# Pathogen infection induces sickness behaviors by recruiting neuromodulatory systems linked to stress and satiety in *C. elegans*

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#### SUMMARY

When animals are infected by a pathogen, peripheral sensors of infection signal to the brain to coordinate a set of adaptive behavioral changes known as sickness behaviors. While the pathways that signal from the periphery to the brain have been intensively studied in recent years, how central circuits are reconfigured to elicit sickness behaviors is not well understood. Here we find that neuromodulatory systems linked to stress and satiety are recruited upon infection to drive sickness behaviors in *C. elegans*. Upon chronic infection by the bacterium Pseudomonas aeruginosa PA14, C. elegans decrease their feeding behavior, then display reversible bouts of quiescence, and eventually die. The ALA neuron and its neuropeptides FLP-7, FLP-24, and NLP-8, which control stress-induced sleep in uninfected animals, promote the PA14-induced feeding reduction. However, the ALA neuropeptide FLP-13 instead acts to delay quiescence and death in infected animals. Cell-specific genetic perturbations show that the neurons that release FLP-13 to delay quiescence in infected animals are distinct from ALA. A brain-wide imaging screen reveals that infection-induced quiescence involves ASI and DAF-7/TGF-beta, which control satiety-induced quiescence in uninfected animals. Our results suggest that a common set of neuromodulators are recruited across different physiological states, acting from distinct neural sources and in distinct combinations to drive state-dependent behaviors.

#### **INTRODUCTION**

The physiology of an animal profoundly influences its behavior<sup>1,2</sup>. For example, when animals are hungry, sick, or injured, they alter their feeding and exploratory behaviors to maximize their chances of survival. The interoceptive pathways that allow physiological signals in the body to influence behavior have been the subject of intense investigation in recent years, revealing several routes of signaling from the body to the brain<sup>3,4</sup>. However, how neural circuits in the brain are modulated by these physiological changes to alter behavior remains poorly understood.

Infection by a pathogen changes many aspects of an animal's behavior, commonly referred to as "sickness" behaviors, that help animals restore bodily homeostasis and adapt their behavior during illness. These commonly include reduced appetite, fever, and lethargy<sup>5–7</sup>. In mammals, multiple pathways relay information about infection to the central nervous system (CNS) to induce sickness behaviors. Peripheral nerve endings in distal tissues, for example those of the vagus nerve, receive

molecular signals pertaining to infection and propagate this information to the CNS<sup>3</sup>. In addition, cytokines produced by circulating immune cells can also act on CNS neurons and cells that line the blood-brain barrier to signal infection<sup>8</sup>. CNS brain regions that are modulated by infection and, in turn, alter behavior include the nucleus of the solitary tract<sup>9</sup>, the insular cortex<sup>10,11</sup>, and subsets of hypothalamic nuclei<sup>12</sup>. Neurons in these brain regions can control aspects of motivated behavior, as well as autonomic functions like the control of body temperature<sup>9,10,12,13</sup>. How these neurons are embedded in a broader circuit architecture that is modulated by infection to control behavior remains an area of active investigation.

The nematode *C. elegans* provides a system where it should be possible to connect physiological changes, like infection, to precise changes in neural circuit function that impact behavior. The *C. elegans* nervous system consists of 302 uniquely identifiable neurons connected through a fully defined wiring diagram<sup>14–16</sup>. These neurons control a well-defined set of motor outputs, including locomotion, feeding, and egg-laying, as well as more complex behaviors like foraging and mating. *C. elegans* modulates its behavior based on its physiological state<sup>17–19</sup>. For example, noxious stressors, like heat shock or UV exposure, lead to bouts of sleep-like quiescence, a behavioral state that depends on the ALA neuron releasing several somnogenic neuropeptides<sup>20–22</sup>. Changes in food availability, ingestion, and metabolism impact the animal's foraging behaviors<sup>23–26</sup>, which depend on neuromodulators like serotonin, octopamine, and insulin-like peptides. In addition, re-feeding after food deprivation induces satiety quiescence, which requires the release of DAF-7/TGF-beta from the ASI neuron<sup>27,28</sup>. Thus, the neuromodulatory systems of *C. elegans* allow the animal to adapt its behavior to its ongoing physiological state.

C. elegans physiology changes as animals become infected by pathogens. When they ingest pathogenic bacteria, these pathogens can populate the C. elegans gut, damage tissues, and cause death<sup>19</sup>. The opportunistic human pathogen *Pseudomonas aeruginosa* strain PA14 has been especially well-studied in this regard. PA14 can release toxins that cause acute injury<sup>29</sup>. In addition, it populates the intestine, where it causes dysbiosis and ruptures the intestinal lining to kill animals<sup>29</sup>. C. elegans has a multi-level defense system to fight infection. Innate immune pathways activated by PA14 infection trigger activation of adaptive stress responses and the production of anti-microbial peptides, which help maintain bodily homeostasis<sup>30,31</sup>. Furthermore, C. elegans exhibits PA14-induced behavioral changes that defend against infection<sup>19</sup>. Infected animals display an avoidance behavior where they leave PA14 bacterial lawns after hours of exposure. This lawn avoidance is triggered by the production of the DAF-7/TGF-beta humoral signal from ASJ neurons<sup>32,33</sup> and FLP-1 neuropeptides from AVK neurons<sup>34</sup>. PA14-exposed animals form associative memories where they learn to avoid the PA14 olfactory cues that were present during infection, so that they navigate away from PA14 in the future<sup>35,36</sup>. This olfactory learning involves serotonin and insulin-like peptides that act on sensory processing neurons to modulate olfactory navigation<sup>35,37</sup>. These studies suggest that neuromodulators play an important role in controlling PA14-induced behavioral changes.

Here, we show that chronic PA14 infection induces sickness behaviors by recruiting neuromodulators that mediate responses to stress and satiety in uninfected animals. *C. elegans* animals chronically infected with PA14 exhibit reduced feeding, followed by quiescence, and then

death. The PA14-induced feeding reduction is mediated by the ALA neuron, which controls stressinduced sleep in uninfected animals. In agreement with ALA's role in suppressing feeding and locomotion in stressed animals, we found that several neuropeptides that are released by ALA suppress feeding in PA14-infected animals. However, one of these ALA-released neuropeptides, *flp-13*, that contributes to stress-induced sleep, is instead released by other neurons in infected animals. In contrast to its previously reported somnogenic roles, FLP-13 acts to delay PA14induced quiescence and death. PA14-induced quiescence requires ASI and DAF-7/TGF-beta, a pathway known to control satiety quiescence in uninfected animals. Taken together, these results suggest that stress- and satiety-associated neuromodulatory systems contribute critically to sickness behaviors. Our results are consistent with a model in which individual neuromodulators do not map onto individual internal states but are deployed from different sources and in different combinations to control distinct internal states, like stress, satiety, and illness.

#### RESULTS

To determine the time course of PA14 infection-induced sickness behaviors and death, we first performed a survival assay to characterize the onset of death after animals were transferred to PA14. Animals started dying after two days of first being exposed to PA14, with more than 90% of animals dead by the fifth day (Fig 1A). Next, we quantified behavioral changes leading up to this point. PA14 infection is known to impact bacterial lawn leaving and olfactory learning<sup>19</sup>, but the impact on other motor programs was unknown. To identify early sickness behaviors, we characterized how PA14 infection alters the different motor outputs of the animal over the first day of infection (Fig 1B, Fig S1A-C). Animals were infected on large lawns of bacteria where they spend most of their time on the lawn, but have the choice of leaving. Given the direct relevance of feeding to pathogen ingestion, we first quantified feeding (or pharyngeal pumping) behavior (Fig 1B). Over the first 20 hours of infection, animals exhibited a progressive decrease in feeding not observed in control animals exposed to non-pathogenic E. coli OP50 bacteria (Fig 1B). We also quantified other motor programs (Fig S1A-C) and found a slight decrease in speed as infection progressed, but no changes in egg-laying or defecation. Notably, starting at two days of infection, we observed that animals frequently exhibited bouts of quiescence (defined as no feeding or movement for >20sec) (Fig 1A, Fig S1D; a more in-depth characterization of this quiescence is below in Fig. 3H-J and Fig. S4D-E). Taken together, these results indicate that PA14 infection suppresses the animal's feeding behavior over the first day of infection, followed by quiescence and eventually death.

We next focused on the PA14-induced decrease in feeding because of its signature as an early behavioral change after PA14 exposure and its possibly relevant role in combating infection. We used multiple approaches to test whether the PA14-induced feeding reduction required an active infection. First, we compared the feeding behavior of animals exposed to live PA14 to animals exposed to UV-killed PA14 for 20 hours (Fig 1C). Animals fed on live PA14 as well as UV-killed PA14 were both transferred to live PA14 plates before assaying feeding rates to control for the acute effects of exposure to PA14 odors or metabolites. Animals previously exposed to live PA14 displayed a reduction in feeding, whereas animals exposed to UV-killed PA14 displayed

high feeding rates matching OP50 controls (Fig 1C). This suggests that only live PA14, capable of infecting *C. elegans*, elicit a decrease in feeding. We also examined the behavioral responses of mutant animals lacking key regulators of innate immunity (*pmk-1* and *tir-1*), which have more severe infections<sup>38,39</sup> (Fig 1D). Both of these mutants displayed exaggerated feeding reduction after PA14 exposure compared to wild-type, providing evidence that innate immunity reduces the impact of PA14 ingestion on feeding behavior. This finding aligns with the established function of innate immunity pathways in defending against PA14 infection in general<sup>30,38</sup>. Finally, we examined the behavioral effects of less infectious variants of *Pseudomonas* and other milder pathogens. Exposure to *Pseudomonas* PA01, which is non-infectious under our culture conditions, and *Serratia* Db11, a bacteria with reduced pathogenicity<sup>29</sup>, did not induce feeding decreases to the same extent as PA14 (Fig 1E). Taken together, these results indicate that a live PA14 infection over 20 hours causes a reduction in feeding in *C. elegans*.

We further characterized this PA14-induced decrease in feeding. First, we tested whether the PA14-induced decrease in feeding is specific to PA14 bacteria or, alternatively, a more general reduction in feeding. To do so, we examined whether transferring infected animals to *E. coli* OP50 could recover feeding rates. We found that PA14-infected animals still displayed reduced feeding on OP50, suggesting that a persisting PA14 infection could still reduce feeding rates on other bacterial food sources (Fig 1F). We also tested whether the reduction in feeding could be due to damage to pharyngeal tissues making animals unable to pump at high rates. We exposed PA14-infected animals to exogenous serotonin, which is known to elevate pumping rates<sup>38</sup>. Exogenous serotonin application caused an increase in pumping in PA14-infected animals, suggesting that these animals are able to pump at higher rates, but do not do so under PA14-infected conditions (Fig 1G).

To identify neural mechanisms that underlie these effects, we examined how PA14 impacts feeding behavior in a panel of 17 mutant strains lacking signaling pathways known to be involved in stress- or pathogen-related behavioral changes (Fig 1H). These included mutants lacking key neuromodulators (*tbh-1*, *tdc-1*, etc.), neuromodulatory receptors (*octr-1*), components of sensory signaling pathways (*tax-4*, etc.), and more. For each mutant, we asked whether PA14 infection reduced their pumping rates compared to baseline feeding rates on OP50. Of all the mutants tested, only *ceh-17* mutants, which lack functional ALA and SIA neurons, failed to reduce their feeding after PA14 infection. Animals lacking *trx-1*, which encodes a Thioredoxin important for PA14 avoidance behaviors<sup>41</sup>, showed an exaggerated decrease in feeding. *ceh-17* animals exhibited normal baseline feeding rates on OP50 bacteria and normal feeding upon initial exposure to PA14 (Fig. S1G). A second null allele of *ceh-17* showed the same phenotype (Fig 1I). Taken together, these data suggest that that the PA14-induced feeding decrease does not require many signaling pathways previously implicated in stress and pathogen responses, but it does require the *ceh-17* gene.

