Cellular/Molecular

Dissecting the Signaling Mechanisms Underlying Recognition and Preference of Food Odors

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Food is critical for survival. Many animals, including the nematode *Caenorhabditis elegans*, use sensorimotor systems to detect and locate preferred food sources. However, the signaling mechanisms underlying food-choice behaviors are poorly understood. Here, we characterize the molecular signaling that regulates recognition and preference between different food odors in *C. elegans*. We show that the major olfactory sensory neurons, AWB and AWC, play essential roles in this behavior. A canonical Gα-protein, together with guanylate cyclases and cGMP-gated channels, is needed for the recognition of food odors. The food-odor-evoked signal is transmitted via glutamatergic neurotransmission from AWC and through AMPA and kainate-like glutamate receptor subunits. In contrast, peptidergic signaling is required to generate preference between different food odors while being dispensable for the recognition of the odors. We show that this regulation is achieved by the neuropeptide NLP-9 produced in AWB, which acts with its putative receptor NPR-18, and by the neuropeptide NLP-1 produced in AWC. In addition, another set of sensory neurons inhibits food-odor preference. These mechanistic logics, together with a previously mapped neural circuit underlying food-odor preference, provide a functional network linking sensory response, transduction, and downstream receptors to process complex olfactory information and generate the appropriate behavioral decision essential for survival.

Key words: **glutamatergic transmission; neuropeptide signaling; olfactory sensory neurons; olfactory sensory signaling; preference of food odors**

Introduction

Olfactory preference among different foods is widely observed in vertebrates and invertebrates and can be influenced by many factors, including nutritional and behavioral states and developmental stage, as well as experience with the food odors (Mandairon et al., 2008; Ha et al., 2010; Fougeron et al., 2011; Saveer et al., 2012; Yoshida et al., 2012). However, the mechanisms whereby olfactory systems process either simultaneously or alternately presented food odors to exhibit behavioral preference remain largely unknown.

Previous work in different organisms, including the nematode *Caenorhabditis elegans,* has revealed the molecular mechanisms underlying primary olfactory sensory response, signaling transduction, and cellular events that are required to generate the appropriate odor-dependent behaviors (Juilfs et al., 1997; Chalasani et al., 2007; Pírez and Wachowiak, 2008; Root et al., 2008, 2011; Ignell et al., 2009; Petzold et al., 2009; Chalasani et al., 2010; Hadley and Halliwell, 2010; Harris et al., 2010). These studies have provided a body of knowledge to address the mechanisms for food-odor preference.

C. elegans senses a plethora of environmental cues, including odorants, salts, pheromones, and temperature (Ward et al., 1975; Dusenbery et al., 1978; Bargmann et al., 1993). The well defined nervous system of the nematode offers an opportunity to study preference of food odors at molecular and cellular levels. Particularly, the AWB and AWC chemosensory neurons have been implicated in sensorimotor responses to food-associated odors. By using intracellular calcium imaging, these two neurons have also been shown to respond to the odors of bacteria, including the laboratory worm food *Escherichia coli* strain OP50 and a pathogenic strain *Pseudomonas aeruginosa* PA14 (Chalasani et al., 2007; Ha et al., 2010). We have previously found that under a standard condition, *C. elegans* prefers the smell of PA14 to the smell of OP50, and AWB and AWC are required for this olfactory preference. Using laser ablation, we have mapped a sensorimotor circuit downstream of AWB and AWC that regulates the olfactory preference of PA14 to OP50 (Ha et al., 2010). These results reveal a neuronal network underlying olfactory preference between two different foods, allowing for characterization of the molecular machinery and signaling pathways for food-odor recognition and preference.

Here, we characterize the sensory transduction underlying the recognition and preference between the smells of two bacterial foods for *C. elegans*, *E. coli* OP50 and *P. aeruginosa* PA14. We

Received Dec. 31, 2013; revised April 24, 2014; accepted May 31, 2014.

Author contributions: G.H. and Y.Z. designed research; G.H., Y.S., H.H., A.D., S.W., and X.Z. performed research; G.H. analyzed data; G.H. and Y.Z. wrote the paper.

We thank the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health (NIH) Office of Research Infrastructure Programs (Grant P40 00010440), Dr. C. Bargmann, Dr. V. Maricq, Dr. R. Komuniecki, and Dr. M. de Bono for strains; and J. Yu for the *tax-4* genomic clone. The work in the Zhang laboratory was supported by the Esther and Joseph Klingenstein Fund, March of Dimes Foundation, the Alfred P. Sloan Foundation, the John Merck Fund, and NIH (Grant R01 DC009852 to Y.Z.).

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DOI:10.1523/JNEUROSCI.0012-14.2014

 0270-6474/14/339389-15$15.00/0

demonstrate that food-odor preference requires the olfactory sensory neurons AWB and AWC that use specific heterotrimeric Gα-proteins, multiple guanylate cyclases, and cGMP-gated channel subunits. We show that downstream of olfactory sensory response, glutamatergic signaling from AWC regulates food-odor recognition and peptidergic transmission from AWB and AWC mediates preference between two different food odors. These results extend our understanding of how olfactory sensory systems process multiple complex odors to produce behavioral responses that are essential for survival and demonstrate that distinct signaling pathways mediate recognition of food odors versus preference between the odors.

Materials and Methods

Culture and maintenance of strains. *C. elegans* hermaphrodites were used in this study. The Bristol N2 (the wild-type reference) strain of *C. elegans* was used as the control for all behavioral analysis and as the parental strain to produce transgenic animals. All animals were raised at 20°C under standard conditions (Brenner, 1974). Mutants used for the study include the following: FK100 *tax-2(ks10)I*, PR691 *tax-2(p691)I*, FK103 *tax-4(ks28)III*, PR678 *tax-4(p678)III*, CX3090 *tax-2(p691)I*; *tax-4(p678)III*, CX10 *osm-9(ky10)IV*, CX2205 *odr-3(n2150)V*, MT4810 *odr-3(n2046)V*, MT6308 *eat-4(ky5)III*, MT150 *egl-3(n150)V*, KP2018 *egl-21(n476)IV*, JT609 *eat-16(sa609)I*, RB1780 *rgs-3(ok2288)II*, MT3113 *tdc-1(n3419)II*, MT9455 *tbh-1(n3247)X*, MT1241 *egl-21(n611)IV*, RB2030 *nlp-3(ok2688)X*, RB1340 *nlp-1(ok1470)X*, RB1668 *c02h7.2(ok2068)X*, RB1429 *t27d1.3(ok1626)III*, RB982 *flp-21(ok889)V*, IC683 *npr-9(tm1652)X*, RB1609 *nlp-5(ok1981)II*, VC1309 *nlp-8(ok1799)I*, RB1372 *nlp-18(ok1557)II*, VC2324 *flp-6(ok3056)V*, DR47 *daf-11(m47)V*, KJ462 *cng-1(ok3292)V*, RB2407 *cng-3(jh113)IV*, CX2065 *odr-1(n1936)X*, PR694 *tax-2(p694)I*, RB1289 *npr-18(ok1388)X*, NL334 *gpa-2(pk16)V*, NL335 *gpa-3(pk35)V*, NL2330 *gpa-13(pk1270)V*, RB658 *glc-4(ok212)II*, VM487 *nmr-1(ak4)II*, RB1808 *glr-2(ok2342)III*, VC350 *glc-2(gk179)I*, CX5019 *glr-1(ky176)III*, KP4 *glr-1(n2461)III*, XA7400 *glc-3(ok321)V*, DA1371 *avr-14(ad1302)I*, DA1051 *avr-15(ad1051)V*, DA1302 *avr-14(ad1302)I*; *avr-15(ad1051)V*, CX03572 *nlp-9(tm3572)V*, and FX02984 *nlp-7(tm2984)X* (gifts from S. Mitani, Tokyo Women's Medical University, Tokyo, Japan).

Transgenic strains used in this study include the following: ZC1626 *eat-4(ky5)III*; *yxEx1519* (*Peat-4:eat-4; Punc-122::gfp*), ZC1631 *eat-4(ky5)III*; *yxEx807* [*Podr-3::eat-4(cDNA)*; *Punc-122::gfp*], ZC1629 *eat-4(ky5)III*; *yxEx805* [*Podr-3::eat-4(cDNA); Punc-122::gfp*], ZC1628 *eat-4(ky5)III*; *yxEx804* [*Podr-3::eat-4(cDNA); Punc-122::gfp*], ZC2419 *eat-4(ky5)III*; *yxEx1263* [*Pstr-1::eat-4(cDNA)*; *Punc-122::gfp*], ZC1964 *eat-4(ky5)III*, *yxEx999* [*Podr-1::eat-4(cDNA); Punc-122::gfp*], ZC2114 *yxEx1127* [*Podr-1::egl-3RNAi*; *Punc-122::gfp*], ZC2112 *yxEx1125* [*Podr-1::odr-1RNAi*; *Punc-122::gfp*], ZC2154 *yxEx1148* [*Podr-1::daf-11RNAi*; *Punc-122::gfp*], ZC2303 *tax-4(ks28)III*; *yxEx1205* [*Ptax-4::tax-4, Punc-122::rfp*].

Generation of transgenes and transgenic animals. Genomic DNA fragments for rescuing experiments were amplified from N2 genomic DNA by PCR using standard protocols and confirmed by sequencing.

To generate strains that express *Podr-1::eat-4* and *Pstr-1::eat-4* transgenes, a 4.7 kb promoter sequence of *Pstr-1* and 2.4 kb promoter sequence of *Podr-1* were amplified by PCR and cloned into pCR8 Gateway entry vector (Invitrogen), which were recombined with the *pSM-rfB-eat-4* destination vector following the manufacturer's instructions (Invitrogen). All transgenes were confirmed by restriction digest and sequencing. Transgenic animals were generated by germ-line transformation (Mello et al., 1991). Transgenes were injected together with *Punc-122::gfp* or *Punc-122::rfp* plasmid, which is expressed in hermaphrodite coelomocytes and serves as a coinjection marker (Miyabayashi et al., 1999), at a total concentration of 20–50 ng/μl. Multiple transgenic lines were examined in each experiment.

Generation of cell-specific RNAi constructs. Neuron-specific RNAi transgenes were constructed as described previously (Esposito et al., 2007; Harris et al., 2009). Briefly, a neuron-specific promoter was fused to an exon-rich region of the target gene (*egl-3, odr-1*, or *daf-11*) to generate both sense and antisense PCR fusion constructs. A 2.4 kb *odr-1* promoter and a 4.7 kb *str-1* promoter were amplified for subsequent PCR fusion reactions (Troemel et al., 1995; L'Etoile et al., 2000). At least three fusion products for each target gene were pooled, and the mixture of the sense and antisense transgenes was injected at 25–100 ng/μl together with 25 ng/μl *Punc-122::rfp* or *Punc-122::gfp* DNA into wild-type animals. Multiple transgenic lines were examined for each RNAi experiment.

