Published in final edited form as:

Invert Neurosci. ; 18(2): 8. doi:10.1007/s10158-018-0211-9.

egl-4* modulates electroconvulsive seizure duration in *C. elegans

Monica G. Risley^{1,2}, Stephanie P. Kelly^{1,2}, Justin Minnerly^{1,2}, Kailiang Jia^{1,2}, and Ken Dawson-Scully^{1,2}

¹Department of Biological Sciences, Florida Atlantic University, Boca Raton, FL 33431, USA

²International Max-Planck Research School (IMPRS) for Brain and Behavior, Boca Raton, FL 33431, USA

Abstract

Increased neuronal excitability causes seizures with debilitating symptoms. Effective and noninvasive treatments are limited for easing symptoms, partially due to the complexity of the disorder and lack of knowledge of specific molecular faults. An unexplored, novel target for seizure therapeutics is the cGMP/protein kinase G (PKG) pathway, which targets downstream K⁺ channels, a mechanism similar to Retigabine, a recently FDA-approved antiepileptic drug. Our results demonstrate that increased PKG activity decreased seizure duration in *C. elegans* utilizing a recently developed electroconvulsive seizure assay. While the fly is a well-established seizure model, *C. elegans* are an ideal yet unexploited model which easily uptakes drugs and can be utilized for high-throughput screens. In this study, we show that treating the worms with either a potassium channel opener, Retigabine or published pharmaceuticals that increase PKG activity, significantly reduces seizure recovery times. Our results suggest that PKG signaling modulates downstream K⁺ channel conductance to control seizure recovery time in *C. elegans*. Hence, we provide powerful evidence, suggesting that pharmacological manipulation of the PKG signaling cascade may control seizure duration across phyla.

Keywords

C. elegans; Seizure; Epilepsy; Protein kinase G; PKG; Electroconvulsive seizure

Introduction

Epilepsy is a neurological disorder characterized by complex physiological and chemical alterations in neuronal firing that affects 1 in 26 people in the USA (Mary Jane et al. 2012). The deteriorating effects of seizures reduce the quality of life and increase healthcare costs for sufferers (Mary Jane et al. 2012; Guerrini et al. 2013). Although the prevalence of seizures is high among Americans, effective and noninvasive treatments are limited for

Correspondence to: Ken Dawson-Scully.

Conflict of interest The authors declare that they have no conflict of interest.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Monica G. Risley and Stephanie P. Kelly co-first authorship.

easing symptoms. Several methods are used to correct this condition; the most common are antiepileptic drug (AED) treatment monotherapies and polytherapies (Kim 2014; Loscher and Schmidt 2011; Szaflarski et al. 2014). While AEDs work sufficiently for some of the population, it is estimated that ~30% of epileptic patients do not respond to current drug therapies (Lee 2014; Brodie et al. 2012). As a result, there is a significant need to a gain better understanding of the cellular and physiological mechanisms that underlie both seizure susceptibility and the response to AED treatment.

Caenorhabditis elegans are a powerful in vivo multicellular human disease model for neurodegenerative research including Parkinson's and Alzheimer's diseases, autism spectrum disorders, and learning and memory studies (Levitan et al. 1996; Link et al. 2003; Hamamichi et al. 2008; Calahorra and Ruiz-Rubio 2011; Zhang et al. 2005; Saeki et al. 2001). Several attractive features, including a fully mapped connectome of 302 neurons and approximately 7000 synapses as well as readily available RNAi libraries and genetic tools, make it possible to insert GFP-tagged human transgenes and observe them through the transparent cuticle (White et al. 1986; Chalfie et al. 1994; Timmons et al. 2001). In addition, its small size, relatively inexpensive maintenance and short generation time provide a powerful platform to explore as a human seizure model. The past research in our laboratory has established a behavioral method to induce an electroconvulsive seizure (ES) in adult *C. elegans* (Risley et al. 2016). With this method, we determined that the behavioral recovery from an ES is partly due to contribution of the major inhibitory neurotransmitter, GABA, in the nervous system.