*ceh-17* encodes a homeobox transcription factor that is required for the specification of ALA and SIA neurons<sup>42,43</sup>. Due to the misspecification of ALA, these animals are known to be defective in stress-induced sleep<sup>22,43,44</sup>, but their responses to PA14 infection had not been previously investigated. To test whether the *ceh-17* phenotype was due to ALA misspecification,

we examined mutant animals lacking *ceh-14*, which have a defect in ALA specification but normal SIA development<sup>43</sup>. Like *ceh-17* mutants, *ceh-14* mutants did not show the PA14-induced suppression of feeding, suggesting that ALA is likely critical for the PA14-induced feeding reduction (Fig 1I). *aptf-1* mutants lacking another stress-induced sleep neuron, RIS<sup>45</sup>, displayed normal feeding decreases upon infection (Fig 1H). We examined whether *ceh-17* animals were defective in other PA14-induced behavioral changes by quantifying PA14-induced lawn leaving in these mutants. This revealed that *ceh-17* mutants display normal PA14 lawn leaving, matching wild-type controls (Fig S1H). This suggests that ALA is not required for PA14 lawn leaving and that the deficit in the feeding response is not due to a difference in PA14-induced feeding reduction.

Stress-induced sleep, which is controlled by ALA, shares a similarity with PA14 infection insofar as feeding rates are reduced during both states, albeit more robustly during stress-induced sleep. Given this parallel, we next tested whether the ALA-expressed neuropeptides that promote stress-induced sleep were required for the PA14-induced feeding decrease (Fig 1I). Three ALA-expressed neuropeptides have been previously shown to have overlapping roles in suppressing motor outputs during sleep: *flp-13*, *flp-24*, and *nlp-8*<sup>20,22</sup>. Mutants lacking *flp-24* or *nlp-8* did not display reduced feeding upon PA14 infection, suggesting that each of these neuropeptides is required for the PA14-induced feeding decrease. In addition, the ALA neuropeptide *flp-7*, previously not reported to have a role in sleep, was also required for the infection-induced feeding decrease (Fig 1I).

In contrast, animals lacking the neuropeptide gene flp-13 displayed an exaggerated reduction in feeding upon PA14 infection. In addition, a large proportion of the flp-13 animals displayed reversible quiescence bouts after 20 hours of infection (Fig 1J, Fig S1E), similar to the quiescence phenotype that was observed in wild-type animals much later in the course of infection (Fig 1A, reversibility characterized in Figs 3H, 3I, 4B). We performed survival assays to characterize the onset of quiescence and death in flp-13 animals and found that animals displayed peak quiescence after 20 hours of infection, and more than 90% animals died by 72 hours, which is greatly accelerated compared to WT animals (Fig 1J, 1A). We also characterized survival and onset of quiescence in *ceh*-17 animals (Fig S1F) and found that *ceh*-17 animals displayed quiescence and death at time points similar to wild-type animal, though the fraction of animals that were quiescent was reduced. Together, these results suggest that the ALA neuron and several neuropeptides are required for the PA14-induced pumping decrease, while the loss of *flp*-13 accelerates the onset of quiescence and death in PA14-infected animals (Fig 1K).

# FLP-13 release from sensory and pharyngeal neurons prevents PA14-induced quiescence and death

We next examined if this accelerated quiescence was dependent on infection by live bacteria. PA14-induced quiescence in *flp-13* mutants was not seen on UV-killed PA14 (Fig 2A), suggesting that this phenotype reflects a response to a live infection. Additionally, infection with a less virulent pathogen, *Serratia* Db11 did not lead to accelerated quiescence like that observed

in *flp-13* mutants (Fig. 2A). Thus, the early onset of quiescence in *flp-13* mutants is a specific consequence of live PA14 infection. We next tested whether specific virulence factors expressed by PA14 were essential to drive this quiescence and indeed found that loss of *Pseudomonas* transcription factors *lasR* and *rhIR*, as well as the quorum sensing signaling molecule  $pqsR^{46-48}$ , led to a suppression of quiescence in *flp-13* animals (Fig S2A). These results indicate that the quiescence observed in infected *flp-13* animals depends on a live infection of PA14 with intact virulence pathways. To further characterize the *flp-13* mutant phenotype, we also examined whether other PA14 behavioral responses such as lawn leaving depended on *flp-13*. However, *flp-13* mutants displayed normal PA14 lawn leaving rates (Fig S2B), suggesting that the acceleration of quiescence and death in *flp-13* animals is not due to a difference in PA14 lawn exposure.

We next determined the functionally relevant sites of FLP-13 release that impact this PA14 response. To test this, we used CRISPR/Cas9 genome editing to generate a conditional knockout allele of *flp-13* in which loxP sites were placed before the second exon and after the last exon of the endogenous gene (Fig 2B). Expression of Cre Recombinase should delete the majority of the gene's coding sequence, attenuating *flp-13* function in cells with Cre expression. In addition, a t2a-mScarlet fluorescent reporter was inserted before the native stop codon of *flp-13* to visualize the gene's expression pattern. In the absence of Cre recombinase, this strain displayed a normal PA14-induced feeding decrease, matching wild-type controls (Fig 2C). Pan-neuronal Cre expression in this strain caused the same phenotype observed in *flp-13(tm2427)* null animals where PA14 infection induced quiescence over an accelerated 20 hour timeframe (Fig 2C; note that all statistical comparisons are between day-matched experimental groups; see figure legend for details). This suggests that *flp-13* expression in neurons inhibits quiescence and death in PA14-infected animals.

We next mapped out the neurons where flp-13 is required to delay quiescence and death. We observed mScarlet expression in 14 neurons across the head region of the animal, with the highest expression in the I5 and ALA neurons (Fig S2C), and lower expression in neurons whose positions matched sites of expression according to CeNGEN, which are mostly sensory and pharyngeal neurons. The same number of neurons showed flp-13 expression in PA14 infected animals, with I5 and ALA again showing the highest levels of expression (Fig. S2C). To map out the neurons where flp-13 expression is functionally required for its PA14-induced phenotypes, we first expressed Cre under broad genetic drivers to knock out flp-13 in different subgroups of neurons. Loss of flp-13 from either sensory or pharyngeal neurons was sufficient to recapitulate the null phenotype (Fig 2D). In contrast, loss of flp-13 from ALA did not lead to any difference compared to wild-type animals and to Cre-negative controls with intact flp-13 expression (Fig 2D, Fig S2C). These results suggest that flp-13 functions in sensory and pharyngeal neurons, but not ALA, to prevent PA14-induced quiescence and death.

We further mapped out which specific neurons were functionally important sites of FLP-13 production using cell-specific Cre drivers. Among the pharyngeal neurons, loss of *flp-13* from 15 or 11 accelerated PA14-induced quiescence and death compared to Cre-negative controls with intact *flp-13* expression (Fig 2E). Among the sensory neurons, loss of *flp-13* from ASH and OLL caused this same phenotype (Fig 2F). The same sets of neurons remained functionally critical when quantifying either quiescent animals or both dead and quiescent animals together, matching the fact that quiescence and death are correlated phenotypes in most strains. These results identify specific sensory and pharyngeal neurons that release FLP-13 to prevent PA14-induced quiescence and death.

The requirement for FLP-13 production in I5, I1, ASH, and OLL for PA14-related phenotypes contrasts with previous results showing that FLP-13 production in ALA is required for stress-induced sleep<sup>20,22</sup>. To confirm this functional difference, we used the same conditional knockout strains that we generated here to identify which neurons need to produce FLP-13 for stress-induced sleep. For these experiments, we used a standard stress-induced sleep assay where animals were exposed to a 30 min heat shock and the fraction of quiescent animals was quantified 30 mins later (Fig 2G, left panel). Consistent with prior literature, deletion of *flp-13* in ALA impaired stress-induced sleep (Fig 2G, right panel). In contrast, deletion of *flp-13* in 15 had no effect (Fig 2G, right panel). The disruption of *flp-13* expression in ALA and I5 in each of the respective conditional knockout strains was confirmed using the mScarlet endogenous reporter (Fig S2C, bottom). Together, these results provide a clear separation between functionally required sites of FLP-13 production for stress-induced sleep versus PA14-related quiescence and death (Fig 2H).

#### DMSR-1 is a FLP-13 receptor that delays PA14-induced quiescence and death

flp-13 has been reported to act through at least two receptors, FRPR-4 and DMSR-1, to control other aspects of behavior<sup>49,50</sup>. We examined PA14-induced behavioral changes in null mutants lacking each of these receptors. Whereas *frpr-4* mutants displayed a normal PA14 feeding decrease (Fig. S3A) but no accelerated onset of quiescence, *dmsr-1* mutants displayed robust quiescence within 20 hours of PA14 infection, matching *flp-13* mutants (Fig 3A). This *dmsr-1* mutant phenotype could be rescued by restoring *dmsr-1* expression with a genomic DNA fragment containing the full *dmsr-1* gene (Fig 3B). In time course assays, no quiescence was observed in *dmsr-1* mutants placed on OP50 for the duration of the assay (Fig S3B). However, upon infection, the time course of quiescence and death in *dmsr-1* was even more accelerated than the *flp-13* phenotype (Fig 3C). This suggests that *flp-13* may act through the *dmsr-1* receptor to prevent quiescence and death in PA14-infected animals. DMSR-1 is a GPCR that has the highest binding affinity to FLP-13 but also has other ligands<sup>51,52</sup>. The stronger phenotype of *dmsr-1* mutants, compared to *flp-13*.

We next tested whether PA14 infection impacts the expression pattern of dmsr-1. To do so, we generated a dmsr-1 reporter strain where t2a-mNeonGreen was inserted into the endogenous dmsr-1 gene just before the stop codon. This strain was crossed to NeuroPAL<sup>53</sup> to facilitate neuron identification. The NeuroPAL transgene has three fluorescent proteins (BFP, OFP, mNeptune) expressed under well-defined genetic drivers, which makes it easy to determine the identities of imaged neurons. We observed expression of dmsr-1 expression in many neurons and reliably identified ~43 neurons per animal with bright reporter expression (Fig 3D, Fig S3C-E). We then compared this set of neurons in control and PA14-infected animals but did not detect any differences (Fig. S3C-E). This suggests that PA14 infection does not change the set of neurons that prominently express dmsr-1.

# *flp-13* is not required for PA14-induced stress responses, and delays quiescence and death through a pathway that is independent of known immune pathways

The accelerated onset of quiescence and death in *flp-13* animals raised the possibility that these animals may have aberrant responses to infection and stress. PA14 infection is known to induce a range of stress responses, which can be measured by quantifying induction of responsive reporter genes: mitochondrial damage response (*hsp-6* reporter)<sup>54</sup>, unfolded protein response (*hsp-6* reporter)<sup>54</sup> 4 reporter)<sup>55</sup>, and innate immune responses (*irg-1* reporter)<sup>56</sup> (Fig 3E-G). We compared induction of these reporters in wild-type and either *flp-13* or *dmsr-1* mutants, based on which gene was unlinked to the integrated stress reporter gene, to ease strain construction. We focused on an early timepoint (6 hours) before there might be indirect effects at later timepoints due to animals transitioning into quiescence. hsp-4 and irg-1 were reliably induced at 6 hours, with no detectable difference between wild-type and *flp-13/dmsr-1* (Fig. 3F and 3G). *hsp-6* induction was only observed at later timepoints (Fig S4A), with no difference observed in baseline expression at 6 hours in wild-type and *flp-13* (Fig. 3E). All three reporters showed increased expression at later stages of infection (20 hours) in both WT and *flp-13/dmsr-1* animals (Fig S4A). *flp-13* animals, which exhibit quiescence at this time point, had elevated expression of hsp-4 and hsp-6. These results suggest that several stress response pathways are activated normally in early stages of infection in *flp-13/dmsr-1* mutants. Related to this, we also noted that *flp-13* animals appear morphologically similar to wild-type animals after 20 hours of PA14 infection, unlike innate immunity-defective *pmk-1* animals, which appear visibly sick at this timepoint (Fig S4B).

