A Neuronal Signaling Pathway of CaMKII and Gq α Regulates Experience-Dependent Transcription of *tph-1*

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Dynamic serotonin biosynthesis is important for serotonin function; however, the mechanisms that underlie experience-dependent transcriptional regulation of the rate-limiting serotonin biosynthetic enzyme tryptophan hydroxylase (TPH) are poorly understood. Here, we characterize the molecular and cellular mechanisms that regulate increased transcription of *Caenorhabditis elegans* *tph-1* in a pair of serotonergic neurons ADF during an aversive experience with pathogenic bacteria, a common environmental peril for worms. Training with pathogenic bacteria induces a learned aversion to the smell of the pathogen, a behavioral plasticity that depends on the serotonin signal from ADF neurons. We demonstrate that pathogen training increases ADF neuronal activity. While activating ADF increases *tph-1* transcription, inhibiting ADF activity abolishes the training effect on *tph-1*, demonstrating the dependence of *tph-1* transcriptional regulation on ADF neural activity. At the molecular level, the *C. elegans* homolog of CaMKII, UNC-43, functions cell-autonomously in ADF neurons to generate training-dependent enhancement in neuronal activity and *tph-1* transcription, and this cell-autonomous function of UNC-43 is required for learning. Furthermore, selective expression of an activated form of UNC-43 in ADF neurons is sufficient to increase ADF activity and *tph-1* transcription, mimicking the training effect. Upstream of ADF, the Gq α protein EGL-30 facilitates training-dependent induction of *tph-1* by functional regulation of olfactory sensory neurons, which underscores the importance of sensory experience. Together, our work elucidates the molecular and cellular mechanisms whereby experience modulates *tph-1* transcription.

Introduction

Serotonin plays important roles in a wide range of neural functions (Azmitia, 2007). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme for serotonin biosynthesis, and transcription of TPH is highly regulated. For example, in the rat raphe nuclei, TPH transcription varies with circadian rhythm (Malek et al., 2005), and the TPH mRNA level is elevated by stress, such as repeated immobilization stress (Chamas et al., 1999) and repeated forced swimming (Shishkina et al., 2008). In addition, the TPH mRNA level in the trigeminal ganglia of female mice oscillates with the natural estrous cycle (Berman et al., 2006). These characterizations underscore the importance of TPH transcriptional regulation; however, the underlying mechanisms are mostly unknown. The mammalian serotonergic neurons are highly heterogeneous in anatomy and function (Jensen et al.,

2008), posing a challenge to the molecular and cellular characterization of TPH regulation.

The *Caenorhabditis elegans* tryptophan hydroxylase, TPH-1, is highly homologous to mammalian TPH and is essential for serotonin biosynthesis. An expression reporter of *C. elegans* *tph-1* is expressed in three pairs of serotonin-producing neurons, NSM, ADF, and HSN (Sze et al., 2000). The anatomical positions and circuit connections of these neurons are well defined (White et al., 1986). Therefore, *C. elegans* is an accessible and suitable model system to study TPH regulation.

The transcription of *C. elegans* *tph-1* in ADF serotonergic neurons is dynamically regulated by conditions including starvation, heat stress, and food quality (Estevez et al., 2004, 2006; Zhang et al., 2005; Moussaif and Sze, 2009), consistent with the modulatory role of serotonin in behaviors that are affected by these conditions (Chase and Koelle, 2007). Our previous work on a form of aversive olfactory learning identified an experience-dependent change in *tph-1* transcription in ADF. We found that, as a bacteria-feeding nematode, *C. elegans* learns to avoid the smell of pathogenic bacteria after ingestion. This learning process requires the serotonergic signal from ADF neurons. Intriguingly, aversive training with pathogenic bacteria increases *tph-1* transcription and the serotonin content in ADF, and the ADF serotonin signal promotes learning through several interneurons (Zhang et al., 2005). These findings further demonstrated the importance of *tph-1* regulation and provided a system to examine its underlying mechanisms.

Here, we found that the activity of ADF serotonergic neurons regulates *tph-1* transcription. Aversive training with pathogenic

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bacteria enhances the sensory-evoked intracellular calcium response of ADF. While activating ADF increases *tph-1* transcription, inhibiting ADF activity suppresses the training-dependent upregulation of *tph-1*. The *C. elegans* homolog of calcium/calmodulin-dependent protein kinase II (CaMKII), UNC-43, acts within ADF to regulate neuronal activity, *tph-1* transcription, and learning. Selective expression of an activated form of UNC-43 in ADF is sufficient to mimic the training effect to generate increased ADF activity and *tph-1* transcription. The Gqα EGL-30 acts in the upstream olfactory sensory neurons to mediate the training effect on *tph-1* transcription by regulating the sensory response of these neurons. Together, these results support a model in which aversive training activates ADF serotonergic neurons via the cell-autonomous function of UNC-43, which acts together with the non-cell autonomous function of Gqα to regulate an experience-dependent increase in *tph-1* transcription (see Fig. 5E,F).

