論文内容の要旨

A Gustatory Neural Circuit of *Caenorhabditis elegans* Generates Memory-Dependent Behaviors in Na^+ Chemotaxis

(線虫 ***Caenorhabditis elegans*** のナトリウムイオンに対する記憶依存の化学走性を制御する神経回路の解明)

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Learning and memory is a fundamental ability imparted to all animals including ourselves. Because of this ability embedded in the nervous system, animals can search for places with plenty of food and water, and move to the environment with adequate temperature and moisture based on previous experiences. The mechanisms for sensing environmental stimuli, storing memory, and executing learned behaviors have been essential subjects of neuroscience. However, there have been difficulties in the study of learning and memory using human and mammals, because of their complicated nervous system and the ethical limitations. On the other hand, learning and memory, as a basic mechanism for survival, is conserved among organisms spanning a long history of evolution. In this respect, invertebrates play important roles in understanding how the neural circuits generate plastic behaviors through the process of learning and memory, because of their experimental accessibility and the simple neural circuits.

The nematode *Caenorhabditis elegans* has only 302 neurons in an adult hermaphrodite, which have been well described, and possesses many genes homologous to those expressed in vertebrate brains, making it an ideal model organism for functional analysis of the nervous system (White et al., 1986). It also has a highly developed chemosensory system that enables it to detect a wide variety of chemicals (volatile and water-soluble) associated with food, danger, or other animals. It is known that ASE neurons, ASE-left (ASEL) and ASE-right (ASER), are responsible for sensing water-soluble chemicals (Bargmann and Horvitz, 1991), but sense different sets of ions. ASEL responds to Mg^{2+}, Li^+, and Na^+, whereas ASER responds to Br^-, I^-, and Cl^- (Fig. 1, Ortiz et al., 2009). *C. elegans* also memorizes and learns to move toward or avoid certain concentrations of chemicals according to previous experience. When nematodes are grown on a medium that contains salt and food, they show attraction to NaCl by using ASE neurons (Kunitomo et al., 2013). However, when they are starved with NaCl, the chemotaxis to NaCl falls dramatically or even becomes negative to avoid NaCl (Tomioka et al., 2006; Adachi et al., 2010).

Two behavioral mechanisms have been reported for salt chemotaxis: klinokinesis, in which

worms change the direction of locomotion quickly with pirouette (sharp turns) (Pierce-Shimomura et al., 1999) and klinotaxis, in which worms gradually curve towards higher (or lower) salt concentrations (Iino and Yoshida, 2009). In klinokinesis, worms increase the rate of pirouette either when salt concentration decreases (driving positive chemotaxis), or when salt concentration increases (driving negative chemotaxis). The Gq/DAG (diacylglycerol)/PKC (protein kinase C) pathway acts in ASER to promote migration to higher salt concentrations, possibly by augmenting the response of downstream AIB neurons (Kunitomo et al., 2013). It was also reported that ASER-evoked curving (klinotaxis) toward lower concentrations of salt is mediated by AIY interneurons (Satoh et al., 2014).

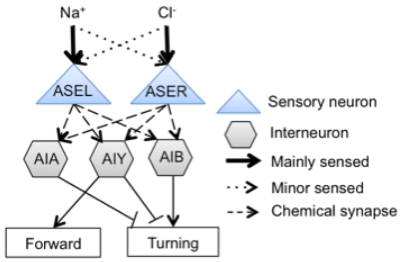

Fig. 1. Sensory specificity of ASE salt-sensing neurons and their first layer interneurons.

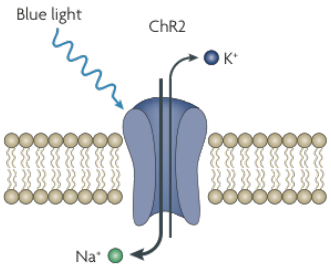

Fig. 2. ChR2 activates the target cell upon blue light illumination (Adapted from Zhang et al., 2007).

In contrast, the role and property of the ASEL neuron in salt chemotaxis plasticity has not been rigorously examined, although our previous study showed that together with ASER, ASEL is important for NaCl chemotaxis learning (Adachi et al., 2010). In this study, chemotaxis assay, optogenetics combining with calcium imaging and behavioral assay were used to systematically investigate the characteristics of ASEL in klinokinesis and its generated Na^+ chemotaxis neuron circuit.

1. ASEL generates memory-dependent behaviors in Na^+ chemotaxis

The chemotaxis assay revealed that after cultivation in Na^+-free conditions, worms showed no Na^+ concentration preference, but after cultivation in the presence of 100 mM Na^+, worms showed positive Na^+ chemotaxis (*i. e.* migrated to higher Na^+ concentrations, Fig. 3). Transgenic worms, in which channelrhodopsin (ChR2) was expressed only in ASEL (Fig. 2), were illuminated by blue-light after cultivation at different Na^+ concentrations, and the behavioral response was quantified by the worm tracking system. Since ASEL is activated by an increase of Na^+ concentration (Suzuki et al., 2008), optogenetic activation of ASEL mimics an increase of Na^+ concentration. When worms were cultivated with Na^+ and tested on test plates at any Na^+ concentration (or without Na^+), the frequency of turning decreased, namely, forward locomotion was stimulated during blue-light stimulation of ASEL (Fig. 4). These results are similar to the previous observation of behavioral responses to step concentration change of NaCl (Miller et al., 2005), and through the klinokinesis mechanism, can generate chemotaxis to a higher salt concentration. These results were consistent with the observation that, during Na^+ chemotaxis assay (Fig. 3), worms that were cultivated with Na^+ migrated to a higher concentration (Fig. 5).

