Presynaptic MAST Kinase Controls Bidirectional Post-Synaptic Responses to Convey Stimulus Valence in *C. elegans*

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24 **Abstract**

25 **Presynaptic plasticity is known to modulate the strength of synaptic transmission.**

26 **However, it remains unknown whether regulation in presynaptic neurons alters the**

27 **directionality –positive or negative- of postsynaptic responses. We report here that the**

28 ***C. elegans* homologs of MAST kinase, Stomatin and Diacylglycerol kinase act in a**

29 **thermosensory neuron to elicit in its postsynaptic neuron an excitatory or inhibitory**

30 **response that correlates with the valence of thermal stimuli. By monitoring neural**

31 **activity of the valence-coding interneuron in freely behaving animals, we show that**

32 **the alteration between excitatory and inhibitory responses of the interneuron is**

33 **mediated by controlling the balance of two opposing signals released from the**

34 **presynaptic neuron. These alternative transmissions further generate opposing**

35 **behavioral outputs necessary for the navigation on thermal gradients. Our findings**

36 **reveal the previously unrecognized capability of presynaptic regulation to evoke**

37 **bidirectional postsynaptic responses and suggest a molecular mechanism of**

38 **determining stimulus valence.**

39 **Introduction**

40 Sensory stimuli that predict the valence of reward or punishment are major drivers of
 41 animal behaviors. For example, odors associated with predators are detrimental to the
 42 animals and elicit fear responses¹, while smells predicting the presence of food or potential
 43 mates evoke different behaviors such as feeding² or mating³. Extracting the valence
 44 information of sensory stimulus – whether the stimulus is attractive or aversive- and
 45 manifesting appropriate behavioral responses are the most vital function of the nervous
 46 system. Deciphering the molecular and circuit mechanisms underlying the valence coding
 47 of sensory information is thus fundamental to understand the principles of how animal
 48 behaviors emerge from the nervous system.

49 The valences of innate odor responses are guided by intrinsic property of the
 50 responding neurons, in which neurons expressing specific odorant receptors are hardwired
 51 in a neural circuit that elicits attractive or aversive behavior^{4,5}. A similar labeled-line circuit
 52 operation was also demonstrated for encoding and responding to tastes such as sweet and
 53 bitter⁶⁻⁸, wherein cells expressing specific taste receptors are embedded in a specialized

neural circuit that promotes or inhibits feeding behaviors.

Contrary to these developmentally programmed, stereotyped behaviors, the valence associated with certain sensory stimuli can vary depending on the past experience, the current environmental context and the stimulus intensity⁹. For example, olfactory preferences to the same odorants can differ depending on the odorant concentration¹⁰. Studies of worms, flies and mammals suggested a common feature of neural mechanism underlying the change in these odorant valences, wherein different concentrations of the same odorants recruit distinct sets of olfactory neurons and consequently change the perception of the same odorants^{11–13}. However, the extent to which the brain utilizes different encoding strategies for alternating stimulus valence remains largely unexplored. In particular, the molecular and circuit mechanisms underlying the perception of altering valence for other sensory modalities are not yet understood.

The compact nervous system of *C. elegans* consisting of only 302 neurons provides an excellent opportunity to explore these questions¹⁴. *C. elegans* exhibits thermotaxis behavior¹⁵, in which the valence of thermal information varies depending on

the past experience, current temperature environment and feeding states. Specifically, the temperature preference of *C. elegans* is plastic and determined by the cultivation temperature, in which animals that are cultivated at a constant temperature with food migrate toward that cultivation temperature on a thermal gradient without food¹⁵. When animals were placed at the temperature below the cultivation temperature they migrate up the thermal gradient, while above the cultivation temperature they move down the gradient, indicating that the valence associated with thermal stimuli alternates in opposing manners depending on the current temperature.

Previous studies identified neurons involved in thermotaxis^{16–18}. Of those neurons, the AFD thermosensory neurons are essential for thermotaxis and are required for migrating up and down the thermal gradient to reach the cultivation temperature^{17,19}. Calcium imaging analyses revealed that the AFD neuron displayed increases in calcium concentration upon warming phases of a temperature ramp both below and above the cultivation temperature^{20,21}. However, it remains to be determined how the AFD neuronal activities with similar calcium dynamics below and above the cultivation temperature are

transformed to encode appropriate valence of temperature information and the consequent manifestation of opposing behavioral regulations.

Here we report that the AFD neuron evokes opposing neuronal responses in its postsynaptic partner AIY interneuron below or above the cultivation temperature: the activation of AFD neuron stimulates the AIY neuron below the cultivation temperature, while it inhibits AIY above that temperature. We identified molecular components important for this process and showed that this alteration of the AFD-AIY communication is regulated by presynaptic actions of the *C. elegans* homologs of MAST (Microtubule-Associated Serine-Threonine) kinase²², Stomatin²³ and Diacylglycerol kinase^{24,25}. Our results further suggest that the alteration of the AFD-AIY synaptic transmission is mediated by the balance of two opposing signals released from the AFD neuron, an excitatory peptidergic signaling and an inhibitory glutamatergic signaling. A high-throughput behavioral analysis revealed that these alternative modes of the AFD-AIY transmission generate opposing behavioral biases in the steering direction of animal locomotion to warmer or colder side of the temperature gradient, thereby driving the

99 animals toward the cultivation temperature. Our results suggest that bidirectional responses
100 in the valence-coding neurons are regulated by presynaptic mechanism whereby the
101 evolutionarily conserved MAST kinase, Stomatin and Diacylglycerol kinase control the
102 presynaptic release of opposing signaling molecules.
103

104

105 **Results**

106 ***kin-4*, *mec-2* and *dgk-1* Regulate the *C. elegans* Thermotaxis Behavior**

107 To elucidate the molecular and neural mechanisms underlying the valence coding of
 108 thermal stimuli during *C. elegans* thermotaxis behavior, we conducted forward genetic
 109 screens and sought mutants that displayed abnormal thermotaxis behavior. We found that
 110 mutations in three genes, *kin-4*, *dgk-1* and *mec-2*, affected this behavior (Fig. 1). While the
 111 wild-type animals that had been cultivated at 20 °C preferred to stay around the cultivation
 112 temperature, loss-of-function mutants of *kin-4*, which encodes the *C. elegans* ortholog of
 113 the mammalian MAST (Microtubule Associated Serine Threonine) kinase, displayed a
 114 cryophilic phenotype and migrated toward a colder temperature region than the wild-type
 115 animals (Fig. 1b and Supplementary Fig. 1a). This defect was rescued by introduction of a
 116 *kin-4* genomic clone (Fig. 1c), indicating that *kin-4* is required for thermotaxis. Animals
 117 carrying mutations in the gene *dgk-1*, which encodes a homolog of Diacylglycerol kinase
 118 $\theta^{24,25}$, also preferred to migrate toward a colder temperature region, as was previously
 119 reported ²⁶ (Fig. 1b and Supplementary Fig. 1a). The cryophilic phenotype of *dgk-1(nj274)*

mutants was rescued by introduction of a *dgk-1* genomic clone (Supplementary Fig. 1b), indicating that *dgk-1* is required for thermotaxis. We also isolated a mutation in *mec-2*, which encodes a *C. elegans* homolog of Stomatin²³. *mec-2(nj89)* animals harbored a mutation that is predicted to alter the glutamic acid 270 to a lysine (*E270K*, see Supplementary Fig. 1a), and displayed a thermophilic phenotype (Fig. 1b). Introduction of a mutant *mec-2(E270K)* clone into the wild-type animals phenocopied *mec-2(nj89)* mutants, while introduction of a wild-type *mec-2* clone into *mec-2(nj89)* mutants did not rescue the thermophilic defect (Fig. 1d). We also generated a null allele of *mec-2(nj251 Δ)* (Supplementary Fig. 1a) and found that *mec-2* null mutants were grossly normal in thermotaxis (Supplementary Fig. 1c), suggesting that some of the nine additional Stomatin genes encoded by the *C. elegans* genome could compensate the deficit of *mec-2*. These results indicate that *mec-2(nj89)* is a gain-of-function mutation and causes a thermophilic phenotype.