Materials and Methods

Strains. The strains were cultivated and maintained under standard conditions at 20°C (Brenner, 1974). *C. elegans* hermaphrodites are used in this study. The expression of *tph-1* was measured using GR1333 *mgIs71* [*tph-1::gfp*, *rol-6(su1006)*] V (Sze et al., 2000). To measure *tph-1* expression in different mutant backgrounds, all of the following strains were crossed with GR1333: MT2605 *unc-43(n498n1186)* IV, MT1092 *unc-43(n498)* IV, DA823 *egl-30(ad805)* I, MT1434 *egl-30(n686)* I, CG21 *egl-30(tg26)* I; *him-5(e1490)* V, KG1278 *unc-73(ce362)* I, RM2221 *egl-8(md1971)* V, and JT734 *goa-1(sa734)* I. The transgenic strains used in this work include CX10281 *kyEx2373* [*Pstr-2::GCaMP2*; *Punc-122::gfp*], ZC405 *yxEx274* [*Pshr-142::YC3.60*; *Punc-122::dsred*], ZC28 *unc-43(n498n1186)* IV; *mgIs71* V; *yxEx12* [*Pshr-142::unc-43::mcherry*; *Punc-122::gfp*], ZC35 *unc-43(n498n1186)* IV; *yxEx19* [*Pshr-142::unc-43::mcherry*; *Punc-122::gfp*], ZC929 *unc-43(n498n1186)* IV; *yxEx334* [*Pshr-142::unc-43::gfp*; *Punc-122::gfp*], ZC949 *unc-43(n498n1186)* IV; *mgIs71* V; *yxEx249* [*Pshr-142::unc-43(E108K)::mcherry*; *Punc-122::gfp*], ZC974 *egl-30(ad805)* I; *mgIs71* V; *yxEx369* [*Pstr-1::egl-30::mcherry*; *Podr-1::egl-30::mcherry*; *Punc-122::gfp*], ZC1240 *egl-30(n686)* I; *mgIs71* V; *yxEx543* [*Pstr-1::egl-30::mcherry*; *Podr-1::egl-30::mcherry*; *Punc-122::gfp*], ZC1145 *yxEx476* [*Pstr-1::GCaMP*; *Punc-122::gfp*], ZC1493 *egl-30(ad805)* I; *kyEx2373* [*Pstr-2::GCaMP2*; *Punc-122::gfp*], ZC1512 *egl-30(ad805)* I; *yxEx476* [*Pstr-1::GCaMP*; *Punc-122::gfp*], ZC1584 *egl-30(ad805)* I; *yxEx476* [*Pstr-1::GCaMP*; *Punc-122::gfp*]; *yxEx772* [*Pstr-1::egl-30::mcherry*; *Punc-122::dsred*], ZC1587 *yxEx775* [*Pstr-1::mcherry*; *Punc-122::dsred*; KP326], ZC1588 *yxEx776* [*Podr-1::mcherry*; *Punc-122::dsred*; KP326], ZC1863 *yxEx944* [*Pshr-142::mcherry*; *Punc-122::dsred*; KP326], ZC947 *egl-30(ad805)* I; *mgIs71* V; *yxEx350* [*Pstr-1::egl-30::mcherry*; *Punc-122::gfp*], ZC371 *egl-30(ad805)* I; *mgIs71* V; *yxEx246* [*Pshr-142::egl-30::mcherry*; *Punc-122::gfp*], ZC1391 *mgIs71* V; *yxEx725* [*Pshr-142::NaChBac::mcherry*; *Punc-122::gfp*], ZC1890 *mgIs71* V; *yxEx960* [*Pshr-142::twk-18(cn110)::mcherry*; *Punc-122::dsred*], ZC2119 *egl-30(ad805)* I; *mgIs71* V; *yxEx249* [*Pshr-142::unc-43(E108K)::mcherry*; *Punc-122::gfp*], ZC2122 *unc-43(n498n1186)* IV; *yxEx249* [*Pshr-142::unc-43(E108K)::mcherry*; *Punc-122::gfp*]; *yxEx274* [*Pshr-142::YC3.60*; *Punc-122::dsred*], ZC2153 *itr-1(sa73)* IV; *yxEx274* [*Pshr-142::YC3.60*; *Punc-122::dsred*], ZC2169 *unc-2(e55)* X; *yxEx274* [*Pshr-142::YC3.60*; *Punc-122::dsred*], ZC2170 *unc-68(e540)* V; *yxEx274* [*Pshr-142::YC3.60*; *Punc-122::dsred*], ZC 2175 *unc-43(n498n1186)* IV; *yxEx12* [*Pshr-142::unc-43::mcherry*; *Punc-122::gfp*]; *yxEx274* [*Pshr-142::YC3.60*; *Punc-122::dsred*]. KP326 is a plasmid that contains a *Pegl-30::gfp* transcriptional reporter (Lackner et al., 1999).

Generation of DNA transgenes and transgenic animals. To generate the *Pshr-142::YC3.60* construct, a 4 kb PCR product of the *srh-142* promoter was cloned into Invitrogen pENTR to make an entry vector, which was used in an LR recombination reaction with a pCVG11 destination vector containing Cameleon YC3.60 and *unc-54* 3'UTR to generate the final construct. A PCR product containing the *srh-142* promoter, YC3.60, and *unc-54* 3'UTR was generated from the plasmid and injected at 100 ng/μl. To generate the *Pshr-142::unc-43::mcherry* construct, the *mcherry* cDNA

was used to replace the *gfp* sequence in pPD95.77 (a gift from Dr. A. Fire, Stanford University School of Medicine, Stanford, CA), and a 4 kb *srh-142* promoter was inserted into the SphI site of the same plasmid to generate *Pshr-142::mcherry*. *unc-43* cDNA, which was isolated from the clone *yk213a11* (a gift from Dr. Y. Kohara, National Institute of Genetics, Mishima, Shizuoka, Japan), was inserted into *Pshr-142::mcherry*. *Pshr-142::unc-43(E108K)::mcherry* was constructed using a QuickChange Site-Directed Mutagenesis kit (Stratagene) from *Pshr-142::unc-43::mcherry*. To generate the *Pshr-142::twk-18(cn110)::mcherry* and *Pshr-142::NaChBac::mcherry* constructs, the coding region of *twk-18(cn110)* (a gift from Dr. E. Jorgensen, The University of Utah, HHMI, Salt Lake City, UT) or *NaChBac* (a gift from Drs. D. Clapham and D. Ren, Harvard Medical School, HHMI, Boston, MA) was amplified with primers engineered with SalI sites and was used to replace the *unc-43* cDNA in the *Pshr-142::unc-43::mcherry* construct. An *egl-30* cDNA was isolated from the clone *yk587a10* (a gift from Dr. Y. Kohara). To generate *Pshr-142::egl-30::mcherry*, a *srh-142* promoter fragment and a PCR product of *egl-30* cDNA were inserted into a *mcherry* vector, which was generated by replacing the *gfp* sequence of pPD95.77 with a *mcherry* coding sequence. The transgenic animals were generated by germline transformation (Mello et al., 1991). The transgenes were injected at 25 ng/μl together with a coinjection marker, *Punc-122::gfp* or *Punc-122::dsred* (Miyabayashi et al., 1999), at 25 ng/μl, unless otherwise noted.

Aversive olfactory training. The animals were trained based on a long-term training procedure as described previously (Zhang et al., 2005). Briefly, the embryos were extracted and placed on nematode growth medium (NGM) plates. For the naive condition, the NGM plate contained a lawn of *Escherichia coli* OP50. For the standard training, the NGM plate contained a lawn of *Pseudomonas aeruginosa* PA14 and a small lawn of OP50. For mutants with locomotory defects, L4 animals on the standard training plate were moved to another training plate that was completely covered by PA14 to ensure sufficient training.

Microscopy to measure the fluorescence intensity of the *tph-1::gfp* reporter. The *gfp* images were collected using an Eclipse TE2000U fluorescence inverted microscope (Nikon) with a 40× NA1.3 oil-immersion objective and a CoolSNAP EZ CCD camera; the quantification was performed using NIS-element software and NIH ImageJ. The mean fluorescence intensity was generated for each neuron by subtracting the background intensity from the average fluorescence intensity of the neuron. Multiple worms were measured for each genotype.

