Behavioral Forgetting of Olfactory Learning Is Mediated by Interneuron-Regulated Network Plasticity in Caenorhabditis elegans

Jamine Hooi-Min Teo,1 Itsuki Kurokawa,1 Yuuki Onishi,1 Noriko Sato,2,3 Tomohiro Kitazono,1 Terumasa Tokunaga,2 Manabi Fujiwara,1,3 and Takeshi Ishihara1,3

Abstract

Forgetting is important for animals to manage acquired memories to enable adaptation to changing environments; however, the neural network in mechanisms of forgetting is not fully understood. To understand the mechanisms underlying forgetting, we examined olfactory adaptation, a form of associative learning, in Caenorhabditis elegans. The forgetting of diacetyl olfactory adaptation in C. elegans is regulated by secreted signals from AWC sensory neurons via the TIR-1/JNK-1 pathway. These signals cause a decline of the sensory memory trace in AWA neurons, where diacetyl is mainly sensed. To further understand the neural network that regulates this forgetting, we investigated the function of interneurons downstream of AWA and AWC neurons. We found that a pair of interneurons, AIA, is indispensable for the proper regulation of behavioral forgetting of diacetyl olfactory adaptation. Loss or inactivation of AIA caused the impairment of the chemotaxis recovery after adaptation without causing severe chemotaxis defects in the naïve animal. AWA Ca$^{2+}$ imaging analyses suggested that loss or inactivation of AIA interneurons did not affect the decline of the sensory memory trace after the recovery. Furthermore, AIA responses to diacetyl were observed in naïve animals and after the recovery, but not just after the conditioning, suggesting that AIA responses after the recovery are required for the chemotaxis to diacetyl. We propose that the functional neuronal circuit for attractive chemotaxis to diacetyl is changed temporally at the recovery phase so that AIA interneurons are required for chemotaxis, although AIA interneurons are dispensable for attractive chemotaxis to diacetyl in naïve animals.

Key words: C. elegans; circuit plasticity; forgetting; memory; olfactory learning

Significance Statement

Forgetting is important to enable animals to adapt to changing environments; however, the mechanisms of forgetting are poorly understood at the molecular and cellular levels. In this study, we found that a pair of interneurons in the olfactory circuit of Caenorhabditis elegans are indispensable for behavioral forgetting, but not for regulation of the sensory memory trace, in simple olfactory learning. These findings suggest that neuronal circuits are important for regulating forgetting by managing memory and also for the generation of appropriate behavioral responses.

Introduction

Animals are able to learn and form memories, depending on the experience they gain from their surroundings; however, to adapt to changing environments, it is essential that dispensable information is discarded to manage accumulating memories. Recent studies reveal that
memories can be actively forgotten by interference with other memories or by activating forgetting in neurons that are important for maintaining memories (Hardt et al., 2013; Davis and Zhong, 2017). However, the molecular mechanisms and neural networks engaged in forgetting are not well understood.

The complexity of brain structure in higher organisms makes studies on active forgetting at molecular and cellular levels challenging; therefore, invertebrates with simple nervous systems, such as Caenorhabditis elegans, have been used (Inoue et al., 2013; Hadziselimovic et al., 2014). Despite a simple neural network, C. elegans shows behavioral plasticity toward various stimuli, such as volatile and water-soluble chemicals (Bargmann et al., 1993; Colbert and Bargmann, 1995; Saeki et al., 2001). In the well studied neural network of C. elegans (White et al., 1986; Cook et al., 2019), most attractive volatile odorants, such as diacetyl and isoamyl alcohol, are sensed by two pairs of amphiid sensory neurons, AWA and AWC, respectively, and these neurons have distinctive sensory mechanisms (Bargmann et al., 1993; Sengupta et al., 1994, 1996; Colbert and Bargmann, 1995; Colbert et al., 1997; L’Etoile and Bargmann, 2000; Bargmann, 2006). These amphiid sensory neurons make synapses to first-layer interneurons, mainly AIA, AIB, and AYI, which also regulate the plasticity of various behaviors, such as associative learning (Rankin et al., 1990; Tomioka et al., 2006; Chalasani et al., 2010; Cho et al., 2016), as well as integrate multiple sensory signals, including contradicting information (Shinkai et al., 2011; Larsch et al., 2015; Dobosiewicz et al., 2019; Wolfe et al., 2019), to generate appropriate cellular responses and animal behavior.

