Title: Olfactory learning modulates the expression of molecular chaperones in *C. elegans*

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Abstract

Learning, a process by which animals modify their behavior as a result of experience, allows organisms to synthesize information from their surroundings to predict danger. Here we show that prior encounter with the odor of pathogenic bacteria not only enhances the ability of *C. elegans* to avoid the pathogen, but also systemically primes the transcription factor, Heat Shock Factor 1 (HSF-1) to increase molecular chaperone expression upon subsequent encounter with the pathogen by promoting its localization to RNA polymerase II enriched nuclear loci. HSF1-dependent chaperone expression ensues, however, only if and when animals encounter the pathogen. This learning-dependent enhancement of chaperone expression requires serotonin. Thus, learning equips *C. elegans* to better survive environmental dangers by pre-emptively and specifically initiating transcriptional mechanisms throughout the organism to maintain homeostasis. These studies provide one plausible basis for the protective role of environmental enrichment in disease.

Main Text

The ability to accurately predict danger and implement appropriate protective responses is critical for survival. Many animals possess neuronal circuits to detect unfavorable conditions such as increasing temperatures and presence of pathogens and implement an avoidance or defense response. In addition, all cells possess conserved mechanisms to repair and protect their macromolecules from damage that occurs under adverse conditions. One such mechanism is the Heat Shock Response (HSR) (*1-4*). The HSR is mediated by the transcription factor Heat Shock Factor 1 (HSF-1), which in response to stress, increases the expression of cytoprotective molecular chaperones, or so-called heat shock proteins (HSPs), to maintain protein stability and help degrade proteins that can misfold and aggregate under stressful conditions (*1-4*). HSF-1 activity is essential for all organisms to adapt to changing environments. The HSR and HSF-1 can be activated autonomously by single cells in response to proteotoxic stressors (*1-3*). However, within a metazoan such as *Caenorhabditis elegans*, HSF-1 and the cellular response to protein damage are not autonomously controlled by individual cells, but instead are under the regulation of the animals' nervous system (*5-11*). The biological role for this regulation is unclear. We recently discovered that one mechanism by which HSF-1 is regulated in *C. elegans* is through the neurosensory release of the bioamine serotonin (5-hydroxytryptamine, 5-HT (*7*)). In vertebrates and invertebrates, serotonergic systems play a central role in neurophysiological processes underlying learning and memory, allowing animals to learn about threats in their environment and form memories that can be later recalled to modify behavior (*12-23*). Therefore, we asked whether control by the serotonergic-based learning circuitry allowed *C. elegans* to modulate HSF-1 activity as a consequence of prior experience, so as to better combat threats in its environment.

To test this, we exploited previous findings that *C. elegans* have an innate aversion to specific pathogens, and display experience-dependent plasticity to avoid ingesting pathogenic bacteria such as *Pseudomonas aeruginosa* PA14 (*15, 24*). Thus, although animals are typically attracted to novel bacteria upon initial encounter, be it pathogenic bacteria such as PA14 or non-pathogenic *E. coli* strains (*25, 26*), animals

previously exposed to a lawn of pathogenic PA14 will avoid PA14 upon subsequent exposure. This learned avoidance behavior requires the olfactory nervous system and 5-HT (*15, 24, 27, 28*). We used this information to set up a paradigm whereby we could train animals to avoid PA14 using odor alone, circumventing any physical damage that could be inflicted by actual exposure (Fig. S1A), and then asked if olfactory training could enhance the transcriptional activity of HSF-1 and promote survival when animals subsequently encountered the pathogen. Animals were trained by exposing them to the odor of PA14 cultures for 30 minutes. Controls were mock-trained by exposure to the odor of the standard *E. coli* OP50 strain on which animals are typically raised. These animals were subsequently tested for their behavioral response to PA14 lawns, HSF1-dependent transcription and survival on PA14. Behavioral avoidance was quantified by calculating a choice index (CI) for PA14 where a CI of 1.0 indicates maximal preference and a CI of -1.0 indicates maximal aversion (Fig. S1A). As previously observed (*15*), naïve animals faced with a choice between OP50, or the novel bacteria, PA14, accumulated within the first 5 minutes on PA14 (Fig. S1B). However, after 45 minutes *C. elegans* began to avoid PA14 and by 4 hours, 80% of the animals have left PA14 and moved to the OP50 lawn (Fig. S1B). When animals were mock-trained on OP50 odor (control animals), they behaved like naïve animals and also initially accumulated on PA14 and began to leave the lawn by 45 minutes (Fig. 1A). In contrast, animals pre-exposed to the odor of PA14 avoided the PA14 lawn significantly earlier and left within 5 minutes (Fig. 1A). This aversive behavior was not observed when animals were trained for similar durations on the odor of another novel, non-pathogenic bacteria, HT115, and were given a choice between HT115 and OP50. In this case, animals did not avoid HT115, but instead stayed on this novel bacteria throughout the analysis, and behaved no different from control animals pre-exposed to the odor of OP50 (Fig. S1C). Thus, the accelerated behavioral response of animals trained on PA14 odor did not appear to be due to adaptation to the PA14 odor, but seemed to represent learned, enhanced avoidance behavior. In addition, this enhanced avoidance response was specific to the pathogen, and pre-exposure of animals to the odor of PA14 did not trigger avoidance of another known *C. elegans* pathogen, *Serratia marcescens* strain DB11 (Fig. S1D).

