1   **A collective modulatory basis for multisensory integration in *C. elegans***

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Abstract

In the natural environment, animals often encounter multiple sensory cues that are simultaneously present. The nervous system integrates the relevant sensory information to generate behavioral responses that have adaptive values. However, the signal transduction pathways and the molecules that regulate integrated behavioral response to multiple sensory cues are not well defined. Here, we characterize a collective modulatory basis for a behavioral decision in *C. elegans* when the animal is presented with an attractive food source together with a repulsive odorant. We show that distributed neuronal components in the worm nervous system and several neuromodulators orchestrate the decision-making process, suggesting that various states and contexts may modulate the multisensory integration. Among these modulators, we identify a new function of a conserved TGF-β pathway that regulates the integrated decision by inhibiting the signaling from a set of central neurons. Interestingly, we find that a common set of modulators, including the TGF-β pathway, regulate the integrated response to the pairing of different foods and repellents. Together, our results provide insights into the modulatory signals regulating multisensory integration and reveal potential mechanistic basis for the complex pathology underlying defects in multisensory processing shared by common neurological diseases.

Author Summary

The present study characterizes the modulation of a behavioral decision in *C. elegans* when the worm is presented with a food lawn that is paired with a repulsive smell. We show that multiple sensory neurons and interneurons play roles in making the decision. We also identify several modulatory molecules that are essential for the integrated decision when the animal faces a choice between the cues of opposing valence. We further show that many of these factors, which often represent different states and contexts, are common for behavioral decisions that

40   integrate sensory information from different types of foods and repellents. Overall, our results

41   reveal a collective molecular and cellular basis for integration of simultaneously present

42   attractive and repulsive cues to fine-tune decision-making.

Introduction

An environment is often represented by numerous sensory cues. For example, a tainted food source can produce both attractive and repulsive odorants. In order to better survive, an animal often needs to detect and process simultaneously present sensory cues to make a behavioral decision [1-8]. Because integrating multiple sensory cues generates a more accurate evaluation of the environment, it provides important adaptive values. Multisensory integration is widely observed in both the vertebrate and invertebrate animals. Previous studies using behavioral and psychophysical approaches show that humans and other organisms can integrate an array of sensory stimuli to generate decisions in every-day life [9-11]. One common characteristic of multisensory behavioral responses and decision-making processes is their ability to be modulated by various internal states and contexts, including arousal, sleepiness versus wakefulness, the motivational or nutritional state of the organism, or the level of the reward paired with the stimuli. Neurotransmitters, such as dopamine, serotonin, glutamate, and neuropeptides, mediate many of these neurological effects on decision-making [3, 4, 12-14]. Intriguingly, patients of several neurological diseases, including autism spectrum disorder, Parkinson's disease, bipolar disorder, depression, schizophrenia, and gambling behaviors, share deficits associated with sensory processing or decision-making when encountering multiple sensory stimuli that evoke certain behavioral choices under normal conditions [15-21]. Together, these studies reveal multisensory integration as a common neuronal and behavioral process modulated by multiple contexts across the animal kingdom and highlight the importance of understanding the underlying mechanisms in normal as well as disease states.

Despite the importance of multisensory integration in animal behavior, our understanding of the underlying signaling mechanisms remains preliminary. The nematode *C. elegans* provides an opportunity to address the question. *C. elegans* feeds on bacteria. A bacterial lawn provides

68   various types of sensory information, including olfactory, gustatory, mechanical, and gaseous

69   cues. The small nervous system (302 neurons) of *C. elegans* generates sensorimotor

70   responses to these modalities [22-33] and many of the responses can be shaped by external

71   and internal contexts that modulate neural activities [4, 34-38]. The *C. elegans* genome encodes

72   the homologues of about 50% of the molecules expressed in the mammalian brains [39], which

73   in combination with a well-defined wiring diagram of the nervous system [40] allows

74   characterizing of the molecular and circuit basis for multisensory integration during decision-

75   making.

76

77   Here, we show that *C. elegans* integrates the information from an attractive food lawn and a

78   simultaneously present repellent to generate a decision on leaving. We show that the decision

79   to leave the lawn depends on the attractiveness of the lawn and the concentration of the

80   repellent. We identify specific neurons and a collection of modulatory molecules that promote or

81   suppress the food-repellant integration underlying the lawn-leaving decision. We further

82   demonstrate that the battery of modulatory molecules and neurons act as common modulators

83   to regulate integrated decisions on different foods paired with different repellants. These

84   findings identify conserved neuronal signals that modulate multisensory processing during

85   decision-making and reveal a collective modulatory basis for multisensory integration.

86

Results

88   ***C. elegans* integrates multiple sensory cues to generate a behavioral decision**

89   To establish a behavioral assay for multisensory integration in *C. elegans*, we presented a

90   repulsive odorant, 2-nonanone, to the animals on a small lawn of the *E. coli* strain OP50 (Figure

91   1A and Experimental Procedures) and assessed the decision of the animals to stay on or leave

92   the lawn over time. Because the OP50 lawn serves as a food source for the worm, under the

93 standard condition *C. elegans* stays on the lawn and leaves only at a low frequency [41, 42].

94 Meanwhile, 2-nonanone strongly repels *C. elegans* at concentrations ranging from 10% to

95 100%. The olfactory sensory neuron AWB detects and mediates the avoidance of 2-nonanone

96 [43-45]. We first presented a drop of 10% 2-nonanone close to the edge of an OP50 lawn, on

97 which 15 - 25 young adults acclimatized for one hour (Figure 1A). We found that within a few

98 minutes the animals migrated to the side of the bacterial lawn away from 2-nonanone, stayed on

99 the edge of the lawn before dispersing throughout the lawn without leaving (Figure 1B). This

100 result indicates that *C. elegans* is able to detect and avoid 10% 2-nonanone even on the food

101 lawn, but the repulsion is not strong enough to suppress the retention of the worm by the food

102 lawn. In contrast, when we presented a drop of higher concentration of 2-nonanone to the

103 worms in the same configuration, the worms migrated to the side of the lawn, started to leave

104 the food lawn in a few minutes, and continued to migrate to the edge of the plate away from the

105 repellent within the one-hour time window of the assay (Figure 1B, 1C and S9 Movie). The food-

106 leaving behavior was robustly evoked with 100% 2-nonanone (Figure 1B-1E), under which

107 condition a significant number of worms already left the lawn after 2-nonanone was presented to

108 the worms for 15 minutes (Figure 1C). In addition, it took a similar amount of time for the worms

109 to reach the edge of the lawn that was paired with either 10% or 100% 2-nonanone (Figure 1D).

110 These results show that *C. elegans* integrates the attraction of a food lawn with the repulsion of

111 2-nonanone to generate a behavioral decision and that increasing concentration of 2-nonanone

112 enhances lawn leaving (Figure 1B and 1C). These findings are consistent with the general rule

113 that governs multisensory integration, where increasing the reliability of a sensory cue, such as

114 increasing the concentration of 2-nonanone, strengthens the weight of the cue in integration

115 [46]. To characterize the regulatory mechanisms underlying multisensory integration, we used

116 100% 2-nonanone as the repellent for the rest of the study unless otherwise described. We

117 quantified the percentage of the worm outside the OP50 lawn 15 minutes after the assay began,

118 because it was an early time point when wild type started to show a robust leaving decision.

119

120 **Sensory neurons that regulate multisensory integration**

121 To characterize how the nervous system regulates the integrated response to the attractive

122 OP50 lawn and the repulsive odorant 2-nonanone, we first probed the amphidal sensory

123 neurons AWB, which mediate avoidance of 2-nonanone via the function of the cGMP-gated

124 channel subunit *tax-2* [43]. Exposure to 2-nonanone suppresses the intracellular calcium

125 transients of AWB [44, 45]. Consistently, we found that the transgenic animals that selectively

126 expressed a hyperactive form of an amiloride-sensitive sodium channel MEC-4 that generated

127 necrosis of AWB [43, 47] did not leave the OP50 lawn when 2-nonanone was present (Figure

128 2A) and that many of the worms remained diffusely distributed on the food lawn by the end of

129 the assay. These transgenic animals were defective in avoiding 2-nonanone in the standard

130 chemotaxis assay (S1 Table and S5 Figure), consistent with previous findings [43]. AWB-killed

131 animals also spent more time to reach the edge of the OP50 lawn when 2-nonanone was

132 present (S2 Table), consistent with the role of AWB in mediating the avoidance of 2-nonanone.

133 Meanwhile, the transgenic animals with genetically killed AWB stayed on OP50 lawn similarly as

134 wild type when 2-nonanone was not present (S3 Table). Together, these results show that AWB

135 regulates the integrated response by mediating the response to the unisensory repellent 2-

136 nonanone.

137

138 Next, we sought additional sensory neurons that regulated the integrated behavioral decision.

139 Previous studies identify several sensory neurons that respond to the smell of the *E. coli* strain

140 OP50 or mediate the behavioral response to the presence or removal of food [22, 44, 48-51]. To

141 examine the potential role of these sensory neurons in our multisensory integration paradigm,

142 we first tested a null mutation *ky4* in *odr-7,* which encoded a putative DNA-binding nuclear

143 receptor that specified the function of the AWA sensory neuron [52], a null mutation *p680* in

144 *che-1,* which encoded a zinc finger transcription factor required for the development and

145   function of the ASE sensory neuron [53], transgenic animals that selectively expressed a cell-

146   death activator EGL-1 [54] in the AQR, PQR and URX neurons or the CO_2-sensing BAG

147   sensory neuron [28-30, 55-57]. We also tested transgenic animals selectively expressing a cell-

148   death inducing caspase, or *twk-18(gf)* that encoded a constitutively active form of the potassium

149   channel TWK-18 [58], or tetanus toxin that eliminated the synaptic release [59] in the ASI, AWC,

150   ASJ, ADL or ASK neuron [49, 55, 60-64]. We found that all except three of the tested strains

151   were normal. The transgenic animals that contained genetically-killed ASK left the OP50 lawn

152   significantly faster than wild type, and the transgenic animals that contained genetically-killed

153   ASI or expressed the tetanus toxin in ADL left the OP50 lawn significantly more slowly than wild

154   type (Figure 2B-2D and S4 Table). Because the transgenic animals defective in the function of

155   ASK or ASI or ADL are not deficient in avoiding 2-nonanone in our standard chemotaxis assay,

156   in their ability to remain on OP50 lawn when 2-nonanone is not present, as well as in moving to

157   the edge of the OP50 lawn with the presence of 2-nonanone (S1-3 Tables), these results

158   together indicate that the sensory neurons ASK, ASI and ADL modulate how rapidly the

159   behavioral decision to leave the repellent-paired food lawn is made.