We also examined interactions between flp-13 and the genetic pathways that have been shown to delay PA14-induced death in *C. elegans*, namely the innate immune pathways. Mutants lacking the immune effectors  $fshr-1^{57}$  and  $sek-1^{38}$ , which are key members of two independent immune pathways, displayed accelerated quiescence and death (Fig. S4C). Deletion of flp-13 in each of these backgrounds led to a stronger, additive phenotype with even further accelerated quiescence and death (Fig. S4C). This suggests that defects in immunity can cause early quiescence and death, but that flp-13 does not function together in a linear pathway with these two well-characterized immune pathways. Mutants lacking the mitochondrial stress regulator  $atfs-1^{58}$ or the immune regulator zip-2, which are both also linked to PA14 immunity, did not display accelerated PA14-induced quiescence, and deletion of flp-13 in these mutant backgrounds yielded phenotypes matching flp-13 single mutants (Fig. S4C). This suggests that defects in mitochondrial stress and the zip-2 immune pathway do not impact chronic PA14-induced quiescence or the flp-13mutant phenotype. Together, these data suggest that flp-13 functions independently of known PA14 innate immunity pathways to delay quiescence and death.

# PA14-induced quiescence is a reversible quiescence state with no change in arousal threshold

Our analysis here of flp-13 mutants revealed a form of quiescence in PA14-infected animals that had not been characterized before. Thus, we next examined whether the PA14induced quiescence state has properties that match other forms of quiescence in *C. elegans*: reversibility, similar genetic requirements, and increased arousal threshold. First, we examined whether this form of quiescence was reversible by testing whether quiescent flp-13 animals could

be acutely aroused out of quiescence. For this, we drove expression of the light-activated adenylyl cyclase BlaC under the pdfr-1 promoter (pdfr-1::BlaC) in flp-13 mutant animals. Activation of pdfr-1::BlaC with blue light has been previously shown to induce robust roaming states<sup>59</sup>, which are sustained bouts of high arousal locomotion (Fig 3H). We examined how quiescent, PA14-infected flp-13; pdfr-1::BlaC animals responded to BlaC activation via blue light illumination. This led to an immediate onset of roaming behavior, peaking at an average speed of 0.15mm/sec, matching typical fast roaming speeds<sup>24,59</sup>. Quiescent flp-13; pdfr-1::BlaC animals also began feeding upon blue light stimulation (Fig 3I). This indicates that flp-13 quiescence is reversible: animals can still exhibit robust locomotion and feeding when optogenetic interventions acutely change the arousal state of their nervous system.

Null mutations in the *egl-4*/PKG gene prevent all known forms of quiescence in *C. elegans*, potentially because these mutations result in high levels of sensory arousal<sup>28,44,60</sup>. To test whether PA14-induced quiescence in *flp-13* or *dmsr-1* animals was also dependent on *egl-4*, we examined PA14 responses in *dmsr-1;egl-4* double mutants. We chose *dmsr-1*, since this gene is unlinked to *egl-4* and eased double mutant construction. Indeed, these animals did not display accelerated PA14-induced quiescence (Fig 3J). However, the elevated PA14-induced death rates observed in *dmsr-1* mutants were still observed in *dmsr-1;egl-4* double mutants (Fig 3J). This result suggests that the death phenotype in *dmsr-1* mutants is not strictly dependent on these mutants displaying quiescence. We also examined the impact of the *egl-4* mutation in an otherwise wild-type background and found that the quiescence displayed after two days in this background was attenuated by the *egl-4* mutation (Fig S4E). This suggests that *flp-13* and *dmsr-1* mutants exhibit accelerated PA14-induced quiescence, which is a reversible behavioral state that requires *egl-4*, like other forms of quiescence in *C. elegans*.

Finally, we tested whether these quiescent animals have an altered arousal threshold. For this, we used a standard assay where animals are exposed to aversive blue light and the latency to respond with reverse movement is quantified. We examined wild-type and *flp-13* animals before and after PA14 infection. Infected *flp-13* mutants that were quiescent after 20 hours of PA14 exposure could still respond to blue light reliably and did not have the increased arousal threshold seen in other forms of quiescence (Fig S4D). These results suggest that PA14-induced quiescence. The suggests that PA14-induced quiescence may be more akin to lethargy than sleep.

#### The PA14-induced quiescence state is mediated by ASI activation and daf-7

We next sought to determine the neural mechanism that drives PA14-induced quiescence. To do so, we performed brain-wide calcium imaging in infected *flp-13* animals as they entered and exited quiescence 20 hours after infection. We performed these studies in freely-moving *flp-13* mutant animals co-expressing pan-neuronal NLS-GCaMP7F and the NeuroPAL transgene to facilitate neuronal identification. Infected animals exhibiting quiescence were selected and were exposed to PA14 during 8-minutes of freely-moving calcium imaging sessions (Fig 4A). At the

end of each imaging session, they were immobilized by cooling and multi-spectral images were captured for NeuroPAL annotations. The live-tracking microscope, software for extracting calcium and behavioral data, and procedure for neuron annotation have been previously described and validated<sup>61</sup>.

We recorded data from eight PA14-infected animals that displayed bouts of quiescence during the recordings. We measured several behavioral variables including feeding rates, locomotion speed, and head curvature, and used these metrics to identify quiescent bouts where there was no movement and feeding (Fig 4B). We recorded 117-176 neurons per animal and determined the identities of 40-121 of these neurons (Fig 4B-C). Bouts of quiescence (quantified as described above) were accompanied by a significant decrease in activity in several (but not all) neuron classes known to encode locomotion (RMD, AVA, OLQ, and others) and feeding (MC, M3, etc.) behaviors (Fig 4D). A smaller subset of neurons displayed elevated activity during quiescence bouts. To determine which neuron classes displayed elevated activity during quiescence, we quantified each neuron class's activity change during quiescence. The neuron classes with the largest activity increase during quiescence were AVB, I4, AVL, ASI, M2 and RIH (Fig. 4C). ASI has been previously shown to be functionally required for satiety-induced quiescence<sup>28,62</sup>, making it an attractive candidate to mediate quiescence in this context. ASI displayed higher activity during quiescence in two of the three datasets where we could identify the neuron (Fig 4E). These results raise the possibility that ASI activity increases during PA14induced quiescence, at a time when many behavioral circuits display diminished activity.

To confirm these results in a larger group of animals, we generated a strain with cellspecific expression of GCaMP7F in ASI (under the *srg-47* promoter) and recorded ASI activity in 17 additional PA14-infected animals. Consistent with the above results, ASI activity was correlated with quiescence bouts in these recordings (Fig 4F). These data indicate that ASI activity is elevated during PA14-induced quiescence in *flp-13* mutants, though we note that its activity is not perfectly predictive of PA14-induced quiescence.

To test whether ASI mediates PA14-induced quiescence, we ablated ASI in a *flp-13* mutant background using a previously characterized ASI::Caspase strain<sup>63</sup>. The loss of ASI delayed the onset of PA14-induced quiescence in *flp-13* animals (Fig 4G and 4J left panel), suggesting that ASI contributes to PA14-induced quiescence in these animals. ASI ablation also delayed PA14-induced quiescence (Fig 4J left panel) and death (Fig S5A-D) in an otherwise wild-type background. Since ASI does not express *flp-13*, but does express *dmsr-1*<sup>64</sup>, FLP-13 could potentially inhibit ASI to delay infection-induced quiescence. However, the additive nature of the *flp-13* and ASI- phenotypes (Fig. 4G) suggests the presence of other factors.

We also examined whether other neurons and genes that control *C. elegans* quiescence in other contexts impact PA14-induced accelerated quiescence in *flp-13* mutants. We found that loss of RIS (via *aptf-1* mutation<sup>65</sup>), or the *nlp-22* neuropeptide-encoding gene (which promotes quiescence<sup>66</sup>) did not alter PA14-induced quiescence in *flp-13* mutants (Fig 4H). Since loss of ALA (via *ceh-17* mutation) suppresses quiescence in *MT* animals (Fig S1F), we tested if it also suppresses quiescence that appears earlier in *flp-13* mutants. Interestingly, loss of ALA dramatically increased the death rate in *flp-13* mutants, indicating that the *ceh-17* mutation is a

genetic enhancer of the *flp-13* mutation. Overall, our results suggest that PA14-induced quiescence in *flp-13* mutants depends in part on the ASI neuron, which has elevated activity during this state.

ASI is known to release the secreted peptide DAF-7/TGF-beta, which impacts many aspects of *C. elegans* behavior and physiology<sup>24,67–69</sup>. Previous work showed that daf-7 expression in ASI is increased upon PA14 infection<sup>32</sup>. In addition, other studies showed that ASI daf-7 expression in fasted animals re-introduced to food is important for satiety quiescence. Moreover, daf-7 is also required for movement quiescence in well-fed animals exposed to noxious stressors such as heat shock or UV exposure<sup>70</sup>. We tested whether daf-7 was required for the quiescence observed in PA14-infected *flp-13* mutants by examining *flp-13;daf-7* double mutant animals. Strikingly, *flp-13;daf-7* double mutants displayed no quiescence or death after 20 hours of infection and attenuated quiescence and death at later time points (Fig 4I and 4J right panel), revealing a strong suppression of the *flp-13* phenotype. We also examined PA14-induced quiescence in *daf-7* single mutants. In contrast to wild-type animals, *daf-7* single mutants did not exhibit quiescence three days after infection (Fig 4J right panel) and showed survival to later time points as well (Fig S5E). The intermediate nature of the daf-7;flp-13 double mutant phenotype suggests that additional PA14-induced quiescence pathways contribute to quiescence in *flp-13* mutants as well (Fig 4I-J, Fig S5E-F). Overall, these results suggest a significant function for *daf*-7 in PA14-induced quiescence and in the accelerated quiescence phenotype of *flp-13*.

#### DISCUSSION

Neuromodulators play pivotal roles in coupling changes in animals' internal states to their behavior. How combinations of neuromodulators released from different neuronal sources control the diverse internal states that animals exhibit remains an open question. In this study, we identified neuromodulatory pathways that control sickness behaviors in pathogen-infected *C. elegans*. In animals that have recently become infected by PA14, feeding behavior is reduced by the release of neuropeptides from ALA, a pathway that is also known to induce stress-induced sleep in uninfected animals. In infected animals, one ALA neuropeptide, FLP-13, takes on a different role and becomes critical for viability. This function of FLP-13 is mediated by its release from sensory and pharyngeal neurons, rather than ALA. The onset of PA14-induced quiescence that is observed in severely infected animals requires ASI and DAF-7/TGF-beta, which also control satiety-induced quiescence in uninfected animals. Our findings show that the neuromodulators that induce sickness behaviors overlap with those that control behavioral states associated with stress and satiety (Fig. 5). This suggests that the states of stress, satiety, and infection are not induced by unique sets of neuromodulators. Instead, one larger set of neuromodulators may be deployed from different sources and in different combinations to specify these different internal states.

#### Infection reconfigures the pathways involved in stress-induced sleep

When uninfected animals are exposed to stressors such as heat-shock or ultraviolet irradiation, the ALA neuron promotes stress-induced sleep by suppressing a suite of behaviors<sup>20,22,44</sup>, including feeding. Our work extends this role to the context of infection, where

ALA suppresses feeding behavior in infected animals. ALA expresses many neuropeptide genes, including *flp-7* as well as *flp-24*, *nlp-8*, and *flp-13*, which collectively induce quiescence . We found that the neuropeptides FLP-24, NLP-8 and FLP-7 are required for feeding reduction upon PA14 infection. These neuropeptides may either be released by ALA or by other co-expressing neurons with infection-specific roles. Interestingly, upon heat shock, *flp-24* and *nlp-8* suppress locomotion and defecation but not feeding<sup>20</sup>, while *flp-7* does not have roles in this context<sup>20</sup>. However, upon infection, each neuropeptide is required for the feeding reduction. Therefore, while two of these neuropeptides continue to suppress behaviors across contexts, all exhibit novel roles in specifically suppressing feeding upon infection. Additional experiments are required to determine whether *flp-24*, *nlp-8* and *flp-7* target overlapping or distinct pathways to suppress feeding upon infection.

