ARTICLES

Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*

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Food can be hazardous, either through toxicity or through bacterial infections that follow the ingestion of a tainted food source. Because learning about food quality enhances survival, one of the most robust forms of olfactory learning is conditioned avoidance of tastes associated with visceral malaise. The nematode *Caenorhabditis elegans* feeds on bacteria but is susceptible to infection by pathogenic bacteria in its natural environment. Here we show that *C. elegans* modifies its olfactory preferences after exposure to pathogenic bacteria, avoiding odours from the pathogen and increasing its attraction to odours from familiar nonpathogenic bacteria. Particular bacteria elicit specific changes in olfactory preferences that are suggestive of associative learning. Exposure to pathogenic bacteria increases serotonin in ADF chemosensory neurons by transcriptional and post-transcriptional mechanisms. Serotonin functions through MOD-1, a serotonin-gated chloride channel expressed in sensory interneurons, to promote aversive learning. An increase in serotonin may represent the negative reinforcing stimulus in pathogenic infection.

Many animals are susceptible to intestinal infections by bacteria. The pathogenic soil bacteria *Pseudomonas aeruginosa* and *Serratia marcescens* can proliferate in the intestine of the soil nematode *C. elegans* after they are ingested, resulting in death of the nematode after several days[1–3]. *C. elegans* protects itself from pathogens through innate immunity pathways[3,4] and through behavioural strategies such as leaving a lawn of pathogenic bacteria[2]. *C. elegans* has a simple nervous system of 302 neurons that facilitates the identification of molecules, neurons and circuits involved in behaviour[5]. One of its most robust behaviours is olfactory chemotaxis towards food-associated odours, an innate behaviour that is highly reproducible among animals[6]. Olfactory preference can be altered by adaptation after prolonged exposure to an odour[7,8] or by starvation[9,10]. Here we use infection by natural pathogens to develop an ecologically relevant olfactory learning assay, with which we identify a circuit and neuronal changes that are associated with learning.

Pathogenic bacteria alter odour preference

The preference of *C. elegans* for different bacterial odours can be measured in a binary choice assay in which animals migrate towards one of two bacterial lawns on opposite sides of a plate (Fig. 1a). In this assay, a choice index of −1.0 represents complete preference for *Escherichia coli* OP50, the control bacterium in all tests, an index of 1.0 represents complete preference for the test bacterium, and an index of 0 represents an equal distribution (Fig. 1a). Animals cultivated on OP50 alone were equally attracted to OP50 and the pathogenic *P. aeruginosa* strain PA14, and were more attracted to the pathogenic bacterium *S. marcescens* ATCC 13880 than to OP50, despite the eventual toxicity of *S. marcescens* infection (Fig. 1b and Supplementary Fig. 1). Animals cultivated from hatching in the presence of both OP50 and PA14, however, strongly preferred OP50 to *P. aeruginosa* PA14; similarly, animals cultivated on OP50 and *S. marcescens* preferred OP50 to *S. marcescens* (Fig. 1b). It was not possible to raise animals on *S. marcescens* or PA14 alone because of the virulence of the infection[1]. These results indicate that *C. elegans* can modify its olfactory preferences to avoid toxic bacteria.

No alteration in olfactory preferences was observed when animals were raised on both OP50 and nonpathogenic *E. coli* HB101, or on OP50 and the harmless soil bacteria *Rhizobium leguminosarum* or *Pseudomonas fluorescens* (Fig. 1b). Three isogenic nonvirulent derivatives of PA14, *12A1*, *50E12* and PA14 (*gacA::Kan*)[1], and two other nonpathogenic strains of *P. aeruginosa*, PAK and PA103, did not modify the olfactory preferences of *C. elegans* (Fig. 1b). These results suggest that pathogenic infection induces the alteration in olfactory preferences.

A learning index was generated by subtracting the choice index of animals exposed to pathogen from the choice index of naive animals. A positive learning index, as shown by wild-type animals exposed to PA14 or *S. marcescens* (Fig. 1c), indicates an acquired avoidance of pathogenic bacteria. When adult animals were acutely exposed to PA14 for only 4 h, the learning index was similar to that of animals that had had lifelong exposure to OP50 and PA14 (Fig. 1d). Adult animals that were grown on *E. coli* OP50 and then starved for 4 h did not show the same change in olfactory preference (Fig. 1e). This result indicates that adult animals rapidly modify their olfactory preferences after exposure to pathogenic bacteria.