Microdroplet assay. Microdroplet assay was performed as previously described with minor modifications (Ha et al., 2010). Briefly, ~50 hermaphrodites of L4-stage larvae were transferred from regular culture plates onto a fresh plate containing a lawn of *E. coli* OP50 during the night before the assay. On the following day, young adults were examined for olfactory behavior using microdroplet assay by comparing their turning rates in response to two alternating air streams. To quantify preference between two food odors, the air streams were saturated by the fresh liquid culture of *E. coli* OP50 or *P. aeruginosa* PA14. To measure the recognition of food odors, one air stream was odorized by passing though nematode growth media (NGM) and the other was saturated by the liquid culture of OP50 or PA14. OP50 and PA14 bacterial cultures were prepared by adding individual OP50 and PA14 bacterial colonies, respectively, into 40 ml of NGM and allowed to grow overnight. The indexes for PA14 preference in the present study show some variations, such as the wild-type preference indexes in Figure 3. These variations are likely due to the variations among different batches of bacteria cultures, because the growth of the bacterial strains depends on environmental conditions, such as temperature and moisture. These variations can result in variations in the smell of bacterial cultures that were used as the odor sources in the microdroplet assays. To analyze the effects of exogenous octopamine or tyramine, NGM plates were prepared to contain either 4 mM tyramine or octopamine hydrochloride (Sigma-Aldrich) as previously described (Horvitz et al., 1982; Alkema et al., 2005; Wragg et al., 2007). On the day of the assay, young adults were transferred from regular OP50 plates to NGM plates containing 4 mM tyramine or octopamine for 30 min before their olfactory responses were measured in the microdroplet assay. Animals were placed on an unseeded plate for 1 min before being transferred to amine-containing plates to remove any adherent OP50 bacteria. Wild-type animals without treatment of tyramine or octopamine were used as controls.

Nonanone assay. Animals were assayed as previously described (Troemel et al., 1997). Briefly, nematodes were grown at 20°C on NGM plates that contained a lawn of *E. coli* OP50. A group of 50–100 adult animals were washed three times in S-basal and once in distilled water, and placed in the center of a square NGM assay plate, on the two opposite ends of which either two individual spots of nonanone (10%, 1 μl each) or ethanol (used as the solvent; Sigma-Aldrich, 1 μl each) were freshly placed. The space of each assay plate was divided into six equal sectors labeled as A–F and the positions of the worms on each assay plate were scored after 1 h. An avoidance index was defined as the number of animals in the two sectors farthest away from nonanone minus the number of animals in the two sectors closest to nonanone and then normalized by the total number of worms in all sectors.

Results

Different sensory neurons combinatorially regulate food-odor preference

We have previously shown that animals cultivated on *E. coli* OP50, the common food for *C. elegans* in laboratories, prefer the smell of the *P. aeruginosa* strain PA14 to the smell of OP50 (Ha et al., 2010). To understand the molecular and circuit mechanisms that are needed to generate olfactory preference of PA14 to OP50, we used an automated assay to measure olfactory responses in individual swimming animals (Ha et al., 2010; see Materials and Methods). We transferred well fed L4-stage larvae onto a freshly prepared regular culture plate 12–15 h before assay to obtain synchronized adult animals (Fig. 1*A*). Each of these animals was then transferred into a 2 μl of droplet of NGM buffer in a semienclosed chamber. These animals, while swimming in the droplets of NGM buffer, were exposed to the two air streams that were saturated with either OP50 smell or PA14 smell and alternated

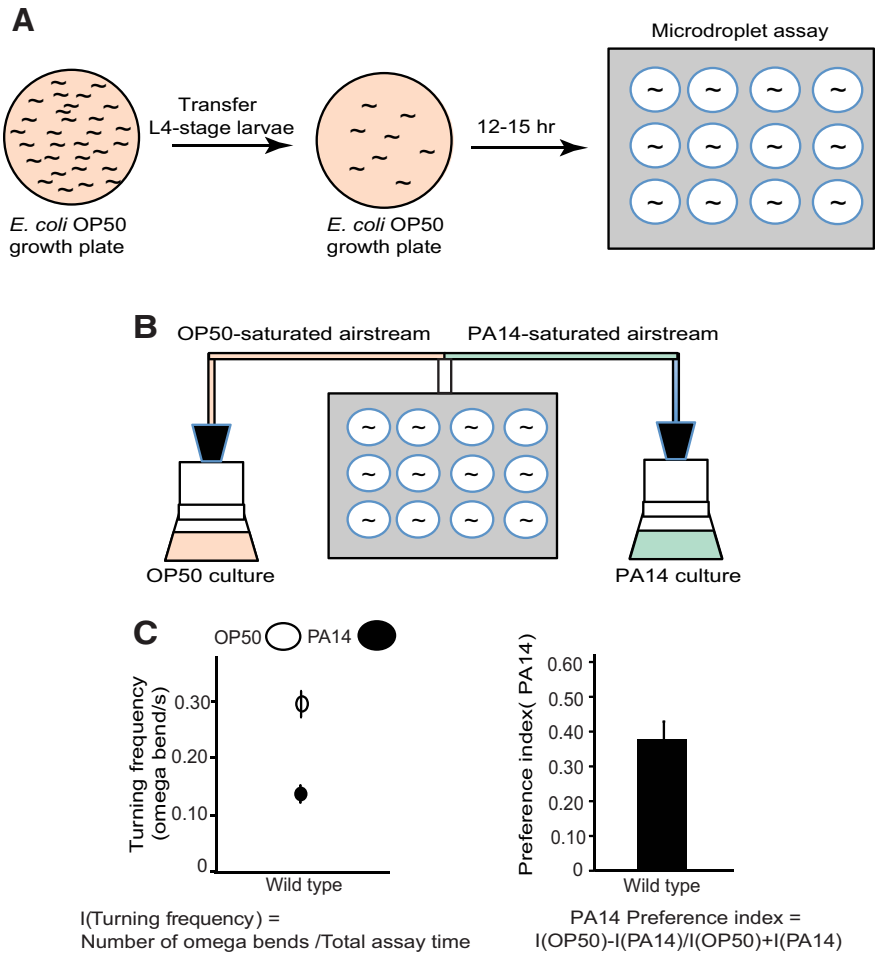

Figure 1. An automated microdroplet assay for sensorimotor responses to food odors. ***A***, The protocol for measuring food-odor preference. ***B***, Schematic of the microdroplet assay for olfactory preference between two bacterial strains, *E. coli* OP50 and *P. aeruginosa* PA14. The sapphire window holds 12 droplets (2 μl each) of NGM buffer that contain individual young adults. The animals are subjected to two air streams that are odorized with the smell of OP50 or the smell of PA14 and alternate every 30 s. The swimming behavior is recorded. The frequency of Ω bends is analyzed by a customized software and the PA14 preference index is calculated as indicated (See Materials and Methods). ***C***, Sample turning frequency and PA14 preference index generated by multiple wild-type animal. Mean $\pm$ SEM.

every 30 s. The locomotory responses of the animals were recorded and measured by a customized software (Fig. 1*B*,*C*). It has been shown that, similar to worms crawling on a solid substrate, swimming worms regulate turning rate during olfactory sensorimotor responses (Pierce-Shimomura et al., 1999; Luo et al., 2008). During swimming, *C. elegans* continuously displays C-shaped body bends that are stochastically interrupted by sharp body bends that resemble the shape of the Greek letter omega (Ω). Attractive odors suppress the rate of Ω bends and removal of attractants increases it. Thus, we used the rate of Ω bends evoked by olfactory cues to measure olfactory preference (Fig. 1*B*,*C*; see Materials and Methods). Consistent with our previous findings, animals raised on OP50 prefer the smell of PA14 in comparison with the smell of OP50 (Fig. 1).

Previously, using similar olfactory assays, we have shown that AWB and AWC olfactory sensory neurons play a critical role in generating the olfactory preference of PA14 over OP50 under standard conditions (Ha et al., 2010). AWC neurons mediate attractive olfactory responses via regulation of reversals and turns (Luo et al., 2008; Tsunozaki et al., 2008; Chalasani et al., 2010). In contrast, AWB neurons mediate avoidance of both repulsive odors and the lawn of certain pathogenic bacteria (Troemel et al., 1997; Chao et al., 2004; Pradel et al., 2007). To understand the role of AWB and AWC in regulating food-odor preference, we examined the primary sensory transduction pathways that are known to regulate AWB-mediated and AWC-mediated sensorimotor behaviors (Coburn and Bargmann, 1996; Roayaie et al., 1998; L'Etoile et al., 2000). First, we examined the effects of mutations in predicted cyclic nucleotide-gated channels in *C. elegans*, including the *tax-2* and *tax-4* genes that encode the α and β subunits of the cGMP-gated cationic channels, respectively, as well as the *cng-1* and *cng-3* genes that encode cyclic nucleotide-gated channels (Coburn and Bargmann, 1996; Coburn et al., 1998; Komatsu et al., 1999; Cho et al., 2004, 2005). The TAX-2/TAX-4 channels are required for a broad range of sensory behaviors, including chemotaxis (Coburn and Bargmann, 1996; Komatsu et al., 1996; Coburn et al., 1998; Hallem et al., 2008; Bretscher et al., 2011; Hellman and Shen, 2011). We found that two loss-of-function mutations, *ks10* in *tax-2* and *ks28* in *tax-4*, significantly disrupted the preference of the smell of PA14 in comparison with the smell of OP50 (Fig. 2*A*,*B*). These defects resulted from the inability of the *tax-2* or *tax-4* mutants to upregulate their turning rate in response to the less preferred smell of OP50 (Fig. 2*A*,*B*). The difference between the phenotypes in *tax-2(ks10)* and *tax-4(ks28)* may be due to the importance of each channel subunit in forming potential functional homodimeric channels that are required to generate PA14 preference. Consistently, the double mutant *tax-2(p691); tax-4(p678)*, as well as each of the single mutants, all generated severely defective preference to PA14 (Fig. 2*I*–*L*). In contrast, both *cng-1* and *cng-3* mutants were wild-type for PA14 preference (Fig. 2*E*,*F*). In addition, a null mutation in *osm-9*, which encodes a TRPV (transient receptor potential vanilloid) channel in sensory neurons that do not express TAX-2/TAX-4 channels (Tobin et al., 2002), exhibited a wild-type PA14 odor preference (Fig. 2*G*,*H*). Expressing a genomic DNA fragment of *tax-4* rescued the preference defect in the *tax-4(ks28)* mutants (Fig. 2*C*,*D*, *Ptax-4::tax-4*) and expressing the wild-type activity of TAX-4 in the AWC, AWB, and AWA neurons using the *odr-3* promoter (Lesch and Bargmann, 2010) fully rescued the defect of the *tax-4(ky791)* mutants (Fig. 2*C*,*D*, *Podr-3::tax-4*). Interestingly, the full-length genomic *tax-4* transgene generated an enhanced PA14 preference, suggesting that potential overexpression of *tax-4* under its endogenous promoter may enhance PA14 preference. These results together demonstrate that the function of the cGMP-gated TAX-2/TAX-4 channels in the AWB and AWC sensory neurons are required to generate olfactory preference of PA14 in comparison with OP50.

