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Developmental history modulates adult olfactory behavioral preferences via regulation of

chemoreceptor expression in *C. elegans*

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ABSTRACT

Developmental experiences play critical roles in shaping adult physiology and behavior. We and others previously showed that adult *C. elegans* which transiently experienced dauer arrest during development (PD: post-dauer) exhibit distinct gene expression profiles as compared to control adults which bypassed the dauer stage. In particular, the expression patterns of subsets of chemoreceptor genes are markedly altered in PD adults. Whether altered chemoreceptor levels drive behavioral plasticity in PD adults is unknown. Here we show that PD adults exhibit enhanced attraction to a panel of food-related attractive volatile odorants including the bacterially-produced chemical diacetyl. Diacetyl-evoked responses in the AWA olfactory neuron pair are increased in both dauer larvae and PD adults, and we find that these increased responses are correlated with upregulation of the diacetyl receptor ODR-10 in AWA likely via both transcriptional and post-transcriptional mechanisms. We show that transcriptional upregulation of *odr-10* expression in dauer larvae is in part mediated by the DAF-16 FOXO transcription factor. Via transcriptional profiling of sorted populations of AWA neurons from control and PD adults, we further show that the expression of a subset of additional chemoreceptor genes in AWA is regulated similarly to *odr-10* in PD animals. Our results suggest that developmental experiences may be encoded at the level of olfactory receptor regulation, and provide a simple mechanism by which *C. elegans* is able to precisely modulate its behavioral preferences as a function of its current and past experiences.

INTRODUCTION

Conditions experienced during early development have profound effects on adult phenotypes. Fetal malnutrition is a major risk factor for metabolic disorders in human adults, and adverse experiences in early life influence adult stress responses in many animal species (Alyamani and Murgatroyd, 2018; Cater and Majdic, 2021; de Gusmao Correia et al., 2012; Hanson and Gluckman, 2014; Smith and Ryckman, 2015). In addition to modulating general life history traits, early experiences can also affect specific adult behavioral phenotypes. For instance, exposure to an odorant during a critical developmental period has been shown to subsequently modulate the responses of adults to that odorant (Hong et al., 2017; Jin et al., 2016; Nevitt et al., 1994; Remy and Hobert, 2005). Adult phenotypic plasticity as a consequence of differential developmental experiences may allow adaptation to variable environments to optimize fitness (Sommer, 2020). Despite the prevalence and critical role of early experiences in shaping adult phenotypes, the underlying mechanisms are not fully understood.

C. elegans adults develop via one of two alternative developmental trajectories based on environmental conditions experienced during their first and second larval stages (Cassada and Russell, 1975). While larvae continue in the reproductive cycle through four larval stages (L1-L4) under favorable conditions, adverse conditions experienced during L1 instead drive larvae into the dauer diapause stage. Dauer larvae undergo extensive morphological, neuroanatomical and behavioral remodeling that maximizes their ability to survive harsh conditions, distinguishing them from their L3 larval counterparts (Albert and Riddle, 1983; Britz et al., 2021; Popham and Webster, 1979; Riddle, 1988). When growth conditions improve, they exit the dauer stage and resume development into reproductive adults. We and others previously showed that adult *C. elegans* that transiently passed through the dauer stage (henceforth referred

68 to as post-dauer (PD) adults) differ markedly from adult animals that bypassed the dauer stage

69 (referred to as control adults) in their life history traits including longevity, stress-resistance, and

70 fecundity (Hall et al., 2010; Hall et al., 2013; Ow et al., 2018; Ow et al., 2021). This phenotypic

71 plasticity is correlated with extensive changes in transcriptional profiles, as well as modification

72 of the chromatin landscape (Bhattacharya et al., 2019; Hall et al., 2010; Hall et al., 2013; Ow et

73 al., 2018; Vidal et al., 2018). Thus, isogenic populations of adult *C. elegans* hermaphrodites

74 retain a molecular and phenotypic memory of their developmental history.

75   Food-seeking is a critical behavioral drive and is subject to extensive modulation as a

76 function of an animal's internal state and external conditions (Flavell et al., 2020; Heisler and

77 Lam, 2017; Kim et al., 2017; Pool and Scott, 2014). Dauer larvae do not feed and the

78 morphologies of their sensory neurons are extensively remodeled (Albert and Riddle, 1983; Britz

79 et al., 2021; Popham and Webster, 1979; Riddle, 1988). Dauer larvae retain the ability to detect

80 and respond to environmental cues including food-related chemical cues in order to assess

81 whether conditions have improved sufficiently to trigger exit from the dauer stage and re-entry

82 into the reproductive cycle. Chemosensory responses of *C. elegans* dauer larvae have been

83 assessed in a limited set of studies and appear to be partly distinct from those of adults or L3

84 larvae (Albert and Riddle, 1983; Hallem et al., 2011; Vertiz et al., 2021; White et al., 2019).

85 Whether PD adults retain these behavioral differences or exhibit further plasticity in food-

86 seeking behaviors as a consequence of their distinctive developmental trajectory is largely

87 unknown. The expression of many chemoreceptor genes in sensory neurons is altered in dauer

88 larvae (Nolan et al., 2002; Peckol et al., 2001; Vidal et al., 2018), and the spatial patterns and/or

89 levels of a subset of these genes is subsequently maintained or further altered in PD adults (Hall

90 et al., 2010; Peckol et al., 2001; Vidal et al., 2018). Altered chemoreceptor expression profiles

91   provide a plausible mechanism for chemosensory behavioral plasticity, but a correlation between

92   changes in chemoreceptor gene expression and altered food-seeking behaviors in PD animals

93   remains to be established.

94   Here we show that PD adults exhibit increased sensitivity to a panel of bacterially-

95   produced attractive odorants including the chemical diacetyl, low concentrations of which are

96   sensed by the AWA olfactory neuron pair. Consistently, both dauer larvae and PD adults also

97   exhibit increased diacetyl-evoked responses in the AWA olfactory neurons. We find that the

98   AWA-expressed *odr-10* diacetyl receptor gene is upregulated in dauer larvae; this upregulation is

99   mediated in part via the DAF-16 FOXO transcription factor. While *odr-10* expression is

100   subsequently downregulated in PD adults relative to levels in dauer larvae, levels of the ODR-10

101   receptor protein in the AWA cilium, the site of primary odorant transduction, are retained at

102   higher levels in PD as compared to control adults. Via transcriptional profiling of sorted

103   populations of AWA neurons from control and PD adults together with examination of

104   expression from endogenous reporter-tagged alleles, we further find that the expression of a

105   subset of additional AWA-expressed chemoreceptor genes is also upregulated in dauers, and is

106   maintained at higher levels in PD adults. Together, our data demonstrate that altered

107   chemoreceptor levels can underlie developmental stage- and history-dependent olfactory

108   behavioral plasticity in *C. elegans*, and highlight the complexity of mechanisms regulating

109   expression of individual chemoreceptor expression genes in this organism.

110

111   **MATERIALS and METHODS**

112   **Strains and growth conditions**

5

113   All *C. elegans* strains were maintained on nematode growth medium (NGM) seeded with

114   *Escherichia coli* OP50 at 20°C unless stated otherwise. Dauer larvae were generated by picking

115   8-10 L4s from continuously growing animal populations onto 10 cm NGM plates and allowing

116   growth and reproduction until food exhaustion (typically 7-9 days) at 25°C. Starved larvae were

117   washed off plates with S-Basal buffer, and dauer larvae were selected by treatment with 1% SDS

118   for 30 mins. To recover PD adults, a droplet containing 100-700 dauer larvae was pipetted onto a

119   10 cm NGM plate seeded with OP50 and allowed to grow for 48 hrs at 20°C. L4 larvae were

120   picked onto fresh, seeded NGM plates 1 day prior to imaging.

121   For all experiments involving growth on plates containing auxin, NGM media was

122   supplemented with the synthetic auxin 1mM 1-Naphthaleneacetic acid (NAA) from a 500 mM

123   stock solution dissolved in 95% ethanol. Corresponding control NGM media was supplemented

124   with an equal volume of 95% ethanol.

125   To collect adult animals that underwent L1 larval arrest, gravid adult hermaphrodites

126   were bleached, and eggs were allowed to hatch overnight in M9 buffer containing 0.1% Triton

127   X-100. 500-1000 L1 larvae were subsequently placed onto 10 cm NGM plates seeded with OP50

128   and grown at 20°C until adulthood.