160

161   **Multisensory integration requires peptidergic and the TGF-β pathways**

162   To characterize the mechanisms underlying multisensory integration of food and 2-nonanone,

163   we examined mutants that were defective in biosynthesis of neurotransmitters. We tested

164   effects of mutating *tph-1* that encoded tryptophan hydroxylase required for the production of

165   serotonin [65], *cat-2* that encoded tyrosine hydroxylase needed for the synthesis of dopamine

166   [66], *tdc-1* that encoded tyrosine decarboxylase required for the synthesis of tyramine and

167   octopamine, or *tbh-1* that encoded tyramine beta hydroxylase required for the production of

168   octopamine [67]. Interestingly, all of these mutants exhibited wild-type behavioral decision when

169   they were exposed to 2-nonanone on an OP50 food lawn (Figure 3A-D and S6 Figure). These

170   results show that serotonin, dopamine, tyramine or octopamine are not required for 2-

171   nonanone-dependent food leaving, although these neurotransmitters regulate many food-

172   dependent sensorimotor responses ([32] and references therein).

173

174   Next, we examined the function of neuropeptide-encoding genes. We first found that mutations

175   in the *kpc-1(gk8)* and *egl-3(n150)* that disabled two of the four known peptide pre-processing

176   enzymes in *C. elegans* [68-70], delayed the decision to leave the food lawn that was paired with

177   2-nonanone (Figure 3E and 3F), suggesting the modulatory role of peptides or growth factors in

178   promoting the integrated decision to leave the tainted food lawn. Next, we screened many

179   mutations in genes encoding peptides or growth factors. We focused on the available mutations

180   that did not generate any gross defect in either development or locomotion and identified three

181   mutations that significantly altered the wild-type decision. The canonical mutations, *e1372*, or a

182   deletion, *ok3125*, in *daf-7* that encoded a TGF-β ligand that regulated development, metabolism

183   and host-pathogen recognition [71-73], significantly delayed the decision to leave the OP50

184   lawn tainted with 2-nonanone (Figure 3G and 3I). A deletion mutation *tm2984* in *nlp-7*, which

185   encoded a neuropeptide-like protein that regulated stress response, egg-laying, life span and

186   modulation of aversive responses to noxious stimuli [74-77], similarly delayed the decision to

187   leave the lawn (Figure 3H). However, the mutations in *daf-7 or nlp-7* did not generate any

188   detectable defect in the chemotactic response to 2-nonanone alone, or the tendency to leave

189   the OP50 lawn when no repellent was present, or the ability to move to the edge of the lawn

190   when 2-nonanone was present (S1-3 Tables). In addition, expressing the genomic fragment

191   containing the regulatory and coding regions of *daf-7* or *nlp-7* fully rescued the defect of the

192   respective mutant animals in making the decision to leave the lawn that was paired with 2-

193   nonanone (Figure 3I and 3J). Together, these results indicate that TGF-β/DAF-7 and NLP-7

194   promote the food-leaving decision when 2-nonanone is present.

195

196   **A new function of the TGF-β/DAF-7 canonical pathway in multisensory integration**

197   The *C. elegans* TGF-β/DAF-7 regulates several physiological processes through the conserved

198   type I and type II TGF-β receptor, DAF-1 and DAF-4, respectively [78, 79]. DAF-7 is found in the

199   sensory neurons OLQ, ADE and ASI, all of which are implicated in sensing bacteria [23, 71, 72,

200   80, 81]. DAF-7 produced by ASI regulates the satiety-induced quiescence, the entry into an

201   alternative developmental stage under the environmental stress, and the modulation of the

202   lifespan by dietary restriction, and responds to the abundance of food [72, 80-83]. The

203   expression of *daf-7* is induced in the sensory neuron ASJ upon exposure to pathogenic bacteria

204   and DAF-7 in ASJ regulates the avoidance of the pathogen through DAF-1 and DAF-4 receptors

205   [71]. In addition, through DAF-1 and DAF-4, DAF-7 regulates metabolism and fat accumulation

206   [73]. Here, we showed that mutating *daf-7* delayed the decision to leave the food lawn when 2-

207   nonanone was present (Figure 3G). To identify the source of the DAF-7 signal regulating

208   multisensory integration, we tested the transgenic animals that selectively expressed a wild-type

209   *daf-7* cDNA in subsets of *daf-7*-expressing neurons in *daf-7* mutant animals for potential

210   rescuing effects. We found that expressing *daf-7* selectively in ASI (*Pstr-3::daf-7* [71]) did not

211   rescue the defects in the integrated response; but expressing *daf-7* in ADE (*Pcat-2::daf-7*) or

212   OLQ (*Pocr-4::daf-7*) sensory neurons using cell-selective promoters [84-86] rescued the

213   delayed leaving phenotype in the *daf-7(e1372)* mutant animals (Figure 3K and 3L, S7 Figure).

214   In addition, we found that the canonical mutation in the type I and type II TGF-β receptor, *daf-*

215   *1(m40)*, similarly delayed the decision to leave the OP50 lawn paired with 2-nonanone (Figure

216   4A). Expressing either the genomic DNA fragment of *daf-1* or the *daf-1* cDNA selectively in the

217   interneurons RIM and RIC (*Pdaf-1::daf-1* or *Ptdc-1::daf-1;* [71, 73]) fully rescued the defect in

218   the *daf-1(m40)* mutant animals (Figure 4B and 4C), while expressing *daf-1* in sensory neurons

219   (*Pbbs-1::daf-1* or *Posm-6::daf-1*; [71]) was not sufficient to rescue (Figure 4D and S8 Figure).

220   Together, these results indicate that the TGF-β/DAF-7 signal produced by the ADE or the OLQ

221   sensory neurons acts through the type I TGF-β receptor DAF-1 in RIM and/or RIC neurons to

222   promote repellent-dependent leaving of the food lawn.

223

224 To further interrogate the role of the RIM/RIC neurons in multisensory integration, we examined

225 the transgenic animals that expressed a histamine-gated chloride channel in the RIM and RIC

226 neurons under the histamine-treated condition [87] or the transgenic animals that expressed

227 tetanus toxin [59] in RIM and RIC. We found that these transgenic animals were normal in

228 leaving the OP50 lawn when 2-nonanone was present (Figure 4E and 4F). Since neither the

229 *tdc-1(n3419)* mutant animals that lacked tyramine and octopamine nor the *tbh-1(n3247)* mutant

230 animals that lacked octopamine is defective in their decisions to leave the OP50 lawn paired

231 with 2-nanone (Figure 3), together, our results suggest that RIM/RIC and the release of the

232 neurotransmitter tyramine and octopamine from these neurons may be suppressed during the

233 integrated response to the simultaneously present food lawn and 2-nonanone. To further

234 interrogate the role of tyramine or octopamine signaling in the *daf-7-* and *daf-1*-dependent

235 integrated response, we tested how removing tyramine and/or octopamine affects the delayed

236 food leaving in the *daf-7(e1372) or daf-1(m40)* mutant animals. Interestingly, both of the *daf-*

237 *1(m40); tbh-1(ok1196)* and the *daf-7(e1372); tbh-1(ok1196)* double mutant animals [19]

238 behaved like the *daf-1(m40)* and the *daf-7(e1372)* single mutants, respectively (Figure 4G). In

239 contrast, the mutation in *tdc-1(ok914)* strongly suppressed the delayed decision phenotype in

240 both *daf-7(e1372)* and *daf-1(m40)* mutant animals (Figure 4H and 4I). While TDC-1 is needed

241 for the production of tyramine and octopamine in both RIM and RIC, TBH-1 is only needed for

242 the biosynthesis of octopamine in RIC [67]. Together, these results show that the TGF-β/DAF-7

243 regulates the decision between staying on a food lawn versus avoiding a repellent through the

244 canonical signaling pathway and that the DAF-7 peptidergic signal produced from ADE or OLQ

245 inhibits the tyramine neurotransmission of RIM and/or RIC to promote the decision to leave the

246 food-lawn that is paired with 2-nonanone.

247

248 **Different interneurons play opposite roles in multisensory integration**

249 To better characterize the neural circuits underlying multisensory integration, we probed the

250 potential interneurons that regulated the decision between staying on the food lawn versus

251 avoiding 2-nonanone. We focused on the interneurons AIY, AIB, and the command

252 interneurons, all of which regulate locomotion [22, 88]. AIY and AIB are also the major

253 interneurons postsynaptic to the sensory neurons that respond to the bacterial food or the

254 repellent 2-nonanone [40]. To disrupt the function of AIY, we selectively expressed in AIY a

255 gain-of-function isoform of a potassium channel TWK-18 [58] to inhibit the activity of AIY (*Pttx-*

256 *3::twk-18(gf)*) or the tetanus toxin (*Pttx-3::TeTx*) to block synaptic release. We also tested the

257 *ttx-3(mg158)* mutants that failed to develop AIY interneurons [89]. All three mutations delayed

258 the decision to leave the lawn (Figure 5A - 5C). However, these manipulations do not disrupt the

259 ability to reach the edge of the food lawn during 2-nonanone-dependent food leaving, to avoid

260 2-nonanone alone, or to stay on OP50 lawn when no repellent was present (S1-3 Tables). In

261 contrast, selectively expressing the tetanus toxin in the AIB interneurons or treating the

262 transgenic animals expressing the histamine-gated chloride channel in AIB with histamine did

263 not significantly alter the decision to leave the OP50 lawn that was paired with 2-nanonone

264 (Figure 5D and 5E). Together, these results indicate that the activity and the synaptic output of

265 the AIY interneurons promote the decision to leave the food lawn paired with 2-nonanone, while

266 AIB is dispensable for the decision-making. Next, we examined transgenic animals that

267 expressed the tetanus toxin with the *nmr-1* promoter or the *glr-1* promoter. The *nmr-1* promoter

268 is expressed in a few command interneurons including AVA, AVB, AVD, AVE and PVC, while

269 the *glr-1* promoter is expressed in several head motor neurons in addition to the *nmr-1-*

270 expressing interneurons [90]. Interestingly, both transgenic lines left the 2-nonanone paired food

271 lawn more than wild type (Figure 5F and 5G). However, these transgenic animals are normal in

272 2-nonanone avoidance in the absence of food or spontaneous food leaving. They also do not

273 reach the edge of the lawn more rapidly than wild type (S1-3 Tables). Together, these results

274 show that different downstream neurons modulate the decision to leave the repellent tainted

275   food lawn in opposite ways by promoting or inhibiting the decision-making process. These

276   neurons may act as the convergent sites to process multiple sensory signals in order to

277   generate specific behavioral outputs.