To further examine the role of chemosensory neurons in food-odor preference, we tested another *tax-2* mutant, *p694*, which has lost the expression of *tax-2* only in a subset of sensory

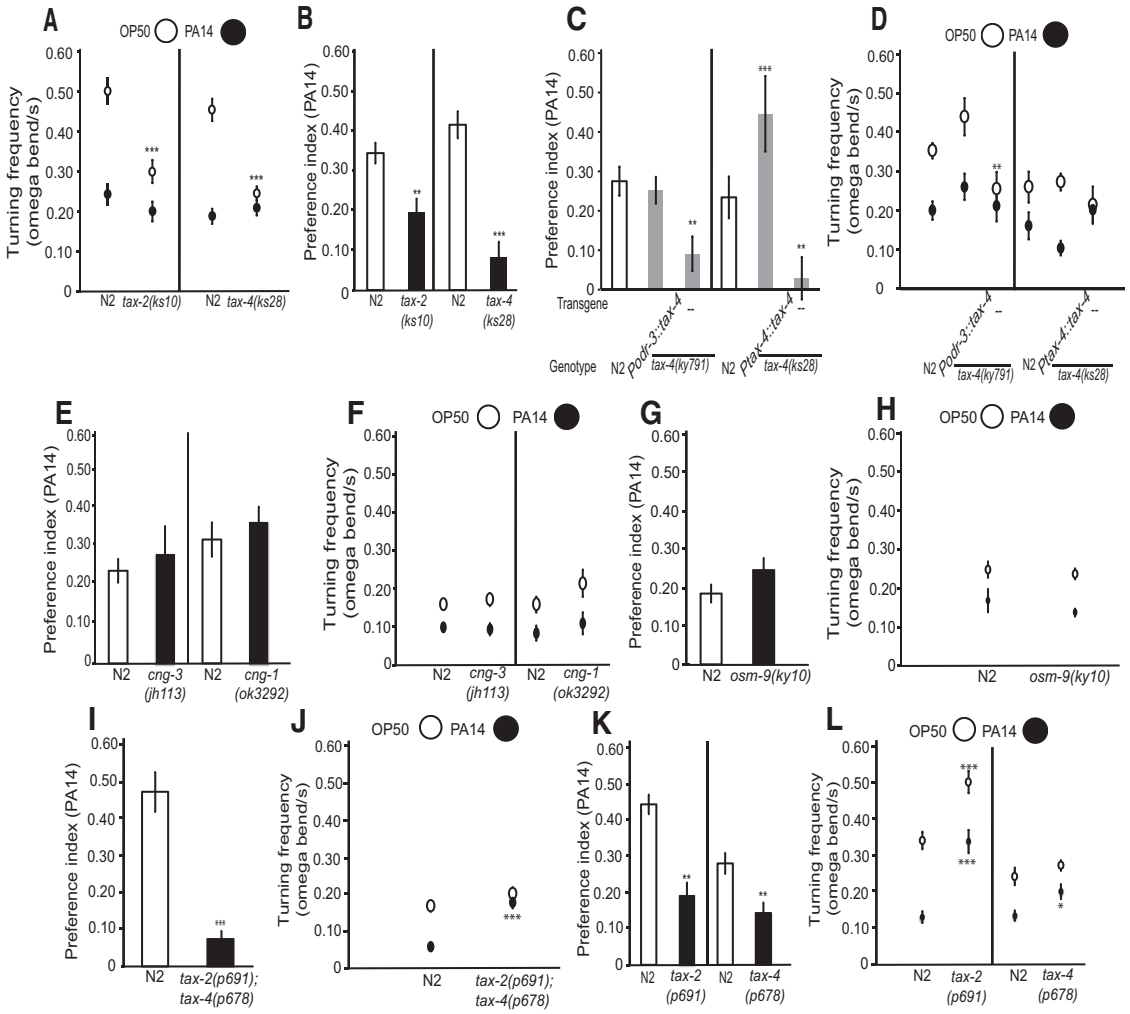

Figure 2. The cGMP-gated TAX-2/TAX-4 channel acts in the AWB and AWC sensory neurons to facilitate food-odor preference of PA14 to OP50. ***A***, ***B***, The *tax-2(ks10)* and *tax-4(ks28)* loss-of-function mutants are defective in preference of the smell of PA14 to the smell of OP50. ***C***, ***D***, Expressing the *tax-4* genomic DNA rescues the preference defect in the *tax-4(ks28)* mutants and selectively expressing a wild-type cDNA of *tax-4* in the AWB and AWC neurons also rescues the preference defect in the *tax-4(ky791)* mutant animals. ***E–H***, The *cng-1(ok3292)* and *cng-3(jh113)* mutants (***E***, ***F***), as well as the *osm-9(ky10)* mutants (***G***, ***H***) are normal in olfactory preference between OP50 and PA14. ***I–L***, The *tax-2(p691);tax-4(p678)* double mutants (***I***, ***J***) and each of the single mutants (***K***, ***L***) are severely defective in the olfactory preference of PA14 over OP50. In ***J***, error bars are smaller than the size of the circles. For all, the transgenic animals and their nontransgenic siblings, as well as mutants, were compared with the wild-type control N2 measured in parallel, two-tailed Student's *t* test. $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$; no asterisk denotes no statistical difference ($p > 0.05$), $n \geq 3$ assays, mean ± SEM.

neurons, including ASE, AQR, AFD, and BAG. The *tax-2(p694)* mutant is defective in foraging on food, CO_2 avoidance, and thermotaxis, but exhibits normal chemosensory responses to volatile chemicals and dauer-inducing cues (Coburn and Bargmann, 1996; Yook and Hodgkin, 2007; Hallem and Sternberg, 2008; Milward et al., 2011). Intriguingly, we found that the *tax-2(p694)* mutant animals were not only capable of preferring the smell of PA14, but also exhibited a significant increase in this preference (Fig. 3*A*,*B*). This increased preference was fully rescued by a full-length *tax-2* genomic transgene or by the expression of a *tax-2* cDNA in the ASE, AQR, BAG, and AFD sensory neurons (Fig. 3*C–F*), indicating that the TAX-2 channel subunit in all or some of these sensory neurons negatively regulates the preference of PA14 under these conditions. To identify the neurons that suppress the olfactory preference of PA14, we performed cell-specific rescuing experiments in the *tax-2(p694)* mutant animals. We found that expression of a *tax-2* cDNA in either BAG or AQR, using the *flp-17* or *gcy-32* promoter, respectively (Milward et al., 2011), rescued the increased PA14 preference in the *tax-2(p694)* mutants (Fig. 3*G–J*). However, expression of *tax-2* in AFD or ASE did not rescue (Fig. 3*K–N*). Further, selective expression of the *egl-1* cDNA, which induces apoptosis (Conradt and Horvitz, 1998), in the BAG neuron also generated an enhanced preference of the PA14 smell, similar to the phenotype of the *tax-2(p694)* mutant animals (Fig. 3*O*). These results together indicate that the TAX-2-mediated signaling in either BAG or AQR negatively regulates the food-odor preference of PA14. Together, our findings indicate that AWB and AWC sensory neurons are required to generate the olfactory preference of PA14 and that BAG or AQR sensory neurons inhibit the preference. Combinatorial effects of these neurons fine-tune the preference between the smells of different foods.

A G-protein signaling pathway mediates recognition of food odors

After identifying the critical role of AWB and AWC olfactory sensory neurons in generating food-odor preference, we characterized the underlying mechanisms. It has been shown that the activity of TAX-2/TAX-4 channels is regulated by cGMP (Komatsu et al., 1999). Thus, we examined animals with mutations in the Gα subunits of heterotrimeric G-proteins that are expressed