The nitric oxide (NO)/cGMP/PKG signaling pathway, which targets downstream ion channels, is conserved from *C. elegans* to humans. A *Drosophila melanogaster* PKG, encoded by the *foraging* gene, has two natural allelic variants that have been attributed to many behavioral responses including foraging patterns, egg-laying and neuronal protection during anoxic and hyperthermic stresses (Krzyzanowski et al. 2013). Drugs, along with genetics, have been shown to alter the endogenous activity levels of cellular PKG (Dawson-Scully et al. 2010). The *C. elegans* ortholog of *foraging* is *egl-4* (Daniels et al. 2000). In worms, this gene is neuronally expressed and has been associated with many parallel characteristics of its fly homolog including food search behaviors, egg-laying, memory association and synaptic transmission (Caplan et al. 2013; Dawson-Scully et al. 2010; Kaun et al. 2007; L'Etoile et al. 2002; Mery et al. 2007; Renger et al. 1999).

With this research, we have successfully demonstrated that behavioral recovery time from an electroconvulsive seizure is reduced when PKG activity is increased both genetically and pharmacologically in an *egl-4* LoF mutant *C. elegans*. *Caenorhabditis elegans* are an ideal system to explore seizure mechanism and novel therapeutic targets that, importantly, respond to human antiepileptic drugs.

Materials and methods

Strains and genetics

Caenorhabditis elegans were maintained on standard NG agar plates seeded with *Escherichia coli*. L4 worms were picked and transferred the evening prior to testing and

maintained overnight at 25 °C. *Caenorhabditis elegans* used in these experiments were Bristol N2 strain, the loss-of-function *egl-4* (n478) IV strain MT1073 and gain-of-function *egl-4* (ad450) IV strain DA521. All *C. elegans* strains were ordered from the Caenorhabditis Genetics Center.

Caenorhabditis elegans electroconvulsive seizure assay

This procedure was performed as described previously (Risley et al. 2016). In brief, 15 µL of standard M9 salt solution was added to 9 mm long segments of clear plastic tubing (Tygon® microbore tubing). Approximately six 1-day-old adult *C. elegans* were transferred to plastic tube using a platinum wire pick and allowed to incubate for 30 min (Fig. 1a). For the pharmacological treatments, the drug was dissolved directly into the M9 and the worms were incubated for a total of 30 min. Following incubation, an insulated copper wire was inserted into either end of the plastic tube and attached with alligator clips to a square-pulse generating stimulator (Grass, SD9). A shock was delivered for three seconds (3 s, 47 V, Fig. 1b), and a microscope camera recorded the shock and recovery phases. Recovery was manually scored when each individual animal resumed a wave-like swimming motion. All data were pooled and averaged.

Pharmaceutical manipulations

Drugs were dissolved directly into the M9 solution, and 15 µL of the solution was aliquot into the clear plastic tubing. The drugs included: 50 mM 8-bromo-cGMP (increases PKG enzymatic activity), 10 mM Rp-8-bromo-cGMP (decreases PKG enzymatic activity) and 0.5 mM Retigabine (K⁺ channel agonist) purchased from Sigma-Aldrich (St. Louis, Missouri, USA), and 0.5 mM sildenafil citrate was purchased from Attix Pharmaceuticals (Toronto, Ontario, Canada).

Statistics

Induction of electroconvulsive seizure was conducted for at least nine worms and experiments were repeated at least three times on different days. Data were analyzed using a Kruskal–Wallis one-way ANOVA on ranks followed by a Dunn’s method post hoc multiple comparisons test or an unpaired Student’s *t* test where indicated in the results. All statistics were performed using SigmaPlot 11.0 (San Jose, California). All bar graphs represent mean ± SEM, and asterisks denote significance between bars were * = *P* < 0.05.

Results

Using a recently developed electroconvulsive seizure assay, we determined that the NO/cGMP/PKG signaling pathway plays a role in recovery from electroconvulsive seizures in *C. elegans*. Behavioral recovery, determined by the ability to resume normal locomotion, is reflected by the ability for the CNS to function after an electroconvulsive shock. This seizure phenotype is based on the strength and duration of the electrical current passed through the animal. Briefly, worms were placed inside a clear plastic tube containing M9 saline and drug solution (Fig. 1a). Both ends of the tube were plugged with copper wire and connected to a square-pulse generating stimulator. This method allowed us to assess approximately ten worms per experiment. During the seizure episode, worms immediately display paralysis,

immediately followed by convulsions. We define convulsions as repeated unilateral body bends and twitching (Fig. 1b).