In invertebrates, despite their simple neural networks, several studies showed that forgetting is actively regulated. In Drosophila, dopamine neurons regulate both learning and active forgetting through distinctive dopamine receptors in mushroom body neurons (Berry et al., 2018, 2012). One of the dopamine receptors in mushroom body neurons, dDA1, leads to memory formation (Berry et al., 2012), while for forgetting, another receptor, DAMB, activates Scribble scaffold to initiate forgetting by actin cytoskeleton remodeling (Shuai et al., 2010; Gervantes-Sandoval et al., 2016; Berry et al., 2018, 2015, 2012). C. elegans is also used to study active forgetting through olfactory adaptation, a form of associative learning (Colbert and Bargmann, 1995; Bargmann, 2006). The forgetting of diacetyl olfactory adaptation, which is sensed by AWA olfactory neurons, is regulated by the TIR-1/JNK-1 pathway in another type of olfactory neuron, AWC. In wild-type and mutant animals, which are defective in the TIR-1/JNK-1 pathway, the sensory Ca$^{2+}$ response of AWA neurons to diacetyl is positively correlated with behavioral change through memory formation and forgetting, suggesting that the sensory response can be considered as the sensory memory trace and that the memory trace in AWAs is actively and non-cell-autonomously regulated by AWCs (Inoue et al., 2013; Kitazono et al., 2017). In addition, a membrane protein, MACO-1, and a tyrosine kinase pathway, SCD-2/HEN-1, regulate the forgetting (Kitazono et al., 2017). Another study showed that, similar to Rac1 in Drosophila, the Arp2/3 complex, which regulates the actin cytoskeleton in AVA interneurons, is important for forgetting downstream of the RNA binding protein Musashi (Hadziselimovic et al., 2014). Although these studies indicate that active forgetting is important, even in simple learning paradigms of model organisms, the corresponding neural network has not been fully revealed.

Here, we demonstrate that a pair of neurons in C. elegans, AIA interneurons, which are the first-layer interneurons in olfactory circuits, is required to regulate forgetting processes of olfactory adaptation. Although absence or inactivation of functional AIA interneurons (AIA$^-$) slightly affects attractive chemotaxis to diacetyl, it caused prolonged retention of the olfactory adaptation to diacetyl, suggesting that AIA$s^-$s accelerate forgetting. Calcium imaging analyses showed that, although the behavioral response in AIA$^-$ animals did not recover after cultivation for 4 h, the calcium responses to diacetyl in AWA animals were recovered. These results suggest that AIA$s^-$s are indispensable for the behavioral response of the olfactory adaptation forgetting mechanism, probably because the functional neuronal circuit is changed temporally so that AIA$s^-$s are required for the chemotaxis.

**Materials and Methods**

**Strains and culture**

All strains were cultured on nematode growth medium (NGM) agar plates seeded with Escherichia coli strain OP50 (Brenner, 1974) and were grown at 20°C before experiments. In all experiments, we used young adult hermaphrodites prepared as described in each section (Table 1).

**Behavioral assay**

Chemotaxis toward attractive odorants was performed on assay plates (2% Bacto agar, 50 mM NaCl, 10 mM K$_2$HPO$_4$, pH 6, 1 mM MgSO$_4$, 1 mM CaCl$_2$) with 1:100 dilutions of odorants (diacetyl and isoamyl alcohol; Bargmann et al., 1993). During behavioral assays, animals were placed in the middle of the assay plate while the odorant and control solution (ethanol, the odorant diluent) were spotted on opposite sides of the plate. The chemotaxis index was calculated as $(A - B)/N$, where $A$ refers to the...
number of animals within 1.5 cm of the odorant spot, B refers to the number of animals within 1.5 cm of the control spot, and N is the total number of animals. In the forgetting assay (Inoue et al., 2013), adult animals were first washed three times with S-basal buffer (100 mM NaCl, 50 mM K₂HPO₄, pH 6, 0.02% gelatin; naive) and pre-exposed to 1:5000 diluted diacetyl or isoamyl alcohol in S-basal buffer with slow rotation for 90 min at room temperature (adaptation). Next, the worms were washed once and allowed to recover on OP50-seeded NGM plates for 4 h (recovery). In the extended forgetting assay, animals were recovered on OP50-seeded NGM plates for 24 h, and behavioral assays after recovery were conducted after 4, 8, and 24 h of recovery.