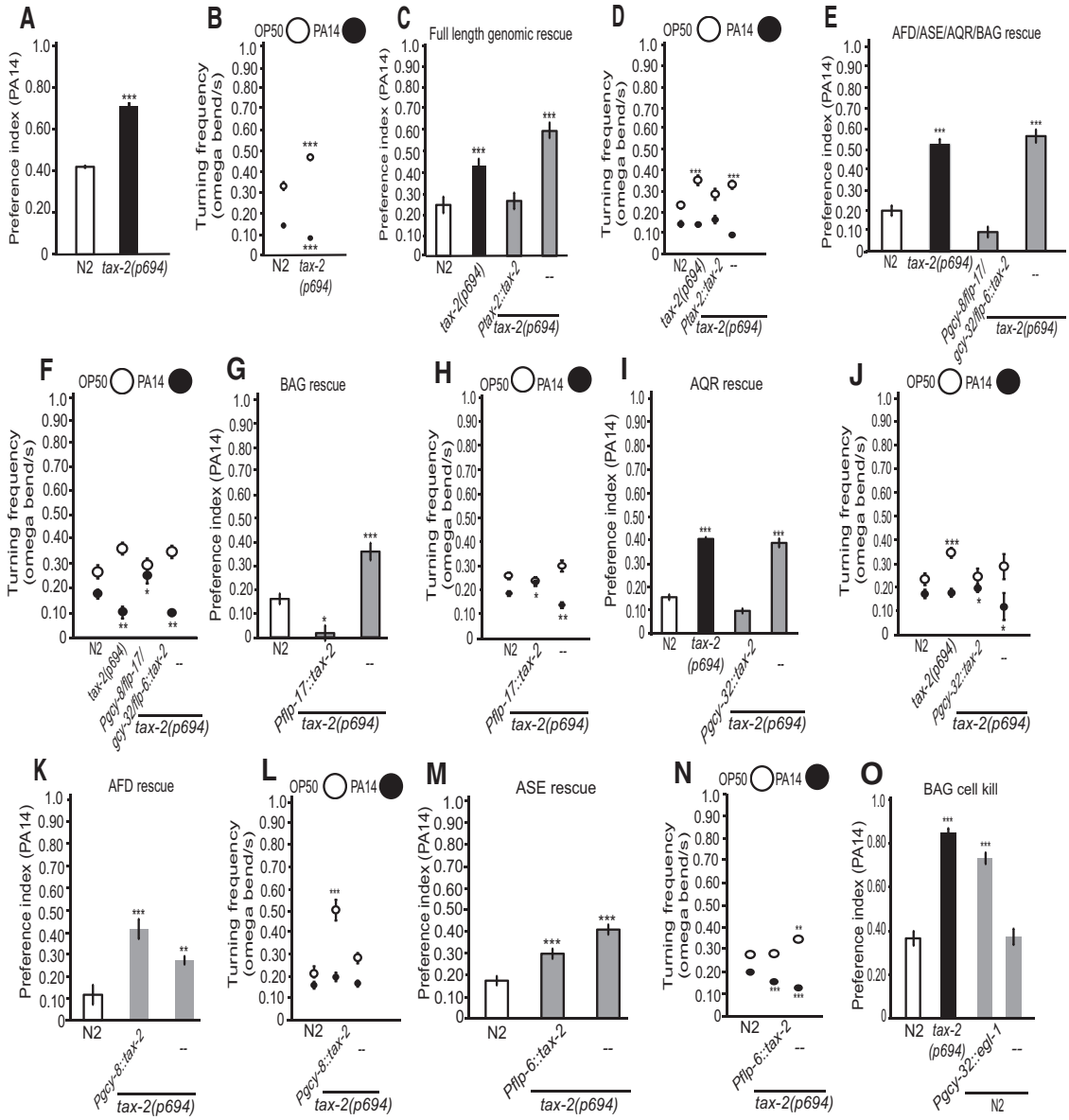

Figure 3. A subset of the *tax-2*-expressing neurons negatively regulates food-odor preference. ***A–D***, The *tax-2(p694)* mutants that have lost the activity of TAX-2 in only a subset of the *tax-2*-expressing sensory neurons (BAG, AQR, AFD, and ASE) exhibit enhanced preference toward the smell of PA14 in comparison with the smell of OP50 (***A***, ***B***) and expressing a full-length *tax-2* genomic transgene rescues the enhanced PA14 preference in *tax-2(p694)* mutants (***C***, ***D***). ***E–N***, Expressing the wild-type *tax-2* cDNA in the ASE (*Pflp-6*), AFD (*Pgcy-8*), BAG (*Pflp-17*), and AQR (*Pgcy-32*) sensory neurons (***E***, ***F***) or in BAG (***G***, ***H***) or AQR (***I***, ***J***) alone rescues enhanced PA14 preference in the *tax-2(p694)* mutants, but expressing the *tax-2* cDNA in either AFD (***K***, ***L***) or ASE (***M***, ***N***) alone does not rescue. In ***N***, error bars are smaller than the size of the circles. ***O***, The animals that express the *egl-1* cDNA in the BAG sensory neuron exhibit enhanced PA14 preference. For all, transgenic and nontransgenic siblings, as well as mutants, were compared with wild-type N2 animals that were examined in parallel, two-tailed Student's *t* test. ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$; no asterisk denotes no statistical difference ($p > 0.05$), $n \geq 4$ assays, mean ± SEM.

in these sensory neurons. AWB sensory neurons strongly express a Gα-protein that is encoded by *odr-3*; AWC expresses many Gα subunits, including *odr-3*, *gpa-2*, *gpa-3*, *gpa-5*, *gpa-6*, and *gpa-13* (Jansen et al., 1999; Lans et al., 2004). We first examined the role of *odr-3*, which is essential for the sensory function of AWC and AWB (Roayaie et al., 1998; Lans et al., 2004; Yoshida et al., 2012). Consistently, two loss-of-function mutants of *odr-3*, *n2150* and *n2046* (Roayaie et al., 1998) were both significantly defective in olfactory preference of PA14 to OP50 (Fig. 4*A*,*B*). We also examined the *odr-3(ky879)* gain-of-function mutant animals, which contained a missense mutation that changed the stability of GTP binding and produced a constitutively active G-protein (Lesch and Bargmann, 2010). Interestingly, the *odr-3(ky879)* mutant animals also exhibited a severely defective ability to prefer the smell of PA14 to the smell of OP50 (Fig. 4*A*,*B*), suggesting that appropriate amount of G-protein signaling is essential to generate odor preference of PA14. To further evaluate this possibility, we examined animals that lacked negative regulators of neuronal G-protein signaling, including *eat-16* and *rgs-3* (Hajdu-Cronin et al., 1999; Ferkey et al., 2007). Interestingly, the *eat-16(sa609)* mutants were severely defective for PA14 preference (Fig. 4*I*,*J*), whereas the *rgs-3(ok2288)* mutants exhibited wild-type olfactory preference (Fig. 4*K*,*L*). These results further characterized the potential G-protein signaling pathway involved in sensory transduction of food odors. In contrast, mutations in *gpa-3* and *gpa-13* (Lans et al., 2004; Burghoorn et al., 2010) did not seem to alter olfactory preference between PA14 and OP50 because both loss-of-function mutants *gpa-3(pk35)* and *gpa-13(pk1270)* (Zwaal et

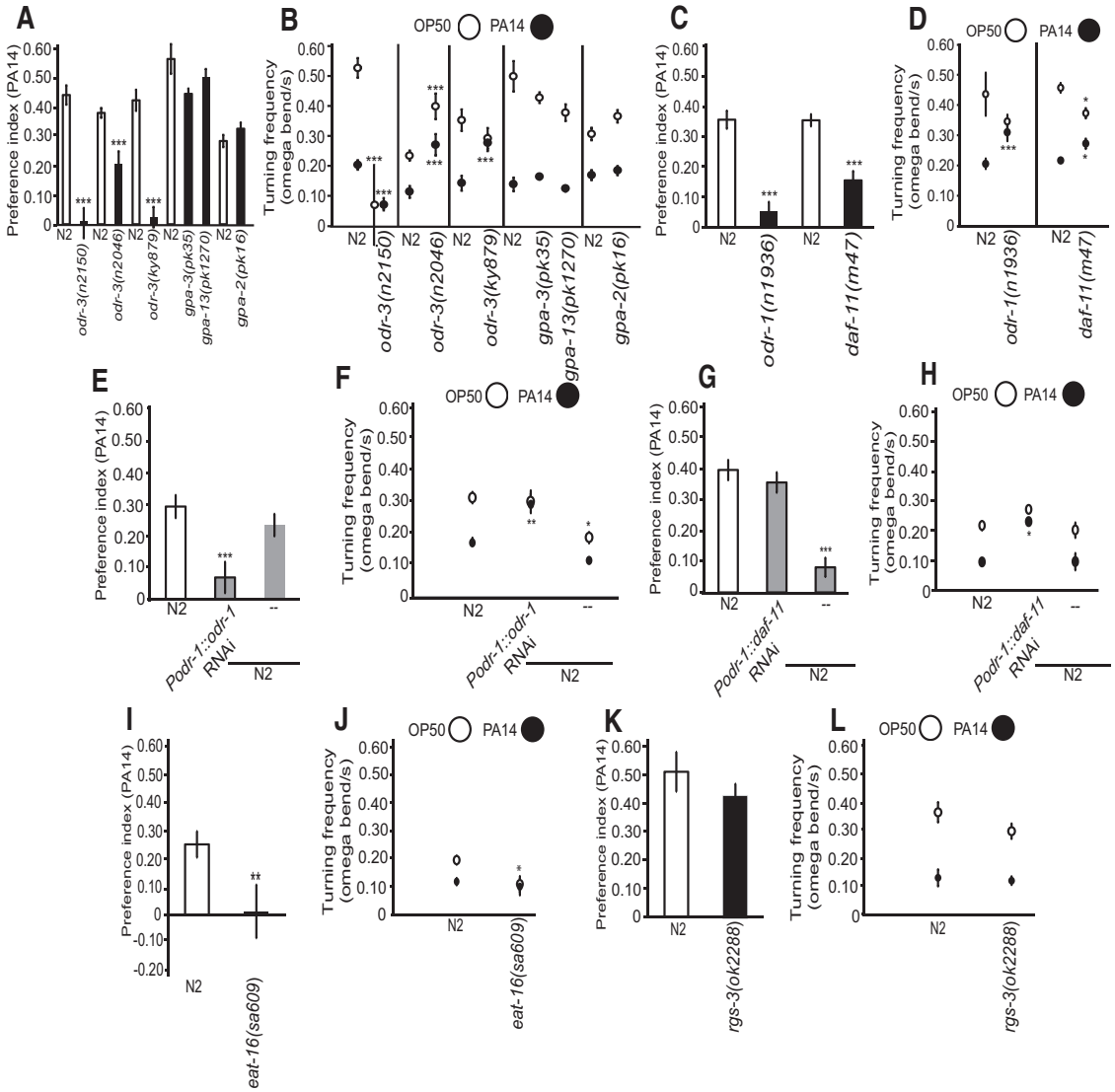

Figure 4. A G-protein signaling pathway regulates food-odor recognition. ***A***, ***B***, Two different mutations in the Gα-encoding gene *odr-3* disrupt olfactory preference of PA14 to OP50, but mutating several other Gα-encoding genes does not alter olfactory preference for PA14. ***C***, ***D***, Mutations in two genes that encode guanylyl cyclases *odr-1* and *daf-11* disrupt the food-odor preference between PA14 and OP50. ***E–H***, Selective knockdown of the *odr-1* activity or the *daf-11* activity in the AWB and AWC sensory neurons in wild-type animals abolishes the preference of PA14 smell to OP50 smell. ***I–L***, Animals containing null mutations in *eat-16* are defective in the olfactory preference of PA14 in comparison with OP50, but deleting *rgs-3* does not alter PA14 preference. For all, transgenic animals and their nontransgenic siblings, as well as mutants, were compared with the wild-type N2 tested in parallel, two-tailed Student's *t* test. ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$; no asterisk denotes no statistical difference ($p > 0.05$), $n \geq 3$ assays, mean ± SEM.

al., 1997; Jansen et al., 1999) generated a wild-type odor preference between PA14 and OP50 (Fig. 4*A*,*B*). In addition, the *gpa-2*-encoded Gα-protein that negatively regulates AWC signaling (Lans et al., 2004) was also not required because the loss-of-function *gpa-2(pk16)* mutants exhibited wild-type PA14 preference (Fig. 4*A*,*B*). Together, these results indicate that the ODR-3-mediated G-protein signaling is required to generate the olfactory preference of PA14 over OP50.

Next, we examined the role of guanylate cyclases. There are 34 guanylate cyclases in the *C. elegans* genome with either distinct or overlapping expression patterns in the nervous system (Yu et al., 1997). We examined two loss-of-function mutations, *odr-1(n1936)* and *daf-11(m47)*. While *odr-1* encodes a receptor guanylate cyclase and *daf-11* encodes a cytoplasmic guanylate cyclase, both of them are expressed in the cilia of AWC and AWB to mediate chemotaxis (Vowels and Thomas, 1992; Schackwitz et al., 1996; Coburn et al., 1998; Bernhard and van der Kooy, 2000; Birnby et al., 2000; L'Etoile and Bargmann, 2000; Murakami et al., 2001; Torayama et al., 2007; Mukhopadhyay et al., 2008; Liu et al., 2010). We found that both the *odr-1(n1936)* and *daf-11(m47)* mutant animals exhibited a defective preference between the PA14 smell and the OP50 smell (Fig. 4*C*,*D*). Consistently, knocking down the function of either *odr-1* or *daf-11* specifically in AWB and AWC generated similar defects in food-odor preference (*Podr-1::odr-1RNAi*, *Podr-1::daf-11RNAi*; Fig. 4*E–H*). Together, our analysis demonstrates that the ODR-1 and DAF-11 guanylate cyclases act together with the Gα-protein ODR-3 and the cGMP-gated TAX-2/TAX-4 channels in the AWB and AWC sensory neurons to produce food-odor preference.

