RESEARCH ARTICLE

SLO BK Potassium Channels Couple Gap Junctions to Inhibition of Calcium Signaling in Olfactory Neuron Diversification

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Abstract

The C. elegans AWC olfactory neuron pair communicates to specify asymmetric subtypes AWC\textsuperscript{OFF} and AWC\textsuperscript{ON} in a stochastic manner. Intercellular communication between AWC and other neurons in a transient NSY-5 gap junction network antagonizes voltage-activated calcium channels, UNC-2 (CaV2) and EGL-19 (CaV1), in the AWC\textsuperscript{ON} cell, but how calcium signaling is downregulated by NSY-5 is only partly understood. Here, we show that voltage- and calcium-activated SLO BK potassium channels mediate gap junction signaling to inhibit calcium pathways for asymmetric AWC differentiation. Activation of vertebrate SLO-1 channels causes transient membrane hyperpolarization, which makes it an important negative feedback system for calcium entry through voltage-activated calcium channels. Consistent with the physiological roles of SLO-1, our genetic results suggest that slo-1\textsuperscript{BK} channels act downstream of NSY-5 gap junctions to inhibit calcium channel-mediated signaling in the specification of AWC\textsuperscript{ON}. We also show for the first time that slo-2\textsuperscript{BK} channels are important for AWC asymmetry and act redundantly with slo-1\textsuperscript{BK} to inhibit calcium signaling. In addition, nsy-5-dependent asymmetric expression of slo-1 and slo-2 in the AWC\textsuperscript{ON} neuron is necessary and sufficient for AWC asymmetry. SLO-1 and SLO-2 localize close to UNC-2 and EGL-19 in AWC, suggesting a role of possible functional coupling between SLO BK channels and voltage-activated calcium channels in AWC asymmetry. Furthermore, slo-1 and slo-2 regulate the localization of synaptic markers, UNC-2 and RAB-3, in AWC neurons to control AWC asymmetry. We also identify the requirement of bkip-1, which encodes a previously identified auxiliary subunit of SLO-1, for slo-1 and slo-2 function in AWC asymmetry. Together, these results provide an unprecedented molecular link between gap junctions and calcium pathways for terminal differentiation of olfactory neurons.
Author Summary

Cell type diversity is important for the nervous system to function properly. Asymmetric differentiation of neurons along the left-right axis is one way to achieve diversity; however, the molecular mechanisms used to establish neuronal asymmetry are only partly understood. In the nematode *C. elegans*, the AWC sensory neuron pair displays stochastic asymmetric identities. Communication between neurons, including the two AWC neurons, through gap junctions inhibits calcium channels in one AWC neuron, resulting in two distinct AWC identities. How gap junctions repress calcium channels in one AWC is not well understood. We show that voltage- and calcium-activated potassium channels provide a molecular link between gap junctions and calcium channels to establish AWC neuronal asymmetry. We show that potassium channels are asymmetrically expressed in AWC neurons, which is dependent on gap junctions. We also find that potassium channels localize close to calcium channels in AWC, suggesting they may functionally couple to establish AWC asymmetry. In addition, our results show that potassium channels regulate the localization of synaptic markers in AWC for asymmetry. Furthermore, we identify an auxiliary subunit of the potassium channels required for their function in establishing AWC asymmetry. These results shed light on mechanisms used to diversify neuronal cell types.

Introduction

The nervous system generates a tremendous diversity of cell types that enable formation of functional neural circuits for information processing and mediating behaviors. Cellular diversity is especially important in the developing sensory system as it allows animals to detect different cues in the environment. However, the molecular mechanisms that generate neuronal diversification are only partly understood. One way to generate cellular diversity in the nervous system is to specify different fates and functions of individual cell types across the left-right axis. Left-right asymmetry in the nervous system is present throughout the animal kingdom [1–3]. For example, anatomical and functional asymmetries in the human nervous system have been described, such as the greater size of the planum temporale in the left hemisphere, and the localization of language to the left hemisphere of the brain [4]. Defects in brain asymmetry have been correlated with various neurological diseases such as dyslexia and schizophrenia [5].

In the *C. elegans* nervous system, two pairs of head sensory neurons display molecular and functional asymmetries: the ASE taste neurons and the AWC olfactory neurons [6–9]. The left and right AWC olfactory neurons appear symmetric at the anatomical and morphological level. However, the two AWC neurons differentiate asymmetrically into two distinct subtypes, one default AWC$_{OFF}$ and one induced AWC$_{CON}$, at both molecular and functional levels in late embryogenesis [10–12]. The AWC$_{CON}$ subtype expresses the G-protein coupled receptor (GPCR) gene *str-2* and functions to detect the odorant butanone [11,12]. The AWC$_{OFF}$ subtype expresses the GPCR gene *srx-3* and functions to sense the odorant 2,3-pentanedione [12,13]. AWC asymmetry is stochastic, such that the AWC$_{CON}$ subtype is induced on the left side of the animal in 50% of the population and on the right side of the animal in the other 50% [11]. AWC asymmetry is maintained throughout the life of an animal [11,14,15].

The default AWC$_{OFF}$ subtype is specified by a calcium-activated protein kinase pathway. In this pathway, calcium entry through voltage-gated calcium channels (the pore-forming α1 subunits UNC-2/N-type or EGL-19/L-type and the regulatory α2β subunit UNC-36) activates a kinase cascade that consists of UNC-43 calcium/calmodulin dependent protein kinase (CaM-KII), the TIR-1 (Sarm1) adaptor protein, NSY-1 MAP kinase kinase kinase (MAPKKK), and
SEK-1 MAPKK [10,11,16,17]. TIR-1 assembles a calcium-signaling complex containing UNC-43 (CaMKII) and NSY-1 (MAPKKK) at postsynaptic sites in the AWC axons, in a manner dependent on microtubules and the kinesin motor protein UNC-104, to promote the AWC^{OFF} subtype [10,18]. Intercellular calcium signaling through a transient embryonic neural network, formed between AWC and other neurons via the NSY-5 gap junction protein innexin, coordinates precise AWC asymmetry [19]. In addition, NSY-5 and the NSY-4 claudin-like protein function in parallel to antagonize calcium signaling through mir-71-mediated downregulation of tir-1 expression in the AWC^{ON} subtype [20–22]. However, the mechanism by which NSY-5 gap junctions and NSY-4 claudin suppress unc-2/unc-36 and egl-19/unc-36 calcium signaling to induce the AWC^{ON} subtype is only beginning to be understood.

The ky389 and ky399 alleles were identified from a forward genetic screen for mutants with two AWC^{ON} neurons (2AWC^{ON} phenotype) [11]. The ky389 and ky399 mutations were revealed as gain-of-function (gf) alleles of slo-1 in a study demonstrating a central role of slo-1 in behavioral response to ethanol [23]. slo-1 encodes a conserved voltage- and calcium-activated large conductance BK potassium channel [24,25]. Activation of SLO-1 (Slo1) channels causes hyperpolarization of the cell membrane, thereby reducing cellular excitability and limiting calcium entry through voltage-gated calcium channels [26]. The 2AWC^{ON} phenotype of slo-1(gf) mutants suggests a sufficient role of slo-1(gf) in promoting AWC^{ON}. However, the effect of slo-1 loss-of-function mutations on AWC asymmetry and the mechanism by which slo-1 functions to control AWC asymmetry remained unaddressed. Here we demonstrate that both slo-1 and slo-2 BK channels are necessary for the establishment of AWC asymmetry. We show that slo-1 and slo-2 act redundantly downstream of nsy-5 (innexin gap junction protein) and in parallel with nsy-4 (claudin) to antagonize the function of unc-2 and egl-19 (voltage-gated calcium channels) in the induced AWC^{ON} subtype. Asymmetric expression of slo-1 and slo-2 in the AWC^{ON} neuron, which is dependent on NSY-5 and NSY-4, is necessary and sufficient for AWC asymmetry. In addition, SLO-1 and SLO-2 BK channels localize close to UNC-2 and EGL-19 voltage-gated calcium channels, suggesting that SLO channels may inhibit calcium channels through functional coupling and negative feedback. Our results also suggest that slo-1 and slo-2 may regulate AWC communication to control AWC asymmetry through modulating UNC-2 synaptic puncta and synaptic vesicle clustering. Thus, our study identifies an unprecedented role of SLO BK potassium channels in mediating transient gap junction signaling for inhibition of a calcium channel-activated kinase cascade in terminal differentiation of olfactory neurons.

Results
slo-1 and slo-2 BK potassium channels act redundantly to establish AWC asymmetry

Wild-type animals have one default AWC^{OFF} neuron, expressing the GPCR gene srsx-3, and one induced AWC^{ON} neuron, expressing the GPCR gene str-2 (Fig 1Ai and 1B). Both slo-1 (ky389gf) and slo-1(ky399gf) mutations resulted in expression of the AWC^{ON} marker str-2 in two AWC neurons (2AWC^{ON} phenotype) (Fig 1Aii, 1B and 1C), as shown previously [11]. Both slo-1(ky389gf)/+ and slo-1(ky399gf)/+ heterozygous animals displayed a less penetrant 2AWC^{ON} phenotype (Fig 1B), confirming their characterization as dominant gain-of-function mutants. We set out to further characterize the AWC phenotypes of slo-1(gf) mutants. We found that the AWC^{OFF} marker srsx-3 was not expressed in either of the AWC neurons of slo-1 (gf) mutants (Fig 1Aii and 1B), consistent with the 2AWC^{ON} phenotype of the mutants (Fig 1Aii and 1B). In addition, overexpression of slo-1(T1001Igf) and slo-1(E350Kgf), containing ky389gf and ky399gf mutations, respectively, in AWC in a wild-type background also caused a
Fig 1. slo-1 and slo-2 act redundantly to promote the AWC<sup>ON</sup> neuronal subtype. (A) Images of wild type (i), slo-1(ky399gf) mutants (ii), and slo-1(eg142lf); slo-2(ok2214lf) double mutants (iii) expressing the transgene str-2p::TagRFP (AWC<sup>ON</sup> marker); srxs-3p::GFP (AWC<sup>OFF</sup> marker) in the adult stage. (i) Wild-type animals have one AWC<sup>ON</sup> neuron and one AWC<sup>OFF</sup> neuron. (ii) slo-1(ky399gf) mutants express str-2p::TagRFP in both AWC neurons, showing a 2AWC<sup>ON</sup> phenotype. (iii) slo-1(eg142lf); slo-2(ok2214lf) double mutants express srxs-3p::GFP in both AWC cells, displaying a 2AWC<sup>OFF</sup> phenotype. srxs-3p::GFP is also expressed in two AWB cells, in addition to the AWC<sup>OFF</sup> cell. Arrows indicate the AWC cell body; asterisks indicate the AWB cell body. Scale bar, 10 μm. Anterior is at left and ventral is at bottom. (B) Expression of AWC markers in slo-1 and slo-2 mutants. (C, D) Schematic representation of SLO-1
strong 2AWCON phenotype (73%-75%) (Fig 1B). However, overexpression of wild-type slo-1 only caused a very weak 2AWCON phenotype (1%) when injected at the same concentration as the slo-1(T1001Igf) and slo-1(E350Kgf) transgenes (Fig 1B). Our results are consistent with the previous electrophysiological study suggesting that slo-1(ky389gf) and slo-1(ky399gf) mutations result in increased SLO-1 channel activity in dopaminergic neurons [23].