278

279   **Multisensory integration is regulated by a common set of modulators**

280   Next, we asked whether the molecular and circuit mechanisms underlying the integrated

281   response to the OP50 food lawn paired with 2-nonanone were shared by the integrated

282   responses to different pairing of attractive foods and repulsive odorants. We paired the OP50

283   lawn with various repellants, including 100% 1-octanol and 100% benzaldehyde. While

284   benzaldehyde is attractive at low concentrations [24], 100% benzaldehyde strongly repels *C.*

285   *elegans* in a way that is dependent on the function of the sensory neuron AWB [91-93]. We

286   found that a drop of 100% benzaldehyde first repelled the animals to the edge of the OP50 food

287   lawn and then in about 10-15 minutes started to repel the animals off the food lawn (Figure 6A

288   and S10 Movie). Interestingly, 1-octanol failed to stimulate food leaving under our experimental

289   conditions (Figure 6A). We also paired a lawn of *Comamonas sp* with 100% 2-nonanone.

290   *Comamonas* is an attractive food source for *C. elegans* [41]. We found that pairing a

291   *Comamonas* bacterial lawn with 100% 2-nonanone repelled *C. elegans* off the lawn similarly as

292   the OP50 lawn paired with 2-nonanone (Figure 7A). Interestingly, we found that several

293   modulators, particularly TGF-β/DAF-7, the TGF-β receptor DAF-1, and the sensory neurons

294   ASK, that regulated the integrated response to an OP50 lawn paired with 100% 2-nonanone

295   also similarly regulated the integrated response to OP50 lawn paired with 100% benzaldehyde

296   and the integrated response to the *Comamonas* lawn paired with 100% 2-nonanone (Figure 6

297   and 7). Together, these results indicate that a common set of modulators and signaling

298   mechanisms regulates integrated behavioral decision on whether to leave or stay on an

299   attractive food lawn paired with an odorant repellent.

300

Discussion

Many organisms can combine information from multiple simultaneously present sensory cues to regulate behavioral outputs [1-8, 32]. While the importance of integrated behavioral responses to multiple sensory stimuli is appreciated, the underlying molecular and signaling mechanisms are not well understood. Using our behavioral paradigm for multisensory integration, we characterize the modulators and signaling pathways that regulate a decision to leave a food lawn that is paired with a repulsive odorant. These findings reveal a new function of a conserved TGF-β that modulates decision-making by regulating the tyramine signal from a set of central neurons. Our results elucidate a set of common molecular and neuronal factors that mediate decision-making when the worm is presented with different pairs of stimuli generated by an attractive food and a repulsive odorant (Figure 8).

Specific sensory neurons regulate multisensory integration

One potential mechanism to regulate a coherent behavioral response to multiple simultaneously present sensory cues is to utilize sensory neurons that are capable of perceiving some or all of the cues. These types of sensory responses can involve either activation or inhibition of certain sensory neurons that detect distinct stimuli. Worms are capable of sensing both food signals and a range of repulsive cues ([32] and the references therein). Here, we characterize the functions of several sensory neurons in regulating the repellent-dependent food leaving when *C. elegans* is exposed to an attractive food lawn concurrently with a repulsive odorant. We confirm the requirement of the AWB sensory neuron that is known to sense repellents, including 2-nonanone and 100% benzaldehyde [43, 45, 91]. AWB also responds to bacterial food [44, 51]. Previous studies identify the role of AWB in promoting food leaving under malnourished conditions [94], suggesting the involvement of AWB in integrating the nutritional state with the

325   food signals. Thus, AWB may regulate the integrated response by simultaneously processing

326   food smells and repulsive odorants.

327

328   Interestingly, we also uncover a novel role of three sensory neurons that modulate the decision

329   to leave a food paired with a repulsive odorant. We show that the ASK sensory neuron

330   suppresses the integrated food-leaving decision, while the ASI and ADL sensory neurons

331   promote it. In contrast, we find that several other sensory neurons previously implicated in

332   mediating responses to food-related cues, including AWA, ASE, AWC, ASJ, BAG, AQR, PQR or

333   URX [44, 48, 49, 51, 71], are dispensable in regulating the integrated behavioral response to the

334   food lawn paired with 2-nonanone (S4 Table). In addition, our results suggest that the effect of

335   removing either ASK or ASI or ADL on the decision-making does not result from the altered

336   chemotactic response to 2-nonanone as a unisensory cue or to the OP50 bacterial lawn alone

337   (S1-3 Tables). These results together reveal a specific function of ASK, ASI and ADL in

338   integrating the food signals with the repellent to generate a decision between two sensory cues

339   of opposing valence.

340

341   Previous studies show that ASK and ASI sensory neurons respond to *E. coli* OP50 by changing

342   the intracellular calcium levels [51, 95]. Both ASI and ASK are involved in evaluating the food

343   environment. ASI also mediates the balance between food intake and fat storage, as well as

344   experience-dependent changes in food response [37, 73, 80-82, 96]. ASK regulates responses

345   to pheromones and plays a role in food leaving in mutants that are significantly food-deprived

346   and modulates locomotion during pre-exposure to unpredictable food environments [49, 63, 64,

347   94]. The ADL sensory neuron has been shown to modulate the responses to octanol,

348   pheromone and the preference for certain food odors [64, 77], suggesting that ADL mediates

349   various context-dependent sensory responses to modulate behavior and decision-making. In

350   our present study, we propose that ASI, ADL and ASK neurons represent the strength of the

351   food signals in an antagonistic manner to mediate a balanced behavioral decision between an

352   attractive food and a repulsive odorant.

353

354   **Neuropeptides and growth factors modulate multisensory integration**

355   Although previous studies characterize the function of neuromodulators, including

356   neuropeptides and growth factors, in modulating olfactory responses and nutrition-dependent

357   state of the nervous system, how neuromodulatory signals regulate a behavioral decision that

358   integrates cues of opposing values is not well understood. Here, we characterize the role of

359   neuromodulatory molecules, including a conserved TGF-β, in modulating the decision to leave a

360   food lawn when a repulsive odorant is presented together with the lawn.

361

362   Neuropeptides and growth factors have been implicated in the context-dependent modulation of

363   several sensorimotor responses in *C. elegans* [3, 34, 77, 96-98]. Here, we identify the

364   neuropeptidergic signaling mechanisms based on the examination of the neuropeptide

365   processing mutants, *egl-3* and *kpc-1*, and identification of the NLP-7 peptide and the TGF-

366   β/DAF-7 that modulate the decision to leave a food lawn paired with a repulsive odorant.

367   Mutating *nlp-7* or *daf-7* delayed the decision to leave the lawn. In contrast, mutating genes that

368   encode several other peptides that are expressed in different sensory neurons and have been

369   shown to signal contextual cues or previous experience or food signals, including *ins-6, ins-7,*

370   *nlp-1, nlp-9, nlp-24* and *flp-19* [62, 77, 96, 97, 99]*,* does not have a significant effect (S4 Table),

371   suggesting a specific function of *nlp-7* and *daf-7* in regulating the decision-making process. *nlp-*

372   *7* is expressed in several amphidial sensory neurons that respond to contextual cues and NLP-7

373   delays the acute avoidance of a noxious stimulus, 1-octanol [74-77, 100]. This effect is in

374   contrast with that of mutating *nlp-7* in the integrated behavioral response, where NLP-7

375   promotes the decision to leave the food in order to avoid the repulsive odorant. Our results

376   together with the previous findings characterize distinct functions of the NLP-7 neuropeptide in

377   regulating multisensory integration versus context-dependent avoidance of noxious stimuli.

378

379   Previous studies show that the DAF-7 pathway regulates dauer formation, food intake, fat

380   storage, as well as avoidance of pathogenic bacteria after prolonged exposure. The functions of

381   DAF-7 in these physiological events depend on its expression in the sensory neurons ASI

382   and/or ASJ [71-73]. Here, we show that DAF-7 promotes the decision to leave the food lawn

383   paired with a repulsive odorant and that different from its previously identified role, the function

384   of DAF-7 in regulating multisensory decision depends on the expression of *daf-7* in either the

385   ADE or the OLQ sensory neurons. Our results are the first to characterize the function of *daf-7*

386   produced by ADE or OLQ. ADE is one of the dopaminergic neurons in the worm nervous

387   system [101]. However, we did not see any phenotype in the *cat-2* mutants that were defective

388   in dopamine synthesis (Figure 3), suggesting that the function of ADE in regulating the

389   integrated response to food and repellent is independent of dopamine. Both ADE and OLQ have

390   been previously implicated in mechanosensation – OLQ is implicated in sensing the gentle

391   touch delivered to the nose and ADE contributes to the slowing response when a worm enters a

392   bacterial lawn [23, 31, 102]. We propose that ADE and OLQ regulate the integrated response to

393   a food lawn paired with a repellent by representing the mechanical stimulus that a worm senses

394   from the food lawn.