Because recognizing food odor is a prerequisite of food-odor preference, we assessed the precise role of the G-protein signaling by analyzing the ability of the mutants in distinguishing food odors versus nonfood odors. Using the microdroplet assay, we found that wild-type *C. elegans* clearly distinguishes the smell of

Table 1. Preference indexes[a]

Genotype	NGMvOP50	NGMvPA14	OP50vPA14
Pair 1			
N2	0.309 (±0.046)	0.334 (±0.032)	0.441 (±0.030)
odr-3(n2150)	0.045 (±0.027)***	0.094 (±0.041)***	0.001 (±0.047)***
Pair 2			
N2	0.343 (±0.046)	0.210 (±0.069)	0.423 (±0.046)
odr-3(ky879)	0.088 (±0.045)***	0.008 (±0.027)***	0.024 (±0.045)***
Pair 3			
N2	0.372 (±0.044)	0.322 (±0.024)	0.355 (±0.020)
daf-11(m47)	−0.051 (±0.031)***	0.184 (±0.028)***	0.156 (±0.031)***
Pair 4			
N2	0.467 (±0.030)	0.340 (±0.030)	0.357 (±0.030)
odr-1(n1936)	0.039 (±0.029)***	0.034 (±0.015)***	0.053 (±0.032)***
Pair 5			
N2	0.347 (±0.041)	0.485 (±0.053)	0.414 (±0.034)
tax-4(ks28)	0.012 (±0.034)***	0.118 (±0.015)***	0.081 (±0.040)***
Pair 6			
N2	0.342 (±0.046)	0.392 (±0.038)	0.30 (±0.020)
eat-4(ky5)	0.115 (±0.027)***	0.101 (±0.036)***	0.132 (±0.020)***
Pair 7			
N2	0.377 (±0.037)	0.497 (±0.022)	0.319 (±0.036)
egl-3(n150)	0.353 (±0.034) NS	0.594 (±0.020) NS	0.169 (±0.040)***
Pair 8			
N2	0.281 (±0.042)	0.339 (±0.039)	0.476 (±0.030)
nlp-1(ok1470)	0.306 (±0.039) NS	0.335 (±0.040) NS	0.239 (±0.043)***
Pair 9			
N2	0.178 (±0.043)	0.182 (±0.031)	0.354 (±0.030)
nlp-9(tm3572)	0.164 (±0.027) NS	0.185 (±0.024) NS	0.181 (±0.033)***
Pair 10			
N2	0.536 (±0.070)	0.335 (±0.030)	0.397 (±0.029)
npr-18(ok1388)	0.644 (±0.052) NS	0.290 (±0.037) NS	0.152 (±0.036)***

[a]The results in the column NGMvOP50 represent the olfactory preference of OP50 over NGM buffer; the results in the column NGMvPA14 represent the olfactory preference of PA14 over NGM buffer; and the results in the column OP50vPA14 represent the olfactory preference of PA14 over OP50. All mutants were compared with N2 tested in parallel, two-tailed Student's *t* test. ***$p \leq 0.001$ represents statistical difference from N2. NS, No statistical difference ($p > 0.05$) from N2. $n \geq 3$ assays, mean (±SEM).

bacteria from the smell of NGM medium and displays strong preference toward the smell of bacteria, whether it is generated by OP50 or PA14 (Table 1; see Materials and Methods). However, mutations in genes encoding the G-protein signaling components, including the Gα subunit ODR-3, the guanylate cyclases ODR-1 and DAF-11, and the cGMP-gated channel subunit TAX-4, severely disrupt the differential olfactory response toward the bacterial smells versus the smell of NGM medium, indicating that the G-protein signaling regulates food-odor preference by enabling recognition of the odors.

The glutamatergic transmission from AWC regulates food-odor recognition

After identifying the G-protein signaling that acts in AWB and AWC neurons to regulate food-odor recognition, we next sought the neurotransmission used by these neurons in mediating the olfactory response. Neurotransmission has been characterized in only a few *C. elegans* sensory neurons. For example, the polymodal ASH sensory neuron primarily uses glutamate to regulate aversive responses to noxious stimuli. Similarly, the olfactory neuron AWC signals via glutamatergic transmission to regulate sensorimotor responses to several attractants and food availability (Hart et al., 1999; Mellem et al., 2002; Chalasani et al., 2007, 2010; Harris et al., 2010). Because the glutamate vesicular transporter EAT-4 is expressed in both AWB and AWC (Lee et al., 1999; Chalasani et al., 2007; Ohnishi et al., 2011), we first examined the olfactory preference between OP50 and PA14 in a putative null mutant *eat-4(ky5)* (Lee et al., 1999). We found that the *eat-4(ky5)* mutant animals displayed a significantly lower preference toward PA14 and the defect was rescued by a wild-type *eat-4* genomic DNA fragment (Fig. 5*A–D*). Thus, glutamatergic neurotransmission is needed to generate food-odor preference of PA14 to OP50.

Next, to specify the role of the glutamatergic transmission in AWB and AWC neurons, we tested the rescuing effect of cell-specific expression of *eat-4*. We found that expressing a wild-type *eat-4* cDNA in both AWB and AWC using the *odr-1* promoter, which is selectively expressed in these neurons (L'Etoile et al., 2000), fully rescued the defect of odor preference in the *eat-4(ky5)* mutant animals (Fig. 5*E*,*F*). However, expressing *eat-4* in AWB alone with the *str-1* promoter did not rescue (Fig. 5*E*,*F*). In addition, knocking down the function of *eat-4* in AWC and ASI, but not AWB, by expressing *eat-4* RNAi using the *nlp-1* promoter (Nathoo et al., 2001; Chalasani et al., 2010; Mills et al., 2012) produced a phenotype similar to the defect in the *eat-4(ky5)* mutant animals (Fig. 5*G*,*H*). Together, these results indicate that the glutamate neurotransmission from AWC sensory neurons is needed to generate the odor preference of PA14 to OP50. Furthermore, the *eat-4(ky5)* animals exhibited significant defects in distinguishing food odors generated by OP50 or PA14 from non-food odors, i.e., the smell of NGM medium (Table 1). These results indicate that the glutamatergic signaling from AWC acts downstream of the G-protein signaling to regulate recognition of food odors, which functions as a prerequisite of odor preference.

Peptidergic signals regulate preference between different food odors

Next, we probed the possibility that AWB employs neuropeptidergic signaling to regulate the food-odor preference of PA14 to OP50. We first examined the phenotypes generated by loss-of-function mutations in *egl-3* and *egl-21*, both of which encode broadly expressed neuropeptide-processing enzymes (Kass et al., 2001; Nathoo et al., 2001; Jacob and Kaplan, 2003). We found that the *egl-3(n150)* mutant animals were significantly defective in the olfactory preference for PA14 in comparison with OP50 (Fig. 6*A*,*B*). Consistently, two different loss-of-function mutations, *egl-21(n476)* and *egl-21(n611)*, strongly reduced the olfactory preference of PA14 to OP50 [Fig. 6*A*,*B*; PA14 preference index: N2, 0.386 ± 0.025; *egl-21(n476)*, 0.228 ± 0.036 ($p \leq 0.001$); turning rate to OP50 (per second): N2, 0.481 ± 0.026; *egl-21(n476)*, 0.212 ± 0.027 ($p \leq 0.01$); turning rate to PA14 (per second): N2, 0.213 ± 0.017; *egl-21(n476)*, 0.133 ± 0.018 ($p > 0.05$); mean ± SE, Student's *t* test, $n > 3$ assays]. Interestingly, *egl-3(n150)* mutants are normal in distinguishing food odors generated from either OP50 or PA14 from nonfood odors (Table 1). Together, these results indicate an essential role of neuropeptidergic signals in generating the food-odor preference of PA14 to OP50, but not in recognizing the food odors. To further implicate the neuropeptide signaling in AWB, we generated cell-specific RNAi of *egl-3* in AWB alone and in both AWB and AWC (*Pstr-1::egl-3RNAi, Podr-1::egl-3RNAi*; Esposito et al., 2007; Mills et al., 2012). These transgenes significantly reduced the preference toward the smell of PA14 in comparison with the smell of OP50 (Fig. 6*C–F*), indicating that neuropeptides transmit the signaling output of AWB to mediate the food-odor preference.

To determine the neuropeptide(s) responsible for AWB output, we examined the effect of deletion mutations in two neuropeptide-encoding genes, *nlp-3(ok2688)* and *nlp-9(tm3572)* (Harris et al., 2010; Mills et al., 2012), because both *nlp-3* and *nlp-9* are expressed in AWB and have been previously demonstrated to modulate aversive responses to noxious stimuli (Nathoo et al., 2001; Harris et al., 2010; Mills et al., 2012). We found

