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The SOL-2/Neto Auxiliary Protein Modulates the Function of AMPA-Subtype Ionotropic Glutamate Receptors

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Summary

The neurotransmitter glutamate mediates excitatory synaptic transmission by gating ionotropic glutamate receptors (iGluRs). AMPA receptors (AMPARs), a subtype of iGluR, are strongly implicated in synaptic plasticity, learning and memory. We previously discovered two classes of AMPAR auxiliary proteins in *C. elegans* that modify receptor kinetics and thus change synaptic transmission. Here, we have identified another auxiliary protein, SOL-2, a CUB-domain protein that associates with both the related auxiliary subunit SOL-1 and with the GLR-1 AMPAR. In *sol-2* mutants, behaviors dependent on glutamatergic transmission are disrupted, GLR-1-mediated currents are diminished, and GLR-1 desensitization and pharmacology are modified. Remarkably, a secreted variant of SOL-1 delivered *in trans* can rescue *sol-1* mutants and this rescue depends on *in cis* expression of SOL-2. Finally, we demonstrate that SOL-1 and SOL-2 have an ongoing role in the adult nervous system to control AMPAR-mediated currents.

Introduction

The AMPA class of iGluRs is intensely studied because of the critical role these receptors play in excitatory neurotransmission and nervous system function. For example, experience dependent changes in AMPAR properties and number are mechanistically linked to learning and memory (Kerchner and Nicoll, 2008; Kessels and Malinow, 2009). Although glutamate-gated currents can be recorded from heterologous cells that express vertebrate AMPAR subunits, recent studies have conclusively demonstrated that these reconstituted currents are significantly different from native neuronal currents (Jackson and Nicoll, 2011). Neuronal AMPARs associate with multiple classes of transmembrane proteins, which serve important auxiliary functions. Some of the auxiliary proteins function as chaperones; but all have some effect on the kinetics and pharmacology of AMPAR gating, thereby providing additional mechanisms for changes in synaptic strength. The first identified auxiliary subunits were the TARPs (Transmembrane AMPAR Regulatory Proteins) (Chen et al., 2000; Milstein and Nicoll, 2008). This was followed by genetic studies in *C. elegans* that identified and characterized SOL-1, a CUB-domain transmembrane protein that defined a second class of AMPAR auxiliary protein (Zheng et al., 2004). *C. elegans* also expresses the TARP proteins STG-1 and STG-2, which have evolutionarily conserved functions (Walker et al., 2006a; Wang et al., 2008). Since then, additional transmembrane proteins have been implicated in AMPAR function (Jackson and Nicoll, 2011; Kalashnikova et al., 2010; Schwenk et al.,

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2012; Schwenk et al., 2009; von Engelhardt et al., 2010). Two outstanding questions are posed by these studies. First, are there additional auxiliary proteins that contribute to receptor function? Second, how do the auxiliary proteins contribute to synaptic transmission and behavior?

In *C. elegans*, the GLR-1 AMPAR mediates glutamate-gated current in a subset of interneurons that control movement and the avoidance of noxious stimuli (Hart et al., 1995; Maricq et al., 1995). Genetic and reconstitution studies have demonstrated that GLR-1 is part of a multi-protein, synaptic complex required for GLR-1-mediated currents and behavior (Wang et al., 2008; Zheng et al., 2004). In addition to GLR-1, this complex contains SOL-1 and at least one of the TARPs, i.e., STG-1 and STG-2. SOL-1 is an evolutionarily conserved type I transmembrane protein that contains protein-protein interaction motifs called CUB-domains (Complement, Urchin EGF, BMP). SOL-1 was shown to regulate the rate of GLR-1 desensitization as well as its rate of recovery from desensitization (Walker et al., 2006a; Walker et al., 2006b; Zheng et al., 2006).

More recently, the CUB-domain-containing transmembrane proteins Neto1 and Neto2 were identified in mice. These proteins contribute to signaling mediated by NMDA (*N*-methyl-D-aspartate) and kainate iGluRs, respectively (Ng et al., 2009; Zhang et al., 2009). However, *C. elegans* SOL-1 and the vertebrate Neto proteins belong to two different classes of CUB domain proteins. Whereas SOL-1 contains four predicted CUB domains, Neto1 and Neto2 contain two CUB domains and a LDLa domain (low-density lipoprotein receptor class A). This raises the question of whether multiple classes of CUB-domain proteins contribute to the function of specific iGluRs.

In an earlier study (Zheng et al., 2006), we found that a secreted form of SOL-1 (s-SOL-1) that lacked the transmembrane domain was sufficient to rescue the behavioral and synaptic signaling defects of *sol-1* mutants. Here, we show that co-expression of s-SOL-1 with GLR-1 and STG-1 in heterologous cells is not sufficient to reconstitute glutamate-gated current. This result led us to the hypothesis that an additional protein, which was missing in heterologous cells, is expressed in neurons and is required for s-SOL-1 function. Presumably, this protein is part of the GLR-1 receptor complex and recruits s-SOL-1 to the complex thus contributing to receptor function. This model also suggests that the protein itself might have a modulatory role in GLR-1 function.

To identify the putative interacting protein, we used an unbiased forward genetic strategy and discovered SOL-2, a CUB-domain transmembrane protein that is the homologue of the vertebrate Neto proteins, with 2 CUB-domains and a LDLa-domain. As predicted, we found that s-SOL-1 function was dependent on SOL-2 and that SOL-2 associates with the GLR-1 signaling complex. We show that surface delivery of GLR-1 and SOL-1 occurs in the absence of SOL-2; however, the stability or function of the complex appears compromised in *sol-2* mutants. In *sol-1* mutants, the remaining components of the GLR-1 complex are also delivered to the postsynaptic membrane, indicating that SOL-1 does not have an essential role in assembly or trafficking of the signaling complex. We demonstrate that GLR-1-mediated currents depend on both SOL-1 and SOL-2, and that currents in *sol-1* and *sol-2* mutants can be rescued in adults, thus demonstrating an ongoing role for these CUB-domain proteins in synaptic transmission.

Remarkably, we found that the extracellular domain of SOL-1 secreted *in trans* is sufficient to rescue glutamate-gated currents in *sol-1* mutants. This rescue depends on *in cis* expression of SOL-2. Finally, we show that glutamate- and kainate-gated currents are differentially disrupted in *sol-1* and *sol-2* mutants, and that SOL-2 contributes to the kinetics of receptor desensitization. In summary, our results demonstrate that SOL-2 is an essential

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component of GLR-1 AMPAR complexes at synapses, and contributes to synaptic transmission and behaviors dependent on glutamatergic signaling.

Results

sol-2 encodes a CUB-domain protein required for AMPAR-mediated signaling

AVA interneurons in *C. elegans* are part of a locomotory control circuit that primarily regulates the direction of a worm's movement. These interneurons receive glutamatergic synaptic inputs and express GLR-1, STG-2 and SOL-1 – essential transmembrane proteins that contribute to a postsynaptic iGluR signaling complex (Brockie et al., 2001a; Maricq et al., 1995; Wang et al., 2008; Zheng et al., 2004). Using *in vivo* patch-clamp electrophysiology, we recorded rapidly activating and desensitizing currents in wild-type worms in response to pressure application of glutamate (Figure 1A). In *sol-1* mutants, glutamate-gated currents rapidly desensitize and consequently we cannot measure the currents using conventional drug application (Figure 1A) (Walker et al., 2006b). A secreted form of SOL-1 that lacks the transmembrane domain (s-SOL-1) can partially rescue the glutamate-gated current when expressed in the AVA neurons of transgenic *sol-1* mutants (Figure 1A) (Zheng et al., 2006). This result suggested that s-SOL-1 formed a functional complex with GLR-1 and STG-2.

To test sufficiency of s-SOL-1, we asked whether we could record glutamate-gated currents from muscle cells that co-expressed GLR-1, STG-1 and s-SOL-1. Muscle cells in *C. elegans* do not express any known iGluRs, STGs or SOL-1 proteins, and thus are ideal for reconstitution studies. We reliably recorded large, rapidly activating inward currents in response to pressure application of glutamate when full-length SOL-1, STG-1 and GLR-1 were co-expressed in muscle cells (Figure 1B). In contrast, we were unable to record appreciable currents in cells that expressed s-SOL-1 instead of full-length SOL-1 (Figure 1B). We found similar results in co-expression studies with STG-2 (data not shown). We next addressed whether the failure to reconstitute function with s-SOL-1 was specific to muscle cells by reconstitution experiments in *Xenopus* oocytes. Again, we were able to measure large glutamate-gated currents when full-length SOL-1 was co-expressed with GLR-1 and STG-1 (Figure 1B), but not when s-SOL-1 replaced SOL-1. These results led us to hypothesize that neurons, but not muscle cells or *Xenopus* oocytes, express a protein that binds to s-SOL-1 and contributes to the function of the GLR-1 complex.

To identify this interacting protein, we turned to a genetic approach that took advantage of the hyper-reversal behavior of transgenic worms that express a gain-of-function variant of GLR-1 (GLR-1(A687T)) (Zheng et al., 1999). The hyper-reversal behavior of these "lurcher" worms is suppressed by mutations in *sol-1* and rescued in transgenic *sol-1; lurcher* mutants that express either full-length SOL-1 or s-SOL-1 (Figure 1C) (Zheng et al., 2006). We hypothesized that mutating the protein predicted to interact with SOL-1 (and s-SOL-1) would also suppress the hyper-reversal phenotype. We therefore mutated lurcher worms, screened approximately 2000 haploid genomes and identified a single mutant, *sol-2*(*ak205*), which partially suppressed the hyper-reversal phenotype (Figure 1C). The *ak205* mutation complemented mutations in *sol-1, stg-1* and *stg-2* (data not shown) indicating that we had mutated a new gene required for signaling mediated by the GLR-1 complex.