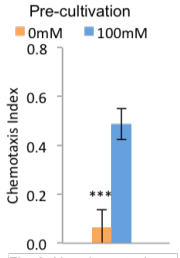

Fig. 3 Na^+ chemotaxis behavioral plasticityof wild type.

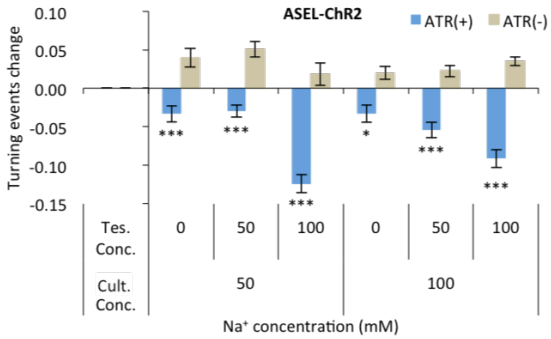

Fig. 4 Worms' behavioral response upon ASEL photostimulation after cultivation with Na^+

2. Neural circuit downstream of ASEL generates behavioral response after cultivation with Na^+

Interneurons AIB, AIY and AIA have synapses with many of the sensory neurons, including

ASER and ASEL, all of the three interneurons are related to chemotaxis to chemical cues (Kunitomo et al., 2013; Satoh et al., 2014). Behavioral response upon stimulation of ASEL was eliminated or reduced significantly in the AIB- or AIY- or AIA-ablated background, indicating that ASEL requires all first-layer interneurons, AIB, AIY, and AIA, for generating the behavioral responses. Besides, behavioral response to ASEL stimulation was similar to AIY stimulation or AIA stimulation but opposite to AIB stimulation, suggesting a positive relationship between ASEL and AIY/AIA, and a negative relationship between ASEL and AIB. Furthermore, similar to ASEL, calcium content of AIY or AIA was increased upon ASEL stimulation, while the calcium level of AIB was decreased significantly upon ASEL photostimulation, indicating that ASEL activates AIY and AIA but inhibits AIB. Thus, all three first-layer interneurons respond to ASEL: AIB was inhibited by ASEL, which is expected to inhibit turning behavior, while AIY and AIA were activated by ASEL, to possibly promote forward locomotion, and therefore all three interneurons may contribute to driving worms to higher Na^+ concentrations (Fig. 5).

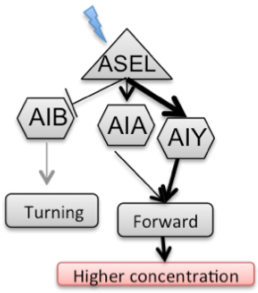

Fig. 5 diagram for neural circuit down-stream of ASEL after cultivation with Na^+

3. Cellular basis of the behavioral plasticity caused by cultivation with/without Na^+

In the chemotaxis assay, we observed that when worms were cultivated in Na^+-free conditions, worms had no Na^+ concentration preference (Fig. 3). We therefore tested the behavioral response to ASEL stimulation after Na^+-free cultivation. When worms were cultivated in Na^+-free conditions, they showed no behavioral response to ASEL photostimulation in Na^+-containing conditions (Fig. 6a). And worms showed similar behavioral response upon ASEL's activation after NaCl or NaAc cultivation. Likewise, replacing NaCl with NaAc in test plates did not cause a significant difference, indicating that Na^+ is responsible for the plasticity in the behavioral response to ASEL stimulation.
We further made use of the *dyf-11* mutant, which has deformed cilia and therefore cannot sense water-soluble chemicals (Kunitomo and Iino, 2008) to test whether ASEL alone can generate Na^+ plasticity or not. When worms were cultivated with Na^+, forward locomotion was not observed any more in *dyf-11* mutant upon ASEL photoactivation, and this defect was rescued by *dyf-11* expression only in ASEL. When worms were cultivated with Na^+-free conditions, they showed increase of turning events upon ASEL photoactivation in the *dyf-11* mutant, and this response was suppressed by *dyf-11* expression in ASEL neuron. These results indicated that ASEL alone could generate the Na^+-dependent plasticity (behavioral response only after cultivation with Na^+) (Fig. 3~6).