Olfactory learning in response to pathogenic bacteria was distinct from known forms of odour adaptation, because the olfactory adaptation-defective mutants *egl-4(ky95)* and *adp-1(ky20)*[7,11] were both proficient in olfactory preference learning after exposure to OP50 and PA14 (Supplementary Figs 2 and 3). Olfactory learning is also distinct from the food-leaving behaviour that *C. elegans* shows in response to *S. marcescens* Db10 or Db11 (ref. 2). Food-leaving requires the Toll-like receptor TOL-1, but *tol-1(nr2033)* animals were proficient in olfactory preference learning (Supplementary Figs 2 and 3). These results suggest that *C. elegans* uses several different behavioural strategies to minimize exposure to pathogens.

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To test whether *C. elegans* associates pathogenesis with simultaneously presented odours, we exposed animals sequentially to pathogenic or nonpathogenic bacterial strains. In the first experiment, adult animals were exposed to pathogenic *P. aeruginosa* PA14 for 4 h, followed by nonpathogenic *S. marcescens* Db1140 for 4 h. In the second experiment, adults were exposed to nonpathogenic *P. aeruginosa 50E12* for 4 h followed by pathogenic *S. marcescens* ATCC 13880 for 4 h. In the third and fourth experiments, the order of presentation for pathogenic and nonpathogenic strains was reversed. Trained animals were then tested for their preference between PA14 and *S. marcescens*. In all tests, animals exposed to pathogenic PA14 and harmless *S. marcescens* showed enhanced avoidance of PA14 as compared with those exposed to pathogenic *S. marcescens* and harmless *P. aeruginosa* (Fig. 1f). This dissociation experiment indicates that *C. elegans* selectively avoids an odour experienced at the same time as pathogenic infection, a criterion for associative learning.

Aversive and attractive aspects of learning

The two-choice preference assay does not distinguish whether trained animals have made a positive association that increases attraction towards the harmless bacterium or a negative association that induces aversion from the pathogenic bacterium. We tested these possibilities by a four-choice maze assay in which animals trained with OP50 and *P. aeruginosa* PA14 were given a choice between OP50, PA14 and two novel bacteria strains: one nonpathogenic strain, *P. fluorescens*; and one pathogenic strain, *S. marcescens* ATCC 13880. Preferences were tested in a partially enclosed eight-arm maze made from polydimethyl siloxane (PDMS) elastomer resting on the surface of an agar plate (Fig. 2a). A decision area in the centre of the maze was connected by slender channels to eight small food chambers, each containing one of the four bacterial strains. Animals were placed in the open decision area and approached food chambers through the channels. Wild-type animals cultivated on OP50 distributed themselves among all four bacteria strains, reproducibly showing strongest attraction towards *S. marcescens* (Fig. 2b).

The four-choice configuration made it possible to compare responses to the two bacteria experienced during training with responses to the novel bacteria. After cultivation on OP50 and PA14, the fraction of animals that approached OP50 was increased, and the fraction of animals that approached PA14 was diminished, as compared with control bacteria (Fig. 2b, c, and Methods), suggesting that olfactory learning on pathogens includes both attractive and aversive components. Similarly, animals cultivated on a different pairing of nonpathogenic and pathogenic bacteria, *P. fluorescens* and *S. marcescens* ATCC 13880, showed increased attraction to *P. fluorescens* and aversion from *S. marcescens* in the four-choice maze assay (Fig. 2d, e).

Exposure to PA14 for 4 h was sufficient to induce aversion from PA14 but not increased attraction towards OP50 (Supplementary Fig. 4). These results suggest that the aversive component of olfactory learning is relatively rapid, whereas attractive changes occur more slowly. Starvation does not elicit these changes in preference (Supplementary Fig. 4).