Using conventional strategies, we mapped the mutation to a small interval on LG I (Figure S1A). We identified an open reading frame (K05C4.11) that rescued the movement of transgenic *sol-2; lurcher* mutants (Figure 1C). Unlike the case for *sol-1; lurcher* mutants, s-SOL-1 did not restore hyper-reversal behavior in *sol-1; sol-2* double mutants that expressed *lurcher*. However, s-SOL-1 did rescue hyper-reversal behavior when co-expressed with

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SOL-2 in transgenic *sol-1; sol-2* double mutants suggesting that the function of s-SOL-1 is dependent on SOL-2 (Figure 1C).

By sequencing the genome of the *sol-2* mutant, we identified a mutation in the K05C4.11 (*sol-2*) gene that causes a frame shift and an early stop, suggesting that the mutation is a null (Figure S1B). The *sol-2* gene is predicted to encode a 436 amino acid, type I transmembrane protein containing two putative CUB domains and a LDLa domain. The SOL-2 protein has closest sequence identity (approximately 20-21%) to the vertebrate Neto proteins, and significant identity to the *C. elegans* CUB domain proteins SOL-1 and LEV-10 (Gally et al., 2004; Zheng et al., 2004) (Figure 1D; Figure S1B). Following our mapping experiments, we discovered an existing mutation in *sol-2* produced by a deletion (*ok1713*) (Wormbase.org). *sol-2(ok1713)* also suppressed the hyper-reversal behavior of lurcher worms, and did not complement the *sol-2(ak205)* mutation (data not shown).

We next addressed whether SOL-2 was the missing protein required for reconstitution of glutamate-gated current using s-SOL-1. We found that we could reconstitute glutamate-gated currents in *Xenopus* oocytes or *C. elegans* muscle cells when s-SOL-1 was co-expressed with SOL-2, STG-1 and GLR-1 (Figure 1E and F), but not in the absence of SOL-2 (Figure 1B). Thus, s-SOL-1 function was dependent on SOL-2. Furthermore, SOL-2 cannot simply replace SOL-1 given that we were unable to reconstitute glutamate-gated current in either oocytes or muscle cells by co-expressing GLR-1, STG-1 and SOL-2 (Figure 1E and F).

GLR-1-mediated behaviors and current are disrupted in *sol-2* mutants

Our reconstitution studies demonstrated that SOL-2 and SOL-1 contribute to the function of the GLR-1 signaling complex. In addition, our finding that mutations in *sol-2* disrupt the behavior of transgenic lurcher mutants (Figure 1C) predicts that glutamatergic neurotransmission is disrupted in *sol-2* mutants. Thus, we evaluated the behavior of *sol-2* mutants using two standard assays that depend on GLR-1 function (Hart et al., 1995; Maricq et al., 1995; Mellem et al., 2002). When tested in an osmotic avoidance assay the *sol-2* mutants were as slow to recoil from the hyper-osmotic stimuli as *glr-1* or *sol-1* mutants (Figure 2A). When tested in a touch-avoidance assay (nose touch response) *sol-2* mutants were significantly impaired, but not to the extent of *glr-1* or *sol-1* mutants (Figure 2B). In both assays, *sol-1; sol-2* double mutants were no more impaired than *sol-1* mutants alone, suggesting that the two gene products act in the same pathway.

The peak amplitude of the glutamate-gated current in AVA was considerably diminished in *sol-2* mutants, and we could only measure a small, rapidly activating and desensitizing current (Figure 2C and D). These currents are distinct from those recorded in *sol-1* mutants where we could not detect a rapidly activating inward current under the same recording conditions (Figure 1A; Figure 2D). Only the GLR-1-mediated current was decreased in *sol-2* mutants; the slower, outwardly rectifying current is mediated by NMDA receptors (Brockie et al., 2001b) and did not appear appreciably different than wild-type current (Figure 2C). Glutamate-gated currents in the AVA neurons of transgenic *sol-2* mutants were rescued by a functional SOL-2::GFP fusion protein that was specifically expressed in AVA using the *rig-3* promoter (Feinberg et al., 2008) (Figure 2C and D). We were also able to rescue current in transgenic *sol-2* mutants that expressed GFP fused to the extracellular N-terminus of full-length SOL-2 (GFP::SOL-2; Figure S2). These results demonstrate that GFP-tagged SOL-2 is functional and acts cell autonomously. However, unlike the case for SOL-1, we did not observe rescue of transgenic *sol-2* mutants that expressed a secreted variant of the fusion protein that lacked the transmembrane domain (GFP::s-SOL-2) (Figure S2).

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SOL-2 associates with the GLR-1 signaling complex

Based on our electrophysiological studies, as well as the known expression pattern of GLR-1 and SOL-1 (Brockie et al., 2001a; Zheng et al., 2004), we predicted that SOL-2 would be expressed in the command interneurons. We therefore used confocal microscopy to determine the cellular and subcellular distribution of SOL-2. The *sol-2* promoter drives expression of GFP in many head and tail neurons, including neurons that express the GLR-1 subunit, as well as the SOL-1 auxiliary subunit (Figure S3A) (Brockie et al., 2001a; Zheng et al., 2004). SOL-2 is also expressed in neurons that do not express either GLR-1 or SOL-1. With respect to avoidance behavior and locomotion, *sol-1; sol-2* double mutants are no more severe than the *sol-1* single mutant (Figure 2A and B), indicating that the role SOL-2 plays in these neurons is not directly relevant to these behaviors. We have not investigated whether SOL-2 contributes to the function of additional GLR receptors (Brockie et al., 2001a) or other behaviors. Importantly, SOL-2 is expressed in the command interneurons, as shown by co-expression of mCherry driven by the *nmr-1* promoter (Figure S3A).

To determine the subcellular localization of SOL-2, we imaged transgenic worms that co-expressed SOL-2::GFP with GLR-1::mCherry in AVA and found that SOL-2 co-localizes with GLR-1 (Figure 3A). To test whether SOL-2 also co-localizes with SOL-1, we co-expressed GFP::SOL-1 and SOL-2::mCherry in AVA and observed GFP and mCherry puncta that co-localized along the length of the AVA processes (Figure 3B).

The co-localization of SOL-2 with both SOL-1 and GLR-1 suggested that SOL-2 was part of the GLR-1/SOL-1 complex (Walker et al., 2006a). To address this possibility, we used BiFC (Bimolecular Fluorescence Complemenation) to probe possible protein interactions. We tagged SOL-1 with the N-terminal half of the fluorescent protein Venus (a YFP variant) (N-YFP::SOL-1) (Chen et al., 2007; Shyu et al., 2008), and SOL-2 with the C-terminal half (C-YFP::SOL-2) and used the *rig-3* promoter to express these constructs along with GLR-1::mCherry in the AVA neurons (Kano et al., 2008). We observed punctate SOL-1/SOL-2 BiFC fluorescence that co-localized with GLR-1::mCherry puncta along the length of the AVA processes in transgenic worms (Figure 3C). We found only minor effects of the BiFC constructs on glutamate-gated current (Figure S3B) and GLR-1::mCherry puncta (Figure S3C), and the intensity of the BiFC signal was somewhat decreased in *glr-1* mutants (Figure S3D). We also observed BiFC fluorescence when C-YFP::SOL-2 was co-expressed in AVA with N-YFP::GLR-1 (Figure 3D). No fluorescence signal was detected when N-YFP::SOL-1, C-YFP::SOL-2, or N-YFP::GLR-1 was expressed alone (data not shown). These results indicate that SOL-2 is in close proximity to SOL-1 and GLR-1 given that BiFC interactions are limited primarily by the length and flexibility of the proteins and linkers (Kerppola, 2006).

To address whether SOL-1 and SOL-2 specifically associate with GLR-1 receptors, we co-expressed C-YFP::SOL-2 with both N-YFP::NMR-2 (NMDA-type iGluR subunit) (Kano et al., 2008) and GLR-1::mCherry in AVA of transgenic worms. We did not observe any BiFC fluorescence in these worms indicating that NMR-2 and SOL-2 do not associate (Figure 3E). This result is consistent with earlier studies showing that neither GLR-1 nor SOL-1 co-localize with NMDARs (Brockie et al., 2001b; Zheng et al., 2004).

The BiFC data suggest a model in which SOL-1 and SOL-2 directly interact. To more rigorously test this hypothesis, we asked whether we could detect SOL-1 and GLR-1 after immunoprecipitation of SOL-2. Because of technical limitations (low abundance of auxiliary proteins and receptors in whole worms), we could not reliably detect these proteins in whole worm lysates. Therefore, we co-expressed these proteins in HEK 293 cells and used these transfected cells for biochemical studies. We found that SOL-2 was associated with GLR-1 and SOL-1 (Figure S4A), but did not associate with the unrelated

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transmembrane protein DCC (Keino-Masu et al., 1996). Together, the biochemical and BiFC data indicate that SOL-1, SOL-2 and GLR-1 physically interact and form a receptor complex.

GLR-1 is expressed at synapses in *sol-2* mutants

We had previously demonstrated that GLR-1 surface expression was not appreciably altered in *sol-1* mutants (Zheng et al., 2004). Is surface expression of GLR-1 also independent of *sol-2*? To test this possibility, we double-labeled GLR-1 with a HA-epitope (extracellular) and GFP (intracellular) and expressed this functional construct (HA::GLR-1::GFP) in transgenic worms (Zheng et al., 2004). We assessed the surface expression of GLR-1 by injecting fluorescently labeled anti-HA antibodies into the pseudocoelomic space of transgenic wild-type worms and *sol-2* mutants (Gottschalk et al., 2005; Zheng et al., 2004). We observed punctate anti-HA antibody fluorescence along the ventral cord in both wild-type worms (Figure 3F) and *sol-2* mutants (Figure 3G) suggesting that GLR-1 is expressed on the cell surface in *sol-2* mutants.

We obtained additional evidence to support the claim that GLR-1 surface expression was not appreciably altered in *sol-2* mutants by generating a transgenic strain that expressed GLR-1 fused to superecliptic phluorin (SEP, a pH-sensitive variant of GFP) (Miesenbock et al., 1998). SEP was inserted in the extracellular N-terminal domain four amino acids from the mature N-terminus (SEP::GLR-1). In agreement with our antibody studies, we found that the fluorescence intensity of surface expressed SEP::GLR-1 appeared similar in transgenic *sol-2* mutants and wild-type worms (Figure 3H). Control experiments (acid wash) indicated that the SEP fluorescence signal represented surface receptors (Figure S4B).