129

130   **Genetics**

131   All strains were constructed using standard genetic methods. Crosses were validated for

132   the presence of the desired mutations using PCR-based amplification and/or sequencing. To

133   generate strains for auxin-induced degradation specifically in AWA, animals were injected with

134   a plasmid driving TIR1 under the AWA specific *gpa-4Δ6* promoter (PSAB1283: *gpa-*

135   *4Δ6*p::*TIR1::SL2::mScarlet*; Table S1) at 5 ng/µl together with the *unc-122*p::*dsRed* co-injection

136   marker at 50 ng/µl. A complete list of strains used in this work is available in Table S2.

137

138   **Molecular biology**

139   Gene editing was performed using CRISPR/Cas9 and repair templates. Plasmids

140   PSAB1279 (*odr-10::splitGFP$_{11}$*) and PSAB1280 (*gpa-4Δ6*p::*splitGFP$_{1-10}$*) (Table S1) were

141   generated using traditional cloning from splitGFP constructs (KP#3315 and KP#3316, gift from

142   J. Kaplan lab). An asymmetric donor template (Dokshin et al., 2018) was amplified from plasmid

143   PSAB1279 to create long and short PCR fragments of 2308 and 600 bp, respectively, containing

144   approximately 1 kb homology arms for CRISPR-mediated insertion. The asymmetric hybrid

145   template was injected (each fragment at 250 ng/µl) together with crRNA (20 ng/µl; IDT

146   Integrated DNA Technologies), tracrRNA (20 ng/µl: IDT), Cas9 protein (25 ng/µl; IDT), and

147   *unc-122*p::*gfp* (40 ng/µl) as the co-injection marker. F1 animals expressing the injection marker

148   were isolated. F2 progeny were screened by PCR for the insertion and confirmed by sequencing

149   to obtain *odr-10(oy158)* (Table S2).

150   To mutate the predicted DAF-16 binding site upstream of *odr-10* in *odr-10(oy158)*, a

151   conserved GTAAACA binding site 815 bp 5' of the *odr-10* start codon (Wexler et al., 2020) was

152   mutated to GTCCCCA to generate *odr-10(oy170)*. The injection mix contained an *odr-10*

153   promoter repair template with the mutated site along with 32 bp 5' and 3' homology arms (590

154   ng/µl; IDT), *dpy-10* repair template (100 ng/µl, IDT), Cas9 protein (25 ng/µl; IDT), crRNA (20

155   ng/µl; IDT), and tracrRNA mix (20 ng/µl: IDT). F1 animals with *dpy* phenotypes were isolated

156   and F1 and F2 progeny were screened by PCR and sequencing. Confirmed mutants were

157   backcrossed to remove the *dpy-10* allele.

158

Chemotaxis behavioral assays

160   Chemotaxis assays were performed essentially as described previously (Bargmann et al.,

161   1993; Troemel et al., 1997). Behavioral attraction and avoidance assays were performed on 10

162   cm round or square plates, respectively. Each assay was performed in duplicate each day, and

163   data are reported from biologically independent assays performed over at least 3 days. Behaviors

164   of control and experimental animals were examined in parallel each day.

165

Calcium imaging

167   Calcium imaging was performed essentially as previously described, using custom

168   microfluidics devices (Chronis et al., 2007; Khan et al., 2022; Neal et al., 2015). Imaging was

169   performed on an Olympus BX52WI microscope with a 40X oil objective and Hamamatsu Orca

170   CCD camera. Video recordings were performed at 4 Hz. All odorants were diluted in filtered S-

171   Basal buffer. 20 μM fluorescein was added to one buffer channel to confirm correct fluid flow in

172   microfluidics devices. 1 mM (-)-tetramisole hydrochloride (Sigma L9756) was used to

173   immobilize animals during imaging. To prevent animals from clogging the microfluidics loading

174   arena and chip, 1 μl of poloxamer surfactant (Sigma P5556) was added to the S-Basal loading

175   buffer. AWA neurons were imaged for one cycle of 30 sec buffer/30 sec odor/30 sec buffer, or

176   for one cycle of 30 sec buffer/10 sec odor/20 sec buffer stimuli.

177   Recorded images were aligned with the template Matching plugin in Fiji (NIH) and

178   cropped to include the AWA neuron soma and surrounding background fluorescence. The region

179   of interest (ROI) was defined by outlining the AWA cell bodies, and an area of background

180   fluorescence was chosen for background subtraction. To correct for photobleaching, an

181   exponential decay was fit to the fluorescence intensity values for the first 30 sec and the last 20

182   sec of imaging. The resulting value was subtracted from original intensity values. Peak

183   amplitude was calculated as the maximum change in fluorescence ($F-F_0$) in the 10 sec following

184   odor addition; F_0 was set to the average $\Delta F/F_0$ value for 5 sec before odor onset. Data

185   visualization was performed using RStudio (Version 1.4.1717). Photomask designs for

186   customized adult and dauer microfluidic imaging chips were adapted from (Chronis et al., 2007)

187   and are available at https://github.com/SenguptaLab/PDplasticity (also see below). AWA neuron

188   mean baseline fluorescence (Figure S1C) was calculated by taking the average $\Delta F/F_0$ during the

189   first 25 sec of imaging (0-25 sec) in each animal. Reported data were collected from biologically

190   independent experiments over at least 2 days.

191

192   **Dauer microfluidics imaging device**

193   Designs were based on olfactory imaging chips previously described (Chronis et al.,

194   2007) with modifications to accommodate dauer larvae. To constrict the thinner dauer larvae in a

195   similar manner to adults, it was necessary to reduce the cross-sectional area of the worm trap.

196   This was accomplished by placing 10 μm-wide posts of decreasing width culminating in a 5 μm

197   gap into which the worm nose was constricted (https://github.com/SenguptaLab/PDplasticity).

198   Posts were used instead of fully narrowing the channel to preclude limiting fluid velocity while

199   loading animals into the channel. AutoCAD drawings were used to generate an ink photomask

200   (outputcity.com) which was subsequently used to generate master molds with 10 μm feature

201   depth using negative photoresist SU-8 3005. To prevent delamination due to small features, a

202   uniform 10 μm layer of SU-8 3005 photoresist was applied first to silicon wafers.

203

Imaging and image analysis

204

205   Animals were mounted on 10% agarose pads set on microscope slides and immobilized

206   using 10 mM (-)-tetramisole hydrochloride. Imaging for expression level quantification was

207   performed on an inverted spinning disk confocal microscope (Zeiss Axiovert with a Yokogawa

208   CSU22 spinning disk confocal head and a Photometerics Quantum SC 512 camera). Optical

209   sections were acquired at 0.27 μM sections using either a 63X oil immersion objective when

210   imaging neuron cell bodies, or a 100X oil immersion objective when imaging the cilia using

211   SlideBook 6.0 software (Intelligent Imaging Innovations, 3i). z-projections of all optical sections

212   at maximum intensity were generated using SlideBook 6.0 or FIJI/ImageJ (NIH).

213   Quantification of GFP or mNeonGreen levels in AWA soma was performed by tracing

214   the ROI of each neuron cell body, and subtracting background fluorescence. The mean

215   fluorescence of each cell body was calculated in FIJI. For quantification of ciliary ODR-10

216   levels in adult animals, an ROI of the cilia base and primary stalk was obtained and the

217   integrated density of total fluorescence in the ROI was calculated using the corrected total cell

218   fluorescence (CTCF) to take into account differences in the sizes of different ROIs and the

219   highly branched structures of AWA cilia. Since the AWA cilia branches are collapsed in dauer

220   larvae and are relatively smaller in L3 larvae, the ROI was traced around the entire cilia base and

221   stalks. Three background ROIs were obtained and averaged to calculate the mean background

222   fluorescence.

223

Collection of AWA neurons

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225   To obtain control animals, adult hermaphrodites expressing the stably integrated

226   transgene *gpa-4Δ6*p*::myrGFP* (PY10421, Table S2) were bleached, and eggs allowed to hatch in

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227   M9 with 0.1% Triton-100. 50,000-100,000 growth-synchronized L1 larvae were plated onto 15

228   cm 8P growth plates seeded with 1 ml of an overnight culture of *E. coli* NA22 grown in 2XYT

229   media. L1 larvae were allowed to grow for 40-48 hrs at 20°C to obtain populations of L4 larvae.

230   To obtain PD animals, SDS-selected dauer larvae were plated onto 15 cm 8P plates seeded with

231   1 ml of an overnight culture of *E. coli* NA22 grown in 2XYT media, and allowed to recover to

232   the L4 stage for 20-24 hrs at 20°C.