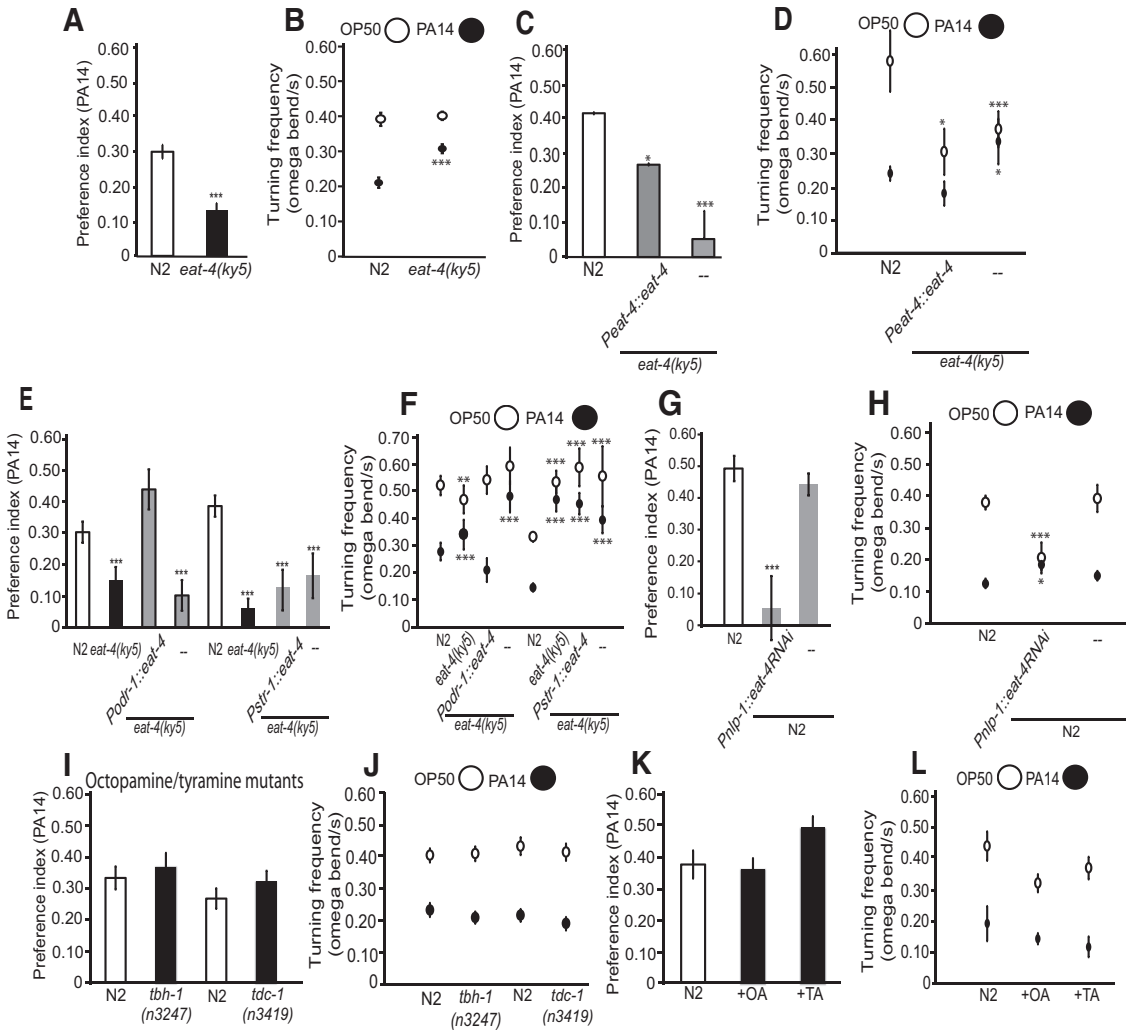

Figure 5. The AWC sensory neuron regulates food-odor recognition through glutamatergic neurotransmission. ***A–F***, The loss of EAT-4 activity in the *eat-4(ky5)* mutants results in a loss of olfactory preference of PA14 to OP50 (***A***, ***B***) and this defect is rescued by the expression of a genomic DNA of *eat-4* (***C***, ***D***) or selective expression of a wild-type *eat-4* cDNA in the AWB and AWC sensory neurons (***E***, ***F***, *Podr-1::eat-4*), but expressing the wild-type *eat-4* cDNA in AWB alone does not rescue (***E***, ***F***, *Pstr-1::eat-4*). ***G, H,*** Selective knockdown of *eat-4* activity in AWC (*Pnlp-1::eat-4RNAi*) abolishes olfactory preference of PA14 to OP50. ***I–L***, Animals lacking *tdc-1* or *tbh-1* are wild-type in the olfactory preference of PA14 in comparison with OP50 (***I***, ***J***), and application of exogenous tyramine or octopamine does not change the preference of PA14 over OP50 (***K***, ***L***). For all, transgenic animals and their nontransgenic siblings, as well as mutants, were compared with the wild-type N2 animals tested in parallel, two-tailed Student's *t* test. ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$; no asterisk denotes no statistical difference ($p > 0.05$), $n \geq 3$ assays, mean ± SEM.

that while *nlp-3(ok2688)* mutants displayed a wild-type preference for the smell of PA14 (data not shown), the *nlp-9(tm3572)* mutants were significantly defective (Fig. 6*G*,*H*). Consistent with the possibility that NLP-9 regulates preference of PA14 odors, the *nlp-9(tm3572)* mutants exhibited normal differential responses between food odors and nonfood odors (Table 1). NLP-9 is expressed in AWB neurons, in ASI neurons, in another four head neurons, in one tail neuron, and in non-neuronal cells (Nathoo et al., 2001; Fox et al., 2005). We found that reducing *nlp-9* activity in AWB neurons by expressing cell-specific RNAi (*Pstr-1::nlp-9RNAi*; Mills et al., 2012) decreased the olfactory preference of PA14 over OP50, suggesting that NLP-9 produced from AWB regulates PA14 preference (Fig. 6*I*,*J*). In addition, several other neuropeptide mutants, *nlp-5*, *nlp-8*, *flp-6*, *nlp-7*, and *flp-21* (Nathoo et al., 2001; Kim and Li, 2004; Bendena et al., 2008), were not defective in odor preference between OP50 and PA14. Together, these results indicate that the NLP-9-mediated neuropeptidergic signal regulates food-odor preference of PA14 over OP50.

In addition, by analyzing animals lacking the neuropeptide NLP-1, which was previously implicated in AWC-mediated olfactory response to attractants (Chalasani et al., 2010), we addressed whether neuropeptide transmission from AWC also regulates PA14 preference. We found that the putative loss-of-function *nlp-1(ok1470)* mutant animals were defective in PA14 preference (Fig. 6*K*,*L*). Similar to the *nlp-9(tm3572)* mutants, the *nlp-1(ok1470)* mutants displayed a wild-type response in distinguishing food odors from nonfood odors (Table 1). In addition, we found that expressing the wild-type NLP-1 activity using the *odr-3* promoter that was primarily expressed in AWC fully rescued the defect of *nlp-1(ok1470)* mutants in generating food-odor preference of PA14 (Fig. 6*M*,*N*). Together, these results reveal that NLP-1 produced by AWC mediates food-odor preference between PA14 and OP50, whereas the glutamatergic signal from AWC regulates recognition of food odors.

Furthermore, we examined additional neurotransmitters that may play a role in olfactory preference between OP50 and PA14. First, we tested the *tdc-1(n3419)* mutants, which harbor a deletion mutation in the tyrosine decarboxylase TDC-1 needed for the biosynthesis of tyramine and octopamine, as well as the *tbh-1(n3247)* mutants, which have a deletion mutation in the gene

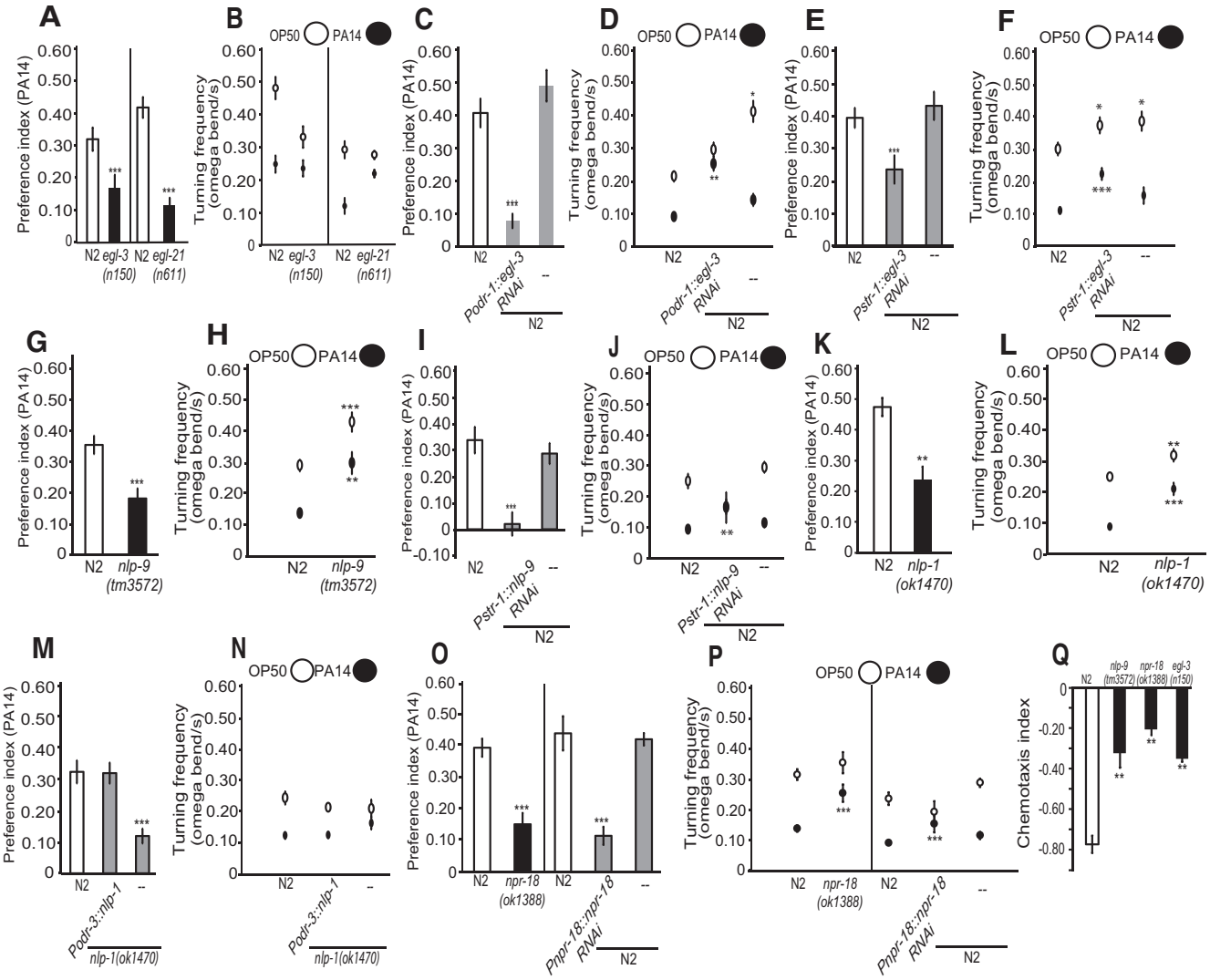

Figure 6. The AWB sensory neuron mediates PA14 odor preference via neuropeptidergic signaling. ***A***, ***B***, Mutations in genes that encode peptide-processing enzymes, *egl-3* and *egl-21*, disrupt food-odor preference of PA14 to OP50. ***C–F***, Selective knockdown of *egl-3* activity in AWB and AWC (***C***, ***D***, *Podr-1::egl-3RNAi*) or in AWB alone (***E***, ***F***, *Pstr-1::egl-3RNAi*) significantly reduces PA14 preference in wild-type animals. ***G***, ***H***, Deleting the AWB-expressing neuropeptide-encoding gene *nlp-9* significantly reduces the olfactory preference of PA14 to OP50. ***I***, ***J***, Selective knockdown of the *nlp-9* activity in AWB alone reduces the preference of the PA14 smell to OP50 smell. ***K***, ***L***, Deleting the AWC-expressing neuropeptide-encoding gene *nlp-1* significantly reduces the food-odor preference of PA14 to OP50. ***M***, ***N***, Expressing the wild-type *nlp-1* activity in AWC olfactory sensory neuron rescues the defect of the olfactory preference of PA14 in comparison with OP50 in *nlp-1(ok1470)* mutants. ***O***, ***P***, Loss of *npr-18* or knocking down *npr-18* activity decreases the food-odor preference of PA14 over OP50. ***Q***, Mutations in *nlp-9, npr-18*, and *egl-3* significantly disrupt the aversive response to the repulsive odorant 2-nonanone, a response mediated by AWB sensory neurons. For all, transgenic animals and their nontransgenic siblings, as well as mutants, were compared with the wild-type N2 animals tested in parallel, two-tailed Student's *t* test. ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$; no asterisk denotes no statistical difference ($p > 0.05$), $n \leq 3$ assays (***A–P***) or $n \geq 2$ separate days (***Q***), mean ± SEM.

