SLO potassium channels antagonize premature decision making in C. elegans

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Animals must modify their behavior with appropriate timing to respond to environmental changes. Yet, the molecular and neural mechanisms regulating the timing of behavioral transition remain largely unknown. By performing forward genetics to reveal mechanisms that underlie the plasticity of thermotaxis behavior in C. elegans, we demonstrated that SLO potassium channels and a cyclic nucleotide-gated channel, CNG-3, determine the timing of transition of temperature preference after a shift in cultivation temperature. We further revealed that SLO and CNG-3 channels act in thermosensory neurons and decelerate alteration in the responsiveness of these neurons, which occurs prior to the preference transition after a temperature shift. Our results suggest that regulation of sensory adaptation is a major determinant of latency before animals make decisions to change their behavior.
One of the central issues in decision-making research is what determines the timing of behavior transition, during which animals learn from a new experience and abandon old knowledge\(^1,2\). Appropriate latency before behavior transition is necessary for animals to discriminate long-lasting environmental changes from temporary changes. For instance, animals keep the state of hibernation irrespective of daily change but emerge from it in response to seasonal change\(^3\). Nevertheless, if the latency is too long, animals lose opportunities to obtain rewards or fail to avoid dangers. Although regulation of the timing of behavior transition in response to environmental changes is critical for animals, the underlying molecular and neuronal principles of this process remain largely unknown.

To understand how the timing of behavior transition is determined, we examined the plasticity of the thermotaxis behavior of *Caenorhabditis elegans* (i.e., association of cultivation temperature with the existence of food and migration toward that temperature on a thermal gradient with no food)\(^4,5\) (Fig. 1a). The thermotaxis of *C. elegans* is essentially regulated by a simple neural circuit\(^6,7\) (Fig. 1b). AFD is a major thermosensory neuron in this circuit that increases the intracellular concentration of Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) in response to a rise in temperature above the past cultivation temperature\(^7-11\). Since this cultivation temperature dependency in the AFD dynamic range is well conserved, even when AFD cells are cultured in isolation from the neural network in vitro, AFD cell-autonomously encodes information regarding cultivation temperature\(^8\). Genetic analyses have revealed the molecular components involved in temperature sensation in AFD. Three receptor-type guanylyl cyclases, GCY-8, GCY-18, and GCY-23, are specifically localized to the sensory ending of AFD and are thought to act as thermosensors\(^12,13\). These guanylyl cyclases synthesize cGMP, which activates cyclic nucleotide-gated (CNG) channels composed of TAX-2 and TAX-4\(^14,15\), leading to an influx of Ca\(^{2+}\) into AFD.

Thermotaxis behavior is plastic; when cultivation temperature shifts, animals change their temperature preference to the new cultivation temperature over the course of a few hours\(^6\) (Fig. 1c, e). Further, AFD neurons that are essential for thermotaxis acclimate to a new temperature by changing the dynamic range of responsive temperature\(^6,16\). However, the molecular mechanisms underlying the transition of the temperature preference and the AFD dynamic range are unknown.

In this study, we performed a forward genetic screen for mutants that were slow to change their temperature preference in thermotaxis and demonstrated that a gain-of-function (gf) mutation in the SLO-2 K\(^{+}\) channel decelerated the preference transition. The *slo-1* gene encodes a Ca\(^{2+}\)-dependent K\(^{+}\) channel, which consists of an N-terminal transmembrane domain and a large C-terminal intracellular regulatory domain\(^20\). The H159Y mutation in *nj131* mutants is within the third helix (S3) of transmembrane domain\(^21\) (Supplementary Fig. 2). To examine the effects of H159Y mutation on the function of the SLO-2 channel, using the patch clamp method, we recorded whole-cell currents from HEK293T cells expressing either wild-type or H159Y mutant of SLO-2 channels. As reported previously\(^20\), depolarization failed to increase outward currents of wild-type and mutant channels when [Ca\(^{2+}\)]\(_o\) = 0 but increased it when [Ca\(^{2+}\)]\(_o\) > 0.6 \(\mu\)M (Fig. 2a, b). At 0.2 \(\mu\)M [Ca\(^{2+}\)]\(_o\), the H159Y mutation was clearly activated in a membrane potential-dependent manner, while the wild-type was activated only slightly. The conductance–membrane potential (GV) relationship of both wild-type and mutant channels was shifted toward hyperpolarized potentials with an increase in [Ca\(^{2+}\)]\(_o\). At the

### Results

**Isolation of *slo-2* gf mutant that is slow to change behavior.** To reveal the molecular mechanisms that determine the timing of behavior transition, we performed a forward genetic screen for mutants that were slow to change their temperature preference after a shift in cultivation temperature (Supplementary Fig. 1a). One of the mutants we isolated was *nj131*. When cultivated first at 17 °C and then re-cultivated at 23 °C for 3 h, wild-type animals migrated to a warm region, suggesting that 3 h of cultivation at a new temperature is sufficient to change their temperature preference. By contrast, a majority of *nj131* animals that were cultivated first at 17 °C and then at 23 °C for 3 h still migrated to a cold region, showing the old temperature preference (Fig. 1d). Nevertheless, *nj131* mutants eventually migrated to a warm region like the wild-type animals approximately 24 h after being transferred from 17 °C to 23 °C (Fig. 1d, e). When *nj131* mutants were cultivated constantly at 23 °C, they migrated to the warm region like the wild-type animals (Fig. 1d, f), suggesting that *nj131* mutants were not simply cryophilic but slow to change their behavior. Hereafter, we designate this abnormality of *nj131* mutants as a “slow-learning” (Slw) phenotype. When *nj131* mutants were first cultivated at 23 °C and then at 17 °C for 3 h, they migrated to a cold region, as did the wild-type animals (Supplementary Fig. 1c–e). This result suggests that *nj131* mutants exhibit the Slw phenotype specifically in response to temperature upshift.

Single-nucleotide polymorphism (SNP) mapping and whole-genome sequencing revealed that *nj131* was a missense mutation in the *slo-2* gene, which alters histidine residue 159 of the gene product to tyrosine (H159Y) (Supplementary Fig. 1b). We then examined whether *slo-2* expression can rescue the Slw phenotype in *nj131* mutants. Whereas injection of a PCR fragment containing *slo-2* genomic DNA into *nj131* animals did not rescue the Slw phenotype, introduction of mutant or wild-type forms of SLO-2 into wild-type animals phenocopied *nj131* mutants (Fig. 1g), suggesting that *nj131* is a gf allele of the *slo-2* gene. When cultivated constantly at 23 °C, both animals expressing the mutant form of SLO-2 and the wild-type animals migrated to a warm region (Supplementary Fig. 1f). The wild-type animals migrated to a warm region like the wild-type animals when cultivated *nj131* mutants is essentially regulated by a simple neural circuit\(^12,13\). These guanylyl cyclases synthesize cGMP, which activates cyclic nucleotide-gated (CNG) channels composed of TAX-2 and TAX-4\(^14,15\), leading to an influx of Ca\(^{2+}\) into AFD.