To address genetic interactions among these genes, we analyzed thermotaxis behaviors of double mutants. Animals carrying mutations in both *kin-4* and *mec-2* showed a

thermophilic phenotype similar to that of *mec-2* single mutants (Fig. 1e), suggesting that *mec-2* acts downstream of or in parallel to *kin-4*. Similarly, *dgk-1* mutations partially suppressed the thermophilic phenotype conferred by *mec-2(nj89gf)* mutation (Fig. 1f), suggesting that *dgk-1* acts downstream of or in parallel to *mec-2*. We hereafter focused on *kin-4(tm1049Δ)*, *mec-2(nj89gf)* and *dgk-1(nj274Δ)* mutants for further analyses.

***kin-4*, *mec-2* and *dgk-1* Function in the AFD Thermosensory Neuron to Regulate Thermotaxis**

To ask where *kin-4* and *mec-2* are expressed, we conducted expression analysis. We generated a functional *kin-4::gfp* translational transgene capable of rescuing the cryophilic phenotype of *kin-4* mutants (Supplementary Fig. 2a). This transgene was broadly expressed in the nervous system, and its expression was observed in neurons previously shown to be involved in thermotaxis^{16,17,27}, including the AFD and AWC thermosensory neurons and the AIY and RIA interneurons (Figs. 2a and b, and Supplementary Fig. 2b). We also assessed expression of *mec-2* and found that a *Pmec-2c::gfp* reporter was expressed in AFD and

AWC (Fig. 2b). As previously reported²³, expression in mechanosensory neurons was also observed when *gfp* was fused to a promoter for another *mec-2* isoform, *mec-2a* (Supplementary Fig. 2c). A previous study also showed that *dgk-1* was ubiquitously expressed in the nervous system²⁵. These results suggest that *kin-4*, *mec-2* and *dgk-1* are expressed in neurons known to be involved in regulation of thermotaxis, including the AFD and AWC thermosensory neurons.

To identify the neuron(s) in which *kin-4*, *mec-2* and *dgk-1* act to regulate thermotaxis, we attempted to express each of these genes in single neurons and assessed their effects on the thermotaxis behavior. Expression of a *kin-4* cDNA in AFD rescued the cryophilic phenotype of *kin-4* mutants, whereas expression in AWC, AIY, AIZ or RIA did not (Fig. 2c), indicating that *kin-4* functions in AFD to regulate thermotaxis. When mutant *mec-2(E270K)* was expressed in AFD, it phenocopied *mec-2(nj89gf)* mutants, while expression in AWC did not (Fig. 2d). Expression of a *dgk-1* cDNA in AFD but not in AWC partially rescued the cryophilic phenotype of *dgk-1* mutants (Fig. 2e). We also observed that simultaneous expression of *dgk-1* in both AFD and AWC fully rescued the *dgk-1*

mutant phenotype. These results indicate that *kin-4*, *mec-2* and *dgk-1* function in AFD to regulate thermotaxis.

***kin-4*, *mec-2* and *dgk-1* Act Downstream of Calcium Influx in AFD**

To assess whether *kin-4*, *mec-2* and *dgk-1* regulate temperature-evoked activity of the AFD thermosensory neuron, we conducted calcium imaging of AFD. We immobilized animals expressing the GCaMP3 calcium indicator in AFD and subjected the animals to a warming stimulus. As previously reported^{20,21}, the AFD neuron showed increases in calcium concentration upon warming stimuli both below and above the cultivation temperature (Figs. 3a and b), and this response required three guanylate cyclase genes, *gcy-8*, *gcy-18* and *gcy-23* exclusively expressed in the AFD neurons^{28–30} (Fig. 3b). The temperature-evoked calcium responses of AFD in *kin-4*, *mec-2* and *dgk-1* mutants were almost indistinguishable from that of the wild-type animals (Fig. 3b-d). These results suggest that *kin-4*, *mec-2* and *dgk-1* regulate a process downstream of the calcium influx in AFD.

180

181 **Valence of Thermal Information Is Encoded by Bidirectional AIY Response**

182 To ask whether *kin-4*, *mec-2* and *dgk-1* regulate the activity of the AIY interneuron, the
 183 sole chemical postsynaptic partner of AFD¹⁴, we monitored calcium dynamics of both AFD
 184 and AIY. We generated the animals expressing a calcium indicator in both AFD and AIY
 185 and subjected them to warming stimuli while they were freely moving under the
 186 microscope with an automated tracking system (Fig. 4). We first subjected animals to a
 187 warming stimulus below the cultivation temperature, in which the temperature was
 188 increasing toward the cultivation temperature (Fig. 4a). When the wild-type animals were
 189 exposed to this warming stimulus, the AFD neuron showed an increase in calcium
 190 concentration, and the AIY neuron also displayed a rise in calcium concentration after a
 191 slight drop observed at the beginning of the warming stimulus (Fig. 4b). By contrast, when
 192 the wild-type animals were subjected to a warming stimulus above the cultivation
 193 temperature, where the temperature was increasing away from the cultivation temperature
 194 (Fig. 4e), the AIY neuron instead showed a decrease in calcium concentration, despite the

increase of calcium level in AFD (Fig. 4f). These results indicate that the bidirectional response of the AIY neuron correlates with the valence of thermal stimuli, with temperature increase toward the cultivation temperature evoking excitatory response and temperature increase away from the cultivation temperature inhibitory response.

***kin-4*, *mec-2* and *dgk-1* Regulate Bidirectional AIY Response**

We next examined the AIY responses in *kin-4*, *dgk-1* and *mec-2* mutants. The AIY neuron in *kin-4* mutants exhibited a decrease in calcium concentration even below the cultivation temperature, the condition in which the wild-type AIY neuron would normally increase its calcium level (Figs. 4b and c). Consistent with the calcium imaging analysis of AFD in immobilized animals (Fig. 3), the AFD neuron of *kin-4* mutants showed increases in calcium concentration both below and above the cultivation temperature (Figs. 4b and c). The defect in the AIY response of *kin-4* mutants was partially rescued by expression of a *kin-4* cDNA solely in the AFD neuron (Figs. 4b and d), indicating that the AIY calcium response is indeed modulated by its presynaptic partner AFD. A similar response profile was

also observed in the cryophilic *dgk-1* mutants: the AIY calcium level dropped upon warming stimuli both below and above the cultivation temperature, while the AFD neuron responded to the warming stimuli by increasing the calcium concentration (Figs. 4b, c, f and g). Furthermore, the thermophilic *mec-2* mutants showed an abnormal increase in the AIY calcium concentration even above the cultivation temperature (Figs. 4f and g). Similarly in the wild type, the AFD neurons in *mec-2* mutants showed increases in calcium concentration under both conditions tested (Figs. 4b and f). These results indicate that the valence-coding bidirectional AIY activity is regulated by *kin-4*, *mec-2* and *dgk-1*. Given that *kin-4* expression only in AFD restored the defect of the AIY response in *kin-4* mutants, these results further suggest that presynaptic regulation is important for determining the bidirectional responses of the AIY neuron.

