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TRPM channels modulate epileptic-like convulsions via systemic ion homeostasis

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Summary

Neuronal networks operate over a wide range of activity levels, with both neuronal and non-neuronal cells contributing to the balance of excitation and inhibition. Activity imbalance within neuronal networks underlies many neurological diseases, such as epilepsy [1]. The *C. elegans* locomotor circuit operates via coordinated activity of cholinergic excitatory and GABAergic inhibitory transmission [2]. We have previously shown that a gain-of-function mutation in a neuronal acetylcholine receptor, *acr-2(gf)*, causes an epileptic-like convulsion behavior [3]. Here, we report that the behavioral and physiological effects of *acr-2(gf)* require the activity of the TRPM channel GTL-2 in non-neuronal tissues. Loss of *gtl-2* function does not affect baseline synaptic transmission, yet can compensate for the excitation-inhibition imbalance caused by *acr-2(gf)*. The compensatory effects of removing *gtl-2* are counter-balanced by another TRPM channel GTL-1, and can be recapitulated by acute treatment with divalent cation chelators, including those specific for Zn^{2+}. Together these data reveal an important role for ion homeostasis in the balance of neuronal network activity and a novel function of non-neuronal TRPM channels in the fine-tuning of this network activity.

Keywords

nicotinic acetylcholine receptor; epileptic-like behavior; neuronal network balance; TRPM channel; ion homeostasis; *C. elegans*; GABA; acetylcholine

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Supplemental information
Included supplemental methods, three figures, two tables, and four movies.

Results

GTL-2 acts in non-neuronal tissues to modulate convulsive behaviors caused by *acr-2(gf)*

In the wild type *C. elegans* locomotor circuit, cholinergic innervation excites muscles to contract alternately on the ventral or dorsal side, while simultaneously activating GABAergic cross-inhibition to relax muscles on the opposite side [2]. A heteromeric acetylcholine receptor, ACR-2R, expressed in the cholinergic neurons plays a key role in the locomotor network [3]. A Valine to Methionine substitution in the transmembrane TM2 domain of the ACR-2 non-alpha subunit increases channel current and causes a gain-of-function effect in the locomotor circuit, manifested as overexcitation accompanied with decreased inhibition. The *acr-2(gf)* animals display a spontaneous convulsive behavior (Figure 1, supplemental movie 1). Similar amino acid substitutions in mammalian neuronal acetylcholine receptors are associated with epilepsy [4].

To search for novel regulators involved in the *acr-2(gf)* convulsive phenotype we isolated and characterized a genetic suppressor, *n2618* (see supplemental methods, Table S1). Double mutants of *n2618;acr-2(gf)* show grossly wild type locomotion and a near complete elimination of spontaneous convulsions (Figures 1A, B, supplemental movie 2). By genetic mapping and whole-genome sequencing we found that *n2618* animals had a single amino acid substitution in a conserved domain of the protein GTL-2, which is a member of the TRPM (for Melastatin family of Transient Receptor Potential) channels (Figure S1) [5]. TRPM channels are multifunctional non-selective cation channels. Those closely related to GTL-2 generally play a role in ion homeostasis in a tissue and organism-specific manner [6–10]. *C. elegans* has three TRPM channels GON-2, GTL-1 and GTL-2, which have previously been implicated in electrolyte Mg^{2+} homeostasis and intestinal Ca^{2+} oscillations [9, 10]. Presumed *gtl-2* null mutations caused similar suppression of *acr-2(gf)*, and failed to complement *n2618* for the suppression activity (Figure 1B). In addition, the suppression effect of *gtl-2(null)* or *gtl-2(n2618)* on *acr-2(gf)* was fully rescued by transgenic expression of the genomic *gtl-2* DNA (Figure 1C). We conclude that inactivation of *gtl-2* antagonizes the locomotor circuit imbalance caused by *acr-2(gf)*.

Other genetic suppressors of *acr-2(gf)* define genes required for the activity of the ACR-2 channel and which act in cholinergic neurons [3]. To determine in which tissues *gtl-2* function was required for suppression, we generated a GTL-2::GFP fusion construct that completely rescued the suppression effects of *gtl-2(lf)* on *acr-2(gf)* (Figure 1C, Table S1, 2). We observed GTL-2::GFP on the epidermal cell surface (Figure 1D, E), as well as in the excretory cell and pharynx, as reported [9], but not in neuronal and muscle cells associated with locomotion. This expression pattern suggests that *gtl-2* may influence the *acr-2(gf)* neuronal phenotype through non-neuronal means. To test this hypothesis we performed cell specific rescue experiments with a *gtl-2cDNA::gfp* construct (Table S2). Expression of *gtl-2* in either the epidermis or the excretory cell, but not in neurons or muscles, showed a rescuing activity comparable to that of *gtl-2* expressed ubiquitously or under the control of its own promoter (Figure 1F). The excretory cell and the epidermis are connected by gap junctions [11]. The ability of either excretory cell or epidermal expression of GTL-2 to

rescue is consistent with functional coupling of these tissues and with our evidence below that GTL-2 regulates neuronal excitability via extracellular ion homeostasis.

GTL-2 specifically affects the activity imbalance caused by *acr-2(gf)*

To investigate how non-neuronal GTL-2 might affect motor neuron activity, we first analyzed neuronal anatomy and physiology in *gtl-2* mutants. Overall synapse and neuronal morphology were grossly normal in these mutants (Figure S2A). *gtl-2* mutants displayed normal sensitivity to the acetylcholinesterase inhibitor Aldicarb [12] (Figure 2A), and exhibited wild type locomotion (supplemental movie 3). Electrophysiological recordings of dissected body-wall muscle preparations showed that the frequencies and amplitudes of endogenous cholinergic and GABAergic activity in *gtl-2(lf)* was similar to that of wild type animals across a range of Ca^{2+} concentrations (Figures 2B, S2D). The expression levels of the postsynaptic GABA receptor UNC-49 and the response to exogenous GABA were also similar to those in wild type animals (Figures S2B,C). Thus, GTL-2 is not essential for most aspects of neuronal and muscle development or physiology.