Thermotaxis behavior is plastic; when cultivation temperature shifts, animals change their temperature preference to the new cultivation temperature over the course of a few hours\(^6\) (Fig. 1c, e). Further, AFD neurons that are essential for thermotaxis acclimate to a new temperature by changing the dynamic range of responsive temperature\(^6,16\). However, the molecular mechanisms underlying the transition of the temperature preference and the AFD dynamic range are unknown.

In this study, we performed a forward genetic screen for mutants that were slow to change their temperature preference in thermotaxis and demonstrated that a gain-of-function (gf) mutation in the SLO-2 K\(^{+}\) channel decelerated the preference transition. The *slo-1* gene encodes the other member of the SLO family of K\(^{+}\) channel in *C. elegans*. Interestingly, the preference transition was accelerated in animals with *slo-2* and *slo-1* loss-of-function (lf) mutations. Calcium imaging of AFD revealed that calcium imaging of AFD revealed that calcium increase the intracellular concentration of Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) in response to a rise in temperature above the past cultivation temperature\(^7-11\). Since this cultivation temperature dependency in the AFD dynamic range is well conserved, even when AFD cells are cultured in isolation from the neural network in vitro, AFD cell-autonomously encodes information regarding cultivation temperature\(^8\). Genetic analyses have revealed the molecular components involved in temperature sensation in AFD. Three receptor-type guanylyl cyclases, GCY-8, GCY-18, and GCY-23, are specifically localized to the sensory ending of AFD and are thought to act as thermosensors\(^12,13\). These guanylyl cyclases synthesize cGMP, which activates cyclic nucleotide-gated (CNG) channels composed of TAX-2 and TAX-4\(^14,15\), leading to an influx of Ca\(^{2+}\) into AFD.

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It was recently reported that gf mutations in a human SLO-2 homolog, Slack/KCNT1, cause early-onset epilepsy\(^17,18\). We found that some epilepsy-related mutations potentiated *C. elegans* SLO-2 in decelerating temperature preference transition. These results imply that the early-onset epilepsy and the latency regulation in *C. elegans* thermotaxis might have similar molecular and physiological mechanisms.
Fig. 1 Gain of SLO-2 function decelerates transition of preference in thermotaxis behavior. a A scheme for a thermotaxis assay is shown. C. elegans cultivated at a certain temperature is placed at the center of a linear thermal gradient without food and is allowed to freely migrate for 1 h. b A neural circuit regulating thermotaxis is shown. c, d Wild-type (c) and slo-2(nj131gf) (d) animals were cultivated at 17 °C for 5 days and then at 23 °C for the time indicated or constantly at 23 °C for 3 days. The animals were then placed on a thermal gradient. The number of animals in each section of the thermal gradient was determined, and the proportion of animals in each region was plotted on a histogram. e The thermotaxis indices at each time point (c, d) were plotted against time after the cultivation temperature was changed to 23 °C. Horizontal bars indicate medians. **p < 0.01, ***p < 0.001 (Welch two-sample t test). f The thermotaxis indices are shown for animals cultivated constantly at 23 °C in c, d, g Genomic PCR fragments covering slo-2 gene locus that were derived from either wild-type or nj131 mutant animals were injected into either wild-type or nj131 animals. Animals were cultivated at 17 °C for 5 days and at 23 °C for 3 h and then subjected to thermotaxis assay, as described above. Animals with extra chromosomal arrays were scored to evaluate thermotaxis. The fractions of animals were plotted on histograms (upper), and the thermotaxis indices were shown on boxplots (lower). n = 24, 5, 14, 14, 9 for each strain. The indices of strains marked with distinct alphabets differ significantly (p < 0.001) according to Tukey-Kramer test. h, i Wild-type and indicated slo-2 mutant animals were cultivated at 17 °C for 5 days and at 23 °C for 3 h and then subjected to thermotaxis assay. n = 4 or 5. p Values are indicated (Dunnett test against wild-type (h) or Tukey-Kramer test (i)). See also Supplementary Fig. 1.

Therefore, H159Y point mutation increases SLO-2 channel activity, which is consistent with the results of genetic analyses of C. elegans behavior (Fig. 1g, h).

slo-1; slo-2 double mutants are faster to change temperature preference. Since a gf mutation in slo-2 decelerated the transition of temperature preference, we examined whether loss of slo-2...
accelerated the transition. We revealed that the preference transition was not accelerated in slo-2(nf101) deletion mutants (Fig. 3c, e). Then we further examined animals lacking both SLO-2 and SLO-1. SLO-1 is structurally homologous to SLO-2 (Supplementary Fig. 2), and SLO-1 and SLO-2 channels constitute the SLO family of the K^+ channel in C. elegans. In slo-1(eg142); slo-2(nf101) double lf mutants, preference transition was accelerated at the time points of 45 min and 1 h after an upshift in cultivation temperature (Fig. 3d, e). Another double lf mutant, slo-1(gk26250); slo-2(ok2214), also showed an accelerated preference transition (Supplementary Fig. 3b and c). These results suggest that endogenous SLO K^+ channels function redundantly in response to an upshift in cultivation temperature. Both slo-1(lf) and slo-2(lf) single mutants were normal, suggesting that both
SLO-1 and SLO-2 act independently. After a downshift in cultivation temperature, slo-1(gq142); slo-2(nf101) double If mutants changed their preference within the same period as the wild-type animals (Supplementary Fig. 3d), suggesting that SLO K+ channels regulate preference transition specifically when temperature is increased. Although vertebrate homologs of SLO-2 and SLO-1 were shown to interact23, slo-2(nj131gf) mutation decelerated preference transition even in the absence of SLO-1 (Supplementary Fig. 3e), indicating that the gf mutant form of SLO-2 can act independently of SLO-1.

SLO K+ channels act in AFD thermosensory neuron. Since slo-2 is expressed broadly in neurons and muscles (Supplementary Fig. 4a)20, we aimed to identify the cells in which SLO K+ channels act during temperature preference transition. Expression of the H159Y gf form of SLO-2 pan-neuronally or specifically in AFD thermosensory neuron photocopied slo-2(nj131gf) mutants, while expression in the AWC chemosensory and thermosensory neuron, in interneurons involved in the regulation of thermotaxis, or in body wall muscles did not (Fig. 4a). These results suggest that SLO-2 acts in AFD to decelerate preference transition.

The abilities of the wild-type and H159Y mutant form of SLO-2 to decelerate preference transition were compared by expressing various concentrations of each form in AFD. Injection of a plasmid containing the H159Y form at 1 ng/μl decelerated the preference transition more than injection of the wild-type at 20 ng/μl, suggesting that the H159Y form is far more potent in decelerating the preference transition (Supplementary Fig. 4b and c). These results are consistent with electrophysiological experiments revealing that mutant channels engage in higher levels of activity (Fig. 2c–e).