A recent study reported that the difference in the AIY calcium responses below or above the cultivation temperature was represented by a difference in the fraction of animals in which AIY showed an increase in calcium concentration upon warming stimulus under the experimental condition where the animals were immobilized³¹. Our results further

suggest that in freely behaving animals, the AFD-AIY transmission can alter between excitatory and inhibitory communication below or above the cultivation temperature.

Alteration of the AFD-AIY Synaptic Transmission Requires Components Essential for Neuropeptide and Glutamate Release

We next asked how the presynaptic regulation in AFD evokes opposing neuronal responses in the AIY postsynaptic neuron. Previous studies indicated that AFD employs two kinds of signaling molecules to communicate with AIY^{27,32}: one is neuropeptide, and the other is glutamate. The peptidergic signaling was shown to be excitatory³², whereas the glutamatergic input is inhibitory due to a glutamate-gated chloride channel acting on AIY²⁷. We hypothesized that the balance of these two opposing signals released from AFD might be modulated, thereby inducing an excitatory or inhibitory postsynaptic response. To test this possibility, we monitored the AIY calcium response in mutants for the gene *unc-31*, which encodes the *C. elegans* ortholog of calcium-dependent activator protein required for secretion of neuropeptides³³. The AIY neurons of *unc-31* mutants failed to increase the

calcium level and instead showed a decrease even below the cultivation temperature, while the AFD neuron showed increases in calcium concentration under both conditions tested (Figs. 5a-c). This defect in the bidirectional AIY response was partially rescued by expression of an *unc-31* cDNA only in AFD (Figs. 5b and d), indicating that the peptidergic signals from AFD is required for the bidirectional AIY response.

We also examined the AIY response in mutants for the *eat-4* gene, which encodes a *C. elegans* homolog of vesicular glutamate transporter required for transporting glutamate into synaptic vesicles³⁴. In *eat-4* mutants, the AIY neurons displayed abnormal increase in the calcium concentration above the cultivation temperature (Figs. 5e-g), and this defect was rescued by expression of *eat-4* only in the AFD neurons (Figs. 5f and h). Like in *unc-31* mutants, the AFD neurons in *eat-4* mutants showed increase in the calcium level under both conditions tested. These results suggest that alteration of the positive and negative modes of the AFD-AIY communication is mediated by the presynaptic control of the glutamatergic and peptidergic outputs.

255 *kin-4*, *mec-2* and *dgk-1* Regulate Curving Bias During Thermotaxis

256 To address how the alteration in the modes of the AFD-AIY communication contributes to
 257 the regulation of thermotaxis behavior, we undertook multi-worm tracking analysis³⁵. We
 258 classified the animal behavior into several behavioral components discernable during *C.*
 259 *elegans* locomotion, such as forward locomotion, turns and reversals³⁶. We sought the
 260 behavioral components that were oppositely biased below or above the cultivation
 261 temperature in the wild-type animals, and were oppositely affected by the cryophilic (*kin-4*
 262 and *dgk-1*) or thermophilic (*mec-2*) mutations. Among the behavioral components we have
 263 examined (Fig. 6), we found that the curve, change in the moving direction during forward
 264 locomotion, was one such component (Fig. 6a): the wild-type animals behaving below the
 265 cultivation temperature showed curving bias toward warmer temperature when migrating
 266 up the thermal gradient, while they curve toward colder temperature above the cultivation
 267 temperature³⁶ (Fig. 6a), suggesting that this regulation of the curve would drive the animals
 268 toward the cultivation temperature. By contrast, the curving rates of the two cryophilic
 269 mutants, *kin-4* and *dgk-1*, below the cultivation temperature were abnormally biased toward

270 the colder side (Fig. 6a). Furthermore, *mec-2* mutants behaving above the cultivation
 271 temperature failed to show a curving bias toward colder temperature (Fig. 6a). These results
 272 indicate that *kin-4*, *mec-2* and *dgk-1* regulate the curve during thermotaxis and suggest that
 273 the alteration of the AFD-AIY synaptic transmission generates opposing curving biases that
 274 drive the animals toward the cultivation temperature.

275

Discussion

When animals encounter environmental stimuli, they need to quickly assess whether the stimuli are beneficial or detrimental. How the brain determines whether the valence of sensory information is attractive or aversive has been a fundamental question in neurobiology. A number of previous studies indicated that the opposing valences of sensory stimuli are encoded by two separate populations of neurons, each of which represents either positive or negative valence of the stimuli^{37,38}. By contrast, recent studies proposed an alternative strategy, wherein a single population of neurons responds to appetitive or aversive stimuli and represents the positive or negative valence of the stimuli by increasing or decreasing neuronal activity^{39,40}. Specifically, CRF (corticotropin-releasing factor)-releasing neurons in the paraventricular nucleus of the hypothalamus are activated by aversive stimuli and inhibited by appetitive stimuli⁴⁰. Likewise, in *C. elegans*, experience-dependent modulation enables a single set of interneurons to elicit bidirectional responses to carbon dioxide, which can be either attractive or aversive, depending on prior experience³⁹. Thus, these observations indicate a previously unrecognized mechanism of valence coding for even a single modality of stimulus, in which the bidirectional activity in

a single population of neurons can be modulated by prior experience and environment to represent stimulus valence. However, little was known about the molecular mechanism and circuit logics for such modulation of valence-coding activity. In this study, we report the molecular components important for determining a valence-coding activity and show that MAST kinase, Stomatin and Diacylglycerol kinase control the activity of the AIY neuron that correlates with the valence of thermal stimuli. Our results also reveal a circuit principle of such valence coding, in which a presynaptic neuron modulates its neuronal outputs and evokes an excitatory or inhibitory postsynaptic response.

We showed that *kin-4*, *mec-2* and *dgk-1* regulate the curving bias during thermotaxis behavior (Fig. 6). A previous study also showed that optogenetic manipulations of the AIY activity evoked biases in the curve: stimulation of AIY caused the animals to turn in the direction in which the head of the animal was bent at the time of the AIY excitation, while inhibition of AIY induced the animals to turn in the opposite direction⁴¹. Given this observation, our results suggest that below the cultivation temperature, a warming stimulus activates both AFD and AIY, leading to curve toward the warmer side of

the temperature gradient, while above the cultivation temperature, a warming stimulus activates AFD, which in turn inhibits AIY, resulting in curving toward the colder side (Supplementary Fig. 3). Thus, the alteration of the AFD-AIY transmission mode would generate the curving bias that drives the animals toward the cultivation temperature.

Recent investigation of the global brain dynamics in *C. elegans* revealed that most interneuron layers represent the motor states of the animals⁴². Indeed, representation of the motor states is pervasive even in the first-layer interneurons that receive direct inputs from sensory neurons. The activity of the AIB interneuron, which is directly innervated by gustatory sensory neurons ASE and olfactory neurons AWC, is correlated with reversals⁴³, and the AIY neuron can represent multiple motor states^{41,43,44}. Because calcium dynamics within the AFD, ASE and AWC neurons represent the respective sensory stimuli^{20,21,45–47}, these observations suggest that transformation of sensory information into motor representation occurs early in the neural circuit and underlies between the sensory neurons and the first-layer interneurons.