We next addressed whether the behavioral suppression of *acr-2(gf)* by *gtl-2(lf)* could be accounted for by restoration of the excitation and inhibition balance in the locomotor circuit. *acr-2(gf)* animals exhibit hypersensitivity to aldicarb, in part due to increased acetylcholine release [3]; and *gtl-2(lf)* partially suppressed this aldicarb hypersensitivity (Figure 2C). We further investigated this effect by electrophysiological recordings of neuromuscular junctions (NMJ). We previously observed that *acr-2(gf)* disrupts the correlated activities between cholinergic and GABAergic transmission in a Ca^{2+} dependent manner [3]. Here, using a modified recording procedure [13], we recorded over a range of extracellular Ca^{2+} concentrations (see supplemental methods). *acr-2(gf)* animals at 0.1 mM extracellular Ca^{2+} showed an increase in both cholinergic and GABAergic transmission, compared to wild type animals (Figure 2D). This observation indicates that the GABAergic neurons in *acr-2(gf)* animals are healthy and capable of responding to synaptic inputs as predicted by their anatomical connectivity [2]. As the extracellular Ca^{2+} concentration was increased, wild type animals showed increases in both cholinergic and GABAergic activities. By contrast, in *acr-2(gf)* mutants, while the cholinergic activity continued to increase, the GABAergic activity began to decrease (Figure 2D), consistent with previous findings [3]. Thus, under physiological calcium levels over 1 mM [14, 15], the increased cholinergic activity caused by the *acr-2(gf)* mutation is accompanied by an anti-homeostatic inhibition of GABAergic neuron activity (Figure 2B), leading to a net over-excitation of the locomotor circuit. Strikingly, the *gtl-2(lf)* mutation not only enhanced GABA release in *acr-2(gf)*, but also restored the Ca^{2+} dependent increase of GABAergic transmission, resulting in correlated cholinergic excitation and GABAergic inhibition in the *acr-2(gf);gtl-2(lf)* double mutant (Figures 2B, D). In these preparations the tissues expressing *gtl-2* were disrupted, therefore the observed physiological effects most likely reflect long-lasting changes in GABAergic presynaptic release as the result of reducing *gtl-2* function.

Although the recordings from the dissociated NMJ preparations did not detect significant effects of *gtl-2(lf)* on the cholinergic activities of *acr-2(gf)*, we wondered whether *gtl-2* might also modulate cholinergic neurons. To address this, we generated

gtl-2(lf);acr-2(gf);unc-25/GAD(0) triple mutant animals in which GABAergic transmission was completely absent due to a null mutation in the glutamic acid decarboxylase UNC-25 [16]. These animals showed significant suppression of convulsions, compared to *acr-2(gf);unc-25/GAD(0)* (Figure 3A), indicating that loss of *gtl-2* activity can alleviate hyperexcitation defects of *acr-2(gf)* in the absence of GABA. These observed *in vivo* effects of *gtl-2(lf)* may reflect acute changes on cholinergic transmission that are not evident in NMJ recordings due to the dissection of GTL-2 expressing tissues and consequent disruption of the extracellular milieu. It is also possible that GTL-2 might also influence ACR-2(gf) independent of both GABAergic and cholinergic activities. To assess whether *gtl-2* modulated other types of neuronal over-excitation, we tested the ability of *gtl-2(lf)* to suppress mutants with increased cholinergic transmission, such as the $G_{o\alpha}$ protein mutant *goa-1(lf)* [17]. Unlike *acr-2(gf)*, loss of *gtl-2* function had no effect on locomotion nor on aldicarb sensitivity of *goa-1(lf)* mutants (Figures 3B, C). These analyses indicate that loss of *gtl-2* function specifically modulates the anti-homeostatic defects in *acr-2(gf)*.

Manipulating ion homeostasis recapitulates the effects of *gtl-2(lf)*

GTL-2 has been reported to regulate fluid Mg^{2+} homeostasis, in conjunction with the other two *C. elegans* TRPM channels, GTL-1 and GON-2 [9, 18]. Specifically, GTL-1 and GON-2 function in the intestine to take up Mg^{2+}, whereas GTL-2 acts in the excretory cell to excrete Mg^{2+}. *gtl-2(lf)* mutants have increased systemic Mg^{2+}, but also reduced systemic Ca^{2+} levels, suggesting other roles for GTL-2 in addition to Mg^{2+} export [9]. To address the basis of *gtl-2* action on the locomotor circuit, we first investigated the roles of these other TRPM channels on *acr-2(gf)*. Loss of *gtl-1* function had no effect on *acr-2(gf)* alone, but completely rescued the suppression of convulsions in the *gtl-2(lf);acr-2(gf)* background (Figure 4A). Moreover, RNAi knockdown of *gtl-1* in *gtl-2(lf);acr-2(gf)* animals in a genetic background in which the neurons are refractory to RNAi also resulted in rescuing of the suppression activity of *gtl-2(lf)* (Figure 4A), suggesting GTL-1 may also function in non-neuronal cells to modulate *gtl-2* suppression of *acr-2(gf)*. Unexpectedly, loss of *gon-2* function attenuated the convulsion frequency of *acr-2(gf)*, similar to mutating *gtl-2* (Figure 4A). This observation suggests that the increased Mg^{2+} in *gtl-2(lf)* may not be directly responsible for the suppression effect on *acr-2(gf)*. To test this, we cultured animals using plates containing varying Mg^{2+} concentration. While increasing Mg^{2+} had a significant effect on brood size in *gtl-2* mutants (Figure S3A), consistent with the previous observations [9], it had no effect on *acr-2(gf)* convulsions nor on the suppression of *acr-2(gf)* by *gtl-2(lf)* (Figure S3B). Soaking animals in high concentrations of Mg^{2+} similarly failed to influence *acr-2(gf)* convulsions. We also performed NMJ recordings using extracellular bath solutions with varying Mg^{2+}, but did not observe any significant differences on cholinergic and GABAergic activities in either wild type or *acr-2(gf)* (Figure S3C,D). Thus, the increased Mg^{2+} levels in *gtl-2(lf)* mutants do not account for the effects on the locomotor circuit. Instead, other divalent cations might be involved in suppression of excitation-inhibition imbalance.