Calcium imaging

Calcium imaging of AWA neuron responses toward diacetyl was performed using AWA-cameleon YC3.60-expressing animals (Inoue et al., 2013). The day before imaging, 30–45 animals (L4 - young adults) were picked and cultured at 20°C. A 1:10/C₀₇ dilution of diacetyl was used for odor stimulation, and a 1:10/C₀₃ dilution of diacetyl was used for adaptation. Adaptation and recovery were conducted as described for the behavioral assay. During Ca²⁺ imaging, odor stimulation was applied to the animal for 60 s (30th to 90th second of recording). Fluorescence images of AWA sensory neurons were acquired using a microscope (model BX53-FL, Olympus) equipped with a 60/C₂ objective lens (UPLSAPO 60XW, Olympus) and a dual CCD camera (model ORDA-D2, Hamamatsu). Cameleon YC3.60 was excited using X-Cite 120 fluorescence lamp illuminators (EXFO). The fluorescence ratio of yellow fluorescent protein (YFP) to cyan fluorescent protein (CFP) in Cameleon YC3.60 was analyzed using an AQUACOSMOS system (version 2.60; Hamamatsu). The

| Table 1: Strain list |
|---------------------|
| **Strain name** | **Genotype** | **Source** |
| N2 | tir-1(tm3036) | CGC |
| RB1085 | tir-1(ok1052) | National Bioresource Project |
| JN578 | peis578[npr-9p::casp1, npr-9p::venus, unc-122p::mCherry] (AI- | CGC |
| JN579 | peis579[txt-3p::casp1, txt-3p::venus, lin-44p::gfp] (AY- | Satoh et al., 2014 |
| JN580 | peis580[ins-1(short)::casp1, ins-1(short)::venus, unc-122p::gfp] (AI′- | Satoh et al., 2014 |
| QD155 | qjEx3[gcyc-28.dp::mec-4(d), gcyc-28.dp::gfp, lin-44p::gfp] | Shinkai et al., 2011 |
| QD156 | qjEx4[gcyc-28.dp::unc-103(gf), myo-3p::gfp] | Shinkai et al., 2011 |
| QD139 | lin-15(n765ts); qjEx3[odr-10p::YC3.60, pBLH98] | Inoue et al., 2013 |
| QD140 | tir-1(tm3036); lin-15(n765ts); qjEx3[odr-10p::YC3.60, pBLH98] | Inoue et al., 2013 |
| QD157 | peis578[npr-9p::casp1, npr-9p::venus, unc-122p::mCherry]; pels579[txt-3p::casp1, txt-3p::venus, lin-44p::gfp] (AI′-; AIY-) | This article |
| QD165 | tir-1(ok1052); qjEx4[gcyc-28.dp::unc-103(gf), myo-3p::gfp] | This article |
| QD164 | tir-1(tm3036); qjEx3[gcyc-28.dp::mec-4(d), gcyc-28.dp::gfp, lin-44p::gfp] | This article |
| QD166 | tir-1(tm3036); qjEx4[gcyc-28.dp::unc-103(gf), myo-3p::gfp] | This article |
| QD153 | lin-15(n765ts); qjEx3[odr-10p::YC3.60, pBLH98]; pels580[ins-1(short)::casp1, ins-1(short)::venus, unc-122p::gfp] | This article |
| QD272 | qjEx52[gcyc-28.dp::GCaMP6F, gcyc-28.dp::paQuasAr3-citrine, lin-44p::gfp] | This article |

CGC, Caenorhabditis Genetics Center.