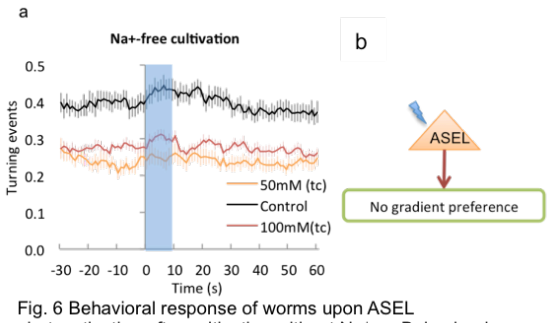

Fig. 6 Behavioral response of worms upon ASEL photoactivation after cultivation without Na^+. *a*, Behavioral response to optical stimulation of ASEL after cultivation Na^+ concentration. *b*, A schematic of worms' behavioral response generated by ASEL after Na^+-free cultivation.

On the other hand, worms cultivated in Na^+-free conditions showed similar response to those cultivated with Na^+, when AIB, AIY or AIA was stimulated by ChR2. It implied that the difference in the behavioral responses after Na^+-containing and Na^+-free cultivation was attributed to neuronal responses upstream of first-layer interneurons, possibly in ASEL. In fact, ASEL showed no calcium response upon ASEL photostimulation after Na^+-free cultivation. This is likely the reason why there was no behavioral response to ASEL stimulation after Na^+-free cultivation, and no Na^+ concentration preference after cultivation in Na^+-free conditions (Fig. 6b).

4. Neurotransmitter glutamate and Gq signaling pathway in ASEL are involved in ASEL-triggered behavioral response

eat-4, which encodes a vesicular glutamate transporter, is necessary for glutamatergic transmission in *C. elegans* (Lee et al., 1999; Rand et al., 2000). *eat-4* is also expressed in ASE neurons (Serrano-Saiz et al., 2013), which implies that ASE neurons release glutamate onto downstream interneurons. An *eat-4* mutation eliminated the behavioral response upon ASEL activation after cultivation with Na^+. Moreover, cell-specific knockdown of *eat-4* in ASEL by RNA interference caused smaller behavioral responses than those in wild type during ASEL stimulation, suggesting that glutamate is used as a neurotransmitter in ASEL for the behavioral responses.

egl-30, which encodes an ortholog of the alpha subunit of heterotrimetric G-protein Gq, positively regulates locomotory movements (Brundage et al., 1996; Lackner et al., 1999; Adachi et al., 2010). The Gq/DAG/PKC pathway modulates NaCl chemotaxis and counteracts the phophatidylinositol 3-kinase signaling (Tomioka et al., 2006; Adachi et al., 2010; Kunitomo et al., 2013). We therefore examined whether the Gq signaling pathway regulates ASEL-dependent chemotaxis (Tomioka et al., 2006; Adachi et al., 2010). In the transgenic strain in which *egl-30(pe914)*, a gain-of-function mutation, was expressed in ASEL, the behavioral response was not observed during and after stimulation of ASEL after cultivation with Na^+, suggesting that *egl-30* negatively regulated the response to ASEL stimulation. After Na^+-free cultivation, the transgenic worms showed a small increase of turning behavior during stimulation of ASEL by ChR2. These results also implied that *egl-30* negatively regulates Na^+ chemotaxis, and possibly the behavioral plasticity in Na^+ chemotaxis.

Conclusion and significance

Unexpectedly, we found ASEL generates a novel type of memory-dependent behavioral plasticity in Na^+ chemotaxis: when worms were cultivated in the presence of Na^+, they showed positive chemotaxis to Na^+; but when worms were cultivated under Na^+-free conditions, they showed no Na^+ concentration preference. ChR2 activation with blue light activated ASEL only after cultivation with Na^+, as judged by the increase in intracellular Ca^{2+}. Under the conditions of cultivation with Na^+, photoactivation of ASEL caused activation of its downstream interneurons AIY and AIA, which stimulate forward locomotion, and inhibition of its downstream interneuron AIB, which inhibits the turning/reversal behavior, overall driving worms towards higher concentrations. We also found that the Gq signaling pathway and the neurotransmitter glutamate are both involved in the behavioral response generated by ASEL.

This is the first report to systematically analyze the character of ASEL, which has an important role in Na^+ chemotaxis. The neural mechanism underlying Na^+ chemotaxis plasticity is different from NaCl chemotaxis plasticity mediated by ASER, in which the plasticity that worms get attracted to NaCl when grown with salt and food (Kunitomo et al., 2013), but avoid to NaCl when starved with NaCl (Tomioka et al., 2006; Adachi et al., 2010), in odor chemotaxis plasticity, when animals are kept with a certain odor without food, they no longer show attraction to that odor (Colbert and Bargmann, 1995), and concentration-dependent odor chemotaxis, in which odor-sensing neurons switch between high-concentration odor avoidance and low-concentration odor attraction behaviors (Yoshida et al., 2012). All these chemosensory behaviors are mediated by overlapping neural circuits and our results will extend the platform for further understanding of the versatile actions of the small neural circuit of *C. elegans*.

As one of the mechanisms for learning and memory, changes in neuronal excitability are well documented, for example, long-term changes in piriform cortex (Saar and Barkai, 2009), amygdala (Sehgal et al., 2014) and hippocampus in rodents (Gruart et al., 2012). In these events voltage-gated and leak cation channels are often involved. Our current findings may lead to recognition of such so far unexplored mechanims in *C. elegans*.