233   Animals were collected and cells dissociated essentially as described previously (Taylor

234   et al., 2021). In brief, animals were washed off plates with M9 buffer and incubated in lysis

235   buffer (200 mM Dithiothreitol (DTT), 0.25% Sodium dodecyl sulfate (SDS), 20 mM HEPES

236   buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 3% sucrose) for 5 mins. Lysed

237   animals were washed 5X with egg buffer (118 mM NaCl, 48 mM KCl, 2 mM $CaCl_2$, 2 mM

238     $MgCl_2$, 25 mM HEPES, pH 7.3) following which the worm pellet was resuspended in 750 μl of

239   15 mg/ml Pronase (Sigma P8811) in egg buffer. To promote dissociation, the mixture was

240   pipetted frequently for 20 mins and progress of dissociation was monitored under a light

241   microscope. Pronase digestion was terminated by adding L-15-10 media (L-15 media + 10%

242   volume FBS, HI) at 4°C. Dissociated worms were centrifuged at 4°C and the worm pellet was

243   resuspended in egg buffer and recentrifuged at 4°C. The supernatant was filtered through a 35

244   μm filter in preparation for FACS sorting. A 25 μl aliquot of the dissociated worm pellet was

245   pipetted directly into Trizol for analysis as the whole worm sample.

246   Prior to performing FACS, 0.5 μl of 1 mg/ml DAPI was added to 1 ml of the cell

247   suspension to exclude dead cells. AWA neurons were captured by isolating the high GFP+ and

248   low DAPI compartment and cells were sorted directly into Trizol. Cells from this compartment

249   were also sorted onto glass slides and imaged under a fluorescence light microscope to confirm

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250   the presence of intact GFP+ cells. Following cell collection, collection tubes were briefly spun

251   down and frozen at -80°C. 1,000 - 10,000 cells were isolated per sorting run.

252

253   **RNA sequencing**

254   RNA was extracted from collection tubes using standard chloroform and isopropanol

255   precipitation. The RNA pellet was resuspended in RNAase-free water and any DNA digested

256   using the RNase-Free DNase kit (Qiagen). RNA was eluted using the RNeasy MinElute Cleanup

257   Kit (Qiagen). mRNA was isolated and libraries amplified using Smart-Seq v4 and library quality

258   checked with Bioanalyzer using Agilent High Sensitivity Gel. Libraries were sequenced on

259   Illumina NextSeq500. Library preparation and sequencing was performed at the MIT BioMicro

260   Center (https://biology.mit.edu/tile/biomicro-center/).

261   Raw RNA-Seq reads were quality checked, adapter trimmed, and aligned to the *C.*

262   *elegans* genome using STAR ALIGNER (https://github.com/alexdobin/STAR). Principal

263   component analyses were performed on the normalized read counts of the 10,000 most variable

264   genes across all samples. Differential expression analysis was performed using DESeq2 with lfc

265   shrinkage on. Differential expression was determined with a significance cut-off of 0.05

266   (adjusted *p*-value; Wald test with Benjamini-Hochberg corrections) and the indicated Log_2 fold

267   change cut off of either -1.5 and 1.5 or -2 and 2. AWA enrichment analysis was performed using

268   the web-based Tissue Enrichment Analysis tool with default parameters

269   (https://www.wormbase.org/tools/enrichment/tea/tea.cgi) (Angeles-Albores et al., 2016).

270

271   **Statistical analyses**

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272   Normal distribution of the data was assessed using the Shapiro-Wilks test (GraphPad

273   Prism v9.0.2). Parametric data were analyzed using a two-tailed Welch's t-test or ANOVA. Non-

274   parametric data were analyzed using a Mann-Whitney t-test or Kruskal-Wallis test across

275   multiple conditions. Post hoc corrections for multiple comparisons were applied to data in which

276   more than two groups were analyzed. The specific tests used and corrections applied are

277   indicated in each Figure Legend.

278

279   **RESULTS**

280   **Post-dauer adult animals exhibit enhanced attraction to a subset of volatile odorants**

281   *C. elegans* adult animals are strongly attracted to a subset of chemicals that indicates the

282   presence of nutritious bacteria, the major food source for these nematodes (Ferkey et al., 2021).

283   Attractive volatile odorants are primarily sensed by the AWA and AWC olfactory neurons pairs

284   in the bilateral head amphid organs of *C. elegans* (Bargmann et al., 1993). Consistently, adult

285   hermaphrodite animals grown continuously under favorable environmental conditions (control

286   adults; Figure 1A) exhibited robust attraction to a range of concentrations of odorants sensed by

287   the AWA and/or AWC neurons (Figure 1B).

288   To test whether passage through the dauer stage influences the olfactory preference

289   behaviors of adult animals, we grew wild-type animals under food-restricted conditions to

290   promote entry into the dauer stage as previously described (Figure 1A) (Ow et al., 2018). Dauer

291   larvae were collected via SDS-mediated selection and subsequently allowed to resume

292   reproductive growth on bacterial food (Figure 1A). Similar to control adults, young post-dauer

293   (henceforth referred to as PD) adult animals were also robustly attracted to both AWA- and

294   AWC-sensed volatile odorants (Figure 1B). We found that PD adults typically exhibited

295   enhanced attraction to lower odorant concentrations relative to control adults (Figure 1B).

296   Animals that experienced starvation-induced developmental arrest at the L1 larval stage did not

297   exhibit similar enhanced attraction responses as adults (Figure S1A). PD animals have

298   previously been shown to exhibit decreased avoidance of the aversive pheromone ascr#3 (Sims

299   et al., 2016). Control and PD animals avoided high concentrations of benzaldehyde to a similar

300   extent (Figure S1B) although the aversion responses of PD adults to high concentrations of

301   octanol were weakly decreased (Figure S1B). We conclude that the behavioral responses of adult

302   animals to multiple attractive volatile chemicals are sensitized upon passage through the dauer

303   diapause stage.

304   We next tested whether increased behavioral attraction correlates with enhanced odorant

305   responses in olfactory neurons by examining odorant-evoked changes in intraneuronal calcium

306   dynamics using a genetically encoded calcium indicator. We focused our attention on the odorant

307   diacetyl, low concentrations of which are sensed by the AWA olfactory neuron pair (Bargmann

308   et al., 1993), and for which the cognate receptor and signal transduction mechanisms have been

309   described (see below) (Colbert et al., 1997; Roayaie et al., 1998; Sengupta et al., 1996). Low

310   concentrations of diacetyl consistently evoked responses of larger amplitude in the AWA

311   neurons of PD as compared to control adults consistent with the observed increased behavioral

312   responses to this chemical (Figure 1C-D). This amplitude difference is unlikely to arise simply

313   due to differences in the expression levels of the AWA-expressed calcium sensor (Figure S1C).

314   Together, these data indicate that neuronal responses of adult animals to the attractant diacetyl

315   are enhanced upon passage through the dauer stage.

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317 **Ciliary levels of the diacetyl receptor ODR-10 are increased as a function of developmental**

318 **history**

319   We and others previously reported that control and PD animals exhibit significant

320 differences in gene expression profiles including in individual sensory neurons (Bhattacharya et

321 al., 2019; Hall et al., 2010; Sims et al., 2016; Vidal et al., 2018). Each chemosensory neuron type

322 in *C. elegans* expresses multiple GPCRs, expression of a subset of which has previously been

323 shown to be regulated by external and internal state (Gruner et al., 2014; Lanjuin and Sengupta,

324 2002; Nolan et al., 2002; Peckol et al., 2001; Ryan et al., 2014; Troemel et al., 1995; Vidal et al.,

325 2018). Low concentrations of diacetyl are sensed by the ODR-10 olfactory receptor which is

326 expressed specifically in the AWA neurons and localizes to their sensory cilia, the primary site

327 of olfactory signal transduction (Sengupta et al., 1996). Behavioral and neuronal responses to

328 diacetyl have previously been shown to be regulated by feeding state, developmental stage and

329 somatic sex via expression changes in *odr-10* (Ryan et al., 2014; Wexler et al., 2020), but

330 whether *odr-10* expression is also modulated via dauer passage is unclear. Since state-dependent

331 regulation of individual olfactory receptor genes provides a simple mechanism for mediating

332 odorant-selective behavioral plasticity, we tested the hypothesis that altered expression of *odr-10*

333 underlies the observed plasticity in diacetyl responses in PD adults.