Although slo-1(gf) mutants caused a strong 2AWCON phenotype, we found that loss-of-function (lf) mutations in slo-1 did not display any defects in AWC asymmetry (Fig 1B and 1C). This suggests that slo-1 may function redundantly with other genes to establish AWC asymmetry. Since slo-2 encodes the only other calcium-activated SLO-like potassium channel in C. elegans and its expression overlaps with slo-1 [24,27,28], we hypothesized that slo-1 and slo-2 have essential and redundant roles in promoting the AWCON subtype.

slo-1 and slo-2 act downstream of nsy-5 to antagonize calcium channel-mediated signaling in promoting the AWCON subtype

The 2AWCON phenotype of slo-1(gf) mutants and the 2AWCOFF phenotype of slo-1(lf); slo-2(lf) double mutants (Figs 1Aii, 1Aiii, 1B and 2A) indicate that the two BK potassium channels function to promote the induced AWCON subtype. To shed light on how slo-1 and slo-2 promote AWCON, we investigated where they are located within the AWC asymmetry pathway by generating double and triple mutants of slo-1, slo-2, and other genes previously implicated in AWC asymmetry (Fig 2A).

slo-1 and slo-2 act downstream of nsy-5 to antagonize calcium channel-mediated signaling in promoting the AWCON subtype

The 2AWCON phenotype of slo-1(lf); slo-2(lf) double mutants (Figs 1Aii, 1Aiii, 1B and 2A) indicate that the two BK potassium channels function to promote the induced AWCON subtype. To shed light on how slo-1 and slo-2 promote AWCON, we investigated where they are located within the AWC asymmetry pathway by generating double and triple mutants of slo-1, slo-2, and other genes previously implicated in AWC asymmetry (Fig 2A).

The slo-1(gf) 2AWCON mutants were crossed with 2AWCOFF mutants including nsy-5/innexin(lf), nsy-4/claudin(lf), unc-43/CaMKII(gf), and tir-1/Sarm1(gf) [11,18,20,21]. The 2AWCOFF phenotype of nsy-5(lf) mutants was completely suppressed by slo-1(gf) mutants (Fig 2A), suggesting that slo-1 acts downstream of nsy-5 gap junctions to specify AWCON (Fig 2B). nsy-4(lf); slo-1(lf) double mutants had mixed 2AWCON and 2AWCOFF phenotypes (Fig 2A), suggesting that slo-1 acts in parallel with nsy-4 claudin in promoting AWCON (Fig 2B). Furthermore, the 2AWCON phenotype of slo-1(gf) was nearly completely suppressed by unc-43(gf) and tir-1(gf) mutations (Fig 2A); the 2AWCOFF phenotype of slo-1(lf); slo-2(lf) double mutants was almost completely suppressed by the unc-43(lf) mutants (Fig 2A), which is consistent with the previous notion that slo-1 acts upstream of unc-43 (CaMKII) [16] (Fig 2B).

Since our genetic results put slo-1 and slo-2 (BK potassium channels) at a position similar to unc-2/unc-36 and egl-19/unc-36 (voltage-gated calcium channels) in the AWC asymmetry
Fig 2. slo-1 and slo-2 act downstream of nsy-5 to antagonize the function of voltage-gated calcium channel-activated kinase cascade in promoting AWCON. (A) Double and triple mutant analysis of slo-1(ky389gf), slo-1(ky399gf), and slo-1(eg142lf); slo-2(ok2214lf) animals with mutants of known genes involved in establishment of AWC asymmetry. 2AWCON: both AWC cells express str-2; 1AWCOFF/AWCON: only one of the two AWC cells expresses str-2; 2AWCOFF, neither AWC cell expresses str-2. (B) The genetic pathway that demonstrates possible relationships between slo-1, slo-2 and other genes required for AWC asymmetry. Genes in green represent AWCOFF promoting, genes in red represent AWCON promoting, and those in grey represent less active or inactive genes.

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pathway, we examined the genetic interaction of slo-1 and slo-2 with unc-36. unc-36(e251lf) mutants have a strong 2AWC\textsuperscript{ON} phenotype \cite{11}(Fig 2A) and were crossed into the slo-1(lf); slo-2(lf) 2AWC\textsuperscript{OFF} mutants. We found that the 2AWC\textsuperscript{ON} phenotype of unc-36(lf) and the 2AWC\textsuperscript{OFF} phenotype of slo-1(lf); slo-2(lf) were significantly mutually suppressed in unc-36(lf); slo-1(lf); slo-2(lf) triple mutants (Fig 2A). These genetic analyses suggest antagonistic and parallel functions of BK potassium channels (slo-1 and slo-2) and voltage-gated calcium channels (unc-36) in AWC asymmetry (Fig 2B). unc-2 and egl-19, both of which encode α1 subunits of voltage-activated calcium channels, were shown to have partially redundant functions in AWC asymmetry \cite{13}. unc-2(lf1lf) mutants had a mixed 2AWC\textsuperscript{ON} and 2AWC\textsuperscript{OFF} phenotype, while a reduction of function (rf) allele of egl-19 did not display any AWC asymmetry defects. However, egl-19(rf); unc-2(lf) double mutants caused a strong 2AWC\textsuperscript{ON} phenotype reminiscent of unc-36(lf) mutants \cite{13}(Fig 2A), which supports partially redundant functions of egl-19 and unc-2 in AWC asymmetry. To test whether unc-2 and egl-19 interact with slo-1 and slo-2 to establish AWC asymmetry, we determined genetic relationship between unc-2, egl-19, slo-1, and slo-2. The 2AWC\textsuperscript{OFF} phenotype of slo-1(lf); slo-2(lf) was only slightly suppressed or not suppressed in slo-1(lf); slo-2(lf) unc-2(lf) or egl-19(rf); slo-1(lf); slo-2(lf) mutants, respectively (Fig 2A). These results suggest that unc-2(lf) or egl-19(rf) alone are not sufficient to suppress the slo-1(lf); slo-2(lf) 2AWC\textsuperscript{OFF} phenotype. However, egl-19(rf); slo-1(lf); slo-2(lf) unc-2(lf) quadruple mutants displayed a high 2AWC\textsuperscript{ON} phenotype, which resembled egl-19(rf); unc-2(lf) mutants (Fig 2A). This result suggests that unc-2 and egl-19 act redundantly to antagonize slo-1 and slo-2 function to promote the AWC\textsuperscript{OFF} identity.

Taken together, these genetic results suggest that slo-1 and slo-2 (BK potassium channels) act downstream of nsy-5 (innexin) and in parallel with nsy-4 (claudin) to antagonize the function of unc-2/unc-36 and egl-19/unc-36 (voltage-gated calcium channels) to induce AWC\textsuperscript{ON} (Fig 2B).

**slo-1 and slo-2 are expressed asymmetrically in the AWC\textsuperscript{ON} neuron**

Both slo-1 and slo-2 are widely expressed in neurons and muscles \cite{25,28}. To determine if slo-1 and slo-2 are expressed in AWC neurons, we crossed odr-1p::TagRFP (expressed in both AWC neurons) with slo-1p::GFP and slo-2p::GFP transgenic strains. We found that GFP expressed from slo-1p and slo-2p was colocalized with the AWC marker odr-1p::TagRFP (Fig 3A and 3C), suggesting that slo-1 and slo-2 are expressed in AWC.

To determine if slo-1 and slo-2 are expressed asymmetrically in AWC neurons, we compared their respective expression level in AWC left (AWCL) and AWC right (AWCR). Although slo-1 and slo-2 are expressed in both AWC neurons in the majority of wild-type animals, both slo-1 and slo-2 are asymmetrically expressed in AWCL or AWCR in a stochastic manner (Fig 3B and 3D, AWCL>AWCR versus AWCL<AWCR are indistinguishable). Random asymmetry of slo-1 and slo-2 expression in AWC neurons is consistent with the stochastic nature of AWC asymmetry. In contrast, nsy-5(ky634lf) and nsy-4(ky627lf) mutants exhibited a significant increase in the percentage of animals that expressed slo-1 and slo-2 symmetrically in AWCL and AWCR (Fig 3B and 3D, AWCL = AWCR). This data suggests that nsy-5 (innexin) and nsy-4 (claudin) are required for the asymmetric expression of slo-1 and slo-2 in AWC neurons, and is consistent with our genetic analysis demonstrating that nsy-5 acts in parallel with nsy-4 to promote AWC\textsuperscript{ON} through slo-1 and slo-2 (Fig 2B). As a control, we examined the asymmetric expression of slo-1 and slo-2 in unc-43(n498gf)/CaMKII mutants, which cause a 2AWC\textsuperscript{OFF} phenotype, similar to that caused by nsy-5(ky634lf) and nsy-4(ky627lf). We found that the asymmetric expression of both slo-1 and slo-2 was unaffected by the unc-43(n498gf) mutation (Fig 3B and 3D). This suggests that unc-43 (CaMKII) does not regulate the...
Fig 3. slo-1 and slo-2 are expressed asymmetrically in the AWC<sup>ON</sup> neuron. (A, C) Images of wild-type L1 animals showing expression of slo-1p::GFP (A) and slo-2p::GFP (C) at a higher level in AWC<sup>CR</sup> (bottom panels) than in AWC<sup>CL</sup> (top panels). Both AWC<sup>CL</sup> and AWC<sup>CR</sup> were labeled by odr-1p::TagRFP. The cell body of both AWC cells is outlined by dashed lines. Scale bar, 5 μm. Anterior is at left and ventral is at bottom. (B, D) Quantification of asymmetric expression of slo-1p::GFP (B) and slo-2p::GFP (D) in AWC<sup>CL</sup> and AWC<sup>CR</sup> in wild type and mutants defective in AWC asymmetry. The single focal plane with the brightest fluorescence in each AWC was selected from the acquired image stack and compared for fluorescence intensity. The fluorescence intensity of slo-1p::GFP and slo-2p::GFP was compared using visual quantitative scoring between AWC<sup>CL</sup> and AWC<sup>CR</sup> in each animal, as previously performed [7,22,58].
expression of slo-1 and slo-2, and is consistent with our genetic results, which place slo-1 and slo-2 upstream of unc-43/CaMKII. The result also supports that the effect of nsy-4 and nsy-5 loss-of-function mutations on asymmetric expression of slo-1 and slo-2 was not due to the 2AWC OFF phenotype.