Another possible explanation for the reduced glutamate-gated current in *sol-2* mutants is reduced surface delivery of SOL-1. However, we also found no appreciable difference in fluorescence intensity when we compared SEP::SOL-1 surface delivery in transgenic wild-type worms and *sol-2* mutants (Figure 3I). Thus, SOL-2 does not appear to have a significant role in the surface delivery of these proteins.

In trans delivery of s-SOL-1 rescues *sol-1* mutants

Our reconstitution experiments also allowed us to begin to address the requirements for the delivery and maintenance of the GLR-1 signaling complex. One possibility is that SOL-1 might have an obligate chaperone role for some critical component of the complex, much like that suggested for a subset of the vertebrate TARPs (Milstein and Nicoll, 2008). In this scenario, the delivery or stability of components of the complex would be compromised in the absence of SOL-1. Alternatively, the components might reside stably at the membrane. To help distinguish between these possibilities, we expressed GFP-tagged secreted s-SOL-1 (GFP::s-SOL-1) in muscle cells of transgenic *sol-1* mutants using the *myo-3* muscle specific promoter. If GLR-1, STGs, SOL-2 and any other necessary components of the complex are stably delivered to the postsynaptic membrane in the absence of SOL-1, then one might predict that s-SOL-1 delivered *in trans* from muscle cells in transgenic *sol-1* mutants would be sufficient to restore a functional signaling complex in AVA.

We first examined whether GFP::s-SOL-1 delivered *in trans* from muscle cells was co-localized with GLR-1 in the processes of the AVA interneurons. We co-expressed muscle-secreted GFP::s-SOL-1 and AVA-specific GLR-1::mCherry in transgenic mutants. We found that GFP::s-SOL-1 and GLR-1::mCherry co-localized at puncta along the length of the AVA processes in *sol-1* mutants (Figure 4A), but not in *sol-1; sol-2* double mutants (Figure 4B). We also observed GFP puncta along the AVA processes when muscle secreted GFP::s-SOL-1 was expressed in the absence of the GLR-1::mCherry transgene (Figure

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S5A). This result indicates that localization of s-SOL-1 to the ventral cord does not require overexpression of GLR-1 or other components of the signaling complex. This localization was also dependent on SOL-2 and thus GFP::s-SOL-1 was not observed along the ventral cord in *sol-1; sol-2* double mutants (Figure S5B). We also found that the hyper-reversal movement of *sol-1; lurcher* mutants was rescued by muscle secreted GFP::s-SOL-1 and that the rescue was dependent on SOL-2 (Figure 4C).

Our behavioral analysis suggested that s-SOL-1 provided *in trans* restored GLR-1-mediated signaling in the command interneurons. To more directly examine signaling, we measured glutamate-gated currents in AVA interneurons of *sol-1* mutants, *sol-1; sol-2* double mutants and transgenic mutants that expressed s-SOL-1 in muscle cells. In either *sol-1* or *sol-1; sol-2* double mutants we could not detect rapidly activating glutamate-gated currents. However, we found partial recovery of the current in transgenic *sol-1* mutants that expressed s-SOL-1 (77.25 ± 28.31 pA, n=4), but not in transgenic *sol-1; sol-2* mutants (n=3), indicating that the function of s-SOL-1 was dependent on SOL-2 in AVA interneurons (Figure 4D). We also found partial recovery of the current in transgenic *sol-1; sol-2* double mutants that expressed s-SOL-1 in muscle cells and SOL-2 only in the AVA interneurons (96.33 ± 23.07 pA, n=6) (Figure 4D). These results indicate that the binding of exogenously delivered s-SOL-1 is sufficient to reconstitute the function of the receptor complex and that SOL-2 is required cell autonomously to recruit s-SOL-1 to the complex. Our results also suggest that in *sol-1* mutants the remaining components of the receptor complex are stably located in the plasma membrane. Presumably, muscle-secreted s-SOL-1 diffuses in the extracellular space and binds to neuronal SOL-2 to reconstitute the GLR-1 receptor complex.

One might imagine that postsynaptic signaling molecules, such as SOL-1, have critical developmental roles in addition to their known signaling functions. Thus, the behavioral defects in *sol-1* mutants might also be a consequence of developmental defects in synaptic morphology or function. To test whether SOL-1 has an obligate developmental role, we generated transgenic *sol-1; lurcher* and *sol-1; sol-2; lurcher* mutants that expressed GFP::s-SOL-1 under the control of a heat-shock inducible promoter (*Phsp::gfp::s-sol-1*). Four hours following heat shock of adult worms, we assessed their behavior. In the absence of the *Phsp::gfp::s-sol-1* transgene, or in the absence of heat shock, *sol-1; lurcher* mutants did not reverse nearly as often as wild-type *lurcher* worms (Figure 4E). In contrast, heat shock induction of GFP::s-SOL-1 rescued reversal behavior in adult *sol-1; lurcher* mutants, and the rescue was dependent on *sol-2* (Figure 4E).

In a complementary set of experiments, we examined whether heat shock driven expression of SOL-2::GFP in adult *sol-2* mutants could similarly rescue the behavioral phenotype. Following heat shock induction, reversal behavior in adult *sol-2; lurcher* mutants was restored to wild-type values (Figure 4F). We extended these studies to examine glutamate-gated currents in heat-shocked worms and found that within four hours of heat shock we could record near wild-type glutamate-gated currents from the transgenic *sol-2* mutants (Figure 4G). These experiments demonstrate that the function of s-SOL-1 is dependent on SOL-2, that the remaining components of the receptor signaling complex are stable in the absence of SOL-1, and that SOL-1 and SOL-2 have ongoing roles in synaptic transmission in the adult nervous system.

Glutamate-gated currents are significantly reduced in *sol-2* mutants, yet paradoxically, SOL-2 is not essential for reconstitution studies in muscle cells or *Xenopus* oocytes (Figure 1) (Walker et al., 2006a; Walker et al., 2006b). One possibility is that the overexpression of SOL-1 in reconstitution studies partially compensates for the absence of SOL-2. This hypothesis predicts that overexpressing SOL-1 in *sol-2* mutants should rescue glutamate-gated currents. Conversely, overexpressing SOL-2 should not rescue *sol-1* mutants. As

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predicted, we found that current was restored in *sol-1; sol-2* double mutants that overexpressed SOL-1, but not in double mutants that overexpressed SOL-2 (Figure 5A and B). The rescue was similar to that observed when SOL-1 was overexpressed in *sol-1* single mutants (Figure 5A and B). Furthermore, overexpressing SOL-1 restored the hyper-reversal locomotion of transgenic *sol-1; sol-2; lurcher* worms, whereas overexpressing SOL-2 did not (Figure 5C). This was in contrast to overexpression of s-SOL-1, which was not sufficient to rescue *sol-1; sol-2* mutants (Figure 1C). These data are in agreement with our studies in heterologous cells demonstrating that SOL-1, GLR-1 and STG-1 (or STG-2) constitute the minimal set of proteins required for reconstitution of glutamate-evoked currents (Figure 1) (Walker et al., 2006a; Walker et al., 2006b; Wang et al., 2008).

SOL-2 modifies the pharmacology and kinetics of GLR-1 receptors

Does SOL-2 directly modify the properties of the GLR-1 signaling complex in addition to its role in recruiting and stabilizing SOL-1 to the complex? To begin to address this question, we examined whether agonist gating of GLR-1 was altered in *sol-2* mutants. AMPARs are gated open by the partial agonist kainate (Mayer and Armstrong, 2004), but the efficacy of this partial agonist depends on a number of factors, including the association of AMPARs with auxiliary subunits (Kato et al., 2010; Shi et al., 2010). Peak currents evoked by kainate were much smaller in *sol-2* mutants than in wild type, indicating that kainate-gating of GLR-1 was also dependent on SOL-2 (Figure 6A and B). We also addressed the question of SOL-1 sufficiency and found that kainate-gated currents were rescued in *sol-1* mutants that overexpressed SOL-1, but only marginally rescued in *sol-1; sol-2* mutants (Figure 6C and D). This was in stark contrast to glutamate-gated currents, which were rescued in *sol-1; sol-2* mutants that overexpressed SOL-1 (Figure 5A and B). These data indicate that not all of SOL-2's effects can be simply explained by recruitment or stabilization of SOL-1. Thus, SOL-2 also appears to directly contribute to the function of the GLR-1 complex.

We next asked whether we could reconstitute the GLR-1 complex by expression in *C. elegans* muscle cells. We found glutamate-gated currents of approximately the same magnitude and kinetics in transgenic muscles that expressed GLR-1, STG-1 and SOL-1, or these three proteins along with SOL-2 (Figure 6E). In contrast, we found that the presence of SOL-2 markedly reduced the magnitude of the kainate-gated current (three independent experiments). These experiments indicate that the receptor complex functions differently in muscles compared to neurons, perhaps because additional factors interact with the complex in neurons.

What explains the reduced glutamate-gated currents in *sol-2* mutants? To better address the mechanism of SOL-2 function, we used rapid perfusion techniques to study glutamate-gated currents from outside-out membrane preparations. Because the receptor complex behaved differently in muscle, we chose to study patches from AVA. When voltage-clamped at –60 mV, the rapid application of glutamate evoked inward currents that desensitized in the continued presence of glutamate (Figure 6F). In wild type, the average tau of desensitization was approximately 4 ms (n=11). In contrast, we found that the current desensitized in less than 0.4 ms in *sol-2* mutants (n=6), which is similar to what we observed in *sol-1* mutants (Figure 6F) (Walker et al., 2006b). These rapid rates of desensitization distort the time-course of glutamate-gated currents, leading to a significant decrease in the peak current elicited by pressure application of agonist (Figure 2). Because the rate of desensitization in these mutants was faster than the rate of piezo-driven solution change, we could not determine whether *sol-1* and *sol-2* mutants exhibit different rates of desensitization.