Calcium imaging. The neuronal calcium response of ADF neurons was measured by quantifying the changes in the YFP/CFP ratio of the ratio-metric calcium indicator Cameleon YC3.60 (Nagai et al., 2004). The neuronal responses of AWB and AWC were measured by quantifying the fluorescence intensity change of the calcium indicator GCaMP (Nakai et al., 2001) or GCaMP2.0 (Tallini et al., 2006). A microfluidic device (Chalasani et al., 2007; Chronis et al., 2007) was used to trap the worm and to deliver fluidic stimuli. The switch between the buffer and the bacteria-conditioned medium was controlled by a Valvebank II Perfusion System (Automate Scientific). To make the bacteria-conditioned medium, *E. coli* OP50 was cultured in NGM overnight until OD 600 reached 0.25–0.30. The bacterial culture was then filtered with a 0.22 μm polyethersulfone membrane filter. ND2 files of fluorescence images were collected using a CoolSNAP EZ CCD camera installed on an Eclipse Ti-U inverted microscope (Nikon) with a 40× NA 1.3 oil-immersion objective. The sampling frequency was 10 Hz. The fluorescence intensity was quantified by analyzing the ND2 files with a customized Matlab script that tracked and measured the average intensity of a designated number of brightest pixels in the selected region of interest in each image throughout the image series, which was then subtracted by a background signal of a nearby region. To correct the photo bleaching in the Cameleon signal, the signal was fitted into a bi-exponential decay function, and the corrected signal was the ratio of the recorded signal over the decay function. To calculate the change in signal, the average signal in a 4 s window before the switch of the stimuli was set as the baseline F_0 . The percentage change relative to F_0 was calculated and plotted.

Slow-killing assay. Slow-killing assays were performed using a procedure similar to the one described previously (Kawli et al., 2010). Briefly, the worms were made sterile by feeding on the *pos-1* RNAi to minimize the effect

Figure 1. Aversive olfactory training enhances ADF neuronal activity to regulate *tph-1* transcription. **A**, Standard and modified training procedure with *P. aeruginosa* PA14. **B**, Schematic for the aversive olfactory learning assay. **C**, Images of *tph-1::gfp* expression in naive or trained animals. Arrowheads indicate ADF; dashed lines outline the worms. **D**, FRET signals of YFP3.60 in ADF neurons in response to medium conditioned with *E. coli* OP50 in naive and PA14-trained wild-type animals. **E**, Heat maps for the FRET signals shown in **D**. **F**, Time integral of the FRET signals in **D** during the last 20 s stimulus presentation. In **D** and **F**, *** $p < 0.001$; * $p < 0.05$, Student's *t* test. Error bars represent SEM. **G**, FRET signals of YFP3.60 in ADF neurons in response to OP50-conditioned medium in naive and PAK-trained wild-type animals. **H–J**, FRET signals of YFP3.60 in ADF neurons in response to OP50-conditioned medium in wild-type and *itr-1(sa73)* mutants (**H**) or in wild-type and *unc-68(e540)* mutants (**I**) or in wild-type and *unc-2(e55)* mutants (**J**). In **D** and **G–J**, solid lines indicate mean values and shading indicates SEM. **K**, Expression of a potassium channel encoded by *twk-18(cn110)* in ADF abolishes training-dependent increase in *tph-1* transcription. A significant genotype $\times$ treatment interaction (** $p < 0.01$) was detected by two-way ANOVA after log transformation ($p < 0.01$ for both genotype and treatment; $n \geq 30$ animals each sample). The data sets were tested as normal distribution by the Shapiro-Wilk test. Fluorescent signals were normalized by the signal of naive wild-type worms measured in parallel; error bars represent SEM.

of progeny hatching inside hermaphrodite bodies during the assays. The killing assays were performed on a 3.5 cm NGM plate covered with PA14 at 25°C. The worm corpses were picked out and scored every 8–10 h, and the worms that had died on the wall of the Petri dish were censored.

Results

Aversive training enhances the sensory-evoked calcium response of ADF neurons

Previously, we have shown that training with pathogenic bacteria, such as *P. aeruginosa* PA14, induces olfactory aversion to the

pathogen (Zhang et al., 2005). Naive animals that are never exposed to the pathogen often prefer the smell of the pathogen, whereas trained animals that have ingested the pathogenic bacteria avoid the bacterial smell (Fig. 1A,B). This form of behavioral plasticity requires the activity of the *C. elegans* TPH-1 in ADF serotonergic neurons, because selective expression of *tph-1* in the ADF neurons of the *tph-1* null mutants rescues the learning defect of the mutants. Interestingly, aversive training increases *tph-1* transcription in ADF, as evidenced by the increased signal

of a *tph-1::gfp* reporter (Sze et al., 2000) in trained animals (Fig. 1C) (Zhang et al., 2005).

To understand how *tph-1* transcription in ADF neurons is regulated by training experience, we first examined the ADF neuronal activity using intracellular calcium imaging. We selectively expressed a genetically encoded calcium sensor, Cameleon YC3.60 (Nagai et al., 2004), in ADF using the *srh-142* promoter and recorded the calcium response of transgenic animals in a microfluidic device (Chronis et al., 2007). We stimulated the transgenic animals with alternating fluid streams of buffer and the medium that was conditioned with the culture of *E. coli* OP50, the common laboratory bacterial food for *C. elegans*. We found that the ratio of YFP-to-CFP intensity increased when the stimulus switched from buffer to the conditioned medium, indicating increased intracellular calcium levels in response to the conditioned medium (Fig. 1D,E).