We also compared expression level of slo-1 and slo-2 in AWC ON and AWC OFF, and found that slo-1 and slo-2 are expressed predominantly in the AWC ON cell (Fig 3E–3H). These results are consistent with the hypothesis that slo-1 and slo-2 promote AWC ON in a cell-autonomous manner.

slo-1 and slo-2 act cell autonomously to specify AWC ON

To determine the site of slo-1 and slo-2 function in promoting AWC ON, we performed genetic mosaic analysis in slo-1(0); slo-2(0) mutants containing an integrated AWC ON marker (str-2p::GFP) transgene and the extrachromosomal array odr-3p::slo-1(overexpressor (OE)); odr-1p::DsRed or odr-3p::slo-2(0E); odr-1p::DsRed. Both odr-3p::slo-1(0E) and odr-3p::slo-2(0E) transgenes rescued the 2AWC OFF phenotype in slo-1(0); slo-2(0) mutants and also caused a slight 2AWC ON overexpression phenotype (Fig 4A and 4C). Since extrachromosomal transgenes are unstable and can be randomly lost at each cell division, the co-injected marker odr-1p::DsRed (normally expressed in both AWC) was used to indicate the presence of the slo-1(0E) or slo-2 (0E) array in AWC. Specifically, we determined if retention of the slo-1(0E) or slo-2(0E) array in only a single AWC cell causes a bias of AWC ON choice in that cell when the mosaic animals exhibited a wild-type 1AWC ON/1AWC OFF phenotype. We found that the slo-1(0E); slo-2(0) AWC became AWC ON and the slo-1(0); slo-2(0) AWC became AWC OFF in the majority of mosaic animals in which slo-1 was expressed only in a single AWC neuron (Fig 4B). Similarly, the slo-1(0); slo-2(0) AWC became AWC ON and the slo-1(0); slo-2(0) AWC became AWC OFF when mosaic animals expressed slo-2 only in a single AWC neuron (Fig 4D). Together, these results support that slo-1 and slo-2 act cell autonomously to specify AWC ON. We did observe a very small percentage of mosaic animals in which the slo-1(0); slo-2(0) AWC became AWC OFF (Fig 4B and 4D). This suggests that although slo-1 and slo-2 have a largely cell-autonomous role in promoting the AWC ON fate, they may also have a nonautonomous role. This is similar to other genes in the AWC asymmetry pathway, such as nsy-5 and nsy-4 which display both autonomous and nonautonomous roles in AWC asymmetry [20,21].

Mosaic analysis was also performed in transgenic lines in which slo-1(T1001Igf), containing the ky389gf mutation, was overexpressed in a wild-type background, resulting in a strong 2AWC ON phenotype (Fig 4E). When the transgene was retained in only one of the two AWC cells, the slo-1(gf) cell became AWC ON and the wild-type cell became AWC OFF in the majority of mosaic animals (Fig 4F). This result is consistent with a largely cell-autonomous function of slo-1 in promoting AWC ON, and also suggests that the AWC with slo-1(gf) activity may become hyperpolarized, allowing the cell to reduce calcium influx and take on the AWC ON subtype.
Fig 4. slo-1 and slo-2 act cell autonomously in promoting the AWC<sup>ON</sup> fate. (A, C) AWC phenotypes in wild type, slo-1<sup>(eg142lf)</sup>; slo-2<sup>(ok2214lf)</sup>, and slo-1<sup>(eg142lf)</sup>; slo-2<sup>(ok2214lf)</sup> expressing extrachromosomal transgenes odr-3<sup>p</sup>::slo-1<sup>(OE)</sup>; odr-1<sup>p</sup>::DsRed (A) or odr-3<sup>p</sup>::slo-2<sup>(OE)</sup>; odr-1<sup>p</sup>::DsRed (C). (B) AWC phenotypes of slo-1<sup>(eg142lf)</sup>; slo-2<sup>(ok2214lf)</sup> mosaic animals containing the extrachromosomal transgene odr-3<sup>p</sup>::slo-1<sup>(OE)</sup> in only one AWC cell, inferred by the presence of the coinjection marker odr-1<sup>p</sup>::DsRed (normally expressed in both AWC). The data was derived from a subset of data in (A). (D) AWC phenotypes of slo-1<sup>(eg142lf)</sup>; slo-2<sup>(ok2214lf)</sup> mosaic animals containing the extrachromosomal transgene odr-3<sup>p</sup>::slo-2<sup>(OE)</sup>; odr-1<sup>p</sup>::DsRed in only one AWC cell. The data was derived from a subset of data in (C). (E) AWC phenotypes in wild-type animals expressing the extrachromosomal transgene nsy-5<sup>p</sup>::slo-1<sup>(T1001lgf)</sup>.
SLO-1 and SLO-2 BK potassium channels are localized in the vicinity of UNC-2 and EGL-19 voltage-gated calcium channels in AWC axons

SLO-1 and SLO-2 have overlapping expression patterns and have been suggested to potentially form heteromeric channels [24,28,29]. In addition, it has been shown that BK channels and N-type voltage-gated calcium channels localize in close proximity to achieve functional coupling of these channels [30]. To determine if SLO-1, SLO-2, UNC-2 (N/P/Q-type calcium channels), and EGL-19 (L-type calcium channels) localize in close proximity in AWC, we generated single copy transgenes expressing functional translational reporters driven by the AWC odr-3 promoter using Mos1-mediated single copy insertion [31–33]. The tagged proteins expressed in these transgenes were functional in rescuing respective mutant phenotypes [34](S1 Fig, Materials and Methods).

These single copy insertion transgenes showed that GFP::UNC-2, SLO-1::TagRFP, SLO-1::GFP, SLO-2::TagRFP, and GFP::EGL-19 were mainly localized on the plasma membrane of AWC cell bodies and also displayed a punctate pattern along AWC axons (Fig 5 and S2 Fig), similar to the previously shown localization pattern of GFP::UNC-2 in AWC [34]. Since these channels were localized throughout the plasma membrane of the AWC cell body and had distinct punctate patterns in AWC axons, we focused on analyzing their localization in relation to each other in AWC axons. We found that both SLO-1::TagRFP and SLO-2::TagRFP were localized adjacent to GFP::UNC-2 and GFP::EGL-19 in AWC axons (Fig 5A and 5B, S2A and S2B Fig). In addition, SLO-2::TagRFP is located close to SLO-1::GFP in AWC axons (Fig 5C). The Coloc 2 plugin in Fiji was used to quantify colocalization of these proteins in AWC axons using three different algorithms (Pearson’s correlation coefficient, Spearman’s rank correlation coefficient, and Li’s ICQ). Each of the algorithms displayed positive correlation indices (Fig 5D and S2C Fig). This further supports that UNC-2 and EGL-19 localize close to SLO-1 and SLO-2, and that SLO-1 and SLO-2 are localized in close proximity as well.

These results support the notion that BK potassium channels (SLO-1 and SLO-2) and voltage-gated calcium channels (UNC-2 and EGL-19) may function in close proximity for rapid activation of SLO-1 and SLO-2 channels by locally increased calcium levels near UNC-2 and EGL-19 calcium channels.

slo-1 and slo-2 regulate the localization of synaptic markers in AWC neurons

It has been shown that communication between the pair of AWC neurons via chemical synapses in axons is important for induction of the AWC\textsuperscript{ON} subtype [11]. Our genetic data suggests that slo-1 and slo-2 are required for the specification of the induced AWC\textsuperscript{ON} subtype. In addition, SLO-1 and SLO-2 displayed distinct punctate localization patterns in AWC axons. Thus, we examined whether slo-1 and slo-2 regulate localization of synaptic markers in AWC neurons. To do so, we generated Mos1-mediated single copy insertion transgenes expressing fluorescently tagged synaptic markers, GFP::UNC-2 and YFP::RAB-3, driven by the AWC odr-3 promoter (Figs 5A, 5B and 6). UNC-2 is localized to presynaptic active zones and RAB-3 is a synaptic vesicle marker [34].

In wild type, GFP::UNC-2 was localized in the AWC axon and cell body, and YFP::RAB-3 was mainly localized in a punctate pattern in the AWC axon as shown previously [34] (Fig 6).
Fig 5. SLO-1 and SLO-2 BK potassium channels are localized in the vicinity of UNC-2 voltage-gated calcium channels in AWC axons. (A-C) Images of wild-type L1 animals expressing single copy insertion transgenes odr-3p::slo-1::TagRFP and odr-3p::GFP::unc-2 (A), odr-3p::slo-2::TagRFP and odr-3p::GFP::unc-2 (B), as well as odr-3p::slo-2::TagRFP and odr-3p::slo-1::GFP (C) in AWC neurons. SLO-1::TagRFP (A), SLO-1::GFP (C), SLO-2::TagRFP (B, C), and GFP::UNC-2 (A, B) were localized in AWC cell bodies (arrows) and in a punctate pattern along AWC axons (arrowheads). In AWC axons, SLO-1::TagRFP was
localized next to GFP::UNC-2 (A); SLO-2::TagRFP was adjacent to GFP::UNC-2 (B); and SLO-2::TagRFP was localized near SLO-1::GFP (C). Insets show higher magnification of the outlined areas that exemplify localization of two translational reporters in close proximity. Scale bar, 5 μm. Anterior is at left and ventral is at bottom. (D) Quantification of mean correlation coefficient between SLO-1 and UNC-2, SLO-2 and UNC-2, as well as SLO-1 and SLO-2 using three algorithms of the Coloc 2 plugin in Fiji. Pearson’s correlation coefficient, Spearman’s rank correlation coefficient, and Li’s ICQ. For each colocalization class, images of three animals were used for quantification. Positive values of each coefficient indicate positive correlation, values close to zero indicate no correlation, and negative values indicate anti-correlation. Pearson’s correlation coefficient ranges from -1 to +1; Spearman’s rank correlation coefficient ranges from -1 to +1; Li’s ICQ value ranges from -0.5 to +0.5. A schematic diagram of the AWC cell body, axon, dendrite, and cilia that represents the approximate region of images in A-C is shown in S2D Fig.

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Fig 6. slo-1 and slo-2 regulate the subcellular localization of synaptic markers in AWC neurons. (A) Left panels: Images of wild type, slo-1(ky399gf), and slo-1(eg142lf); slo-1(ok2214lf) mutants expressing the single copy insertion transgene odr-3p::GFP::unc-2 (the same transgene as shown in Fig 5A and 5B) in AWC cell bodies (arrows) and axons (arrowheads) in L1. Right panel: Quantification of GFP::UNC-2 fluorescence intensity in AWC axons and cell bodies. slo-1(eg142lf); slo-1(ok2214lf) mutants displayed a significant decrease in GFP::UNC-2 intensity in AWC axons and cell bodies. (B) Left panels: Images of wild-type, slo-1(ky399gf), and slo-1(eg142lf); slo-1(ok2214lf) mutants expressing the single copy insertion transgene odr-3p::YFP::rab-3 in AWC cell bodies (arrows) and axons (arrowheads) in L1. Right panel: Quantification of YFP::RAB-3 fluorescence intensity in AWC axons and cell bodies. slo-1(eg142lf); slo-1(ok2214lf) mutants had a significant decrease in YFP::RAB-3 intensity in AWC axons and cell bodies. (A, B) Anterior is at left and ventral is at bottom. Scale bar, 5 μm. Student’s t-test was used for statistical analysis. ns, not significant. Error bars, standard error of the mean. AU, arbitrary unit.