Like the other ALA neuropeptides, FLP-13 suppresses behaviors to promote quiescence in uninfected animals exposed to stress<sup>20,22,44,72</sup>. In PA14-infected animals, however, we find that FLP-13 instead delays quiescence and death, a phenotype that is typically classified as an innate immune function. We identified the pharyngeal I1 and I5 neurons, and the sensory ASH and OLL neurons, but not ALA, as critical sites of infection-induced FLP-13 function. It is currently unclear why these specific neurons play a role since we were unable to identify evident changes in the expression patterns of *flp-13* upon infection. One explanation might have been a dosage-dependent effect of *flp-13* where neurons expressing higher levels of *flp-13* play a more critical role in its functions. However, our results are not fully consistent with this explanation: I5 and ALA display the highest *flp-13* expression levels in uninfected and infected animals, but disrupting *flp-13* in ALA did not impact infection-induced quiescence and deleting *flp-13* in I5 did not impact heat shock-induced quiescence. Thus, the functional relevance of the neural source does not fully map onto neurons with the highest *flp-13* receptor encoding gene, *dmsr-1*.

Other untested hypotheses may explain why specific pharyngeal and sensory neurons, and not ALA, become critical FLP-13 sources on infection. One possibility is that chronic infection may increase I5, I1, ASH and OLL activity, resulting in increased FLP-13 release by these neurons. Alternatively, the I1, I5, ASH and OLL neurons may release co-transmitters with FLP-13 that modulate FLP-13 function. Such co-release and co-transmission modulation is pervasive, evolutionary conserved, and lends greater flexibility to neural circuits. Additional studies will be necessary to determine the mechanism at work here, as well as the complex interactions between neuromodulators that give rise to different internal states. Similar complexities are likely to exist in mammalian systems where internal states are best decoded from combinations of neuromodulator-producing cell types, rather than individual cells types<sup>73,74</sup>.

#### Distinct mechanisms underlie different forms of behavioral quiescence

Reversible behavioral quiescence is a conserved component of animal behavior, from jellyfish<sup>75</sup> to humans. The reversibility of quiescence differentiates it from immobile states such as paralysis, coma or death. Diverse environmental and homeostatic cues induce quiescence, which despite being behaviorally homogenous can be caused by distinct neural sub-programs to serve different functions. For instance, in reptiles, birds and mammals, behavioral quiescence is

observed during both rapid eye movement sleep (REM) and non-REM (NREM) sleep<sup>76–78</sup>. However, REM and NREM have distinguishable brain activity patterns<sup>79</sup> and functions in memory consolidation and the regulation of emotion<sup>80,81</sup>. In *Drosophila*, quiescence observed in response to sleep deprivation versus that during natural sleep cycles are mechanistically and functionally distinct<sup>82,83</sup>. Similarly, in the compact nervous system of *C. elegans*, the neural circuits that generate quiescence during developmentally timed sleep at larval transitions<sup>60,66</sup>, stress-induced sleep in adults<sup>20,22,44</sup> or upon satiation<sup>62</sup>, are largely separate<sup>84</sup>.

Our study characterizes a novel form of quiescence in adult *C. elegans* that is displayed upon PA14 infection, is reversible, and is accompanied by reduced activity in many neuronal cell types that encode behavior. However, in contrast to other forms of quiescence, it does not correlate with an increased arousal threshold, suggesting that it may be more akin to lethargy than sleep. In addition, we did not observe increased activity upon infection-quiescence in the ALA and RIS neurons which were reported to be sleep-active in other forms of sleep in *C. elegans* (Fig. 4C). This quiescence is mediated by the selective recruitment of key players involved in other quiescence pathways, i.e., stress-induced sleep and satiety-induced quiescence. Interestingly, the onset of PA14-induced quiescence is often followed by death. Whether infection-induced quiescence functions as a last resort for homeostatic recovery or is regulated by additional non-neuronal endocrine signals remains to be explored.

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#### **AUTHOR CONTRIBUTIONS**

Conceptualization, S.P, G.K.M, and S.W.F. Methodology, S.P and G.K.M, Software, S.P, G.K.M., A.A., A. K-L., L., J.P., and S.W.F. Formal analysis, S.P., G.K.M, and D.K. Investigation, S.P, G.K.M., D.K., E.B., U.D., T.K., M.A.G., J.D.L., and S.W.F. Writing – Original Draft, S.P, G.K.M, and S.W.F. Writing – Review & Editing, S.P, G.K.M, and S.W.F. Funding Acquisition, S.P. and S.W.F.

#### **DECLARATION OF INTERESTS**

The authors have no competing interests to declare.

#### MATERIALS AND METHODS

### **Key Resources Table**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains	1	
Escherichia coli: Strain OP50	<i>Caenorhabditis</i> Genetics Center	OP50
Pseudomonas aeruginosa: Strain PA14	Bob Horvitz Lab	PA14
<i>P. aeruginosa</i> : Strain PA01	Dianne Newman Lab	PA01
Serratia marcescens: Strain Db11	<i>Caenorhabditis</i> Genetics Center	Db11
<i>P.aeruginosa</i> : Strain PA14 $\Delta lasR$	Lars Dietrich Lab	PA14 lasR
P.aeruginosa: Strain PA14 ΔrhIR	Dianne Newman Lab	PA14 rhIR
P.aeruginosa: Strain PA14 ΔpqsR	Dianne Newman Lab	PA14 pqsR
P.aeruginosa: Strain PA14 $\Delta$ hcnABC	Lars Dietrich Lab	PA14 hcn
P.aeruginosa: Strain PA14 Δphz1/2	Lars Dietrich Lab	PA14 phz
<i>P.aeruginosa</i> : Strain PA14 $\Delta$ <i>rhIA</i>	Lars Dietrich Lab	PA14 rhlA
<i>P.aeruginosa</i> : Strain PA14 $\Delta soxR$	Dianne Newman Lab	PA14 soxR
Experimental Models: Organisms/Strains		
<i>C. elegans:</i> Wild-type Bristol N2	<i>Caenorhabditis</i> Genetics Center (CGC)	N2
C. elegans: flp-13 (tm2427)	Sternberg Lab	PS6994
C. elegans: dmsr-1(qn45)	CGC	NQ915
C. elegans: flp-13(syb6180 syb6395)	This paper	PHX6395
C. elegans: flp-13(syb6180 syb6395); flvEx424(osm-6p::Cre, ceh-34p::Cre, elt-2p::GFP)	This paper	SWF896
C. elegans: flp-13(syb6180 syb6395); flvEx421(eat-4p::Cre, elt-2p::GFP)	This paper	SWF893

C. elegans: flp-13(syb6180 syb6395); flvEx431(tag-168p::Cre, elt-2p::GFP) line 1	This paper	SWF921
C. elegans: flp-13(syb6180 syb6395); flvEx431(tag-168p::Cre, elt-2p::GFP) line 2	This paper	SWF922
C. elegans: flp-13(syb6180 syb6395); flvEx444(flp-24p::Cre, elt- 2p::GFP)	This paper	SWF934
C. elegans: flp-13(syb6180 syb6395); flvEx426 (ceh-34p::Cre, elt-2p::GFP)	This paper	SWF943
C. elegans: flp-13(syb6180 syb6395); flvEx426(osm-6p::Cre, elt- 2p::GFP)	This paper	SWF944
C. elegans: flp-13(syb6180 syb6395) IV; flvEx465(ceh-53p::Cre, elt-2p::GFP)	This paper	SWF973
C. elegans: flp-13(syb6180 syb6395) IV ; flvEx468(trh-1p::Cre, elt-2p::GFP)	This paper	SWF975
C. elegans: flp-13(syb6180 syb6395) IV; flvEx471(ceh-45p::Cre, elt-2p::GFP)	This paper	SWF979
C. elegans: flp-13(syb6180 syb6395) IV ; flvEx506(ser-2dp::Cre, sra-6p::Cre, elt-2p::GFP)	This paper	SWF1031
C. elegans: flp-13(syb6180 syb6395) IV; flvEx508(tax-4p::Cre, elt-2p::GFP )	This paper	SWF1034
C. elegans: flp-13(syb6180 syb6395) IV; flvEx511(sra-9p::Cre, gcy-5::Cre, gcy-7p::Cre; gcy-33p::Cre, gcy-36p::Cre, elt- 2p::GFP)	This paper	SWF1037
C. elegans: flp-13(syb6180 syb6395) IV; flvEx515(ceh-28p::Cre, elt-2p::GFP)	This paper	SWF1041
C. elegans: flp-13(syb6180 syb6395) IV; flvEx566(degt-1p::Cre, elt-2p::GFP)	This paper	SWF1134
C. elegans: flp-13(syb6180 syb6395) IV; flvEx564(sra9p::Cre, gcy-5::Cre, gcy-33p::Cre, gcy-36p::Cre, elt-2p::GFP)	This paper	SWF1132
C. elegans: flp-13(syb6180 syb6395) IV; flvEx565(gcy-7p::Cre, elt-2p::GFP)	This paper	SWF1133
C. elegans: flp-13(syb6180 syb6395) IV; flvEx562(sra-6p::Cre, elt-2p::GFP)	This paper	SWF1130
C. elegans: flp-13(syb6180 syb6395) IV; flvEx563(ser-2d::Cre, elt-2p::GFP)	This paper	SWF1131
C. elegans: dmsr-1(qn45); flvEx460(dmsr-1p(3.5kB)::dmsr1- T2a-GFP; myo2p::mCherry) line 1	This paper	SWF968
C. elegans: C. elegans: dmsr-1(qn45); flvEx460(dmsr- 1p(3.5kB)::dmsr1-T2a-GFP; myo2p::mCherry) line 2	This paper	SWF969

C. elegans: zcls13 [hsp-6p::GFP + lin-15(+)] V.	CGC	GL347
C. elegans: flp-13(tm2427); zcls13 (hsp-6p::GFP + lin-15(+))	This paper	SWF995
C. elegans: agls17 [myo-2p::mCherry + irg-1p::GFP] IV	CGC	AU133
C. elegans: dmsr-1(qn45); agls17 (myo-2p::mCherry + irg- 1p::GFP)	This paper	SWF1052
C. elegans: zcls4 (hsp-4::GFP)	CGC	SJ4005
C. elegans: flp-13(tm2427); zcls4 (hsp-4::GFP)	This paper	SWF1114
C. elegans: kyEx4984 (pdfr-1p::BlaC); flp-13(tm2427) IV	This paper	SWF1030
C. elegans: flp-13(tm2427);ceh-17(np1)	This paper	SWF861
C. elegans: flp-13(tm2427); sek-1 (km4)	This paper	SWF982
C. elegans: flp-13(tm2427); nlp-22(gk509904)	This paper	SWF1046
C. elegans: flp-13(tm2427); aptf-1(tm3287)	This paper	SWF1047
C. elegans: flp-13(tm2427); oyls84(gpa-4p::TU#813 + gcy- 27p::TU#814 + gcy-27p::GFP +unc-122p::DsRed)	This paper	SWF1053
flp-13; otls670 [NeuroPAL] V; SWF196-6[MT21793[tag- 168::nls-gcamp7f, gcy-28::tagRFP, ceh-36::tagRFP, inx- 1::tagRFP, mod-1::tagRFP, tph-1(s)::tagRFP, gcy5::tagRFP, gcy7::tagRFP]] back-crossed 5x to MT21793	This paper	SWF994
C. elegans: flp-13(tm2427); daf-7(e1372ts)	This paper	SWF1055
C. elegans: flp-13(tm2427) IV; flvEx535[srg-47p::GCaMP7f(60 ng/uL), myo3p::mCherry(5 ng/uL)] line 4	This paper	SWF1071
C. elegans: oyls84(gpa-4p::TU#813 + gcy-27p::TU#814 + gcy- 27p::GFP +unc-122p::DsRed)	CGC	PY7505
C. elegans: daf-7(e1372ts)	CGC	CB1372
C. elegans: egl-4(n478)	CGC	MT1073
C. elegans: dmsr-1(qn45); egl-4(n478)	This paper	SWF1054
C. elegans: flp-13(tm2427);atfs-1(cmh15)	This paper	SWF880
C. elegans: atfs-1(cmh15)	CGC	SWF879
C. elegans: pmk-1 (km25)	CGC	KU25
C. elegans: tir-1(qd4)	CGC	ZD101
C. elegans: tax-4 (p678)	CGC	CX13078
C. elegans: tbh-1(n3247)	CGC	MT9455