| Table 2: Statistical table |
|---------------------------|
| **Data structure** | **Type of test** | **Power (α = 0.05)** |
| a | Figure 1B,C | Normal distribution | Two-way ANOVA | Strains: <0.0001 |
| b | Fig. 1D | Normal distribution | Two-way ANOVA | Strains: <0.0001 |
| c | Fig. 1E | Normal distribution | Student’s t test | 1:10⁻²: 0.0201 |
| d | Fig. 2 | Normal distribution | Two-way ANOVA | Strains: <0.0001 |
| e | Fig. 3B | Normal distribution | Two-way ANOVA | Strains: 0.0271 |
| f | Fig. 4B | Normal distribution | Student’s t test | Adaption: 0.13 |
| g | Fig. 5 | Normal distribution | Two-way ANOVA | Strains: <0.0001 |
| h | Fig. 6 | Normal distribution | Two-way ANOVA | Strains: <0.0001 |
calculation \((R_{\text{max}} - R_0)/R_0\) was performed as the peak amplitude of the YFP/CFP ratio during the first 10 s interval after stimulation \((R_{\text{max}})\) relative to the mean basal ratio \((R_0)\) during the 10 s interval before stimulation. The relative \(\text{Ca}^{2+}\) response was evaluated by normalized \((R_{\text{max}} - R_0)/R_0\) with respect to the average naive value.

For calcium imaging on AIA neurons, we used animals expressing GCaMP6f in AIA neurons by gcy-28.d promoter. Animals were cultivated as for AWA imaging.

A 1:10/C0 \(7\) dilution of diacetyl was used for odor stimulation, and a 1:10/C0 \(3\) diacetyl was used for adaptation. Fluorescent images were acquired using a microscope (model BX53-FL, Olympus) equipped with a 60× objective lens (UPLSAPO60XS2, Olympus) and an ORCA-Flash camera with extended focus device of Gemini-2c (Hamamatsu). GCaMP6f was excited using a 470 nm laser (LDI, 89 North) with a dichroic mirror (488/543/635, Semrock) and fluorescence images were captured at 50 ms through an emission filter (512/25, Semrock). The neurite of AIA neurons was analyzed. The fluorescent intensities were normalized by the average response \((R_0)\) of a 5 s time period prior the stimulation.

**Experimental design and statistical analyses**

For all experiments, adult hermaphrodites were used. In the behavioral assays, the stage of animals was synchronized by removing adult animals from OP50-seeded NGM.
markers, such as myo-3p::gfp test with Bonferroni t of recovery was analyzed (animals, and after adaptation (0 h), and after 4, 8, and 24 h of recovery was analyzed (n \geq 6, two-way ANOVA, F_{\text{strain}(2,9)} = 61.26, p = 4.84 \times 10^{-18}, \eta^2 = 0.5531; \text{mean} \pm \text{SEM})^d. Post hoc t test with Bonferroni’s correction. Error bars represent the SEM.

plates 16–20 h after transfer (Ishihara et al., 2002). Injection markers, such as myo-3p::gfp, lin-44p::gfp, unc-122p::mCherry, and unc-122p::gfp, were used to distinguish transgenic animals with extrachromosomal transgenes, and the values of animals with extrachromosomal transgenes were compared with those of animals without transgenes on the same plates as internal controls.

All values are presented as either the mean \pm SEM in a line graph or box plot. Data analyses were performed using Bell Curve for Excel (version 3.22; Social Survey Research Information Co., Ltd.). Statistical significance between means was determined by Student’s t test or two-way ANOVA followed by a post hoc t test with Bonferroni’s correction. Sample sizes and statistical values are noted in the figure legends (Table 2).

