Neuronal plasticity regulated by the insulin-like signaling pathway underlies salt chemotaxis learning in *Caenorhabditis elegans*

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Oda S, Tomioka M, Iino Y. Neuronal plasticity regulated by the insulin-like signaling pathway underlies salt chemotaxis learning in *Caenorhabditis elegans*. *J Neurophysiol* 106: 301–308, 2011. First published April 27, 2011; doi:10.1152/jn.01029.2010.—Quantification of neuronal plasticity in a living animal is essential for understanding learning and memory. *Caenorhabditis elegans* shows a chemotactic behavior toward NaCl. However, it learns to avoid NaCl after prolonged exposure to NaCl under starvation conditions, which is called salt chemotaxis learning. Insulin-like signaling is important for this behavioral plasticity and functions in one of the salt-sensing sensory neurons, ASE right (ASER). However, how neurons including ASER show neuronal plasticity is unknown. To determine the neuronal plasticity related to salt chemotaxis learning, we measured Ca2+ response and synaptic release of individual neurons by using in vivo imaging techniques. We found that response of ASER increased whereas its synaptic release decreased after prolonged exposure to NaCl without food. These changes in the opposite directions were abolished in insulin-like signaling mutants, suggesting that insulin-like signaling regulates these plasticities in ASER. The response of one of the downstream interneurons, AIB, decreased profoundly after NaCl conditioning. This alteration in AIB response was independent of the insulin-like signaling pathway. Our results suggest that information on NaCl is modulated at the level of both sensory neurons and interneurons in salt chemotaxis learning.

LEARNING AND MEMORY ARE CRUCIAL for animals to cope with a constantly changing environment. Previous studies suggested that insulin is involved in learning and memory in mammals (Dou et al. 2005; Zhao et al. 1999). Indeed, studies using mammalian cultured neurons or *Xenopus* tadpoles suggested that insulin regulates neuronal plasticities such as long-term depression (LTD), internalization of DL-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) receptors, and changes in the number of synapses (Chiu et al. 2008; Man et al. 2000). However, how insulin actually regulates learning and memory is still obscure.

*Caenorhabditis elegans* also shows learning and memory such as thermotaxis learning, food-odor associative learning, and salt chemotaxis learning (Mori et al. 2007; Nuttley et al. 2002; Saeki et al. 2001; Tomioka et al. 2006). The molecular mechanisms underlying these behavioral plasticities have been studied well. Insulin-like signaling, for instance, regulates several types of starvation-associated learning (Kodama et al. 2006; Lin et al. 2010; Tomioka et al. 2006). However, the plasticities of neuronal activity underlying these behavioral plasticities are mostly unknown.

Salt chemotaxis learning is one of the starvation-associated learning types. In this behavioral plasticity, worms show aversive behavior toward the attractant NaCl after prolonged exposure to NaCl for 10–60 min without food. However, in the presence of food or serotonin (5-HT), the putative food mediator (Avery and Horvitz 1990; Horvitz et al. 1982), salt chemotaxis learning does not occur (Saeki et al. 2001). These previous studies suggested that two pieces of information, NaCl and starvation, are integrated in salt chemotaxis learning. Previously we showed (Tomioka et al. 2006) that insulin-like signaling is required for salt chemotaxis learning. The insulin-like signaling pathway is composed of *ins-1*, *daf-2*, and *age-1*, which encode an insulin-like peptide, insulin receptor, and phosphatidylinositol 3-kinase (PI3-kinase), respectively (Kimura et al. 1997; Morris et al. 1996; Pierce et al. 2001). The insulin-like peptide INS-1 is secreted from several neurons including AIA, and the downstream signaling proteins DAF-2 and AGE-1 function in one of the major NaCl-sensing sensory neurons, ASE right (ASER). However, how neurons including ASER show neuronal plasticities remained to be elucidated.

Here we focused on salt chemotaxis learning and conducted physiological analyses of neuronal plasticity related to learning and memory of *C. elegans* by using imaging techniques. We first observed the dynamics of ASE after prolonged NaCl exposure. The changes in Ca2+ responses of ASER and ASE left (ASEL) were asymmetric: the response of ASER increased whereas that of ASEL decreased after NaCl conditioning. Interestingly, in the case of ASER, synaptic release did not correlate with the Ca2+ response; that is, synaptic release from ASER decreased. These contradictory phenomena were regulated by insulin-like signaling. We also measured Ca2+ responses in downstream interneurons including AIA and AIB (White et al. 1986). Responses of both interneurons decreased after NaCl conditioning, especially in AIB. In contrast to the change in ASER response, the strong decrease in Ca2+ response of AIB was independent of insulin-like signaling. These observations indicate that memory traces are found at the first step of information transmission in the neural circuit, the salt-sensing neuron ASER and downstream interneurons AIA and AIB, in salt chemotaxis learning.