We next examined whether the accelerated preference transition in slo-1; slo-2 double mutants was caused by the loss of SLO channels in AFD. Expression of SLO-1 or SLO-2 in AFD reversed the accelerated preference transition in slo-1; slo-2 mutants (Fig. 4b, c), indicating that loss of SLO-1 and SLO-2 causes accelerated preference transition and that SLO-1 and SLO-2 act in AFD. Together with the results of the cell-specific expression experiments (Fig. 4a), it can be concluded that SLO K+ channels act in AFD to decelerate preference transition.

CNG-3 CNG channel functions with SLO-2. To investigate how SLO K+ channels decelerate preference transition, we performed a genetic screen for the suppressors of slo-2(nj131gf) mutants to isolate molecules that functionally interact with SLO-2 (Supplementary Fig. 5a). Of the mutagenized slo-2(nj131gf) mutant animals, we isolated nj172 mutation, which partially suppressed the Slw phenotype of slo-2(nj131gf) (Fig. 5a, b, d, g). When cultivated at a constant temperature of 17°C, both nj172; slo-2(nj131gf) double mutants and wild-type animals migrated to a cold region (Fig. 5d, 0 h), suggesting that nj172 does not make animals simply thermophilic but suppresses the Slw phenotype in slo-2(nj131gf) animals.

SNP mapping and subsequent rescue experiments showed that nj172 is an allele of cng-3 (Fig. 5i, Supplementary Fig. 5h and i). The cng-3 gene encodes a subunit of the CNG cation channel. Whole-genome sequencing revealed that nj172 mutants carried a missense mutation in the cng-3 gene, which alters methionine residue 467 to isoleucine (M467I) within a conserved C-terminal cyclic nucleotide-binding domain of the gene product24. When cultivated constantly at 23°C, nj172; slo-2(nj131gf) animals expressing cng-3 migrated to a warm region similarly to wild-type animals (Supplementary Fig. 5i), suggesting that cng-3 expression does not make animals simply cryptophilic but cancels the suppression of slo-2(nj131gf) by cng-3(nj172).

We also characterized cng-3(jh113) deletion mutants. When first cultivated at 17°C and then at 23°C for 3 h, cng-3(jh113); slo-2(nj131gf) animals migrated toward the warm region on a thermal gradient (Fig. 5i, g), suggesting that cng-3(jh113) deletion mutation also suppresses the Slw phenotype of slo-2(nj131gf) animals. The extent of suppression seemed stronger than that of cng-3(nj172) mutation, suggesting that cng-3(nj172) is a reduction-of-function allele. When cultivated constantly at 17°C or 23°C, both cng-3(jh113); slo-2(nj131gf) and cng-3(jh113) mutants did not show a clear preference for a cultivation temperature (Fig. 5e–h)25. The abnormal thermotaxis in cng-3(jh113) mutants cultivated constantly at 17°C or 23°C were rescued by expressing CNG-3 in AFD (Supplementary Fig. 6a and b). These results indicate that CNG-3 is necessary for thermotaxis after cultivation under constant temperature but unnecessary for acquisition of a new preference after a shift in cultivation temperature. Other CNG subunits, TAX-2 and TAX-4, are critical for thermosensation; animals lacking either TAX-2 or TAX-4 were completely athermotactic, regardless of whether cultivation temperature was shifted (Supplementary Fig. 6c).

It was reported that CNG-3 is expressed in sensory neurons, including AFD26,27. To determine the site(s) at which CNG-3 acts, we cell-specifically expressed CNG-3 in cng-3(nj172); slo-2(nj131gf) mutants. Expression of CNG-3 in AFD but not in AWC canceled suppression by cng-3(nj172) (Fig. 5j), suggesting that, like SLO channels, CNG-3 functions in AFD (Fig. 4).
became comparable to that for wild-type animals (Fig. 6h). These results suggest that slo-2(nj131gf) mutation slowed down transition of the AFD dynamic range (i.e., AFD adaptation). AFD adaptation preceded behavioral transition in both wild-type animals and slo-2(nj131gf) mutants (Figs. 1e and 6h). These results indicate that a mechanism is acting either downstream of the Ca2+ influx in AFD or in downstream neural circuits, which may explain that the transition of the AFD dynamic range is transformed onto the behavior with delay. Moreover, expression of the gf form of SLO-2 specifically in AFD neurons lowered the onset temperature for animals cultivated first at 17 °C and then at 23 °C for 3 h (Fig. 6e, g), suggesting that enhanced SLO-2 currents in AFD slow down AFD adaptation cell-autonomously.

Since the Slw phenotype in slo-2(nj131gf) mutants was suppressed by cng-3 mutation, we next examined whether the slowed AFD adaptation in slo-2(nj131gf) mutants after an upshift in cultivation temperature was also suppressed by cng-3 mutation. When cultivated first at 17 °C and then at 23 °C for 3 h, cng-3(jh113) suppressed the lowered onset temperature of AFD in slo-2(nj131gf) animals to a level comparable to that of wild-type animals (Fig. 6d, g). Moreover, AFD-specific expression of CNG-3 in cng-3(jh113); slo-2(nj131gf) double mutants canceled the suppression of decelerated adaptation by cng-3 if mutation (Fig. 6f, g). These results suggest that CNG-3 functionally interacts with SLO-2 in AFD to slow down AFD adaptation and thereby to decelerate behavioral transition. Although cng-3(jh113) mutants showed aberrant thermotaxis behavior when cultivated constantly at 17 °C or at 23 °C (Fig. 5e), AFD in cng-3(jh113) mutants responded to a temperature increase at around the cultivation temperature, like the wild-type animals, except for a slower decrease in the level of Ca2+ (Fig. 6c). This is in a sharp contrast to tax-2 or tax-4 mutants, in which AFD is totally inactive.