Genetic PKG activation reduces seizure duration

The increased duration of the electroconvulsive seizure in worms was directly correlated with the decreased concentration of PKG enzymatic activity. Initially, N2, *egl-4* [encodes the worm PKG gene; homologous to the *foraging* gene in flies (Krzyzanowski et al. 2013; L'Etoile et al. 2002)] loss-of-function (LoF; -PKG) and *egl-4* gain-of-function (GoF) were explored to determine if PKG plays a role in seizure recovery. After stimulation (3 s, 47 V), worms resumed normal sinusoidal thrashing locomotion. N2 resumed normal locomotion in approximately 47 ± 6.6 s and *egl-4* (n478) LoF recovered in 107 ± 17.8 s (one-way ANOVA on ranks, $F_{(2,55)} = 15.906$, $P < 0.001$; Dunn, $P < 0.05$, Fig. 2). *egl-4* (ad450) GoF rescued recovery time to N2 levels of 46 ± 18.2 s (Dunn, $P < 0.05$, Fig. 2).

Pharmacological PKG modulation alters seizure duration in wild-type

Next, we investigated whether drugs that increase and decrease PKG enzymatic activity in wild-type animals would reflect the results of the genetic manipulations. N2 worms were pretreated with two PKG activators, 50 mM 8-bromo-cGMP and 0.5 mM sildenafil citrate and one PKG inhibitor Rp-8-bromo-cGMP (Fig. 3). When exposed to the PKG inhibitor, N2 worms took an average of 71 ± 12.4 s to recover compared to the control N2 at 52 ± 9.8 s. While this average is trending in the direction we hypothesized, and it is not significant compared to control, it was significantly different from both of the PKG activators, 8-bromo-cGMP and sildenafil citrate (Student's *t* test, $P < 0.05$) which recovered at 38 ± 6.6 and 36 ± 7.2 s, respectively.

PKG activation reduces recovery time in *egl-4* LoF

We explored the idea that PKG activation would rescue the time to recovery of the *egl-4* LoF. We pretreated N2, *egl-4* LoF and *egl-4* GoF worms for 30 min with 50 mM 8-bromo-cGMP (Fig. 4). As anticipated, the *egl-4* GoF treated with the PKG activator was not significantly different from the control. However, the LoF worms took significantly less time to recover compared to the control, 74 ± 16.5 s and 134 ± 16.7 s, respectively (Student's *t* test, $P < 0.05$).

Antiepileptic drug reduces seizure duration in *egl-4*

Previous investigations from our laboratory have determined that *C. elegans* susceptible to seizures respond to human AEDs; therefore, we wanted to test if the PKG mutant could also be rescued with an AED. N2 and *egl-4* LoF worms were treated with 0.5 mM Retigabine, an FDA-approved AED that targets a voltage-gated potassium channel. This treatment rescued seizure duration of the PKG mutant down to wild-type levels where the wild-type recovered in approximately 37 ± 3.6 s and the Retigabine-treated mutant recovered in approximately 26 ± 4.4 s (oneway ANOVA on ranks, $F_{(3,133)} = 50.299$, $P < 0.001$; Dunn, $P > 0.05$, Fig. 5). This is significantly less than the *egl-4* LoF control (134 ± 13.6 s) (Dunn, $P < 0.05$, Fig. 5).

Discussion

Therapies to control epileptic seizures primarily involve mechanisms that modulate ion channel conformation, thereby decreasing neuronal excitability. We demonstrated that by modulating cGMP-dependent protein kinase (PKG), we can alter electroconvulsive seizure duration in *C. elegans*. Given these results, we have proposed that the cGMP/PKG pathway is a potential new target for investigating seizure mechanism.

In the worm, *egl-4* regulates many important behavioral and developmental processes, such as entry into dauer formation, egg-laying, chemosensory function and synaptic transmission. Previous work has determined that *egl-4* LoF is sensitive to the acetylcholinesterase inhibitor, aldicarb, suggesting that *egl-4* works to regulate presynaptic neurotransmission (Daniels et al. 2000). While there is ample evidence that *egl-4* is a strong LoF mutant with non-functional PKG receptors for cGMP (L'Etoile et al. 2002; Fujiwara et al. 2002), we showed that using 8-bromo-cGMP reduced recovery time back to control levels. This may be due to cGMP directly activating cGMP-gated ion channels (Liu et al. 2010), or the exogenous cGMP could have an effect on transcription factors leading to multiple physiological reactions (Hahm et al. 2009). This further supports our idea that the cGMP/PKG pathway might function in modulating neuronal excitability.