### Results

#### AIA interneurons are required to regulate forgetting in AWA olfactory adaptation

*C. elegans* shows strong attractive chemotaxis to diacetyl, which is mainly sensed by AWA sensory neurons. After animals are exposed to diacetyl without food for 90 min, they show significantly weaker responses to diacetyl (olfactory adaptation; Colbert and Bargmann, 1995). The conditioned animals are able to recover the attractive chemotaxis toward diacetyl to a level similar to that of naive animals after cultivation with food for 4 h (recovery), and we consider this recovery as forgetting (Inoue et al., 2013). Consistent with the behavioral change, the Ca^{2+} responses to diacetyl in AWA neurons are decreased after conditioning and recover with cultivation. This correlation between behavior and sensory responses is also observed in mutants defective in the TIR-1/JNK-1 pathway, which function in AWC sensory neurons. In the tir-1 (tm3036)-null mutant, naive animals show the sensory response to diacetyl and, after conditioning, the Ca^{2+} responses decrease to levels similar to those of wild-type animals. However, in tir-1-null animals, similar to the behavioral changes, the Ca^{2+} responses in AWAs to diacetyl do not recover with cultivation. Therefore, the forgetting of diacetyl olfactory adaptation in AWA neurons is regulated by AWC sensory neurons via the TIR-1/JNK-1 pathway (Inoue et al., 2013). AWC neurons do not make direct connections to AWAs; therefore, other neurons may be involved in this regulation.

Figure 1A shows the olfactory circuit including olfactory sensory neurons, AWAs, and AWCs, and their downstream interneurons (White et al., 1986; Chalasani et al., 2010, 2007; Cook et al., 2019; Dobosiewicz et al., 2019). As shown in Figure 1A, AWCs and AWAs mainly relay signals to the first-layer interneurons AIA, AIB, and AIY. Among these, we first examined whether AIB and AIY interneurons, the main synaptic target of AWCs, are involved in forgetting by using animals with genetically ablated AIB and AIY, in which cell-specific cell death is promoted by expressing mouse Caspase 1 (Casp1; Satoh et al., 2014). However, in animals without AIBs and/or AIYs, we detected no significant differences in changes of chemotaxis to diacetyl among naive, conditioned, and recovered animals (Fig. 1B,C). This indicated that AIB and AIY interneurons are dispensable for the regulation of forgetting in this olfactory adaptation.

Next, we examined whether AIA is important for forgetting using several AIA malfunction strains (AIA⁺). By using an AIA-specific gcy-28.d promoter (Shinkai et al., 2011) and an ins-1 (short) promoter (Satoh et al., 2014), we expressed (1) a hyperactive form of the DEG (degenerin)/epithelial sodium channel MEC-4 [MEC-4(d)] to cause neural toxicity (Harbinder et al., 1997; Shinkai et al., 2011), (2) a constitutively active form of the ERG-like potassium channel UNC-103 [gain of function (gf)] to hyperpolarize and consequently inactive neurotransmission (Shinkai et al., 2011), and (3) Casp1 for genetic ablation (Satoh et al., 2014). In naive animals, chemotaxis to diacetyl in AIA⁺ strains was weakly defective (Fig. 1D,E), probably because AIA interneurons are involved in diacetyl perception (Larsch et al., 2015). Despite this weak naive chemotactic defect, we could detect more prominent decreases in chemotaxis to diacetyl after recovery from adaptation in AIA⁺ animals (Fig. 1D), indicating that AIA interneurons are required for forgetting diacetyl olfactory adaptation.

#### AIA interneurons accelerate forgetting of olfactory adaptation

Next, we examined whether AIA⁻ animals completely lost the ability to forget, or decelerated the forgetting progress, as in tir-1 (tm3036)-null animal (Fig. 2; Inoue et al., 2013). To test this, we analyzed the time course of memory retention for up to 24 h of recovery (4, 8, and 24 h after conditioning; Fig. 2). In the first 4 and 8 h of recovery, although wild-type animals showed full recovery of chemotaxis, AIA⁻ and tir-1 (tm3036)-null animals showed very weak recovery. After 24 h, AIA⁻ animals, similar to tir-1 (tm3036)-null mutants, showed almost full recovery to diacetyl, suggesting that, even without AIALs, animals can...
slowly forget the memory. AIA interneurons, therefore, accelerate the forgetting of olfactory adaptation.