334   An endogenous *odr-10* allele tagged with *t2A::mNeonGreen* (*odr-10::t2A::mNG*) was

335 specifically expressed in both AWA neurons (Figure 2A) (McLachlan et al., 2022). However,

336 expression levels of the fluorescent reporter protein were not significantly altered in PD as

337 compared to control adults (Figure 2B). Since this reporter does not allow assessment of ciliary

338 ODR-10 protein levels, we next quantified ODR-10 protein levels in AWA cilia from the

339 endogenous *odr-10* allele tagged with the split-GFP reporter GFP$_{11}$ (*odr-10(oy158)*), and

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340   reconstitution of GFP via expression of the GFP$_{1-10}$ fragment under a constitutive AWA-specific

341   promoter (Kamiyama et al., 2016). We confirmed that tagging *odr-10* with GFP$_{11}$ had no effect

342   on behavioral responses to diacetyl, and that these animals continued to exhibit enhanced

343   attraction to diacetyl upon passage through the dauer stage (Figure S2A). The reconstituted

344   ODR-10 fusion protein was localized to the extensively branched AWA cilia in both control and

345   PD animals (Figure 2C). Since the complex architecture of the AWA cilium precluded precise

346   quantification of overall ciliary protein levels in adult animals, we restricted our analysis to

347   assessing ODR-10 protein levels at the ciliary base and in the primary ciliary stalk. We found

348   that reconstituted ODR-10::GFP levels in AWA cilia were significantly higher in PD than in

349   control animals (Figure 2C-D). These observations raise the possibility that increased ciliary

350   levels of ODR-10 protein in PD animals may contribute to the increased diacetyl responses of

351   PD animals.

352   To establish whether increasing ODR-10 levels alone in AWA is sufficient to enhance

353   diacetyl response sensitivity even in control animals, we overexpressed *odr-10* from the

354   constitutive *gpa-4Δ6* promoter and examined diacetyl responses via both behavioral assays and

355   imaging of diacetyl-evoked intracellular calcium dynamics. As shown in Figure 2E, the

356   behavioral responses of control animals overexpressing *odr-10* were higher than those of wild-

357   type control animals across multiple concentrations of diacetyl. Overexpression of *odr-10* was

358   also sufficient to increase diacetyl-evoked calcium responses in AWA in control adults (Figure

359   2F, Figure S2B). We conclude that increased ODR-10 levels may be sufficient to account for the

360   enhanced diacetyl responses of PD animals.

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362   ***odr-10* expression and diacetyl behavioral responses are increased in dauer larvae**

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363   *C. elegans* dauer larvae as well as the analogous infective juvenile larvae of parasitic

364   nematodes have been shown to exhibit distinct olfactory behaviors (Vertiz et al., 2021). The

365   expression of many neuronal genes including chemoreceptor genes is also markedly altered in

366   dauer larvae (Bhattacharya et al., 2019; Hall et al., 2010; Nolan et al., 2002; Peckol et al., 2001;

367   Vidal et al., 2018). The dauer-specific expression patterns of a subset of these genes is

368   maintained in PD adults, while the expression of other genes is further altered to a PD-specific

369   pattern or restored to the pattern observed in control adults (Hall et al., 2010; Peckol et al., 2001;

370   Vidal et al., 2018). We asked whether *odr-10* expression is modulated in dauers, following which

371   ciliary protein levels may be subsequently maintained at higher levels in PD animals.

372   Expression of the *odr-10::t2A::mNG* reporter remained restricted to the AWA neurons in

373   dauer larvae (Figure 3A). In contrast to our observations in PD adults, *odr-10::t2A::mNG*

374   expression was strongly upregulated in dauer animals as compared to levels in L3 larvae (Figure

375   3A-B). Consistently, reconstituted ODR-10::GFP protein levels were also increased in AWA

376   cilia in dauers (Figure 3C-D).

377   We tested whether upregulation of *odr-10* expression in dauers correlates with increased

378   diacetyl responses in these animals. To examine diacetyl-evoked calcium responses in AWA, we

379   modified the microfluidics imaging device typically used for imaging *C. elegans* adults (Chronis

380   et al., 2007) to accommodate the thinner and smaller dauer larvae (see Materials and Methods),

381   although we were unable to use these or the adult imaging devices to examine L3 larvae. In

382   response to a pulse of 10^{-5} dilution of diacetyl, dauer animals exhibited markedly increased

383   responses as compared to control or PD adults (Figure 3E-F). Altered expression levels of the

384   calcium sensor in AWA are unlikely to account for the observed increase in diacetyl responses in

385   dauer larvae (Figure S1C). We could not reliably assess the behavioral responses of dauers to

386   diacetyl in our behavioral assays in part due to their unique locomotory patterns (Bhattacharya et

387   al., 2019; Gaglia and Kenyon, 2009), although we note that a previous report indicated that

388   dauers exhibit decreased behavioral responses to diacetyl (Vertiz et al., 2021). We conclude that

389   *odr-10* may be upregulated upon entry into the dauer stage possibly via transcriptional

390   mechanisms and correlates with increased diacetyl responses. While this transcriptional

391   upregulation is not maintained in PD adults, increased ODR-10 protein levels in AWA cilia

392   correlates with enhanced diacetyl responses in PD adults.

393

394   **Upregulation of *odr-10* expression in dauer larvae is mediated in part via the DAF-16**

395   **FOXO transcription factor**

396   The TGF-β and insulin signaling pathways act in parallel to regulate dauer formation in

397   response to adverse environmental conditions (Fielenbach and Antebi, 2008; Riddle and Albert,

398   1997). Insulin signaling inhibits nuclear translocation of the DAF-16 FOXO transcription factor,

399   and DAF-16 is nuclear-localized in dauer larvae (Aghayeva et al., 2021; Lin et al., 1997; Ogg et

400   al., 1997). Since this molecule has been implicated in the altered regulation of sensory gene

401   expression in dauer larvae (Aghayeva et al., 2021; Bhattacharya et al., 2019; Wexler et al.,

402   2020), we tested whether the observed upregulation of *odr-10* expression in dauers is mediated

403   in part via DAF-16-dependent transcriptional regulation.

404   Since *daf-16* mutants cannot enter the dauer stage, we assessed the effects of *daf-16*

405   depletion via auxin-induced degradation specifically in AWA (Nishimura et al., 2009; Zhang et

406   al., 2015). We expressed the auxin receptor TIR1 in AWA in a strain in which the endogenous

407   *daf-16* locus has been edited to include a degron tag (Aghayeva et al., 2020; Aghayeva et al.,

408   2021). To obtain larger numbers of dauer larvae, these experiments were performed in *daf-2*

18

409   insulin receptor mutants that constitutively enter the dauer stage even under favorable

410   environmental conditions (Figure 4A) (Gems et al., 1998; Riddle et al., 1981). As in wild-type

411   animals, *odr-10::t2A::mNG* expression levels were upregulated in *daf-2* dauer larvae as

412   compared to expression levels in control or PD *daf-2* adults in the absence of auxin treatment

413   (Figure 4B). We found that auxin-mediated depletion of DAF-16 in AWA decreased, although

414   did not fully abolish, the upregulated *odr-10* expression observed in dauer animals (Figure 4Ai,

415   4B). Growth on auxin had little effect on *odr-10* expression levels in adult animals that bypassed

416   the dauer stage (Figure 4Aii, 4B), although we note that *odr-10* was previously identified as a

417   putative DAF-16-regulated gene in a comparison of the neuronal transcriptomes of *daf-2* and

418   *daf-16; daf-2* adult animals (Kaletsky et al., 2015). Addition of auxin only during dauer recovery

419   also did not affect *odr-10* expression in adult animals (Figure 4Aiii, 4B). We infer that DAF-

420   function in AWA is partly necessary during dauer entry to transcriptionally upregulate *odr-10*

421   expression, although we are unable to exclude the possibility that DAF-16 is not fully depleted in

422   AWA under these conditions.

423   A putative DAF-16 binding site in the proximal regulatory sequences of *odr-10* was

424   previously shown to be necessary for starvation-dependent upregulation of *odr-10* expression

425   driven from a transcriptional reporter in *C. elegans* males (Wexler et al., 2020). In contrast to the

426   expression of the endogenous *odr-10::t2A::mNG* reporter, transgenic expression of GFP driven

427   by ~1kb of the *odr-10* promoter containing the predicted DAF-16 binding site retained high

428   levels of expression in PD adults (Figure 4C). Mutating the predicted DAF-16 binding site in this

429   reporter construct decreased expression levels in PD adults (Figure 4C). However, mutating this

430   site in the endogenous *odr-10* locus via gene editing had no effect on the upregulated levels of

431   ciliary ODR-10 protein in either dauer or PD animals (Figure 4D). These results suggest that

19

432   multiple DAF-16 binding sites in *odr-10* regulatory sequences may contribute redundantly in the

433   context of the endogenous promoter to the upregulation of *odr-10* expression in dauer larvae.

434   Alternatively, DAF-16 may act indirectly to regulate endogenous *odr-10* expression.

435

436   **AWA neurons exhibit distinct gene expression profiles in control and PD adults**

437   In addition to diacetyl, PD adults also exhibit increased responses to the AWA-sensed

438   odorants pyrazine and 2,4,5-trimethylthiazole (Figure 1B). Although the receptors for these

439   chemicals are as yet unidentified, this observation suggests that the expression of

440   chemoreceptors in addition to *odr-10* in AWA may also be altered as a function of dauer

441   passage. To test this notion, we dissociated control and PD L4 larvae expressing GFP

442   specifically in AWA, collected GFP-labeled populations of AWA neurons via fluorescence-

443   activated cell sorting (FACS), and performed transcriptional profiling (Taylor et al., 2021). In

444   order to obtain large populations of growth-synchronized L4 animals for cell sorting, the control

445   population was grown from L1-arrested larvae (see Materials and Methods). In parallel, we also

446   transcriptionally profiled populations of dissociated but unsorted cells from control and PD L4

447   animals.