We next examined how endogenous SLO channels affect the timing of changes in the AFD dynamic range. The dynamic range of AFD changes within a few hours after a shift in cultivation temperature, whereas AFD is responsive to an increase in temperature beyond the temperature 5–6 °C higher than the cultivation temperature. Thus animals cultivated at 17 °C were investigated when their AFD regained the responsiveness after an upshift in cultivation temperature. To do so, we used a temperature program that produced short-term temperature oscillations (20 s, 0.05 Hz) around 17 °C followed by a shift to 23 °C, which mimicked an upshift in cultivation temperature. In wild-type animals, AFD exhibited phase-locked responses to oscillations around 17 °C, a large response to a temperature upshift and gradual Ca2+ decrease, and then faintly regained phase-locked responses to oscillations around 23 °C 20 min after the upshift (Fig. 7a). In contrast, in slo-2(nj131gf) animals, AFD showed a sharp decrease in Ca2+ level and profound silencing after the temperature upshift and did not regain the ability for phase-locked response in 20 min (Fig. 7b). On the other hand, in slo-1; slo-2 double lf mutants, AFD showed clear phase-locked responses to oscillations from an earlier time point than wild-type animals (Fig. 7e). Use of the Fourier transform to examine the AFD responses further supported these observations (Fig. 7f–j). First, Fourier transform of the sequence of temperature revealed a clear peak at 0.05 Hz. The peak at 0.05 Hz in the AFD responses of slo-1; slo-2 double lf mutants was much more explicit than that of wild-type animals (Fig. 7f, j). In time courses of the Fourier transform, the Fourier components of AFD response other than those at or near 0.05 Hz disappeared earlier in slo-1; slo-2 double lf mutants than in wild-type animals (Fig. 7k, o). Taken together, these results further support that SLO K+ channels
slow down AFD adaptation after an upshift in cultivation temperature.

**Epilepsy-related mutations potentiate SLO-2.** Early-onset forms of epilepsy such as malignant migrating partial seizures of infancy and autosomal-dominant nocturnal frontal lobe epilepsy can be caused by gl mutations in the *slo-2* human homolog Slack/KCNT1, which encodes a Na+-gated K+ channel mainly expressed in the brains. Epilepsy-related mutant forms of the Slack channel feature enhanced channel activity. Analogously, the H159Y mutant form of *C. elegans* SLO-2 isolated in this study featured higher channel activity, suggesting that both human epilepsy and decelerated temperature preference transition in thermotaxis are caused by excess K+ efflux through

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**Fig. 4** SLO K+ channels act in the AFD thermosensory neuron to decelerate preference transition in thermotaxis. a Animals expressing the H159Y mutant form of SLO-2 isoform b under the control of promoters indicated were cultivated at 17 °C for 5 days and then at 23 °C for 3 h and subjected to thermotaxis assay. The fractions of animals and thermotaxis indices are plotted. n = 17, 17, 4, 5, 4, 4. ***p < 0.001 (Dunnett test against wild type). b Wild-type animals, wild-type animals that express SLO-1 in AFD after insertion of a single copy of a transgene into a genome, *slo-1*(eg142); *slo-2*(nf101) animals, and *slo-1*(eg142); *slo-2*(nf101) animals that express SLO-1 in AFD after insertion of a single copy of a transgene were cultivated at 17 °C for 5 days and then at 23 °C for 1 h. Animals were then subjected to thermotaxis assay. n = 4. p Values are indicated (Tukey-Kramer test). c Wild-type animals, wild-type animals that express SLO-2 in AFD after insertion of a single copy of a transgene into a genome, *slo-1*(eg142); *slo-2*(nf101) animals, and *slo-1*(eg142); *slo-2*(nf101) animals that express SLO-2 in AFD after insertion of a single copy of a transgene were cultivated at 17 °C for 5 days and then at 23 °C for 45 min. Animals were then subjected to thermotaxis assay. n = 8. p Values are indicated (Tukey-Kramer test). See also Supplementary Fig. 4
**Fig. 5** cng-3 loss-of-function suppresses decelerated preference transition in slo-2(nj131gf) mutants. **a–f** Wild-type (a, n = 3, 3, 5, 3, 6, 8), slo-2(nj131gf) (b, n = 3, 9, 5, 5, 3, 8), cng-3(nj172) (c, n = 3, 3, 3, 3, 4), cng-3(nj172); slo-2(nj131gf) (d, n = 3, 3, 3, 3, 3, 4), cng-3(jh113) (e, n = 5, 3, 3, 3, 5, 6), and cng-3(jh113); slo-2(nj131gf) (f, n = 3, 3, 3, 3, 5, 6) animals were cultivated at 17 °C for 5 days and then at 23 °C for the time indicated or constantly at 23 °C for 3 days. Animals were then subjected to thermotaxis assay. The fractions of animals are plotted. **g** The means of the thermotaxis indices at each time point (a–f) are plotted against time after cultivation temperature was changed to 23 °C. The error bars represent the SEM. *p < 0.05, **p < 0.01, ***p < 0.001 between wild-type and slo-2(nj131gf) animals, and †p < 0.05 between slo-2(nj131gf) and cng-3(nj172); slo-2(nj131gf) animals (Tukey–Kramer test). **h** The thermotaxis indices for animals cultivated constantly at 23 °C in a–f are shown. The indices of strains marked with distinct alphabets differ significantly (p < 0.001) according to Tukey–Kramer test. **i** Genomic PCR fragments covering the cng-3 locus were injected into cng-3(nj172); slo-2(nj131gf) animals. Animals were cultivated first at 17 °C, then at 23 °C for the time indicated, and then subjected to thermotaxis assay. n = 5, 8, 5. ***p < 0.001 (Tukey–Kramer test). **j** cng-3(nj172); slo-2(nj131gf) animals expressing cng-3 under control of the indicated promoters were cultivated at 17 °C for 5 days, then at 23 °C for 12 h, and then subjected to thermotaxis assay. n = 8, 8, 6, 5. **p < 0.001 (Dunnett test against cng-3(nj172); slo-2(nj131gf) animals). See also Supplementary Figs. 5 and 6.
SLO-2 channels. Therefore, we tested whether introduction of epilepsy-related mutations in C. elegans SLO-2 (Supplementary Figs. 2 and 7) could phenocopy the gf nature of H159Y for decelerating the preference transition in thermotaxis.

We introduced each of six epilepsy-related mutations into C. elegans slo-2 DNA and injected each DNA into slo-2(nf101) deletion mutants. Animals expressing wild-type or mutant SLO-2 in AFD were cultivated first at 17 °C, then at 23 °C for 3 h, and then were subjected to thermotaxis assay. Expression of two of six epilepsy-related mutant forms of SLO-2 more greatly decelerated preference transition than did expression of wild-type SLO-2 (Fig. 8a, b). These results imply that, although slow preference transition in C. elegans thermotaxis and early-onset epilepsy in humans are macroscopically divergent, both events result from common cellular processes initiated by a gf mutation in the SLO-2 channel. Excessive activity of SLO channels and resulting excessive repolarization after depolarization either in AFD thermosensory neurons in C. elegans or in neurons in the human...
brains seem to cause the respective event. These results also indicated that these two mutations potentiate SLO-2/Slack channels, regardless of their differences such as gating by Ca\(^{2+}\) or Na\(^{+}\). We also found that cng-3 mutation suppressed deceleration of preference transition caused by the expression of SLO-2(R376Q) in AFD (Fig. 8c), implying that inhibition of CNG channels might attenuate epilepsy symptoms. It is of clinical interest to examine whether Slack(gf) rodent models show epilepsy-related phenotypes and whether CNG inhibitors attenuate those phenotypes.