395

396   We further show that the canonical TGF-β receptor DAF-1 acts in the interneurons RIM and RIC

397   to regulate the decision to leave the food lawn paired with a repulsive odorant. Interestingly,

398   inhibiting the activity of RIM and RIC, or blocking the synaptic outputs of these neurons, or

399   disrupting the biosynthesis of the common neurotransmitter of these neurons, tyramine, does

400   not significantly change the decision-making process. However, disrupting the production of

401   tyramine, but not octopamine, in these neurons suppresses the slow-decision phenotype in the

402   *daf-1* or *daf-7* mutant animals (Figure 3 and 4). Together, these results indicate that the DAF-

403   7/DAF-1 pathway promotes the decision to leave by inhibiting the tyramine signaling from these

404   interneurons. This regulatory mechanism of DAF-7 is reminiscent of that in feeding, where DAF-

405   7 promotes the pumping rate by inhibiting the output from the RIM and/or RIC neurons.

406   However, different from the function of DAF-7 in regulating feeding that is dependent on

407   tyramine and/or octopamine [73], DAF-7 modulates the signal of tyramine, but not octopamine,

408   to regulate the decision to leave a food paired with the repellant 2-nanonone.

409

410   The RIM and RIC neurons have been previously implicated in various sensorimotor responses,

411   as well as the context-dependent locomotory and feeding behaviors [67, 73, 87, 100, 103]. Our

412   results that characterize RIM/RIC as the downstream neurons of the TGF-β /DAF-7 signal in

413   regulating the decision to leave a tainted food lawn further reveal RIM/RIC as one of the central

414   sites where different sensory signals converge to generate appropriate behavioral outputs.

415   Previously, TGF-β signals have been implicated in various neuronal functions, including learning

416   and memory, neural plasticity in the forms of LTP and LTD, synaptic formation, dendritic

417   development, and regulation of the function of the neural-muscular junctions [104-107]. Defects

418   in TGF-β pathways have been implicated in the pathology of neurological disorders, such as

419   schizophrenia, depression, anxiety and Alzheimer's disease [108-110]. Our work reveals a new

420   role for TGF-β signals in regulating decision-making, when sensory cues of opposing valance

421   are simultaneously present.

422

423   **Specific interneurons modulate 2-nonanone-dependent food leaving in *C. elegans*.**

424   The ability to integrate multiple types of sensory stimuli requires not only the responses across

425   peripheral sensory areas, but also the signal processing in downstream network of interneurons

426   [1, 3, 5-8, 111]. In *C. elegans*, a number of sensorimotor responses are modulated by specific

427   contexts via the functions of several interneurons [34, 97, 100, 112]. However, how interneurons

428   mediate decision-making during multisensory behavior is not fully characterized. Here, by

429   examining a number of interneurons that are downstream of the sensory neurons that detect the

430   food-related cues and the repulsive odorant, we find that the AIY interneuron and command

431   interneurons, as well as motor neurons, play a modulatory role in 2-nonanone-dependent food

432   leaving. Disrupting the function of AIY significantly delays the decision to leave the food paired

433   with 2-nonanone, without altering the response to either of the two cues that is presented alone.

434   The AIY interneuron receives synaptic inputs from sensory neurons that detect olfactory,

435   gustatory and thermal information. Previous studies implicate AIY in integrating simultaneously

436   present aversive and attractive cues in olfactory plasticity and in food and serotonin-dependent

437   modulation of sensorimotor responses [34, 97, 112-114]. We propose that AIY may act as an

438   integrating site that receives and processes signals from the food and the repellent 2-nonanone

439   during multisensory integration. In contrast, we did not detect a role for the interneuron AIB with

440   our assay, suggesting the functional diversity among the interneurons in modulating 2-

441   nonanone-dependent food leaving. Our study also implicates the *glr-1-* and *nmr-1*-expressing

442   neurons in promoting the repellent-dependent food leaving. It is conceivable that some of the

443   *nmr-1*-expressing command interneurons and the *glr-1*-expressing command interneurons or

444   head motor neurons may serve as the downstream-modulated targets for the integrated

445   behavioral response.

446

447   **A common set of modulators regulate repellent-dependent food leaving**

448   For freely feeding animals, such as *C. elegans,* appropriate behavioral responses to food

449   sources paired with other sensory cues are critical for survival, because food can be easily

450   contaminated with toxins. To understand to what extent the identified modulators generally

451   regulate integrated responses to foods and repellents, we paired the *E. coli* strain OP50 with

452   either 100% 2-nonanone or 100% benzaldehyde. We also paired 100% 2-nonanone with a

453   second food, *Comamonas.* Interestingly, we found that the TGF-β/DAF-7 pathway and the ASK

454   sensory neuron regulate the integrated responses to these two different pairs of foods and

455   repellents. Avoidance of both 100% 2-nonanone and 100% benzaldehyde depends on the

456   function of the olfactory sensory neurons AWB [43, 45, 91, 115]. Meanwhile, *Comamonas sp*

457   also serves as an attractive food source to the worms [41]. It is conceivable that a common set

458   of modulators represent the contexts where the worm needs to evaluate the opposing values

459   provided by a source of nutrients and a potential threat to generate a behavioral decision.

460

461   **Figure legends**

462   **Fig 1. *C. elegans* performs multisensory integration to leave food paired with a repulsive**

463   **odorant 2-nonanone.**

464   **(A)** A schematic of 2-nonanone-dependent food leaving assay.

465   **(B)** The time course for worms leaving OP50 lawn that is paired with 2-nonanone of different

466   concentrations over 60 minutes, n = 2 assays for 10% and n = 3 assays each for 30%, 50% and

467   100%.

468   **(C)** More worms leave the OP50 food lawn paired with 100% 2-nonanone (n = 4 assays) than

469   the OP50 lawn paired with 10% 2-nonanone (n = 5 assays). Bar graph represents the

470   percentage of worms outside the lawn 15 minutes after the assay starts.

471   **(D)** The time taken for worms to reach the edge of the OP50 food lawn when the lawn is paired

472   with either 10% or 100% 2-nonanone, n = 2 assays each.

473   **(E) I – IV,** Sample images of wild-type animals leaving an OP50 lawn that is paired with 100% 2-

474   nonanone at different time points of the 60-minute assay.

475   For **B-D**, Mean ± SEM, Student's *t* test, ** p $\leqslant$ 0.01, n.s., not significant.

476

477   **Fig 2. Several sensory neurons modulate 2-nonanone-dependent food leaving.**

478 **(A-D)** The transgenic animals that either lack the functional AWB sensory neuron by selectively

479 expressing the gain-of-function isoform of an amiloride-sensitive sodium channel MEC-4 (**A**,

480 *pAWB::mec-4(d)*, n = 5 assays each) or lack the ASI sensory neuron by expressing a cell death

481 promoting molecule caspase (**B**, *pASI::caspase*, n = 7 assays for wild type and 8 assays for the

482 transgenic animals) or are defective in the synaptic transmission of the ADL sensory neuron by

483 expressing the tetanus toxin (**D,** *pADL::TeTx*, n = 5 assays for wild type, 4 assays for the

484 transgenic animals, and 3 assays for non-transgenic siblings) display a delayed decision to

485 leave the OP50 lawn paired with 100% 2-nonanone; while the transgenic animals that express

486 caspase in the ASK sensory neuron (**C**, *pASK::caspase*, n = 9 assays each) display a faster

487 decision to leave.

488 Each bar graph reports the average percentage of worms outside the lawn 15 minutes after the

489 assay starts. Mean ± SEM, Student's *t-t*est, * p ⩽ 0.05, ***p ⩽ 0.001.

490

491 **Fig 3. NLP-7 and TGF-β/DAF-7 modulate the decision to leave the OP50 food lawn paired**

492 **with 2-nonanone.**

493 **(A-D)** The mutant animals that are defective in the biosynthesis of the neurotransmitter

494 serotonin (**A,** *tph-1(mg280)*, n = 2 assays each), or dopamine (**B,** *cat-2(e1112),* n = 4 assays

495 each), or tyramine and octopamine (**C,** *tdc-1(n3419),* n = 2 assays each), or octopamine (**D,** *tbh-*

496 *1(n3247),* n = 3 and 4 assays for wild type and *tbh-1* mutants, respectively) display a normal

497 decision to leave the OP50 food lawn that is paired with 2-nonanone.

498 **(E-K)** Mutations in the genes encoding the neuropeptide processing enzymes, *kpc-1* (**E,** n = 4

499 and 5 assays for wild type and *kpc-1* mutant, respectively), or *egl-3* (**F**, n = 4 and 6 assays for

500 wild type and *egl-3* mutant, respectively), or a TGF-β -encoding gene *daf-7* (**G,** n = 6 and 5

501 assays for wild type and *daf-7* mutant, respectively), or a neuropeptide-encoding gene *nlp-7* (**H,**

502 n = 7 and 8 assays for wild type and *nlp-7* mutant, respectively) generate a delayed decision to

503   leave the OP50 food lawn paired with 2-nonanone, and expressing the genomic DNA of *nlp-7* (**I,**

504   n = 6, 7 and 4 assays for wild type, transgenic animals and non-transgenic siblings,

505   respectively**)** or *daf-7* (**J,** n = 4 assays each for wild type, transgenic animals and non-transgenic

506   siblings) rescues the delayed food leaving phenotype of the respective mutant animals.

507   **K)** Expressing the wild-type *daf-7* cDNA in the sensory neurons ADE rescues the delayed

508   decision in the *daf-7(e1372)* mutant animals, n = 4 assays each for wild type, transgenic

509   animals and non-transgenic siblings, respectively.

510   **(L)** Expressing the wild-type *daf-7* cDNA in the sensory neurons OLQ also rescues the delayed

511   decision in the *daf-7(e1372)* mutant animals, n = 3 assays for wild type, 3 assays for transgenic

512   animals and 2 assays for non-transgenic siblings, respectively.

513   Each bar graph reports the average percentage of worms outside the lawn 15 minutes after the

514   start of the assay, mutants are compared with wild-type animals and transgenic animals are

515   compared with non-transgenic siblings using Student's *t-te*st, * $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq$

516   0.001, n.s., not significant.