448   Principal component analyses indicated that with the exception of one sample, the RNA-

449   Seq profiles of all biologically independent replicates of sorted control and PD AWA neurons

450   were present in a cluster distinct from that obtained from cells collected from whole animals

451   (Figure S3A). AWA-expressed genes were the most enriched in the dataset collected from sorted

452   AWA neurons (Figure S3B). Moreover, the 20 most highly expressed genes in AWA as

453   predicted by the CeNGEN neuronal profiling project (Taylor et al., 2021) were robustly

454   represented as upregulated in the AWA RNA-Seq data as compared to data from whole animals

20

455   (Figure S3C). These observations indicate that we successfully enriched and profiled populations

456   of AWA neurons.

457   Comparison of the gene expression changes in the datasets from control and PD whole

458   animals indicated that the expression of multiple G protein-coupled receptor (GPCR) genes was

459   altered as a function of dauer passage (Figure 5A-B, File S1). There was minimal overlap with

460   previously published datasets of genes differentially regulated in PD vs control whole animals

461   (Hall et al., 2010; Ow et al., 2018), likely due to differences in conditions of animal growth and

462   sample preparation although we note that genes predicted to be involved in GPCR signaling were

463   also previously identified as a differentially expressed category (Hall et al., 2010). Up- or

464   downregulated chemoreceptor genes are predicted to be expressed in multiple chemosensory

465   neuron types, indicating that modulation of chemoreceptor gene expression as a function of

466   developmental history is mediated at the level of individual chemoreceptor genes and not

467   sensory neuron types. However, despite the established importance of neuropeptide and

468   hormonal signaling in regulating dauer entry (Fielenbach and Antebi, 2008; Riddle and Albert,

469   1997), the expression of only a few predicted neuropeptide genes appeared to be altered in PD as

470   compared to control animals (Figure 5B, File S1).

471   In contrast to the gene expression changes in the whole animal dataset, the expression of

472   only a small number of GPCRs appeared to be altered in PD vs control AWA neurons (Figure

473   5C-D, File S2). As expected, *odr-10* transcript levels were not significantly changed in PD

474   adults. However, we noted that the expression of several neuropeptide genes and in particular,

475   multiple transcription factors was significantly altered in PD vs control adults (Figure 5D, File

476   S2). Affected transcription factors belong to multiple subfamilies including the greatly expanded

477   nuclear hormone receptor family, members of which are predicted to be co-expressed in sensory

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478   neurons along with putative chemoreceptor GPCRs, and which have been suggested to act as

479   receptors for external and internal cues (Sural and Hobert, 2021; Taylor et al., 2021). Together,

480   these results indicate that passage through the dauer stage alters the expression of genes from

481   multiple families, and that the expression of genes in individual sensory neurons such as AWA is

482   also affected.

483

484   **The expression of multiple AWA-expressed chemoreceptor genes is upregulated in dauers**

485   **and is maintained at higher levels in PD adults**

486   As described above, while *odr-10* expression is not altered in PD adults, expression of

487   this gene is transcriptionally upregulated in dauer larvae following which ciliary ODR-10::GFP

488   protein levels are maintained at higher levels in PD adults. Since our RNA-Seq experiments

489   indicated that the mRNA levels of only a small number of GPCR genes are altered in PD as

490   compared to control AWA neurons, we tested whether additional AWA-expressed

491   chemoreceptor genes are regulated similarly to *odr-10*.

492   Since we were unable to efficiently dissociate dauer larvae likely due to their modified

493   cuticle (Cassada and Russell, 1975) and thus could not profile populations of sorted AWA

494   neurons from dauer larvae, we instead examined the expression of a subset of endogenously

495   tagged chemoreceptor genes. Similar to *odr-10*, mRNA levels of the putative AWA-expressed

496   chemoreceptors *srd-27*, *srd-28*, and *str-44* were also not significantly upregulated in AWA in PD

497   adults (Figure 5C-D, File S2) although *srd-28* was identified as a gene upregulated in PD adults

498   in the whole animal dataset (Figure 5B). *srd-27, srd-28* and *str-44* endogenously tagged with

499   *t2A::mNG* were specifically expressed bilaterally in the AWA olfactory neurons in L3 larvae and

500   control adults (Figure 6A) (McLachlan et al., 2022). Expression of all three genes was

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501   upregulated in AWA in dauer larvae as compared to L3 larvae with *srd-28* and *str-44* showing

502   stronger changes (Figure 6A-D). However, unlike *odr-10* whose expression remained restricted

503   to AWA in all examined stages, all three chemoreceptor genes were expressed in additional

504   neurons in the head in dauer larvae (Figure 6A). Expression in all neurons including in AWA

505   was subsequently downregulated in PD adults relative to dauer larvae, although *srd-28*, *str-44*,

506   and *srd-27* retained higher expression levels in PD as compared to control adults (Figure 6A-D).

507   As in the case of *odr-10*, depletion of DAF-16 during dauer entry via auxin-induced degradation

508   (Figure 4Ai) also decreased but did not abolish upregulation of *srd-28::t2A::mNG* expression in

509   dauer larvae (Figure 6E). We conclude that the expression of multiple AWA-expressed

510   chemoreceptor genes is likely transcriptionally upregulated in dauer larvae in part via DAF-16-

511   dependent mechanisms, and that increased levels of a subset of these receptors may be

512   maintained in PD adults possibly via non-transcriptional mechanisms.

513

514   **DISCUSSION**

515   We show here that adult *C. elegans* hermaphrodites that transiently passed through the

516   dauer developmental stage exhibit enhanced olfactory responses associated with food-seeking as

517   compared to adult animals that bypassed this stage. Increased sensitivity to the odorant diacetyl

518   is correlated with upregulated expression of the diacetyl receptor ODR-10 in dauer larvae, and

519   higher levels of the ODR-10 protein in AWA olfactory cilia in PD adults. Via transcriptional

520   profiling, we further show that levels of a subset of additional AWA-expressed chemoreceptors

521   are also modulated by developmental stage and trajectory. Our results suggest that state- and

522   experience-dependent cues are integrated to differentially modulate individual chemoreceptor

523   levels in a single sensory neuron type likely via both transcriptional and post-transcriptional

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524　mechanisms, thereby providing a possible mechanism underlying developmental history-

525　dependent olfactory behavioral plasticity.

526　　　　Upon entry into the dauer stage, *odr-10* expression is transcriptionally upregulated in part

527　via DAF-16. However, activation of DAF-16 alone appears to be insufficient to upregulate *odr-*

528　*10* expression since *odr-10* expression is not upregulated in *daf-2* insulin receptor mutants that

529　did not enter the dauer stage. Although we are unable to exclude the possibility that DAF-16 is

530　only partially depleted in AWA upon auxin treatment, it is likely that additional factors including

531　transcription factors such as the DAF-3 SMAD or the DAF-12 nuclear hormone receptor that act

532　downstream of dauer-promoting hormonal signals also play a role in regulating *odr-10*

533　expression (Aghayeva et al., 2021; Fielenbach and Antebi, 2008; Sims et al., 2016). Similarly,

534　altered expression of innexins and other chemoreceptors in dauers were shown to be only partly

535　DAF-16-dependent (Aghayeva et al., 2021). Upon exit from the dauer stage, *odr-10* expression

536　is downregulated possibly due to inactivation of DAF-16 and the altered chromatin profile of

537　these animals (Hall et al., 2010; Sims et al., 2016), but levels of ciliary ODR-10 protein continue

538　to be maintained at higher levels than in control animals. Ciliary GPCRs are trafficked to the

539　cilia base and further trafficked into and out of the cilium via diffusion and motor-driven

540　transport (Mukhopadhyay et al., 2017; Nachury and Mick, 2019). We previously showed that

541　ciliary trafficking of chemoreceptors in different *C. elegans* sensory neurons is regulated by

542　multiple neuron- and receptor-specific mechanisms, and that these trafficking mechanisms are

543　further modulated by sensory signaling (Brear et al., 2014; DiTirro et al., 2019). Sensory neuron

544　cilia including those of AWA are extensively remodeled in dauers but their morphologies appear

545　to be restored to those resembling those in control animals in PD adults (Albert and Riddle,

546　1983; Britz et al., 2021) (this work). We propose that one or more ciliary trafficking mechanisms

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547   are altered in PD animals resulting in increased ODR-10 protein trafficking into, or decreased

548   removal from, the AWA cilia in PD animals. In addition to transcriptional changes in

549   chemoreceptor expression, regulated trafficking of ciliary GPCRs provides an additional

550   mechanism to fine tune sensory responses as a consequence of developmental experience.