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In slo-1(ky399gf) animals, the intensity of GFP::UNC-2 or YFP::RAB-3 was not significantly affected in the AWC axon and cell body (Fig 6). However, slo-1(eg142lf); slo-2(ok2214lf) mutants displayed significant reduction in intensity of GFP::UNC-2 and YFP::RAB-3 in the AWC axon and cell body (Fig 6). These results suggest that slo-1 and slo-2 are required for localization and/or stability of synaptic markers, UNC-2 and RAB-3, in AWC neurons, which may contribute to the 2AWC^OFF phenotype caused by the slo-1(eg142lf); slo-2(ok2214lf) mutations. Our genetic mosaic analysis suggests a minor role of nonautonomous function of slo-1 and slo-2 in regulating synaptic communication of AWC neurons. In addition, autofluorescence of the gut found in wild-type animals was visibly decreased in slo-1(eg142); slo-2(ok2214lf) mutants (S3A Fig), suggesting that the SLO channels are required for gut autofluorescence.

As a control, the intensity of GFP expressed from the transgene odr-3p::GFP was analyzed in wild-type and mutant backgrounds, and no significant effect was observed in slo-1(ky399gf) and slo-1(eg142lf); slo-2(ok2214lf) mutants (S3B Fig). This result rules out the possibility that the activity of the odr-3 promoter is regulated by slo-1 and slo-2, and also supports the notion that the effect of slo-1(lf); slo-2(lf) mutations on UNC-2 and RAB-3 is mainly at the subcellular localization level. It is also possible that slo-1(lf); slo-2(lf) mutations may affect unc-2 and rab-3 at post-transcriptional levels, such as translation efficiency, mRNA and/or protein stability.

Previous studies have shown that slo-1(lf) or slo-2(lf) mutations result in increased neurotransmitter release at the neuromuscular junction in the ventral nerve cord [25,35]. However, a recent study showed that UNC-2 localization is not affected at the presynaptic terminals of neuromuscular junctions in slo-1(lf) mutants [36]. Thus, previous findings did not demonstrate a correlation between increased neurotransmitter release and increased localization of UNC-2 or RAB-3 at presynaptic sites of the neuromuscular junction in slo-1(lf) or slo-2(lf) mutants. To examine whether the localization of UNC-2 and RAB-3 is affected in ventral cord motor neurons in slo-1(lf); slo-2(lf) mutants, we quantified the intensity of GFP::UNC-2 and RAB-3::mCherry driven by the unc-25 promoter, which is expressed in ventral cord motor neurons [34]. We examined the axons located anterior to VD5 and DD3 neurons in wild type and slo-1(lf); slo-2(lf) mutants at the L4 stage, but no significant difference was observed (S4 Fig). This suggests that slo-1 and slo-2 do not play an apparent role in the localization of these presynaptic markers in the ventral nerve cord. The different effects of slo-1 and slo-2 mutations on the localization of synaptic markers in AWC neurons and ventral cord motor neurons suggest that slo-1 and slo-2 take on a different function in AWC neurons than in the ventral cord motor neurons.

Although no apparent effect of slo-1(lf); slo-2(lf) mutations on the localization of UNC-2 and RAB-3 was observed in ventral cord motor neurons, the effect of slo-1 and slo-2 mutations on locomotion was performed by analyzing the wavelength and wave width of body wave tracks of wild type, slo-1(1f); slo-2(1f), and slo-1(1f); slo-2(1f) animals. We found that the wavelength of the worm track was not affected in the mutants, however the wave width was significantly increased in slo-1(1f), slo-2(1f), and slo-1(1f); slo-2(1f) mutants (S5 Fig). These results suggest that slo-1 and slo-2 are required for normal locomotion.

**bkip-1 is required for slo-1 and slo-2 function in promoting AWC**

Previous studies have identified several modulators of SLO-1 activity in muscles using forward genetic screens. Since genes may interact in similar pathways in different tissues, we chose these candidate genes to determine whether they may also modulate SLO-1 activity in AWC neurons. bkip-1 mutants were identified from a screen for suppressors of the lethargic
phenotype of slo-1(gf) mutants. BKIP-1 (BK channel Interacting Protein), a single pass membrane protein, functions as an auxiliary subunit of SLO-1 to assist in regulating neurotransmitter release and regulate the surface expression of the channel [37]. Similar to bkip-1, ctn-1 (α-catenin), identified from two independent screens for suppressors of the slo-1(gf) lethargic phenotype, also regulates surface localization of SLO-1 in both muscles and ventral nerve cord motor neurons [38,39]. In addition, components of the dystrophin-associated protein complex (DAPC), including dys-1 (dystrophin), dyb-1 (dystrobrevin), strn-1 (syntrophin), and dyc-1 (C-terminal PDZ-domain ligand of nNOS), control the localization of SLO-1 in muscles but not in neurons [40,41]. Furthermore, islo-1, encoding a transmembrane protein, functions as an adaptor protein that links the DAPC to SLO-1 for SLO-1 localization in muscles [40].

To determine whether bkip-1, ctn-1, dys-1, and islo-1 play a role in AWC asymmetry, we first examined expression of the AWC\textsuperscript{ON} marker str-2p::GFP in their respective loss-of-function mutants, but did not see any defects in AWC asymmetry (Fig 7A). We then determined whether bkip-1(1f), ctn-1(1f), dys-1(1f), and islo-1(1f) mutants suppress the slo-1(gf) 2AWC\textsuperscript{ON} phenotype in AWC asymmetry by performing double mutant analysis. We found that dys-1 (cx18); slo-1(ky399gf), ctn-1(eg116); slo-1(ky399gf), and islo-1(eg978); slo-1(ky399gf) all displayed the same 2AWC\textsuperscript{ON} phenotype as slo-1(ky399gf) animals (Fig 7A). This suggests that dys-1, ctn-1, and islo-1 are not required for slo-1 function in AWC asymmetry. However, bkip-1(zw2) completely suppressed the 2AWC\textsuperscript{ON} phenotype of both slo-1(ky389gf) and slo-1(ky399gf) mutants to wild type (Fig 7A), indicating that bkip-1 is required for slo-1 function in promoting AWC\textsuperscript{ON}. As shown by our results, slo-1(1f) and slo-2(1f) single mutants did not display AWC asymmetry defects (Figs 1B and 7A). However, both bkip-1(1f); slo-1(1f) and bkip-1(1f); slo-2(1f) displayed a 2AWC\textsuperscript{OFF} phenotype (Fig 7A), supporting a role of bkip-1 in both slo-2 and slo-1 function, respectively. However, the 2AWC\textsuperscript{OFF} phenotype of bkip-1(1f); slo-1(1f) and bkip-1(1f); slo-2(1f) was not 100% as seen in slo-1(1f); slo-2(1f) double mutants (Fig 7A), suggesting that bkip-1 is not the only factor required for slo-1 and slo-2 function in AWC asymmetry. We also determined whether slo-2 is required for slo-1 function by crossing slo-2(1f) mutants into both slo-1(ky389gf) and slo-1(ky399gf) alleles. We found that slo-2(1f) did not suppress the slo-1(gf) 2AWC\textsuperscript{ON} phenotype (Fig 7A), suggesting that slo-2 is not required for slo-1 function in AWC asymmetry.

Previous work demonstrates a role of bkip-1 in regulating the surface expression of SLO-1 in muscle dense bodies and the nerve ring [37]. We therefore determined whether bkip-1 affects SLO-1 localization in AWC neurons by examining a functional SLO-1::GFP translational reporter driven by the AWC odr-3 promoter in wild type and bkip-1(zw2) mutants (Fig 7B). We found that in bkip-1(zw2) mutants, SLO-1::GFP intensity was significantly reduced in AWC axons (Fig 7B and 7C) but is not significantly affected in cell bodies (Fig 7D). This suggests that bkip-1 is required for appropriate localization of SLO-1 in AWC axons but not in the AWC cell body. Consistent with the result suggesting that bkip-1 is not the only factor required for slo-1 function in AWC asymmetry (Fig 7A), this result also suggests that slo-1 activity could be required in both the AWC axons (dependent on bkip-1) and cell bodies (independent of bkip-1). We also examined whether bkip-1(zw2) mutants display altered the localization of SLO-2::GFP in AWC axons, but did not find a significant effect (S6 Fig). This result suggests that slo-2 may require bkip-1 in a manner independent of appropriate localization. bkip-1 may be required for appropriate slo-2 expression levels, or BKP1 may physically interact with SLO-2.

Together, our results showed that bkip-1 is the only one of the known modulators of slo-1 activity in muscles to be also required for slo-1 and slo-2 function in AWC asymmetry. Thus, our results suggest that slo-1 and slo-2 need a different set of regulators for their function in AWC asymmetry.
Fig 7. bkip-1 modulates slo-1 and slo-2 activity in AWC neurons. (A) Genetic analysis of known modulators of SLO-1 in AWC asymmetry. (B) Images of wild type and bkip-1(zw2) L1 animals expressing odr-3p::slo-1::GFP in AWC axons and cell bodies. Scale bar, 5 μm. (C, D) Quantification of SLO-1::GFP fluorescence intensity in AWC axons (C) and AWC cell body (D). In bkip-1(zw2) mutants, SLO-1::GFP intensity is significantly decreased in AWC axons, but is not significantly affected in AWC cell body. Anterior is at left and ventral is at bottom. Student’s t-test was used for statistical analysis. ns, not significant. Error bars, standard error of the mean. AU, arbitrary unit.

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Additional voltage-gated potassium channel EGL-2 (EAG) plays a role in AWC asymmetry

The voltage-dependent activation of SLO-1 and SLO-2 channels is modulated by calcium (for SLO-1 and SLO-2) and chloride (for SLO-2) \[24,26\]. To determine whether any chloride channels or other voltage-gated potassium channels might be involved in establishing left-right AWC asymmetry, we examined AWC asymmetry in mutants of selective channels that have been shown to be expressed in the nervous system (WormBase). Although the majority of mutants examined did not display an AWC asymmetry defect (S7A Fig), a gain of function mutation in unc-103 (ERG voltage-gated potassium channel) resulted in a slight 2AWC\textsuperscript{ON} phenotype (S7B Fig). In addition, a gain of function mutation in egl-2 (EAG voltage-gated potassium channel) caused a high penetrance of the 2AWC\textsuperscript{ON} phenotype (S7B Fig), as previously shown \[11\]. We found that the egl-2(n693gf) mutation suppressed the 2AWC\textsuperscript{OFF} phenotype observed in slo-1(eg142); slo-2(ok2214) double mutants, nsy-5(ky634lf), unc-43(n498gf), and tir-1(ky648gf) single mutants (S7B Fig). This suggests that egl-2 may function downstream of these genes to promote the AWC\textsuperscript{ON} fate. Alternatively, it is possible that egl-2 may function at the same level as slo-1 and slo-2; and that the production of 2 AWC\textsuperscript{ON} neurons by egl-2(gf) in the slo-1(eg142); slo-2(ok2214) mutants is because egl-2(gf) is sufficient to cause enough membrane hyperpolarization to induce AWC\textsuperscript{ON} even in the absence of slo-1 and slo-2. Like slo-1(lf) and slo-2(lf) mutants, loss-of-function mutations in egl-2 did not cause a significant effect on AWC asymmetry nor did slo-1(lf); egl-2(lf) double mutants (S7B Fig), suggesting that egl-2 may act redundantly with other factor(s) in promoting AWC\textsuperscript{ON}.