Discussion
In this study, we demonstrated that, upon an upshift in cultivation temperature, SLO K\(^{+}\) channels function with the CNG-3 channel in the AFD thermosensory neuron to slow down the transition of the AFD dynamic range and thereby generate latency for transition of the temperature preference. An upshift in the temperature results in a large influx of Ca\(^{2+}\) into AFD, and consequently K\(^{+}\) efflux through SLO channels repolarizes the membrane potential and decreases [Ca\(^{2+}\)]\(_{i}\), which antagonize premature AFD adaptation (Fig. 8d). Although the expression levels of AFD-specific guanylyl cyclases are shown to increase when cultivation temperature is increased, this induction was unaffected by slo-2(nj131gf) mutation (Supplementary Fig. 8a–c).

We also found a molecular and physiological link between early-onset epilepsy and preference transition in thermotaxis, providing a model system that can be used to understand the pathogenesis of this type of epilepsy and to screen for drugs.

Although CNG channels are generally thought to depolarize the membrane potential, our results implied that CNG-3 acts with SLO K\(^{+}\) channels to hyperpolarize AFD (Fig. 5). Considering that SLO-1 and SLO-2 of C. elegans are both gated by Ca\(^{2+}\) and that CNG channels are generally permeable to Ca\(^{2+}\), it is reasonable to hypothesize that SLO and CNG channels are co-localized in a limited region in which local Ca\(^{2+}\) influx through CNG channels, including CNG-3, activates SLO K\(^{+}\) channels.

While the onset temperature of AFD in slo-2(nj131gf) animals cultivated at 17 °C was comparable with that in wild-type animals, the Ca\(^{2+}\) level decreased sharply beneath the base line in slo-2(nj131gf) animals (Fig. 6b). This result is consistent with the important roles of SLO-2 in post-excitatory repolarization of the membrane potential in body wall muscles\(^{29,34,35}\) and in motor neurons\(^{36}\) in C. elegans and in mammalian neurons\(^{37,38}\). This altered AFD response accompanied with the diffused distribution during thermotaxis behavior of animals cultivated at 17 °C in both slo-2(nj131gf) animals (Figs. 6b, 1d) and animals with AFD-specific SLO-2 expression (Fig. 6e and Supplementary Fig. 7f, 7i, 0h and 7j). In addition, both the altered AFD response and the diffused distribution of the animals were suppressed by cng-3 mutation (Figs. 6d and 5d). Thus the sharp decrease in Ca\(^{2+}\) in AFD may result in insufficient navigation during thermotaxis.

Although a number of studies have focused on alteration of the relationships between unconditional stimuli (US) and conditional stimuli (CS)\(^{39}\), our study focused on CS transition. C. elegans associate CS such as temperature or chemicals with preferable US such as the existence of food, and the conditioned animals are attracted to the CS. Similarly, they can also associate CS with unpreferable US and no longer be attracted to the CS or even avoid it. For instance, the role of the insulin–phosphoinositide-3 kinase–Akt pathway was intensively studied during association between CS with starvation as a negative US\(^{40–42}\). The mechanisms underlying decay of the US–CS association have also been reported\(^{43–45}\). In this study, we performed a forward genetic screen that focused on CS transition and newly identified molecules that do not have any reported roles in learning. dgk-3 mutants were previously reported to be slow to change temperature preference in thermotaxis after cultivation temperature was shifted\(^{16}\). However, dgk-3 mutants in our experimental set-up were simply cryptic. In this study, our results suggested that the SLO and CNG channels act together to modulate transition of the dynamic range of a sensory neuron that can encode the CS.

Unveiling the mechanisms underlying short- and long-term memory (STM and LTM, respectively) and consolidation of STM into LTM is an important issue in neuroscience. The role of the CAMP–protein kinase A–CREB pathway in consolidation of Pavlovian conditioning is conserved among Aplysia, flies, and mammals. Our results may provide a foundation for further genetic research to reveal the mechanisms underlying consolidation. Since the original report on thermotaxis\(^{5}\), it has been known that temperature preference reaches a local maximum 2–3 h after a shift in cultivation temperature, is slightly weakened, and then reaches a plateau (Fig. 1e). We found that slo-2(nj131gf) animals were slow to change their behavior, whereas cng-3(jh113) animals could transiently acquire an ability to perform thermotaxis immediately after a shift in cultivation temperature but gradually lost the ability when cultivated for longer at the new temperature. One possibility is that two different components, one of which is fast but transient and the other of which is delayed but persistent, might help shape the transition of temperature preference (Supplementary Fig. 8d). Our results suggested that SLO and CNG channels suppress the fast component and that the delayed component is dependent on CNG-3 but not on SLO channels, since slo-1; slo-2 double If mutants exhibited normal thermotaxis when cultivated for a long time at a certain temperature. Revealing the mechanisms by which each component is regulated, through screening for suppressors for cng-3 mutants or analyzing animals in a transient state of consolidation, might lead to a comprehensive understanding of consolidation of STM into LTM.

Vertebrate CNG channels, which are essential for the functions of sensory neurons such as rod and cone photoreceptors and...
**Fig. 7** Endogenous SLO K⁺ channels slow down AFD adaptation. 

- **a–e** Wild-type (a), slo-2(nj131gf) (b), slo-1(eg142) (c), slo-2(nf101) (d), and slo-1(eg142); slo-2(nf101) (e) animals that express GCaMP3 and tagRFP in AFD were cultivated at 17 °C for 5 days and subjected to Ca²⁺ imaging analysis with a temperature stimulus involving 20 s (0.05 Hz) oscillations around 17 °C followed by a temperature upshift to 23 °C and oscillations around 23 °C. The intensity of green fluorescence was divided by that of red fluorescence, and the ratio was normalized to a range between 0 and 1 and plotted against time. Gray and red lines indicate traces from individual animals and the average, respectively. Data were collected from distinct animals (n = 6).

- **f–j** The Fourier transform was computed with the Hanning window on the temperature program and ratio of fluorescence intensity between 401 and 1390 s for each animal in a–e. Averaged power spectra are plotted against frequency. k–o Time evolutions of averaged spectrograms are plotted in a color map against the centric time of each segment. Trends detected by Butterworth filter were removed from the ratio of fluorescence intensity of each animal in a–e. The resulting signals were divided into segments of 128 s, and the Fourier transform was separately computed for each segment.
olfactory receptor neurons, are also expressed in the central nervous system and play versatile roles. CNG channels with different subunit composition have different properties, such as sensitivity to cNMP and ion permeability, and function in different cell types. The results of this study and others provide clues that improve our understanding of how CNG channels with distinct subunit compositions contribute to different cellular processes. Among the CNG channels in *C. elegans*, TAX-2 and TAX-4 are essential for thermotaxis as well as for chemotaxis, but CNG-3 seems to play an auxiliary role. Although an α subunit TAX-4 can form a functional homo-tetramer that is far more sensitive to cGMP than the heteromer consisting of TAX-4 and TAX-2, the specific contributions of different subunits to their functional properties require further investigation.