517

518   **Fig 4. The TGF-β receptor DAF-1 acts in the RIM and RIC neurons to mediate 2-**

519   **nonanone-dependent food leaving.**

520   **(A-D)** Mutating *daf-1* that encodes the type I TGF-β receptor delays the decision to leave the

521   OP50 lawn paired with 100% 2-nonanone (**A,** n = 8 assays each), and expressing the genomic

522   DNA of *daf-1* (**B,** n = 6 assays each) or a wild-type *daf-1* cDNA in the RIM and RIC neurons (**C,**

523   n = 6, 6 and 5 assays for wild type, transgenic animals and non-transgenic siblings,

524   respectively) in the *daf-1(m40)* mutant animals rescues the delayed decision, but expressing

525   wild-type *daf-1* in the sensory neurons (**D**, n = 3, 5 and 2 assays for wild type, transgenic

526   animals and non-transgenic siblings, respectively) does not rescue. Mutants are compared with

527   wild type and transgenic animals are compared with non-transgenic siblings with Student *t*-test.

528   **(E-F)** Inhibiting the activity of the RIM and RIC neurons by selectively expressing a histamine-

529   gated chloride channel (**E,** n = 2 and 4 assays for wild type and transgenic animals,

530   respectively) or blocking the synaptic release from these neurons by selectively expressing the

531   tetanus toxin (**F,** n = 2 assays each) does not alter the decision to leave the OP50 food lawn

532   paired with 100% 2-nonanone. Transgenic animals are compared with wild type.

533   **(G-I)** Removing octopamine signaling in the *daf-7(e1372)* or *daf-1(m40)* mutants with a mutation

534   that disrupt biosynthesis of octopamine *tbh-1(ok1196)* does not suppress the delayed leaving

535   from the OP50 lawn paired with 100% 2-nonanone (**G,** n = 6 assays for wild type; n = 4 assays

536   for *daf-7* mutants; n = 2 assays for *daf-1* mutants; n = 3 assays for *daf-7;tbh-1* double mutants;

537   n = 2 assays for *daf-1;tbh-1* double mutants), but removing the tyramine and the octopamine

538   signals with the mutation in *tdc-1(ok914)* in either the *daf-7(e1372)* (**H,** n = 6, 5 and 4 assays for

539   wild type, *daf-7* mutants and *daf-7;tdc-1* double mutants, respectively) or the *daf-1(m40)* (**I,** n =

540   5, 4 and 5 assays for wild type, *daf-1* mutants and *daf-1;tdc-1* double mutants, respectively)

541   mutant animals suppresses the delay-decision phenotype in either of the mutant animals.

542   Double mutants were compared with the respective single mutants using student's *t* test.

543   Each bar graph reports the average percentage of worms outside the lawn 15 minutes after the

544   start of the assay. Mean ± SEM, ** p $\leqslant$ 0.01, *** p $\leqslant$ 0.001, n.s.; not significant.

545

546   **Fig 5. Downstream circuit that regulates 2-nonanone-dependent food leaving**

547   **(A-C)** Inhibiting the activity of the AIY interneuron by expressing the gain-of-function isoform of

548   the potassium channel TWK-18 (**A,** *Pttx-3::twk-18(gf)*, n = 3 assays each) or by blocking the

549   synaptic outputs of AIY by expressing tetanus toxin (**B,** *Pttx-3::TeTx,* n = 4 assays each), or the

550   mutation *ttx-3(mg158)* that generates development defects in AIY (**C,** n = 3 assays each) delays

551   the decision to leave the OP50 lawn paired with 100% 2-nonanone.

552   **(D, E)** Selectively expressing tetanus toxin (**D,** *Pinx-1::TeTx*, n = 2 assays each) or the inhibitory

553   HisCl channel (**E,** *Pinx-1::HisCl*, n = 3 and 4 assays for wild type and transgenic animals,

554   respectively) in the AIB interneuron does not significantly alter the lawn-leaving decision, when

555   the OP50 lawn is paired with 100% 2-nonanone.

556   **(F, G)** Blocking synaptic outputs from the *nmr-1*-expressing neurons (**F,** *Pnmr-1::TeTx*, n = 3

557   assays each) or the *glr-1*-expressing neurons (**G,** *Pglr-1::TeTx*, n = 4 and 3 assays for wild type

558   and transgenic animals, respectively) enhanced the 2-nonanone-dependent lawn leaving.

559   Each bar graph reports the average percentage of worms outside the lawn 15 minutes after the

560   start of the assay. Mean ± SEM, mutants are compared with wild-type animals with student's *t*

561   test, transgenic animals are compared with non-transgenic siblings with student's *t*-test, * p $\leqslant$

562   0.05, ** p $\leqslant$ 0.01, *** p $\leqslant$ 0.001, n.s., not significant.

563

564   **Fig 6. Integrated response to a repellent-paired food lawn is regulated by a common set**

565   **of factors.**

566   **(A)** Wild-type animals also leave the lawn of OP50 paired with 100% benzaldehyde; in contrast,

567   paring an OP50 lawn with either 100% octanol does not repel worms (n = 2 assays for each

568   condition).

569   **(B-E)** Genetic ablation of the sensory neuron AWB (**B,** *pAWB::mec-4(d)*, n = 3 assays each) or

570   ASK (**C,** *pASK::caspase*, n = 3 assays each) or mutating the genetic components of the TGF-

571   β/DAF-7 pathway (**D,** *daf-7(e1372)*, n = 3 assays each; **E,** *daf-1(m40)*, n = 4 assays each) alters

572   the decision to leave the benzaldehyde-paired OP50 lawn.

573   Each bar graph reports the average percentage of worms outside the lawn 25 minutes (**B, D, E**)

574   or 5 minutes (**C**) after the start of the assay. Mean ± SEM, mutants are compared with wild-type

575   animals with Student's *t* test, ** p $\leqslant$ 0.01, *** p $\leqslant$ 0.001, n.s., not significant.

576

577 **Fig 7. Integrated response to a repellant-paired food lawn requires a common set of**

578 **factors.**

579 **(A-D)** Genetic ablation of the sensory neuron AWB (**A,** *pAWB::mec-4(d)*, n = 2 assays each) or

580 ASK (**B,** *pASK::caspase*, n = 2 assays each), or mutating the genetic components of the TGF-

581 β/DAF-7 pathway (**C,** *daf-7(e1372)*, n = 4 assays each; **D,** *daf-1(m40),* n = 2 assays each) alters

582 the decision to leave the 2-nonanone-paired *Comamonas* lawn.

583 Each bar graph reports the average percentage of worms outside the lawn 25 minutes (**A**) or 20

584 minutes (**B-D**) after the start of the assay. Mean ± SEM, mutants or transgenic animals are

585 compared with wild type with Student's *t* test, * p $\leqslant$ 0.05, ** p $\leqslant$ 0.01.

586

Methods

587

588 **Strains**

589 *C. elegans* strains were cultivated under the standard conditions [116]. Hermaphrodites were

590 used in this study. The strains that were used in the study include: PR680 *che-1(p680)I,*

591 CX14394 *npr-5(ok1583)V*, MT15434 *tph-1(mg280)II,* CB1112 *cat-2(e1112)II, MT9455 tbh-*

592 *1(n3247)X, RB1161 tbh-1(ok1196)X, RB993 tdc-1(ok914)II, MT13113 tdc-1(n3419)II*, DR40 *daf-*

593 *1(m40)IV, PR691 tax-2(p691)I, PR671 tax-2(p671)I*, RB859 *daf-22(ok693)II*, OH8 *ttx-*

594 *3(mg158)X, MT150 egl-3(n150)V*, CX4 *odr-7(ky4)X, CX03572 nlp-9(tm3579)V*, ZC2685 *npr-*

595 *2(ok419)IV,* VC48 *kpc-1(gk8)I,* RB1341 *nlp-1(ok1470)X,* RB1289 *npr-18(ok1388)X,* CB1372

596 *daf-7(e1372)III,* ZC2673 *gcy-33(ok232)V,* SM2322 *daf-7(ok3125)III,* AX1295 *gcy-35(ok769)I,*

597 QZ81 *ins-6(tm2416)II,* QZ126 *ins-7(tm2001)IV,* FX02105 *nlp-24(tm2105)V,* RB1902 *flp-*

598 *19(ok2460)x,* CX10 *osm-9(ky10)IV,* FX02984 *nlp-7(tm2984)X,* RB1161 *tbh-1(ok1196)X,* RB993

599 *tdc-1(ok914)II,* KQ361 *tdc-1(ok914)II; daf-7(e1372)III,* KQ363 *tdc-1(ok914)II; daf-1(m40)IV,*

600 KQ364 *daf-1(m40)IV; tbh-1(ok1196)X,* KQ362 *daf-7(e1372)III; tbh-1(ok1196)X,* ZC1952

601 *yxIs25[Pttx-3::TeTx::mCherry; Punc-122::gfp],* KQ280 *daf-1(m40)IV; ftEx98[Pdaf-1::daf-1::gfp;*

602   *Podr-1::dsRed]*, KQ380 *daf-1(m40)IV; ftEx205[Ptdc-1::daf-1::gfp; Podr-1::dsRed]*, KQ252 *daf-*

603   *1(m40)IV; ftEx70[Pbbs-1::daf-1::gfp; Podr-1::dsRed]*, ZD736 *daf-7(ok3125)III;qdEx44[Pstr-*

604   *3p::daf-7; Pges-1::gfp]*, ZD729 *daf-7(ok3125)III;qdEx37[Pdaf-7::daf-7; Pges-1::gfp]*, PY7502

605   *yxIs34[Pceh-36▽::TU813; Pceh-36▽::TU814; Psrtx-1::gfp; Punc-122::dsRed]*, ZC2393

606   *yxEx1248 [Pttx-3::twk-18(gf)::mCherry; Punc-122::RFP]*, CX14848 *kyEx4866[Pinx-*

607   *1::HisCl1::SL2mCherry; Punc-122::dsRed]*, CX16040 *kyEx5464[Ptdc-1::HisCl1::SL2mCherry]*,

608   ZC1451 *yxEx699[Pnmr-1::TeTx::mCherry; Punc-122::dsRED]*; QS4 *qrIs2[Psra-9::mCasp1;*

609   *Psra-9::gfp; Pelt-2::gfp]*, PS6025 *qrIs2[Psra-9::mCasp1; Psra-9::gfp; Pelt-2::gfp]*; ZC1552