Discussion

Here we identify an essential role of SLO BK potassium channels in asymmetric differentiation of one pair of olfactory neurons. Our findings reveal a functional link between gap junctions and SLO channels in inhibition of voltage-gated calcium channels for diversification of olfactory neurons. To the best of our knowledge, stochastic AWC asymmetry is the first system in which SLO channels are implicated in terminal neuron differentiation, stochastic cell fate determination, and left-right patterning.

Our results suggest antagonistic and parallel functions of BK potassium channels (SLO-1 and SLO-2) and voltage-gated calcium channels (UNC-2/UNC-36 and EGL-19/UNC-36) downstream of NSY-5 gap junctions in AWC asymmetry. UNC-2/UNC-36 and EGL-19/UNC-36 activate a CaMKII-MAP kinase cascade to specify the default AWC\textsuperscript{OFF} subtype, while SLO-1 and SLO-2 inhibit the calcium channel-activated kinase cascade to promote the induced AWC\textsuperscript{ON} subtype (Fig 8). Calcium and voltage are potential signals that mediate intercellular communication between the two AWC neurons and other neurons in the NSY-5 gap junction network to coordinate stochastic AWC asymmetry \[19,20\]. In addition, both SLO BK channels and voltage-gated calcium channels generate voltage and calcium signals, and are subject to calcium- and voltage-dependent activation and inactivation \[26,42\]. The regulatory loop between gap junctions, SLO BK channels, and voltage-gated calcium channels can potentially generate sustained differences in calcium-regulated signaling outputs between the two AWC cells through positive and negative feedback mechanisms, leading to asymmetric differentiation of AWC cells. This extends the previous model of NSY-5 function in AWC asymmetry by identifying SLO BK channels as the mediators of transient gap junction signaling for antagonizing voltage-gated calcium channel pathways.

Signaling via NSY-5 gap junctions may lead to transcriptional regulation of slo-1 and slo-2 in order to ensure that these genes are expressed asymmetrically in the AWC neurons. Studies have shown that connexin gap junction proteins are capable of regulating gene expression. For
example, gap junction communication mediated by Cx43 is required for ERK phosphorylation of the transcription factor Sp1, which in turn leads to appropriate expression of an osteocalcin transcriptional element [43]. It has been suggested that gap junctions may allow diffusion of second messengers such as calcium and cyclic nucleotides, which subsequently can influence gene transcription [43, 44]. It has also been suggested that C-terminal tails of connexins may bind to particular proteins, which can then contribute to regulating gene expression [44]. It is possible that NSY-5 gap junctions use similar mechanisms to regulate slo-1 and slo-2 gene expression.

SLO-1 is 55% identical to its mouse orthologue Slo1 and SLO-2 is 41% identical to its mammalian orthologue Slack, while SLO-1 is only 18% identical to its nematode paralogue SLO-2 along the entire channel peptide [28]. SLO-1/Slo1 and SLO-2/Slack have overlapping expression patterns and may form heteromeric channels [24, 28, 29]. However, functional relationships between SLO-1/Slo1 and SLO-2/Slack have not yet been demonstrated in any biological contexts. Our results show that SLO-1 localizes in close proximity to SLO-2 in AWC neurons. In addition, our results suggest that slo-1 and slo-2 have complete functional redundancy in AWC asymmetry, since loss-of-function mutations in either gene alone did not cause any defects in AWC asymmetry while slo-1(lf); slo-2(lf) double mutants displayed a complete penetrance of the 2AWCOFF phenotype. Functional redundancy between SLO-1/Slo1 and SLO-2/Slack may represent one of the general mechanisms for their roles in other systems.
The voltage range of activation of BK channels is modulated by different intracellular factors including calcium (for SLO-1, Slo1, and SLO-2), chloride (for SLO-2 and Slack), sodium (for Slack), pH, and phosphorylation [24,26]. None of the mutants of chloride channels we examined displayed any AWC asymmetry defects. In addition, although SLO-2 shares a complete redundant function with SLO-1 in AWC asymmetry, it has not been shown that the activation of SLO-1 channels is sensitive to chloride. These findings suggest that SLO-2’s redundant role with SLO-1 in establishing AWC asymmetry may be more dependent on sensitivity to calcium than to chloride.

Calcium-activated BK channels and voltage-gated calcium channels have been shown to localize in close proximity to ensure selective and rapid activation of BK channels by a local increase in cytosolic calcium level [30]. The sensitivity of vertebrate Slo1 channels to calcium provides an important negative feedback for calcium entry in many cell types. For example, activation of Slo1 channels causes transient membrane hyperpolarization, which limits calcium entry through voltage-gated calcium channels to control the burst of calcium action potentials in cerebellar Purkinje cells and to regulate synaptic transmission in presynaptic terminals [26]. Our genetic results and findings that SLO-1 and SLO-2 localize close to UNC-2 and EGL-19 voltage-gated calcium channels are consistent with the physiological roles of vertebrate Slo1 channels in inhibiting voltage-gated calcium channels through functional coupling and negative feedback. By analogy to functional coupling between Slo1 and voltage-gated calcium channels in vertebrates, SLO-1 and SLO-2 may couple with UNC-2/UNC-36 and EGL-19/UNC-36 to generate oscillation of cytosolic calcium and voltage signals to coordinate stochastic AWC asymmetry through a feedback loop. In this hypothetical feedback loop, an increase in voltage triggers voltage-gated calcium channels to open, leading to an increase in intracellular calcium levels. High calcium levels allow the coupled calcium-activated BK channels to open, resulting in a decrease in voltage. The decreased voltage causes the voltage-gated calcium channels to close, leading to a decrease in intracellular free calcium levels and the subsequent closure of calcium-activated BK channels and an increase in voltage. This would initiate another cycle of calcium and voltage oscillation. Previous studies identified two forms of intercellular communication important for AWC asymmetry: one is mediated by NSY-5 gap junctions between the cell body of AWC and other neurons in a network [19,20]; the other is by synaptic connection between two AWC axons [10,11]. Since SLO-1 and SLO-2 are localized in proximity to UNC-2 and EGL-19 at the AWC axons, functional coupling between BK channels (SLO-1 and SLO-2) and voltage-activated calcium channels (UNC-2 and EGL-19) may occur at AWC axons.

Our study has revealed that SLO-1 and SLO-2 have different functions and interacting partners in AWC olfactory neurons than in ventral cord motor neurons. Our genetic analysis suggests that BK potassium channels (SLO-1 and SLO-2) act to antagonize calcium channels (UNC-2/UNC-36 and EGL-19/UNC-36) to promote the AWCON identity. A recent report suggests that in M4 motor neurons, UNC-2 and UNC-36 function to activate SLO-1, which in turn antagonizes the EGL-19 calcium channel to inhibit synaptic transmission at the M4 neuromuscular junction [45]. A recent study showed that UNC-2 localization is not affected at the presynaptic terminals of neuromuscular junctions in slo-1(lf) mutants [36]. However, our results suggest that slo-1 and slo-2 are required for appropriate localization or stability of presynaptic markers UNC-2 and RAB-3 in AWC axons. We also show that SLO-1 and SLO-2 localize in close proximity to both UNC-2 and EGL-19 calcium channels in AWC neurons, in contrast to a report that SLO-2 exclusively couples with EGL-19 but not with UNC-2 in ventral cord motor neurons [35].

BK channels are ubiquitously expressed and have a staggering repertoire of functions in different tissues. To achieve functional diversity, BK channels, which assemble as tetramers of
pore-forming α-subunits, can form complexes with various auxiliary β-subunits. For example, the β1 subunit changes gating and calcium sensitivity of Slo1 α subunits, and β2 subunits promote fast inactivation of Slo1 channels [26]. In addition, functional diversity of Slo1 channels can be achieved by alternative splicing, posttranslational modifications, and heteromultimer formation [26]. In *C. elegans*, several modulators have been identified for surface expression and activity of SLO-1 channels in muscles and neurons [37–41]. Our results show that the auxiliary subunit BKIP-1 is the only previously identified modulator of SLO-1 to be required for SLO-1 and SLO-2 function in asymmetric AWC differentiation. AWC asymmetry may provide the ease of unbiased forward genetic screens in identifying biologically relevant genes and robust phenotypic readouts of SLO channel activity.

**Materials and Methods**

**Strains and transgenes**

Wild type is strain N2, *C. elegans* variety Bristol. Strains were maintained by standard methods [46]. Mutants used were as follows: *nys-5(ky634)* I [20], *dys-1(cx18)* I [47], *ctr-1(eg116)* I [38], *avr-14(ad1302)* I [48], *bkip-1(zw2)* II [37], *chl-1(qa900)* II, *chl-1(qa901)* II, *chl-2(ok636)* II, *chl-3(ok763)* II, *unc-36(e251)* III [46], *tir-1(ky648)* III [18], *nys-4(ky627)* IV [21], *unc-103 (el1597)* III, *egl-19(n582)* IV [49], *islo-1(eg978)* IV [40], *unc-43(n498)* IV [50], *unc-43(n1186)* IV, *slo-1(ky399)* V [11], *slo-1(ky389)* V [11], *slo-1(eg142)* V [40], *slo-1(js118)* V [25], *slo-1(js379)* V [25], *egl-2(n693)* V, *egl-2(n693n904)* V, *exp-2(n26ad1462)* V, *shw-3(ok1884)* V, *dhl-6(ok791)* V, *slo-2(ok2214)* X (C. elegans knockout consortium), *slo-2(nf100)* X [51], *e36(n728)* X, *e36(n728n398)* X, and *chl-4(ok1162)* X, *unc-2(lj1)* X [52].