encoding the tyramine β-hydroxylase needed for the biosynthesis of octopamine (Alkema et al., 2005; Wragg et al., 2007). Interestingly, both the *tdc-1(n3419)* and *tbh-1(n3247)* mutants and the wild-type animals preincubated with 4 mM exogenous octopamine or tyramine (see Materials and Methods) showed wild-type olfactory preference between OP50 and PA14 (Fig. 5*I–L*). Dopamine and serotonin have been previously examined in PA14 preference (Ha et al., 2010). While serotonin plays a significant role in learned olfactory avoidance of PA14, it is not required for PA14 preference under the naive condition. The *cat-2* mutants, which lack the dopamine synthesis enzyme, also did not affect PA14 preference (Ha et al., 2010). Together, our findings reveal the specific roles of glutamate neurotransmission and neuropeptide signaling in food-odor recognition and preference, respectively.

Multiple glutamate receptors mediate olfactory preference for food odors

Having identified glutamate as a neurotransmitter of AWC that regulates recognition of food odors generated by OP50 and PA14, we next sought the downstream signaling. We examined the olfactory preference between OP50 and PA14 in the loss-of-function mutants of a series of AMPA/kainate-like glutamate receptor subunits, NMDA-like receptor subunits, or glutamate-gated chloride channel subunits (*glr-1*, *glr-2*, *nmr-1*, *glc-2, glc-3*, *glc-4*, *avr-14*, and *avr-15*). However, none of these single mutants exhibited any significant defect (Fig. 7*A*,*B*). Interestingly, the double mutants *glr-1(ky176); glr-2(ak10)* and *glr-1(ky176); nmr-1(ak4)* (Mellem et al., 2002) were severely defective in generating the olfactory preference of PA14 in comparison with OP50 (Fig. 7*C*,*D*). In contrast, the double mutant *glr-1(n2461); glc-3(ok321)*

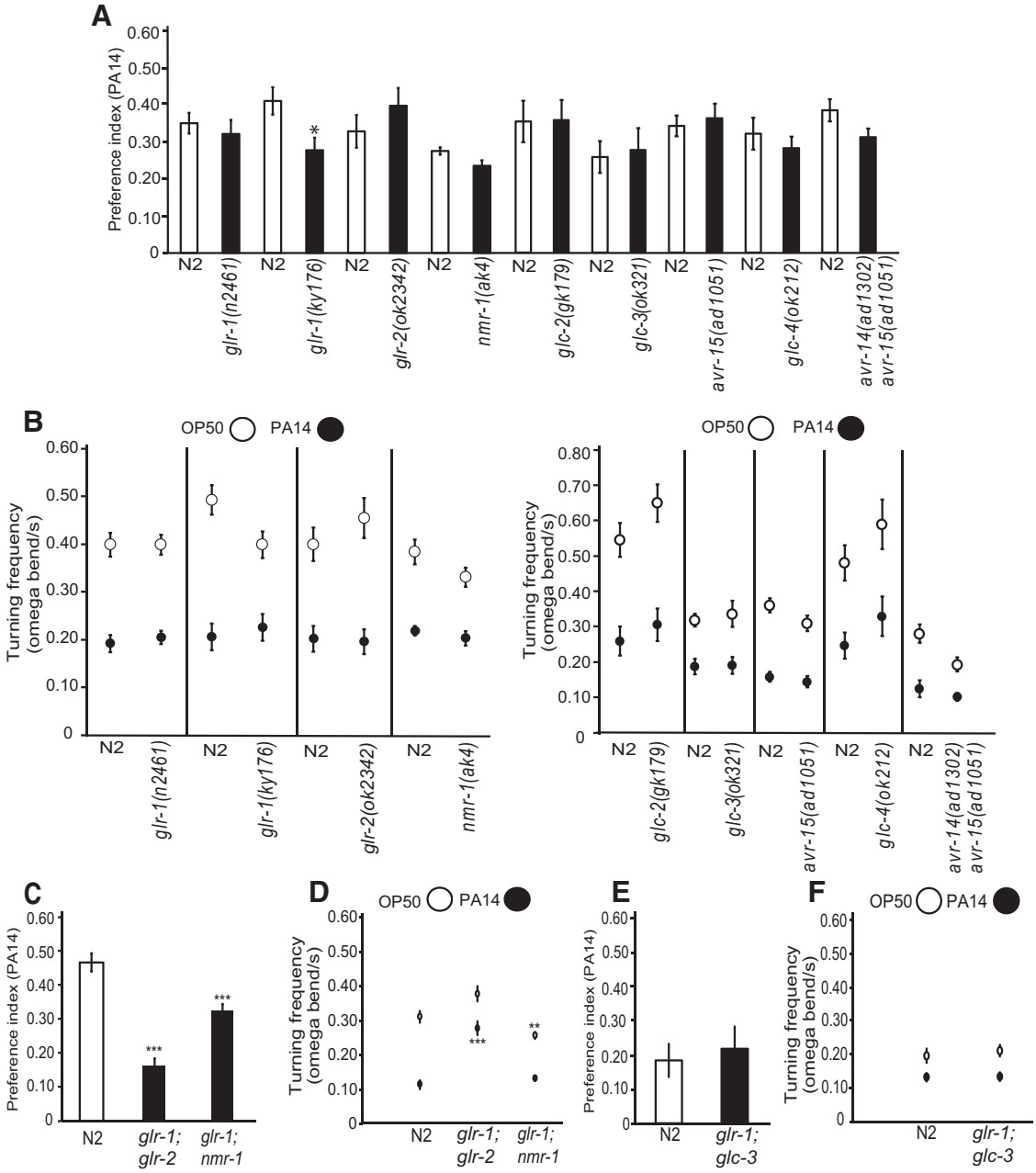

Figure 7. Combinatorial effects of multiple glutamate receptors on food-odor response to PA14 and OP50. ***A***, ***B***, Single mutations in a series of genes that encode glutamate receptors do not alter food-odor preference for PA14. ***C***, ***D***, The double mutants *glr-1;glr-2* and *glr-1;nmr-1* reduce PA14 preference. ***E***, ***F***, The double mutant *glr-1;glc-3* exhibits wild-type PA14 preference. For all, transgenic animals and their nontransgenic siblings, as well as mutants, were compared with wild-type N2 animals tested in parallel, two-tailed Student's *t* test. $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$; no asterisk denotes no statistical difference ($p > 0.05$), $n \leq 3$ assays, mean ± SEM.

(Chalasani et al., 2007) displayed normal olfactory preference of PA14 to OP50, suggesting that specific disruption of glutamatergic transmission in *glr-1(ky176); glr-2(ak10)* or *glr-1(ky176); nmr-1(ak4)* mutants disrupts the food-odor preference and the general reduction in the glutamate signaling cannot account for the defects in these receptor mutants. These results suggest that glutamatergic signaling mediates the food-odor response via multiple glutamate receptor subunits.

Neuropeptide receptor NPR-18 mediates food-odor preference

Having identified NLP-9 as the peptidergic output of AWB sensory neuron in mediating food-odor preference between OP50 and PA14 bacteria strains, we next sought the downstream neuropeptide receptor. We first examined the effect of a putative loss-of-function mutation in *npr-18*, which has been shown to act as a potential receptor for NLP-9 (Mills et al., 2012). We found that the *npr-18(ok1388)* mutants were defective in generating the preference of the smell of PA14 in comparison with the smell of OP50 (Fig. 6*O*,*P*). Consistently, wild-type animals that expressed an *npr-18*-RNAi with the *npr-18* promoter (Esposito et al., 2007; Mills et al., 2012) were also defective for PA14 preference (*Pnpr-18::npr-18RNAi*; Fig. 6*O*,*P*). In contrast, mutations in other neuropeptide receptor-encoding genes known to be expressed in sensory neurons, such as *npr-9(tm1652)* and *npr-19(ok2068)*, showed wild-type preference for food odors (data

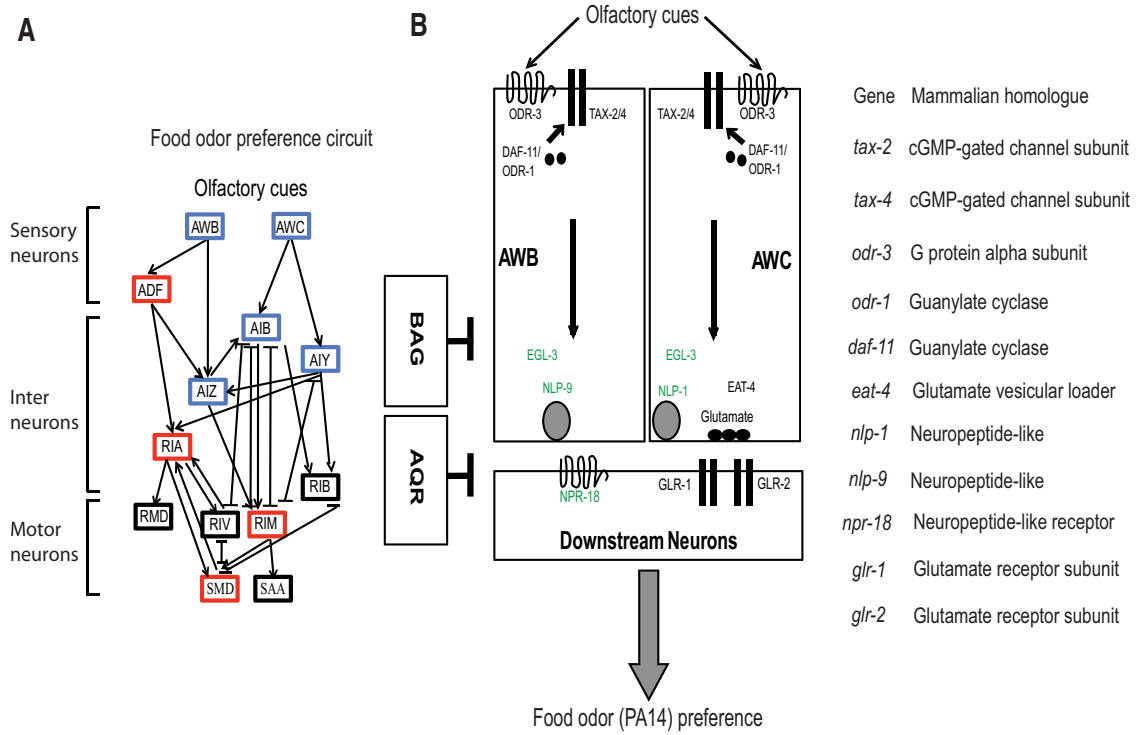

Figure 8. The signaling pathways underlying the olfactory preference of PA14 to OP50. ***A***, Diagram represents the neuronal network for naive and learned olfactory preference for PA14 over OP50 (Ha et al., 2010). Anatomically, the network consists of the major olfactory sensory neurons AWB and AWC, as well as the downstream interneurons and motor neurons that mediate head bending. Functionally, our analysis has shown that neurons highlighted in blue regulate olfactory preference of PA14 in naive animals (i.e., animals cultivated with OP50 as food) and neurons highlighted in red mediate learned preference of PA14 after training with PA14. Arrows and lines denote chemical and electrical synapses, respectively. ***B***, The AWC/AWB signal transduction and neurotransmission pathways that regulate the recognition and preference of the odors generated by *E. coli* OP50 and *P. aeruginosa* PA14. *C. elegans* genes implicated in food-odor recognition and preference and their mammalian homologues are shown on the right. In the schematic, the genes in black font are required for food-odor recognition and the genes in red font are specifically required for only food-odor preference.