To further address the involvement of ion homeostasis in modulating the excitation-inhibition imbalance caused by *acr-2(gf)*, we soaked *acr-2(gf)* and *gtl-2(lf);acr-2(gf)* animals in multiple cation chelators (Figure 4B,C). Bathing *acr-2(gf)* animals in the divalent

cation chelators ethylenediaminetetraacetic acid (EDTA) resulted in significant suppression of convulsions and improved locomotor ability (supplemental movie 4). However, EDTA had no effects on *gtl-2(lf);acr-2(gf)*, consistent with the above observation of culturing *acr-2(gf)* on high concentration Mg^{2+} plates and *gtl-2(lf);acr-2(gf)* on plates with no Mg^{2+} added. Treatment with ethylene glycol tetraacetic acid (EGTA) had no effect on *acr-2(gf)* single or *gtl-2(lf);acr-2(gf)* double mutants. As EGTA has relatively high specificity to Ca^{2+} [19], these results suggest that reducing extracellular cations, but not necessarily Ca^{2+}, specifically mimicked the effects of reducing *gtl-2* activity. As some TRPM channels show a high level of conductance for trace metals, particularly Zn^{2+} [20], we tested the heavy metal specific chelators dietylenetriaminepentaacetic acid (DTPA), a membrane impermeant chelator [21], and tetrakis(2-pyridylmethyl)ethlyenediamine (TPEN), a membrane permeant chelator [22], and found that both suppressed the *acr-2(gf)* phenotype comparable to EDTA treatment (Figures 4B,C). All together, these findings demonstrate that divalent cationic balance of heavy metals is critical for the expression of the convulsive behavior caused by *acr-2(gf)*.

Discussion

Epilepsy is one of the most common neurological diseases today, affecting approximately one percent of the population [23]. Common to all forms of epilepsy is an imbalance of excitation and inhibition [1]. A distinct physiological feature of the *acr-2(gf)* mutation is that while increasing cholinergic excitation, it creates an anti-homeostatic decrease in GABAergic activity cell non-autonomously [3] (this study). How are TRPM channels, GTL-2, GON-2 and GTL-1 influencing this neuronal activity imbalance? Our data suggest two possible mechanisms that are not mutually exclusive, one involving local action of GTL-2 and the other involving systemic ion homeostasis (Figure 4D). Local ionic composition fluctuates as the result of neuronal activity. The epidermis is adjacent to motor neurons and their axons [2]. As GTL-2 is expressed in the epidermis, it could influence this local ionic balance (Figure 4D). The role of GTL-2 and the epidermis may be analogous to that of glial cells in the mammalian nervous system. Glial cells maintain normal levels of network activity through their roles in K^+ buffering, glutamate uptake, and glia specific release of neurotransmitter, and have been implicated in epilepsy [24]. A number of TRPM channels are highly expressed in the brain, and TRPM1 and TRPM7 have been shown to play cell autonomous roles in neuronal function [25–28]. However, non-cell autonomous roles of TRPM channels in the nervous system remain to be examined.

GON-2 and GTL-1 function in fluid ion homeostasis through ion uptake in the intestine [9]. Thus, their effects on *acr-2(gf)* indicate a role of systemic ion regulation on neuronal function (Figure 4D). Although the three *C. elegans* TRPM channels have been linked to Mg^{2+} homeostasis [9] based on their effects on fertility and growth rate, our results suggest they influence neuronal excitability independent of Mg^{2+}. In *gtl-2(lf)* animals, systemic Mg^{2+} levels are increased. However, chelation of divalent cations, including Mg^{2+}, suppressed *acr-2(gf)*, and did not reverse the suppression effects of *gtl-2(lf)* on *acr-2(gf)*. Instead, treatment using heavy metal chelators DTPA and TPEN mimicked loss of *gtl-2* function. These observations indicate that the action of these TRP channels influences cation homeostasis beyond simple import and export of Mg^{2+}. In fact, Teramato et al. also showed

Ca^{2+} levels were decreased in *gtl-2(lf)* mutants, which they attributed to the inhibition of GON-2 by high Mg^{2+} [9]. This interpretation could explain our observation that loss of GON-2 partially suppressed *acr-2(gf)* in a manner similar to *gtl-2(lf)*.

Studies of several TRPM channels in other organisms, such as TRPM7 channels, show that they can be permeable to multiple divalent ions, including the heavy metal Zn^{2+} [20]. Additionally, independent studies on the *Drosophila* TRPM channel reported changes in larval Zn^{2+} and Mg^{2+} homeostasis [6, 7]. Interestingly, altered Zn^{2+} levels have been reported in epilepsy patients, and it is thought that disturbed Zn^{2+} homeostasis can be proconvulsant, whereas Zn^{2+} can act as an anticonvulsant when its homeostasis is maintained [29]. Manipulation of Zn^{2+} can also potentiate acetylcholine receptors and inhibit certain GABA receptors [30, 31]. We have shown here that altering homeostasis of heavy metals including Zn^{2+} by chelation has an anticonvulsant effect. We speculate that altered neuronal activity by ACR-2(gf) could cause local ionic imbalance, possibly involving Zn^{2+}. Conversely, alteration in systemic Zn levels may directly affect the activity of *acr-2(gf)*, restoring neuronal network balance.

Current treatments for epilepsy focus on normalizing the neuronal network excitation-inhibition imbalance by targeting mostly neuronal proteins [32]. Yet, many epilepsy patients are refractory to pharmacological treatments [33], prompting an increased effort to search for epilepsy treatments that go beyond direct effects on neurotransmission. One long-standing treatment that has proven effective in epilepsy patients who are refractory to traditional medications is the ketogenic diet [34]. Systemic metabolic changes can have long-lasting effects on neurotransmission [35]. Our study reveals a critical role for ion homeostasis in balancing neuronal excitation and inhibition in a network, as well as introducing TRPM channels as novel molecular players in this pathway.