Integrated transgenes used include *kyls140 [str-2p::GFP; lin-15(+)]* I [11], *vyIs76 [ceh-36p::myrTagRFP; ofm-1p::DsRed]* I, *vyIs58 [odr-1p::TagRFP]* I, *vyIs56 [odr-1p::TagRFP]* III, *vyIs68 [str-2p::TagRFP; srx3-3p::GFP]* II [7], *vyIs8 [odr-3p::slo-2c::TagRFP; unc-119(+)] I, *vyIs18 [odr-3p::GFP::unc-2; unc-119(+)] I, *vyIs23 [odr-3p::slo-1a::TagRFP; unc-119(+)] I, *vyIs38 [odr-3p::slo-1a::GFP; unc-119(+)] I, *vyIs39 [odr-3p::YFP; rab-3; unc-119(+)] I, *vyIs58 [odr-3p::slo-2::TagRFP; unc-119(+)] IV, *vyIs51 [str-2p::xnsTagRFP; ofm-1p::DsRed]* V [18], *vyIs74 [ceh-36p::myrTagRFP; ofm-1p::DsRed]* V, *otls264 [ceh-36p::TagRFP]* [53], *kyIs479 [unc-25p::GFP; unc-25::mCherry; rab-3; odr-1p::mCherry]* [34], *vyTi2 [odr-3p::GFP; egl-19]*, and *vyTi6 [odr-3p::GFP; egl-19]*. Transgenes maintained as extrachromosomal arrays include *vyEx842, 843 [nys-5p::slo-1 (7.5 ng/μl); odr-1p::DsRed (15 ng/μl); ofm-1p::DsRed (30 ng/μl)]; vyEx1573, 1574, 1575, 1576 [nys-5p::slo-1(T1001Igf) (7.5 ng/μl); odr-1p::DsRed (15 ng/μl); ofm-1p::DsRed (30 ng/μl)]; vyEx822, 823 [nys-5p::slo-1(E350Kgf) (7.5 ng/μl); odr-1p::DsRed (15 ng/μl); ofm-1p::DsRed (30 ng/μl)]; vyEx1539, 1540 [slo-1p::GFP (15 ng/μl); ofm-1p::DsRed (30 ng/μl)]; vyEx1684 [slo-1p::xnsGFP (5 ng/μl)]; vyEx1701 [slo-1p::xnsGFP (2 ng/μl); pRF-4(rol-6/su1006) (50 ng/μl)]; sEx10749 [slo-2p::GFP; pCeh361]; vyEx1122, 1151 [odr-3p::slo-1 (30 ng/μl)]; vyEx1682 [ceh-36p::myrTagRFP (5 ng/μl)]; vyEx1239 [odr-3p::slo-2c (30 ng/μl)]; odr-1p::DsRed (15 ng/μl)]; odr-1p::DsRed (30 ng/μl)]; vyEx1572 [odr-3p::slo-2d (30 ng/μl)]; odr-1p::DsRed (15 ng/μl)]; odr-1p::DsRed (30 ng/μl)]; vyEx1418 [odr-3p::slo-1::GFP (20 ng/μl)]; ofm-1p::DsRed (30 ng/μl)]; vyEx1393, 1367 [slo-1p::slo-1 (15 ng/μl); odr-1p::DsRed (15 ng/μl); ofm-1p::DsRed (30 ng/μl)]; vyEx1266 [slo-1p::slo-1::GFP (7.5 ng/μl)]; odr-1p::DsRed (30 ng/μl)]; vyEx1594 [odr-3p::slo-1::TagRFP (30 ng/μl)]; odr-1p::DsRed (30 ng/μl)]; vyEx1325, 1326 [odr-3p::slo-2c::TagRFP (30 ng/μl)]; elt-2p::GFP (5 ng/μl)]; and vyEx611 [odr-3p::GFP (7.5 ng/μl); elt-2p::CFP (7.5 ng/μl)].
Plasmid construction

To make nsy-5p::slo-1, a 3420 bp fragment of full-length slo-1a cDNA was amplified from snb-1p::slo-1a (pBK3-1) [25] and cloned into a vector containing a 5556 bp of nsy-5 promoter [20]. nsy-5p::slo-1(T1001Igf) and nsy-5p::slo-1(E350Kgf) were generated by site directed mutagenesis of nsy-5p::slo-1 using a QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). slo-1p::GFP was made by replacing slo-1a::GFP in the slo-1p::slo-1a::GFP vector containing a 5239 bp of slo-1 promoter [25] with GFP. slo-1p::slo-1 was generated by subcloning the 3420 bp of slo-1a coding region into the vector containing a 5239 bp of slo-1 promoter. odr-3p::slo-1 was made by subcloning the 3420 bp of slo-1a coding region into a vector containing the odr-3 promoter (Roayaie et al. 1998). odr-3p::slo-2c and odr-3p::slo-2d were generated by cloning 3261 bp of slo-2c and 3351 bp of slo-2d, respectively, into the odr-3p vector.

odr-3p::slo-1::GFP was made by subcloning the slo-1a::GFP translation fusion from slo-1p::slo-1a::GFP [25] into the odr-3p vector. To make odr-3p::slo-1::TagRFP, TagRFP was inserted into the slo-1a cDNA at a location corresponding to a region of the protein between S8 and S9, the same insertion site as GFP in slo-1p::slo-1a::GFP [25], using fusion PCR. odr-3p::slo-2c::TagRFP was made by inserting TagRFP into the slo-2c cDNA at a location corresponding to a region of the protein between the last 16th and 17th amino acids from the C-terminus, the same insertion site as GFP in a slo-2::GFP translation fusion construct [28], using fusion PCR. For Mos1-mediated single copy insertion (MosSCI) of these translational reporter transgenes, a pAB1 construct [14], derived from a pCF151 MosSCI insertion vector for integration on chromosome II [31], was modified to generate pAB1.1 that includes a new set of restriction enzyme sites. odr-3p::slo-1::TagRFP, odr-3p::slo-2::TagRFP, and odr-3p::YFP::rab-3 fragments were sub-cloned into pAB1.1 to generate pAB1.1::odr-3p::slo-1::TagRFP, pAB1.1::odr-3p::slo-2::TagRFP, and pAB1.1::odr-3p::YFP::rab-3 respectively. To make pAB1.1::odr-3p::GFP::unc-2, partially overlapping fragments of linearized pAB1.1 vector backbone as well as odr-3p::GFP and GFP::unc-2, both of which were PCR amplified from odr-3p::GFP::unc-2 [34], were assembled and ligated using Gibson Assembly (New England Biolabs; Ipswich, MA). pAB1.1::odr-3p::slo-1::GFP was made by Gibson Assembly of 3 partially overlapping fragments of pAB1.1::odr-3p::slo-1::TagRFP, odr-3p::slo-2::TagRFP, and unc-54 3’UTR. odr-3p::slo-2::TagRFP fragment was cloned into the pCFJ356 MosSCI insertion vector for integration on chromosome IV [33] to generate pCFJ356::odr-3p::slo-2::TagRFP. odr-3p::GFP::egl-19 miniMos construct was generated by replacing snb-1p::HALO in the snb-1p::HALO::egl-19 miniMos construct (pSAM354), containing a section of egl-19 gDNA (exons 5–9 and introns in between the exons) sandwiched between two stretches of cDNA (exons 1–4 and 10–17), with an odr-3p::GFP fragment from odr-3p::GFP::unc-2 [33]. We found that a set-18p::GFP::egl-19 transgenic array rescued the locomotory phenotypes of the egl-19(n582) hypomorph mutant, supporting that GFP::EGL-19 translational reporter is functional.

Germ line transformation

Transgenic strains were generated by injecting DNA constructs into the syncytial gonad of adult worms (P0) as previously described [54]. F1 worms expressing fluorescent transgenes were picked and cloned (1 worm per plate). The F1 clones that have F2 progeny containing fluorescent transgenes were selected as transgenic lines and analyzed.

MosSCI integrations

MosSCI lines were generated using the direct insertion protocol as previously described [31,33]. Briefly, pAB1.1::odr-3p::GFP::unc-2 (22 ng/µl), pAB1.1::odr-3p::slo-2::TagRFP (71 ng/µl), pAB1.1::odr-3p::slo-1::TagRFP (107 ng/µl), pAB1.1::odr-3p::slo-1::GFP (43 ng/µl), or
pAB1.1::odr-3p::YFP::rab-3 (26 ng/μl) was injected along with hsp16.4p::peel-1 (10 ng/μl), eft-3p::mos-1 (50 ng/μl), rab-3p::mCherry (10 ng/μl), myo-2p::mCherry (2.5 ng/μl), and myo-3p::mCherry (5 ng/μl) into ~100 EG4322 (ttTi5605 II; unc-19(ed3) III) worms cultured at 15 or 20°C. pCFJ356:odr-3p::slo-2::TagRFP (34 ng/μl) was injected along with hsp16.4p::peel-1 (10 ng/μl), eft-3p::mos-1 (50 ng/μl), rab-3p::mCherry (10 ng/μl), myo-2p::mCherry (2.5 ng/μl), and myo-3p::mCherry (5 ng/μl) into ~100 EG6703 (unc-19(ed3) III; cxTi10816 IV) worms cultured at 15 or 20°C. Three injected worms were picked to one plate and cultured at 25°C until starvation (~1 week). The starved worms were heat shocked at 34°C for two hours to activate the negative selection marker PEEL-1, which kills animals carrying extrachromosomal arrays. After recovery at 25°C for four hours, worms that were rescued for the unc-119 phenotype and lacked the three mCherry co-injection markers were cloned out from separate plates. The presence of single copy inserts was verified by PCR.

**miniMOS single copy insertion**

miniMos integration was done as previously described [55]. Briefly, odr-3p::GFP::egl-19 mini-Mos construct containing a hygromycin resistance cassette (17.5 ng/μl) was injected along with hsp16.4p::peel-1 (10 ng/μl), eft-3p::mos-1 (50 ng/μl), rab-3p::mCherry (10 ng/μl), myo-2p::mCherry (2.5 ng/μl), and myo-3p::mCherry (10 ng/μl) into ~100 vvSi8 II; unc-119(ed3) III and vvSi23 II; unc-119(ed3) III worms cultured at 15–20°C. Three injected animals were picked per plate and cultured at 25°C. Three days after injection, hygromycin was added directly onto the plate to a final concentration of 0.25 mg/ml and cultured further until starvation (~1 week). The starved worms were heat shocked at 34°C for two hours to kills animals carrying extrachromosomal arrays. After recovery at 25°C for four hours, worms that survived and lacked the three mCherry co-injection markers were cloned out to determine homozygosity.

**Imaging of transgenic worms expressing fluorescent proteins**

Transgenic strains expressing fluorescent markers or fluorescently tagged proteins were mounted onto 2% agarose pads and anesthetized with 5mM sodium azide (Sigma) or 7.5mM levamisole (Sigma). Z-stack images were acquired at room temperature (20–22°C) using Zeiss Axio Imager Z1 or M2 microscopes, each of which is equipped with a motorized focus drive, a Zeiss objective EC Plan-Neofluar 40x/1.30 Oil DIC M27, a Piston GFP bandpass filter set (41025, Chroma Technology), a TRITC filter set (41002c, Chroma Technology), and a Zeiss AxioCam CCD digital camera (MRm for Z1 and 506 mono for M2) driven by the Zeiss imaging software. Since the background autofluorescence varies between some genetic backgrounds (S3A Fig), fluorescence intensity of reporter transgenes was subtracted by background fluorescence intensity to obtain corrected fluorescence intensity. Images shown in Figs 1A, 3A, 3C, 3E, 3G, 5A, 5B, 5C, 6A, 6B and 7B, as well as S2A, S2B, S3A, S3B, S4A and S6A Figs were processed with Adobe Photoshop; the same degree of brightness and contrast adjustment was applied to all images in each set of experiments for comparison of fluorescence intensity (Figs 6A, 6B and 7B, as well as S3A, S3B and S6A Figs).