MATERIALS AND METHODS

Strains. All strains were cultivated with *Escherichia coli* OP50 at 20°C except *daf-2 (e1370)*. As *daf-2 (e1370)* is a temperature-sensitive...
mutant, it was cultivated at 15°C. GCaMP2, SNB-1::pHluorin, and TRPV1 were expressed by using the following promoters: gcy-5 (for ASER expression), flp-6 (for ASE expression), and ins-1 (for AIA expression). The extrachromosomal arrays, peEx776[ASERp::gcamp2 lin-44p::gfp], peEx777[flp-6p::gcamp2 lin-44p::gfp], kyEx903[odr-2bp::gcamp unc-122p::gfp], and peEx778[ASERP::snb-1::pHluorin lin-44p::gfp], were introduced into ins-1(m2901), daf-2(e1370), age-1(lt546), unc-13(e51), and peEx780[ASERP::trpv1 unc-122p::mCherry] backgrounds by crossing with wild-type animals (N2) carrying each extrachromosomal array. The GCaMP strain for AIB imaging (CX7469 kyEx903[odr-2bp::gcamp unc-122p::gfp]) was a kind gift from C. I. Bargmann (Chalasani et al. 2007). The ASER-ablated strain with AIAp::gcamp2 was made by crossing two strains: JN79 (peEx779[AIAp::gcamp2]) and OH8593[nls[gcy-5p::gfp] V oEx5830[ceh-3p::CZ-caspase3(17) gcy-5p::caspase3(p12)-NZ myo-3p::mCherry]) (Ortiz et al. 2009). The ASER-ablated strain (OH8593) was a kind gift from O. Hobert.

Calcium imaging. Worms expressing GCaMP were placed in a microfluidic device fabricated from polydimethylsiloxane (PDMS) attached with inlet tubes. The design was the same as the "olfactory chip" designed by Chronis et al. (Chronis et al. 2007). The compositions of buffers for calcium imaging. Worms expressing pHuorin (Miesenböck et al. 1998; Sankaranarayanan et al. 2000), a pH-sensitive green fluorescent protein (GFP) variant, fused with synaptoprevinin SNB-1 (SNB-1::pHluorin) were placed in the PDMS chips, and stimuli were given in the same manner as that for calcium recordings, nose tips of the worms were washed with the NaCl-free buffer for 5 min.

Time-lapse imaging was conducted on an Olympus upright microscope (BX51). The fluorescence images were captured by using a ×60 Olympus oil immersion objective and a CoolSnap CCD camera (Photometrics). Exposure time was 500 ms at 1.25 Hz. The images were acquired and analyzed with Metamorph software (Molecular Devices) and Excel software (Microsoft). For the background subtraction of each image, the region of interest (ROI) was set in an adjacent area of the desired area and average intensity within the ROI was calculated (Supplemental Fig. S1A). This background value was subtracted from the average intensity of the ROI, which was set at a desired area. The position of each ROI was corrected, if necessary, with a ROI correction program that was a kind gift from Takeshi Ishihara. The fluorescence intensity during the 8-s period before delivery of the stimulus was averaged and defined as F0.

Observation of synaptic release. Worms expressing pHuorin (Miesenböck et al. 1998; Sankaranarayanan et al. 2000), a pH-sensitive green fluorescent protein (GFP) variant, fused with synaptoprevinin SNB-1 (SNB-1::pHluorin) were placed in the PDMS chips, and stimuli were given in the same manner as that for calcium