Experimental Procedures

Genetics, molecular and transgenic analysis

C. elegans strains were grown on NGM plates at room temperature (20–22°C) as described [36]. Isolation and cloning of *n2618* were detailed in supplemental methods. Double mutants were constructed using standard procedures, and genotypes were confirmed by allele sequence determination (Table S1). Molecular biology, transgenic analysis and RNAi were detailed in supplemental methods. Table S2 lists the information of all constructs.

Behavior, Pharmacology and Chelation analysis

Convulsion was quantified as detailed in supplemental methods [3]. All drugs were from Sigma-Aldrich, and manipulations were performed according to published procedures [3, 12, 38]. For chelator soaking assays L4 larvae were put into 200 μl of M9 solution containing the chosen chelator and concentrated bacteria. For each chelator, a range of concentration was tested, and the concentration that did not cause noticeable sickness to animals was used in the final quantification analysis. Soaking was performed overnight at room temperature. The treated animals were transferred to regularly seeded plates the following morning, and were then video recorded to quantify convulsions.

Electrophysiology

We adapted a recording procedure developed from previous studies [13, 39]. Detailed information is in the supplemental methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. McCormick DA, Contreras D. On the cellular and network bases of epileptic seizures. Annu Rev Physiol. 2001; 63:815–846. [PubMed: 11181977]
2. White JG, Southgate E, Thomson JN, Brenner S. The structure of the ventral nerve cord of Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci. 1976; 275:327–348. [PubMed: 8806]
3. Jospin M, Qi YB, Stawicki TM, Boulin T, Schuske KR, Horvitz HR, Bessereau JL, Jorgensen EM, Jin Y. A neuronal acetylcholine receptor regulates the balance of muscle excitation and inhibition in Caenorhabditis elegans. PLoS Biol. 2009; 7:e1000265. [PubMed: 20027209]
4. Marini C, Guerrini R. The role of the nicotinic acetylcholine receptors in sleep-related epilepsy. Biochem Pharmacol. 2007; 74:1308–1314. [PubMed: 17662253]
5. West RJ, Sun AY, Church DL, Lambie EJ. The C. elegans gon-2 gene encodes a putative TRP cation channel protein required for mitotic cell cycle progression. Gene. 2001; 266:103–110. [PubMed: 11290424]
6. Georgiev P, Okkenhaug H, Drews A, Wright D, Lambert S, Flick M, Carta V, Martel C, Oberwinkler J, Raghu P. TRPM channels mediate zinc homeostasis and cellular growth during Drosophila larval development. Cell Metab. 2010; 12:386–397. [PubMed: 20889130]
7. Hofmann T, Chubanov V, Chen X, Dietz AS, Gudermann T, Montell C. Drosophila TRPM channel is essential for the control of extracellular magnesium levels. PLoS One. 2010; 5:e10519. [PubMed: 20463899]
8. Schlingmann KP, Waldegger S, Konrad M, Chubanov V, Gudermann T. TRPM6 and TRPM7--Gatekeepers of human magnesium metabolism. Biochim Biophys Acta. 2007; 1772:813–821. [PubMed: 17481860]
9. Teramoto T, Sternick LA, Kage-Nakadai E, Sajjadi S, Siembida J, Mitani S, Iwasaki K, Lambie EJ. Magnesium excretion in C. elegans requires the activity of the GTL-2 TRPM channel. PLoS One. 2010; 5:e9589. [PubMed: 20221407]
10. Xing J, Yan X, Estevez A, Strange K. Highly Ca2+-selective TRPM channels regulate IP3-dependent oscillatory Ca2+ signaling in the C. elegans intestine. J Gen Physiol. 2008; 131:245–255. [PubMed: 18299395]
11. Hall, DH.; Altun, ZF. *C. elegans* Atlas. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2008. Excretory System; p. 131-143.
12. Rand JB. Acetylcholine. WormBook. 2007:1–21. [PubMed: 18050502]
13. Vashlishan AB, Madison JM, Dybbs M, Bai J, Sieburth D, Ch'ng Q, Tavazoie M, Kaplan JM. An RNAi screen identifies genes that regulate GABA synapses. Neuron. 2008; 58:346–361. [PubMed: 18466746]