**Genetic mosaic analysis**

Genetic mosaic analysis was performed with various unstable extrachromosomal transgenic arrays in either wild type or slo-1(egl142lf); slo-2(ok2214lf) mutants. Three different experiments were performed to determine the sites of slo-1 and slo-2 function in AWC asymmetry. odr-3p::
slo-1 was injected into slo-1(lf); slo-2(lf) mutants to determine whether slo-1 acts cell autonomously or nonautonomously to rescue the 2AWC\textsuperscript{OFF} mutant phenotype. A similar experiment was performed using the odr-3p::slo-2 extrachromosomal array. In the third experiment, nsy-5p::slo-1(T1001Igf) was injected into wild-type animals. In all three experiments, the odr-1p::DsRed marker (expressed in AWC and AWB) was included in the injection mix to serve as an indicator for presence or absence of the extrachromosomal transgene in AWC. The AWC\textsuperscript{ON} and AWC\textsuperscript{OFF} neurons were determined using expression of a stable integrated str-2p::GFP (AWC\textsuperscript{ON} marker) transgene. Transgenic strains were passed for minimum of six generations to allow the transgenes to stabilize before scoring for mosaic animals.

**Colocalization analysis**

Colocalization was quantified using the Coloc 2 plugin (http://fiji.sc/Coloc_2) in Fiji [56]. Three different algorithms were used: Pearson’s correlation coefficient, Spearman’s rank correlation coefficient, and Li’s ICQ. For each colocalization class, images of at least three animals were used for quantification. Positive values of each coefficient indicate positive correlation, values close to zero indicate no correlation, and negative values indicate anti-correlation. Pearson’s correlation coefficient ranges from -1 to +1; Spearman’s rank correlation coefficient ranges from -1 to +1; Li’s ICQ value ranges from -0.5 to +0.5

**Locomotion analysis**

Locomotion analysis was performed on L4 animals in wild type, slo-1(eg142), slo-2(ok2214), and slo-1(eg142); slo2(ok2214) animals. Single animals of each genotype were placed on a bacterial lawn and allowed to make tracks. The worm tracks as well as individual worms were imaged and analyzed in ImageJ [57]. All animals were placed on the same batch of NGM plates seeded with the same batch of HB101 and were imaged on the same day. Wavelength was measured as the distance between wave peaks, and at least 3 wavelengths were measured and averaged per animal. The wavelength was normalized by the body length of the animal. Wave width was measured as the distance from the peak to the trough of the worm wave. At least 3 wave widths were measured and averaged per animal. The wave width was normalized by the body length of the animal.

**Supporting Information**

S1 Fig. (Related to Fig 5) Rescue of slo-1(lf); slo-2(lf) 2AWC\textsuperscript{OFF} phenotype by different transgenes. 2AWC\textsuperscript{ON}, both AWC cells express str-2; 1AWC\textsuperscript{OFF}/AWC\textsuperscript{ON}, only one of the two AWC cells expresses str-2; 2AWC\textsuperscript{OFF}, neither AWC cell expresses str-2.

S2 Fig. (Related to Fig 5) EGL-19 localizes in close proximity to SLO-1 and SLO-2. (A-B) Images of wild-type L1 animals expressing single copy insertion transgenes odr-3p::slo-1::TagRFP and odr-3p::GFP::egl-19 (A) as well as odr-3p::slo-2::TagRFP and odr-3p::GFP::egl-19 (B) in AWC neurons. SLO-1::TagRFP (A), SLO-2::TagRFP (B), and GFP::EGL-19 (A, B) were localized in AWC cell bodies (arrows) and in a punctate pattern along AWC axons (arrowheads). In AWC axons, SLO-1::TagRFP was localized next to GFP::EGL-19 (A); SLO-2::TagRFP was adjacent to GFP::EGL-19 (B). Insets show higher magnification of the outlined areas that exemplify localization of two translational reporters in close proximity. Scale bar, 5 μm. Anterior is at left and ventral is at bottom. (C) Quantification of mean correlation coefficient between SLO-1 and EGL-19 as well as SLO-2 and EGL-19 using 3 algorithms of the Coloc 2 plugin in Fiji: Pearson’s correlation coefficient, Spearman’s rank correlation coefficient,
and Li's ICQ. Images of four (SLO-1 and EGL-19) or six (SLO-2 and EGL-19) animals were used for quantification. Positive values of each coefficient indicate positive correlation, values close to zero indicate no correlation, and negative values indicate anti-correlation. Pearson's correlation coefficient ranges from -1 to +1; Spearman's rank correlation coefficient ranges from -1 to +1; Li's ICQ value ranges from -0.5 to +0.5. (D) Schematic diagram of the AWC cell body, axon, dendrite, and cilia. The outlined region represents the approximate region of images shown in Fig 5A, 5B and 5C, S2A and S2B Fig.

S3 Fig. (Related to Fig 6) slo-1 and slo-2 mutants alter gut autofluorescence but have no effect on expression of GFP from an AWC odr-3 promoter in AWC cells. (A) Representative images taken at identical exposure times of wild-type, slo-1(ky399gf), and slo-1(eg142lf); slo-2(ok2214lf) animals expressing the single copy insertion odr-3p::GFP::unc-2 (partial head images of the same animals were shown in Fig 6A). The gut autofluorescence of the worm is noticeably decreased in slo-1(eg142lf); slo-2(ok2214lf) mutants as compared to wild-type and slo-1(ky399gf) mutants. Scale bar, 20 μm. (B) Left panels: Images of wild type, slo-1(ky399gf), and slo-1(eg142lf); slo-2(ok2214lf) mutants expressing odr-3p::GFP in AWC in L1. Right panel: Quantification of GFP fluorescence intensity in AWC cell bodies. For each animal, GFP intensity was quantified from the single focal plane with the brightest GFP expression in the AWC cell body and subtracted by background fluorescence intensity. Scale bar, 5 μm. Student’s t-test was used for statistical analysis. ns, not significant (p = 0.6). Error bars, standard error of the mean. AU, arbitrary unit.

S4 Fig. (Related to Fig 6) slo-1 and slo-2 mutations do not affect localization of UNC-2 and RAB-3 in axons of ventral cord motor neurons. (A) Representative L4 images of wild type and slo-1(eg142); slo-2(ok2214) expressing unc-25p::GFP::unc-2 (left panels) and unc-25p::rab-3::mCherry (right panels). Scale bar, 20 μm. Arrows indicate the axon section analyzed in the left graph of panel (B), arrowheads indicate the axon section analyzed in the right graph of panel (B). Anterior is at left and ventral is at bottom. (B) Quantification of GFP::UNC-2 and RAB-3::mCherry in axons anterior to the VD5 neuron (left graph) and DD3 neuron (right graph) in wild type and slo-1(eg142); slo-2(ok2214) mutants. Student’s t-test was used for statistical analysis. ns, not significant. Error bars, standard error of the mean. AU, arbitrary unit.

S5 Fig. (Related to Fig 6) slo-1 and slo-2 are required for locomotion behavior. (A) Quantification of normalized wavelength of wild type, slo-1(eg142), slo-2(ok2214), and slo-1(eg142); slo-2(ok2214) mutants. Student’s t-test was used for statistical analysis. Error bars, standard error of the mean. ns, not significant. (B) Quantification of normalized wave width of wild type, slo-1(eg142), slo-2(ok2214), and slo-1(eg142); slo-2(ok2214) mutants. slo-1(eg142), slo-2(ok2214), and slo-1(eg142); slo-2(ok2214) have significantly greater wave widths than wild-type animals. Student’s t-test was used for statistical analysis. Error bars, standard error of the mean. (C) Schematic of body wave worm tracks and indications of wave width (red) and wavelength (green). Scale bar, 0.1 mm. All animals quantified were at the L4 stage.

S6 Fig. (Related to Fig 7) The bkip-1(lf) mutation does not affect the localization of SLO-2 in AWC. (A) Representative images of wild type and bkip-1(zw2) L1 animals expressing odr-3p::slo-2::TagRFP in AWC axons and cell bodies. Scale bar, 5 μm. (B, C) Quantification of SLO-2::TagRFP fluorescence intensity in AWC axons (B) and AWC cell body (C). In bkip-1(zw2) mutants, SLO-2::TagRFP intensity is not significantly decreased in AWC axons or AWC
cell body. Anterior is at left and ventral is at bottom. Student’s t-test was used for statistical analysis. ns, not significant. Error bars, standard error of the mean. AU, arbitrary unit.

**S7 Fig.** Other voltage-gated potassium and chloride channels in AWC asymmetry. (A) Analysis on the effect of mutations in additional voltage-gated potassium channels and chloride channels. 2AWC<sup>ON</sup>, both AWC cells express str-2; 1AWC<sup>OFF</sup>/AWC<sup>ON</sup>, only one of the two AWC cells expresses str-2; 2AWC<sup>OFF</sup>, neither AWC cell expresses str-2. (B) Analysis on the effect of mutations in egl-2 (EAG voltage-gated potassium channel) and unc-103 (ERG voltage-gated potassium channel) on AWC asymmetry.

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**Author Contributions**

Conceived and designed the experiments: AA YWH JAS CFC. Performed the experiments: AA YWH JAS XW GM CFC. Analyzed the data: AA YWH JAS XW CFC. Contributed reagents/materials/analysis tools: AA YWH JAS SAM BB EMJ CFC. Wrote the paper: AA YWH JAS EMJ CFC.

**References**

1. Alqadah A, Hsieh YW, Chuang CF (2013) microRNA function in left-right neuronal asymmetry: perspectives from C. elegans. Front Cell Neurosci 7: 158. doi: 10.3389/fncel.2013.00158 PMID: 24065887

2. Galaburda AM, Sherman GF, Rosen GD, Aboltiz F, Geschwind N (1985) Developmental dyslexia: four consecutive patients with cortical anomalies. Ann Neurol 18: 222–233. PMID: 4037763

3. Oertel-Knochel V, Linden DE (2011) Cerebral asymmetry in schizophrenia. Neuroscientist 17: 456–467. doi: 10.1177/1073858410386493 PMID: 21518811

4. Sun T, Walsh CA (2006) Molecular approaches to brain asymmetry and handedness. Nat Rev Neurosci 7: 655–662. PMID: 16858393

5. Taylor RW, Hsieh YW, Gamse JT, Chuang CF (2010) Making a difference together: reciprocal interactions in C. elegans and zebrafish asymmetric neural development. Development 137: 681–691. doi: 10.1242/dev.038695 PMID: 20147373

6. Alqadah A, Hsieh YW, Chuang CF (2014) A molecular link between distinct neuronal asymmetries. Cell Cycle 13: 1515–1516. doi: 10.4161/cc.29010 PMID: 24769883

7. Cochella L, Tursun B, Hsieh YW, Galindo S, Johnston RJ, et al. (2014) Two distinct types of neuronal asymmetries are controlled by the Caenorhabditis elegans zinc finger transcription factor die-1. Genes Dev 28: 34–43. doi: 10.1101/gad.233643.113 PMID: 24361693

8. Hobert O (2014) Development of left/right asymmetry in the Caenorhabditis elegans nervous system: From zygote to postmitotic neuron. Genesis.

9. Hsieh YW, Alqadah A, Chuang CF (2014) Asymmetric neural development in the Caenorhabditis elegans olfactory system. Genesis.