It is interesting to note that the GoF response to ES is not different from the wild-type in several of our results, and we hypothesize this could be due to several different reasons. Interestingly, according to the researchers that characterized the allele, the *egl-4* GoF protein levels are significantly reduced as compared to the wild-type worms, which would appear opposite from what is expected (Raizen et al. 2006). The group reported many physiological processes that were opposite of the expected LoF phenotypes and proposed that perhaps there is less stability in protein folding or there is altered subcellular distribution of PKG in the GoF strain (Raizen et al. 2006). Additionally, there have been other studies investigating both GoF and LoF alleles where LoF appears to differ from the wild-type phenotype, unlike the GoF. In one particular study that investigated *C. elegans* tastant behavior, the *egl-4* LoF allele was more sensitive to an aversive tastant, in this case 1 mM quinine, and the *egl-4* GoF was not different from wild-type (Krzyzanowski et al. 2013). These findings provide evidence that our results are not out of the ordinary regarding the GoF allele.

Lastly, we have demonstrated that the *egl-4* LoF seizure phenotype can be rescued by pretreatment with an FDA-approved AED. Our laboratory has used Retigabine with *C. elegans* in the past to show that human seizure therapeutics can successfully be used to alter recovery time in invertebrates; however, regarding N2, the drug must be used in conjunction with a chemical convulsant to induce an effect (Opperman et al. 2017; Risley et al. 2016). We hypothesize this is due to the increased seizure threshold for N2. The main target of Retigabine in mammals is a voltage-gated potassium channel, KCNQ2/3 (Barrese et al. 2010; Friedman et al. 2015; Rundfeldt 1997). The *C. elegans* ortholog of is *kqt-1/2/3*; therefore, it is possible that this drug works in a similar mechanism. These results and previous results suggest, however, that Retigabine reduces excitability and lessens the time to recovery. Additionally, we found that modulating PKG in vivo alters recovery time from

an electroconvulsive seizure. Using genetic and pharmacological manipulation in the ES assay, the data support an unexplored target for seizure exploration.

Acknowledgements

Research was supported by a compound transfer grant (CTP) Grant from Pfizer WI225058 for KD-S. Some strains were provided by the CGC, which is funded by National Institute of Health (NIH) Office of Research Infrastructure Programs (P40 OD010440).

Funding Research was supported by a compound transfer grant (CTP) grant from Pfizer WI225058 for KD-S.