14. Conway EJ. Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. Physiol Rev. 1957; 37:84–132. [PubMed: 13419551]
15. Hille, B. Ionic Channels of Excitable Membranes. 2 Edition. Sunderland, MA: Sinauer Associates, Inc.; 1992. Introduction; p. 15
16. Jin Y, Jorgensen E, Hartwieg E, Horvitz HR. The Caenorhabditis elegans gene unc-25 encodes glutamic acid decarboxylase and is required for synaptic transmission but not synaptic development. J Neurosci. 1999; 19:539–548. [PubMed: 9880574]
17. Miller KG, Emerson MD, Rand JB. Goalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in C. elegans. Neuron. 1999; 24:323–333. [PubMed: 10571227]
18. Teramoto T, Lambie EJ, Iwasaki K. Differential regulation of TRPM channels governs electrolyte homeostasis in the C. elegans intestine. Cell Metab. 2005; 1:343–354. [PubMed: 16054081]
19. Smith GL, Miller DJ. Potentiometric measurements of stoichiometric and apparent affinity constants of EGTA for protons and divalent ions including calcium. Biochim Biophys Acta. 1985; 839:287–299. [PubMed: 2985122]
20. Monteilh-Zoller MK, Hermosura MC, Nadler MJ, Scharenberg AM, Penner R, Fleig A. TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. J Gen Physiol. 2003; 121:49–60. [PubMed: 12508053]
21. Dawson, RMC.; Elliott, DC.; Elliott, WH.; Jones, KM. Data for biochemical research. 3rd Edition. New York: Oxford Science; 1986.
22. Arslan P, Di Virgilio F, Beltrame M, Tsien RY, Pozzan T. Cytosolic Ca2+ homeostasis in Ehrlich and Yoshida carcinomas. A new, membrane-permeant chelator of heavy metals reveals that these ascites tumor cell lines have normal cytosolic free Ca2+ J Biol Chem. 1985; 260:2719–2727. [PubMed: 3919006]
23. Hauser WA. The prevalence and incidence of convulsive disorders in children. Epilepsia. 1994; 35(Suppl 2):S1–S6. [PubMed: 8275976]
24. Seifert G, Carmignoto G, Steinhauser C. Astrocyte dysfunction in epilepsy. Brain Res Rev. 2010; 63:212–221. [PubMed: 19883685]
25. Aarts M, Iihara K, Wei WL, Xiong ZG, Arundine M, Cerwinski W, MacDonald JF, Tymianski M. A key role for TRPM7 channels in anoxic neuronal death. Cell. 2003; 115:863–877. [PubMed: 14697204]
26. Inoue K, Branigan D, Xiong ZG. Zinc-induced neurotoxicity mediated by transient receptor potential melastatin 7 channels. J Biol Chem. 2010; 285:7430–7439. [PubMed: 20048154]
27. Krapivinsky G, Mochida S, Krapivinsky L, Cibulsky SM, Clapham DE. The TRPM7 ion channel functions in cholinergic synaptic vesicles and affects transmitter release. Neuron. 2006; 52:485–496. [PubMed: 17088214]
28. Morgans CW, Zhang J, Jeffrey BG, Nelson SM, Burke NS, Duvoisin RM, Brown RL. TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. Proc Natl Acad Sci U S A. 2009; 106:19174–19178. [PubMed: 19861548]
29. Bitanihirwe BK, Cunningham MG. Zinc: the brain's dark horse. Synapse. 2009; 63:1029–1049. [PubMed: 19623531]
30. Hsiao B, Dweck D, Luetje CW. Subunit-dependent modulation of neuronal nicotinic receptors by zinc. J Neurosci. 2001; 21:1848–1856. [PubMed: 11245669]
31. Smart TG, Hosie AM, Miller PS. Zn2+ ions: modulators of excitatory and inhibitory synaptic activity. Neuroscientist. 2004; 10:432–442. [PubMed: 15359010]
32. Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. Nat Rev Neurosci. 2004; 5:553–564. [PubMed: 15208697]
33. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000; 342:314–319. [PubMed: 10660394]
34. Bailey EE, Pfeifer HH, Thiele EA. The use of diet in the treatment of epilepsy. Epilepsy Behav. 2005; 6:4–8. [PubMed: 15652725]
35. Valdez G, Tapia JC, Kang H, Clemenson GD Jr, Gage FH, Lichtman JW, Sanes JR. Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. Proc Natl Acad Sci U S A. 2010; 107:14863–14868. [PubMed: 20679195]

36. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974; 77:71–94. [PubMed: 4366476]
37. Finney M, Ruvkun G. The unc-86 gene product couples cell lineage and cell identity in C. elegans. Cell. 1990; 63:895–905. [PubMed: 2257628]
38. Lewis JA, Wu CH, Levine JH, Berg H. Levamisole-resistant mutants of the nematode Caenorhabditis elegans appear to lack pharmacological acetylcholine receptors. Neuroscience. 1980; 5:967–989. [PubMed: 7402460]
39. Richmond JE, Jorgensen EM. One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction. Nat Neurosci. 1999; 2:791–797. [PubMed: 10461217]
40. Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. Genome Biol. 2001; 2 RESEARCH0002.

Highlights

- TRPM channels are modulators of neuronal excitability
- Non-neuronal action of TRPM channel GTL-2 affects excitation-inhibition imbalance
- Epileptic-like activity can be modulated by extracellular divalent ion balance