**Fig. 8** Epilepsy-related mutations potentiate SLO-2. a slo-2(nf101) animals expressing either wild-type or the indicated mutant form of SLO-2b in AFD were cultivated at 17 °C for 5 days and then at 23 °C for 3 h and then subjected to thermotaxis assay. Fractions of animals are plotted (upper). n = 9, 17, 16, 12, 4, 2, 4, 2, 4. Thermotaxis indices of strains marked with distinct alphabets differ significantly (p < 0.05) according to Tukey-Kramer test (lower). b slo-2 (nf101) animals expressing either wild-type or the indicated mutant form of SLO-2b in AFD were cultivated at 17 °C for 5 days and then at 23 °C for the indicated time points. The animals were then subjected to thermotaxis assay. The means of thermotaxis indices are shown. The error bars represent the SEM. Data at 3 h are identical to those in a. *p < 0.01, **p < 0.001 (Tukey-Kramer test, compared with animals expressing SLO-2b(+)). The fractions of animals and individual indices at each time point are shown in Supplementary Fig. 7. c Animals expressing SLO-2b(R376Q) in AFD with either a wild-type or cng-3(nj172) background were cultivated at 17 °C for 5 days, then at 23 °C for the indicated time points, and then subjected to thermotaxis assay. Thermotaxis indices at each time point are shown. The horizontal bars represent the medians. n = 2, 6, 3, 5, 3, 3 for each time point. *p < 0.05, **p < 0.01 (Welch two-sample t test between two strains at each time point). The fractions of animals are shown in Supplementary Figs. 2, 3, 7, and 8.
and a β subunit TAX-2(18) and TAX-2 cannot form a functional homomer by itself, TAX-2 is essential for thermosensation and chemosensation, indicating that heteromer-specific functions exist. CNG-3 was suggested to form a hetero-tetramer with TAX-4 and TAX-2 in AWC(27), implying that a similar heteromer also forms in AFD. Our results suggest that CNG-3 is necessary for steady, but not for transient, thermotaxis. Thus investigating how CNG channels with different compositions function in specific behavioral or cellular contexts in combination with the analysis of their electrophysiological properties would provide further understanding of how CNG channels function in the nervous system.

This study, focusing on a transient state of decision making, not only provided scientific insights into the mechanisms that determine the timing of decision making but also revealed possible clinical applications that may contribute to the treatment of early-onset epilepsy. Our results suggested that the preference transition in thermotaxis might be useful as a model system for early-onset epilepsy caused by Slack/KCNT1 mutations. Given that quinidine blocks the Slack channel(49,50), we investigated how C. elegans would be valuable. Since Slack is expressed in cardio-myocytes(19), identification of molecules that functionally interact with Slack only in neurons by screening suppressors for CNG channels with different compositions function in the nervous system.

Methods

Experimental model and subject details. C. elegans strains were maintained as described(24). Briefly, animals were cultivated on nematode growth medium (NGM) plates seeded with an Escherichia coli OP50 strain (Caenorhabditis Genetics Center [CGG], Twin Cities, MN, USA). N2 (Bristol) was used as the wild-type strain unless otherwise indicated. Transgenic lines were generated as described(21). Briefly, plasmid DNA or PCR fragments were directly injected into the hermaphrodite gonad. Strains used in this study are listed in Supplementary Table 2.

Behavioral assays. Population thermotaxis assays were performed as described previously(26). Briefly, 50–250 animals cultivated at 17 °C or 23 °C were subjected to a shift in cultivation temperature were placed at the center of the assay plates without food and with a temperature gradient of 17-23 °C. The animals were allowed to freely move for 60 min. The assay plate was divided into eight sections along the temperature gradient, and the number of adult animals in each section was scored. The ratio of animals in each section was plotted on histograms. Thermotaxis indices were calculated as shown below:

$$\sum_{i=1}^{8} n_i \times N$$

where \(N\) is the number of animals in each section i (i = 1–8) and \(N_i\) is the total number of animals on the test plate.

Forward genetic screen for Slw mutants. For mutagenesis, wild-type animals were treated with 50 mM ethyl methanesulfonate (EMS; Nacalai, Kyoto, Japan) for 4 h at room temperature. The F1 generation of the mutagenized animals was cultivated at 17 °C for 5 days and was allowed to self-fertilize and give rise to the F2 generation. F2 animals were cultivated at 23 °C for 3 h and then put on a thermal gradient for thermotaxis assay. Most animals migrated to the warm region of the thermal gradient. Animals that still migrated to the cold region and thus were putatively slow to change behavior were isolated. To discriminate between animals that are slow to change behavior and those that constitutively prefer cold temperature, the strains isolated above were cultivated constantly at 23 °C and subjected to thermotaxis assay. Animals that migrated to the cold region when cultivated at 17 °C and then at 23 °C for 3 h but migrated to the warm region when cultivated constantly at 23 °C were considered to be slow to change behavior.

Suppressor screen against slo-2(nj131gf). As described above, slo-2(nj131gf) animals were mutagenized with EMS. Mutagenized animals were cultivated at 17 °C for 5 days for 3 h and then were subjected to thermotaxis assay as described above. Most animals still migrated to the cold region of the thermal gradient. Animals that migrated to the warm region to the wild-type animals were isolated.

Mapping of nj131 and nj172. We crossed nj131 animals with a wild-type poly-morphic CB4858 strain(27). Since the Sw phenotype of nj131 was inherited semi-dominantly, we isolated F2 animals that exhibited Sw or wild-type phenotypes and identified crossover sites as described(58). We mapped nj131 to a 1.3-Mb interval between nucleotides 11,136,567 and 12,419,537 on linkage group (LG) X. To map nj172, we first crossed slo-2(nj131gf) animals with CB4858 four times to obtain a strain in which the slo-2(nj131gf) allele exists on the CB4858 genome (JK2092). We then crossed nj172 slo-2(nj131gf) with JK2092, isolated F2 animals in which the Sw phenotype was suppressed and identified crossover sites. We mapped nj172 to a 1.1-Mb interval between nucleotides 8,723,437 and 9,847,895 on LG IV.

Whole-genome sequencing. Genomic DNA was purified with the PureGene Core Kit A (Qagen, Hilden, Germany). The genome was sequenced with MiSeq (ILLUS- trion, San Diego, CA, USA), and the sequence was analyzed with CLC Genomics Workbench (CLC Bio, Aarhus, Denmark).