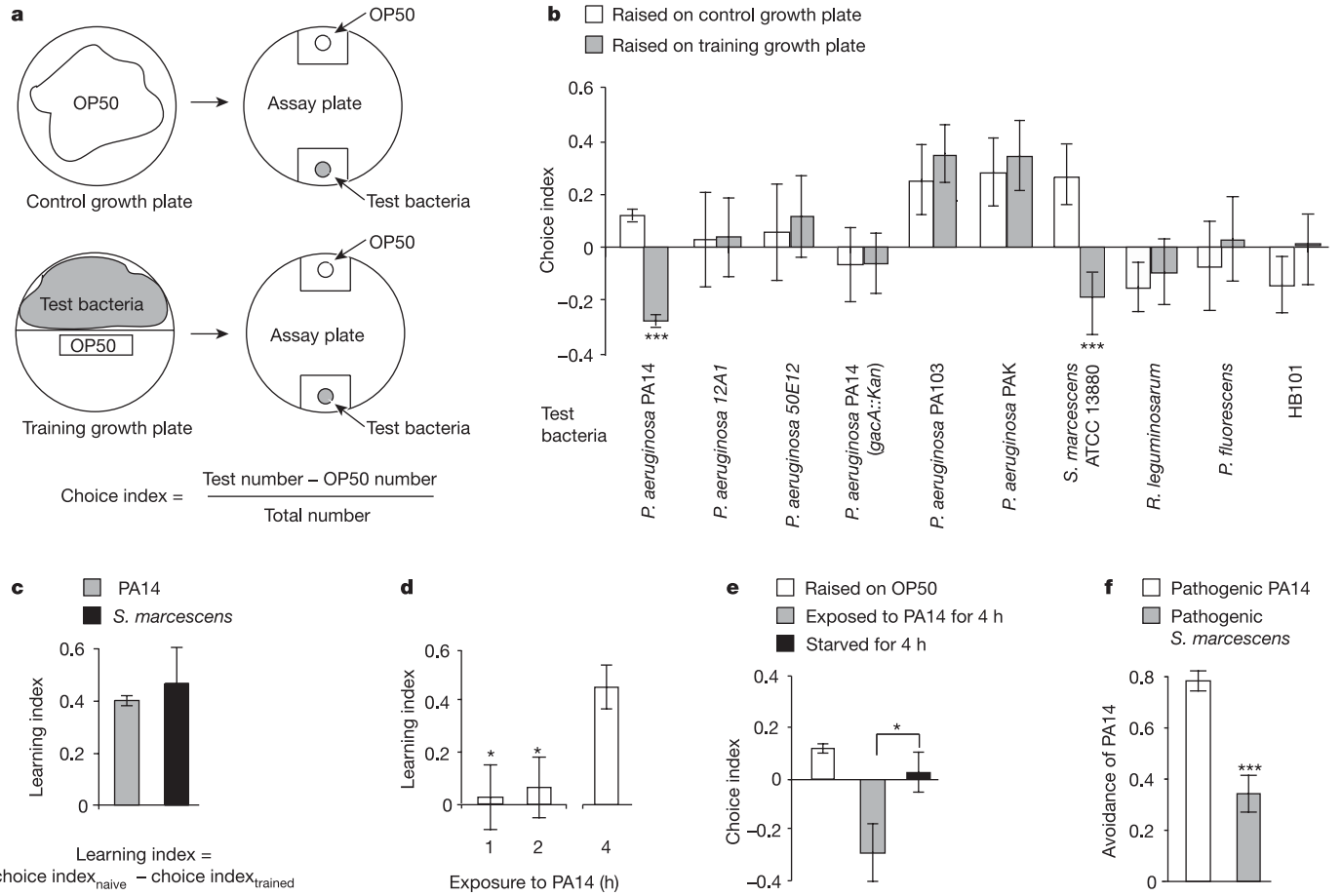


Figure 1 | *C. elegans* learns to avoid pathogenic bacteria. **a**, Training protocol and two-choice olfactory preference assays. **b**, Olfactory preferences after exposure to pathogenic *P. aeruginosa* PA14 or *S. marcescens*, isogenic nonvirulent *P. aeruginosa*, or nonpathogenic bacteria. **c**, Learning index after training with pathogens. **d**, Rapid learning in adults transferred from OP50 to PA14. **e**, Starvation and pathogens cause different changes in olfactory preference. **f**, Animals exposed to pathogenic *P. aeruginosa* PA14 and harmless *S. marcescens* avoid PA14 more than do animals exposed to harmless *P. aeruginosa* and pathogenic *S. marcescens*. $^{***}P < 0.001$, $^{*}P < 0.05$, $n \geq 4$ assays. Error bars indicate the s.e.m.

Serotonin induces aversive learning

The neurotransmitter serotonin is essential for pathogen-induced olfactory learning. The general catecholamine-defective mutants *cat-1* (ref. 12) and *cat-4* (ref. 13) showed significantly reduced learning in the two-choice assay (Fig. 3a and Supplementary Fig. 3), as did the more specific mutant *tph-1*, which is deficient in a tryptophan hydroxylase required for biosynthesis of serotonin but not other catecholamines[14] (Fig. 3a and Supplementary Fig. 3). *cat-2* mutants lacking dopamine[15] but not serotonin were proficient in learning. In the four-choice maze assay, *tph-1* mutants were defective in both aversive and attractive components of olfactory learning (Fig. 3b and Supplementary Fig. 9a, b).

tph-1 mutants were normal in their basal preference for bacterial strains and in their tendency to leave a lawn of pathogenic *S. marcescens* (Supplementary Figs 5 and 9a) and showed no alteration in their susceptibility to pathogenic infection and killing by PA14 (Supplementary Fig. 6). Thus, the learning defects of *tph-1* mutants are not due to changes in general olfactory ability, recognition of bacteria or innate immunity. Instead, *tph-1* is selectively unable to associate the physiological responses to pathogens with olfactory cues.

