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Olfactory plasticity is regulated by pheromonal signaling in *Caenorhabditis elegans*

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Abstract

Population density-dependent dispersal is a well-characterized strategy of animal behavior in which dispersal rate increases when population density is higher. *C. elegans* shows positive chemotaxis to a set of odorants, but the chemotaxis switches from attraction to dispersal after prolonged exposure to the odorants. We show here that this plasticity of olfactory behavior is dependent on population density and this regulation is mediated by pheromonal signaling. We show that a peptide SNET-1 negatively regulates olfactory plasticity and its expression is down-regulated by the pheromone. NEP-2, a homologue of the extracellular peptidase neprilysin, antagonizes SNET-1 and this function is essential for olfactory plasticity. These results suggest that population density information is transmitted through the external pheromone and endogenous peptide signaling to modulate chemotactic behavior.

C. elegans is attracted to a series of odorants such as benzaldehyde (1). However, after extended exposure to the odor in the absence of food, worms stop approaching the odorant and disperse from it, a behavioral plasticity called olfactory adaptation or food-odor associative learning (2–4) (hereafter called olfactory plasticity). The odorant likely serves as a signal of remote food, and the naive chemotaxis to the odorant is probably beneficial for *C. elegans* in nature, but if worms fail to reach food after certain period of odor sensation, they no longer approach the odorant. For the best chance of survival, animals may have to optimize the extent of this behavioral switching by assessing environmental conditions, including the population density of the species, which can critically affect the survival strategy (5–7). Over the course of our olfactory plasticity assays (fig. S1), we noticed that the extent of olfactory plasticity shows positive correlation with the density of animals on the cultivation plates (Fig. 1A).

Population density is known to regulate the developmental decision of dauer larva formation, and for this regulation, the population density information is transmitted by the dauer pheromone which is continuously secreted by the animals (8). Mutants of *daf-22*, which encodes an enzyme in the pheromone biosynthesis pathway (9), showed a defect in olfactory plasticity (Fig. 1B, fig. S2 and S3). The chemical nature of dauer pheromones was

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recently described as a set of sugar derivatives called ascarosides (10–13). Addition of either crude pheromone extract or individual synthetic pheromones restored olfactory plasticity to the *daf-22* mutants (Fig. 1B and fig. S4). Long-term incubation with the pheromone (more than 24 hours) before the assay is needed to exert full effect of the pheromone (fig. S5). We therefore conclude that population density information is transmitted via the dauer pheromone ascarosides, which enhance olfactory plasticity.

To learn how olfactory plasticity is regulated, we screened for mutants defective in the plasticity of chemotaxis to benzaldehyde (14). Genetic fine mapping and rescue experiments revealed that one of the mutants, *pe356*, had a mutation in a splicing acceptor of the *nep-2* gene, which encodes a *C. elegans* homologue of the mammalian peptidase family neprilysin (Fig. 2, A to C, fig. S2, S3, S6A and S8). Neprilysin is a type II transmembrane protein with a zinc metalloprotease activity in the C-terminal ectodomain and is known to degrade extracellular peptides (15,16). A deletion mutant of *nep-2*, *pe379*, also showed a severe defect in olfactory plasticity (Fig. 2, A and B). Metalloprotease activity is probably essential for regulation of olfactory plasticity because a mutant form of *nep-2*, *nep-2(FExxF)*, in which zinc-binding amino acids at the active center were mutated (17), failed to rescue the mutant phenotype (fig. S7).

Observation of the fluorescent reporter Venus fused to the *nep-2* promoter suggested that *nep-2* is mainly expressed in muscles, glia-like cells (GLR) and several classes of neurons (Fig. 3, B and C). Expression in each of these tissues is driven by separate cis-acting elements in the promoter region (Fig. 3, A, D to F). The translational fusion protein NEP-2::Venus was localized to the cell surface in both muscles and neurons (fig. S7, D to H). Tissue- and cell-specific rescue experiments show that expression of *nep-2* in the nervous system is important for olfactory plasticity (Fig. 2D) and *nep-2* can be functional when expressed in a variety of neurons (Fig. 2E). These observations are consistent with NEP-2 acting as an ecto-peptidase that degrades paracrine or endocrine peptides, such that accumulation of the peptide in extracellular space in the *nep-2* mutant causes inhibition of olfactory plasticity.