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**Fig. 1. Ca**²⁺ responses and synaptic transmission of ASE right (ASER) and ASE left (ASEL) are altered after NaCl conditioning. Calcium responses of ASE after exposure to 20 mM NaCl (NaCl cond.) or 0 mM NaCl (mock cond.) for 10 min without food in the polydimethylsiloxane (PDMS) chips are shown. For quantification, values of change in fluorescence (ΔF/F0) during the 15-s period (boxed) after the stimulus (NaCl upstep or downstep) were averaged. A and C: calcium responses of ASER to downstep of NaCl after NaCl (n = 11) or mock (n = 10) conditioning. In the case of mock conditioning, after exposure to NaCl-free buffer for 10 min a worm was exposed to buffer containing 20 mM NaCl for a minute and then reexposed to NaCl-free buffer. B and D: calcium responses of ASEL to upstep of NaCl after NaCl (n = 10) or mock (n = 9) conditioning. After NaCl conditioning, NaCl concentration was shifted from 20 to 0 mM and maintained for a minute before the upshift stimulus was given. E and F: synaptic release from ASE measured by SNB-1::pHluorin after exposure to 20 mM NaCl (NaCl cond.) or 0 mM NaCl (mock cond.) for 10 min without food. The stimuli (downstep of NaCl concentrations from 20 mM to 0 mM for ASER or upstep of NaCl concentrations from 0 mM to 20 mM for ASEL) were applied in the same manner as that for Ca**²⁺** imaging at 0 s, and 3D stacks were acquired every 60 s (n = 8–10). Error bars in trace data represent SE. Horizontal bars in quantified data represent SE. Significant difference from control (mock cond.): *P < 0.05, **P < 0.01 (Mann-Whitney U-test).
imaging. The fluorescence image stack was obtained with a Leica confocal microscope (TCS SP5) and analyzed with the software attached to the confocal microscope (LAS AF). Before delivering the stimulus, we acquired the image of the axonal region of ASE sensory neurons, where synapses are distributed (White et al. 1986), and then photobleached the axonal region by a 488-nm argon laser with the highest laser intensity until intensity of its fluorescence dropped by ~30% (Supplemental Fig. S1B). The images of the axonal region were captured at 1-min intervals with low power intensity to minimize further photobleaching. The fractional recovery was calculated with the following equation: \[ \frac{[f(t) - f_p]}{[f_0 - f_p]} \], where \( f_0 \) is the fluorescence at the axonal region before photobleaching, \( f_p \) is the fluorescence at the axonal region after photobleaching, and \( f(t) \) is the fluorescence at the axonal region at time \( t \) after photobleaching (Samuel et al. 2003).

RESULTS

Calcium responses and synaptic outputs of ASE sensory neurons are altered after prolonged NaCl exposure. C. elegans shows an attractive behavior toward NaCl (Bargmann and Horvitz 1991). However, after exposure to NaCl without food for 10–60 min it comes to avoid NaCl (salt chemotaxis learning) (Saeki et al. 2001; Tomioka et al. 2006). We reasoned that Ca\(^{2+}\) response or synaptic output of sensory neurons might be altered in salt chemotaxis learning. Therefore, we first measured Ca\(^{2+}\) responses and release of synaptic vesicles in ASE neurons in response to NaCl stimulus after prolonged exposure to NaCl, by using the genetically encoded Ca\(^{2+}\) indicator GCaMP2 (Tallini et al. 2006) and the synaptic release-indicator SNB-1::pHluorin. The latter is a fusion of pHluorin, a pH-sensitive GFP variant (Miesenböck et al. 1998; Sankaranarayanan et al. 2000), with SNB-1, which is a homolog of synaptobrevin localizing to synaptic vesicles (Nonet et al. 1998). Several salts such as NaCl are mainly sensed by ASE chemosensory neurons (Bargmann and Horvitz 1991; Suzuki et al. 2008; Thiele et al. 2009), which consist of a pair of neurons, ASER and ASEI, located on the right and left side, respectively, of the body. These neurons are functionally distinct: ASER responds to a downshift of NaCl concentration, whereas ASEI responds to an upshift of it (Suzuki et al. 2008). Previous studies characterized a behavioral plasticity in which exposure to a buffer containing 20 mM NaCl for 10 min leads to a significant decrease of NaCl chemotaxis (Tomioka et al. 2006). To determine whether the responses of ASE are altered by prolonged exposure to NaCl, we exposed the worms trapped in a microfluidic chip to 20 mM NaCl for 10 min and observed the Ca\(^{2+}\) dynamics and synaptic transmission of ASE in response to NaCl upshift or downshift (see MATERIALS AND METHODS). In the salt chemotaxis learning assay, 20 mM NaCl-conditioned worms were directly placed at the center of an assay plate with the NaCl concentration close to 0 mM (Tomioka et al. 2006) and then experienced different kinds of NaCl concentration change while they crawled over the assay plate. Because we wanted to reproduce this situation in a PDMS chip, in the case of upshift stimulus after NaCl exposure for 10 min we shifted NaCl concentration from 20 to 0 mM for 1 min after prolonged NaCl exposure and gave an upshift stimulus from 0 to 20 mM.