C. elegans: tdc-1(3419)	CGC	MT13113
C. elegans: cat-2(n4547)	CGC	M15620
C. elegans: mod-1(ok103)	CGC	MT9668
C. elegans: nmr-1(ak4)	CGC	VM487
C. elegans: octr-1(ok371)	CGC	CX13079
C. elegans: olm-1(ut305)	CGC	JC2209
C. elegans: trx-1(jh127)	CGC	KJ912
C. elegans: aex-5(sa23)	CGC	JT23
C. elegans: npr-1(ky13)	CGC	CX4148
C. elegans: ins-11(tm1053)	CGC	FX1053
C. elegans: daf-2(m41)	CGC	DR1564
C. elegans: daf-16(mu86)	CGC	CF1038
C. elegans: aptf-1(tm3287)	CGC	HBR232
C. elegans: ceh-17(np1)	CGC	IB16
C. elegans: ceh-17(tm250)	<i>C. elegans</i> National BioResource Project	
C. elegans: ceh-14(ot900)	CGC	OH15422
C. elegans: flp-7(ok2625) BX2	CGC	SWF1135
C. elegans: flp-24(gk3109)	Paul Sternberg Lab	PS6814
C. elegans: nlp-8(ok1799)	Paul Sternberg Lab	PS6911
C. elegans: nlp-8(ok1799);flp-24(gk3109)	Paul Sternberg Lab	PS6991
C. elegans: flp-13(tm2427);flp-24(gk3109);nlp-8(ok1799)	Paul Sternberg Lab	PS6992
C. elegans: flp-13(tm2427);nlp-8(ok1799)	Paul Sternberg Lab	PS6993
C. elegans: flp-13(tm2427);flp-24(gk3109)	Paul Sternberg Lab	PS6994
C. elegans: flp-13(tm2427);flp-7(ok2625)	Paul Sternberg Lab	PS7084

Software and Algorithms		
MATLAB (2021)	Mathworks	https://www.mathworks.com
Streampix (v7.0)	Norpix	https://www.norpix.com
Adobe Illustrator	Adobe	https://www.adobe.com
NIS Elements	Nikon	www.nikoninstruments.com/prod ucts/software
Datavyu	Datavyu	https://datavyu.org/
Zeiss Zen 3.7	Zeiss	https://www.zeiss.com/microscop y/en/products/software/zeiss- zen.html
Fiji (ImageJ)	Schindelin <i>et al.</i> (2012) <sup>85</sup>	https://imagej.net/software/fiji/
Other		1
SP-20000M-USB3 CMOS camera	JAI	N/A
Micro-NIKKOR 55mm f/2.8 lens	Nikon	N/A
10x25 White Panel LED backlight, 24VDC	Metaphase Technologies	Cat#MS-BL10X25-W-24-ILD-PS
Precision LED Spot Light, 625nm, 40W, Type H	Mightex	Cat#BLS-PLS-0625-030-40-S
BioLED Light Source Control Module	Mightex	Cat#BLS-13000-1
JMP 16	SAS Institute Inc.	https://www.jmp.com/
GraphPad Prism 9.5.1	GraphPad	www.graphpad.com

#### Plasmids

The pSM-nCre expression plasmid was previously described<sup>86</sup>. Promoters used for nCre expression were: *ceh-45* (2.5 kB), *ceh-53* (1.5 kB), *tax-4*<sup>87</sup>, *osm-6* (2.4 kB), *trh-1* (2.0 kB), *ceh-34* (3.7 kB), *sra-6* (3.8 kB), *ser-2d*<sup>88</sup>, *ceh-28* (650 bp), *sra-9*<sup>87</sup>, *gcy-33*, and *gcy-36*<sup>87</sup>.

The *dmsr-1* genomic rescue plasmid was constructed by inserting a region of *dmsr-1* genomic locus (spanning from 3.4 kB upstream of start codon up to the stop codon; including introns and exons) into pSM-t2a-GFP.

# New alleles generated in this study

The *flp-13* conditional knockout allele was generated through two iterative CRISPR/Cas9 genome editing steps. In one step, a loxP site was inserted 62 bp after exon 1 of the gene. In a second step, a t2a-mScarlet-loxP sequence was inserted immediately before the stop codon of *flp-13*.

We constructed two *dmsr-1* fluorescent reporter strains via CRISPR/Cas9 genome editing. For the *dmsr-1a* reporter, a t2a-mNeonGreen sequence was inserted immediately before the stop codon of the *dmsr-1a* isoform. For the *dmsr-1b*, the same strategy was used, inserting t2a-mNeonGreen immediately before the *dmsr-1b* stop codon. We failed to observe any fluorescence in the *dmsr-1b* reporter line. The images reported here are exclusively from the *dmsr-1a* reporter.

# Preparation of bacteria for experiments

*E. coli* OP50 was grown overnight in Luria Broth (LB) cultures at 37°C under shaking conditions. *Pseudomonas aeruginosa* strains (PA14 and other mutants) and *Serratia* Db11 single colonies were inoculated in 6-8ml of LB in a 15ml round bottom tube with loose cap to allow for adequate aeration and grown at 37°C under shaking conditions for 16 hours. All bacteria were stored at 4°C and used within one week.

UV-killing was performed by exposing plates seeded with PA14 to a UV transilluminator (302nm) for 30 mins. The exposure time needed to kill bacteria depended on the amount of bacteria seeded on the plate. For a 200ul lawn grown for 20 hours at room temperature (RT, ~22°C), 30 mins was sufficient for bacterial killing. This was verified by culturing bacteria from random zones on the plate to check for overnight growth at 37°C. Plates were kept facing the UV light source with lids off. After exposure, plates were left on the bench to come back to RT before adding animals.

# **Behavior Experiments**

All behavioral experiments were done over at least two days and over multiple experimental replicates. All animals were added onto experimental bacteria as adults for specific durations of time. All animals were staged as L4s the day before.

# Feeding and quiescence assays

Low peptone NGM plates (3g NaCl, 22g agar, 0.75g peptone, 1ml cholesterol (5mg/ml) per 1L media) were used as experimental plates across all experiments to slow down growth of bacteria. 200ul of OP50 or PA14 were seeded on 10cm low peptone NGM plates and spread using a bacterial spreader to make a large lawn which did not completely cover the plate. Plates were left at RT for 20 hours stacked horizontally. Five animals were added per plate per bacterial condition and left undisturbed in a box inside a 21°C incubator for 20 hours after which feeding/pumping behavior was measured. Grinder movements or pharyngeal pumps were counted for each animal with a manual tally counter (Digi 1st TC-890 Digital Tally Counter) under a benchtop microscope for 20 secs. Pharyngeal pump counts are multiplied by 3 and plotted as pumps/min across figures. Standard pumping experiments were performed at least on 2 different days with 3 plates per condition (15 animals) for each strain/genotype.

Quiescence assays were performed using the same plates described above. An animal showing complete lack of pumping, body movement or locomotion for the 20 secs of observation time were marked as quiescent. Plates were handled gently so as to not to rouse any potential quiescent animals. Animals that did not respond at all after poking with a platinum wire pick were considered as dead.

For experiments with the *flp-13;pdfr-1::BlaC* strain, 0.5mW blue light (stage backlight) was used on the Leica Fluocombi III stereomicroscope using the 5X objective to quantify feeding rates. BlaC is extremely sensitive to blue light and hence a filter was placed on the microscope stage with the stage backlight to prevent blue light from the backlight from stimulating the animals at baseline. The filter was carefully removed from under the experimental plate taking care not to mechanically disturb the animals. Feeding was quantified for 20 seconds as in previous experiments immediately after exposure to the blue light.

# **Survival Assays**

Low peptone NGM plates were seeded with 200ul of bacteria same as above. 10 animals were added per plate, and number of animals that were quiescent (as defined previously) or dead were measured at specific time points (0 hour to 120 hours). Animals were moved to plates with equivalent lawns of bacteria (seeded at the same time) every 2 days to ensure that the animals were not confused with their adult progenies.

# **Egg-laying assay**

10 animals were added to plates seeded as above. Number of eggs on the plate were measured after 1 hour of exposure (0-1 hour). For the 20-21 hour quantification, animals were transferred to an equivalently seeded plate and number of eggs laid after 1 hour was quantified.

#### **Defection measurement**

Five animals were added on plates seeded similarly as above. For each animal, observation started after the onset of defecation and the inter-defecation interval was measured for 5 consecutive defecation events for each animal.

# Pumping with exogenous serotonin

Animals were exposed to PA14 for 20 hours as described above. Feeding was quantified after 20 hours of infection. These animals were then transferred to 6cm low peptone plates with added 15mM serotonin (Sigma Aldrich) for 10 mins, and pumping was quantified as above. Serotonin plates were made the day before, seeded with 50ul OP50 and kept at room temperature.

#### Lawn leaving assays

10ul lawns of OP50 or PA14 were seeded on 10 cm low peptone plates for 20 hours at 25°C. Fresh overnight cultures of bacteria were used. 10 animals were added per plate, taking care to not disturb the bacterial lawn during transfer. Animals were recorded on Streampix software at 1 frame every 30 minutes for 20.5 hours using JAI SP-20000M-USB3 CMOS cameras (41mm, 5120x3840, Mono) with Nikon Micro-NIKKOR 55mm f/2.8 lenses. Backlighting was provided by a white panel LED (Metaphase Technologies Inc. White Metastandard 10" X 25," 24VDC). Number of animals on the lawn were measured at each 30 minute interval manually from the recorded images.

# *flp-13* and *dmsr-1* reporter imaging

Animals were added on OP50 or PA14 low peptone plates for 20 hours as described above and imaged using a Zeiss LSM900 confocal microscope system. Animals were immobilized on a flat

agar pad using 100uM sodium azide (Sigma Aldrich). For *flp-13::mScarlet* expression, all neurons expressing *flp-13* were observed using a Z stack, and maximum intensity projections were used to observe all neurons in one frame. For the *dmsr-1:T2A-mNeonGreen* imaging, all neurons expressing *dmsr-1* were overlaid with NeuroPAL expression. The atlas from Yemini et al.<sup>53</sup> was referred to for neuronal identification. A subset of neurons with the highest *dmsr-1* expression were selected across the head region of the animal to quantify levels of expression in infected and uninfected conditions. For neuron classes that had L/R pairs, the brighter cell was chosen.

#### Stress reporter imaging

Animals were placed on OP50 or PA14 low peptone plates for 6 hours or 20 hours as described above. Multiple animals were added onto 500ul agar pads and immobilized with 100uM sodium azide (Sigma Aldrich). For each reporter strain, animals on OP50 and PA14 were imaged using a custom Andor spinning disk confocal system, containing a 5000 rpm Yokogawa CSU-X1 spinning disk unit and Borealis upgrade, built on a Nikon ECLIPSE Ti microscope<sup>61</sup> at the same laser intensity using a 10X objective (CFI Plan Fluor 10x, Nikon). Images were analyzed using Fiji where a region of interest (ROI) was drawn around the region of expression, extending from the region immediately posterior to the pharyngeal bulb along the full length of the animal's body. Expression was mostly seen in different parts of the intestine for the tested strains. Mean fluorescence intensity of a circle drawn outside the animal was subtracted to perform background subtraction.

### Multi-animal behavioral recordings

For recordings of animal speed, multi-worm tracking recordings were performed essentially as previously described<sup>89</sup>. Animals were pre-exposed to bacteria as described in the text and in the bacteria methods section above. Animals were recorded on Streampix software at 3 fps. JAI SP-20000M-USB3 CMOS cameras (5120x3840, mono) with Nikon Micro-NIKKOR 55mm f/2.8 were used for recordings. Backlighting was provided by IR LEDs (Metaphase). Data analysis was conducted using previously-described custom MATLAB scripts (Rhoades *et al.*, 2019)<sup>89</sup>. For optogenetic BlaC stimulation, light was supplied from a 470nm (at 0.5mW/mm<sup>2</sup>) Mightex LED at defined times in the video. Custom Matlab scripts were used to analyze videos to derive animal speed.

#### Heat shock experiments

Heat shock experiments were derived from protocols used by Nath *et al.*, 2016<sup>20</sup> and Nelson *et al*, 2014<sup>22</sup>. Young adult animals were added on 200ul OP50 lawns on 10 cm low peptone plates as described above for 20 hours. For the heat shock, 10 animals were transferred to 6cm plates which were seeded overnight with 50ul OP50 and pre-warmed in a 35°C incubator. These plates were immediately sealed with parafilm and kept immersed in a 35°C circulating water bath for 30 mins. The lids were exchanged with dry lids and plates were kept inverted to minimize condensation after taking out of the water bath and left undisturbed for 30 mins. Plates were then handled very carefully to not rouse animals out of quiescence. Quiescence was then measured as described above using a 20 sec observation period. Heat shock times were staggered to be able to assay individual plates exactly at 30 mins after heat shock.