610   *yxEx749[Pglr-1::TeTx::mCherry; Punc-122::gfp]*, PY7505 *oyIs84[Pgpa-4::TU813; Pgcy-*

611   *27::TU814; Pgcy-27::gfp; Punc-122::dsRed]*, CX3830 *kyIs102V; kyIs104[Pstr-1::mec-4(d); Pstr-*

612   *1::gfp]*; CX14637 *kyEx4779[Pinx-1::TeTx::mCherry; Punc-122::gfp]*, CX14993 *kyEx4962[Ptdc-*

613   *1::TeTx::mCherry]*, AX2051 *Ex[Pgcy-33::egl-1; Punc-122::dsRed]*, CX12330 *Ex[Psre-*

614   *1::TeTx::mCherry; Punc-122:RFP]*, CX7102 *lin-15B(n765)X; qaIs2241[Pgcy-36::egl-1; Pgcy-*

615   *35::gfp; lin-15(+)]*, ZC2752 *nlp-7(tm2984)X; yxEx1420[Pnlp-7::nlp-7; Punc-122::gfp]*, ZC2731

616   *daf-7(e1372)III; yxEx1409[Pcat-2::daf-7; Punc-122::gfp]*; ZC2734 *daf-7(e1372)III,*

617   *yxEx1412[Pocr-4::daf-7; Punc-122::gfp]*

618

619   **Behavioral assay for multisensory integration**

620   On a 5 cm-diameter NGM (Nematode Growth Medium) plate, 20-25 young adult worms were

621   placed on a small 1 cm-diameter round-shaped bacterial lawn made of freshly cultivated *E. coli*

622   OP50 strain and left to acclimatize on the lawn for 1-2 hour. Next, a drop of 1 µl 2-nonanone

623   (Sigma Aldrich, Cat # 821-55-6), either 10% (v/v in 100% ethanol) or 100%, was placed on the

624   right-hand side of the lawn and 3 mm away from the lawn. The number of worms on the lawn

625   was counted every 5 minutes for a total of 60 minutes, and the percentage of worms outside the

626   lawn was calculated (Figure 1A and 1B). In some assays, 1 µl of 100% benzaldehyde (Sigma

627   Aldrich, Cat # 100-52-7) was used, instead of 2-nonanone. The OP50 culture was prepared

628   freshly each day by culturing at 27°C for 12-15 hours in NGM medium. For assays using

629   *Comamonas sp* for the food lawn, the experiments were performed in the same way, except

630   that the bacterial strain was cultured with Luria Broth. To determine the time taken to reach the

631   edge of the food lawn, the food lawn was divided into 5 columns with each being 2 mm wide (S5

632   Fig). The time taken for 90% of the worms to crawl into the column furthest away from the

633   repellent was recorded. Mutants were compared with wild-type animals tested in parallel, and

634   transgenic animals were compared with non-transgenic siblings or wild-type animals tested in

635   parallel on the same day.

636

637   The bar graphs in the figures report the percentage of worms outside the lawn at the time point

638   when the significant difference between the tested genotypes was first observed. When there

639   was no significant difference, the bar graphs report the percentage of worms outside the lawn

640   15 minutes after the start of the assay.

641

642   **Transgenes and transgenic animals**

643   To generate a *nlp-7* genomic rescue fragment, a 4.7 kb PCR product was amplified from

644   genomic DNA that included 2.5 kb 5' upstream sequence, the *nlp-7* coding region, and 1 kb 3'

645   downstream sequence (NLP-7F: 5'-CATGTTTTTGATCATTTTCGAAC-'3 and NLP-7R3'UTR: 5'-

646   AATATCGTATGCCAACTTGAAC-'3). The *nlp-7* genomic PCR product was injected into the *nlp-*

647   *7(tm2984)* animals. To generate the construct expressing a wild-type *daf-7* cDNA in the OLQ or

648   ADE sensory neurons, the *daf-7* cDNA was amplified from PJM016 (Gift from Dr. Dennis Kim

649   and Dr. Joshua Meisel [71]). The *daf-7* cDNA product was cloned into a gateway destination

650   vector that contained an *unc-54* 3'UTR using the Nhe-1 and Kpn-1 sites. Both the promoter

651   regions of *ocr-4* (4.0 kb promoter for expression in OLQ) and *cat-2* (1.1 kb promoter for

652   expression in ADE) were amplified from genomic DNA (CAT-2F:

653   CTAGCAGGCCCAATCTTTTCTG and CAT-2R: TCCTCTTCCAATTTTTCAAGGGGT/OCR-4F:

654   5'-TTCTAATATTGCTCCATCAAC-'3 and OCR-4R: 5'-TAATACAAGTTAGATTCAGAGAATA-'3)

655   and cloned into the entry-TOPO vector PCR8 (Invitrogen). The expression clones, *Pcat-2::daf-7*

656   and *Pocr-4::daf-7,* were generated using LR recombination reactions (Invitrogen). Each

657   transgene was injected at 30-50 ng/µl with the co-injection marker as previously described

658   [117].

659

Lawn-leaving assay

661   Lawn-leaving assay was performed and analyzed similarly as the assay for multisensory

662   integration, except that no repulsive chemical was present. Briefly, animals were placed on a 1

663   cm-diameter round-shaped bacterial lawn of OP50 and left for 10 minutes to acclimatize before

664   examining food leaving over a period of one hour by counting the number of worms that were

665   present on the food lawn every 5 minutes for a total of 60 minutes.

666

2-nonanone avoidance assay

668   To examine the avoidance of 2-nonanone, chemotaxis assays were performed essentially as

669   previously described [43]. Briefly, animals were placed in the center of a square plate that was

670   divided into sectors A - F and 2 drops of 1 µl of 2-nonanone was added to one side and 2 drops

671   of 1 µl ethanol was added to the opposite side of the plate as control. Approximately 100 worms

672   were used in each assay. Chemotactic avoidance was analyzed by counting the number of

673   worms in the sectors A-B, C-D and E-F with E-F being furthest away from the 2-nonanone point

674   sources (S5 Fig). The avoidance index was calculated as the number of animals in sectors A

675   and B minus the number of animals in the sectors E and F and normalized with the total number

676   of animals in all 6 sectors on plate.

677

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683

684   **The authors declare no competing interest.**

685

686   **References**

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1085

Supporting information

1087   **S1 Table. 2-nonanone avoidance assay.**

1088   Wild type, mutants and transgenic animals are examined for avoiding 100% 2-nonanone as

1089   previously described (Troemel et al., 1997 and S5 Figure). Avoidance Index was calculated as

1090   described in S5 Figure. The avoidance in each genotype is represented by the average

1091   avoidance index of individual assays. n = 2 – 4 assays each genotype, 75-100 animals tested in

1092   each assay, mutants and transgenic animals are compared with wild-type animals or the non-

1093   transgenic siblings tested on the same days with student's t test, Mean ± SEM.

1094

1095   **S2 Table. Time to reach the edge of the food lawn during multisensory integration**

1096   Wild type, mutants and transgenic animals are examined for the time taken to reach the edge of

1097   the food lawn away from the repellent. The average time taken for 90% of the worms in one

1098   assay to reach the edge of a *E. coli* OP50 food lawns during exposure to 100% 2-nonanone is

1099   presented for each genotype (Experimental Procedures). n = 2 – 4 assays for each genotype,

1100   20-25 animals tested in each assay, mutants or transgenic animals are compared with wild-type

1101   animals or non-transgenic siblings tested on the same days with student's t test, Mean ± SEM.

1102

1103 **S3 Table. Spontaneous food leaving from an OP50 lawn without pairing with 2-nonanone.**

1104 Wild type, mutant animals and transgenic animals are examined for food leaving for 1 hour.

1105 Young adult worms are placed on an OP50 food lawn for 1 hour and the number of worms on

1106 food lawn is counted every 5 minutes for a total assay time of 60 minutes. The percentage of

1107 worms off the food lawn at 15 minutes is reported. n = 2 – 4 assays for each genotype and 20-

1108 25 worms in each assay, mutants or transgenic animals are compared with wild-type animals

1109 tested in parallel with student's *t* test, Mean $\pm$ SEM.

1110

1111 **S4 Table. Many signaling mutants show no phenotype in 2-nonanone-dependent food**

1112 **leaving.** Wild type, mutant and transgenic animals are examined for leaving an *E. coli* OP50

1113 food lawn paired with 100% 2-nonanone. The average percentage of worms outside the food

1114 lawn at 15 minutes is reported. Mutants or transgenic animals are compared with the wild-type

1115 control tested on the same days with student's *t* test, n = 2 – 4 assays for each genotype, 20-25

1116 animals in each assay, Mean ± SEM.

1117

1118 **S5 Fig. Schematics of assays**

1119 **(A)** Assay to measure time taken to reach the edge of the food lawn (Experimental Procedures).

1120 **(B)** Chemotaxis assay for avoidance of 100% 2-nonanone (Experimental Procedures)

1121

1122 **S6 Fig. Additional alleles of *tdc-1* and *tbh-1* mutants are also wild-type for 2-nonanone-**

1123 **dependent food leaving.**

1124 Each bar graph shows the percentage of animals outside the food lawn 15 minutes after the

1125 start of the assay, mutants are compared with wild type tested in parallel with Student's *t* test,

1126 n= 3 assays each; mean ± SEM, n.s., not significant.

1127

1128 **S7 Fig. DAF-7 produced by the sensory neurons ASI does not regulate 2-nonanone-**

1129 **dependent food leaving.**

1130 Expressing a wild-type *daf-7* cDNA in the sensory neurons ASI does not rescue the delayed

1131 decision phenotype in the *daf-7(e1372)* mutant animals (n = 4, 3 and 4 assays for wild type,

1132 transgenic animals and non-transgenic siblings, respectively).