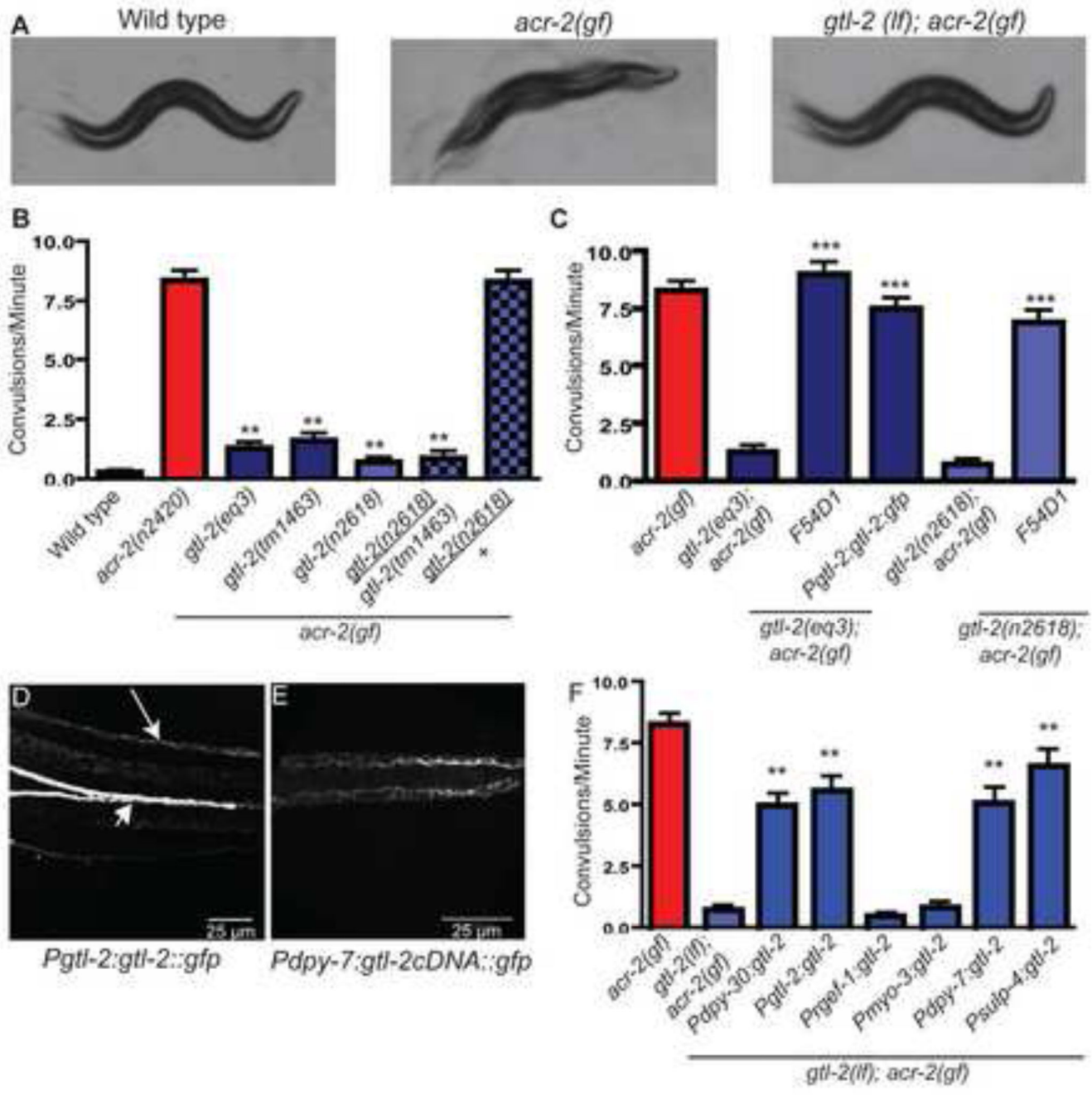

Figure 1. *gtl-2* acts in non-neuronal tissues to modulate *acr-2(gf)* convulsions

(A) Images of wild type, *acr-2(gf)* and *gtl-2(lf);acr-2(gf)* locomotion. (B) Quantification of convulsion frequency in mutants of genotype as indicated. **, p<0.01, compared to *acr-2(gf)*, ANOVA and Dunnett's post hoc test. (C) Transgenic rescue of the suppression activities in *gtl-2(0);acr-2(gf)* and *gtl-2(lf);acr-2(gf)*. ***, p<0.001, ANOVA and Bonferroni post hoc test. (D) Confocal image of anti-GFP immunostaining of GTL-2::GFP in an adult shows expression in the excretory cell (arrowhead) and epidermis (arrow). (E) Confocal image of anti-GFP immunostaining of GTL-2::GFP in a L4 larva expressing

Pdpy-7:gtl-2cDNA::gfp. (F) Cell specific rescue of *gtl-2* shows requirement in epidermis and excretory cell. Cell types are as follows: *Pdpy-30* – all cells, *Prgef-1* – pan-neuronal, *Pmyo-3* – body muscles, *Psulp-4* – excretory cell, and *Pdpy-7* – epidermis. **, $p<0.01$, compared to *gtl-2(lf);acr-2(gf)*, ANOVA and Dunnett's post hoc test.