# Latency to reversal

Young adult animals were infected on PA14 or kept on OP50 for 20 hours as described above. A Leica Fluocombi III stereomicroscope was used with a 5X objective to observe onset of reversals. Blue light intensity was kept at 50%, which corresponds to 0.5 mW/cm<sup>2</sup>. A stopwatch was started coincident with switching on the blue light and time required for onset of a reversal was measured. An inherent latency of about a second by the experimenter handling the two buttons is likely. Only quiescent infected animals and quiescent heat shocked (heat shock was performed as described above) were tested. If animals were right next to each other on the plate and were exposed to similar levels of blue light, only one was tested. A period of 30 seconds was given between testing multiple animals on the plate.

# Single neuron calcium imaging

Calcium imaging of ASI was conducted as previously described for other neurons<sup>89</sup>. Animals with ASI-specific expression of GCaMP7f were infected with PA14 for 20 hours starting on their first day of adulthood, as described above. After the 20 hour infection period they were placed on imaging slides. Imaging slides were prepared by placing a 3uL drop of PA14 on a minimally thick NGM pad. Then a custom cut PDMS corral was placed on the pad. Five to eight animals were picked (without food) from their PA14 infection plates to the center of the corral and covered in a coverslip. Animals were allowed to acclimate for 5 mins after which they were imaged on PA14. Individual slides were imaged for no longer than 20 mins. Animals were imaged in a widefield epifluorescence configuration. Exposures alternated between GCaMP imaging and brightfield imaging, controlled by NI-DAQ triggering. The pattern of acquisitions was a frame rate of 16 fps for behavior and 4 fps for calcium signals. Exposures were a maximum of 10ms to avoid motion blur. Data were acquired through a 4X/0.2NA objective and Andor Zyla 4.2 Plus sCMOS camera. Neurons were segmented through custom ImageJ scripts. Behavior was quantified using custom Image J macros and datavyu.

# **Brain-wide Calcium Imaging**

# Mounting and recording

Young adult SWF994 animals were added to 200ul PA14 lawns on low peptone plates as described before. A concentrated PA14 culture to be used in the mounting buffer was grown as mentioned before (bacterial culture section) on the day before the experiment. 1mL of the fresh PA14 culture was pelleted and then re-suspended in 80uL of M9. This was used as the mounting buffer. A thin, flat agar pad (2.5cm x 1.8cm x 0.8mm) was made from low peptone NGM immediately prior to each animal being imaged. 4ul of a concentrated solution of 100um microbeads in M9 was pipetted on the four corners of the pad in a single layer to maintain a gap between the pad and cover-slip so that free movement of the animal was possible. An infected quiescent animal was transferred to the center of the agar pad and 9.5ul mounting buffer was added on top. A glass cover-slip (#1.5) was then carefully positioned on top taking care to not insert any bubbles.

We started each imaging session while the infected animals were aroused in order to observe frequent transitions between quiescence and active behavior. Each recording lasted for 8 minutes.

Animals that were quiescent for more than half of the recording were discarded since 1) their postures during quiescence and active behavior were dramatically different, which prevented high-quality registration, and 2) calcium dynamics with low variability across time were difficult to distinguish from noise.

Since infected animals generally expressed dimmer fluorescence, we excited each fluorophore with higher laser intensities during freely-moving recordings than previously described<sup>61</sup>. During freely-moving recording, we excited GCaMP7f with a 488 nm laser at 14% of its maximal intensity and TagRFP (together with CyOFP and mNeptune) with a 561 nm laser at 19% of its maximal intensity, respectively. As for the RGB composite image that enabled neuron identification with NeuroPAL, we excited mTagBFP2 using a 405nm laser at 18% intensity under a 447/60 bandpass filter, CyOFP1 using a 488nm laser at 32% intensity under a 585/40 bandpass filter, and mNeptune2.5 using a 637nm laser at 50% intensity under a 655LP-TRF filter.

The rest of the image setup and experimental procedures followed the description of our previous study<sup>61</sup>.

# Image processing

Behavioral feature extraction and neuron registration were performed in the same way as described in our previous paper<sup>61</sup>, with a few improvements that enabled faster and more robust extraction of calcium dynamics from dimmer infected animals:

1) Improvement on the 3D segmentation U-Net:

We used a 3D U-Net for simultaneous semantic and instance segmentation. Our original U-Net<sup>61</sup> was trained only on the fluorescent images from SWF415 animals, which lacked the NeuroPAL construct. While it was largely able to generalize to SWF702, we were able to improve its performance further by adding four manually annotated SWF702 images to the training data. This led to an increase in the number of segmented and registered neurons in the SWF702 animals, by an average of 13 new neuron traces per animal.

2) Improvement on the clustering algorithm:

In our previous work<sup>61</sup>, we constructed a similarity matrix from the neurons detected at each time point to estimate their likelihood of being same neuron, and clustered the rows of this similarity matrix to construct the neural traces. This used to take multiple days per animal. We observed that this similarity matrix, as well as the distance for hierarchical clustering, were sparse. It would therefore be much more efficient to list out and sort all the nonzero entries of the matrix, and use a Union-Find data structure to store the clustering outputs (code available at https://github.com/flavell-lab/SparseClustering.jl). The new clustering algorithm took less than a minute per animal.

3) Improvement on channel alignment of the NeuroPAL RGB composite images:

Previously<sup>61</sup>, we performed rigid-body Euler registration using the gradient descent-based package elastix to align each channel of the NeuroPAL RGB composite images . This process was susceptible to failure when the original images were initialized too far apart for the gradient to descend properly. In this current work, we implemented a custom package (code available at <u>https://github.com/flavell-lab/euler\_gpu</u>) to perform GPU-accelerated grid search across all possible combinations of rigid-body parameters to ensure that the optimal solution was always found regardless of the initial conditions. This rigid-body registration was very accurate, enabling us to skip the rest of the rigid-body and affine registrations, and use it to directly initialize the B-spline registrations, which were carried out as described previously<sup>61</sup>.

#### Neuron identification

Each registered neuron was identified as a genetically defined class using NeuroPAL manually by two independent annotators. In the case of disagreement, a third annotator would make a final decision. Neurons identified with a confidence level below 3 (on a 1-5 scale) were excluded from further analysis.

#### Statistical analysis

Left/ right pair of the same neuron class was considered as the same neuron, whereas each dorsal/ ventral pair of the same neuron class was considered separately due to their distinct connection to body wall muscles. Quiescent bouts were defined as >8 consecutive seconds (or >13 consecutive fluorescent volumes) during which the animal's locomotion speed was subthreshold to measurement noise (0.01 mm/s) and no pharyngeal pumping was visible to a manual annotator. We used this bout duration threshold specifically for our brain-wide calcium imaging experiments due to the higher spatial resolution of near-infrared behavioral imaging under a 10X air objective<sup>61</sup> compared to a stereo microscope. The new threshold also allowed us to capture more frequent transitions between quiescence and active behavior in infected animals that were aroused from the recent mounting procedure.

For each neuron that was successfully registered across imaging time points and annotated as a genetically defined neuron class, we computed a quiescence-modulation index (QMI):

$$QMI = \frac{1}{Q} \sum_{q=0}^{Q} F_q - \frac{1}{A} \sum_{a=0}^{A} F_a$$

where F refers to the GCaMP fluorescence at each imaging time point normalized by mean fluorescence of that neuron in the same recording, Q refers to the total number of time points that the animal was in a quiescent bout, and A refers to the total number of time points that the animal was performing active behavior. Neurons that were consistently more active during quiescence would have a positive QMI, whereas neurons that were inhibited during quiescence would have a negative QMI.

To assert statistical significance on the QMI of each neuron class, we considered the neuron classes that were observed for more than 3 times across 8 animals. We first took the mean QMI across all observations for each neuron class, and then compared this mean value against a distribution of mean simulated QMIs computed from real neural traces of the same neuron class in relation to synthetic binary quiescence vectors. These synthetic binary quiescence vectors were generated from a hidden Markov model trained on real behavioral data of these 8 animals such that the synthetic behavioral data retained the temporal structures of the real quiescence bouts. Each neuron class then received a p-value based on the rank of its real mean QMI among 10,000 simulated mean QMIs (for instance, if the real value is either higher or lower than all simulated values, the p-value for that neuron classes simultaneously, we corrected for multiple testing using the Benjamini-Hochberg method. Neuron classes were tested significant for quiescence modulation at a false detection rate of 5%.

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Figure 1. PA14 infection reduces feeding behavior via the neuropeptidergic neuron ALA

- (A) Time course analysis of how WT animal behavior and viability changes over the course of infection. Experimental design (top); time indicated on the x-axis is the number of hours since animals were first placed on PA14 bacterial lawns as one day old adults (bottom). The indicated line plots depict the fraction of animals that exhibited the indicated phenotypes at that time point. Each data point indicates mean fraction of the indicated phenotype, and surrounding error bars indicate standard deviation. For each genotype, 10 animals were placed on a single plate; two replicates with three plates each were performed. n=10 animals per replicate, 6 replicates. Median quiescence observation was 60 hrs in WT animals. Median time point at which 50% animals died was 72 hrs in WT animals.
- (B) Experimental design (top); feeding rates of wild-type animals exposed to the indicated bacterial strains for the indicated durations of time (bottom). Feeding was quantified as pharyngeal pumping rates. Feeding rate on PA14 was compared to that on OP50 for each time point. Dots indicate individual animals; dark bar indicates mean. n= 15-87 animals; \*\*\*\*p<0.0001, n.s. indicates not significant with Wilcoxon rank sum test.</p>
- (C) Feeding rates of animals exposed to the indicated bacteria for 20 hours, and then assayed on the indicated bacteria after 1 hour of exposure. PA14 killing was accomplished by UV light treatment (see Methods). Dots indicate individual animals; dark bar indicates mean. n=34-47 animals per condition; \*\*\*\*p<0.0001, n.s. indicates not significant with Wilcoxon rank sum test.
- (D) Feeding rates of wild-type animals or two mutant strains with defects in innate immune activation (*pmk-1* and *tir-1*). Dots indicate individual animals; dark bar indicates mean. N2, n= 18-37 animals per genotype per bacterial condition. Asterisks above each mutant indicate whether the PA14-induced feeding decrease for that mutant was significantly different from the decrease observed in day matched wild-type animals. \*\*\*\*p<0.0001 represents empirical P-value with Bonferroni correction.</p>
- (E) Feeding rates of wild-type animals exposed to *P.aeruginosa* PA14, *P. aeruginosa* PAO1, or *Serratia marcescens* Db11. Asterisks above each bacterial condition indicate whether the feeding decrease for that bacteria was significantly different from the decrease observed for PA14 in day matched wild-type animals. Dots indicate individual animals; dark bar indicates mean. n=30 animals per bacterial condition; \*\*\*\*p<0.0001 represents empirical P-value with Bonferroni correction.</p>
- (F) Feeding rates of animals exposed to the indicated bacterial strains for 20 hours and then transferred and assayed on the indicated bacterial strain after 1 hour of exposure. Dots indicate individual animals; dark bar indicates mean. n=45-47 per condition; \*\*\*\*p<0.0001 with Wilcoxon rank sum test. The same OP50 to OP50 and PA14 to PA14 controls were used across 1C and 1F.