1133 Each bar graph shows the average percentage of worms outside the lawn 15 minutes after the

1134 start of the assay, transgenic animals are compared with non-transgenic siblings using

1135 Student's *t-t*est, n.s., not significant.

1136

1137 **S8 Fig. Expressing the *daf-1* cDNA in sensory neurons with *osm-6* promoter does not**

1138 **rescue the delayed-decision phenotype in the *daf-1(m40)* mutants.** The transgenic animals

1139 (n = 3 assays) are compared with non-transgenic siblings (n = 4 assays) with Student's *t* test,

1140 wild type = 3 assays; bar graph shows the percentage of worms outside of lawn 15 minutes

1141 after the start of the assay, mean ± SEM, n.s., not significant.
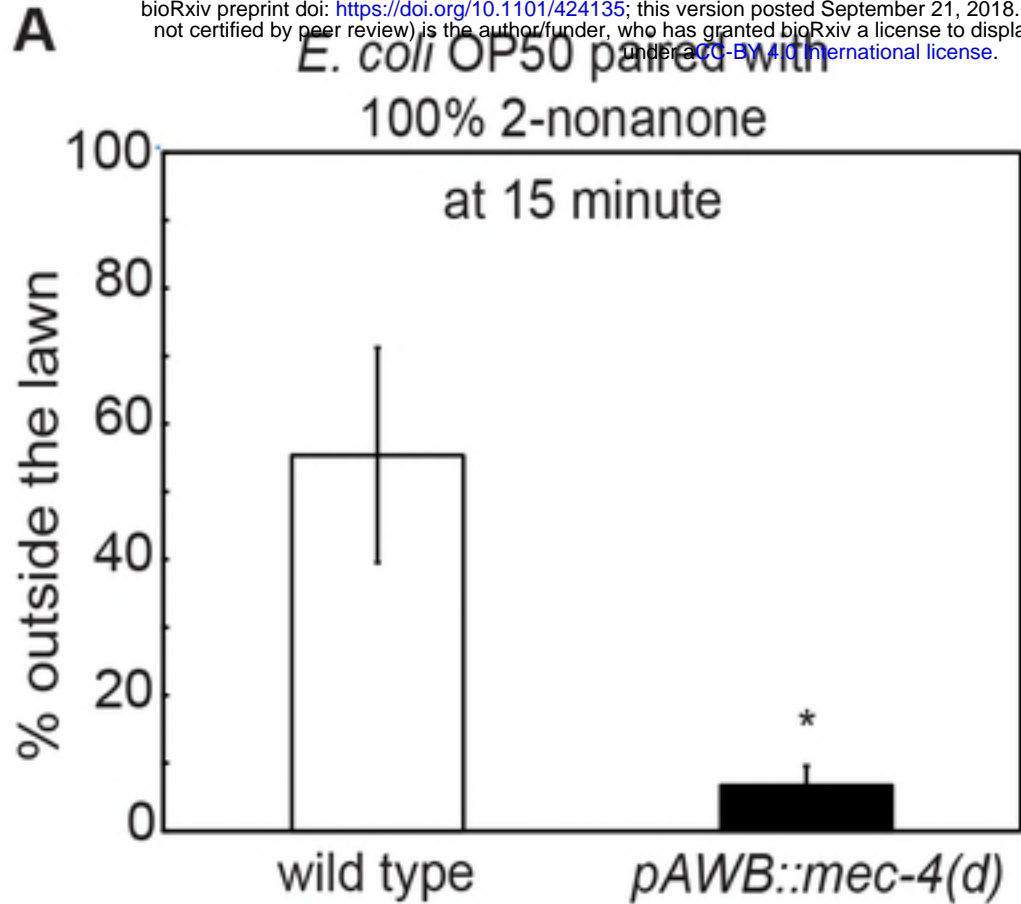
1142

1143 **S9 Movie. Wild-type worms performing food leaving on an *E. coli* OP50 lawn paired with**

1144 **100% 2-nonanone.**

1145

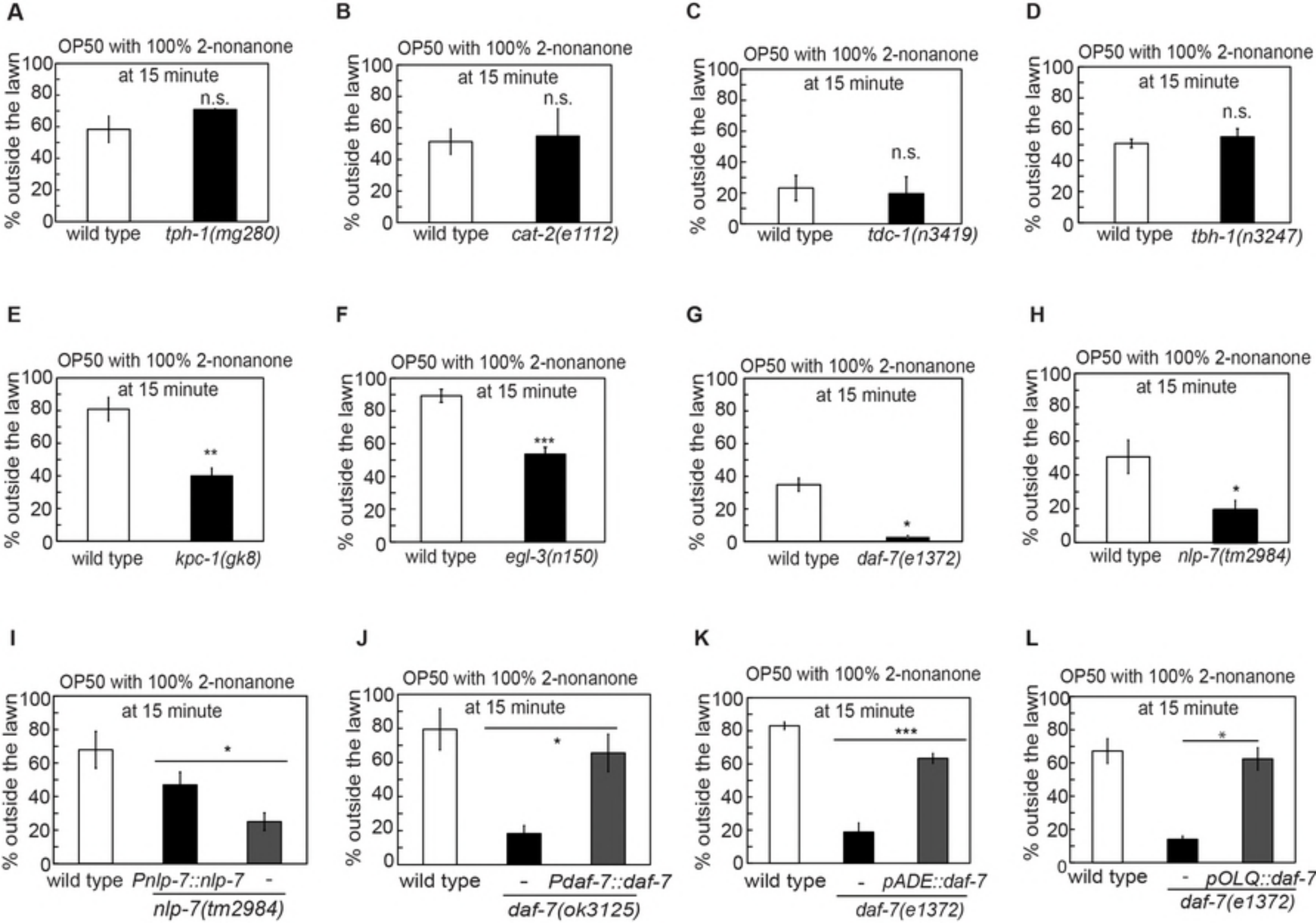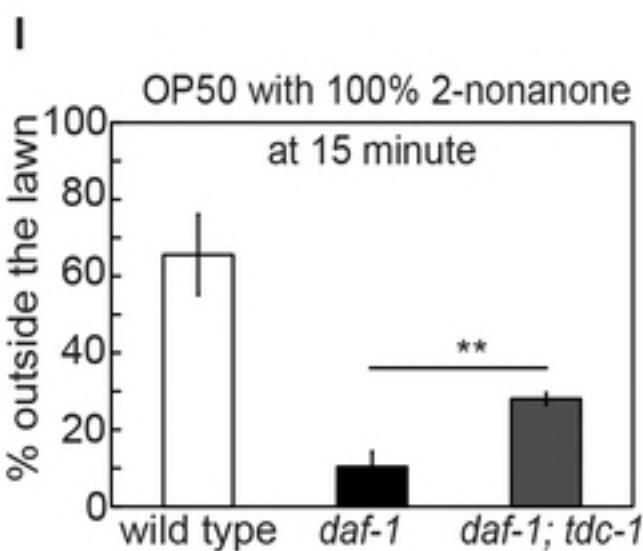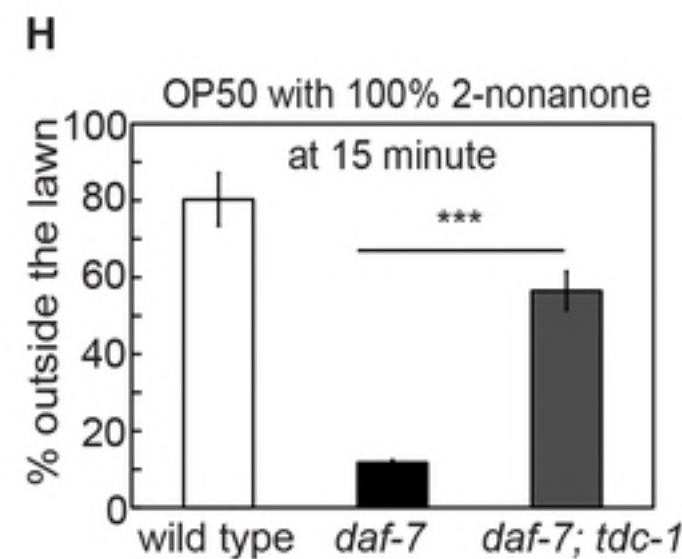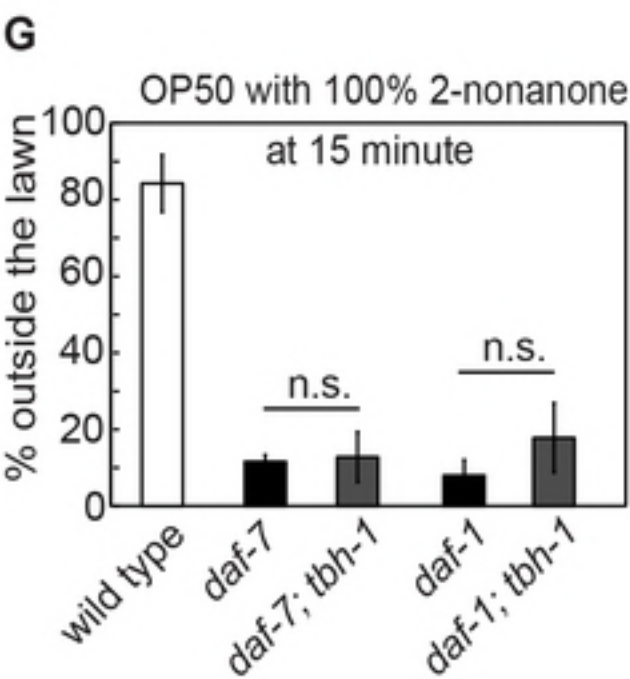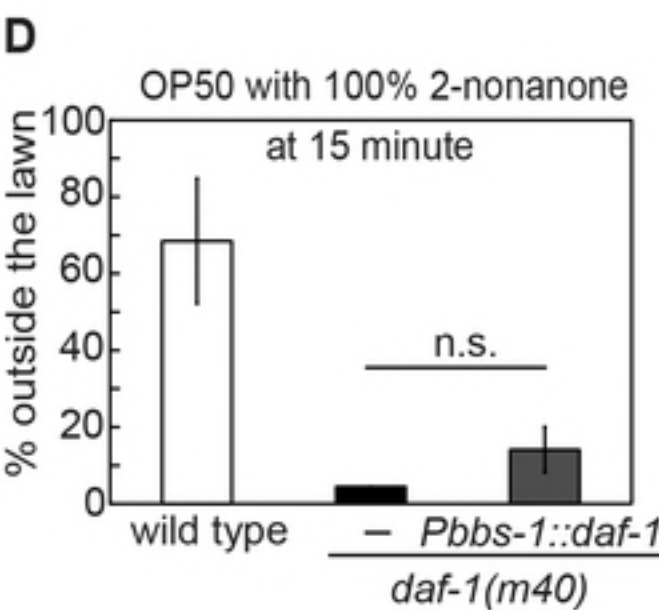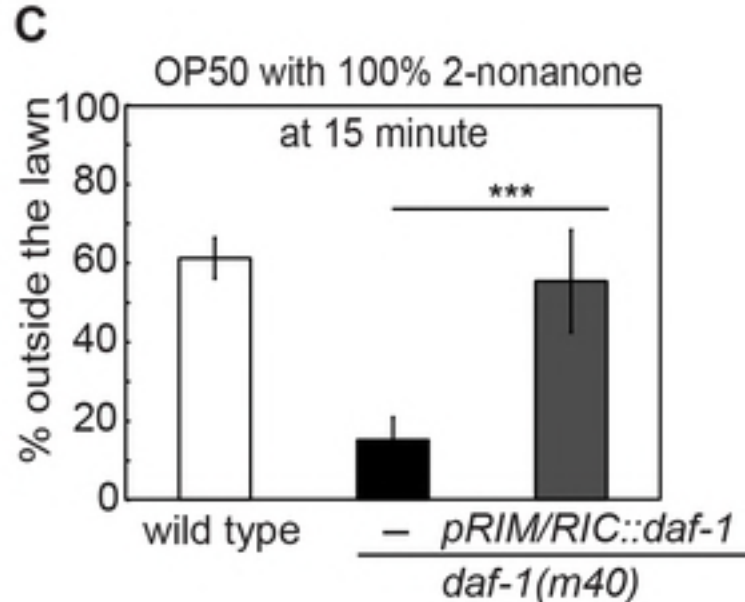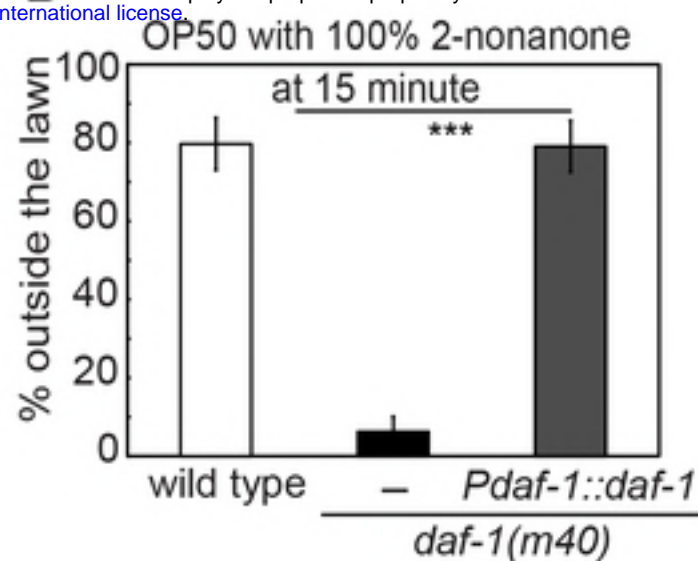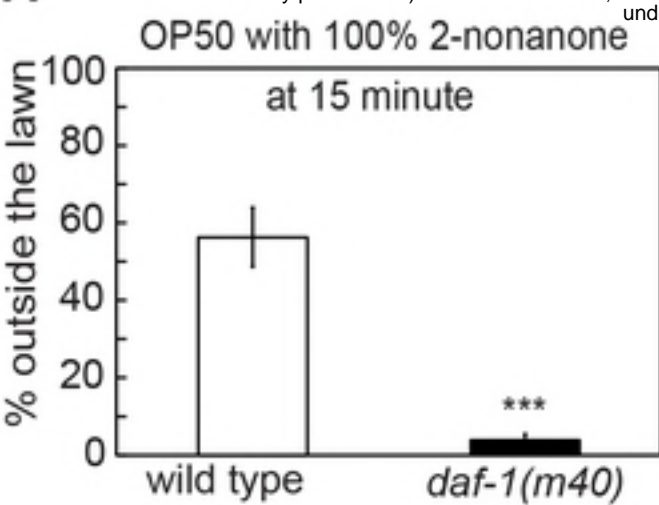1146 **S10 Movie. Wild-type worms performing food leaving on an *E. coli* OP50 food lawn paired**