To better address the functional effects of SOL-2, we turned to reconstitution of GLR-1 function in *Xenopus* oocytes. We recorded both glutamate- and kainate-gated currents from

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oocytes in which GLR-1 and STG-2 were co-expressed with either SOL-1, or both SOL-1 and SOL-2 (Figure 7A). The kainate-gated current appeared faster and smaller with co-expression of SOL-2. This can be appreciated by examining the ratio of peak kainate- to glutamate-gated current. SOL-2 decreased this ratio by approximately 50% (Figure 7B). These results suggest that in our reconstitution studies, SOL-2 acts to increase the rate of desensitization. One way to examine this possibility is by studying a GLR-1 variant in which the rate of desensitization is greatly slowed by the introduction of a single amino acid change (Q552Y) in the GLR-1 ligand-binding domain (Brockie et al., 2001b; Stern-Bach et al., 1998; Walker et al., 2006b). The glutamate-gated current recorded from *Xenopus* oocytes that expressed GLR-1(Q552Y), STG-2, and SOL-1 did not desensitize (Figure 7C). In contrast, there was considerable desensitization when SOL-2 was co-expressed (Figure 7C), indicating that the function of the receptor was modified by SOL-2.

Additional evidence for modification of receptor function by SOL-2 could be observed following treatment by Concanavalin-A, a lectin that strongly blocks the desensitization of kainate receptors, but only weakly blocks desensitization of AMPARs (Partin et al., 1993). In the neuron AVA, Concanavalin-A only weakly modifies glutamate-gated currents (data not shown). However, in reconstitution studies in oocytes, we previously found that receptor desensitization was dramatically slowed by Concanavalin-A (Walker et al., 2006b). We now find that the efficacy of Concanavalin-A depends on the composition of the receptor complex. Thus, the block of desensitization of either glutamate- or kainate-gated currents was greatly diminished if SOL-2 was part of the receptor complex (Figure 7D).

SOL-2 is most closely related to the vertebrate Neto2 protein, which modifies the function of kainate receptors (Zhang et al., 2009), thus raising the question of whether GLR-1 is more closely related to kainate receptors or AMPARs. To address this question we first expressed Neto2 under control of the *sol-2* promoter in transgenic *sol-2; lurcher* mutants. We did not find rescue of the *lurcher* phenotype nor did we find rescue of glutamate-gated currents in AVA (data not shown). These negative results are not interpretable because it is difficult to evaluate protein expression in transgenic worms. Therefore, we turned to reconstitution experiments in *Xenopus* oocytes. First, we compared the effects of SOL-2 and Neto2 on GLR-1 mediated currents. Consistent with our transgenic experiments, we did not find an obvious effect of Neto2 on glutamate-gated currents (Figure S6). Although SOL-2 dramatically changed the sensitivity to Concanavalin-A, we observed no such effects with Neto2 (Figure S6). Second, we examined the effects of SOL-2 and Neto2 on vertebrate GluA1(flip). As found previously (Zhang et al., 2009), we did not observe an obvious effect of Neto2 on GluA1-mediated current, and there was no obvious effect of SOL-2 on these currents (Figure S7A). Finally, we examined the effects of SOL-2 and Neto2 on vertebrate GluK2. Again, as previously observed (Zhang et al., 2009), we found that Neto2 dramatically increased GluK2-mediated current. However, we observed no such effect with SOL-2 (Figure S7B). Thus, in contrast to the evolutionarily conserved function of TARP proteins, i.e., vertebrate TARPs can contribute to GLR-1 function and *C. elegans* TARPs (STG-1, STG-2) can contribute to vertebrate AMPAR function (Walker et al., 2006a; Wang et al., 2008), we do not observe conservation of function with the SOL-2 and Neto2 CUB-domain proteins. Our data suggest that additional interacting proteins might contribute to Neto2, SOL-1 and SOL-2 function.

Discussion

Our study has identified the SOL-2/Neto CUB-domain protein, which is part of the GLR-1 signaling complex, thus defining a third class of AMPAR auxiliary proteins. SOL-2/Neto contributes to the GLR-1 complex by its interactions with SOL-1, and by modifying GLR-1 kinetics and pharmacology. Consequently, in *sol-2* mutants GLR-1-mediated current and

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behaviors are disrupted. Our search for SOL-2 was motivated by our observation that the secreted extracellular domain of SOL-1 (s-SOL-1) was functional when expressed in neurons *in vivo*, but not in reconstitution studies (Figure 1). These conflicting results suggested that neurons express a specific protein required for s-SOL-1 function that is not expressed in *C. elegans* muscle cells or *Xenopus* oocytes. Because of our past success with a genetic strategy to identify components of the GLR-1 complex (SOL-1 and STG-2) (Wang et al., 2008; Zheng et al., 2004), we predicted that this protein could be identified using the same strategy, i.e., screening for mutations that suppress the hyper-reversal phenotype of transgenic lurcher worms. As predicted, we identified a new gene product, the SOL-2 CUB-domain transmembrane protein, that is homologous to vertebrate Neto proteins. This discovery further increases the complexity of the GLR-1 AMPAR postsynaptic signaling complex, which now contains members of at least four classes of proteins: AMPAR subunits, TARPs, SOL-1, and SOL-2/Neto, all of which have been validated by genetic perturbation, electrophysiology, cell biology and behavioral studies.

In support of our model that SOL-2 is part of the GLR-1 receptor complex, we found that SOL-2 co-localized and associated with SOL-1 and GLR-1 at synaptic sites. We also found that overexpressing SOL-1, but not SOL-2, in *sol-1; sol-2* double mutants was sufficient to rescue both behavior and glutamate-gated current. These results indicate that SOL-2 likely functions as an adaptor protein that contributes to the interaction between SOL-1 and the receptor complex. However, in reconstitution studies, we also found that SOL-2 modifies relative agonist efficacy and the rate of receptor desensitization. Thus, SOL-2 has at least two roles: interacting with SOL-1 and modifying receptor function.

We were able to exclude an obligate role for SOL-1 in the biosynthesis, trafficking or stability of the GLR-1 signaling complex by demonstrating that s-SOL-1 provided *in trans* rescues glutamate-gated currents in *sol-1* mutants. We also excluded an obligate developmental role for SOL-1 by showing that glutamate-gated current and GLR-1-dependent behavior was rescued in adult *sol-1* mutants following heat shock induction of s-SOL-1 in adult worms. This result provides additional evidence that the receptor complex is stable in the absence of SOL-1. We were also able to rescue the behavioral and electrophysiological defects of *sol-2* mutants by heat shock induction of SOL-2 in adult worms, indicating that SOL-2 has an ongoing function in adult animals and does not play an essential developmental role.

Components of the complex are also present in the absence of SOL-2 because GLR-1-mediated currents, although diminished, were observed in *sol-2* mutants. However, the function of the complex is altered as shown by the differential rescue of glutamate- and kainate-gated currents in *sol-2* mutants by overexpression of SOL-1. These data, together with the rapid perfusion experiments, where we could record rapidly desensitizing glutamate-gated currents in the absence of either SOL-1 or SOL-2, indicate that the components of the GLR-1 receptor complex are not degraded in *sol-1* or *sol-2* mutants. Thus, these proteins do not serve essential chaperone functions, suggesting that the identified components of the signaling complex might be independently regulated. Our results also suggest that dynamic changes in the composition of the complex could modulate the glutamate-gated postsynaptic current.

SOL-2 shares significant domain homology with the CUB-domain protein LEV-10, which is required for clustering of a subset of acetylcholine receptors at the neuromuscular junction in *C. elegans* (Gally et al., 2004). However, LEV-10 binds the CCP (sushi)-domain protein LEV-9 and does not appear to regulate receptor function (Gally et al., 2004; Gendrel et al., 2009). By sequence identity and domain structure, SOL-2 is homologous to the mammalian CUB-domain-containing transmembrane proteins Neto1 and Neto2. Both Neto proteins

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serve as auxiliary proteins for kainate receptors (Straub et al., 2011; Tang et al., 2011; Zhang et al., 2009) and modify receptor kinetics and kainate binding. Neto1 also appears to interact with NMDARs (Ng et al., 2009).

C. elegans GLR-1 was first defined as an AMPAR based on sequence identity (Brockie et al., 2001a); however, our demonstration that a Neto protein contributes to its function might suggest that GLR-1 is functionally more similar to kainate receptors. Although GLR-1 appears to share some characteristics with both AMPARs and kainate receptors, the bulk of the evidence indicates that GLR-1 is more like an AMPAR subunit: GLR-1 interacts with TARPs (which are AMPAR-specific auxiliary proteins) (Jackson and Nicoll, 2011; Milstein and Nicoll, 2008); the vertebrate TARP, stargazin, modulates GLR-1 function and *C. elegans* TARPs modulate vertebrate AMPARs (Walker et al., 2006a; Wang et al., 2008); and a conserved amino acid that dramatically influences AMPAR gating is found in GLR-1 (Brockie et al., 2001b; Stern-Bach et al., 1998; Walker et al., 2006b). Because we have found a homolog of vertebrate Neto proteins (SOL-2) that is required for SOL-1 and thus AMPAR function, we predict that there should also be Neto proteins and SOL-1 homologues in the vertebrate nervous system that function as AMPAR auxiliary proteins.

Our studies revealed several surprises when comparing loss of function in mutant worms to overexpression in reconstitution studies. Thus, in *sol-2* mutants, the loss of SOL-2 increased the rate of receptor desensitization. Contrary to our expectations, co-expressing SOL-2 with components of the GLR-1 complex in reconstitution studies also increased the rate of desensitization. This was particularly striking when recording GLR-1(Q552Y)-mediated currents that switched from non-desensitizing in the absence of SOL-2 to desensitizing in the presence of SOL-2. The most likely explanation for these conflicting results is that additional proteins contribute to receptor function and these proteins are not present in the heterologous cells used for reconstitution.