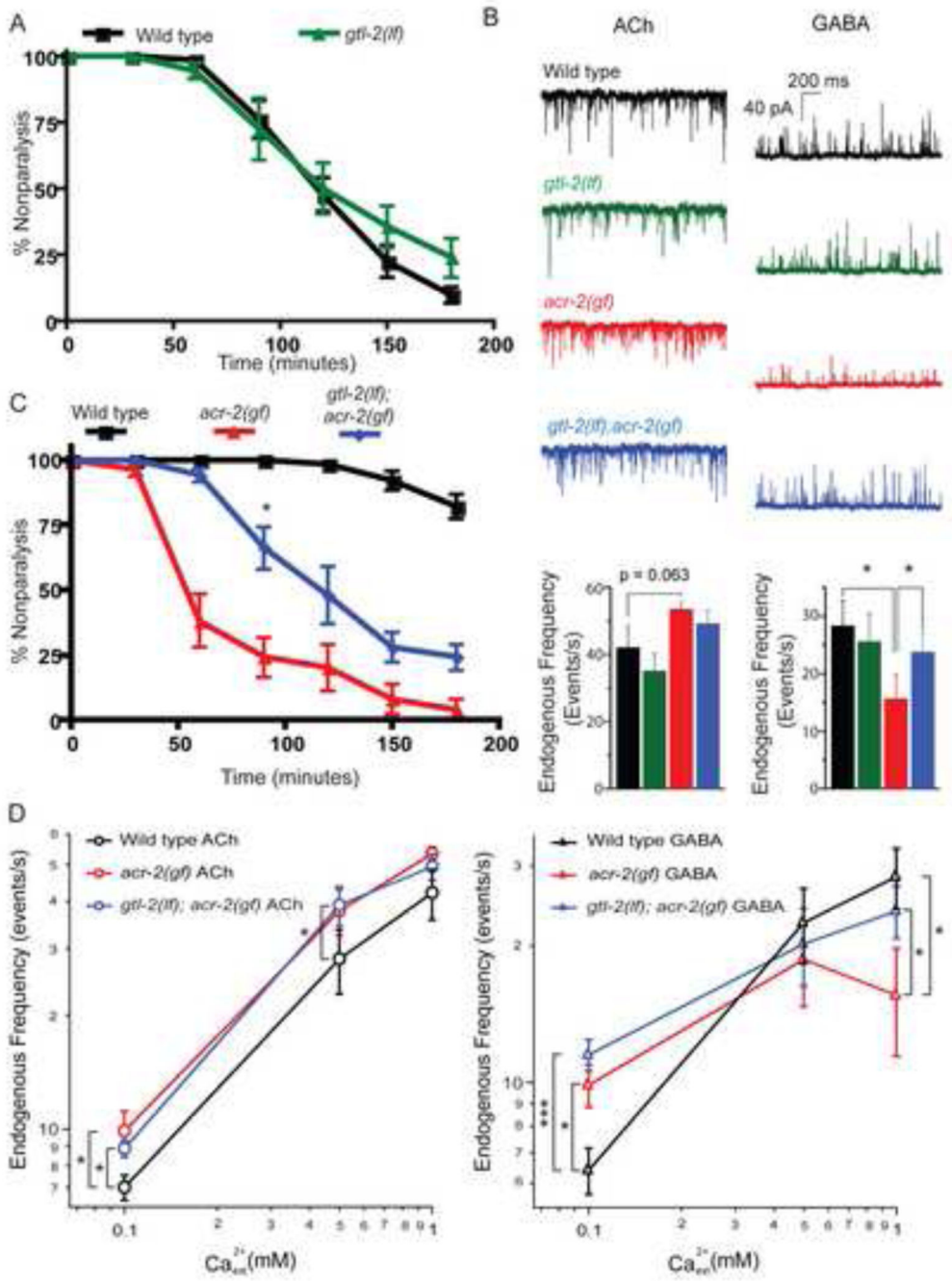

Figure 2. Physiology of *gtl-2(lf)* and its effects on *acr-2(gf)*

(A) Paralysis response to 500 μM aldicarb in *gtl-2(lf)* compared to wild type. (B) Representative traces and frequencies of endogenous acetylcholine and GABA postsynaptic currents from wild type (n=25), *gtl-2(lf)* (n=12), *acr-2(gf)* (n=14), and *gtl-2(lf);acr-2(gf)* (n=12) animals in 1 mM external $CaCl_2$ bath solution. Error bars indicate SEM. (C) Paralysis response to 200 μM aldicarb in wild type, *acr-2(gf)*, and *gtl-2(0);acr-2(gf)* animals. *, p<0.05, compared to *acr-2(gf)*, two-way ANOVA and Bonferroni post hoc tests. (D) Summary of frequencies of endogenous acetylcholine (left) and GABA (right) release in

$CaCl_2$ bath solutions of 0.1 mM (wild type (n=14), *acr-2(gf)* (n=9), and *gtl-2(lf);acr-2(gf)* (n=10)), 0.5 mM (wild type (n=12), *acr-2(gf)* (n=14), and *gtl-2(lf);acr-2(gf)* (n=11)), and 1 mM (wild type (n=25), *acr-2(gf)* (n=14), and *gtl-2(lf);acr-2(gf)* (n=12)). Error bars indicate SEM. Statistics in B, D used SigmaStat 3.5 (Aspire Software International): *, $p<0.05$, ***, $p<0.001$ by Student's t-test, or the Mann-Whitney rank sum test for GABA data in 0.5 mM $CaCl_2$ according to the normality of datum distribution for data.

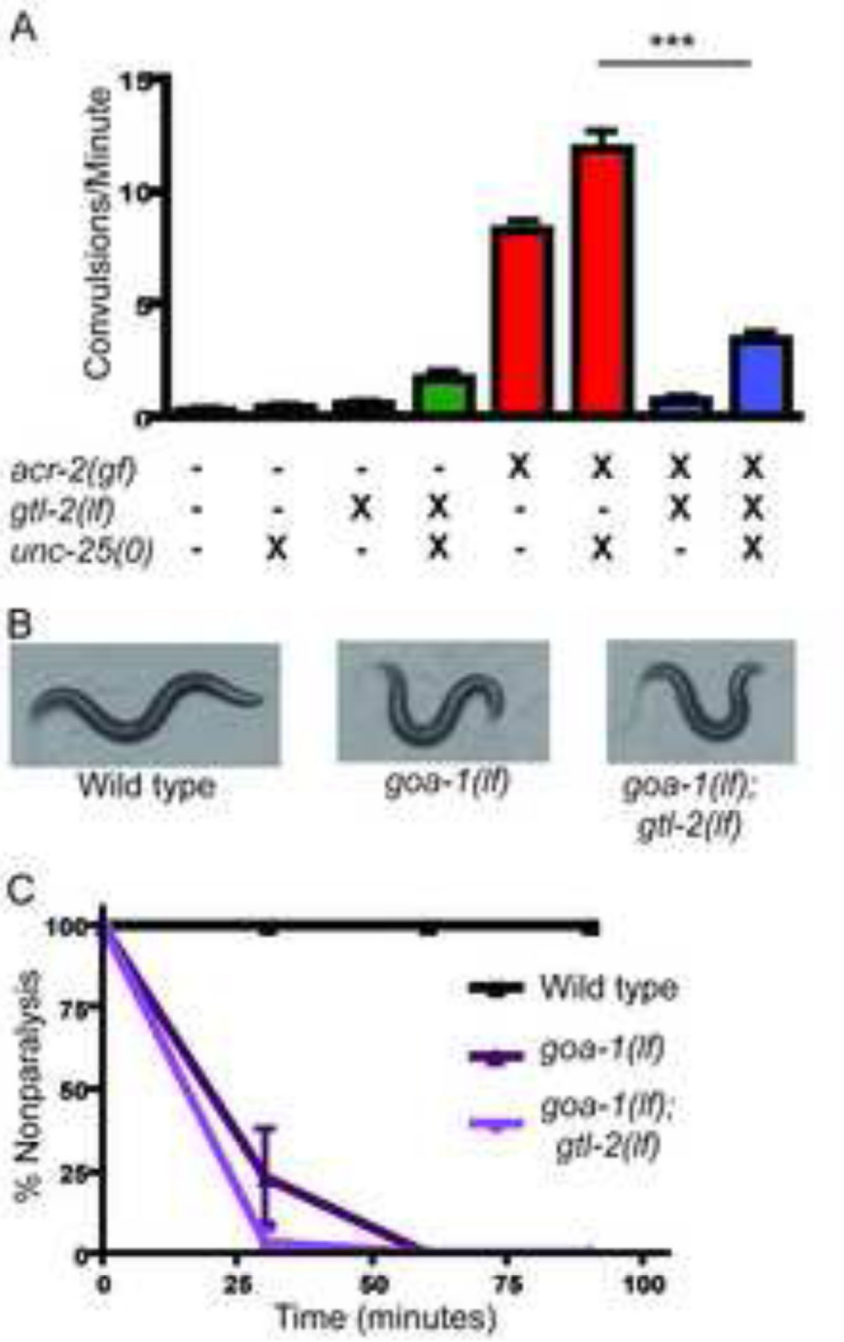

Figure 3. *gtl-2* specifically influences the overexcitation phenotype in *acr-2(gf)*, but not of *goa-1(lf)* mutants with increased cholinergic transmission

(A) Quantification of convulsion frequency in mutants of genotypes indicated. -, no mutation; x, has mutation. ***, $p<0.001$, compared to *gtl-2(lf);acr-2(gf)*, ANOVA and Bonferroni post hoc test. (B) Images of animals of genotype indicated. *gtl-2(lf)* does not alter locomotion behavior of *goa-1(ep275)* animals. (C) Paralysis response to 200 μM aldicarb in animals of genotypes indicated. n=3 trials of 10 animals per genotype.