In *C. elegans* hermaphrodites, *tph-1* is expressed in the serotonergic neurons ADF, NSM and HSN, and occasionally AIM and RIH[14]. Expression of a *tph-1* complementary DNA in ADF chemosensory neurons partially rescued the learning defects of *tph-1* mutants in the two-choice assay, but expression of *tph-1* in NSM pharyngeal neurons did not (Fig. 3c and Supplementary Fig. 3). Expression of *tph-1* in NSM neurons did partially rescue a different serotonin-dependent behaviour, namely the enhanced slowing of starved animals in response to fresh food, but this behaviour was not rescued by *tph-1* expression in ADF neurons[16] (Supplementary Fig. 7). These results suggest that serotonin from ADF and NSM neurons has different functions: serotonin from ADF neurons has a stronger role in olfactory learning, whereas that from NSM neurons has a stronger role in the enhanced slowing response.

In the four-choice maze assay, expression of *tph-1* in ADF neurons alone fully rescued aversive but not attractive learning (Fig. 3d and Supplementary Fig. 9d, e). Expression of *tph-1* in NSM neurons did not rescue learning, but expression in both ADF and NSM neurons restored both aversive and attractive learning (Fig. 3d and Supplementary Fig. 9c). We propose that ADF neurons may evaluate aversive and NSM neurons attractive components of food-related signals.

The *C. elegans* genome encodes at least 12 potential serotonin receptors, including MOD-1, a serotonin-gated chloride channel that regulates the enhanced slowing of starved animals on fresh food[16,17]. *mod-1* mutants showed significantly decreased olfactory learning to PA14 in the two-choice learning assay (Fig. 4a and Supplementary Fig. 3). In the four-choice maze assay, *mod-1* mutants were specifically defective in aversive but not attractive learning when trained either on OP50 and PA14, or on *P. fluorescens* and *S. marcescens* (Fig. 4b and Supplementary Fig. 10a, b). Thus, the partial defect in *mod-1* seems to result from its role in aversive learning, the same component that is affected by ADF sensory neurons.

Like *tph-1* mutants, *mod-1* mutants were killed by PA14 infection with the same kinetics as wild-type animals and were able to discriminate between bacteria in maze assays (Supplementary Figs 6 and 10a). The specific defect of *mod-1* mutants in aversive learning suggests that MOD-1 is the downstream target of the serotonin signal from ADF neurons.

mod-1 promoter fusions are expressed in AIA, AIB, AIY, RID and probably AIZ interneurons, as well as in other neurons in the head, ventral cord and tail[17,18]. The aversive learning defect of *mod-1(ok103)* mutants was completely rescued by expression of a

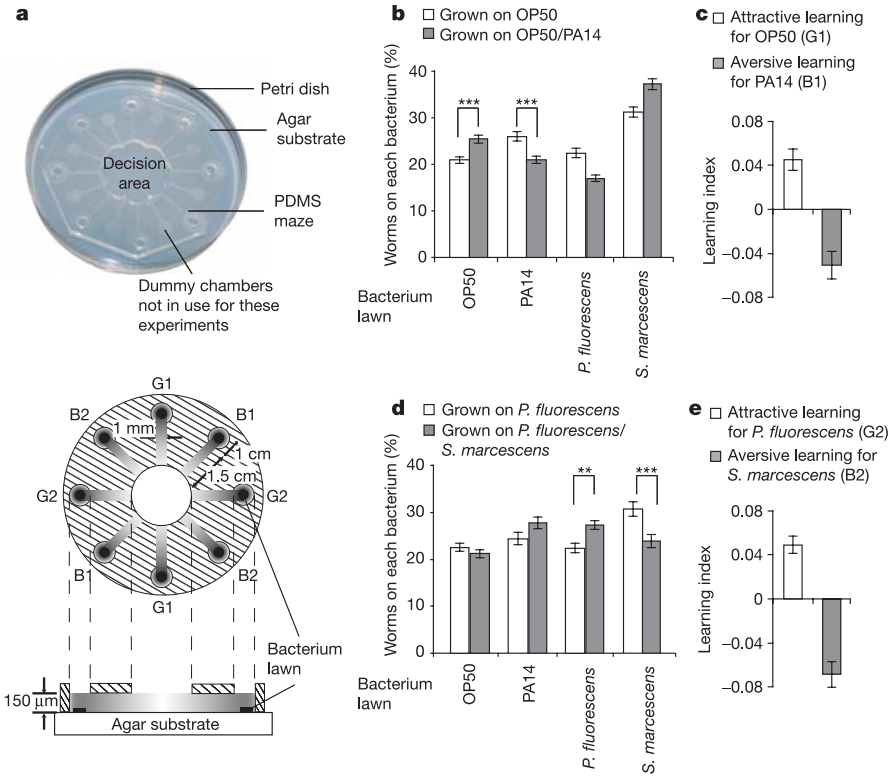


Figure 2 | Olfactory maze assay. a, Photograph and scheme of the four-choice maze assay for olfactory preference. Each test bacterium was placed in two of the eight chambers (G1, *E. coli* OP50; B1, *P. aeruginosa* PA14; G2, *P. fluorescens*; B2, *S. marcescens* ATCC 13880). **b**, **c**, Training with PA14 and OP50 results in both increased attraction towards OP50 and aversion from PA14. **d**, **e**, Training with *S. marcescens* and *P. fluorescens* results in both increased attraction towards *P. fluorescens* and aversion from *S. marcescens*. $^{***}P < 0.001$, $^{**}P < 0.01$, $n \geq 23$ assays. Error bars indicate the s.e.m.