To identify the source of ADF calcium transients, we examined the effects of several mutations that disrupt either intracellular calcium release from *endoplasmic reticulum* or calcium influx from extracellular space. First, we imaged ADF neurons in *itr-1(sa73)* and *unc-68(e540)* mutant animals, which carried a reduction-of-function mutation in the inositol-1,4,5-trisphosphate receptor ITR-1 (Dal Santo et al., 1999) and a splicing-acceptor site mutation in the ryanodine receptor UNC-68 (Sakube et al., 1997), respectively. We quantified the sensory-evoked ADF calcium transients by calculating the time integral of the YFP/CFP ratio change during the 30 s exposure to the conditioned medium. We did not observe any obvious defect in ADF calcium responses to OP50-conditioned medium in these two mutants compared with wild-type animals ($p > 0.05$ for time integrals over 30 s stimulus exposure, Student's *t* test) (Fig. 1H,I). Because previous studies have indicated important roles of ITR-1 and UNC-68 in regulating intracellular calcium release (Maryon et al., 1996; Sakube et al., 1997; Clandinin et al., 1998; Baylis et al., 1999; Dal Santo et al., 1999; Busch et al., 2012), these results suggest that intracellular calcium stores are not likely to be the main source for the sensory-evoked calcium transients in ADF. Next, we tested ADF calcium response in *unc-2(e55)* animals, which carry a nonsense mutation in the *C. elegans* non-L-type voltage-gated calcium channel UNC-2 (Schafer and Kenyon, 1995; Estevez et al., 2004). We found that compared with wild-type animals, *unc-2(e55)* mutants displayed weaker ADF calcium transients in response to OP50-conditioned medium (Fig. 1J), and the integral of the calcium response during 30 s exposure of the stimulus is smaller in *unc-2(e55)* than in wild-type animals ($p < 0.05$, Student's *t* test). The partial defect of *unc-2(e55)* could be attributable to potential redundant roles played by other calcium channels. For example, the L-type voltage-gated calcium channel EGL-19 (Lee et al., 1997) has been shown to regulate sensory-evoked calcium transients in several types of sensory neurons (Suzuki et al., 2003; Hilliard et al., 2005; Frøkjær-Jensen et al., 2006). However, we could not assess the effect of *egl-19(ad1006)* mutants because of weak expression of Cameleon YC3.60 in the ADF neurons of the *egl-19* mutants. Both intracellular and extracellular sources of calcium contribute to the calcium responses of *C. elegans* neurons (Suzuki et al., 2003; Hilliard et al., 2005; Frøkjær-Jensen et al., 2006; Busch et al., 2012; Hendricks et al., 2012). Our results together suggest that influx of extracellular calcium regulates the sensory-evoked calcium transients in ADF.

Next, we examined whether the aversive training alters ADF activity by comparing the calcium responses of ADF neurons in the naive and trained animals. We found that trained animals exhibited calcium transients for an extended amount of time, ranging from 10 to 30 s (Fig. 1D,E), in response to the conditioned medium. To quantify this change, we calculated the time

integral of the YFP/CFP ratio change during the last 20 s exposure to the conditioned medium and found that PA14-trained animals displayed a significant increase compared with naive animals (Fig. 1F). In contrast, training with PAK, a nonpathogenic strain of *P. aeruginosa*, did not induce similar changes ($p > 0.05$ for time integrals of the calcium transients over the last 20 s stimulus presentation, Student's *t* test) (Fig. 1G), suggesting that the training-dependent enhancement in ADF activity is contingent on the aversive experience with the pathogenic bacterium PA14. Together, these results indicate that the sensory-evoked calcium response in ADF is enhanced in the PA14-trained animals, suggesting that aversive training activates ADF neurons.

Activating ADF neurons increases *tph-1* transcription, whereas inhibiting ADF abolishes training-dependent induction of *tph-1*

Because aversive training increases both ADF neural activity and *tph-1* transcription in these cells, we examined whether increased ADF activity can lead to upregulation of *tph-1* transcription. First, we activated ADF neurons by selectively expressing a bacterially derived voltage-gated sodium channel, NaChBac (Ren et al., 2001), in ADF. Ectopic expression of NaChBac depolarizes and increases the excitability of several types of neurons in flies and mice (Nitabach et al., 2006; Kelsch et al., 2009). We found that compared with nontransgenic siblings (1.0 ± 0.06 , intensity normalized by the signal in nontransgenic animals; $n \geq 49$ animals), the transgenic animals that expressed NaChBac only in ADF neurons displayed significantly enhanced *tph-1* expression in these neurons even in the absence of training (1.34 ± 0.08 , intensity normalized by the signal in non-transgenic animals; $p < 0.01$, Student's *t* test; $n \geq 50$ animals; mean $\pm$ SEM), thus supporting the hypothesis that increased ADF neuronal activity during aversive training induces *tph-1* expression.

Next, we examined whether the training-dependent increase in *tph-1* transcription depends on ADF neuronal activity. We inhibited ADF neurons by selectively expressing an activated form of potassium channel *twk-18(cn110)* in ADF neurons. The expression of *twk-18(cn110)* in *Xenopus* oocytes generates a potassium channel that conducts significantly larger outward currents than the wild-type channels (Kunkel et al., 2000). We found that training-induced *tph-1* transcription was abolished in the transgenic animals, whereas non-transgenic wild-type siblings displayed a normal level of induction (Fig. 1K). Together, these results support our hypothesis that activation of ADF neurons during the aversive experience leads to increased *tph-1* transcription.

CaMKII/UNC-43 functions in ADF neurons to regulate ADF neuronal activity, *tph-1* transcription, and aversive learning

Next, we sought the molecular mechanisms whereby *tph-1* transcription in ADF neurons is upregulated by training experience. Because UNC-43, the only *C. elegans* homolog of CaMKII (Reiner et al., 1999; Rongo and Kaplan, 1999), regulates ADF *tph-1* expression under normal conditions (Zhang et al., 2004), we examined whether UNC-43 also regulates training-dependent *tph-1* expression in ADF.

First, we found that the training-dependent upregulation of *tph-1* transcription in ADF neurons was completely abolished in the *unc-43(n498n1186)* null mutants, measured by the expression of the transcriptional reporter *tph-1::gfp* in both naive and trained animals (Fig. 2A). In addition, the *unc-43(n498n1186)* mutants failed to learn to avoid the smell of PA14 after training (Fig. 2C). UNC-43 is ubiquitously expressed in the nervous sys-