Interestingly, the response of ASER to NaCl downshift and that of ASEI to NaCl upshift increased and decreased, respectively, after the prolonged NaCl exposure (NaCl conditioning; Fig. 1 and Supplemental Fig. S2, A–D). By contrast, the response of ASER to NaCl upshift and that of ASEI to NaCl downshift did not show significant changes (Supplemental Fig. S3A and B).

We next observed synaptic release from ASE. Fluorescence of SNB-1::pHluorin at a synaptic region was photobleached by high-power laser, and the fluorescence recovery after downshift or upshift of NaCl concentrations was measured (Supplemental Fig. S1B). To confirm that synaptic release was measured properly in our experimental conditions, we performed the same experiments on ASER, using the synaptic release-
defective mutant unc-13(e51) (Richmond et al. 1999). Compared with wild type, fluorescence recovery after downshift of NaCl was strongly reduced, suggesting that the fluorescence recovery indeed reflects synaptic release (see Fig. 3D), although there may be some background signal. After prolonged exposure to NaCl, we found that synaptic release of ASER decreased significantly whereas that of ASEL was on a downward trend, although the difference was not significant (Fig. 1, E and F).

Insulin-like signaling is involved in regulating both increase in Ca\(^{2+}\) response and decrease in synaptic release of ASER. We previously reported (Tomioka et al. 2006) that insulin-like signaling is required in ASER for salt chemotaxis learning. Therefore, we examined how much of the observed changes in ASER and ASEL depended on the function of insulin-like signaling. We measured Ca\(^{2+}\) response and synaptic release of ASER after prolonged NaCl exposure in the mutants ins-1(nr2091) (Pierce et al. 2001), daf-2(e1370) (Kimura et al. 1997), and age-1(hx546) (Morris et al. 1996), which encode an insulin-like peptide, a homolog of insulin/IGF-1 receptor, and a homolog of PI3-kinase acting downstream of DAF-2, respectively. All these mutants show defects in salt chemotaxis learning (Tomioka et al. 2006). We found that the Ca\(^{2+}\) responses of ASER in these mutants did not increase as much as wild type after prolonged NaCl exposure (Fig. 2, A–C). Likewise, in the presence of 5-HT, which has been reported to mimic food (Avery and Horvitz 1990; Horvitz et al. 1982) and inhibit salt chemotaxis learning (Saeki et al. 2001), the increase of ASER response was smaller (Fig. 2D). On the other hand, the decreased Ca\(^{2+}\) response of ASEL was observed after NaCl conditioning in ins-1 mutants as well as wild type (Supplemental Fig. S4A). These results suggest that alterations in Ca\(^{2+}\) response of ASER, but not of ASEL, after NaCl exposure require the normal activity of insulin-like signaling. The decrease in synaptic release from ASER after NaCl conditioning was also significantly affected in these mutants, suggesting that the alteration in synaptic release of ASER is also regulated by insulin-like signaling (Fig. 3, A–C).

One of the possible causes of the contradictory phenomena, increased Ca\(^{2+}\) response and decreased synaptic release of ASER, would be a feedback regulation via synapses from downstream neurons, because the ASER neuron receives synaptic inputs as well as sending synaptic outputs (White et al. 1986). For example, if there is a negative feedback from downstream neurons that are activated by excitatory synaptic transmission from ASER, then decreased synaptic release from ASER caused by prolonged NaCl exposure will result in a decreased negative feedback regulation leading to increased sensory response of ASER. To test this possibility, we examined the synaptic release-defective mutant, unc-13(e51). As Suzuki et al. reported (Suzuki et al. 2008), ASER neurons of unc-13(e51) mutants responded to downshift of NaCl concentrations to the same extent as those in wild-type animals (Supplemental Fig. S5A). The increased Ca\(^{2+}\) response of ASER after prolonged NaCl exposure was also observed in unc-13 as observed in wild-type animals (Supplemental Fig. S5B). These data suggest that the increased Ca\(^{2+}\) response of ASER was not caused by feedback regulation via chemical synapses.