We also found that Concanavalin-A, a drug known to block desensitization of kainate receptors, also blocked desensitization of the GLR-1-mediated currents recorded from *Xenopus* oocytes in the absence of SOL-2. However, the effect on desensitization was greatly attenuated when SOL-2 was co-expressed with the other known members of the GLR-1 complex. This result suggests that the GLR-1 complex containing SOL-2 behaves more like an AMPAR and is consistent with Concanavalin-A's known differential effect on AMPA and kainate receptors (Partin et al., 1993). Another surprise was that the rates of desensitization measured in outside-out patches from *sol-1* and *sol-2* mutants were quite similar. Because currents in response to pressure application of glutamate were larger in *sol-2* mutants, we would have expected a rate of desensitization that was intermediate between *sol-1* mutants and wild type. However, we might not have detected differences between *sol-1* and *sol-2* mutants due to limitations in the rate at which we could exchange solutions during glutamate application. Alternatively, the similar rates of desensitization might be due to the apparently unstable association between SOL-1 and the receptor complex in the absence of SOL-2. Modification of receptor kinetics by formation of outside-out patches has been previously reported (Li and Niu, 2004). Thus, SOL-1 might dissociate from the complex in outside-out patches from *sol-2* mutants.

Our results help provide a new mechanistic view of postsynaptic function, where GLR-1 and the associated TARP proteins interact at the plasma membrane with a protein complex containing SOL-1 and SOL-2. The absence of any one component markedly changes the properties of the receptor suggesting that the presence of AMPARs at the postsynaptic membrane is necessary, but not sufficient, for normal glutamate-gated current. Additionally, our findings suggest that glutamate-gated current might be modified by activity-dependent changes in the relative numbers of auxiliary proteins present at the postsynaptic membrane,

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or in the association of these auxiliary proteins with AMPAR subunits. SOL-1 has a large extracellular region that could conceivably span the synaptic cleft and interact with active zone presynaptic proteins, thus maintaining the postsynaptic receptor complex in register with presynaptic release sites. In this scenario, SOL-2 and SOL-1 might contribute to functional slots predicted by electrophysiological analysis (Shi et al., 2001). In turn, the number of these slots, and their residency by AMPARs, might be regulated by activity and contribute to synaptic plasticity.

The phenomenon of long term potentiation (LTP) is most simply explained by the movement of receptor complexes from either extracellular regions or intracellular compartments to the synaptic membrane (Jackson and Nicoll, 2011; Kerchner and Nicoll, 2008). In our view, a major challenge for a deeper understanding of LTP is the role of activity-dependent changes in the number of auxiliary proteins. In this view, a subset of AMPARs might be silent because they are not associated with the proper complement of auxiliary proteins.

Experimental procedures

General methods, genetics and plasmids

All *C. elegans* strains were raised under standard laboratory conditions at 20 °C. Transgenic strains were generated using standard microinjection into the gonad of adult hermaphrodite worms. All fluorescently labeled proteins were found to be functional in transgenic rescue experiments of the relevant mutant phenotype. Plasmids, transgenic arrays and strains are described in **Supplemental Experimental Procedures**.

Antibody staining, SEP acid washes, and microscopy

Live transgenic worms that expressed HA::GLR-1::GFP in neurons (*akIs101*) were immunolabelled as previously described (Zheng et al., 2004). Briefly, anti-HA polyclonal sera (Molecular Probes) was diluted (1:1000) in worm injection buffer and injected into the pseudocoelomic space. To test the pH sensitivity of superecliptic phluorin (SEP), transgenic worms that expressed SEP::GLR-1 in AVA neurons were dissected to expose the ventral nerve cord (VNC). Dissected worms were bathed in extracellular fluid pH 7.4 (Mellem et.al., 2002) and the VNC was imaged both before and after solution exchange to ECF pH 6.5. Dissected worms were then bathed in ECF pH 7.4 containing 50 mM NH_4Cl to change intracellular pH. Images were acquired using a Roper Cascade 512B CCD camera and a Zeiss 100X 1.0 NA water immersion lens. Confocal images were acquired using Nikon Ti-eclipse equipped with a Yokogawa CSU10 spinning disc head, and captured by a Cascade 1224B EMCCD camera.

Electrophysiological studies

Electrophysiological recordings from *Xenopus* oocytes were performed using standard two-electrode voltage clamp techniques (Walker et al., 2006a). Plasmids for cRNA are described in **Supplemental Experimental Procedures**. Recordings from AVA interneurons and muscle cells from dissected transgenic worms were performed as previously described (Mellem et al., 2002). Rapid perfusion experiments were performed on outside-out patches obtained from AVA interneurons in dissected *C. elegans* preparations. Control extracellular fluid (ECF) and 3 mM glutamate ECF solutions were delivered via theta tubing mounted on a piezoelectric manipulator (MXP ZT-300, Siskiyou). The rate of solution exchange was measured as the 10-90% change in open-tip potential. Statistical significance was determined using Student's T-test.

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Behavioral analysis

Nose touch response and osmotic avoidance assays were performed as described in (Mellem et al., 2002). Reversal frequency was recorded manually and quantified using a computer program written in python. A reversal was defined as a switch from forward to backward movement. Statistical significance was determined by using the standard Student's t test. Error bars throughout represent the SEM.

Immunoprecipitation

HEK 293 cells were cultured in DMEM medium with 10% bovine fetal serum and transiently transfected using Lipofectamine 2000 (Life Technologies) in the presence of Opti-MEM. Plasmids for transfection are described in **Supplemental Experimental Procedures**. 48 hrs post-transfection, cells were lysed in ice-cold immunoprecipitation (IP) buffer (25 mM Tris, pH 7.5; 150 mM NaCl; 10% glycerol; 1% Triton X-100; 1mM EDTA; and Complete Protease Inhibitor Cocktail (Roche Diagnostics)). Cell lysates were spun at 16,100 g for 20 min and 79,000 g for 1 hr. The supernatants were incubated with rabbit polyclonal antibodies (Santa Cruz Biotechnology, Inc.) on ice for 1 hour followed by the addition of Protein A-Agarose (Santa Cruz Biotechnology, Inc.) and gentle mixing at 4 °C for 2 hours in ice-cold IP buffer. Samples were subject to 6 washes in ice-cold IP buffer and then denatured in 5x SDS sample buffer for 5 min at 95 °C. Proteins were resolved on 10% Mini-PROTEAN TGX precast gels (Bio-Rad), transferred to nitrocellulose membranes, and blocked in Odyssey® Blocking Buffer (LI-COR). Blots were probed with mouse monoclonal antibodies (Santa Cruz Biotechnology, Inc.) then IRDye 800CW (LI-COR) as primary and secondary antibodies, respectively. Signal was detected by the Odyssey® Infrared Imaging System (LI-COR).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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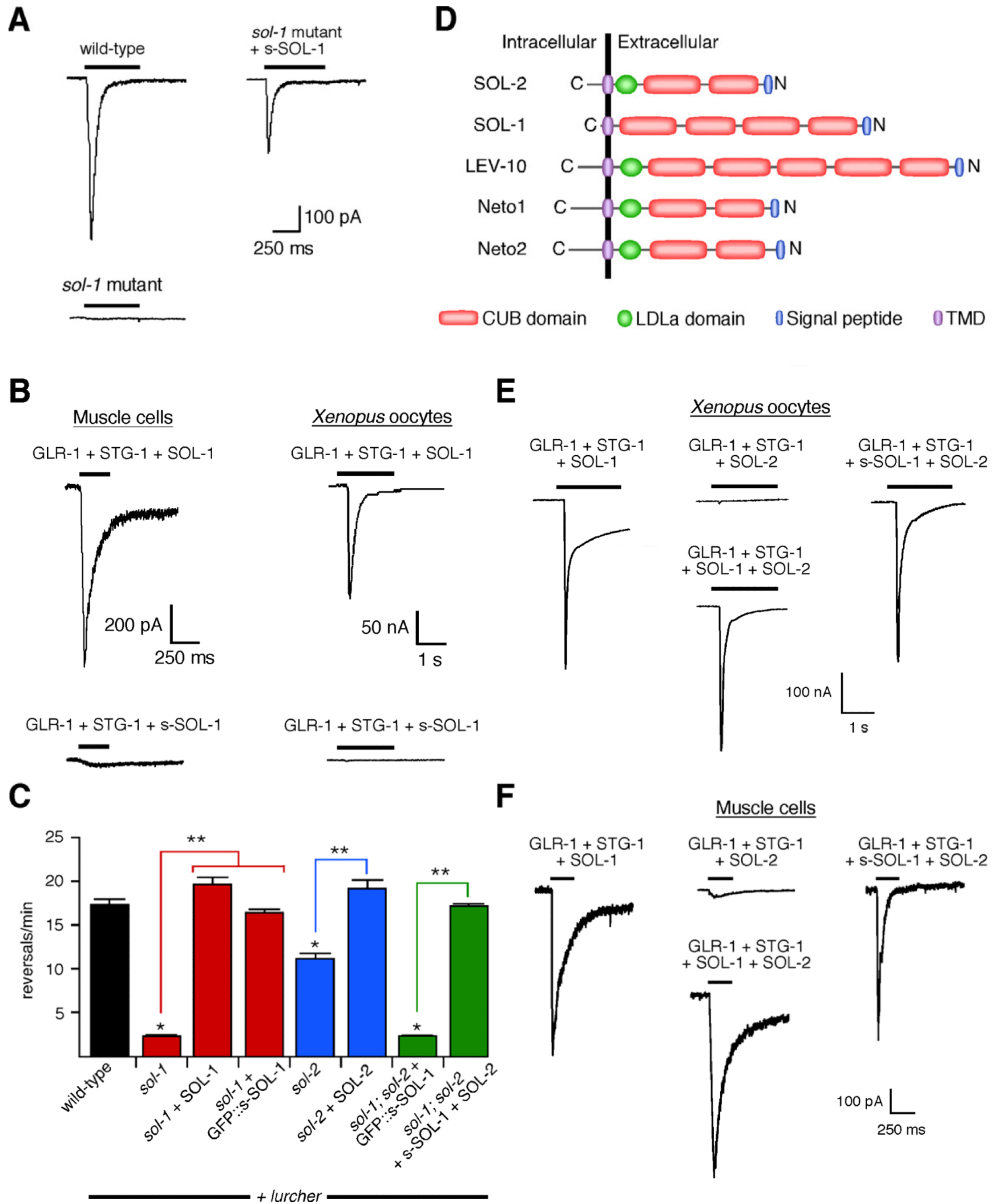

Figure 1.

sol-2 encodes a CUB-domain transmembrane protein that is required for s-SOL-1 function. (A) Currents in response to 3 mM glutamate in the AVA neuron of a wild-type worm, a *sol-1* mutant, and a transgenic *sol-1* mutant that expressed s-SOL-1 in AVA. (B) Glutamate-gated current in muscle cells of transgenic worms (left) and *Xenopus* oocytes (right) that expressed GLR-1 and STG-1 with either SOL-1 or s-SOL-1. (C) Reversal frequency in transgenic wild-type worms, *sol-1* and *sol-2* single and double mutants, and mutants that overexpressed specific auxiliary proteins. All worms expressed the GLR-1(A687T) lurcher variant of GLR-1. n=10 for all genotypes. *, significantly different from wild-type, p<0.001. **, p<0.001. (D) Domain organization of transmembrane CUB-domain proteins from *C.*

elegans (SOL-1, SOL-2 and LEV-10) and vertebrates (Neto1 and Neto2). (E and F) Glutamate-gated currents recorded in *Xenopus* oocytes (E) and the muscle cells of transgenic worms (F). All Cells were voltage-clamped at –60 mV.
See also Figure S1.