1147 **with 100% benzaldehyde.**

A Assay

15 - 25 worms

Repellant

1 cm

6 cm plate

B *E. coli* OP50 with 2-nonanone

% outside the lawn

Time (minute)

- 10%
- 30%
- 50%
- 100%

C *E. coli* OP50 with 2-nonanone at 15 minute

% outside the lawn

**

10%   100%

D *E. coli* OP50 with 2-nonanone

time to reach the edge (s)

n.s.

10%   100%

E

I) at 2 minute

2-nonanone

OP50 lawn

C. elegans

100% 2-nonanone

II) at 5 minute

100% 2-nonanone

III) at 10 minute

100% 2-nonanone

IV) at 15 minute

100% 2-nonanone

A *E. coli* OP50 paired with 100% 2-nonanone at 15 minute

B *E. coli* OP50 paired with 100% 2-nonanone at 15 minute

C *E. coli* OP50 paired with 100% 2-nonanone at 15 minute

D *E. coli* OP50 paired with 100% 2-nonanone at 15 minute

A OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, *daf-1(m40)* ***

B OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, −, *Pdaf-1::daf-1*, *daf-1(m40)* ***

C OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, −, *pRIM/RIC::daf-1*, *daf-1(m40)* ***

D OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, −, *Pbbs-1::daf-1*, *daf-1(m40)* n.s.

E OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, *pRIM/RIC::HisCl* n.s.

F OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, *pRIM/RIC::TeTx* n.s.

G OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, *daf-7*, *daf-7; tbh-1*, *daf-1*, *daf-1; tbh-1* n.s. n.s.

H OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, *daf-7*, *daf-7; tdc-1* ***

I OP50 with 100% 2-nonanone at 15 minute — % outside the lawn; wild type, *daf-1*, *daf-1; tdc-1* **

A OP50 with 100% 2-nonanone
at 15 minute

% outside the lawn

wild type | pAIY::twk-18 | –
wild type

B OP50 with 100% 2-nonanone
at 15 minute

% outside the lawn

wild type | pAIY:TeTx

***

C OP50 with 100% 2-nonanone
at 15 minute

% outside the lawn

wild type | ttx-3(mg158)

***

D OP50 with 100% 2-nonanone
at 15 minute

% outside the lawn

wild type | pAIB::TeTx

n.s.

E OP50 with 100% 2-nonanone
at 15 minute

% outside the lawn

wild type | pAIB::HisCl

n.s.

F OP50 with 100% 2-nonanone
at 15 minute

% outside the lawn

wild type | Pnmr-1::TeTx

**

G OP50 with 100% 2-nonanone
at 15 minute

% outside the lawn

wild type | Pglr-1::TeTx

*

A OP50 with 100% repellent

% outside the lawn vs Time (min)
- 2-Nonanone (black)
- 1-Octanol (blue)
- Benzaldehyde (red)

B OP50 with 100% Benzaldehyde at 25 minute

% outside the lawn: wild type, *pAWB::mec-4(d)* ***

C OP50 with 100% Benzaldehyde at 5 minute

% outside the lawn: wild type, *pASK::caspase* **

D OP50 with 100% Benzaldehyde at 25 minute

% outside the lawn: wild type, *daf-7(e1372)* **

E OP50 with 100% Benzaldehyde at 25 minute

% outside the lawn: wild type, *daf-1(m40)* **

A *Comamonas* with 100% nonanone
at 25 minute

% outside the lawn

wild type   *pAWB::mec-4(d)*

B *Comamonas* with 100% nonanone
at 20 minute

% outside the lawn

wild type   *pASK::caspase*

C *Comamonas* with 100% nonanone
at 20 minute

% outside the lawn

wild type   *daf-7(e1372)*

D *Comamonas* with 100% nonanone
at 20 minute

% outside the lawn

wild type   *daf-1(m40)*