10. Chuang CF, Bargmann CI (2005) A Toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. Genes Dev 19: 270–281. PMID: 15625192
11. Troemel ER, Sagasti A, Bargmann CI (1999) Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in C. elegans. Cell 99: 387–398. PMID: 10571181

12. Wes PD, Bargmann CI (2001) C. elegans odour discrimination requires asymmetric diversity in olfactory neurons. Nature 410: 698–701. PMID: 11287957

13. Bauer Huang SL, Saheki Y, VanHoven MK, Torayama I, Ishihara T, et al. (2007) Left-right olfactory asymmetry results from antagonistic functions of voltage-activated calcium channels and the Raw repeat protein OLRN-1 in C. elegans. Neural Dev 2: 24. PMID: 17986337

14. Lesch BJ, Bargmann CI (2010) The homeodomain protein hmbx-1 maintains asymmetric gene expression in adult C. elegans olfactory neurons. Genes Dev 24: 1802–1815. doi: 10.1101/gad.1932610 PMID: 20713521

15. Lesch BJ, Gehrke AR, Bulyk ML, Bargmann CI (2009) Transcriptional regulation and stabilization of left-right neuronal identity in C. elegans. Genes Dev 23: 345–358. doi: 10.1101/gad.1763509 PMID: 19204119

16. Sagasti A, Hisamoto N, Hyodo J, Tanaka-Hino M, Matsumoto K, et al. (2007) Left-right olfactory asymmetry results from antagonistic functions of voltage-activated calcium channels and the Raw repeat protein OLRN-1 in C. elegans. Neural Dev 2: 24. PMID: 17986337

17. Lesch BJ, Gehrke AR, Bulyk ML, Bargmann CI (2009) Transcriptional regulation and stabilization of left-right neuronal identity in C. elegans. Genes Dev 23: 345–358. doi: 10.1101/gad.1763509 PMID: 19204119

18. Chang C, Hsieh YW, Lesch BJ, Bargmann CI, Chuang CF (2011) Microtubule-based localization of a synaptic calcium-signaling complex is required for left-right neuronal asymmetry in C. elegans. Development 138: 3509–3518. doi: 10.1242/dev.069740 PMID: 21771813

19. Schumacher JA, Hsieh YW, Chen S, Pirri JK, Alkema MJ, et al. (2012) Intercellular calcium signaling in a gap junction-coupled cell network establishes asymmetric neuronal fates in C. elegans. Development 139: 4191–4201. doi: 10.1242/dev.083428 PMID: 23093425

20. Vanhoven MK, Bauer Huang SL, Albin SD, Bargmann CI (2006) The claudin superfamily protein nsy-4 biases lateral signaling to generate left-right asymmetry in Caenorhabditis elegans olfactory neurons. Neuron 51: 291–302. PMID: 16880124

21. Hsieh YW, Chang C, Chuang CF (2012) The microRNA mir-71 inhibits calcium signaling by targeting the TIR-1/Sarm1 adaptor protein to control stochastic L/R neuronal asymmetry in C. elegans. PLoS Genet 8: e1002864. doi: 10.1371/journal.pgen.1002864 PMID: 22876200

22. Davies AG, Pierce-Shimomura JT, Kim H, VanHoven MK, Thiele TR, et al. (2003) A central role of the BK potassium channel in behavioral responses to ethanol in C. elegans. Cell 115: 655–666. PMID: 14675531

23. Saltzberg, A, Foe, E, Sulston, J (1988) WormBook: 1–15.

24. Wang ZW, Saltzberg, A, Sulston, J, Hart, B (2000) SLO-1 potassium channels control quantal content of neurotransmitter release at the C. elegans neuromuscular junction. Neuron 32: 867–881. PMID: 11738032

25. Salkoff L, Butler A, Ferreira G, Santi C, Wei A (2006) High-conductance potassium channels of the SLO family. Nat Rev Neurosci 7: 921–931. PMID: 17115074

26. Lim HH, Park BJ, Choi HS, Park CS, Eom SH, et al. (1999) Identification and characterization of a novel voltage-dependent potassium channel gene (Ce-slo-2) in C. elegans. Gene 240: 35–43. PMID: 10564810

27. Yuan A, Dourado M, Butler A, Walton N, Wei A, et al. (2000) SLO-2, a K+ channel with an unusual Cl-dependence. Nat Neurosci 3: 771–779. PMID: 10903569

28. Joiner WJ, Tang MD, Wang LY, Dworetzky SI, Boissard CG, et al. (1998) Formation of intermediate-conductance calcium-activated potassium channels by interaction of Slack and Slo subunits. Nat Neurosci 1: 462–469. PMID: 10196543

29. Marrion NV, Tavolini SJ (1998) Selective activation of Ca2+-activated K+ channels by co-localized Ca2 + channels in hippocampal neurons. Nature 395: 900–905. PMID: 9804423

30. Frokjaer-Jensen C, Davis MW, Hopkins CE, Newman BJ, Thummel JM, et al. (2008) Single-copy insertion of transgenes in Caenorhabditis elegans. Nat Genet 40: 1375–1393. doi: 10.1038/ng.248 PMID: 18953339
32. Roayaie K, Crump JG, Sagasti A, Bargmann CI (1998) The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilia morphogenesis in C. elegans olfactory neurons. Neuron 20: 55–67. PMID: 9459442
33. Frokjaer-Jensen C, Davis MW, Allion M, Jorgensen EM (2012) Improved Mos1-mediated transgenesis in C. elegans. Nat Methods 9: 117–118. doi: 10.1038/nmeth.1865 PMID: 22290181
34. Saheki Y, Bargmann CI (2009) Presynaptic CaV2 calcium channel traffic requires CALF-1 and the alpha(2)delta subunit UNC-36. Nat Neurosci 12: 1257–1265. doi: 10.1038/nn.2383 PMID: 19718034
35. Liu P, Chen B, Wang ZW (2014) SLO-2 potassium channel is an important regulator of neurotransmitter release in Caenorhabditis elegans. Nat Commun 5: 5155. doi: 10.1038/ncomms5155 PMID: 25300429
36. Oh KH, Abraham LS, Gegg C, Silvestri C, Huang YC, et al. (2015) Presynaptic BK channel localization is dependent on the hierarchical organization of alpha-catulin and dystrobrevin and fine-tuned by CaV2 calcium channels. BMC Neurosci 16: 26. doi: 10.1186/s12868-015-0166-2 PMID: 25907097
37. Chen B, Ge Q, Xia XM, Liu P, Wang SJ, et al. (2010) A novel auxiliary subunit critical to BK channel function in Caenorhabditis elegans. J Neurosci 30: 16651–16661. doi: 10.1523/JNEUROSCI.3211-10.2010 PMID: 21148004
38. Abraham LS, Oh HJ, Sancar F, Richmond JE, Kim H (2010) An alpha-catulin homologue controls neuromuscular function through localization of the dystrophin complex and BK channels in Caenorhabditis elegans. PLoS Genet 6.
39. Chen B, Liu P, Wang SJ, Ge Q, Zhan H, et al. (2010) alpha-Catulin CTN-1 is required for BK channel subcellular localization in C. elegans body-wall muscle cells. EMBO J 29: 3184–3195. doi: 10.1038/embj.2010.194 PMID: 20700105
40. Kim H, Pierce-Shimomura JT, Oh HJ, Johnson BE, Goodman MB, et al. (2009) The dystrophin complex controls bk channel localization and muscle activity in Caenorhabditis elegans. PLoS Genet 5: e1000780. doi: 10.1371/journal.pgen.1000780 PMID: 20019812
41. Sancar F, Touroutine D, Gao S, Oh HJ, Gendrel M, et al. (2011) The dystrophin-associated protein complex maintains muscle excitability by regulating Ca(2+)-dependent K(+) (BK) channel localization. J Biol Chem 286: 33501–33510. doi: 10.1074/jbc.M111.227678 PMID: 21795674
42. Catterall WA (2000) Structure and regulation of voltage-gated Ca2+ channels. Annu Rev Cell Dev Biol 16: 521–555. PMID: 11031246
43. Stains JP, Civitelli R (2005) Gap junctions regulate extracellular signal-regulated kinase signaling to affect gene transcription. Mol Biol Cell 16: 64–72. PMID: 15525679
44. Giepmans BN (2004) Gap junctions and connexin-interacting proteins. Cardiovasc Res 62: 233–245. PMID: 15094344
45. Steciuk M, Cheong M, Walte C, You YJ, Avery L (2014) Regulation of synaptic transmission at the Caenorhabditis elegans M4 neuromuscular junction by an antagonistic relationship between two calcium channels. G3 (Bethesda) 4: 2535–2543.
46. Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77: 71–94. PMID: 4366476
47. Bessou C, Giugia JB, Franks CJ, Holden-Dye L, Segalat L (1998) Mutations in the Caenorhabditis elegans dystrophin-like gene dys-1 lead to hyperactivity and suggest a link with cholinergic transmission. Neurogenetics 2: 61–72. PMID: 9933302
48. Flavell SW, Pokala N, Macosko EZ, Albrecht DR, Larsch J, et al. (2013) Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in C. elegans. Cell 154: 1023–1035. doi: 10.1016/j.cell.2013.08.001 PMID: 23972393
49. Lee RY, Lobel L, Hengartner M, Horvitz HR, Avery L (1997) Mutations in the alpha1 subunit of an L-type voltage-activated Ca2+ channel cause myotonia in Caenorhabditis elegans. EMBO J 16: 6066–6076. PMID: 9231396
50. Park EC, Horvitz HR (1986) Mutations with dominant effects on the behavior and morphology of the nematode Caenorhabditis elegans. Genetics 113: 821–852. PMID: 3744028
51. Santi CM, Yuan A, Fawcett G, Wang ZW, Butler A, et al. (2003) Dissection of K+ currents in Caenorhabditis elegans muscle cells by genetics and RNA interference. Proc Natl Acad Sci U S A 100: 14391–14396. PMID: 14612577
52. Tam T, Mathews E, Snutch TP, Schafer WR (2000) Voltage-gated calcium channels direct neuronal migration in Caenorhabditis elegans. Dev Biol 226: 104–117. PMID: 10993677
53. Patel T, Tursun B, Rahe DP, Hobert O (2012) Removal of Polycomb repressive complex 2 makes C. elegans germ cells susceptible to direct conversion into specific somatic cell types. Cell Rep 2: 1178–1186. doi: 10.1016/j.celrep.2012.09.020 PMID: 23103163
54. Mello C, Fire A (1995) DNA transformation. Methods Cell Biol 48: 451–482. PMID: 8531738
55. Frokjaer-Jensen C, Davis MW, Sarov M, Taylor J, Flibotte S, et al. (2014) Random and targeted transgene insertion in Caenorhabditis elegans using a modified Mos1 transposon. Nat Methods 11: 529–534. doi: 10.1038/nmeth.2889 PMID: 24820376

56. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, et al. (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9: 676–682. doi: 10.1038/nmeth.2019 PMID: 22743772

57. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9: 671–675. PMID: 22930834

58. Didiano D, Hobert O (2008) Molecular architecture of a miRNA-regulated 3’ UTR. RNA 14: 1297–1317. doi: 10.1261/rna.1082708 PMID: 18463285