Our results indicate that the initial step of information processing that transforms

322 thermal information into stimulus valence occurs within the AFD sensory neuron and
 323 suggest that information processing for this transformation resides in cellular processes
 324 between calcium influx and neurotransmitter release. These observations underscore neural
 325 computation at axonal regions in a single neuron. In addition to the current view that neural
 326 computations take place at the synaptic communication and dendritic computations, our
 327 results highlight the importance of axonal computation in information processing in the
 328 nervous system. These observations, in turn, raise a future challenge in neuroscience.
 329 Although electrophysiological and calcium imaging analyses undoubtedly reveal certain
 330 aspects of neuronal properties, understanding the axonal computation requires the
 331 development of methods to quantitatively measure the release of the signaling molecules,
 332 including small neurotransmitters, neuropeptides and biogenic amines^{48,49}, preferably in
 333 behaving animals with high spatiotemporal resolution. Utilization of a pH-sensitive green
 334 fluorescent protein, pHluorin⁵⁰ has been successfully used to monitor the release and
 335 recycle of synaptic vesicles⁵¹⁻⁵⁴. However, since synaptic vesicle neurotransmitter content
 336 can be dynamically regulated in both vertebrates and invertebrates^{55,56}, direct measurements

of signaling molecules rather than that of vesicle release are required to fully understand the neuronal output. Development of such methods would unveil the dynamics of axonal computations and help dissect the mechanisms by which neurons execute this type of neural computation.

Neurons expressing multiple neurotransmitters are present in virtually all animal species. Co-transmission of multiple transmitters from single neurons can influence the same target neurons (convergent actions) or different targets (divergent actions), thereby providing additional flexibility to the circuit functions^{57,58}. A few studies reported co-transmission of multiple transmitters with opposing actions⁵⁹⁻⁶². In mammals, orexin and dynorphin neuropeptides exert opposing actions on excitability of ventral tegmental area dopamine neurons^{59,60}, and in *Aplysia* neuromuscular junction, multiple co-transmitted peptides exert antagonistic actions, with one peptide promoting muscle contraction and the other increasing relaxation rate^{61,62}. In these cases, multiple transmitters are co-packaged and co-released in a fixed ratio. The apparently antagonistic actions of multiple transmitters likely facilitate rhythmic muscle contraction, thereby providing temporal flexibility in the

352 circuit function^{61,62}.

353 Our results suggest that by altering the balance of multiple transmitters with
 354 opposing actions, a single presynaptic neuron can evoke excitatory and inhibitory
 355 postsynaptic responses, convey the valence information of sensory stimulus to the circuit,
 356 and induce an appropriate behavior. Our observations provide a sharp contrast to current
 357 views of presynaptic plasticity, in which presynaptic regulation facilitates or depresses the
 358 strength of the postsynaptic response^{63,64} but does not change the mode of transmission
 359 from an excitatory (inhibitory) to an inhibitory (excitatory) communication. We speculate
 360 that such mechanism of presynaptic control might function in other systems⁶⁵ and be
 361 particularly effective in assigning the stimulus valence over a range of stimulus intensity.
 362 Since previous studies suggested that *dgk-1* regulates synaptic transmission at
 363 neuromuscular junction in *C. elegans*^{24,25,66}, *kin-4*, *mec-2* and *dgk-1* might also regulate the
 364 release of glutamate and/or neuropeptide from AFD to control the bidirectional AIY
 365 response. Thus, presynaptic control of multi-transmitter release could be a fundamental
 366 mechanism of generating plasticity without extensive structural modification on the defined

367 neural circuitry, thereby extending the computational repertoire employed by the nervous

368 system.

369

Methods

C. elegans strains

All *C. elegans* strains were cultivated at 20 °C on nematode growth medium plates seeded with *E. coli* OP50 bacteria⁶⁷. N2 (Bristol) was the wild-type strain. The following mutations, extrachromosomal arrays and integrated transgenes were generated or described previously.

LGI: *njIs24[gcy-8p::GCaMP3, gcy-8::TagRFP]*⁶⁸. LG III: *eat-4(ky5)*³⁴. LG IV: *kin-4(tm1049Δ), kin-4(nj170Δ), unc-31(e928)*^{67,69}. LG V: *njIs110[gcy-8p::4xNLS::YCX1.6, AIYp::YCX1.6]*. LG X: *mec-2(nj89gf), mec-2(nj251Δ), dgk-1(nj271), dgk-1(nj274Δ)*.

Extrachromosomal arrays: *njEx682[kin-4(+), ges-1p::gfp], njEx683[kin-4::gfp, ges-1p::TagRFP], njEx753[gcy-8p::kin-4d cDNA, ges-1p::gfp]* (used for rescuing the cryophilic phenotype of *kin-4(tm1049Δ)*, *njEx759[ttx-3p::kin-4d cDNA, ges-1p::gfp]*, *njEx760[lin-11p::kin-4d cDNA, ges-1p::gfp], njEx763[ceh-36p::kin-4d cDNA, ges-1p::gfp], njEx764[glr-3p::kin-4d cDNA, ges-1p::gfp], njEx1029[mec-2(E270K), ges-1p::gfp], njEx1035[mec-2(+), ges-1p::gfp], njEx1158[Pmec-2a::gfp, ges-1p::TagRFP], njEx1159[Pmec-2c::gfp, ges-1p::TagRFP], njEx1220[ceh-36p::mec-2a cDNA (E270K), ceh-36p::mec-2c cDNA (E195K), ges-1p::gfp], njEx1231[gcy-8p::mec-2a*

386 *cDNA (E270K), gcy-8p::mec-2c cDNA (E195K), ges-1p::gfp], njEx1317[gcy-8p::dgk-1b*
 387 *cDNA, ges-1p::gfp], njEx1319[odr-1p::dgk-1b cDNA, ges-1p::gfp], njEx1330[dgk-1(+),*
 388 *ges-1p::gfp], njEx1331[gcy-8p::dgk-1b cDNA, odr-1::dgk-1b cDNA, ges-1p::gfp],*
 389 *njEx1435[gcy-8p::kin-4d, ges-1p::TagRFP]* (used for rescuing AIY calcium response),
 390 *njEx1438[gcy-8p::unc-31 cDNA, ges-1p::TagRFP], njEx1439[gcy-8p::eat-4 cDNA,*
 391 *ges-1p::TagRFP].* We used following promoters for cell-specific expression of transgenes
 392 and cDNAs: *gcy-8* promoter for AFD; *ttx-3* promoter for AIY; *ceh-36* or *odr-1* promoters
 393 for AWC; *lin-11* promoter for AIZ; *glr-3* promoter for RIA; *ges-1* promoter for intestinal
 394 cells.

395

396 Thermotaxis behavioral tests

397 Thermotaxis assays were performed as previously described⁶⁸. Animals that had been
 398 cultivated at 20 °C were placed on the center of thermotaxis assay plate with a temperature
 399 gradient ranging from 17 °C to 23 °C. The steepness of the temperature gradient was set at
 400 0.45 ~ 0.5 °C / cm. Animals were allowed to freely move on the plate for 1 hour. The

thermotaxis assay plate was divided into 8 sections along the temperature gradient, and the number of adult animals in each section was counted.

Expression analysis of *kin-4* and *mec-2*

To construct *kin-4::gfp*, we modified the fosmid containing the *kin-4* locus, WRM0635dF07. We inserted a *gfp* coding sequence immediately before the stop codon of *kin-4* using bacterial recombineering. To construct *Pmec-2a::gfp* and *Pmec-2c::gfp*, a 3.5 kb and a 3.2 kb fragments upstream of the start codon of *mec-2a* and *mec-2c* were cloned into the *gfp* expression vector pPD95.75, respectively. Animals carrying *kin-4::gfp*, *Pmec-2a::gfp* or *Pmec-2c::gfp* were anesthetized with 50 mM sodium azide and were observed under Nomarski optics equipped with epifluorescence. Identification of the AFD, ALM, AVM, AWC, AIY, PLM, PVM and RIA neurons was conducted by observing the positions and sizes of the nuclei and the patterns of neuronal processes.