Plasmids. POX nSlo2 was a gift from Larry Salkoff (Addgene plasmid #16207). A DNA clone including a part of cng-3 cdna (yk33849) was provided by Yuji Kohara. To generate plasmids to cell-specifically express slo-2 or cng-3, we fused promoter sequences of gcy-8, ceh-36, ttx-3, lin-11, glr-3, or myo-3; the cDNA of slo-2 or cng-3; and the unc-5 3′UTR sequence to Multisite Gateway Technology (Thermo Fisher Scientific, Waltham, MA, USA). To generate plasmids to express SLO-2 in the HEK293T cells, slo-2 cdna was inserted into a pcAG vector. Details regarding the plasmid constructs can be obtained from the authors. Pgf-3::Cas9-SV40 NLS:ibb-2-3′UTR (Addgene plasmid #46168) and Punc-unc-119 sgRNA (#46169) were gifts from John Calarco(50). The unc-119 single-guide RNA (sgRNA) sequence in Pgf-3::Cas9-SV40 NLS:ibb-2-3′UTR was replaced with the sequences of sgRNA(unc-22, slo-2 k1, and slo-2 k4), which were GAAGGCGTTCTCGAAGACTAACG(AGG)(GAA), GAACTCGTTTCTCGGCG(TG), and GCACAGTACGATGCGGCGG(TG), respectively. Erik Jorgensen gifted pcF150 (pDESTTr16505/#R3J-13J) (Addgene plasmid #19329), pcF601 Pgf-3::Mot1 transposase (#438874), pcm122 Pphp:peel-1 (#438873), pcF190 myo-2p::mCherry (#19327), pcF104 myo-3p::mCherry (#19328), and pcG88 rab-3p::mCherry (#193595). A plasmid containing slo-1 cdna was a gift from Jonathan T. Pierce(63). The gcy-8 promoter sequence, slo-1 cdna, and unc-5 3′UTR sequence were fused into pcF150 to make pcIA109. Plasmids used in this study are listed in Supplementary Table 3.

Knockout of slo-2 by CRISPR/Cas9. The slo-2 gene was knocked out by CRISPR/Cas9 as described(64). First, slo-2(nj131gf) animals were injected with Pgf-3::Cas9- SV40 NLS:ibb-2-3′UTR, prF4 rad-51(k0906, gfi), psN680 unc-22 sgRNA, plAI09 slo-2 sgRNA k1, and plAI054 slo-2 sgRNA k4. Then unc-22+/– and unc-22 unc-22 F1 twitcher animals were subjected to genotyping for the slo-2 gene locus. Although deletion between the sites of sgRNA k1 and k4 was not detected, small indels around the sgRNA #1 site were detected by polymerase chain reaction following PCR that amplified 60 bp region including the site of sgRNA#1 with primers TCTGAGAGTCCCTGGAAATTTCT and ttggttgctggctgctttc. A strain that homozygously carried a small deletion at the slo-2 locus was isolated from the progeny of a unc-22+/+ animal with a small deletion. Sequencing of the genomic slo-2 locus revealed that in this strain, two nucleotides in the third exon are deleted, causing a frame shift after His344. No indel was found adjacent to the site of sgRNA #4. Then unc-22 mutation was knocked out by selecting non-twitcher animals.

Single-copy insertion by MosSCI. A single copy of gcy-8p::slo-la(+); or gcy-8p:: slo-2(+) was inserted into a genome as described(21). First, tf15605, unc-119(ed3) animals derived from the EG6699 strain, which feature uncoordinated (unc) locomotion, were injected with pIA109 or pIA110 in addition to pCF601, pMA122, pGHR, pcF190, and pcF104. Then NGM plates with non-unc animals were heat-shocked at 42 °C for 2 h. Non-unc animals without mCherry fluorescence were selected and subjected to genotyping. PCR was performed to confirm insertion of a full-length transgene.

Imaging analyses. Calcium imaging was performed as described elsewhere(65). A single adult animal that expressed the genetically encoded calcium indicators R-GECI or FRET-2(8) was imaged using a Microscope (Olympus, Tokyo, Japan). The fixed and green fluorescent was separated by the dualView optics system (Molecular Devices, Sunnyvale, CA, USA), and images were captured by an ImageEM EM-CCD camera (C9100-13, Hamamatsu Photonics, Japan) at a frame rate of 1 Hz. Excitation pulses were generated by a SPECTRA light engine (Lumencor, Beaverton, OR, USA). Fluorescence measurements were measured using the MetaMorph imaging system (Molecular Devices).

Expression of SLO-2::mCherry was observed with a BXX3 upright microscope (Olympus).
Electrophysiology. HEK293T cells were transfected with plasmids encoding yellow fluorescent protein (YFP) and either the wild-type or H395Y mutant form of SLO-2b with Lipofectamine 2000 (Invitrogen). The macroscopic current in HEK293T cells expressing YFP in a whole-cell patch clamp configuration was recorded using the Axopatch 200B amplifiers, Digidata 1322 A, and pClamp 9 software (Axon Instruments, Foster City, CA, USA) at room temperature, as previously described\(^{20}\). The bath solution contained (in mM) 140 NaCl, 4 KCl, 10 HEPES, 0.1 MgCl\(_2\), and 0.3 MgCl\(_2\) (pH = 7.4). Pipette resistances ranged from 1 to 3 M\(\Omega\) when the glass pipette was filled with the pipette solution, which contained (in mM) 130 KCl, 10 NaCl, 10 HEPES, 5 K\(_2\)ATP, 4 MgCl\(_2\), 3 EGTA, 0.3 GTP, and various concentrations of CaCl\(_2\) (pH = 7.3). To achieve 0, 0.2, 0.6, 2, and 20 mM of free [Ca\(^{2+}\)]\(_i\), 1.1, 1.56, 2.55, 2.86, 2.99, and 3.10 mM of CaCl\(_2\), respectively, were added to the pipette solution, according to the Ca-Mg-ATP-EGTA Calculator v1.0 using constants from the NIST database #46 v8 (http://mashelcher.stanford.edu/CaMgATPEGTA-NIST.htm). After rupture of the sealed membrane, cells were held at −60 mV for at least 3 min to dialyze the pipette solution into the cell, and then depolarizing step pulses (10 mV increment for 200 ms) were applied. Immediately after application of the step pulse, cells were held at 0 mV (50 ms) for tail current analysis. The tail currents were measured at the end of the step pulses and normalized by the membrane capacitance of each cell. Then the current densities were averaged and plotted as a function of the membrane potentials. To analyze the voltage dependence of the channels, the tail current amplitudes at the beginning of 0 mV were measured and normalized by the maximal amplitude at the most depolarized potential (G/G\(_{\text{max}}\)). G/G\(_{\text{max}}\) values were plotted against the membrane potential of half-maximal activation (V\(_{1/2}\)) in each cell. The activation kinetics of the channels was analyzed by fitting the traces of outward current to double exponential functions. Several traces that were not fitted to the double exponential functions were eliminated from the kinetic analyses.