- (G) Feeding rates of PA14-infected or uninfected wild-type animals before and after exposure to exogenous serotonin (15 mM) for 10 minutes. Raincloud distributions of the groups are also shown on the right. n=45 animals per condition; \*\*\*p<0.001, \*\*\*\*p<0.0001 with Wilcoxon rank sum test.</p>
- (H) Effects of PA14 infection on feeding for a panel of mutant strains related to neuromodulation, stress responses, sensory responses, or sleep pathways. Alleles used are detailed in Methods. N2, n= 22-391 animals per genotype per bacterial condition. Asterisks above each mutant indicate whether the PA14-induced feeding decrease for that mutant was significantly different from the decrease observed in day matched wildtype animals. Multiple mutants were tested alongside the same control wild-type animals on a given experimental day (See methods). \*\*\*\*p<0.0001 represents empirical P-value with Bonferroni correction.
- (I) Effects of PA14 infection on a panel of mutants related to ALA development and function. n= 15-175 animals per genotype per bacterial condition. Asterisks above each mutant indicate whether the PA14-induced feeding decrease for that mutant was significantly different from the decrease observed in day matched wild-type animals.
  \*\*\*\*p<0.0001 represents empirical P-value with Bonferroni correction. Multiple mutants were tested alongside the same control wild-type animals on a given experimental day (See methods). The same animals for the *ceh-17* genotype (n=175) is plotted across both Fig 1H and I.
- (J) Time course analysis of how animal behavior and viability changes over the course of infection. Time indicated on the x-axis is the number of hours since animals were first placed on PA14 bacterial lawns as one day old adults. The indicated line plots depict the fraction of animals that exhibited the indicated phenotypes at that time point. Data are shown for *flp-13* animals. Each data point indicates mean fraction of the indicated phenotype, and surrounding error bars indicate standard deviation. For each genotype, 10 animals were placed on a single plate; two replicates with three plates each were performed. n=10 animals per replicate, 6 replicates. Median quiescence observation was 34 hrs in *flp-13*, which was significantly different relative to WT animals in Fig 1A. Two-tailed Mann-Whitney test,\*p<0.05. Median time point at which 50% animals died was 48 hrs in *flp-13* animals, which was not significantly different from WT animals in Fig 1A. Two-tailed Mann-Whitney test, n.s. (p=0.057).
- (K) Diagrammatic representation of infection-induced roles of ALA-expressed neuropeptides: the FLP-24, FLP-7 and NLP-8 neuropeptides function in the same pathway as the ALA neuron to control feeding reduction, whereas the FLP-13 neuropeptide delays the onset of quiescence.



# Figure 2. *flp-13* functions in sensory and pharyngeal neurons to prevent PA14-induced quiescence and death

(A) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. As in Fig 1F, these animals were grown on *E. coli* OP50 until they were one day old adults, and then transferred to plates containing the indicated bacteria. PA14 killing was accomplished by UV light treatment as in Fig 1G. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*p<0.01, fraction quiescence compared via Chi-square test with Bonferroni correction.

(B) Cartoon depicting CRISPR/Cas9-engineer flp-13 conditional knockout allele. In this allele (*syb6180 syb6395*), loxP sites were positioned in the indicated locations and a t2a-mScarlet fluorescent reporter cassette was also inserted. This allows for visualization of sites of flp-13 expression and Cre-dependent deletion of flp-13 and mScarlet. Blue boxes indicate exons, intervening black lines indicate introns, yellow box indicates t2A sequence, and red box indicates mScarlet sequence.

(C) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. The pan-neuronal promoter used was *Primb-1*. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p<0.001, fraction quiescence compared via Chi-square test with Bonferroni correction.

(D) PA14-induced quiescence and death in animals of the indicated genotypes, displayed as in (C). Here, a series of broadly-expressed promoters were used to drive expression of Cre recombinase in order to delete *flp-13* expression from broad subsets of neurons. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p<0.001, fraction quiescence compared via Chi-square test with Bonferroni correction.

(E & F) PA14-induced quiescence and death in animals of the indicated genotypes, displayed as in (C). Cell-specific promoters were used to drive expression of Cre recombinase. The neurons listed are those with intersecting expression of *flp-13* and the promoter driving Cre expression. I5, I1, ASH and OLL neurons were inferred as sites of function based on cross-intersection of different promoters. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p<0.001, fraction quiescence compared via Chi-square test with Bonferroni correction.

(G) Stress-induced sleep assays for animals of the indicated genotypes. Data are shown as fraction of quiescent animals 30 min after a 35min heat shock at 30C. Note that the genotypes used here are identical to a subset of those used in panels (E-F). Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p<0.001, fraction quiescence compared via Chi-square test with Bonferroni correction.

(H) Diagrammatic representation of context-dependent functions of FLP-13. On heat shock, FLP-13 is released from ALA to promote quiescence, while on infection FLP-13 is released from a distinct set of neurons to delay quiescence.



Figure 3. Mutants lacking *flp-13* and its cognate receptor *dmsr-1* display reversible quiescence upon PA14 infection

(A) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. Alleles used are *frpr-4(ok2376)* and *dmsr-1(qn45)*. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p< 0.001, fraction quiescence compared via Chi-square test with Bonferroni correction.

(B) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. A genomic fragment of *dmsr-1* was expressed under a 3.5kB endogenous *dmsr-1* promoter in *dmsr-1(qn45)* mutants. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p<0.001, \*\*p<0.01, fraction quiescence compared via Chi-square test with Bonferroni correction.

(C) Time course analysis of how *dmsr*-1 mutant behavior and viability changes over the course of infection, displayed as in (2A). Time indicated on the x-axis is the number of hours since animals were first placed on PA14 bacterial lawns as one day old adults. The indicated line plots depict the fraction of animals that exhibited the indicated phenotypes at that time point. Each data point indicates mean fraction of the indicated phenotype, and surrounding error bars indicate standard deviation. For each genotype, 10 animals were placed on a single plate; two replicates with three plates each were performed. n=10 animals per replicate, 6 replicates. Median quiescence observation was 20 hrs in *dmsr-1* vs 60 hrs in WT animals (Fig 1A). Two-tailed Mann-Whitney test,\*\*p<0.005. Median time point at which 50% animals died was 48 hrs in *dmsr-1* mutants vs 72 hrs in WT (Fig 1A), \*p<0.05. two-tailed Mann-Whitney test.

(D) Representative images of expression of *dmsr-1* transgene (*syb6591*) overlaid with NeuroPal expression in animals exposed to either OP50 or PA14 for 20 hours. No difference was found in expression patterns of *dmsr-1* in the head region of the animal. Refer Fig S3 for further quantification.

(E) Quantification of fluorescent intensities of GFP tagged *hsp-6* in WT and *flp-13* animals 6 hours after being placed on indicated bacteria. n=10-20 animals per genotype per bacterial condition; n.s. indicates not significant with Wilcoxon rank sum test.

(F) Quantification of fluorescent intensities of GFP tagged *hsp-4* in WT and *flp-13* animals 6 hours after being placed on indicated bacteria. n=11-14 animals per genotype per bacterial condition; \*\*\*p<0.001, n.s. indicates not significant with Wilcoxon rank sum test.

(G) Quantification of fluorescent intensities of GFP tagged *irg-1* in WT and *flp-13* animals 6 hours after being placed on indicated bacteria. n=10-17 animals per genotype per bacterial condition; \*p<0.05, \*\* p<0.01, \*\*\*p<0.001, n.s. indicates not significant with Wilcoxon rank sum test.

(H) Locomotion speed of animals over continuous behavioral recordings. Blue light (0.5mW) was applied during the indicated time window to activate the light-activated adenylyl cyclase BlaC, expressed under the *Ppdfr-1* promoter. *flp-13* animals lacking the *pdfr-1::BlaC* transgene provide a control for the generic effects of blue light on behavior. n= 27-63 tracks over the 90 min recording period, Wilcoxon rank sum test was run on n=17-19 tracks in the 5 min period right after blue right stimulation to test for differences in speed upon blue light stimulation.

(I) Pumping rate of quiescent animals before and after blue light (0.5mW) exposure for 10 secs. Blue light was applied during the indicated time window to activate the light-activated adenylyl cyclase BlaC, expressed under the *Ppdfr-1* promoter. n=8-14; \*\*\*p<0.001, Paired t-test.

(J) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. Alleles used are *egl-4(n478)* and *dmsr-1(qn45)*. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p< 0.001, fraction quiescence compared via Chi-square test with Bonferroni correction.



# Figure 4. Brain-wide imaging identifies ASI and DAF-7/TGF-Beta as critical components of infection-induced quiescence

- (A) (Left) Diagrammatic representation of the experimental setup of brain-wide imaging of *flp-13* animals following hours of infection. (Right) Near-infrared image of a typical animal under the 10X objective and maximum intensity projection of fluorescent images of a typical animal under the 40X objective (to be added).
- (B) (Top) Behavioral features extracted from the near-infrared images of infected *flp-13* animals recorded over 8 minutes. Quiescence on the Y axis represents the onset and offset of quiescent bouts, which are defined as periods of time no less than 8 seconds during which speed < 0.01 mm/s, head curvature derivative < 0.1 rad/s and pumping = 0 Hz. (Bottom) Heatmap that displays the activity of 159 neurons, as indicated by z-scored GCaMP fluorescence, of the same animal, with warmer colors indicating higher activity.</p>
- (C) Heatmap that summarizes the modulation of neural activity by quiescence bouts. Rows represent neuron classes and columns represent individual animals. Color palette of quiescence modulation index indicates the change in mean activity during quiescent bouts from mean activity outside quiescent bouts, relative to the range of neural activity for each neuron where warmer colors indicate more correlation with quiescence; \*p<0.05, two-tailed empirical P value with multiple correction (See methods). This is overlaid with circles depicting whether *dmsr-1* expression is identified in that neural class as measured in Figs 3D and S3).
- (D) Event triggered averaged activity traces of motor and pharyngeal neural classes which significantly decrease during quiescence bouts.
- (E) Neural activity of ASI, from a single infected *flp-13* animal from the brain-wide imaging dataset is represented as  $F/F_{20}$  and is overlaid with quiescence bouts observed in the simultaneously recorded behavior.
- (F) Normalized mean ASI GCaMP7f signal in PA14-infected *flp-13* mutants. Each dot indicates normalized mean GCaMP signal during non-quiescence vs quiescence bouts for a single animal; lines connect dots from non-quiescence bouts of one animal to quiescence bouts of the same animal within the same recording. n=17 animals. \*\*p<0.01, two-tailed Wilcoxon matched-pairs signed rank test.
- (G) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. An ASI::Caspase strain was used. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*p< 0.05, n.s. indicates not significant, fraction quiescence compared via Chi-square test with Bonferroni correction.</p>

- (H) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. Alleles used include *ceh-17(np1)*, *aptf-1(tm3286)*, *nlp-22(gk509904)* and *flp-13(tm2427)*. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p< 0.001, n.s. indicates not significant, fraction quiescence compared via Chi-square test with Bonferroni correction.
- (I) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. Alleles used include *daf-7(e1372ts)* and *flp-13(tm2427)*. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescent, and dead, respectively. Graphically represented data was collected across multiple days but statistical comparisons were computed between day-matched experimental groups. \*\*\*p< 0.001,\*\*p<0.01, n.s. indicates not significant, fraction quiescence compared via Chi-square test with Bonferroni correction.</p>
- (J) Time course analysis of how animal quiescence changes over the course of infection. Time indicated on the x-axis is number of hours since animals were first placed on PA14 bacterial lawns as one day old adults. The indicated line plot depicts the fraction of animals that exhibited quiescence at that time point. Data are shown for wild-type, *flp-13(tm2427)*, (left) ASI::Caspase, *flp-13(tm2427)*;ASI::Caspase, (right) *daf-7(e1372ts)* and *flp-13(tm2427)*;*daf-7(e1372ts)* animals. For each genotype, 10 animals were placed on a single plate; three replicates with three plates each were performed. Median quiescence observation was significantly accelerated in *flp-13* animals compared to wild-type (WT), \*\*\*p<0.0001. (Left) Median quiescence observation was not different between WT and ASI- animals (n.s.), and (right) *daf-7* and WT animals (n.s.), although peak fraction quiescence observation was significantly different between WT and *daf-7* animals, \*\*\*p<0.001. Median quiescence observation was significantly delayed in ASI-; *flp-13* animals relative to *flp-13*, \*p<0.05. Median quiescence values compared via two-tailed Mann-Whitney test.</p>



Fig 5. Model for PA14-infection-induced sickness behaviors in C. elegans.