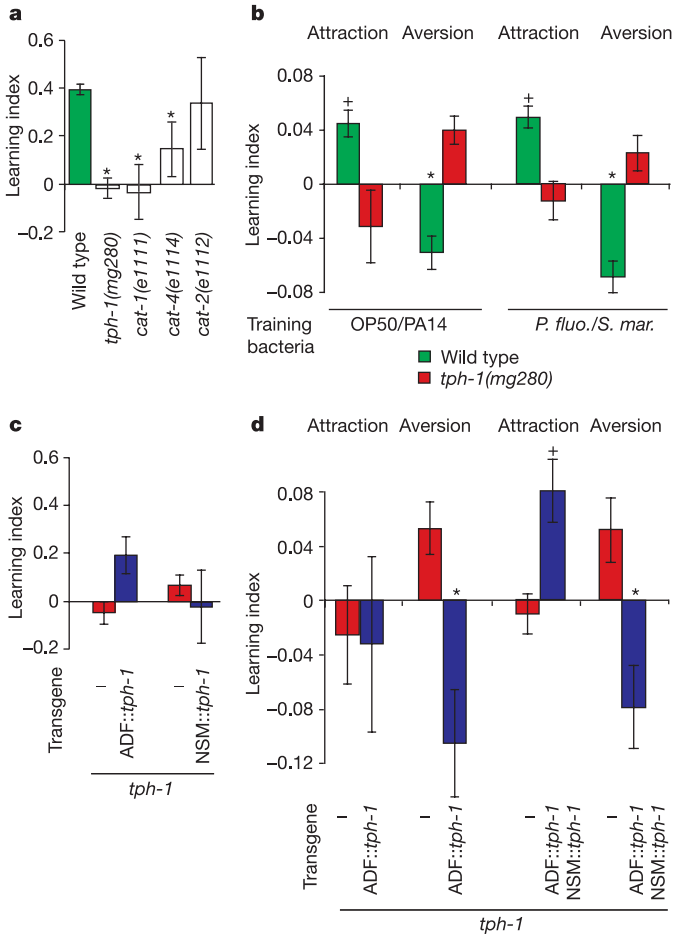


Figure 3 | ADF serotonergic neurons regulate aversive learning. **a**, Two-choice learning to PA14 in catecholamine biosynthesis mutants. **b**, Defective aversive and attractive learning in four-choice maze assays of *tph-1(mg280)* animals trained with PA14 or *S. marcescens*. **c**, *tph-1* expression in ADF neurons partially rescues *tph-1(mg280)* in two-choice assays. Expression in NSM and other pharyngeal neurons does not rescue. **d**, *tph-1* expression in ADF neurons in *tph-1(mg280)* rescues aversive learning to PA14, but not attractive learning to OP50. *tph-1* expression in both ADF and NSM neurons rescues both attractive and aversive learning. $^{*}P < 0.05$, $^{+}P < 0.05$, $n \geq 6$ assays. Error bars indicate the s.e.m.