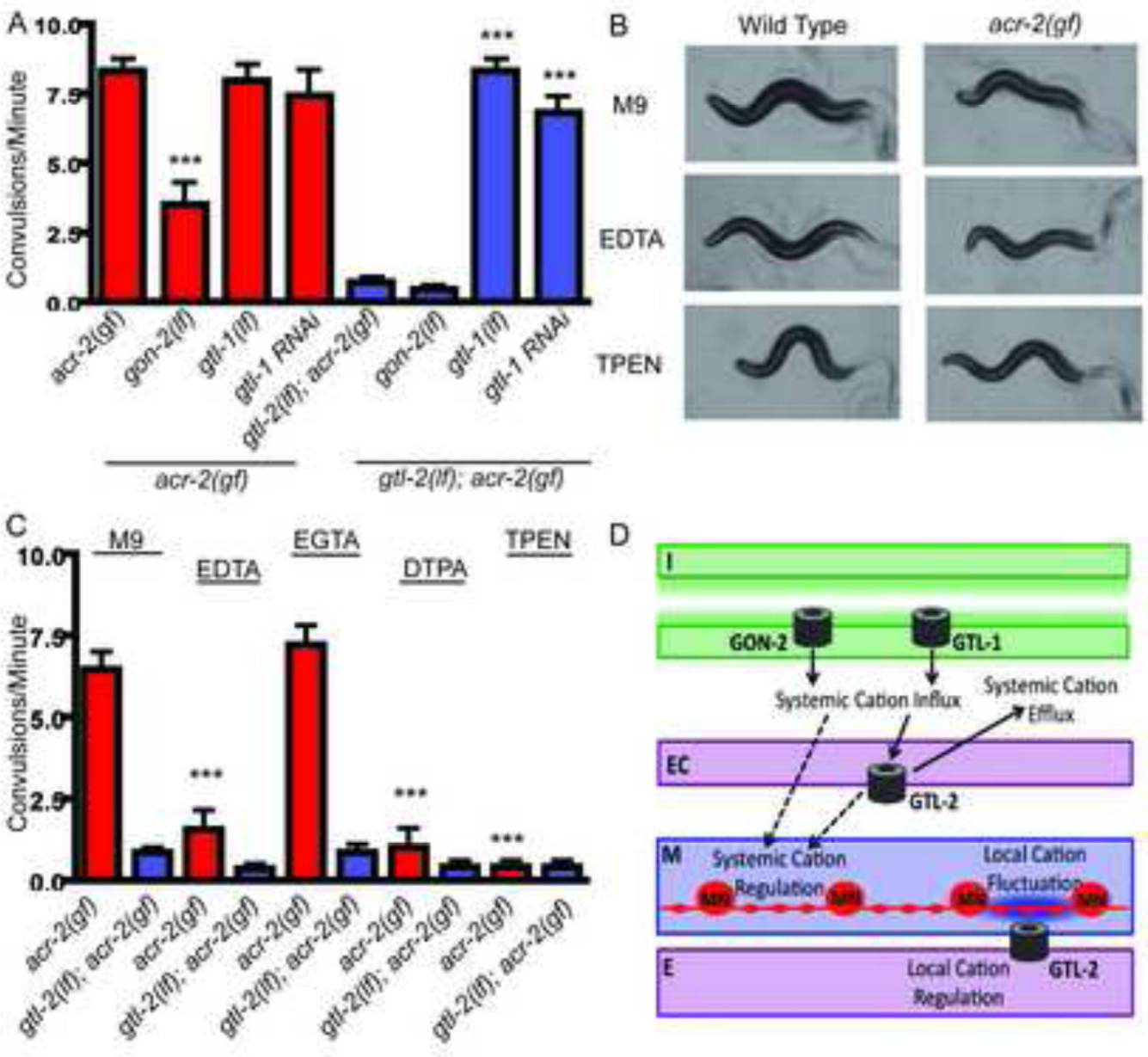

Figure 4. Ion homeostasis plays a key role in the *acr-2(gf)* phenotype

(A) Quantification of convulsion frequency of *acr-2(gf)* and *gtl-2(lf);acr-2(gf)* with genetic mutations in *gon-2* and *gtl-1* or treated with *gtl-1* RNAi to reduce the function of different TRPM channels. *** = p<0.001, ANOVA and Bonferroni post hoc test. (B) Images of *acr-2(gf)* animals soaked in either M9 (control), EDTA or TPEN. (C) Convulsion frequency of *acr-2(gf)* and *gtl-2(lf);acr-2(gf)* soaked in the cation chelators EDTA (75 mM), EGTA (75 mM), DTPA (75mM), and TPEN (100 μM). Statistics in A, C, *** = p<0.001, ANOVA and Bonferroni post hoc test. (D) Model of the regulation of locomotor circuit by ion homeostasis and the three TRPM channels. GON-2 and GTL-1 act in the intestine (I) to allow divalent cation influx, whereas GTL-2 acts in the excretory cell (EC) for cation efflux [9](and this study). Neuronal activity (MN) likely influences local cation levels (dark blue

oval). Our data suggest that both the systemic cation fluctuations due to the function of the three TRPM channels as well as local ion fluctuations involving GTL-2 in the epidermis (E) modulate the excitability of the locomotor circuit, hence contractions of muscles (M).