Figure 2. UNC-43 functions cell-autonomously in ADF neurons to regulate training-dependent increase in ADF neuronal activity and *tph-1* transcription, as well as aversive olfactory learning. **A**, The null mutant *unc-43(n498n1186)* is defective in training-dependent upregulation of *tph-1::gfp* expression in ADF neurons. **B**, Expression of *unc-43* in the ADF neurons of *unc-43(n498n1186)* mutants fully rescues the defect in *tph-1::gfp* regulation. The fluorescent signals were normalized by the signal of naive wild-type worms (**A**) or naive rescued animals (**B**) measured in parallel. Significant genotype $\times$ treatment interactions (*** p < 0.001) were detected by two-way ANOVA after log transformation (p < 0.001 for both genotype and treatment; $n \geq 59$ animals each sample). The data sets were tested as normal distribution by the Shapiro-Wilk test. Error bars represent SEM. **C**, The null mutant *unc-43(n498n1186)* is defective in learning to avoid the smell of PA14 and expression of *unc-43* in the ADF neurons of *unc-43(n498n1186)* mutants partially rescues the learning defect. ** p < 0.01; * p < 0.05, Student's t test; $n \geq 6$ assays. Error bars represent SEM. **D**, The gain-of-function mutant *unc-43(n498)* exhibits increased *tph-1::gfp* expression in ADF neurons in naive animals (*** p < 0.001, Student's t test) and is defective in training-dependent increase in ADF *tph-1* expression [a significant genotype $\times$ treatment interaction (*** p < 0.001) was detected by two-way ANOVA after log transformation; p < 0.01 for genotype, p < 0.001 for treatment; $n \geq 57$ each sample; error bars represent SEM; the data sets were tested as normal distribution by the Shapiro-Wilk test]. **E**, FRET signals of YC3.60 in ADF neurons in response to OP50-conditioned medium in *unc-43(n498n1186)* mutants and in the *unc-43(n498n1186)* mutant animals that selectively express wild-type UNC-43 in ADF under both naive and PA14 training conditions. **F**, Time integral of FRET signals in **E** during the last 20 s stimulus presentation. A significant genotype $\times$ treatment interaction (* p < 0.05) was detected by two-way ANOVA (p > 0.05 for genotype, p < 0.01 for treatment; $n \geq 13$ each sample; the data sets were tested as normal distribution by the Shapiro-Wilk test; error bars represent SEM). **G**, FRET signals of YC3.60 in ADF neurons in response to OP50-conditioned medium in *unc-43(n498n1186)* mutants and in *unc-43(n498n1186)* mutants that selectively express the activated UNC-43(E108K) in ADF under both naive and PA14-training conditions. **H**, Time integral of the FRET signals in **G** and of the FRET signals in wild-type animals during the last 20 s stimulus presentation. A significant genotype $\times$ treatment interaction (* p < 0.05) was detected by two-way ANOVA (p < 0.01 for genotype, p > 0.05 for treatment; $n \geq 11$ each sample; the data sets were tested as normal distribution by the Shapiro-Wilk test). Student's t test was used to compare wild-type animals ($n = 17$) and *unc-43(n498n1186)* mutants that selectively expressed UNC-43(E108K) in ADF under the naive condition (*** p < 0.001). Error bars represent SEM.

tem (Reiner et al., 1999; Rongo and Kaplan, 1999). We found that selective expression of a wild-type *unc-43* cDNA in the ADF neurons of *unc-43(n498n1186)* mutants fully restored the training-dependent upregulation of *tph-1* expression in ADF and partially rescued the learning defects in the mutants (Fig. 2B,C). One potential reason for the partial rescue of learning is that UNC-43 also regulates the function of motor neurons and muscles (Reiner et al., 1999; Rongo and Kaplan, 1999), and the transgenic animals with UNC-43 activity restored only in ADF were still defective in locomotion. Thus, UNC-43 functions cell-autonomously in ADF neurons to regulate the training-dependent enhancement of *tph-1* transcription and aversive olfactory learning.

The mammalian CaMKII isoform α_B is localized in the nucleus (Brooke et al., 1995). However, we did not observe any clear

signal in the nuclei of transgenic animals that expressed a translational reporter *unc-43::mcherry* only in ADF neurons under either the naive or the trained condition (data not shown). The mammalian α_B -CaMKII possesses a nuclear localization signal (Brooke et al., 1995) that is absent in UNC-43. This notion is consistent with the imaging results. Therefore, UNC-43 likely functions through further downstream signaling events to regulate *tph-1* transcription in ADF neurons.

Next, we investigated how UNC-43 functions cell-autonomously to regulate training-dependent *tph-1* transcription in ADF neurons. First, we found that *tph-1* transcription in the ADF neurons of a gain-of-function mutant, *unc-43(n498)* (Reiner et al., 1999; Rongo and Kaplan, 1999), was significantly elevated under the naive condition (Fig. 2D), consistent with previous

studies (Zhang et al., 2004). In addition, this elevated *tph-1* level was not further increased by training (Fig. 2D). The *unc-43(n498)* mutant exhibits opposing phenotypes to the *unc-43* loss-of-function mutant in both locomotion and egg laying (Reiner et al., 1999; Rongo and Kaplan, 1999; Robatzek and Thomas, 2000), and the amino acid substitution E108K in *unc-43(n498)* results in constitutive activation of UNC-43 that is calcium independent (Umemura et al., 2005). These results suggest the possibility that activation of UNC-43 in ADF neurons during aversive training leads to increased *tph-1* expression. To further test this possibility, we selectively expressed the constitutively active form UNC-43(E108K) in the ADF neurons of the *unc-43(n498n1186)* null mutants and found that this ectopic expression enhanced *tph-1* transcription [1.84 ± 0.14 , intensity normalized by the signals in *unc-43(n498n1186)* mutants that expressed wild-type UNC-43 only in ADF; $n \geq 37$ animals; mean $\pm$ SEM] compared with transgenic animals that expressed the wild-type UNC-43 in the ADF neurons of the same mutants (1.0 ± 0.03 , normalized intensity; $n \geq 37$ animals; $p < 0.001$, Student's *t* test; mean $\pm$ SEM). This enhancement strongly mimicked the training effect. The transgenic animals that expressed ADF-specific UNC-43(E108K) appeared to exhibit higher expression of *tph-1* in ADF neurons than the *unc-43(n498)* gain-of-function mutants (Fig. 2D). This difference may result from a higher expression level of UNC-43(E108K) in the ADF neurons of the transgenic animals and/or some unidentified defects in the *unc-43(n498)* mutant animals. Together, these findings support that activated UNC-43 in ADF neurons increases *tph-1* transcription during aversive training.

UNC-43 regulates training-dependent enhancement of ADF calcium responses

Having characterized the roles of UNC-43 and ADF neuronal activity in regulating *tph-1* transcription in ADF, we examined whether UNC-43 regulates ADF activity. We performed calcium imaging on the ADF neurons of *unc-43(n498n1186)* null mutants (Fig. 2E,F). We found that loss of UNC-43 activity abolished training-induced increase in ADF calcium transients and that this defect was fully rescued by selective expression of wild-type UNC-43 in the ADF neurons (Fig. 2E,F). Conversely, ADF-specific expression of the activated UNC-43(E108K) isoform in the *unc-43(n498n1186)* null mutants is sufficient to increase ADF calcium transients, mimicking the training effect (Fig. 2G,H). Moreover, training did not further enhance ADF calcium response in these transgenic animals. Together, our results indicate that aversive training experience activates ADF serotonergic neurons via activated function of UNC-43 in ADF, which leads to upregulation of *tph-1* transcription (Fig. 5E,F).