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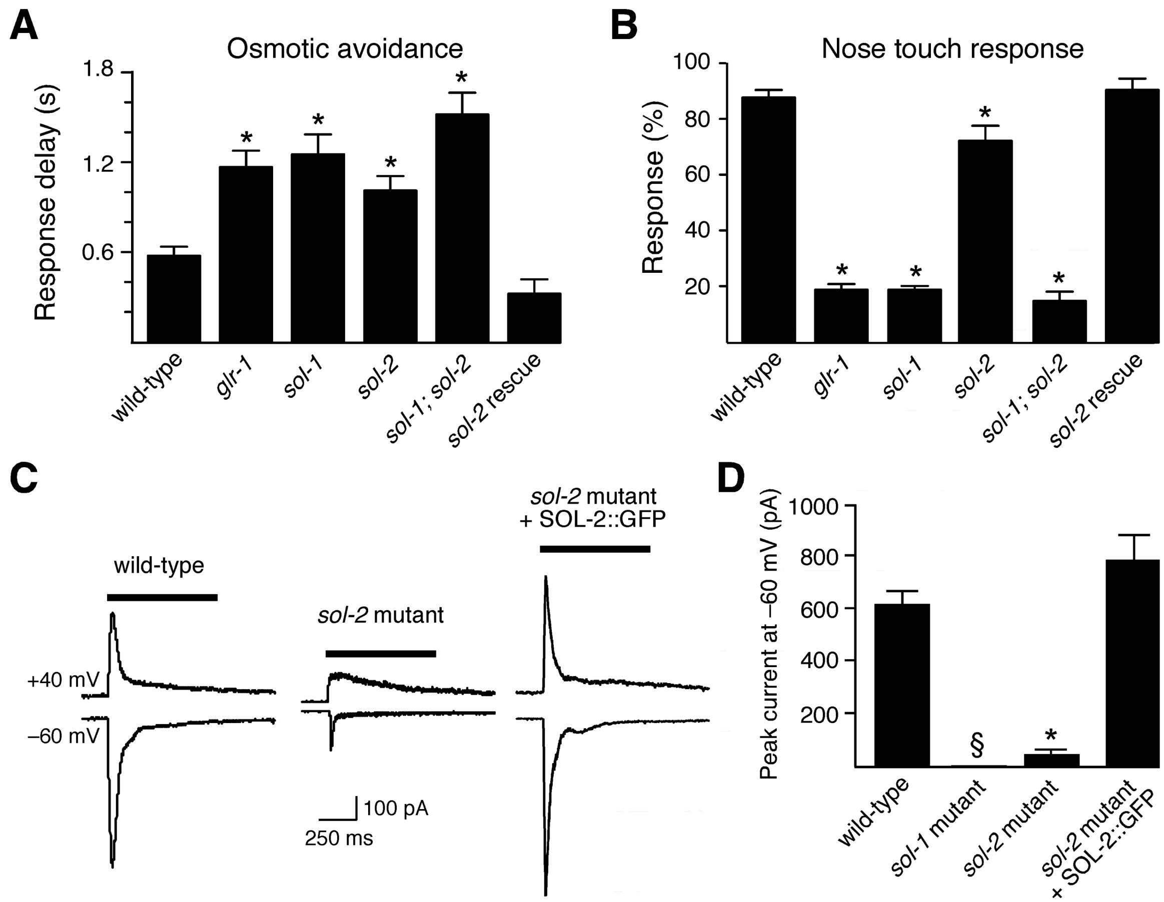

Figure 2.

SOL-2 is required for GLR-1 dependent behaviors and glutamate-gated current. (A) Quantification of the delay in response to hyper-osmotic stimuli. Wild type, n=41; *glr-1*, n=21; *sol-1*, n=9; *sol-2*, n=24; *sol-1; sol-2*, n=11; *sol-2* rescue, n=15. *, significantly different from wild-type, $p<0.01$. (B) The response to nose touch stimulation (percentage response of 10 trials per worm). Number of worms, wild-type, n=10; *glr-1*, n=10; *sol-1*, n=5; *sol-2*, n=10; *sol-1; sol-2*, n=5; *sol-2* rescue, n=8. *, significantly different from wild-type, $p<0.05$. (C) Currents in response to 3 mM glutamate in AVA interneurons of wild-type worms, *sol-2* mutants, and transgenic *sol-2* mutants that expressed SOL-2::GFP in AVA. (D) Average peak glutamate-gated current in wild-type worms (n=19), *sol-1* mutants (n>10), *sol-2* mutants (n=10), and transgenic *sol-2* mutants that expressed SOL-2::GFP (n=7). § indicates that rapidly activating currents were not detected in *sol-1* mutants. *, significantly different from wild-type, $p<0.001$.

See also Figure S2.

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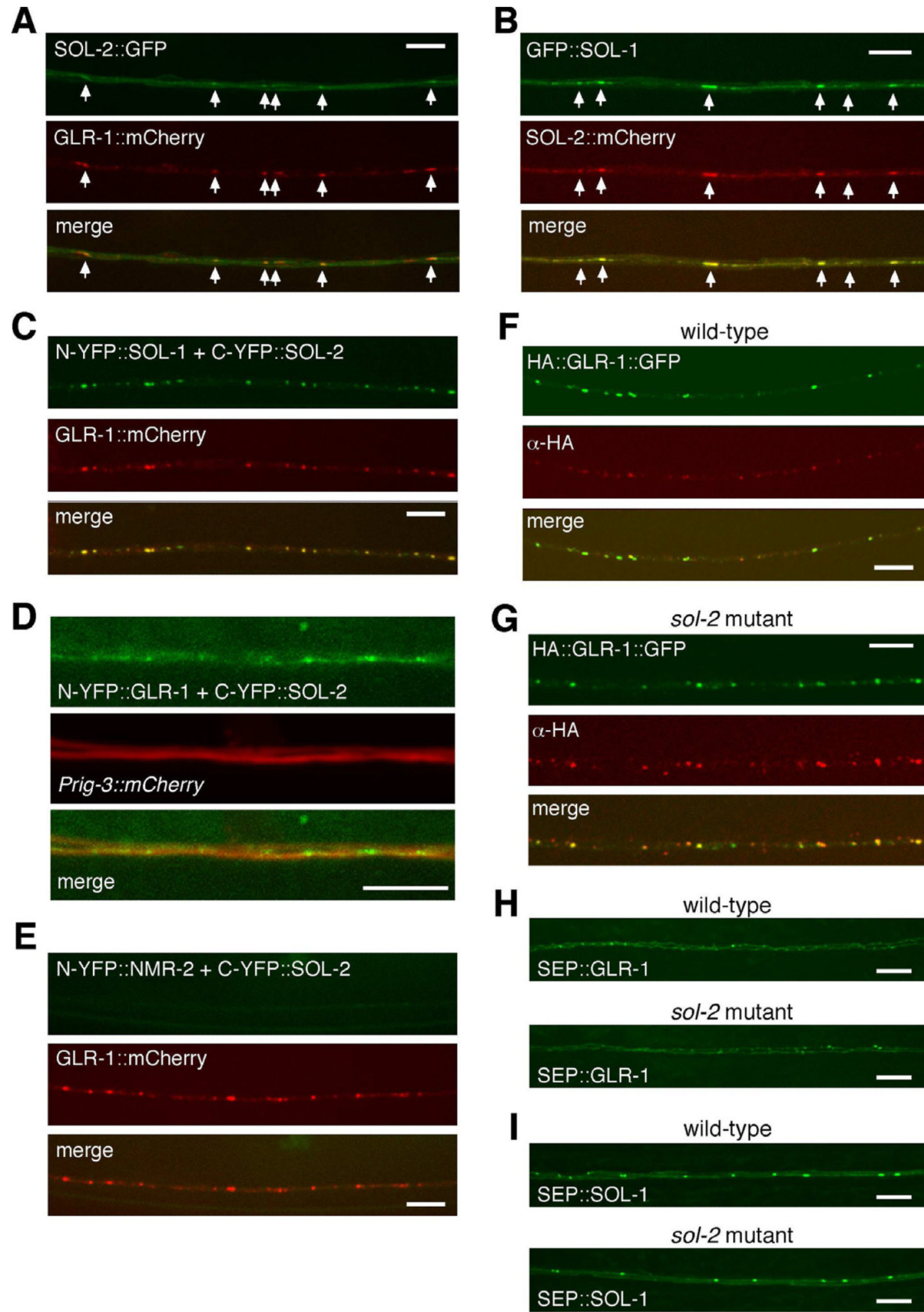

Figure 3.

