAMS, S. N. et al. Epileptic-like convulsions associated with LIS-1 in the cytoskeletal control of neurotransmitter signaling in Caenorhabditis elegans. **Human molecular genetics,** v. 13, n. 18, p. 2043-2059, 2004. ISSN 0964-6906.

ZARGHI, A. et al. Synthesis and pharmacological evaluation of new 2-substituted-5-{2-[(2-halobenzyl) thio) phenyl}-1, 3, 4-oxadiazoles as anticonvulsant agents. **Scientia pharmaceutica,** v. 76, n. 2, p. 185, 2008. ISSN 0036-8709.

ZARGHI, A. et al. Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1, 3, 4-oxadiazoles. **Bioorganic & medicinal chemistry letters,** v. 15, n. 7, p. 1863-1865, 2005. ISSN 0960-894X.

ZHANG, H. et al. Maternal cocaine administration causes an epigenetic modification of protein kinase Cepsilon gene expression in fetal rat heart. **Mol Pharmacol,** v. 71, n. 5, p. 1319-1328, 2007. ISSN 0026-895X (Print) 0026-895X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17202284 >.

7. APÊNDICE A: Esquema de Tratamentos



Indica a transferência de uma população de *C. elegans* de uma placa para outra