Multi worm tracking analysis

Multi worm tracking analysis was performed as described³⁶. Animals were cultivated at 20 °C and were placed on the thermal gradient with the center temperature of 18.5 °C or 21.5 °C to monitor their behaviors below or above the cultivation temperature, respectively. The behaviors during the first 10 minutes of the thermotaxis assay were captured by a CMOS camera and were analyzed by multi worm tracker to obtain the coordinates of animal's centroids and spines³⁵. The data were further analyzed by a custom-written program in MATLAB to classify the behaviors into behavioral components.

Calcium imaging of AFD in immobilized animals

Calcium imaging of the AFD neuron was performed as described⁶⁸. Animals expressing GCaMP3 and TagRFP in AFD were cultivated at 20 °C and placed on a 5 – 10 % agarose pad with polystyrene beads to immobilize the animals. Animals were subjected to a warming stimulus, and the fluorescence intensities from the AFD cell body were captured and analyzed by the MetaMorph software. The ratio of the fluorescence intensity (GCaMP3/TagRFP) was used to calculate ratio change, which was defined as (ratio –

baseline ratio)/baseline ratio, where baseline ratio was the mean of the ratio values during the first 30 seconds of the experiment. The response temperature was previously defined⁶⁸ as the temperature at which the ratio change first exceeded 1.

Calcium imaging of AIY in freely moving animals

We generated animals expressing the ratiometric calcium probe YCX1.6 in AFD and AIY. YCX1.6 in AFD was localized to the nucleus to separate the fluorescence signals from these neurons. Animals were cultivated at 20 °C and placed on a 2 – 2.5 % agarose pad on a cover glass. The sample was covered by another cover glass and was placed onto a transparent temperature-controlled device (TOKAI HIT Co. Ltd., Fujinomiya). This device was installed onto a motorized stage (HawkVision Inc., Fujisawa) that keeps the target image of animals in the field of view. Controlling the stage movement was achieved by real-time analysis of transmitted infrared light images. The fluorescence images were captured twice a second and split into YFP and CFP channels by W-VIEW GEMINI (Hamamatsu photonics K.K., Hamamatsu). The YFP and CFP fluorescence intensities were

446 obtained from the cell body of AFD and an axonal region of AIY⁶⁸. The fluorescent images
 447 were analyzed by a custom-written program in MATLAB with manual inspection of region
 448 of interest in every frame, and the fluorescent intensities of YFP and CFP were determined.
 449 We eliminated from the analysis the trials in which the temperature program failed to
 450 activate AFD. For this purpose, we applied a low-pass filter to the YFP/CFP ratio with the
 451 cut-off frequency at 0.05 Hz, and the resulting ratio data were used to calculate the ratio
 452 change. We eliminated from the analysis the trials in which the maximum ratio changes
 453 were smaller than 0.25 for recordings below the cultivation temperature or 0.22 for above
 454 the cultivation temperature. These threshold values were determined from experiments in
 455 which the temperature was kept constant. For the reminder of the trials, the ratio of
 456 fluorescence intensities (YFP/CFP) was used to calculate the standardized ratio change of
 457 AFD and AIY, which was defined as $(\text{ratio} - \text{minimum ratio}) / (\text{maximum ratio} - \text{minimum}$
 458 $\text{ratio})$. The baseline standardized ratio, which was the mean of the standardized ratio values
 459 of 5 consecutive frames immediately before the onset of warming stimulus, was subtracted
 460 from the standardized ratio change of each frame. For comparison of the peak standardized

ratio change, the maximum standardized ratio change (positive or negative) in a 2-second time window centered at the peak of the mean control standardized ratio change was used.

Statistics

Normality of the data was assessed by Shapiro-Wilk test. Equal variance among data sets was assessed by *F*-test or Bartlett test. When both normality and equal variance were assumed for the data set, we used two-tailed student *t*-test for pairwise comparison and one-way analysis of variance (ANOVA) with Tukey-Kramer or Dunnett's test for multiple comparisons. In other cases, we applied Wilcoxon rank sum test for pairwise comparison and Kruskal-Wallis rank sum test with Steel method for multiple comparisons.

Data availability

All raw images, source data and custom scripts are available from the authors upon reasonable request.

References

1. Zangrossi, H. & File, S. E. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. *Brain Res. Bull.* **29**, 381–388 (1992).
2. Terry, L. M. & Johanson, I. B. Olfactory influences on the ingestive behavior of infant rats. *Dev. Psychobiol.* **20**, 313–331 (1987).
3. Johnston, R. E. Effects of female odors on the sexual behavior of male hamsters. *Behav. Neural Biol.* **46**, 168–188 (1986).
4. Min, S., Ai, M., Shin, S. A. & Suh, G. S. B. Dedicated olfactory neurons mediating attraction behavior to ammonia and amines in *Drosophila*. *Proc. Natl. Acad. Sci.* **110**, E1321–E1329 (2013).
5. Kobayakawa, K. *et al.* Innate versus learned odour processing in the mouse olfactory bulb. *Nature* **450**, 503–508 (2007).
6. Mueller, K. L. *et al.* The receptors and coding logic for bitter taste. *Nature* (2005). doi:10.1038/nature03352
7. Zhao, G. Q. *et al.* The receptors for mammalian sweet and umami taste. *Cell* **115**,

- 491 255–266 (2003).
- 492 8. Marella, S. *et al.* Imaging taste responses in the fly brain reveals a functional map of
- 493 taste category and behavior. *Neuron* **49**, 285–295 (2006).
- 494 9. Laing, D. G., Panhuber, H. & Baxter, R. I. Olfactory properties of amines and
- 495 n-butanol. *Chem. Senses* **3**, 149–166 (1978).
- 496 10. Charro, M. J. & Alcorta, E. Quantifying relative importance of maxillary palp
- 497 information on the olfactory behavior of *Drosophila melanogaster*. *J. Comp. Physiol.*
- 498 *A* **175**, 761–766 (1994).
- 499 11. Malnic, B., Hirono, J., Sato, T. & Buck, L. B. Combinatorial receptor codes for
- 500 odors. *Cell* **96**, 713–723 (1999).
- 501 12. Semmelhack, J. L. & Wang, J. W. Select *Drosophila* glomeruli mediate innate
- 502 olfactory attraction and aversion. *Nature* **459**, 218–223 (2009).
- 503 13. Yoshida, K. *et al.* Odour concentration-dependent olfactory preference change in *C.*
- 504 *elegans*. *Nat. Commun.* **3**, 711–739 (2012).
- 505 14. White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. The Structure of the

- 506 Nervous System of the Nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. B*
507 *Biol. Sci.* **314**, 1–340 (1986).
- 508 15. Hedgecock, E. M. & Russell, R. L. Normal and mutant thermotaxis in the nematode
509 *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* **72**, 4061–4065 (1975).
- 510 16. Kuhara, A. *et al.* Temperature sensing by an olfactory neuron in a circuit controlling
511 behavior of *C. elegans*. *Science* **320**, 803–807 (2008).
- 512 17. Mori, I. & Ohshima, Y. Neural regulation of thermotaxis in *Caenorhabditis elegans*.
513 *Nature* **376**, 344–348. (1995).
- 514 18. Beverly, M., Anbil, S. & Sengupta, P. Degeneracy and Neuromodulation among
515 Thermosensory Neurons Contribute to Robust Thermosensory Behaviors in
516 *Caenorhabditis elegans*. *J. Neurosci.* **31**, 11718–11727 (2011).
- 517 19. Luo, L. *et al.* Bidirectional thermotaxis in *Caenorhabditis elegans* is mediated by
518 distinct sensorimotor strategies driven by the AFD thermosensory neurons. *Proc.*
519 *Natl. Acad. Sci.* **111**, 2776–2781 (2014).
- 520 20. Kimura, K. D., Miyawaki, A., Matsumoto, K. & Mori, I. The *C. elegans*