SOL-2 associates with the GLR-1 signaling complex. (A and B) Confocal images of the ventral nerve cord in transgenic worms that expressed either SOL-2::GFP and GLR-1::mCherry (A), or GFP::SOL-1 and SOL-2::mCherry (B) in the AVA interneurons. Arrows indicate co-localized GFP and mCherry puncta. (C) SOL-1/SOL-2 BiFC fluorescence and GLR-1::mCherry in the AVA processes. (D) GLR-1/SOL-2 BiFC fluorescence and soluble mCherry in AVA processes. (E) Confocal image showing the absence of NMR-2/SOL-2 BiFC fluorescence in the AVA processes of transgenic worms that also expressed GLR-1::mCherry. (F and G) Anti-HA (red) and GFP fluorescence in the ventral nerve cord of transgenic wild-type worms (F) or *sol-2* mutants (G) that expressed

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HA::GLR-1::GFP. (H and I) SEP::GLR-1 (H) and SEP::SOL-1 (I) fluorescence in the AVA processes of transgenic wild-type worms and *sol-2* mutants. Scale bars represent 5 μm. See also Figure S3 and S4.

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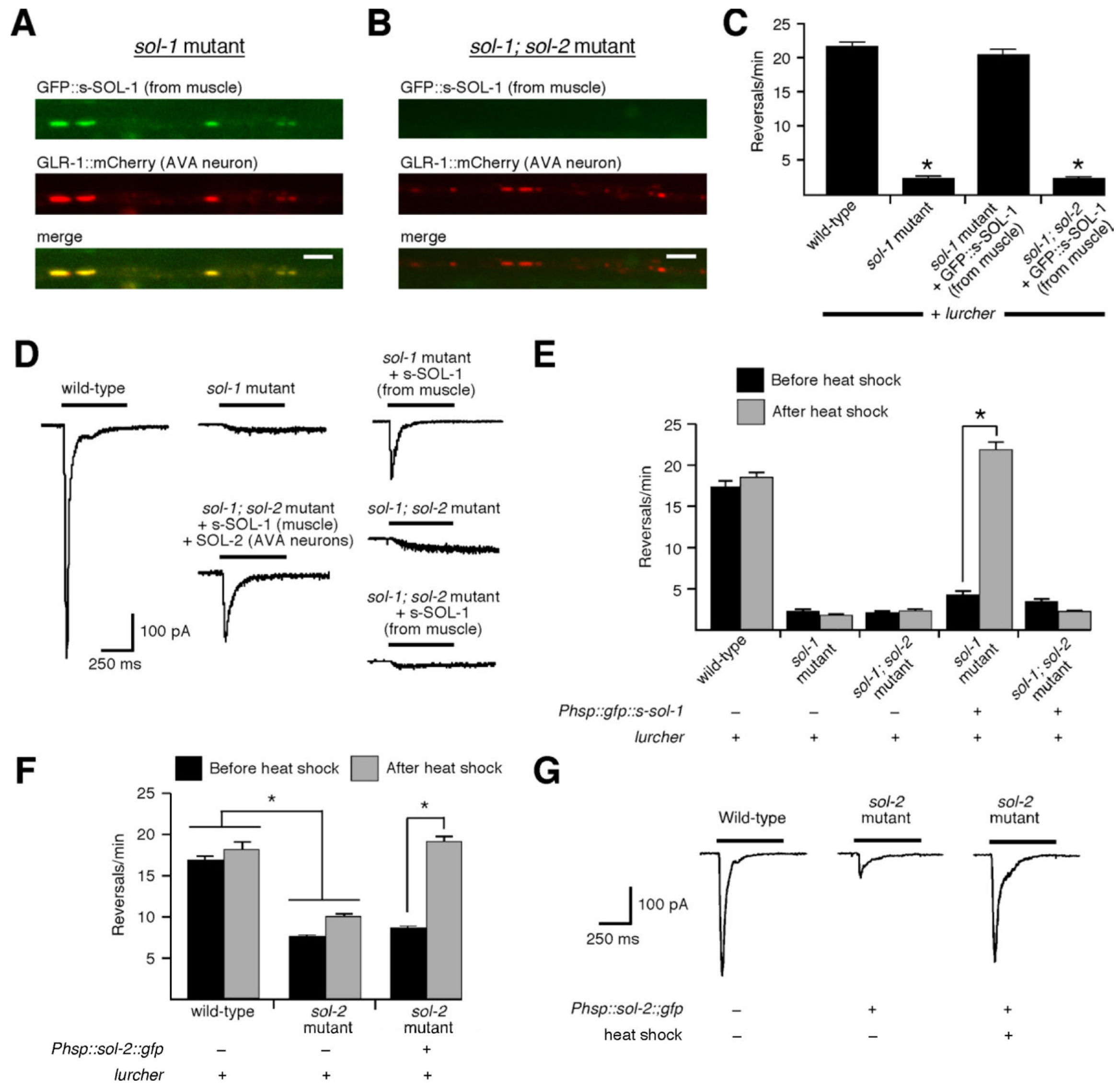

Figure 4.

sol-1 mutants can be rescued by *in trans* delivery of s-SOL-1. (A and B) Confocal images of the ventral nerve cord in transgenic *sol-1* mutants (A) and *sol-1; sol-2* double mutants (B) that expressed GLR-1::mCherry in the AVA interneurons and GFP::s-SOL-1 secreted by muscle cells. (C) Reversal frequency in transgenic wild-type worms and *sol-1* mutants, and transgenic *sol-1* mutants or *sol-1; sol-2* double mutants with GFP::s-SOL-1 secreted by muscle cells. All worms expressed the GLR-1(A687T) lurcher variant of GLR-1. n=10 for all genotypes; *, significantly different from wild-type, $p<0.001$. (D) Currents in response to 3 mM glutamate in the AVA neuron of a wild-type worm, *sol-1* mutant, *sol-1; sol-2* double mutant, a transgenic *sol-1* or *sol-1; sol-2* mutant with s-SOL-1 secreted by muscle cells, and a transgenic *sol-1; sol-2* mutant with s-SOL-1 secreted by muscle cells and SOL-2 expressed specifically in the AVA interneurons. Cells were held at –60 mV. (E) Reversal frequency in transgenic wild-type worms, *sol-1* mutants, *sol-1; sol-2* double mutants, and transgenic mutants that carried a heat-shock inducible GFP::s-SOL-1 transgene (*Phsp16-2::gfp::s-sol-1*). All worms expressed the GLR-1(A687T) lurcher variant of GLR-1. (+) and (–) indicate the presence or absence of the transgene and when heat shock was used to induce GFP::s-SOL-1 expression. n=10 for all genotypes; *, $p<0.001$. (F and G) Reversal frequency

(F) and glutamate-gated current (G) in wild-type worms and transgenic *sol-2* mutants with a heat-shock inducible SOL-2::GFP transgene (*Phsp::sol-2::gfp*). n=10 for all genotypes; *, $p<0.001$.

See also Figure S5.

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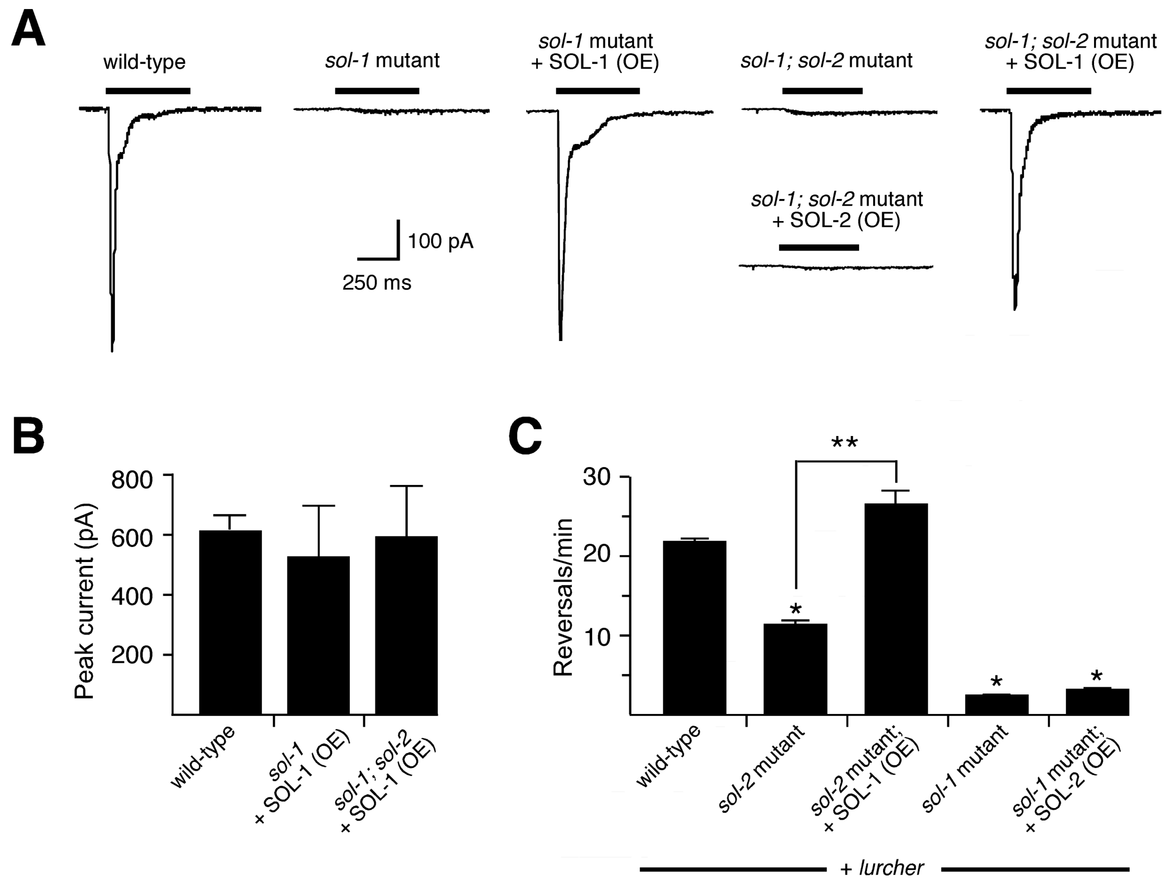

Figure 5.