AWA neurons recovered their sensory response to diacetyl after adaptation even in the absence of AIA+ animals

The diacetyl-evoked Ca2+ response in AWAs is correlated with behavioral change in naive, conditioned, and recovered animals. Therefore, the weakened Ca2+ response in AWAs after conditioning can be considered a sensory memory trace. Consistent with this, in tir-1 (tm3036)-null mutants, similar to its behavioral response, a weakened Ca2+ response in AWAs after conditioning did not recover after 4 h of recovery (Fig. 3; Inoue et al., 2013). To examine whether the ablation of AIA+ causes prolonged weakened Ca2+ responses in AWAs after
conditioning, we analyzed Ca$^{2+}$ responses of AWAs to diacetyl in AIA$^{-}$ animals (naive, adapted, and recovered). In contrast to the behavioral response, the Ca$^{2+}$ response in AIA$^{-}$ animals was recovered after recovery for 4 h (Figs. 1D, 3), suggesting that the loss of AIA neurons decelerates forgetting but not through the inhibition of sensory recovery in AWAs.

**AIA neurons can respond to diacetyl in naive animals and after the recovery**

The loss of the functional AIA neurons caused a defect in chemotaxis to diacetyl after the recovery, but not before the conditioning. Recently, Larsch et al. (2015) reported that AIA neurons respond to diacetyl stimulation. Therefore, by using animals expressing GCaMP6f specifically in AIA, we analyzed the responses of AIA neurons to diacetyl in naive animals, immediately after conditioning, and after the recovery. As shown in Figure 4, we found that in naive animals and after the recovery, the fluorescent intensities of AIA neurons responding to the diacetyl stimulation were significantly increased, but such changes were not seen in those immediately after conditioning. This result is consistent with the importance of AIA in the chemotaxis to diacetyl after the recovery.

**AIA interneurons regulate forgetting downstream of the TIR-1 pathway**

AIA interneurons are not required to regulate the Ca$^{2+}$ responses in AWAs after recovery; therefore, we suspected that AIA interneurons might regulate forgetting independently of the TIR-1/JNK-1 pathway. We examined...
the genetic relationship between AIA interneurons and the TIR-1/JNK-1 pathway in the forgetting mechanism, by analyzing genetic epistasis using tir-1(ok1052 gf) animals, which show weak adaptation after conditioning probably because of hyperforgetting (Chuang and Bargmann, 2005; Inoue et al., 2013). Consistent with previous studies, tir-1(ok1052 gf) animals showed weak adaptation after conditioning (Fig. 5; Inoue et al., 2013). We made tir-1(ok1052 gf) animals without AIA interneurons and found that the animals showed normal adaptation and also prolonged retention of the adaptation (Fig. 5). These phenotypes cannot be distinguished from those of AIA–animals, suggesting that AIA interneurons regulate forgetting downstream of the TIR-1/JNK-1 pathway.

**Discussion**

Forgetting is important for animals to manage information to properly respond to changing environments. Yet, the neuronal mechanisms for forgetting are not fully understood. In this study, we discovered that a pair of interneurons, AIA interneurons, is required to regulate behavioral forgetting of olfactory adaptation.

**Interneurons accelerate forgetting of olfactory adaptation**

We found that AIA interneurons are required to accelerate forgetting of olfactory adaptation (Figs. 2, 6). Without functional AIAs, conditioned animals were unable to regain chemoattraction toward diacetyl after cultivation with food for 4 h. However, after cultivation with food for 24 h, chemoattraction was recovered in AIA–animals, suggesting that, even in the absence of the functional AIA interneurons, animals can slowly forget. Therefore, AIAs are important to accelerate forgetting of olfactory adaptation.

AIA interneurons are indispensable for chemotactic behavior to diacetyl only after recovery and, thereby, for behavioral forgetting