EGL-30 regulates increased *tph-1* transcription during aversive training

Next, we investigated the molecular pathways in which UNC-43 acts to regulate *tph-1* induction during training. Previously, it was shown that UNC-43 regulates locomotion and egg laying through a pathway of GOA-1 and EGL-30, the *C. elegans* Go α and Gq α , respectively (Miller et al., 1999; Robatzek and Thomas, 2000; Lee et al., 2004). To test whether UNC-43 signals in the same pathway to regulate *tph-1* in ADF neurons during aversive learning, we analyzed the effect of mutations in the Go α /Gq α pathway on this process. Because mutants in this pathway are often deficient in locomotion, to ensure sufficient training we transferred trained animals from a standard training plate to a plate that was fully covered by a lawn of PA14 during the L4 larval stage and measured ADF *tph-1* levels 12 h later (Fig. 1A).

First, we found that both *egl-30(ad805)* and *egl-30(n686)* (Brundage et al., 1996), two different mutations that disrupt EGL-30 function, abolished the training-dependent upregulation of *tph-1* transcription in ADF (Fig. 3A,B). Surprisingly, the gain-of-function mutant *egl-30(tg26)* (Doi and Iwasaki, 2002; Bastiani et al., 2003) is similarly defective (Fig. 3C), suggesting that proper activity level of Gq α /EGL-30 is important in regulating *tph-1* transcription. In contrast, a canonical mutant, *goa-1(sa734)*, exhibited normal training-dependent induction of *tph-1* despite an increased level of *tph-1* expression under the naive condition (Fig. 3D). These results suggest that the functions of GOA-1 and EGL-30 in regulating training-dependent expression of *tph-1* are different from their antagonistic roles in locomotion and egg laying and thus suggest a new mechanism of EGL-30 in regulating *tph-1* transcription.

Previous studies have implicated the Rho guanine nucleotide exchange factor (GEF) domain of UNC-73 (Williams et al., 2007) and the phospholipase C β EGL-8 as downstream effectors of EGL-30 in regulating locomotion and egg laying (Lackner et al., 1999; Miller et al., 1999). We found that a nonsense mutation in *egl-8(md1971)*, which produces strong defects in locomotion and egg laying (Miller et al., 1999), displayed normal training-dependent induction of *tph-1* expression in ADF neurons (Fig. 3E), suggesting that EGL-30 functions mostly independently of EGL-8 to enhance *tph-1* transcription during aversive training. This result is consistent with a previous finding that EGL-8 acts as a minor downstream effector or in a parallel pathway of EGL-30 to regulate egg-laying behavior (Bastiani et al., 2003). Similarly, the *unc-73(ce362)* mutant that contains a mutation in the Rho GEF domain was also not significantly defective in *tph-1* induction (Fig. 3F), implicating an unidentified or novel effector(s) of Gq α /EGL-30 in regulating training-dependent *tph-1* transcription. Together, our results indicate that UNC-43 functions together with EGL-30 to modulate *tph-1* expression in ADF neurons during aversive training.

EGL-30 mediates sensory response of AWB and AWC olfactory neurons to regulate *tph-1* induction during aversive training

Next, we examined the mechanisms whereby EGL-30 regulates *tph-1* transcription in ADF neurons during aversive training. *egl-30* is known to express widely in the nervous system (Lackner et al., 1999). First, we confirmed the expression of *egl-30* in ADF neurons (Fig. 4A–C) and the major olfactory sensory neurons AWB and AWC (Fig. 4D–F) by examining the coexpression of a transcriptional reporter, KP326 (*Pegl-30::gfp*) (Lackner et al., 1999), with mCherry reporters expressed in these neurons. Next, we found that, different from the cell-autonomous role of UNC-43, selective expression of a wild-type *egl-30* cDNA in ADF neurons did not rescue the defect of *egl-30(ad805)* mutants in regulating training-dependent induction of *tph-1* expression in ADF (Fig. 4G). This defect and the similar defect in the other *egl-30* mutant, *n686*, required the activity of *egl-30* in both AWB and AWC sensory neurons to rescue (Fig. 4H,I). AWB synapse onto ADF with multiple chemical synapses (White et al., 1986), but expression of *egl-30* in AWB alone did not rescue (Fig. 4J). Together, our results reveal a small neuronal network in which *unc-43* functions cell-autonomously in ADF neurons and *egl-30* functions non-cell-autonomously in the upstream sensory neurons to produce activity-dependent upregulation of *tph-1* transcription in ADF (Fig. 5E,F). Consistently, ADF-specific expression of the activated UNC-43(E108K) in *egl-30(ad805)* mutants significantly increases ADF *tph-1* expression [Student's *t*

Figure 3. *egl-30* regulates *tph-1* transcriptional induction in ADF neurons during training. **A–D**, The *egl-30(ad805)* (**A**), *egl-30(n686)* (**B**), and *egl-30(tg26)* (**C**) mutants are defective in *tph-1* transcriptional upregulation induced by aversive training; however, the *goa-1(sa734)* mutant animals (**D**) are normal in this training-dependent change. **E**, **F**, The *egl-8(md1971)* mutants (**E**) and *unc-73(ce362)* mutants (**F**) are not significantly defective in training-dependent *tph-1* upregulation in ADF neurons. In **A**, **B**, **D**, and **F**, genotype $\times$ treatment interactions (** $p < 0.001$; * $p < 0.01$; n.s., $p \geq 0.05$) were tested by two-way ANOVA after log transformation ($p < 0.001$ for both genotype and treatment; $n \geq 40$ for each sample; the data sets were tested as normal distribution by the Shapiro–Wilk test). In **C**, a significant genotype $\times$ treatment interaction (** $p < 0.01$) was detected by the Scheirer–Ray–Hare nonparametric test ($p < 0.001$ for genotype and treatment; $n \geq 54$ animals for each sample), because the data sets were tested as deviated from normal distribution by the Shapiro–Wilk test. In **E**, genotype $\times$ treatment interaction was tested by two-way ANOVA after log transformation (n.s., $p > 0.05$ for interaction, $p = 0.902$ for genotype, and $p < 0.001$ for treatment; $n \geq 40$ for each sample; the data sets were tested as normal distribution by the Shapiro–Wilk test). For all, the worms were trained with a modified protocol as in Figure 1A. The fluorescent signals were normalized by the signal of naive wild-type worms measured in parallel. Error bars represent SEM.

test, $p < 0.001$ between transgenic animals (3.26 ± 0.18) and nontransgenic siblings (1 ± 0.08); *tph-1* signals were normalized by the signals in nontransgenic animals; $n \geq 25$ animals each group; mean $\pm$ SEM], further supporting the possibility that UNC-43 acts downstream of EGL-30 in regulating *tph-1* expression in ADF.