Overexpression of SOL-1 partially compensates for the loss of SOL-2. (A) Currents in response to 3 mM glutamate in the AVA interneurons of wild-type worms, *sol-1* mutants, *sol-1; sol-2* double mutants, and transgenic mutants that overexpressed either SOL-1 or SOL-2. Cells were held at –60 mV. (B) Average peak glutamate-gated current in wild-type worms (n=19) and transgenic *sol-1* mutants (n=5) or *sol-1; sol-2* double mutants (n=4) that overexpressed SOL-1 in AVA. (C) Reversal frequency in transgenic wild-type and mutant worms that expressed the GLR-1(A687T) lurcher variant of GLR-1 with or without overexpression of SOL-1 or SOL-2. n=10 for all genotypes; *, significantly different from wild-type, p<0.01; **, p<0.001.

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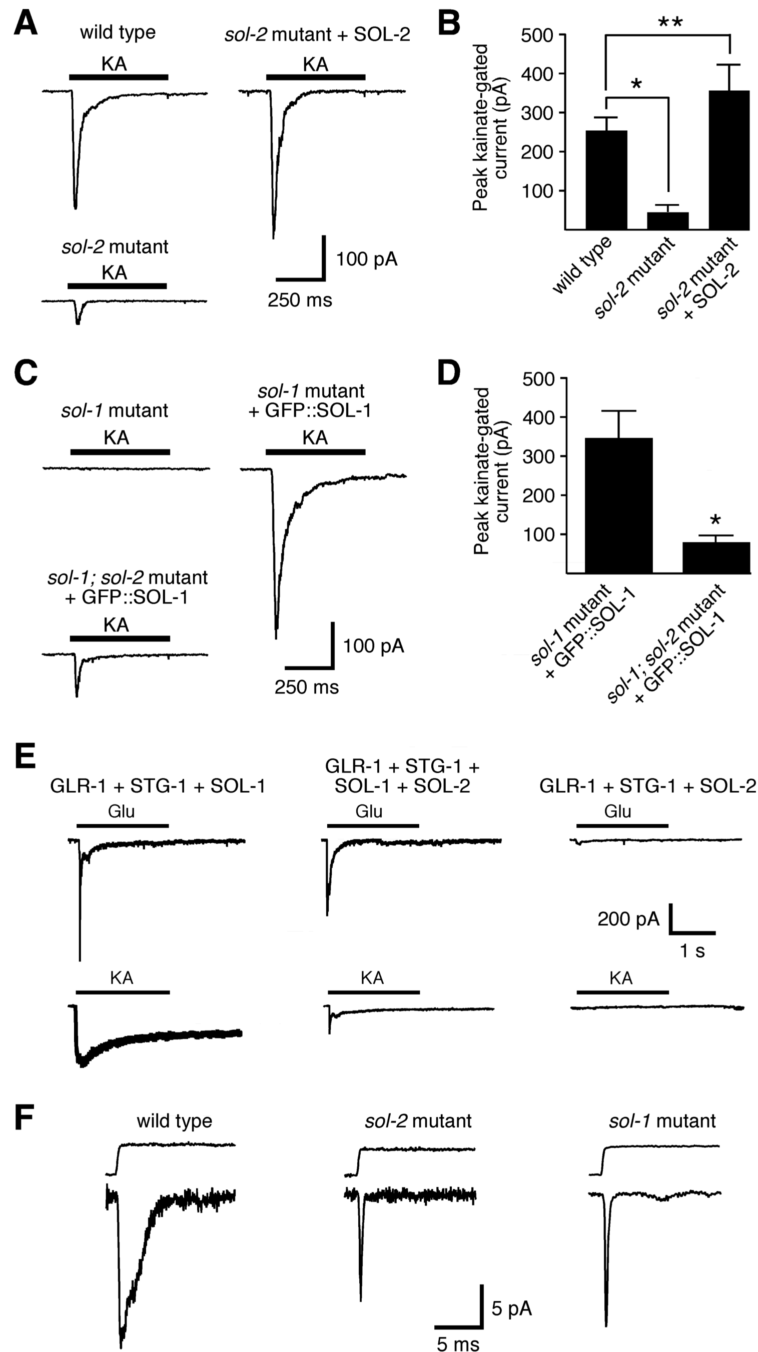

Figure 6.

SOL-2 contributes to the pharmacology and kinetics of GLR-1. (A) Currents in response to 100 μM kainate (KA) in the AVA interneurons of wild-type worms, *sol-2* mutants, and transgenic *sol-2* mutants that overexpressed wild-type SOL-2 in AVA. (B) Average peak kainate-gated current in wild-type worms (n=14), *sol-2* mutants (n=5) and transgenic *sol-2* mutants that overexpressed SOL-2 in AVA (n=5). *, p<0.0001. (C) Kainate-gated current in a *sol-1* mutant, a transgenic *sol-1* mutant, or a *sol-1; sol-2* double mutant that overexpressed GFP::SOL-1 in AVA. (D) Average peak kainate-gated current in transgenic *sol-1* mutants (n=5) and *sol-1; sol-2* mutants (n=9) that overexpressed GFP::SOL-1 in AVA. *, p<0.02. (E) Currents evoked in muscle cells in response to 3 mM glutamate and 100 μM kainate in

transgenic worms that ectopically expressed various combinations of GLR-1, STG-1, SOL-1 and SOL-2 in muscles. (F) Currents measured in response to rapid application of 3 mM glutamate to an outside-out patch pulled from an AVA interneuron from a wild-type worm, a *sol-1* mutant, or a *sol-2* mutant. All cells were voltage-clamped at–60 mV.

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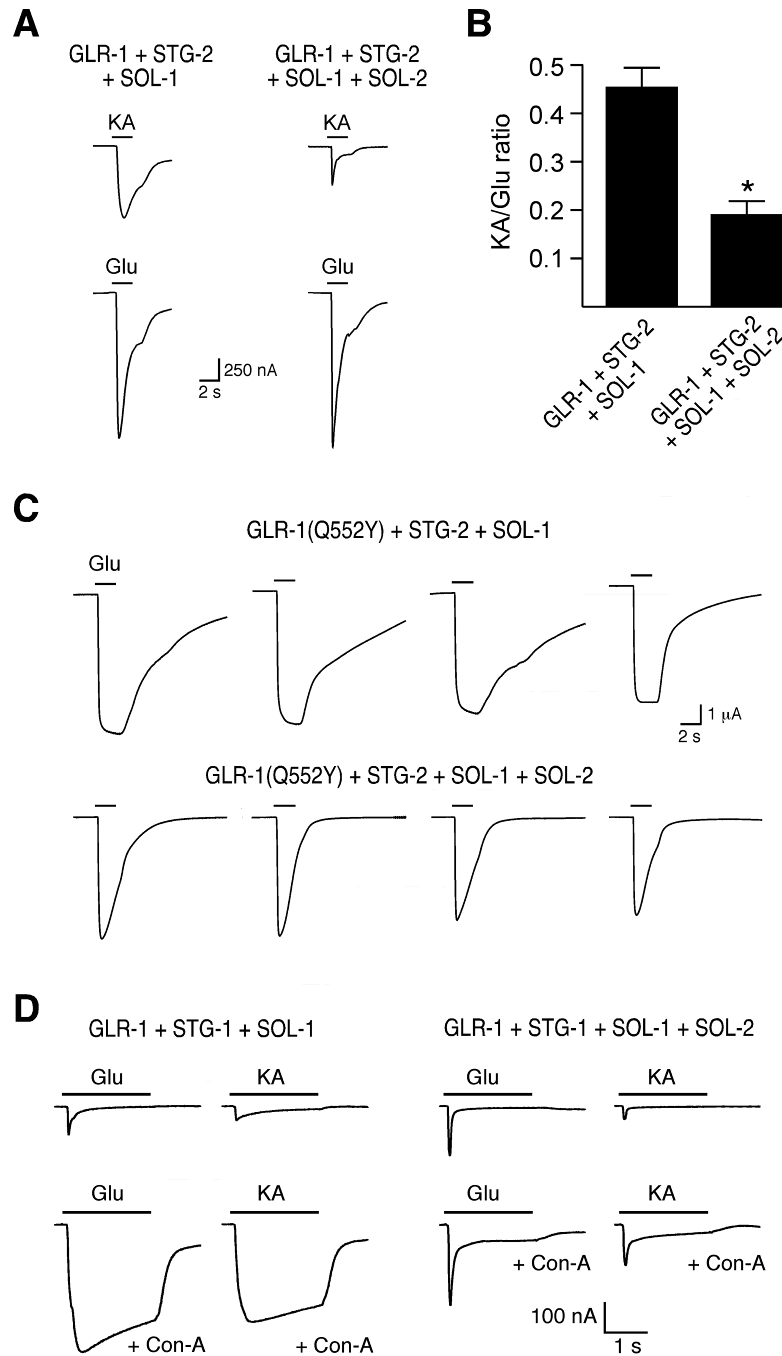

Figure 7.

SOL-2 modifies kainate sensitivity and receptor desensitization in reconstitution studies. (A) Currents in response to 100 μM kainate (KA, top) and 1 mM glutamate (Glu, bottom) in *Xenopus* oocytes that expressed GLR-1, STG-2 and SOL-1 either with (left) or without (right) SOL-2. (B) The ratio of peak kainate to glutamate-gated current in *Xenopus* oocytes that expressed GLR-1, STG-2 and SOL-1, or GLR-1, STG-2, SOL-1 and SOL-2. For all conditions, n=11. *, $p<0.001$. (C) Examples of current recorded in response to 1 mM glutamate in *Xenopus* oocytes that expressed GLR-1(Q552Y), STG-2 and SOL-1 either with (bottom) or without (top) SOL-2. (D) Currents evoked in *Xenopus* oocytes in response to 1 mM glutamate and 100 μM kainate in either the presence or absence of Conconavalin-A.

See also Figure S6 and S7.

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