- thermosensory neuron AFD responds to warming. *Curr. Biol.* **14**, 1291–1295 (2004).
21. Clark, D. A., Biron, D., Sengupta, P. & Samuel, A. D. T. The AFD Sensory Neurons Encode Multiple Functions Underlying Thermotactic Behavior in *Caenorhabditis elegans*. *J. Neurosci.* **26**, 7444–7451 (2006).
22. Walden, P. D. & Cowan, N. J. A Novel 205-Kilodalton Testis-Specific Serine/Threonine Protein Kinase Associated with Microtubules of the Spermatid Manchette. *Mol. Cell. Biol.* **13**, 7625–7635 (1993).
23. Huang, M., Gu, G., Ferguson, E. L. & Chalfie, M. A stomatin-like protein necessary for mechanosensation in *C. elegans*. *Nature* **378**, 292–295 (1995).
24. Miller, K. G., Emerson, M. D. & Rand, J. B. Gqalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in *C. elegans*. *Neuron* **24**, 323–333 (1999).
25. Nurrish, S., Ségalat, L. & Kaplan, J. M. Serotonin inhibition of synaptic transmission: $g\alpha(o)$ decreases the abundance of *unc-13* at release sites. *Neuron* **24**, 231–242. (1999).
26. Okochi, Y., Kimura, K. D., Ohta, A. & Mori, I. Diverse regulation of sensory

- 536 signaling by *C. elegans* nPKC-epsilon/eta TTX-4. *EMBO J.* **24**, 2127–2137 (2005).
- 537 27. Ohnishi, N., Kuhara, A., Nakamura, F., Okochi, Y. & Mori, I. Bidirectional
- 538 regulation of thermotaxis by glutamate transmissions in *Caenorhabditis elegans*.
- 539 *EMBO J.* **30**, 1376–1388 (2011).
- 540 28. Inada, H. *et al.* Identification of guanylyl cyclases that function in thermosensory
- 541 neurons of *Caenorhabditis elegans*. *Genetics* **172**, 2239–2252 (2006).
- 542 29. Ramot, D., MacInnis, B. L. & Goodman, M. B. Bidirectional temperature-sensing by
- 543 a single thermosensory neuron in *C. elegans*. *Nat. Neurosci.* **11**, 908–915 (2008).
- 544 30. Takeishi, A. *et al.* Receptor-type Guanylyl Cyclases Confer Thermosensory
- 545 Responses in *C. elegans*. *Neuron* **90**, 235–244 (2016).
- 546 31. Hawk, J. D. *et al.* Integration of Plasticity Mechanisms within a Single Sensory
- 547 Neuron of *C. elegans* Actuates a Memory. *Neuron* 356–367 (2018).
- 548 doi:10.1016/j.neuron.2017.12.027
- 549 32. Narayan, A., Laurent, G. & Sternberg, P. W. Transfer characteristics of a
- 550 thermosensory synapse in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci.* **108**,

- 551 9667–9672 (2011).
- 552 33. Speese, S. *et al.* UNC-31 (CAPS) Is Required for Dense-Core Vesicle But Not
- 553 Synaptic Vesicle Exocytosis in *Caenorhabditis elegans*. *J. Neurosci.* **27**, 6150–6162
- 554 (2007).
- 555 34. Lee, R. Y. N., Sawin, E. R., Chalfie, M., Horvitz, H. R. & Avery, L. EAT-4 , a
- 556 Homolog of a Mammalian Sodium-Dependent Inorganic Phosphate Cotransporter ,
- 557 Is Necessary for Glutamatergic Neurotransmission in *Caenorhabditis elegans*.
- 558 *Neuron* **19**, 159–167 (1999).
- 559 35. Swierczek, N. A., Giles, A. C., Rankin, C. H. & Kerr, R. A. High-throughput
- 560 behavioral analysis in *C. elegans*. *Nat. Methods* **8**, 592–598 (2011).
- 561 36. Ikeda, M. *et al.* Circuit Degeneracy Facilitates Robustness and Flexibility of
- 562 Navigation Behavior in *C. elegans*. *bioRxiv* (2018). doi:10.1101/385468
- 563 37. Bruchas, M. R., Calhoon, G. G., Al-Hasani, R., Namburi, P. & Tye, K. M.
- 564 Architectural Representation of Valence in the Limbic System.
- 565 *Neuropsychopharmacology* **41**, 1697–1715 (2015).

- 566 38. Knaden, M. & Hansson, B. S. Mapping odor valence in the brain of flies and mice.
567 *Curr. Opin. Neurobiol.* **24**, 34–38 (2014).
- 568 39. Guillermin, M. L., Carrillo, M. A. & Hallem, E. A. A Single Set of Interneurons
569 Drives Opposite Behaviors in *C. elegans*. *Curr. Biol.* **27**, 2630–2639.e6 (2017).
- 570 40. Kim, J. *et al.* Rapid, biphasic CRF neuronal responses encode positive and negative
571 valence. *Nat. Neurosci.* (2019). doi:10.1038/s41593-019-0342-2
- 572 41. Kocabas, A., Shen, C. H., Guo, Z. V. & Ramanathan, S. Controlling interneuron
573 activity in *Caenorhabditis elegans* to evoke chemotactic behaviour. *Nature* **490**,
574 273–277 (2012).
- 575 42. Kato, S. *et al.* Global Brain Dynamics Embed the Motor Command Sequence of
576 *Caenorhabditis elegans*. *Cell* **163**, 656–669 (2015).
- 577 43. Luo, L. *et al.* Dynamic encoding of perception, memory, and movement in a *C.*
578 *elegans* chemotaxis circuit. *Neuron* **82**, 1115–1128 (2014).
- 579 44. Li, Z., Liu, J., Zheng, M. & Xu, X. Z. S. Encoding of both analog- and digital-like
580 behavioral outputs by one *C. elegans* interneuron. *Cell* **159**, 751–765 (2014).

- 581 45. Suzuki, H. *et al.* Functional asymmetry in *Caenorhabditis elegans* taste neurons and
582 its computational role in chemotaxis. *Nature* **454**, 114–117 (2008).
- 583 46. Chalasani, S. H. *et al.* Dissecting a circuit for olfactory behaviour in *Caenorhabditis*
584 *elegans*. *Nature* **450**, 63–70 (2007).
- 585 47. Kunitomo, H. *et al.* Concentration memory-dependent synaptic plasticity of a taste
586 circuit regulates salt concentration chemotaxis in *Caenorhabditis elegans*. *Nat.*
587 *Commun.* **4**, 1–11 (2013).
- 588 48. Marvin, J. S. *et al.* An optimized fluorescent probe for visualizing glutamate
589 neurotransmission. *Nat. Methods* **10**, 162–170 (2013).
- 590 49. Sun, F. *et al.* A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific
591 Detection of Dopamine in Flies, Fish, and Mice. *Cell* **174**, 481–496 (2018).
- 592 50. Miesenbock, G., Rothman, D. A. D. A. & Rothman, J. E. Visualizing secretion and
593 synaptic transmission with pH-sensitive green fluorescent proteins. *Nature* **394**,
594 192–195 (1998).
- 595 51. Bozza, T., McGann, J. P., Mombaerts, P. & Wachowiak, M. In vivo imaging of

596 neuronal activity by targeted expression of a genetically encoded probe in the mouse.