Next, we investigated the function of *egl-30* in AWB and AWC sensory neurons. First, we found that expression of *egl-30* in AWB and AWC neurons did not significantly alter locomotory speed (transgenic animals, 0.13 ± 0.04 pixels s^{-1} ; nontransgenic siblings, 0.27 ± 0.13 pixels s^{-1} ; Student's *t* test, $p > 0.05$; $n = 5$ animals each group; mean $\pm$ SEM), suggesting that *egl-30* function in these neurons is not essential for locomotion. Using slow-killing assays (Kawli et al., 2010) to measure resistance to PA14 infection, we found that the activity of *egl-30* in AWB and AWC also did not significantly change the susceptibility of the *egl-30(n686)* mutants (Fig. 5A), consistent with the previous finding on the role of intestinal EGL-30 in regulating pathogen resistance (Kawli et al., 2010).

Finally, we examined whether *egl-30* mediates the sensory response of AWB and AWC, the major sensory neurons that detect repellents and attractants, respectively (Bargmann et al., 1993). The sensory ability of these two pairs of neurons is essential for worms to learn to avoid the smell of pathogenic bacteria (Ha et al., 2010), and both AWB and AWC cells are activated by the removal of medium conditioned by the worm food *E. coli* OP50 (Chalasani et al., 2007; Ha et al., 2010). Using intracellular calcium imaging and transgenic animals that selectively expressed the calcium-sensitive fluorescent protein GCaMP (Nakai et al., 2001) or GCaMP2.0 (Tallini et al., 2006), we found that compared with wild-type animals, *egl-30(ad805)* mutants displayed a significantly weaker calcium response in AWB neurons after the removal of OP50-conditioned medium (Fig. 5B). This defect was fully rescued by selective expression of *egl-30* cDNA in AWB neurons (Fig. 5C). Similarly, *egl-30(ad805)* mutants showed a mild defect in the calcium response of AWC neurons (Fig. 5D). Together, these results indicate that EGL-30 functions in AWB and AWC neurons to regulate olfactory sensory response, suggesting an important role of sensory experience in the upregulation of *tph-1* transcription during aversive training.

Discussion

Regulated expression of TPH, the rate-limiting biosynthetic enzyme of serotonin, is essential for serotonergic signaling. Here, we characterize a neuronal signaling network that regulates the experience-dependent transcription of *C. elegans* *tph-1* during aversive olfactory learning. We show that aversive training activates ADF neurons and that increased ADF neuronal activity results in enhanced *tph-1* expression. This experience-dependent increase in ADF activity and *tph-1* expression requires the function of UNC-43 in ADF. ADF-selective expression of an activated form of UNC-43 produces the training effect on ADF neuronal activity and *tph-1* transcription in the absence of training. We also show that Gqα/EGL-30 regulates the sensory function of AWB and AWC olfactory neurons, the sensory neurons upstream of ADF, to mediate the training-dependent enhancement of *tph-1* expression, implicating the importance of sensory experience. Thus, our work provides mechanistic insights into experience-dependent regulation of *tph-1* expression.

L-type voltage-gated calcium channel EGL-19 regulates sensory-evoked calcium transients in several *C. elegans* mechanosensory neurons (Suzuki et al., 2003; Frøkjær-Jensen et al., 2006); nociceptive neuron ASH (Hilliard et al., 2005); and

oxygen-sensing neurons, such as URX (Busch et al., 2012). Although we could not assess the role of *egl-19* in ADF calcium transients because of weak expression of the calcium indicator Cameleon YC3.60 in *egl-19(ad1006)* mutants, we have identified the role of UNC-2, a non-L-type voltage-gated calcium channel, in regulating sensory-evoked calcium response in ADF. Previous studies have shown that mutations in *unc-2* decrease *tph-1* expression in ADF (Estevez et al., 2004). Together, these results suggest that the UNC-2-mediated intracellular calcium level is important for *tph-1* expression in ADF neurons.

Intracellular calcium levels are known to regulate serotonergic signaling. In mammals, an increase in the intracellular calcium concentration in brain tissues leads to an increase in serotonin synthesis (Elks et al., 1979; Hamon et al., 1979). Here, we found that the sensory-evoked calcium response of ADF serotonergic neurons is enhanced after aversive training and increased ADF neuronal activity generates elevated transcription of *tph-1*. Thus, we propose that activity-dependent regulation of *tph-1* transcription is one of the mechanisms that underlie calcium-regulated serotonin production. In rats, repeated immobilization or repeated forced swimming stress elevated the mRNA level of TPH in the raphe nuclei (Chamas et al., 1999; Shishkina et al., 2008). We speculate that increased serotonergic neuronal activity may underlie the experience-dependent regulation of TPH in these cases.

Importantly, we have identified a cell-autonomous role of CaMKII/UNC-43 in regulating training-dependent activity increase in ADF neurons. We have also shown that increased ADF activity enhances *tph-1* transcription. Thus, we propose that aversive training experience upregulates *tph-1* transcription in ADF neurons via UNC-43-mediated neuronal activity enhancement (Fig. 5E,F). In the mammalian serotonergic system, TPH can be directly phosphorylated and activated by CaMKII (Ehret et al., 1989). Here, we show that CaMKII also enhances *tph-1* transcription during aversive olfactory learning, providing a new mechanism whereby CaMKII regulates serotonin signaling. Previous studies have shown that UNC-43 is required to maintain the basal level of *tph-1* transcription under normal conditions (Estevez et al., 2004, 2006; Zhang et al., 2004). Our findings reveal several novel functions of UNC-43 in regulating *tph-1*. First, we show that UNC-43 regulates *tph-1* transcription in a way that is dependent on training experience. Second, we

Figure 4. EGL-30 functions in the AWB and AWC sensory neurons to regulate training-dependent induction of *tph-1* transcription in ADF neurons. **A–C**, *egl-30* is expressed in the ADF neurons, as shown by colocalization of the fluorescent signals generated by the *Psh-142::mcherry* (**A**) and the *Pegl-30::gfp* (**B**) transcriptional reporters. **D–F**, *egl-30* is expressed in the AWB and AWC neurons, as shown by colocalization of the fluorescent signals generated by the *Podr-1::mcherry* (**D**) and the *Pegl-30::gfp* (**E**) transcriptional reporters. **G**, Expression of *egl-30* in ADF neurons does not rescue the mutant phenotype of *egl-30(ad805)* in *tph-1* expression. Two-way ANOVA after log transformation, n.s., $p > 0.05$ for genotype $\times$ treatment interaction, $p < 0.001$ for genotype, $p = 0.07$ for treatment; $n \geq 28$ for each group; the data sets were tested as normal distribution by the Shapiro-Wilk test. Error bars represent SEM. **H**, **I**, Expression of *egl-30* in both AWB and AWC fully rescues the defect of *egl-30(ad805)* (**H**) and *egl-30(n686)* (**I**) in *tph-1* regulation. A significant genotype $\times$ treatment interaction ($***p < 0.001$) was detected by the Scheirer-Ray-Hare nonparametric test (**H**) or by two-way ANOVA after log transformation (**I**), $p < 0.001$ for genotype and treatment (**H**, **I**); $n \geq 35$ for each group; the data sets were tested as deviated from normal distribution (**H**) or normal distribution (**I**) by the Shapiro-Wilk test. Error bars represent SEM. **J**, Expression of *egl-30* in AWB neurons alone does not rescue the mutant phenotype of *egl-30(ad805)* in *tph-1* expression. The genotype $\times$ treatment interaction (n.s., $p > 0.05$) was tested by the Scheirer-Ray-Hare nonparametric test ($p = 0.12$ for genotype, $p < 0.05$ for treatment; $n \geq 40$ for each sample), because the data sets were tested as deviated from normal distribution by the Shapiro-Wilk test. Error bars represent SEM.