References

- Barrese V, Miceli F, Soldovieri MV, Ambrosino P, Iannotti FA, Cilio MR et al. (2010) Neuronal potassium channel openers in the management of epilepsy: role and potential of retigabine. *Clin Pharmacol* 2:225–236. 10.2147/cpaa.s15369 [PubMed: 22291509]
- Brodie MJ, Barry SJ, Bamagous GA, Norrie JD, Kwan P (2012) Patterns of treatment response in newly diagnosed epilepsy. *Neurology* 78(20):1548–1554. 10.1212/WNL.0b013e3182563b19 [PubMed: 22573629]
- Calahorra F, Ruiz-Rubio M (2011) *Caenorhabditis elegans* as an experimental tool for the study of complex neurological diseases: Parkinson's disease, Alzheimer's disease and autism spectrum disorder. *Invert Neurosci* 11(2):73–83. 10.1007/s10158-011-0126-1 [PubMed: 22068627]
- Caplan SL, Milton SL, Dawson-Scully K (2013) A cGMP-dependent protein kinase (PKG) controls synaptic transmission tolerance to acute oxidative stress at the *Drosophila* larval neuromuscular junction. *J Neurophysiol* 109(3):649–658. 10.1152/jn.00784.2011 [PubMed: 23136350]
- Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC (1994) Green fluorescent protein as a marker for gene expression. *Science* 263(5148):802–805 [PubMed: 8303295]
- Daniels SA, Ailion M, Thomas JH, Sengupta P (2000) *egl-4* acts through a transforming growth factor-beta/SMAD pathway in *Caenorhabditis elegans* to regulate multiple neuronal circuits in response to sensory cues. *Genetics* 156(1):123–141 [PubMed: 10978280]
- Dawson-Scully K, Bukvic D, Chakabarty-Chatterjee M, Ferreira R, Milton SL, Sokolowski MB (2010) Controlling anoxic tolerance in adult *Drosophila* via the cGMP-PKG pathway. *J Exp Biol* 213(Pt 14):2410–2416. 10.1242/jeb.041319 [PubMed: 20581270]
- Friedman LK, Slomko AM, Wongvavut JP, Naseer Z, Hu S, Wan WY et al. (2015) Efficacy of retigabine on acute limbic seizures in adult rats. *J Epilepsy Res* 5(2):46–59. <https://doi.org/10.14581/jer.15010> [PubMed: 26819936]
- Fujiwara M, Sengupta P, McIntire SL (2002) Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron* 36(6):1091–1102 [PubMed: 12495624]
- Guerrini R, Rosati A, Giordano F, Genitori L, Barba C (2013) The medical and surgical treatment of tumoral seizures: current and future perspectives. *Epilepsia* 54(Suppl 9):84–90. 10.1111/epi.12450
- Hahm JH, Kim S, Paik YK (2009) Endogenous cGMP regulates adult longevity via the insulin signaling pathway in *Caenorhabditis elegans*. *Aging Cell* 8(4):473–483. 10.1111/j.1474-9726.2009.00495.x [PubMed: 19489741]
- Hamamichi S, Rivas RN, Knight AL, Cao S, Caldwell KA, Caldwell GA (2008) Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. *Proc Natl Acad Sci USA* 105(2):728–733. 10.1073/pnas.0711018105 [PubMed: 18182484]
- Kaun KR, Hendel T, Gerber B, Sokolowski MB (2007) Natural variation in *Drosophila* larval reward learning and memory due to a cGMP-dependent protein kinase. *Learn Mem* 14(5):342–349. 10.1101/lm.505807 [PubMed: 17522025]
- Kim JB (2014) Channelopathies. *Korean J Pediatr* 57(1):1–18. 10.3345/kjp.2014.57.1.1 [PubMed: 24578711]