551   Why do PD adults enhance their responses to food-related odors? Stress, including

552   starvation, experienced during early larval stages may indicate that food availability is unreliable.

553   Increased attraction to food in PD adults may be a bet-hedging strategy that optimizes growth

554   and survival in a variable environment. However, although animals also retain a cellular memory

555   of starvation-induced L1 arrest (Jobson et al., 2015; Webster et al., 2018), this arrest does not

556   appear to alter examined olfactory behaviors in adults, suggesting that stress assessed during a

557   distinct period in development and/or dauer entry is required to drive behavioral plasticity in

558   ensuing adults. Interestingly, we and others previously showed that expression of the *osm-9*

559   TRPV channel gene is strongly downregulated in the ADL nociceptive but not AWA neurons in

560   PD adults, resulting in decreased responses to the ADL-sensed aversive ascr#3 pheromone (Sims

561   et al., 2016). Decreased aversion to this pheromone by PD adults has been suggested to be a

562   mechanism that inhibits dispersion in a crowded environment and promotes outcrossing (Sims et

563   al., 2016). Coordinated differential modulation of responses in defined subsets of chemosensory

564   neurons likely allow PD adults to optimize survival and reproduction.

565   In contrast to the main olfactory systems of vertebrates in which each olfactory sensory

566   neuron expresses one or very few olfactory receptors (Chess et al., 1994; Ressler et al., 1993;

567   Vassar et al., 1993), the co-expression of as many as 100 chemoreceptors in each chemosensory

568   neuron type in *C. elegans* (Taylor et al., 2021; Troemel et al., 1995; Vidal et al., 2018) raises a

569   unique challenge for this organism. Since co-expressed chemoreceptors are likely tuned to

25

570   distinct odorants, modulating synaptic transmission from a single chemosensory neuron in *C.*

571   *elegans* as a function of internal state would be expected to coordinately alter responses to a

572   broad range of chemicals sensed by that neuron type. Regulation of individual chemoreceptors

573   instead enables the animal to precisely target and modulate defined chemosensory behaviors.

574   Consistent with this notion, expression of chemoreceptor genes is subject to complex modes of

575   regulation. *odr-10* expression is higher in hermaphrodites and juvenile male larvae than in adult

576   males, and expression is upregulated in adults of both sexes upon starvation (Ryan et al., 2014;

577   Wexler et al., 2020). The expression of multiple chemoreceptors in many sensory neuron types is

578   dramatically altered in dauer larvae and subsequently further modulated in PD animals, whereas

579   the expression of other receptors is sexually dimorphic (Nolan et al., 2002; Peckol et al., 1999;

580   Troemel et al., 1995; Vidal et al., 2018). In the ADL nociceptive neurons, expression of the *srh-*

581   *234* chemoreceptor has been shown to be regulated via cell-autonomous and non cell-

582   autonomous mechanisms (Gruner et al., 2016; Gruner et al., 2014). Thus, the expression of each

583   chemoreceptor gene may be modulated by multiple regulatory modules that act combinatorially

584   to integrate distinct inputs and appropriately calibrate the behavioral response.

585   Individual chemosensory neurons in *Aedes aegypti* also express multiple receptors from

586   different receptor subfamilies (Younger et al., 2020). The expression of subsets of these

587   receptors has been shown to be modulated by feeding and reproductive state although whether

588   receptor expression is causative to altered olfactory behavioral profiles is unclear (Matthews et

589   al., 2016). In rodents, a subset of specialized olfactory neurons in rodents express multiple

590   receptors of the MS4A family, each of which responds to ethologically relevant chemical stimuli

591   (Greer et al., 2016). It will be interesting to assess whether expression of these receptors is also

592   subject to extensive state-dependent modulation, and whether this modulation drives sensory

593   behavioral plasticity as a function of current and past external and internal conditions.

594

DATA AVAILABILITY STATEMENT

All plasmids used in this work are listed in Table S1. All strains used in this work are listed in Table S2. Strains and plasmids are available upon request. Data underlying this article are available at https://github.com/SenguptaLab/PDplasticity. Sequencing files of the RNA-Seq experiments have been deposited in the Array Express database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-11823.

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811
812

813 **FIGURE LEGENDS**

814 **Figure 1.** Olfactory responses to a panel of volatile attractants is increased in PD adults.

815 **A)** Cartoon of growth conditions for the generation of control and post-dauer (PD) adults.

816 **B)** Behavioral responses of wild-type control and PD adults to a panel of volatile odorants at the

817 indicated dilutions. Chemotaxis index = (Number of animals at the odorant – number of animals

818 at the diluent ethanol) / (Number of animals at the odorant + number of animals at ethanol). Each

819 dot is the chemotaxis index of a single assay plate containing ~50-300 adult hermaphrodites.

820 Bars represent the mean; error bars are SEM. The behaviors of control and PD animals were

821 assayed in parallel in duplicate; ≥3 independent experiments. *, **, *** indicate different at

822 $P<0.05$, 0.01, and 0.001, respectively (two-tailed Welch's t-test).

823 **C)** Average changes in GCaMP2.2b fluorescence in AWA soma to a 30 sec pulse of 10^{-5} diacetyl

824 in control and PD adults. Shaded regions indicate SEM. n ≥ 16 animals (1 neuron per animal)

825 each.

826 **D)** Quantification of peak fluorescence intensity changes in AWA soma expressing GCaMP2.2b

827 to a 30 sec pulse of diacetyl at the indicated concentrations. Bars represent mean, error bars are

828 SEM. n ≥ 16 animals (1 neuron per animal) each. Control and PD adults were examined in

829 parallel over at least three days. ** and *** indicate different at each concentration at $P<0.01$

830 and 0.001, respectively (two-tailed Welch's t-test); ns – not significant.

831

832 **Figure 2.** Ciliary ODR-10 levels are increased in PD adults.

833 **A)** Representative images of AWA neurons expressing *odr-10*::*t2A*::*mNeonGreen* from the

834 endogenous *odr-10* locus in control and PD adults. Arrows indicate AWA cell bodies. Anterior is

835 at left. Scale bars: 5 μm.

34

836   **B)** Quantification of ODR-10::t2A::mNeonGreen fluorescence in AWA soma in control and PD

837   adults. Each dot is a measurement from an individual neuron. Bars represent the mean; error bars

838   are SEM. n ≥ 39 neurons (≥ 21 animals). Control and PD adults were imaged in parallel on the

839   same day; ≥3 independent experiments. ns – not significant.

840   **C)** Representative images of ODR-10::splitGFP$_{11}$ expression in AWA cilia of control and PD

841   adults also expressing *gpa-4Δ6*p::splitGFP$_{1-10}$. MKS-5::tagRFP marks the ciliary transition

842   zones. Arrows indicate the ciliary base. Anterior is at top. Scale bars: 5 μm.

843   **D)** Quantification of total ciliary ODR-10::splitGFP$_{11}$ fluorescence in AWA cilia base and

844   primary stalk in control and PD adults also expressing *gpa-4Δ6*p::splitGFP$_{1-10}$. Each dot is a

845   measurement from an individual animal. Bars represent the mean, error bars are SEM. n ≥ 29

846   animals. Control and PD adults were imaged in parallel; ≥3 independent experiments. ***

847   indicates different at *P*<0.001 (two-tailed Welch's t-test).

848   **E)** Behavioral responses of wild-type control and wild-type control animals overexpressing

849   ODR-10::tagRFP under the *gpa-4Δ6* promoter (OX ODR-10; 5 ng/μl) to the indicated dilutions

850   of diacetyl. Each dot represents the chemotaxis index of a single assay plate containing ~50-300

851   adult hermaphrodites. Bars represent the mean; error bars are SEM. A subset of control and

852   experimental chemotaxis assays were performed in parallel over at least three days. * and **

853   indicate different at each concentration at *P*<0.05 and <0.01, respectively (two-tailed Welch's t-

854   test).

855   **F)** Quantification of peak fluorescence intensity changes in AWA to a 10 sec pulse of 10^{-7}

856   diacetyl. Responses of wild-type control animals injected with *gpa-4Δ6p::odr-10::tagRFP* at 5

857   and 30 ng/μl are shown. Bars represent mean, error bars are SEM. n ≥ 16 animals (1 neuron per

858   animal) each. Animals were examined over two days with the exception of OX ODR-10 (30

859   ng/µl). * and ** indicate different at *P*<0.05 and <0.01, respectively (Kruskal-Wallis with

860   Dunn's multiple comparisons test).

861

862   **Figure 3.** Dauer larvae exhibit upregulated *odr-10* expression and enhanced diacetyl responses.