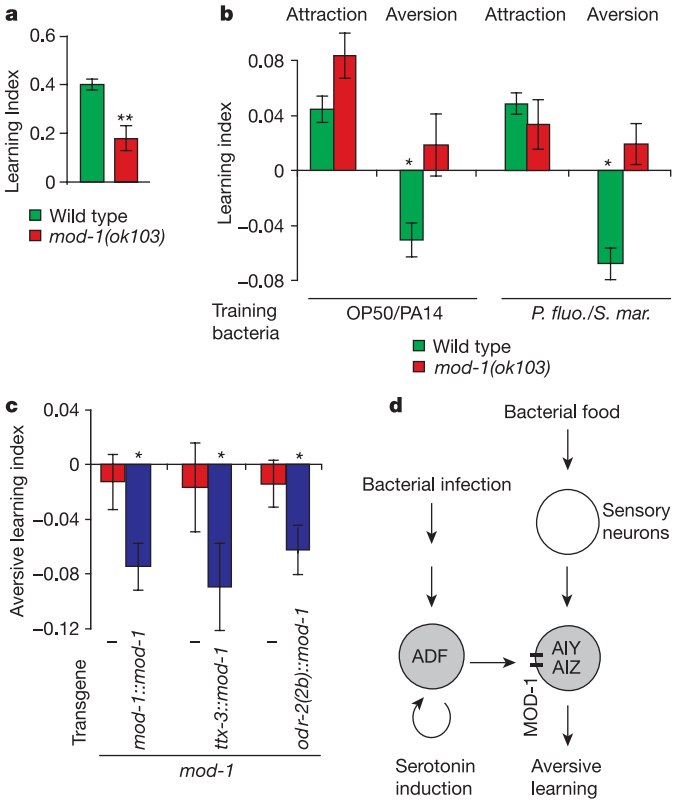
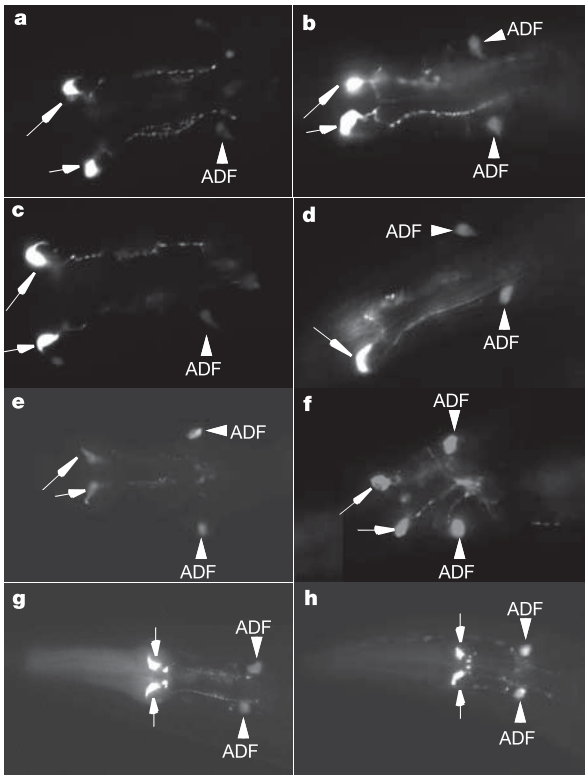


Figure 4 | MOD-1 regulates aversive learning. **a**, Two-choice learning assays to PA14. *mod-1(ok103)* is partly defective. **b**, Four-choice maze assays. *mod-1(ok103)* animals are defective in aversive learning when trained either with OP50 and PA14 or with *P. fluorescens* and *S. marcescens*. **c**, Four-choice maze assays for aversive learning after training with OP50 and PA14. Expression of *mod-1* in several subsets of *mod-1*-expressing neurons rescues aversive learning. *ttx-3::mod-1* is expressed in AIY neurons; *odr-2(2b)::mod-1* is expressed in AIB, AIZ and other neurons. **d**, Model of aversive olfactory learning on pathogenic bacteria. $^{**}P < 0.01$, $^{*}P < 0.05$, $n \geq 5$ assays. Error bars indicate the s.e.m.

mod-1 cDNA from a *mod-1* promoter[18] (Fig. 4c and Supplementary Fig. 10c, d). The principal interneurons downstream of chemosensory neurons such as ADF are AIA, AIB, AIY and AIZ[5]. We used a *ttx-3* promoter to express *mod-1* in AIY and possibly AIA interneurons[18], and an *odr-2(2b)* promoter to express wild-type *mod-1* in AIZ and AIB interneurons, as well as in a few other neurons[19]. Both the *ttx-3::mod-1* and *odr-2(2b)::mod-1* transgenes rescued aversive learning in *mod-1(ok103)* mutants (Fig. 4c and Supplementary Fig. 10e–h), suggesting that the serotonin receptor can function in several interneurons to modulate olfactory preference. Because ADF neurons synapse onto AIZ and perhaps AIY interneurons[5], these two neurons are potential sites of MOD-1 action in aversive learning.

To determine how serotonin signalling changes during learning, we examined serotonin immunoreactivity in OP50-fed naive animals and PA14-trained animals by staining with polyclonal antibodies against serotonin. Exposure to PA14 resulted in a 3.3 ± 0.3 (s.e.m.) fold increase in serotonin immunostaining in ADF neurons, but no change in NSM neurons (Fig. 5a, b, i). A significant increase in serotonin in PA14-trained animals as compared with OP50-fed animals was also detected by directly measuring serotonin in dialysed *C. elegans* homogenates by high-performance liquid chromatography (HPLC; data not shown). No serotonin was detected by HPLC or antibody staining in PA14-trained *tph-1* mutants. Similarly, animals exposed to *S. marcescens* showed a 2.2 ± 0.2 fold increase in ADF immunoreactivity as compared with animals fed on nonpathogenic *P. fluorescens* (Fig. 5c, d, i). Serotonin immunoreactivity in ADF neurons was not induced by three isogenic nonvirulent PA14 derivatives that did not induce olfactory learning (Supplementary Fig. 8). These results suggest that exposure to pathogenic bacteria specifically increases serotonin in ADF neurons.