- Krzyzanowski MC, Brueggemann C, Ezak MJ, Wood JF, Michaels KL, Jackson CA et al. (2013) The *C. elegans* cGMP-dependent protein kinase EGL-4 regulates nociceptive behavioral sensitivity. *PLoS Genet* 9(7):e1003619 10.1371/journal.pgen.1003619 [PubMed: 23874221]
- Lee SK (2014) Old versus new: why do we need new antiepileptic drugs? *J Epilepsy Res* 4(2):39–44 [PubMed: 25625087]
- L'Etoile ND, Coburn CM, Eastham J, Kistler A, Gallegos G, Bargmann CI (2002) The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. *Neuron* 36(6):1079–1089 [PubMed: 12495623]
- Levitan D, Doyle TG, Brousseau D, Lee MK, Thinakaran G, Slunt HH et al. (1996) Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 93(25):14940–14944 [PubMed: 8962160]
- Link CD, Taft A, Kapulkin V, Duke K, Kim S, Fei Q et al. (2003) Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. *Neurobiol Aging* 24(3):397–413 [PubMed: 12600716]
- Liu J, Ward A, Gao J, Dong Y, Nishio N, Inada H et al. (2010) *C. elegans* phototransduction requires a G protein-dependent cGMP pathway and a taste receptor homolog. *Nat Neurosci* 13(6):715–722. 10.1038/nn.2540 [PubMed: 20436480]
- Loscher W, Schmidt D (2011) Modern antiepileptic drug development has failed to deliver: ways out of the current dilemma. *Epilepsia* 52(4):657–678. 10.1111/j.1528-1167.2011.03024.x [PubMed: 21426333]
- Mary Jane E, Catharyn TL, Andrea MS, Larisa MS (2012) *Epilepsy across the spectrum: promoting health and understanding*. The National Academies Press, Washington, DC
- Mery F, Belay AT, So AK, Sokolowski MB, Kawecky TJ (2007) Natural polymorphism affecting learning and memory in *Drosophila*. *Proc Natl Acad Sci USA* 104(32):13051–13055. 10.1073/pnas.0702923104 [PubMed: 17640898]
- Opperman KJ, Mulcahy B, Giles AC, Risley MG, Birnbaum RL, Tulgren ED et al. (2017) The HECT family ubiquitin ligase EEL-1 regulates neuronal function and development. *Cell Rep* 19(4):822–835. 10.1016/j.celrep.2017.04.003 [PubMed: 28445732]
- Raizen DM, Cullison KM, Pack AI, Sundaram MV (2006) A novel gain-of-function mutant of the cyclic GMP-dependent protein kinase egl-4 affects multiple physiological processes in *Caenorhabditis elegans*. *Genetics* 173(1):177–187. 10.1534/genetics.106.057380 [PubMed: 16547093]
- Renger JJ, Yao WD, Sokolowski MB, Wu CF (1999) Neuronal polymorphism among natural alleles of a cGMP-dependent kinase gene, foraging, in *Drosophila*. *J Neurosci* 19(19):RC28 [PubMed: 10493773]
- Risley MG, Kelly SP, Jia K, Grill B, Dawson-Scully K (2016) Modulating behavior in *C. elegans* using electroshock and antiepileptic drugs. *PLoS ONE* 11(9):e0163786 10.1371/journal.pone.0163786 [PubMed: 27668426]
- Rundfeldt C (1997) The new anticonvulsant retigabine (D-23129) acts as an opener of K⁺ channels in neuronal cells. *Eur J Pharmacol* 336(2–3):243–249 [PubMed: 9384239]
- Saeki S, Yamamoto M, Iino Y (2001) Plasticity of chemotaxis revealed by paired presentation of a chemoattractant and starvation in the nematode *Caenorhabditis elegans*. *J Exp Biol* 204(Pt 10):1757–1764 [PubMed: 11316496]
- Szaflarski JP, Nazzari Y, Dreier LE (2014) Post-traumatic epilepsy: current and emerging treatment options. *Neuropsychiatr Dis Treat* 10:1469–1477. 10.2147/ndt.s50421 [PubMed: 25143737]
- Timmons L, Court DL, Fire A (2001) Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* 263(1–2):103–112 [PubMed: 11223248]
- White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 314(1165):1–340 [PubMed: 22462104]
- Zhang Y, Lu H, Bargmann CI (2005) Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* 438(7065):179–184. 10.1038/nature04216 [PubMed: 16281027]

Fig. 1.

A schematic that represents the position of the worms before and during an ES. **a**

Approximately six worms are added to a plastic tub plugged on either end with copper wire (represented in black). The worms habituated in the aqueous solution for 30 min before the electric shock. During this time, they display normal thrashing motion. **b** During the shock, some worms will exhibit unilateral body bends, and some will elongate. This is represented by the various positions of the cartoon worms inside the time. The time to resume normal thrashing is recorded as recovery

Fig. 2.

Seizure recovery can be mediated by PKG concentrations in *C. elegans*. N2 wild-type worms and *egl-4* GoF mutants took significantly less time to recover from an electroconvulsive seizure when compared to the worms with decreased levels of PKG. Error bars represent $\pm$ SEM and $n = 15$

Fig. 3. Wild-type worms are treated with PKG activators and inhibitors. Pretreatment of wild-type worms with 10 mM of Rp-8-bromo-cGMP (PKG inhibitor) causes a significantly increased time to recovery compared to the worms that are treated with 50 mM 8-bromo-cGMP and 0.5 mM sildenafil citrate (PKG activators). Error bars represent $\pm$ SEM and $n = 24$

Fig. 4.

Treatment with a PKG activator, 8-bromo-cGMP. When *egl-4* LoF were pretreated with 50 mM 8-bromo-cGMP, they took significantly less time to recover compared to the control. There was no significant difference between the treatment and control for the wild-type or the *egl-4* GoF. Error bars represent $\pm$ SEM and $n = 9$

Fig. 5.

Treatment with an FDA-approved antiepileptic drug. N2 and *egl-4* LoF were pretreated with an FDA-approved human AED, 0.5 mM Retigabine, which significantly lowered the time to recovery in the *egl-4* LoF. Error bars represent $\pm$ SEM and $n = 20$