863   **A)** Representative images of AWA neurons in L3 and dauer larvae expressing ODR-

864   10::t2A::mNeonGreen from the endogenous *odr-10* locus. Arrows indicate AWA cell bodies.

865   Anterior is at left. Scale bar: 5 µm.

866   **B)** Quantification of ODR-10::t2A::mNeonGreen fluorescence in AWA neurons of the indicated

867   animals. Each dot is a measurement from a single neuron. Bars represent the mean; error bars are

868   SEM. n ≥ 31 neurons; (≥ 18 animals). L3 and dauer larvae were imaged on the same day, ≥3

869   independent experiments. *** indicates different between indicated at *P*<0.001 (two-tailed

870   Welch's t-test).

871   **C)** Representative images of reconstituted ODR-10::GFP expression in AWA cilia of L3 and

872   dauer larvae. MKS-5::tagRFP marks the ciliary transition zones. Arrows indicate the AWA cilia

873   base. Anterior at top. Scale bars: 5 µm.

874   **D)** Quantification of total reconstituted ODR-10::GFP fluorescence in the AWA ciliary base and

875   primary stalk in L3 and dauer animals. Each dot is a measurement from an individual animal.

876   Bars represent the mean total fluorescence, error bars are SEM. n ≥ 23 animals. *** indicates

877   different between indicated at *P*<0.001 (two-tailed Welch's t-test).

878   **E)** Average changes in GCaMP2.2b fluorescence in AWA to a 30 sec pulse of 10^{-5} dilution of

879   diacetyl in control and PD adults, and dauer larvae. Shaded regions indicate SEM. Control and

880   PD adult data are repeated from Figure 1D. n ≥ 16 neurons (1 neuron per animal).

881 **F)** Quantification of peak fluorescence intensity changes in AWA expressing GCaMP2.2b to a

882 30 sec pulse of 10^{-5} dilution of diacetyl. Each dot is a measurement from a single neuron. Bars

883 represent mean fluorescence, error bars are SEM. $n \geq 16$ neurons (1 neuron per animal). Control

884 and PD adult data are repeated from Figure 1D. * and *** indicate different at $P<0.05$ and

885 <0.001, respectively (one-way ANOVA with Tukey's multiple comparisons test).

886

887 **Figure 4.** DAF-16 FOXO may be partly necessary for upregulation of *odr-10* expression in

888 dauer larvae.

889 **A)** Cartoons of different growth conditions of *daf-2(e1368)* animals expressing *daf-*

890 *16::mNeptune::AID, odr-10::t2A::mNeonGreen* and *gpa-4Δ6*p::TIR1 with or without 1 mM

891 auxin.

892 **B)** Quantification of mean *odr-10::t2A::mNeonGreen* fluorescence in AWA in animals grown

893 with or without 1 mM auxin in conditions indicated in **Ai-Aiii**. Each point is a measurement

894 from an individual AWA neuron. Bars represent mean fluorescence, error bars are SEM. $n \geq 46$

895 neurons (≥ 23 animals) for dauers; $n \geq 40$ neurons (≥ 22 animals) for adults. Dauer larvae and

896 control and PD adults grown with or without 1 mM auxin were imaged in parallel; 3 independent

897 experiments. *** indicates different between indicated at $P<0.001$ (two-tailed Welch's t-test); ns

898 – not significant.

899 **C)** Quantification of GFP fluorescence in AWA neurons expressing GFP under 1.0 kb of wild-

900 type or mutant *odr-10* promoter sequences in which a conserved DAF-16 binding site has been

901 mutated (mut) (Wexler et al., 2020). Each point is a measurement from an individual neuron.

902 Bars represent mean fluorescence, error bars are SEM. $n \geq 21$ neurons (≥ 27 animals). ** and

37

903   *** indicate different at *P*<0.01 and <0.001, respectively (Kruskal-Wallis test with Dunn's

904   multiple comparisons test); ns – not significant.

905   **D)** Quantification of total reconstituted ODR-10::GFP fluorescence in AWA ciliary base and

906   stalk. mut indicates animals in which a conserved DAF-16 binding sequence within the

907   endogenous *odr-10* promoter has been mutated. Each dot is a measurement from an individual

908   neuron. Bars represent the mean total fluorescence, error bars are SEM. n ≥ 29 animals. Control

909   and PD wild-type data are repeated from Figure 2D. *** indicates different at *P*<0.001 (one-way

910   ANOVA with Tukey's multiple comparison test); ns – not significant.

911

912   **Figure 5.** Gene expression is altered in AWA following passage through the dauer stage.

913   **A)** MA plot showing enrichment and log2 fold changes of differentially expressed genes in PD

914   vs control whole worm RNA-Seq libraries. The names of a subset of genes from different gene

915   families are indicated. Up- and down-regulated genes were determined by differential expression

916   analysis with a log2 fold change cut off > 1.5, padj < 0.05.

917   **B)** Waterfall plot of differentially expressed genes from indicated gene families in control and

918   PD whole animal RNA-Seq data. Up- and down-regulated genes were determined by differential

919   expression analysis with a log2 fold change cut off > 1.5, padj < 0.05.

920   **C)** MA plot showing enrichment and log2 fold changes of differentially expressed genes in PD

921   vs control AWA RNA-Seq libraries. The names of a subset of genes from different gene families

922   are indicated. Up- and down-regulated genes were determined by differential expression analysis

923   with a log2 fold change cut off > 1.5, padj < 0.05.

924   **D)** Waterfall plot of differentially expressed genes from indicated gene families in PD vs control

925   AWA RNA-Seq libraries. Up- and down-regulated genes were determined by differential

926   expression analysis with a log2 fold change cut off > 1.5, padj < 0.05.

927

928   **Figure 6.** The expression of a subset of AWA-expressed chemoreceptor genes may be regulated

929   similarly to *odr-10* upon dauer passage.

930   **A)** Representative images of receptor::t2A::mNeonGreen expression from the corresponding

931   endogenous loci in L3, dauer, control, and post-dauer adult stage animals. AWA neurons are

932   marked via expression of *gpa-4Δ6*p::tagRFP. Arrows indicate AWA neurons; asterisks indicate

933   ectopic expression in other cell types. Anterior is at left. Scale bars: 5 µm.

934   **B,C,D)** Quantification of mean receptor::t2A::mNeonGreen fluorescence in AWA neurons in

935   animals of at the indicated developmental stages. Each dot is a measurement from an individual

936   neuron. Bars represent mean; error bars are SEM. n ≥ 28 neurons (≥ 18 animals). L3 and dauer

937   larvae were imaged in parallel; 3 independent experiments. Control and PD adults were imaged

938   in parallel; 3 independent experiments. *, **, *** indicates different at *P*<0.05, <0.01, and

939   <0.001 (Kruskal-Wallis with Dunn's multiple comparisons test); ns – not significant.

940   **E)** Quantification of mean SRD-28::t2A::mNeonGreen fluorescence in AWA in *daf-2(e1368)*

941   animals expressing *daf-16::mNeptune::AID* and *gpa-4Δ6p::TIR1* grown with or without 1 mM

942   auxin. Animals were grown as indicated in the cartoon in Figure 4Ai. Each dot is a measurement

943   from an individual neuron. Bars represent mean fluorescence, error bars are SEM. n ≥ 37

944   neurons (≥ 22 animals). Control and dauers grown on plates with and without 1 mM auxin plates

945   were imaged in parallel; 3 independent experiments. *** indicates different at *P*<0.001 (two-

946   tailed Welch's t-test); ns – not significant.

Kyani-Rogers et al., Figure 1

Kyani-Rogers et al., Figure 2

A

B

C

D

E

F

Kyani-Rogers et al., Figure 3

A

i

daf-2;daf-16::AID;
odr-10::t2A::mNG;
Ex[AWAp::TIR1]

L4s → Dauer larvae

4 Days 25°C

L4s → Dauer larvae

+1 mM Auxin

ii

daf-2;daf-16::AID;
odr-10::t2A::mNG;
Ex[AWAp::TIR1]

L4s → Control Adults

4 Days 20°C

L4s → Control Adults

+1 mM Auxin

iii

daf-2;daf-16::AID;
odr-10::t2A::mNG;
Ex[AWAp::TIR1]

L4s → 4 Days 25°C → Dauer larvae → 2 Days 20°C → Post-dauer Adults

+1 mM Auxin → Post-dauer Adults

B

Genotype of strain:
daf-2;daf-16::AID;
odr-10::t2A::mNG;
Ex[AWAp::TIR1]

C

D

Kyani-Rogers et al., Figure 4

Kyani-Rogers et al., Figure 5

Kyani-Rogers et al., Figure 6

1   **SUPPLEMENTAL FIGURE LEGENDS**

2   **Figure S1.** Adults that experienced L1 arrest do not exhibit increased diacetyl responses.