Serotonin in ADF neurons could rise either through increased transcription of *tph-1*, the rate-limiting enzyme for serotonin biosynthesis, or through post-transcriptional mechanisms such as changes in TPH-1 enzymatic activity. The transcription of *C. elegans tph-1* in ADF neurons is increased by neuronal activity[20], recovery from the dauer stage, and heat stress[21]. The biochemical activity of mammalian tryptophan hydroxylase is activated by phosphorylation mediated by Ca^{2+}/calmodulin-mediated kinase II and protein kinase A (ref. 22). To characterize the mechanism of serotonin upregulation in ADF neurons, we examined serotonin immunoreactivity in a *tph-1;srh-142::tph-1* strain in which TPH-1 was expressed from a heterologous, ADF-specific promoter. The *srh-142* promoter was not regulated by exposure to PA14 (data not shown). In this strain, exposure to PA14 caused a 2.1 ± 0.15 fold increase in serotonin immunoreactivity in ADF as compared with naive OP50-fed animals (Fig. 5e, f, j). Serotonin immunoreactivity in NSM neurons was also detected in these animals, probably owing to the reuptake of serotonin released from ADF neurons. These results suggest that pathogen exposure increases TPH-1 activity or decreases serotonin turnover in



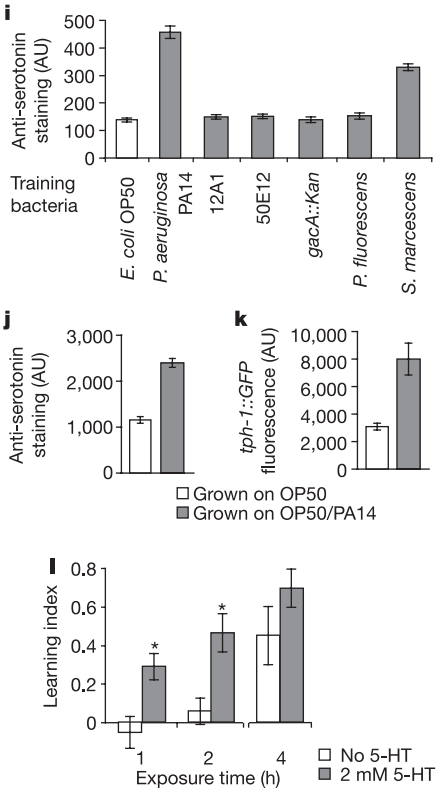


Figure 5 | Pathogenic bacteria increase serotonin in ADF neurons. **a**–**f**, Serotonin immunoreactivity in either wild-type animals fed OP50 (**a**), OP50 and PA14 (**b**), *P. fluorescens* (**c**) or *P. fluorescens* and *S. marcescens* (**d**), or *tph-1;srh-142::tph-1* animals fed OP50 (**e**) or OP50 and PA14 (**f**). Arrowheads indicate ADF neurons; arrows indicate NSM neurons. **g**, **h**, *tph-1::GFP* expression in wild-type animals fed OP50 (**g**), or OP50 and PA14 (**h**). **i**, ADF serotonin immunoreactivity in wild-type animals fed OP50 with other bacteria. **j**, ADF serotonin immunoreactivity in *tph-1;srh-142::tph-1* animals. **k**, ADF *tph-1::GFP* fluorescence in wild-type animals. **l**, Exogenous serotonin (5-HT) accelerates olfactory learning. $^*P < 0.05$, $n \geq 4$ assays or ≥ 14 animals. Error bars indicate the s.e.m. AU, arbitrary fluorescence units.

ADF neurons. The rescue of aversive learning by *srh-142::tph-1* indicates that post-transcriptional mechanisms are sufficient for learning (Fig. 3d). Notably, exposure to PA14 also induced a 2.6 ± 0.42 fold increase in expression of a *tph-1::GFP* reporter gene lacking most of the TPH-1 protein in ADF neurons (Fig. 5g, h, k). Thus, pathogens increase serotonin in ADF neurons by both transcriptional and post-transcriptional mechanisms.

We next tested whether increased serotonin facilitates olfactory learning directly. Taking advantage of the ability of *C. elegans* to take up exogenous serotonin[16]; we raised wild-type animals on OP50 and transferred them to PA14 with or without 2 mM exogenous serotonin. In the presence of exogenous serotonin, significant learning was observed within 1 h of pathogen exposure, and full aversive learning within 2 h (as compared with 4 h in untreated animals; Figs 1d and 5l). These results suggest that an increase in serotonin directly promotes olfactory learning in pathogen-exposed animals, perhaps by encoding the unconditioned stimulus of pathogenic infection.