597 *Neuron* **42**, 9–21 (2004).

598 52. Li, Z. *et al.* Synaptic vesicle recycling studied in transgenic mice expressing

599 synaptotHluorin. *Proc. Natl. Acad. Sci.* **102**, 6131–6136 (2005).

600 53. Ventimiglia, D. & Bargmann, C. I. Diverse modes of synaptic signaling, regulation,

601 and plasticity distinguish two classes of *C. elegans* glutamatergic neurons. *Elife* **6**,

602 1–25 (2017).

603 54. Sankaranarayanan, S. & Ryan, T. A. Calcium accelerates endocytosis of vSNAREs

604 at hippocampal synapses. *Nat. Neurosci.* **4**, 129–136 (2001).

605 55. Aguilar, J. I. *et al.* Neuronal Depolarization Drives Increased Dopamine Synaptic

606 Vesicle Loading via VGLUT. *Neuron* **95**, 1074–1088.e7 (2017).

607 56. Steinert, J. R. *et al.* Experience-Dependent Formation and Recruitment of Large

608 Vesicles from Reserve Pool. *Neuron* **50**, 723–733 (2006).

609 57. Nusbaum, M. P., Blitz, D. M. & Marder, E. Functional consequences of

610 neuropeptide and small-molecule co-transmission. *Nat. Rev. Neurosci.* **18**, 389–403

- 611 (2017).
- 612 58. Vaaga, C. E., Borisovska, M. & Westbrook, G. L. Dual-transmitter neurons:
- 613 Functional implications of co-release and co-transmission. *Curr. Opin. Neurobiol.* **29**,
- 614 25–32 (2014).
- 615 59. Baimel, C., Lau, B. K., Qiao, M. & Borgland, S. L. Projection-Target-Defined
- 616 Effects of Orexin and Dynorphin on VTA Dopamine Neurons. *Cell Rep.* **18**,
- 617 1346–1355 (2017).
- 618 60. Muschamp, J. W. *et al.* Hypocretin (orexin) facilitates reward by attenuating the
- 619 antireward effects of its cotransmitter dynorphin in ventral tegmental area. *Proc. Natl.*
- 620 *Acad. Sci.* **111**, E1648–E1655 (2014).
- 621 61. Vilim, F. S., Price, D. a, Lesser, W., Kupfermann, I. & Weiss, K. R. Costorage and
- 622 corelease of modulatory peptide cotransmitters with partially antagonistic actions on
- 623 the accessory radula closer muscle of *Aplysia californica*. *J. Neurosci.* **16**, 8092–104
- 624 (1996).
- 625 62. Vilim, F. S., Cropper, E. C., Price, D. A., Kupfermann, I. & Weiss, K. R. Peptide

626 Cotransmitter Release from Motorneuron B16 in *Aplysia californica*: Costorage,
627 Corelease, and Functional Implications. *J. Neurosci.* **20**, 2036–2042 (2000).

628 63. Monday, H. R. & Castillo, P. E. Closing the gap: long-term presynaptic plasticity in
629 brain function and disease. *Curr. Opin. Neurobiol.* **45**, 106–112 (2017).

630 64. Regehr, W. G. Short-term presynaptic plasticity. *Cold Spring Harb. Perspect. Biol.* **4**,
631 a005702 (2012).

632 65. Tsunozaki, M., Chalasani, S. H. & Bargmann, C. I. A Behavioral Switch: cGMP and
633 PKC Signaling in Olfactory Neurons Reverses Odor Preference in *C. elegans*.
634 *Neuron* **59**, 959–971 (2008).

635 66. McMullan, R., Hiley, E., Morrison, P. & Nurrish, S. J. Rho is a presynaptic activator
636 of neurotransmitter release at pre-existing synapses in *C. elegans*. *Genes Dev.* **20**,
637 65–76 (2006).

638 67. Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94 (1974).

639 68. Kobayashi, K. *et al.* Single-Cell Memory Regulates a Neural Circuit for Sensory
640 Behavior. *Cell Rep.* **14**, 11–21 (2016).

69. Charlie, N. K., Schade, M. A., Thomure, A. M. & Miller, K. G. Presynaptic UNC-31 (CAPS) is required to activate the Gas pathway of the *Caenorhabditis elegans* synaptic signaling network. *Genetics* **172**, 943–961 (2006).

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Author Contributions

S.N. and I.M. designed the experiments. S.N., R.A., A.S. and R.K. conducted experiments.

S.N. and M.I. wrote custom codes for the analyses. Y.T., X.F. and K.H. developed and set up the tracking system for calcium imaging of freely moving animals. T. S., K. I. and T. H. conducted whole genome sequencing of mutants isolated in this study. S.N. and I. M. wrote the manuscript.

Competing Interests

The authors declare no competing interests.

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669

670 **Figure Legends**

671 **Figure 1. *kin-4*, *mec-2* and *dgk-1* Regulate the *C. elegans* Thermotaxis Behavior**

672 (a) Procedure of thermotaxis assay is shown. Animals were cultivated at 20 °C and were
 673 placed on the center of a thermal gradient ranging from 17 °C to 23 °C. Each assay
 674 typically contains 100 ~ 200 animals. Distribution of the animals in each section of the
 675 assay plate was determined. We also use thermotaxis (TTX) index to quantify the behavior.
 676 The formula of TTX index is shown. (b-f) Thermotaxis behavior of the wild type, *kin-4*,
 677 *mec-2* and *dgk-1* mutant animals. Distributions of the animals in each section of the assay
 678 plate (top) are shown as means $\pm$ s.e.m. TTX indices (bottom) are shown as dots. Lines
 679 indicate the means. *n* represents the number of independent experiments. *P* values were
 680 determined by Kruskal-Wallis test with Steel method for comparison to the wild-type
 681 animals in (b), one-way ANOVA with Dunnett's test for comparison to *kin-4(tm1049Δ)*
 682 mutants in (c), two-tailed Student's *t*-tests in (d) and one-way ANOVA with Tukey-Kramer
 683 test in (e) and (f).

684

Figure 2. *kin-4*, *mec-2* and *dgk-1* Function in the AFD Thermosensory Neurons to Regulate Thermotaxis

(a) Neural circuit involved in thermotaxis. Arrows indicate chemical synapses. Triangles and hexagons represent sensory and interneurons, respectively. (b) Expression analyses of *kin-4::gfp* (Top) and *Pmec-2c::gfp* (Bottom). Head regions of animals are shown. The arrowheads indicate the AFD and AWC sensory neurons. Scale bars, 10 μ m. (c-e) Thermotaxis behaviors of animals in which *kin-4*, *mec-2(E270K)* or *dgk-1* cDNA was specifically expressed in AFD and other neurons. Animals were cultivated at 20 °C. Distributions of animals in each section of the assay plates are shown as means $\pm$ s.e.m. TTX indices are shown as dots, and the lines indicate means. *n* indicates the number of the independent experiments. Animals were cultivated at 20 °C. *P* values were determined by one-way ANOVA with Dunnett's test for multiple comparison to *kin-4(tm1049Δ)* in (c) or *dgk-1(nj274Δ)* in (e). Kruskal-Wallis test with Steel method was used for comparison to the wild-type animals in (d).