Ca\(^{2+}\) responses of AIA and AIB interneurons are decreased after prolonged exposure to NaCl. To evaluate the effect of prolonged NaCl exposure on interneurons downstream of ASER, we observed Ca\(^{2+}\) responses in AIA, AIB, and AIY, which receive relatively large numbers of synaptic inputs from

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**Fig. 3.** Insulin-like signaling is required for the decrease in synaptic transmission from ASER. A–C: synaptic transmission from ASER in ins-1(nr2091), daf-2(e1370), and age-1(hx546) measured by SNB-1::pHluorin after mock or NaCl conditioning. The stimulus (downstep of NaCl concentrations from 20 mM to 0 mM) was applied at 0 s after exposure to 0 or 20 mM NaCl for 10 min without food, and 3D stacks were captured every 60 s (n = 7–9). No significant differences were observed (Mann-Whitney U-test). D: naive synaptic transmission from ASER in wild type (WT) (N2) and unc-13(e51). The stimulus (downstep of NaCl concentration from 20 mM to 0 mM) was applied at 0 s, soon after the nose tip of the worm was washed with NaCl-free buffer for 5 min (n = 9–11). Significant difference from control (WT): **p < 0.01 (Mann-Whitney U-test). Error bars represent SE.
ASER (White et al. 1986). The responses of these neurons to NaCl had not been examined before. Therefore, we first measured the Ca^{2+} responses of these neurons in the naive state. We found that AIA and AIB responded to upstep and downstep of NaCl, respectively (Fig. 4, A, B, D, E). However, we could not observe a significant response of AIIY after changes of NaCl concentration even after prolonged NaCl exposure in our experimental conditions (data not shown). We could measure Ca^{2+} responses of AIB at the soma and confirmed there was no laterality in AIB in our experimental conditions (data not shown). On the other hand, Ca^{2+} response could be only detected at neurites in AIA. Because it was very difficult to distinguish left and right neurites, we could not test laterality in AIA. After prolonged NaCl exposure, the AIB responses were decreased dramatically (Fig. 4C and Supplemental Fig. S2, E and F). The AIA responses were slightly but significantly decreased after NaCl exposure: the average of ΔF/F<sub>0</sub> values during the 15-s period after delivery of the stimulus did not show a significant difference, while those during the 60-s period showed a significant difference in AIA responses after NaCl conditioning (Fig. 4F and Supplemental Fig. S2, G and H). The responses of these neurons to the opposite NaCl...
concentration steps did not change significantly after prolonged NaCl exposure (Supplemental Fig. S3, C and D). Because AIA neurons responded to upstep of NaCl concentration, which was opposite to ASER, we wondered whether AIA response requires the ASER neuron. When ASER was genetically ablated, AIA showed almost normal responses (Supplemental Fig. S6), suggesting that the AIA response to NaCl and the decreased response after NaCl conditioning are caused by inputs from sensory neurons other than ASER, possibly ASEL.

To confirm the connectivity between ASER and AIB, we specifically activated ASER expressing the mammalian capsaicin receptor TRPV1 (Caterina et al. 1997; Tobin et al. 2002) by delivering capsaicin and then monitored the Ca\(^{2+}\) responses of ASER and AIB neurons. As expected, the addition of capsaicin activated ASER. AIB neurons were also activated by this treatment, suggesting that ASER can excite AIB interneurons through direct synaptic connections and/or indirect neuronal connections between ASER and AIB (Fig. 5).

We next investigated the dependence of the dramatic decrease of AIB responses after NaCl conditioning on insulin-like signaling by measuring AIB responses in ins-1 mutants. The dramatic decrease of AIB responses was still observed in ins-1 mutants (Supplemental Fig. S4B). These results suggest that the decrease in AIB responses after NaCl conditioning is caused by an ins-1-independent pathway.

**DISCUSSION**

A neuronal plasticity is considered to underlie behavioral plasticity in various behaviors. In salt chemotaxis learning of worms, chemotaxis to NaCl is dramatically altered by prolonged exposure to NaCl in the absence of food. In this study, we examined the change of property of the major salt-sensing chemosensory neurons, ASE, and downstream interneurons after prolonged exposure to NaCl. The Ca\(^{2+}\) responses of ASE, which consist of right and left members, were altered after NaCl exposure without food: the ASER response increased and the ASE response decreased (Fig. 1, A–D). In a previous study (Tomioka et al. 2006), we could not detect a change of Ca\(^{2+}\) response in ASER after NaCl conditioning. We consider the difference in Ca\(^{2+}\) indicators and methods used to immobilize worms used in the two studies as possible causes for this discrepancy. GCaMP2 was used as the Ca\(^{2+}\) indicator in this study, while YC2.12 was used in the previous study. Worms were trapped in a PDMS chip in this study, while they were glued to agarose pads in the previous study. Chronis et al. (Chronis et al. 2007) found (Chronis et al. 2007) that ASH sensory neurons responded to both addition and removal of high osmotic solution (1 M glycerol) by using a PDMS chip, while this property of ASH was not found when the worm was glued to agarose pads (Hilliard et al. 2005).