To identify neuropeptides that are negatively regulated by NEP-2, we further screened for mutations that suppress the olfactory plasticity defect of *nep-2(pe379)*. One of these mutations, *pe1063*, strongly suppressed the defect of *nep-2* (Fig. 4A). Fine mapping and rescue experiments showed that the causative gene was *C02F12.3*, which we named *snet-1* (suppressor of *nep-2*, Fig. 4B, fig. S2, S3, S6B and S8). *snet-1* is predicted to encode a small protein of 101 amino acids, with a signal sequence and dibasic cleavage sites typical of neuropeptide precursors (Fig. 4C). The predicted peptide region had partial similarity with *Aplysia* L11 peptide, a peptide secreted from the L11 neuron (18) (fig. S9A). Rescue experiments with truncated constructs suggested that the peptide region is important for regulation of olfactory plasticity (fig. S9, B and C). When Venus was fused to the coding sequence, the fluorescence was observed in coelomocytes, the scavenger cells located in the body cavity, indicating that the protein is secreted by the action of the signal sequence (fig. S10, A, B, and G). Intracellular localization of SNET-1::Venus fusion protein and its dependence on UNC-104 kinesin-3 suggest that SNET-1 is transported to the neurites by kinesin-dependent anterograde transport and probably secreted there (19) (fig. S10, C to F). Olfactory plasticity defect of *nep-2* re-appeared when *snet-1* was expressed by various cell-specific promoters in the *nep-2*; *snet-1* double mutant (Fig. 4D). These observations are consistent with SNET-1 being a secreted peptide and a substrate of the NEP-2 peptidase. Because null mutation of *snet-1* may not be fully epistatic to *nep-2* (Fig. 4A) there may be also other targets of NEP-2.

The *snet-1p::Venus* promoter fusion reporter was expressed in several neurons including the sensory neurons ASK and the interneurons AIB, AIM and PVQ (Fig. 3, G to I), suggesting that SNET-1 may be regulated by sensory inputs such as sensation of the pheromone. Because overexpression of *snet-1* by increased gene dosage leads to defect in olfactory plasticity, quantitative regulation of the *snet-1* gene expression may be important (fig. S11). By quantitative RT-PCR, we indeed found that the transcript of *snet-1* was increased in the adult animals of *daf-22* pheromone-less mutants compared to the wild type (fig. S12). In the *daf-22* mutant background, *snet-1p::Venus* was expressed in ASI sensory neurons in addition to the cells observed in the wild type background, and the supernumerary expression was suppressed by the addition of the pheromone to the culture plates (Fig. 3, J and K, and Fig. 4E). ASI neurons are known to regulate dauer larva formation and expression of the genes *daf-7* TGF β , *daf-28* insulin and several chemoreceptors such as *srd-1*, *str-2* and *str-3* in ASI is regulated by the dauer pheromone (20–22). Of particular interest is that expression of *str-3* and *daf-7* is regulated through the pheromone receptor SRBC-64/SRBC-66 on ASK sensory neurons in larvae (23), but mutants of these receptors did not show any defect in olfactory plasticity (fig. S13). These observations suggest that down-regulation of *snet-1* expression by the pheromone, probably in an SRBC-64/66-independent manner, results in promotion of olfactory plasticity by reducing the SNET-1 peptide signaling. In support of the notion that SNET-1 conveys the pheromonal signal, null mutation of *snet-1* suppressed the olfactory plasticity-defective phenotype of *daf-22* to the wild type level (Fig. 4F).