Figure 3. *kin-4*, *mec-2* and *dgk-1* Regulate a Process Downstream of Calcium Influx in

AFD

(a) Temperature program used for calcium imaging of the AFD neurons in immobilized animals. (b) Ratio changes of the AFD calcium dynamics are shown. Grey lines are results for individual traces; thick colored lines represent mean values. *N* indicates the number of animals observed. (c) Comparison of the maximum ratio changes in each strain. Individual data points are shown as dots. Boxes display the first and third quartiles; lines inside the boxes are the medians; the whiskers extend to 1.5-time interquartile range from the box. Kruskal-Wallis test with Steel method was used to compare to the wild type animals. (d) Comparison of the response temperatures in the wild type, *kin-4*, *mec-2* and *dgk-1* mutants. The response temperature was defined previously⁶⁸ as the temperature at which the ratio change first exceeded 1. Individual data points are shown as dots. Boxes display the first and third quartiles; lines inside the boxes are the medians; the whiskers extend to 1.5-time interquartile range from the box. *P* values were determined by one-way ANOVA with Dunnett's test for comparison to the wild type.

715

716 **Figure 4. The Bidirectional AIY Response Encodes Stimulus Valence and Is**
 717 **Regulated by *kin-4*, *mec-2* and *dgk-1***

718 Calcium imaging of AFD and AIY neurons in freely moving animals. **(a-d)** Calcium
 719 imaging below the cultivation temperature. **(e-g)** Calcium imaging above the cultivation
 720 temperature. **(a, e)** Neurons imaged and the temperature program used. **(b, f)** Standardized
 721 ratio changes of AFD (top) and AIY (bottom) calcium dynamics. Grey lines are results for
 722 individual traces; colored lines represent mean values. Time 0 corresponds to the onset of
 723 the warming stimulus. *n* indicates the number of trials. *N* indicates the number of animals
 724 observed. Note that the scales for the mean values are on the right axis. **(c, d, g)**
 725 Comparisons of peak AIY standardized ratio changes. Individual data points are shown as
 726 dots. Boxes display the first and third quartiles; lines inside the boxes are the medians; the
 727 whiskers extend to 1.5-time interquartile range from the box. *P* values were determined by
 728 Kruskal-Wallis test with Steel method to compare to the wild type animals in (c) and (g) or
 729 by Wilcoxon rank sum test in (d).

730

731 **Figure 5. Alteration of the AFD-AIY Synaptic Valence Requires Components**
 732 **Essential for Neuropeptide and Glutamate Release**

733 (a-d) Calcium imaging below the cultivation temperature. (e-h) Calcium imaging above
 734 the cultivation temperature. (a, e) The temperature program used. (b, f) Standardized ratio
 735 changes of AFD (top) and AIY (bottom) calcium dynamics. Grey lines are results for
 736 individual traces; colored lines represent mean values. Time 0 corresponds to the onset of
 737 the warming stimulus. *n* indicates the number of trials. *N* indicates the number of animals
 738 observed. Note that the scales for the mean values are on the right axis. (c, d, g, h)
 739 Comparisons of peak AIY standardized ratio changes. Individual data points are shown as
 740 dots. Boxes display the first and third quartiles; lines inside the boxes are the medians; the
 741 whiskers extend to 1.5-time interquartile range from the box. *P* values were determined by
 742 Kruskal-Wallis test with Steel method was used to compare to the wild type animals in (c)
 743 and (g) or by Wilcoxon rank sum test in (d) and (h). Note that the wild-type data are
 744 identical to those shown in Fig. 4 and indicated here for comparison to *unc-31* and *eat-4*

mutants.

Figure 6. *kin-4*, *mec-2* and *dgk-1* Regulate Curving Bias During Thermotaxis

Multi-worm tracking analyses of curve (a), shallow turn (b), omega turn (c), reversal (d) and reversal turn (e). Schematics of the behavioral components are shown (left). Animals were cultivated at 20 °C (T_c), and their behaviors within the temperature ranges from 17.0 °C to 20.0 °C (middle) or from 20 °C to 23.0 °C (right) were monitored. (a) Curve is characterized by two angles ϕ and θ , where ϕ corresponds to the change in the moving direction during forward locomotion, and θ animal's previous moving direction relative to the vector pointing to the warm side of the temperature gradient. ϕ is given as a positive value if the angle change is directed toward the warmer side and a negative value if directed toward the colder side (left). Dot plots of mean curving rate of animals migrating up the temperature gradient below (middle) or above (right) the cultivation temperature. The curving rates of all animals during the first 10 minutes of thermotaxis assay were averaged and shown as dots. n indicates the number of independent experiments. P values were

determined by one-way ANOVA with Dunnett's test for comparison to the wild-type animals. **(b-e)** Turns were classified into shallow turn (b) and omega turn (c), and reversals were divided into reversal (d) and reversal turn (e). The angle ψ , which represents the change in the direction after the turns or reversals, was used to classify the turns and reversals (left). Dot plots of frequencies of each behavioral component while animals were migrating up the temperature gradient below (middle) or above (right) the cultivation temperature. The frequencies of the behavioral component of all animals during the first 10 minutes of thermotaxis assay were averaged and shown as dots. n indicates the number of independent experiments. P values were determined by one-way ANOVA with Dunnett's test or by Kruskal-Wallis test with Steel method for comparison to the wild-type animals.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Supplemental Information

Supplemental Figure Legends

Supplemental Figure 1. *kin-4*, *mec-2* and *dgk-1* Regulate Thermotaxis Behavior.

(a) Gene structures of *kin-4* (top), *mec-2* (middle) and *dgk-1* (bottom) and mutations associated with each mutant are shown. Filled boxes and triangles represent exons, empty boxes and triangles untranslated region, and lines introns. (b, c) Thermotaxis behaviors of *dgk-1(nj274Δ)* carrying a genomic *dgk-1(+)* transgene (b) and *mec-2(nj251Δ)* mutants (c). Animals were cultivated at 20 °C and were placed on a thermal gradient ranging from 17 °C to 23 °C. Distributions of the animals (top) in each section of the assay plates are shown as means ± s.e.m. TTX indices (bottom) are shown as dots. Lines indicate the means. *P* values were determined by one-way ANOVA with Dunnett's test for comparison to *dgk-1(nj274Δ)* mutants (b) and two-tailed Student's *t*-tests in (c).

Supplemental Figure 2. Expression analysis of *kin-4::gfp* and *Pmec-2::gfp*.

(a) Thermotaxis behavior of animals carrying a translational *kin-4::gfp* fusion. Wild-type, *kin-4(tm1049Δ)* mutants and *kin-4(tm1049Δ)* animals carrying the *kin-4::gfp* transgene were cultivated at 20 °C and subjected to thermotaxis assay. Distributions of the animals in each section of the assay plates are shown as means $\pm$ s.e.m (top). *n* indicates the number of the independent experiments. TTX indices are shown as dots. Lines indicate the means. *P* values were determined by one-way ANOVA with Dunnett's test for comparison to *kin-4(tm1049Δ)* animals. (b) Expression analysis of the *kin-4::gfp* transgene. Head regions of animals are shown. The *kin-4::gfp* fusion was expressed in the AIY (left) and RIA (right) interneurons. Scale bars, 10 μ m. (c) Expression analysis of *Pmec-2a::gfp*. The entire body of an animal is shown. The *Pmec-2a::gfp* reporter was expressed in the ALM, AVM, PLM and PVM neurons. Scale bar, 50 μ m.

Supplemental Figure 3. Model of curve regulation by alteration of AFD-AIY neuronal communication.

Below the cultivation temperature (left), a head bending toward warmer side of the temperature gradient activates both AFD and AIY, leading to curve toward warmer side. Above the cultivation temperature (right), a temperature increase activates AFD, which in turn, inhibits AIY, leading to curve toward colder side.

Supplementary Figure 1

a

b

c

Supplementary Figure 2

Supplementary Figure 3