Quantification and statistical analysis. The error bars in histograms and line charts indicate the standard error of mean (SEM). In the boxplots, the bottom and top of boxes represent the first and third quartiles, and the band inside the box represents the median. The ends of the upper and lower whiskers represent the lowest datum still within the 1.5 interquartile range (IQR), which is equal to the difference between the third and first quartiles, of the lower quartile, and the highest datum still within the 1.5 IQR of the upper quartile, respectively. For multiple-comparison tests, one-way analyses of variance were performed, followed by Tukey-Kramer or Dunnett tests, as indicated in each figure legend. The Welch two-sample t-test was used to compare two values.

Data availability. The datasets generated during the current study, including source data for the figures in the paper, are available in the figshare repository (https://doi.org/10.6084/m9.figshare.c.4168931\(^{30}\)).

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References

1. Doya, K. Modulators of decision making. Nat. Neurosci. 11, 410–416 (2008).

2. Wang, X.-J. in Neuroeconomics 2nd edn (eds Glimcher, P. W. & Fehr, E.) Ch. 23 (Elsevier, 2014).

3. Williams, C. T., Barnes, B. M., Kenagy, G. J. & Buck, C. L. Pheno...
41. Kodama, E. et al. Insulin-like signaling and the neural circuit for integrative behavior in C. elegans. Genes Dev. 20, 2955–2960 (2006).
42. Chalasani, S. H. et al. Neuropeptide feedback modifies odor-evoked dynamics in Caenorhabditis elegans olfactory neurons. Nat. Neurosci. 13, 615–621 (2010).
43. Vukovic, V. et al. A role for alpha-adducin (ADD-1) in nematode and human memory. EMBO J. 31, 1453–1462 (2012).
44. Hadziselimovic, N. et al. Forgetting is regulated via musashi-mediated translational control of the Arp2/3 complex. Cell 156, 1153–1166 (2014).
45. Inoue, A. et al. Forgetting in C. elegans is accelerated by neuronal communication via the TIR-1/JNK-1 pathway. Cell Rep. 3, 808–819 (2013).
46. Podda, M. V. & Grassi, C. New perspectives in cyclic nucleotide-mediated functions in the CNS: the emerging role of cyclic nucleotide-gated (CNG) channels. Pfugers Arch. 466, 1241–1257 (2014).
47. O’Halloran, D. M. et al. Contribution of the cyclic nucleotide gated channel subunit, CNG-3, to olfactory plasticity in Caenorhabditis elegans. Sci. Rep. 7, 169 (2017).
48. Komatsu, H. et al. Functional reconstitution of a heteromeric cyclic nucleotide-gated channel of Caenorhabditis elegans in cultured cells. Brain Res. 821, 160–168 (1999).
49. Bhattacharjee, A. et al. Slick (Slo2.1), a rapidly-gating sodium-activated potassium channel inhibited by ATP. J. Neurosci. 23, 11681–11691 (2003).
50. Yang, B. et al. Pharmacological activation and inhibition of Slack (Slo2.2) channels. Neuropharmacology 51, 896–906 (2006).
51. Bearden, D. et al. Targeted treatment of migratory partial seizures of infancy with quinidine. Ann. Neurol. 76, 457–461 (2014).
52. Mikai, M. A. et al. Quinidine in the treatment of KCNT1-positive epilepsies. Ann. Neurol. 78, 995–999 (2015).
53. Chong, P. F., Nakamura, R., Saito, H., Matsumoto, N. & Kira, R. Ineffective quinidine therapy in early onset epileptic encephalopathy with KCNT1 mutation. Brain 146, 503–508 (2013).
54. Brenner, S. The genetics of Caenorhabditis elegans. Genetics 77, 71–94 (1974).
55. Mello, C. C., Kramer, J. M., Stinchcomb, D. & Ambros, V. Efficient gene transfer in C. elegans: extrachromosomal maintenance and integration of transforming sequences. EMBO J. 10, 3959–3970 (1991).
56. Ito, H., Inada, H. & Morii, I. Quantitative analysis of thermotaxis in the nematode Caenorhabditis elegans. J. Neurosci. Methods 154, 45–52 (2006).
57. Hiller, L. W. et al. Whole-genome sequencing and variant discovery in Caenorhabditis elegans. EMBO J. 20, 1182–1192 (2001).
58. Wicks, S. R., Yeh, R. T., Gish, W. R., Waterston, R. H. & Plasterk, R. H. Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map. Nat. Genet. 28, 160–164 (2001).
59. Friedland, A. E. et al. Heritable genome editing in C. elegans via a CRISPR-Cas9 system. Nat. Methods 10, 741–743 (2013).
60. Kim, H. et al. A Co-CRISPR strategy for efficient genome editing in Caenorhabditis elegans. Genetics 197, 1069–1080 (2014).
61. Frejkaer-Jensen, C., Davis, M. W., Allion, M. & Jorgensen, E. M. Improved Mos1-mediated transgenesis in C. elegans. Nat. Methods 9, 117–118 (2012).
62. Frejkaer-Jensen, C. et al. Single copy insertion of transgenes in C. elegans. Nat. Genet. 40, 1375–1383 (2008).
63. Davis, S. J., Scott, L. L., Hu, K. & Pierce-Shimomura, J. T. Conserved single residue in the BK potassium channel required for activation by alcohol and intoxication in C. elegans. J. Neurosci. 34, 9562–9573 (2014).
64. Farboud, B. & Meyer, B. J. Dramatic enhancement of genome editing by CRISPR/Cas9 through improved guide RNA design. Genetics 199, 959–971 (2015).
65. Yoshida, A. et al. A glial K+/-Cl\textsuperscript{-} cotransporter modifies temperature-evoked dynamics in Caenorhabditis elegans sensory neurons. Genes Brain Behav. 15, 429–440 (2016).
66. Inoue, M. et al. Rational design of a high-affinity, fast, red calcium indicator R- CaMP2. Nat. Methods 12, 64–70 (2014).
67. Tian, L. et al. Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat. Methods 6, 875–881 (2009).
68. KimE., SunL., GabelC. V. & Fang-YenC. Long-term imaging of Caenorhabditis elegans using nanoparticle-mediated immobilization. PLoS ONE 8, e53419 (2013).
69. Tatemaya, M. & Kudo, Y. Binding of Gq protein stabilizes the activated state of the muscarinic receptor type 1. Neuropharmacology 65, 173–181 (2013).
70. Aoki, I. et al. SLO potassium channels antagonize premature decision making in C. elegans. (2018). https://doi.org/10.6084/m9.ﬁgsshare.c.4168931.v1

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I.A. designed and performed experiments, wrote the manuscript, and secured funding. M.T., T.S., and K.I. performed experiments. Y.K. and S.N. provided expertise and feedback. I.M. supervised and secured funding.

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