Throughout the animal kingdom, greater population density leads to increased rates of dispersal behavior, because high population density usually leads to competition for limited resources within a habitat (5–7). Population density-dependent olfactory plasticity may serve as one of such adaptive mechanisms. Our results indicate that *nep-2* regulates olfactory plasticity by acting in a cell-nonautonomous manner. Mammals possess seven neprilysin family proteins, neprilysin, ECE1, ECE2, PHEX, neprilysin-2, DINE and KELL (15). Although little is known about the loss-of-function phenotypes of the neprilysin family proteins, recent biochemical studies revealed that neprilysin scavenges amyloid beta peptide efficiently and can prevent Alzheimer's disease by this mechanism (16). Based on our observations, we propose a model in which SNET-1 peptide is secreted as an environmental signal that prevents olfactory plasticity and NEP-2 turns off the SNET-1 signals by degrading the excess peptides, creating a balance sensitive to environmental signals. By acting on an unknown receptor in the olfactory sensory circuit, SNET-1 negatively regulates olfactory plasticity (Fig. 4G and fig. S14). Given that *daf-22*, *nep-2* and *snet-1* regulate the plasticity of the response to several types of odorants (fig. S3), the pheromonal information may also regulate other behaviors through the peptide signaling. Our identification of the SNET-1 pathway that regulates olfactory plasticity sheds light on the complexity of the regulatory network underlying simple sensory behavior in *C. elegans* and suggests similar regulation might be present in other animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Olfactory plasticity is regulated by population density and dauer pheromone. **(A)** Two to twelve parental adults per plate were put on the culture plates and the progeny was tested for olfactory plasticity. ($n \geq 6$) **(B)** Olfactory plasticity of *daf-22* mutants was tested with or without adding 0.03 unit/ml of crude dauer pheromone to the culture plates. ($n \geq 6$) (Open bars and filled bars represent mock pre-exposed condition and bz pre-exposed condition, respectively. *, $P < 0.01$, **, $P < 0.001$ for all figures, Student's *t* test (B) and Dunnett's test for multiple comparisons (A). Error bars represent SEM in all figures)

Figure 2. The *nep-2* neprilysin gene is essential for olfactory plasticity. **(A)** Olfactory plasticity of *nep-2* mutants, *pe356* and *pe379*. ($n \geq 3$) **(B)** Gene model of *nep-2* and lesions in *nep-2* mutants. **(C)** Domain structure and motifs in the predicted NEP-2 protein. **(D and E)** Tissue- and cell-specific rescue experiments. *nep-2* cDNA was expressed by tissue- or cell-specific promoters in *nep-2(pe356)* mutants and the transgenic animals were subjected to olfactory plasticity assay. ($n \geq 4$, E : $n \geq 5$) (Open bars and filled bars represent mock pre-exposed conditions and bz pre-exposed conditions, respectively. Dunnett's test for multiple comparisons.)

Figure 3.

Expression patterns of *nep-2* and *snet-1*. **(A)** Structure of the truncated *nep-2* promoters, *nep-2p*, *nep-2p(H)*, *nep-2p(S)*, and *nep-2p(HS)*, used for the expression of Venus reporter. **(B to F)** Expression pattern of Venus reporter driven by *nep-2p*, *nep-2p(H)*, *nep-2p(S)*, or *nep-2p(HS)*. Bright field image of B is shown as an inset. C is a magnified image of the depicted region of B. D to F show the same head region as shown in C. **(G to I)** Expression pattern of the Venus reporter driven by *snet-1p*. Bright field image of G is shown as an inset. H shows a bright field image of the head region observed in I. **(J and K)** Expression pattern of Venus reporter driven by *snet-1p* in the *daf-22* background with or without addition of 0.1 unit/ml of dauer pheromone to the culture plate. “?” indicates an unidentified pair of lateral neurons.

Figure 4. *snet-1* encodes a peptide and regulates olfactory plasticity. **(A)** Olfactory plasticity of *nep-2*, *snet-1*, and *nep-2; snet-1* mutants ($n \geq 5$) **(B)** Gene model of *snet-1* and the *pe1063* mutation. **(C)** Domain structure in the predicted SNET-1 protein. **(D)** *snet-1* cDNA was expressed in the *nep-2(pe379); snet-1(pe1063)* double mutant background by cell-specific promoters and the transgenic animals were subjected to olfactory plasticity assay. ($n \geq 5$) **(E)** Relative levels of *snet-1p::venus* fluorescence in adult *daf-22* mutant animals with or without 0.1 unit/ml of crude pheromone in the culture plates. ($n = 80$ for ASK and $n = 40$ for other neurons) **(F)** Olfactory plasticity of the *daf-22(m130); snet-1(pe1063)* double mutant. ($n \geq 5$) **(G)** A possible role for NEP-2 and SNET-1 in pheromone-dependent regulation of olfactory plasticity. (In A, D and F, open bars and filled bars represent mock pre-exposed conditions and bz pre-exposed conditions, respectively. Tukey's test (A and F), Dunnet's test (D) and Student's t test with Bonferroni correction (E) for multiple comparisons.)