not shown), supporting the specific effect of *npr-18(ok1388)*. Consistently, the *npr-18(ok1388)* mutants distinguished food odors from nonfood odors similarly to wild-type animals (Table 1). In addition, both *nlp-9(tm3572)* and *npr-18(ok1388)* mutants are defective in avoiding 2-nonanone, an aversive response mediated by AWB (Troemel et al., 1997), further demonstrating the role of NLP-9 and NPR-18 in AWB-mediated sensorimotor responses (Fig. 6*Q*). Together, these results show that AWB mediates food-odor preference through the NLP-9 peptide signal and the NPR-18 receptor.

Discussion

Many animals use olfactory cues to locate preferred food sources. While the conserved signaling pathway underlying olfactory sensorimotor response is well characterized, how the signaling pathways process more naturalistic olfactory cues, such as those generated by foods, has not been systematically examined. Here, we combine quantitative behavior analysis with genetic approaches to characterize the signaling molecules and neurotransmitters that regulate recognition and preference of food odors generated by two different bacteria strains, *E. coli* OP50 and *P. aeruginosa* PA14, in *C. elegans*. Our results elucidate the molecular and cellular attributes that allow the nervous system to generate behavioral preference among different food odors (Fig. 8).

Olfactory preference for food odors requires a combination of olfactory sensory neurons

Odorants may stimulate or inhibit different sensory neurons to generate appropriate behavioral responses, which depend on the nature, concentration, and, sometimes, context of the stimuli (de Bruyne et al., 1999; Vogler and Schild, 1999; Kuebler et al., 2011; Yoshida et al., 2012). Previously, we demonstrated that AWB and AWC neurons are required for the preference of the smell of *P. aeruginosa* PA14 in comparison with the smell of *E. coli* OP50 (Ha et al., 2010). Here, we show that AWB and AWC sensory neurons facilitate this preference by mediating the recognition of food odors through the cGMP-gated TAX-2/TAX-4 channel and Gα subunit ODR-3, which are known to regulate sensory function of both neurons. Previously, it has been shown that AWC regulates the rate of reversals and turns in response to food availability (Bargmann et al., 1993; Wakabayashi et al., 2004; Gray et al., 2005; Chalasani et al., 2007, 2010; Ha et al., 2010; Yoshida et al., 2012) and AWB regulates avoidance of pathogenic bacterial lawns (Troemel et al., 1997; Pradel et al., 2007; Ha et al., 2010). Together, these results suggest that AWB and AWC sense complex bacterial odors to coordinate behavioral responses. Intriguingly, we have identified a negative role of the sensory neurons BAG and AQR in generating olfactory preference of PA14 over OP50, which may regulate the sensory neurons AWB and AWC and/or downstream circuit to produce the preference (Fig. 8). These results reveal a mechanism by which a network of olfactory neurons processes olfactory inputs to generate preference.

A Gα signaling pathway regulates food-odor recognition

Previous studies in both invertebrates and vertebrates have characterized the molecular mechanisms that mediate sensory response to olfactory stimuli. However, the function of these signaling molecules for detecting and distinguishing complex ol-

factory cues, such as food odors, is not clear (Root et al., 2008, 2011; Ignell et al., 2009; Martin and Hildebrand, 2010; Das et al., 2011). In this study, we characterize the role of transduction molecules within the AWB and AWC sensory neurons in generating behavioral response to the smells of two worm foods, PA14 and OP50.

The *C. elegans* ODR-3 Gα subunit, the DAF-11 and ODR-1 guanylate cyclases, and the TAX-2/TAX-4 cGMP-gated channels are part of the primary machinery that regulates olfactory responses to attractive volatiles, such as benzaldehyde and isoamyl alcohol, which are detected by the AWC neurons. These molecules are also required for AWB-mediated aversive responses to a volatile repellent, 2-nonanone, and to a bacterially produced surfactant, the serrawettin W2 (Coburn and Bargmann, 1996; Coburn et al., 1998; Roayaie et al., 1998; Birnby et al., 2000; L'Etoile and Bargmann, 2000; Pradel et al., 2007). Our results show that these same signaling transduction molecules in AWB and AWC are required for the olfactory preference of PA14 over OP50 by mediating the recognition of the food odors. Disrupting the signaling components in this pathway not only disrupts preference between different food odors, but also disrupts the ability to distinguish food odors from nonfood odors. Particularly, we propose that the ODR-3 Gα subunit is the primary olfactory Gα subunit protein in this food-odor response. These results reveal the function of a G-protein-dependent cGMP-signaling pathway in regulating behavioral response to complex food odors.

Glutamatergic neurotransmission mediates food-odor recognition

In *C. elegans*, glutamate is the primary synaptic neurotransmitter for various chemotactic responses that are mediated by sensory neurons, including AWC and ASH (Hart et al., 1995; Mellem et al., 2002; Chalasani et al., 2007, 2010). Here, we show that the EAT-4-dependent glutamatergic transmission from the AWC sensory neuron regulates recognition of food odors, a requirement for the generation of food-odor preference. In crawling animals, the glutamatergic neurotransmission from AWC suppresses reversals in response to attractive odors (Chalasani et al., 2007). Here, we show that AWC-glutamate signal also plays a critical role in suppressing turning rate in swimming animals in response to the preferred PA14 smell, suggesting a general role for EAT-4-dependent glutamate signaling in regulating attractive sensorimotor responses. Further, we have shown that two AMPA receptor-like glutamate receptors, GLR-1 and GLR-2, are required to regulate food-odor preference between OP50 and PA14. Whereas *glr-1* has been implicated in many sensory-evoked behaviors, *glr-2* appears to play a role in only a few (Hart et al., 1995, 1999; Zheng et al., 1999; Brockie et al., 2001; Mellem et al., 2002; Chao et al., 2004; Chalasani et al., 2007). Combination of *glr-1* and *glr-2* regulates dopamine-dependent increase in turning rate acutely induced by removal of food (Hills et al., 2004; Gray et al., 2005). Here, we show that *glr-1* and *glr-2* play redundant roles in generating food-odor preference of PA14. While removing the function of either *glr-1* or *glr-2* does not alter the food-odor preference, losing both receptors abolishes the response. Further, similar to the effect of removing *eat-4*, combining mutations in *glr-1* and *glr-2* generates defects in suppressing turns in response to PA14 (Fig. 7*C*,*D*), consistent with the possibility that GLR-1 and GLR-2 act downstream of the EAT-4-dependent signaling in generating PA14 preference. The expression patterns of *glr-1* and *glr-2* partially overlap (Brockie et al., 2001; Hills et al., 2004), suggesting that GLR-1 and GLR-2 may act independently of each other to provide functional redundancy during integration of food odors.

Glutamate acts as a primary transmitter in olfactory systems to convey sensory information to downstream projecting neurons; and neuropeptides often modulate olfactory sensorimotor response by regulating sensitivity, acuity, and plasticity (Chalasani et al., 2007, 2010; Ignell et al., 2009; Harris et al., 2010; Root et al., 2011). In rodents, glutamatergic signaling conveys odor information to mitral cells, tufted cells, and local inhibitory neurons (Berkowicz et al., 1994; Ennis et al., 1996, 2006). In fruit flies, glutamate acts as an inhibitory neurotransmitter to coordinate antennal lobe responses (Liu and Wilson, 2013). In zebrafish, glutamate acts as an excitatory neurotransmitter to transmit olfactory signaling to the olfactory bulb (Edwards and Michel, 2002). Here, we show that glutamate plays a critical role in distinguishing complex food odors from nonfood odors, expanding the signaling function of glutamate in regulating olfactory sensorimotor responses.

Neuropeptides mediate food-odor preference, but not recognition

Neuropeptides have been implicated in olfactory behavior and plasticity in both invertebrates and vertebrates. For example, in rats, insulin and leptin modulate spontaneous and odorant-evoked activity of olfactory sensory neurons (Savigner et al., 2009). In *Ambystoma mexicanum*, neuropeptide Y regulates the activity of the olfactory epithelium in response to hunger (Mousley et al., 2006). In *Drosophila melanogaster*, neuropeptide F and insulin regulate nutrition-dependent effects on avoidance of noxious foods and olfaction-driven food searching (Wu et al., 2005; Root et al., 2011). Tackykinin-related neuropeptides mediate presynaptic inhibition of olfactory sensory neurons during odor-evoked responses (Winther et al., 2006; Ignell et al., 2009). Similarly, in *C. elegans*, neuropeptides are critical modulators of olfaction (Bendena et al., 2008; Cohen et al., 2009; Chalasani et al., 2010; Harris et al., 2010, 2011; Mills et al., 2012; Chen et al., 2013). Here, we show that the NLP-9 signal from the AWB neurons and the somatostatin-like neuropeptide receptor NPR-18 regulate olfactory preference for PA14 in comparison with OP50. In addition, AWC signals through the buccalin-like neuropeptide NLP-1 to regulate the olfactory preference. We further extended the significance of these findings by demonstrating that NLP-1, NLP-9, and NPR-18 are not required for the recognition of food odors. These results together reveal that AWC sensory neurons employ glutamate neurotransmission to process sensorimotor response to food odors and AWB/AWC neurons use neuropeptides to modulate olfactory response to generate preference between the odors (Fig. 8*B*). Thus, our study provides a framework for understanding how sensory systems integrate complex odors at the molecular and cellular level to generate defined behavioral outputs.

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