3   **A)** Behavioral responses of wild-type control (con) and post-L1 (post-L1 arrest) arrested adults

4   to indicated dilutions of diacetyl. Each dot is the chemotaxis index of a single assay plate

5   containing ~50-300 adult hermaphrodites. Bars represent the mean; error bars are SEM. The

6   behaviors of control and post-L1 arrested animals were assayed in parallel in duplicate; ≥3

7   independent experiments; ns – not significant (two-tailed Welch's t-test).

8   **B)** Behavioral responses of wild-type control and PD adults to the indicated concentrations of

9   aversive volatile odorants. Each dot represents the chemotaxis index of a single assay plate

10   containing ~50-300 adult hermaphrodites. Bars represent the mean; error bars are SEM. Control

11   and PD behaviors were assessed in parallel; ≥ 3 independent experiments. * indicates different at

12   *P*<0.05 (two-tailed Welch's t-test); ns – not significant.

13   **C)** Baseline GCaMP2.2b fluorescence in AWA soma in the indicated wild-type animals.

14   Baseline measurements were collected from experiments reported in Figures 1D and 3F. Each

15   dot is the measurement from a single neuron. Bars represent the mean; error bars are SEM. n ≥

16   17 animals (1 neuron per animal). * indicates different at *P*<0.05 (one-way ANOVA with

17   Tukey's multiple comparisons test); ns – not significant.

18

19   **Figure S2.** An endogenously tagged *odr-10::gfp₁₁* strain retains dauer passage-dependent

20   olfactory behavioral plasticity as adults.

21   **A)** Behavioral responses of *odr-10(oy158)* control and PD animals expressing *odr-10::gfp₁₁* from

22   the endogenous *odr-10* locus to the indicated dilutions of diacetyl. Each dot represents the

23   chemotaxis index of a single assay plate containing ~50-300 adult hermaphrodites. Bars

1

24   represent the mean; error bars are SEM. Chemotaxis assays at each concentration were

25   performed in parallel over at least three days. ** and *** indicate different at each concentration

26   at $P<0.01$ and 0.001, respectively (two-tailed t-test with Welch's correction).

27   **B)** Average changes in GCaMP2.2b fluorescence in AWA soma in control animals of the

28   indicated genotypes to a 10 sec pulse of 10^{-7} diacetyl. Shaded regions indicate SEM. n $\geq$ 16

29   animals (1 neuron per animal) each.

30

31   **Figure S3.** Transcriptional profiling of sorted populations of AWA neurons.

32   **A)** PCA clustering of RNA-Seq libraries from sorted populations of AWA neurons and

33   dissociated cells from whole animals based on the 10,000 most differentially expressed genes.

34   **B)** Tissue Enrichment Analysis with the web-based Tissue Enrichment Analysis tool

35   (https://www.wormbase.org/tools/enrichment/tea/tea.cgi) (Angeles-Albores et al., 2016) shows

36   enrichment of AWA-expressed genes in AWA RNA-Seq libraries. log2 fold change cut off > 2,

37   padj < 0.05.

38   **C)** MA plot showing differentially expressed genes in RNA-Seq data from FACS-sorted

39   populations of control and PD AWA neurons as compared to dissociated but unsorted cells from

40   control and PD whole animals. 20 of the most highly AWA-expressed genes (Taylor et al., 2021)

41   are indicated. Up- and down-regulated genes were determined by differential expression analysis

42   with a log2 fold change cut off > 2, padj < 0.05.

43

44   **File S1.** RNA-Seq data of differentially expressed genes from control and PD whole animals.

45   **File S2.** RNA-Seq data of differentially expressed genes from sorted control and PD AWA

46   neurons.

47 **Table S1.** Plasmids used in this work.

Plasmid	Construct	Source	Relevant Figures
PSAB1144	*gpa-4Δ6*p::*mks-5::rfp*	This work	2C, 2D, S2A, 3C, 3D, 4D
PSAB1280	*gpa-4Δ6*p:: *gfp₁₋₁₀*	This work	2C, 2D, S2A, 3C, 3D, 4D
PSAB1281	*gpa-4Δ6*p::*odr-10::tagRFP*	This work	2E, 2F, S2B
PSAB1282	*gpa-4Δ6*p::*tagRFP*	This work	6A, 6B, 6C, 6D
PSAB1283	*gpa-4Δ6*p::*tir1::SL2::mScarlet*	This work	4A, 4B, 6E

48
49

50   **Table S2.** Strains used in this work.

Strain	Genotype	Source	Relevant Figures
WT	N2 (Bristol)	CGC	1A, 1B, S1A, S1B
CX14887	*kyIs598[gpa-6*p*::GCaMP2.2b, unc-122*p*::dsRed]*	(Larsch et al., 2013)	1C, 1D, 2F, 3E, 3F, S1C, S2B
PHX3508	*odr-10(syb3508) (odr-10::t2A::mNeonGreen)*	(McLachlan et al., 2022)	2A, 2B, 3A, 3B
PY12004	*odr-10(oy158) [odr-10::gfp$_{11}$]; oyEx681 [gpa-4Δ6*p*::gfp$_{1-10}$; gpa-4Δ6*p*::mks-5::tagRFP, unc-122*p*::dsRed]*	This work; gift from J. Kaplan	2C, 2D, 3C, 3D, 4D, S2A
PY12009	*oyEx683 [gpa-4Δ6*p*::odr-10::tagRFP, unc-122*p*::gfp]* 5 ng/μl	This work	2E
PY12010	*oyEx683 [gpa-4Δ6*p*::odr-10::tagRFP, unc-122*p*::gfp; kyIs598[gpa-6*p*::GCaMP2.2b, unc-122*p:dsRed]* 5 ng/μl	This work	2F, S2B
PY12412	*oyEx684 [gpa-4Δ6*p*::odr-10::tagRFP, unc-122*p*::gfp; kyIs598[gpa-6*p*::GCaMP2.2b, unc-122*p:dsRed]* 30 ng/μl	This work	2F, S2B
PY12400	*odr-10(syb3508) (odr-10::t2A::mNeonGreen); daf-2(e1368); daf-16(ot975) [daf-16::mNeptune2.5::AID]; oyEx687 [gpa-4Δ6*p*::tir1::SL2::mScarlet, unc-122*p*::dsRed]*	This work; (Aghayeva et al., 2020; Aghayeva et al., 2021; McLachlan et al., 2022)	4A, 4B
CX3260	*kyIs37 [odr-10*p*::gfp + lin-15*(+)]	(Sengupta et al., 1996)	4C
PY12401	*fsEx571 [odr-10*p*::Δdaf-16::gfp + myo-3*p*::mCherry]* Line 2, 3x backcrossed	(Wexler et al., 2020)	4C
PY12402	*odr-10(oy170) odr-10*p*Δdaf-16; odr-10(oy158); oyEx681 [gpa-4Δ6*p*:: gfp$_{1-10}$; gpa-4Δ6*p*::mks-5::tagRFP, unc-122*p*::dsRed*	This work	4E
PY10421	*oyIs88 [gpa-4Δ6*p*::myrGFP]*	(Maurya and Sengupta, 2021)	5A, 5B, 5C, 5D, S3
PY12403	*srd-27(syb2465) (srd-27::t2A::NeonGreen); oyEx688 [gpa-4Δ6*p*::tagRFP, unc-122*p*::gfp]*	(McLachlan et al., 2022)	6A, 6B
PY12404	*srd-28(syb2320) (srd-28::t2A::NeonGreen); oyEx688 [gpa-4Δ6*p*::tagRFP, unc-122*p*::gfp]*	(McLachlan et al., 2022)	6A, 6C
PY12405	*str-44(syb1869) (str-44::t2A::NeonGreen); oyEx688 [gpa-4Δ6*p*::tagRFP, unc-122*p*::gfp]*	(McLachlan et al., 2022)	6A, 6D
PY12406	*srd-28(syb2320) (srd-28::t2A::NeonGreen); daf-2(e1368); daf-16(ot975 [daf-*	This work; (Aghayeva et al., 2020; Aghayeva	6E

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16::mNeptune2.5::AID]; oyEx687[gpa-4Δ6p::tir1::SL2::mScarlet, unc-122p::dsRed]	et al., 2021; McLachlan et al., 2022)	

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Kyani-Rogers et al., Figure S1

Kyani-Rogers et al., Figure S2

Kyani-Rogers et al., Figure S3