AIA interneurons are part of the olfactory sensory circuit (Larsch et al., 2015; Dobosiewicz et al., 2019). We observed a minor defect in chemoattraction of naive AIA–animals to diacetyl (Fig. 1D,E), indicating that the neuronal circuit for chemotaxis can function in naive animals in the absence of AIA interneurons. However, after recovery for 4 h, AIA–animals still showed a defect in chemotaxis to diacetyl (Fig. 1D), observed as a defect in behavioral forgetting, although the sensory memory trace declined normally in AWA sensory neurons (Fig. 3). These observations suggest that, in AIA–animals, the sensory response of AWAs cannot induce attractive chemotaxis to diacetyl after conditioning. These results raise two possibilities. One is that although redundant neuronal circuits can regulate chemotaxis to diacetyl in naive animals, after conditioning, the circuit that does not include AIAs becomes nonfunctional so that the AIAs become indispensable for the chemotaxis (Fig. 7A). Another one is that, only after conditioning does the neuronal circuit for chemotaxis to diacetyl require the activity of AIAs, which is distinct from the naive circuit (Fig. 7B). In these hypotheses, the functional neuronal circuit that does not include AIAs may recover slowly so that chemotaxis to diacetyl recovers after conditioning for 24 h. Our Ca2+ imaging analyses of AIA might support the model for the redundant neuronal circuits in naive animals (Fig. 7A) because AIA responses are similar to those after the recovery (Fig. 4). These kinds of circuit plasticity, which are based on internal states, are important for behavioral plasticity in higher organisms (Herry et al., 2008; Ramaswami, 2014; Joshua Kim et al., 2017; Kuchibhotla et al., 2017). Furthermore, we suspect that such circuit plasticity involving AIA interneurons...
might also be used by other olfactory adaptation mechanisms because we also observed that AIA\textsuperscript{−} animals displayed a defective forgetting phenotype toward AWC-sensed isoamyl alcohol without causing a severe chemotactic defect (Fig. 6). To clarify the precise role of AIA interneurons in both circuit and behavior plasticity, additional experiments including optogenetic inactivation or activation of the olfactory circuits in naive and conditioned animals are required to examine these hypotheses.

Our genetic epistasis experiment indicates that AIAs function downstream of the TIR-1 pathway in the regulation of forgetting (Fig. 5). TIR-1 is required to accelerate the forgetting of olfactory adaptation of diacetyl; therefore, the adaptation defect to diacetyl in the tir-1(gf) mutant might be caused by forced chemotactic recovery from adaptation during conditioning (Inoue et al., 2013). If this is the case, the suppression of the adaptation defect by AIA\textsuperscript{−} is consistent with the role of AIAs in chemotaxis during the recovery phase.

Our study shows that AIA interneurons in C. elegans are required to regulate behavioral forgetting of olfactory adaptations. This indicates that intact neural circuits are important for simple forgetting regardless of the simplicity of the neural system. Studies that reveal learning, memory formation, and forgetting pathways in invertebrates might be conserved across species (Stein and Murphy, 2014; Vorster and Born, 2015; Lipina et al., 2016; Costa et al., 2020; Rahmani and Chew, 2021); therefore, we believe that studies in invertebrates are important to elucidate the mechanisms of forgetting in higher organisms.

Figure 6. Chemotaxis to AWC-sensed isoamyl alcohol in AIA\textsuperscript{−} animals. Chemotaxis to isoamyl alcohol was analyzed in two AIA\textsuperscript{−} transgenic animals, AIA\textsuperscript{p}::mec-4(d) and AIA\textsuperscript{p}::unc-103 (gf), in naive, adapted, and 4 h recovery phases. Boxes, First to third quartiles (Q1 25th to Q3 75th percentile) of each dataset; black line in the boxes, medians; black dots, mean; whiskers, minimum and maximum, excluding outliers (beyond 1.5-fold interquartile range from Q3 and Q1); x, outliers (n = 8, two-way ANOVA, $F_{\text{strain}}(3,82) = 11.4853, p = 2.31 \times 10^{-6}, \eta^2 = 0.2959$). Post hoc t test with Bonferroni’s correction.

Figure 7. AIAs may regulate forgetting of diacetyl olfactory adaptation via circuit plasticity. A, B, Two hypothetical neural circuits of AIA-dependent behavioral plasticity in the forgetting of olfactory adaptation to diacetyl. In the two models, naive chemotactic behavior might be regulated along with (A) or independent from (B) an AIA-dependent functional neural circuit. In both models, after conditioning, the AIA-dependent functional neural circuit is required to regulate the corresponding behavioral output after the animal recovers from adaptation.

A  Redundant Circuit

B  Switching Circuit

[Diagram showing two models of neural circuitry]
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