The insulin-like signaling acting in ASER is important for salt chemotaxis learning (Tomioka et al. 2006). However, how ASER function is altered physiologically by insulin-like signaling remained to be elucidated. In this study, we found that the insulin-like signaling pathway is required for two kinds of changes of ASER properties, increased Ca\(^{2+}\) response and decreased synaptic release, after prolonged NaCl exposure (Figs. 2, A–C, and 3, A–C). Decreased outputs from ASER could cause the increased Ca\(^{2+}\) response through feedbacks from downstream interneurons. However, our results using the synaptic transmission-defective mutant unc-13 ruled out this possibility (Supplemental Fig. S5). Although how insulin-like signaling regulates both changes is still obscure, we can speculate that insulin-like signaling could regulate both increased Ca\(^{2+}\) response and decreased synaptic transmission. In rat hypothalamic glucose-responsive neurons, it was reported that the insulin pathway regulates K\(^{+}\) channel functions (Spanswick et al. 2000). Also in *C. elegans*, the insulin-like pathway might regulate ASER excitability through the negative regulation of K\(^{+}\) channel functions, resulting in increased Ca\(^{2+}\) response in ASER. On the other hand, the DAF-2/AGE-1 pathway in ASER was previously suggested to negatively regulate synaptic transmission in salt chemotaxis learning (Tomioka et al. 2006). Further analyses of downstream targets of the insulin-like signaling pathway are required to unveil regulatory mechanisms of neuronal plasticity in ASER.
ASEL is also involved in salt chemotaxis learning, although it is less important than ASER. In this report, we found that the Ca\(^{2+}\) response of ASEL is altered after NaCl conditioning in an ins-1-independent manner (Supplemental Fig. S4A). Therefore, the neuronal plasticity in ASEL might also contribute to salt chemotaxis learning in combination with ASER plasticity. We also measured Ca\(^{2+}\) responses in AIB and AIA after prolonged NaCl exposure. Ca\(^{2+}\) responses of both neurons were decreased, especially in AIB. Previous cell ablation experiments suggested that AIB interneurons act to increase turning frequency (Gray et al. 2005). In addition, a dramatic decrease in the probability of pirouette (a bout of sharp turns) occurs after NaCl conditioning (Iino and Yoshida 2009). Therefore the decreased turning frequency followed by decreased AIB activity may be one of the components contributing to the behavioral change after NaCl conditioning.

Because the dramatic decrease of AIB response after NaCl conditioning did not depend on insulin-like signaling and was not directly correlated with the response of ASER (Supplemental Fig. S4B, Fig. 3, A–C), plasticity of AIB neurons might be caused by altered responsiveness of AIB to sensory neurons by an unknown mechanism independent of the insulin-like signaling pathway. Because ablation of AIB was not sufficient for the dramatic change in salt chemotaxis (Iino and Yoshida 2009; Tomioka et al. 2006) and ablation of interneurons downstream of ASE neurons other than AIB also affects mechanisms of chemotaxis such as the weather vane mechanism, the coordinated and parallel functions of several interneurons including AIA and AIB regulated by ins-1-dependent and -independent pathways might be required for the behavioral change after NaCl conditioning.

Salt chemotaxis learning is inhibited by the presence of food during NaCl conditioning (Saeki et al. 2001), suggesting that information on starvation and NaCl are integrated. The ins-1 mutant also shows defects in starvation-associated plasticity of thermotaxis and odor-starvation associative plasticity (Kodama et al. 2006; Lin et al. 2010). By contrast, ins-1 shows no defect in food-associated plasticity (Kodama et al. 2006; Lin et al. 2010). These results imply that INS-1 transmits the starvation signal. We now show that the information on NaCl was modulated at ASER where insulin-like signaling acts. In light of these findings, one of the places for integration of this information might be ASER.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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J Neurophysiol • VOL. 106 • JULY 2011 • www.jn.org 307