(A) Neuromodulatory pathways associated with stress (blue panel, left) and satiety (yellow panel, right) are recruited upon infection to drive sickness behaviors. Upon heat shock, the ALA neuron induces quiescence through the action of its neuropeptides, including the FMRFamide peptide FLP-13 (red rectangle)<sup>20,22</sup>. In satiated animals, the neuromodulator TGF-beta/DAF-7 is released by the ASI neurons to promote quiescence. These neuromodulatory pathways are recruited in the infection context (center panel). While ALA continues to suppress behaviors like feeding, FLP-13 delays quiescence and is released from other neurons (red circles). The effects of FLP-13 to delay quiescence are suppressed by the quiescence-promoting ASI and TGF-beta/DAF-7 pathway.

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#### Figure S1, Related to Figure 1

(A) Mean speed (mm/sec) of wild-type animals exposed to indicated bacterial strains for 30 mins and 20 hours, Y axis is on a log scale. These animals, as well as all others in the study, were grown on *E. coli* OP50 until they were one day old adults. They were then transferred to plates containing the indicated bacteria and time point of the transfer was considered t=0. Dots indicate

individual animals; dark bar indicates mean. n = 346-396 animals per condition; \*\*\*\*p<0.0001, n.s. indicates not significant with Wilcoxon rank sum test.

(B) Egg-laying rate of wild-type animals exposed to indicated bacteria for 1 hour right after transfer to said bacteria or for 1 hour after 20 hours of exposure to bacteria. Each data point indicates one plate containing 10 animals, dark bar indicates mean. n=16; \*\*\*\*p<0.0001, n.s. indicates not significant with Wilcoxon rank sum test.

(C) Defecation rate represented as inter-DMP (defecation motor program) interval for wild-type animals exposed to indicated bacteria for 20 hours. Dots indicate individual animals; dark bar indicates mean. n=113-125 animals; n.s. indicates not significant with Wilcoxon rank sum test.

(D-E) Time course analysis of how animal behavior changes on OP50 bacterial controls for Fig 1A and 1J. Time indicated on the x-axis is the number of hours since animals were first placed on OP50 bacterial lawns as one-day old adults. The indicated line plots depict the fraction of animals that exhibited the indicated phenotypes at that time. Each data point indicates the mean fraction of the indicated phenotype, and surrounding error bars indicate the standard deviation. For each genotype, 10 animals were placed on a single plate; two replicates with three plates each were performed. No quiescence or death was observed when WT or *flp-13* mutants were placed on OP50, as indicated by 100% non-quiescence throughout the time course. This is in contrast to their phenotypes in Fig 1A and 1J, respectively.

(F) Time course analysis of how behavior and viability changes over the course of infection in *ceh-17* (left) and WT (right) animals. The time indicated on the x-axis is the number of hours since animals were first placed on OP50 or PA14 bacterial lawns as one day old adults. The indicated line plots depict the fraction of animals that exhibited the indicated phenotypes at that time point. Data are shown for wild-type and *ceh-17(np1)* animals. For each genotype, 10 animals were placed on a single plate; two to three replicates with three plates each were tested. Median time point of quiescence observations was not significantly different between PA14infected WT and *ceh-17* animals n.s. Despite no change in the onset of quiescence or death, peak fraction quiescence was significantly higher in WT animals compared to *ceh-17* mutants (0.39 vs 0.2), \*\*p<0.01. Median time point for 50% death was not significantly different between PA14infected WT and *ceh-17* animals, n.s. Median values compared via two tailed Mann-Whitney test.

(G) Feeding rates of animals on indicated bacteria upon initial exposure. Animals were left on the plate for 30 minutes for acclimatization before testing. n=45 animals per genotype per bacterial condition. n.s. indicates not significantly different as tested with Wilcoxon rank-sum tests.

(H) Lawn leaving rates of wild-type vs *ceh-17* animals on PA14 over 20 hrs. For each genotype, animals were placed on a lawn of PA14, and the percent animals on the lawn were quantified over time (n=5-6 replicates with 10 animals each for each genotype). No significant difference observed between lawn leaving rates based on on two-way ANOVA.

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# Figure S2, Related to Figure 2

(A) Quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals on indicated bacterial mutants. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent,

non-quiescence, and dead, respectively. \*\*p < 0.01, fraction quiescence compared via Chi-square test with Bonferroni correction.

(B) Lawn leaving rates of wild-type vs *flp-13* animals on PA14 over 20 hrs. For each genotype, animals were placed on a lawn of PA14, and the percent animals on the lawn were quantified over time (n=9 replicates with 10 animals in each replicate for each genotype). n.s. indicates not significant interaction based on two-way ANOVA.

(C) (Top) Fluorescence images of *flp-13(syb6180 syb6395)* animals, after exposure to either OP50 or PA14 for 20 hours, beginning at the first day of adulthood. Images are shown for the head region. No fluorescence was observed in the ventral cord or tail. Dotted white circles indicate distinct neurons, some of which have been identified. In addition, no non-neuronal expression of mScarlet was detected.

(Bottom) Fluorescence images of *flp-13(syb6180 syb6395)* animals expressing *Pflp-24::Cre* (left) and *Pceh-53::Cre* (right) indicating absence of mScarlet fluorescence in ALA and I5 respectively. The top panel is overexposed to be able to visualize all potential sites of *flp-13* expression.



		OP50	PA14
<i>.</i>	ADA	4/4	4/4
	AIM	4/4	4/4
	AIN	4/4	4/4
	AIZ	4/4	4/4
	ALN	3/3	3/3
ł	ASI	4/4	4/4
	ASJ	4/4	4/4
	ASK	4/4	4/4
	AVD	4/4	4/4
	AVE	4/4	4/4
	AVH	4/4	4/4
	AVJ	4/4	4/4
	BAG	4/4	4/4
	CEP DV	4/4	4/4
	DA9	4/4	4/4
	11	4/4	4/4
3	3	4/4	4/4
ŝ	10	4/4	4/4
ŝ	1	4/4	4/4
ŝ		4/4	4/4
2		4/4	4/4
	LUA M1	4/4	4/4
5	M3	4/4	4/4
5	MC	4/4	4/4
		4/4	1/4
		3/3	2/2
		3/3	2/2
		3/3	2/2
	POP	3/3	3/3
	PVC	2/2	4/4
		3/3	3/3
		4/4	4/4
-	RIC	4/4	4/4
	RID	4/4	4/4
		4/4	4/4
		4/4	4/4
		4/4	4/4
		4/4	4/4
		4/4	4/4
	SIVID	4/4	4/4
		4/4	4/4
	LIRY	4/4	4/4 4//
	ν/Δ12	4/4	4/4
	VA12	דוד	4/4

### Figure S3, Related to Figure 3

(A) Feeding rates in response to PA14 infection, in relation to the quiescence phenotypes observed in the same animals as reported in Fig 3A. Asterisks above each mutant indicate whether the PA14-induced feeding decrease for that mutant was significantly different from the decrease observed in day matched wild-type animals. \*\*\*\*p<0.0001 represents empirical P-value with Bonferroni correction. Multiple mutants were tested alongside the same control wild-type animals on a given experimental day. n.s. indicates not significantly different.

(B) Time course analysis of how animal behavior changes on OP50 bacterial controls for Fig 1A and 1J. Time indicated on the x-axis is the number of hours since animals were first placed on OP50 bacterial lawns as one day old adults. The indicated line plots depict the fraction of animals that exhibited the indicated phenotypes at that time point. Each data point indicates mean fraction of the indicated phenotype, and surrounding error bars indicate standard deviation. For each genotype, 10 animals were placed on a single plate; two replicates with three plates each were performed. No quiescence or death was observed when *dmsr*-1 mutants were placed on OP50, as indicated by 100% non-quiescence throughout the time course. This is in contrast to its phenotype in Fig 3C.

(C) List of neurons expressing *dmsr-1* identified in animals exposed to OP50 or PA14 for 20 hours. Each row depicts a neuron class, columns indicate bacterial conditions with number of times said neuron was observed over total number of observations.

(D) Representative fluorescent images on OP50 (top) and PA14 (bottom) after 20 hours. Top panel for each bacterial condition indicates the expression of *dmsr-1* tagged with mNeonGreen without the overlay of the NeuroPAL transgene (refer Fig 3D for image with NeuroPal overlay). Middle panel indicates the mid-body of the worm, and bottom panel indicates the tail region for each bacterial condition with an overlay of NeuroPAL.

(E) Expression levels of a subset of neurons showing highest expression of dmsr-1 across the head region of the animal in infected (PA14) and uninfected (OP50) contexts (as tabulated in Fig S3C). For some neurons with L/R/D/V pairs, the brighter cell (closer to the objective) was chosen. n.s. indicates no difference in dmsr-1 expression was found for that neuron class upon infection, as tested by Wilcoxon rank sum test.

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#### Figure S4, Related to Figure 3

(A) Quantification of fluorescent intensities of GFP tagged *hsp-4*, *irg-1* and *hsp-6* in WT and *flp-13* or *dmsr-1* animals 20 hours after being placed on indicated bacteria. n = 16-23 (for *hsp-4::GFP)*, 13-14 (for *irg-1::GFP)*, 13-24 (for *hsp-6::GFP)* animals per genotype per bacterial condition; \*p<0.05, \*\* p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, n.s. indicates not significant with Wilcoxon rank sum test.</p>

- (B) Representative images of wild-type, immune pathway defective mutant *pmk-1*, and *flp-13* animals after 20 hours of exposure to PA14. Unlike *pmk-1* animals which look visibly sick, *flp-13* animals look similar to wild-types.
- (C) PA14-induced quiescence and death in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. Alleles used include *sek-1(km4)*, *sek-1(km4)*;*flp-13(tm2427)*, *atfs-1(null)*, *atfs-1(null)*;*flp-13(tm2427)*; *zip-2(ok3730)*, *zip-2(ok3730)*;*flp-13(tm2427)*, *fshr-1(ok778)*, *and fshr-1(ok778)*;*flp-13(tm2427)*. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, non-quiescence, and dead, respectively. \*p<0.05, \*\*p<0.01,\*\*\*p<0.0001, fraction quiescence compared via Chi-square test with Bonferroni correction.</p>
- (D) Latency to arousal measured by exposing animals to blue light which leads to a robust aversive response with onset of reversals in wild-type uninfected animals, wild-type animals post heat shock and quiescent *flp-13* animals post PA14 infection. n=14-26 animals per genotype per condition; \*\*\*p<0.001 with Wilcoxon rank sum test.
- (E) PA14-induced quiescence and death forty-eight hours (2 days) of infection in animals of the indicated genotypes, shown as fraction of the total population of assayed animals. Alleles used are *egl-4(n478)* and *dmsr-1(qn45)*. Error bars represent the 95% confidence interval for the bootstrapped mean fraction values of animals that were quiescent, nonquiescence, and dead, respectively. \*\*\*p< 0.001, \*p<0.01 fraction quiescence and death compared via Chi-square test with Bonferroni correction.



Figure S5, Related to Figure 4

(A-F) Time course analysis of how animal behavior and viability changes over the course of infection. The time indicated on the x-axis is the number of hours since animals were first placed on OP50 or PA14 bacterial lawns as one-day old adults. The line plots depict the fraction of animals that exhibited the indicated phenotypes at that time. Data are shown for wild-type, *flp-13(tm2427)*, (left) ASI::Caspase, *flp-13(tm2427)*; ASI::Caspase, (right) *daf-7(e1372ts)* and *flp-13(tm2427)*; *daf-7(e1372ts)* animals. For each genotype, 10 animals were placed on a single plate; three replicates with three plates each were performed.

(A and B) Median time point by which 50% animals died was significantly accelerated in PA14-infected *flp-13* animals, compared to wild-type (WT) (Fig S5A vs S5B,). \*\*\*\*p<0.0001, two tailed Mann-Whitney test.

(A, C, E) Median time point by which 50% animals died was not significantly different between PA14-infected WT and ASI- animals (S5A vs S5C,), and between WT and *daf-7* animals (Fig S5A vs S5E). n.s., two tailed Mann-Whitney test.

(B, D, F) Median time point by which 50% animals died was not different between *flp-13* and ASI-*;flp-13* animals (Fig S5B vs S5D), but was significantly delayed in *daf-7; flp-13* compared to *flp-13* animals (Fig S5B vs S5F). \*\*p<0.01., two tailed Mann-Whitney test. Time course for quiescence from these graphs was also represented in Fig 4J.