Discussion

In its natural soil habitat, *C. elegans* interacts with many different bacteria. Some are good food sources, some are poor food sources, and some are pathogenic hazards[1–3,23]. Here we have shown that *C. elegans* learns to avoid the odours of pathogenic bacteria after interacting with the pathogens. Exposure to pathogens upregulates expression of serotonin in the ADF chemosensory neurons, and aversive learning requires serotonin from ADF neurons and the serotonin receptor MOD-1 (Fig. 4d). The induced avoidance of pathogenic bacteria is analogous to conditioned taste aversion, a learning behaviour that has been described in mammals, snails, cuttlefish and fish[24–26] in which animals avoid food flavours associated with intestinal distress. Olfactory learning may allow *C. elegans* to distinguish among natural food sources on the basis of relevant experiences.

In both vertebrates and invertebrates, catecholamines including serotonin function as reinforcing signals during learning[27]. In mammals, dopamine and norepinephrine are reinforcing signals in learning and addiction[28]. In insect olfactory learning, octopamine functions as a positive reinforcing signal and dopamine as a negative reinforcing signal[29]. Serotonin, particularly that from NSM neurons, has been considered to be a positive, food-related signal in *C. elegans*: serotonin falls when *C. elegans* is removed from food, and its absence is associated with starvation-induced behaviours[9,10,14,16,30]. Our results identify another role of serotonin: in ADF neurons, serotonin is increased under noxious conditions of infection by pathogenic bacteria. The rapid transcriptional and post-transcriptional induction of serotonin after pathogen exposure provides a signal that could be used to modify various behaviours.

Serotonin in the mammalian intestine functions in signalling malaise, specifically the nausea associated with chemotherapy, by activating the 5-HT_3 receptor, a serotonin-gated ion channel[31]. Our results indicate that MOD-1, a serotonin-gated ion channel of *C. elegans*, signals the presence of aversive intestinal pathogens. The similarity of these vertebrate and invertebrate pathways might result from an ancient role of serotonin in signalling between the viscera and the brain; 95% of the serotonin in the human body is made by intestinal cells, not neurons[31].

METHODS

Nematode strains, molecular biological methods, immunohistochemistry and statistics are described in the Supplementary Information.

Binary choice assays. Embryos were collected by bleaching and were grown at room temperature. 'Naive' animals were grown on a standard nematode growth medium (NGM) plate that was evenly spread with 300 µl of *E. coli* OP50 suspension. For training, a suspension of ~200 µl of the test bacteria was spread on a plate and ~50 µl of OP50 suspension was used to make a small lawn on the side. Plates were incubated at 26 °C for 48 h before use.

The two-choice olfactory preference assays were based on standard chemotaxis assays[6] except that bacterial suspensions were used as odour sources (Fig. 1a). Bacteria grown overnight in NGM at 26 °C were resuspended at an absorbance of 1.0 at 600 nm, and 25 µl of each bacterial suspension was spotted onto the plate and air-dried for 5 h at room temperature. Animals were washed twice in S-basal buffer and once in assay buffer, and 50–200 animals were placed near the centre of the plate, equidistant from the two bacteria. Animals were allowed to move freely for 1–2 h before being immobilized by 1 µl of 10 mM

sodium azide applied at the bacteria spots. In most cases, animals quickly entered one lawn and remained there for the duration of the assay.

Microfabrication and four-choice maze assays. We fabricated microdevices using the PDMS rapid prototyping technique[32]. Photolithography masks were laser-printed on emulsion films with 5,080 d.p.i. resolution and used to produce prototype masters in a photo-patternable epoxy resin (SU-8-50, Microchem) on silicon wafers by ultraviolet photolithography. Masters were silanized by vapour-phase tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (United Chemical Technologies). PDMS devices were micro-moulded using two-part Sylgard 184 silicone elastomer (Dow Corning). Small holes were punched out above the decision area and microwells for loading *C. elegans* and bacteria. A maze was placed on an assay plate immediately before bacteria suspensions were spotted onto the plate. Bacteria were prepared as in the two-choice assays, except that suspensions were 10 times as concentrated for *S. marcescens* and 20 times as concentrated for the other bacteria. We used 1.25 μl of bacteria suspension in each chamber.

An attractive learning index for OP50 (G1) was calculated as (percentage of animals at $G1)_{trained}$–(percentage of animals at $G1)_{naive}$. An aversive learning index for PA14 (B1) was calculated as (percentage of animals at $B1)_{trained}$–(percentage of animals at $B1)_{naive}$. An attractive learning index for *P. fluorescens* (G2) and an aversive learning index for *S. marcescens* (B2) were calculated in the same way as described for the OP50 and PA14 training.

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