show that UNC-43 regulates the training-dependent change in ADF activity and *tph-1* transcription in a cell-autonomous manner, linking the signal transduction of UNC-43 and *tph-1* transcription within the same cells. Third, we show that ADF-specific

Figure 5. EGL-30 mediates the sensory function of the AWB and AWC olfactory neurons to regulate training-dependent increase in *tph-1* transcription in ADF neurons. **A**, The resistance of *egl-30*(n686) mutants to PA14 is not significantly rescued by the expression of *egl-30* in AWB and AWC neurons ($n = 3$ assays, 3 plates per assay; no significant difference among the *egl-30*(n686) groups; the difference between wild-type and all other genotypes is significant; log-rank test with Bonferroni correction; error bars represent SEM). **B, C**, GCaMP signals of AWB neurons in wild-type and *egl-30*(ad805) mutant animals (**B**) and in transgenic animals that express *egl-30* selectively in AWB and nontransgenic siblings (**C**). **D**, GCaMP2 signals of AWC neurons in wild-type and *egl-30*(ad805) mutant animals. In **B–D**, solid lines indicate mean, and shading indicates SEM. $*p < 0.05$, Student's t test. **E, F**, Working model that describes the role of CaMKII/UNC-43 and Gqα/EGL-30 signaling pathway in regulating *tph-1* transcription in ADF neurons in the naive (**E**) and trained (**F**) animals.

expression of a constitutively active form of UNC-43 mimics the training effect on ADF neuronal activity and *tph-1* transcription, suggesting that activation of UNC-43 in ADF is a functional consequence of training that leads to increased ADF neuronal activity and *tph-1* expression. Meanwhile, we have also shown that increased ADF neuronal activity, measured by intracellular calcium levels, leads to increased *tph-1* transcription, causally linking the UNC-43-mediated neuronal activity change with increased *tph-1* transcription within ADF (UNC-43 → neuronal activity → *tph-1* transcription). During hippocampal long-term potentiation, high-frequency stimulation activates postsynaptic CaMKII, which in turn alters neuronal activity by modulating the conductance and localization of ionotropic receptors (Lisman et al., 2012). We propose that training experience activates the worm CaMKII, UNC-43, in ADF neurons, which enhances ADF neuronal activity by increasing intracellular calcium levels. In the mammalian nervous system, elevated intracellular calcium levels activate kinase pathways, including those of PKA, CaMK, and RSK/MSK, which results in transcriptional changes via modulation of transcriptional regulators (Flavell and Greenberg, 2008). Our study suggests that the signal of UNC-43-mediated calcium

increase in trained ADF neurons is conveyed into the nucleus to enhance *tph-1* transcription (Fig. 5E,F).

CaMKII is a calcium-activated enzyme that plays an essential role in calcium signaling and regulates activity-dependent changes in a variety of neuronal functions (Lisman et al., 2002). Mutations in *unc-43*, which encodes the *C. elegans* CaMKII, generate pleiotropic defects, including abnormal locomotion, egg laying, and defecation (Reiner et al., 1999; Rongo and Kaplan, 1999; Robatzek and Thomas, 2000), and UNC-43 regulates these different behaviors through different effectors. Particularly, in locomotion and egg laying, UNC-43 acts with a $G\alpha$ (GOA-1) and a $Gq\alpha$ (EGL-30), which play antagonistic roles (Mendel et al., 1995; Segalat et al., 1995; Lackner et al., 1999; Miller et al., 1999; Nurrish et al., 1999; Robatzek and Thomas, 2000; Lee et al., 2004). Here, we show that GOA-1 is not essential for the training-induced *tph-1* upregulation in ADF neurons, whereas both UNC-43 and EGL-30 are required for this regulation. Furthermore, before our study, it was not clear whether UNC-43 and EGL-30 act in the same cells for many of their signaling functions. Here, we show that UNC-43 plays a cell-autonomous role in ADF neu-

rons, whereas EGL-30 plays a non-cell-autonomous role in the upstream olfactory sensory neurons, to induce *tph-1* transcription during training. Thus, our results reveal another mechanism for the diverse function of the UNC-43 and EGL-30 signaling pathway.

Our study also identifies an unexpected role of Gq α /EGL-30 in mediating the sensory response of olfactory neurons. Similar to vertebrates, *C. elegans* olfaction also signals through GPCR and G-protein pathways. At least 20 G α subunits are encoded in the *C. elegans* genome (Jansen et al., 1999). One G α , ODR-3, plays an important role in mediating olfactory sensation of the AWB and AWC neurons (Roayaie et al., 1998), but a strong mutant of *odr-3* is only partially defective in long-range avoidance of 2-nonanone, a repellent detected by AWB, suggesting potential additional G-proteins that mediate AWB sensation (Troemel et al., 1997). Because each *C. elegans* olfactory sensory neuron can express multiple GPCRs, more than one G α protein may be used for signal transduction in individual neurons. In this study, we show that EGL-30 acts in AWB to mediate its sensory response and mutations in *egl-30* also impair the sensory response of AWC. Therefore, it is possible that Gq α /EGL-30 is one of the G α proteins involved in olfactory sensation of AWB and AWC. Intriguingly, the activity of EGL-30 in AWB and AWC is essential for aversive training-enhanced *tph-1* expression in ADF neurons, which receive many synapses from AWB (White et al., 1986). These results implicate the importance of sensory experience during training in the upregulation of *tph-1* expression. AWB and AWC may perceive either sensory inputs from a training pathogen or internal signals generated by infection; the nature of these sensory cues that elicit AWB and AWC responses and mediate *tph-1* regulation will require further investigation.

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