Exposure to PA14 is known to be toxic (*29, 30*), causing increased protein damage (*31, 32*) and ultimately, death. Animals exposed to PA14 for only 10 minutes activated HSF-1 (Fig. S1E), and upregulated several molecular chaperones such as the inducible *hsp70* F44E5.4/F44E5.5, and the small heat shock proteins *hsp-16.2* and *hsp-16.41* as measured using qRT-PCR (Fig. S1E and Figs. 1B-D), and their survival on PA14 was HSF1-dependent (Fig. 1E). Pre-exposure to the odor of PA14 enhanced the HSF1-dependent transcriptional response (Figs. 1B-D): the mRNA levels of all three chaperones were significantly higher when animals were first pre-exposed to the odor of PA14, compared to control animals pre-exposed to the smell of OP50. Of note, pre-exposure to the odor of PA14 did not, in itself, induce chaperone expression: animals exposed to the odor of PA14 had low basal chaperone levels that were similar to control animals (Figs. 1B-D). These data suggested that pre-exposure to PA14 odor was, in some unknown way, priming HSF-1 to enhance its transcriptional output upon subsequent exposure to the threat. Such priming was advantageous as pre-exposure to PA14 conferred a consistent and significant survival advantage to the animals when they were subsequently exposed to PA14: while only 46% of control animals survived on PA14 after 18 hours, 63% of the animals pre-exposed to PA14 odor survived at the same time point (Fig. 1F). Pre-exposure to PA14 once again conferred stressor-specific benefits, only enhancing survival upon exposure to PA14 but not under conditions of lethal heat stress (Fig. S1F).

A "grape-like" odor from the compound 2-aminoacetophenone (2AA) made by *Pseudomonas aeruginosa*, is responsible for food-related olfactory behavior of other invertebrate species such as *Drosophila melanogaster* (*33, 34*). We tested whether this compound was, at least in part, responsible for the learned enhanced aversion of *C. elegans* to PA14. Pre-exposure to 2AA mimicked the results observed in our choice assay: animals that were pre-exposed to the smell of 2AA (compared to control animals exposed to the 'odor' of water) avoided PA14 lawns by 15 minutes compared to control animals that only began to show avoidance of PA14 lawns by 45 minutes (Fig. 1G). Pre-exposure to 2AA odorant also enhanced the expression of *hsp70* mRNA when animals were subsequently exposed to PA14 lawns, while in itself not inducing *hsp70* mRNA (Fig. 1H). Moreover, olfaction of the aversive PA14-derived odor appeared to

facilitate a mechanism by which animals could 'decide' to activate HSF-1 if PA14 was indeed present. This was seen when *C. elegans* that were pre-exposed to 2AA odorant encountered an OP50 lawn instead of a PA14 lawn: under these conditions, they did not activate HSF1-dependent *hsp* gene expression (Fig. 1H). These data taken together suggest that the prior experience of PA14 odor, mimicked in large part by the odorant 2AA, was enhancing the organism's ability to survive, not only by hastening the avoidance behavior of the animal from the pathogen, but also by enhancing the expression of cytoprotective HSF-1 transcriptional targets upon an actual encounter with the pathogen. Moreover, since prior olfactory experience did not in itself cause avoidance behaviors to other pathogenic bacteria (Fig. S1D), nor induce the transcription of the protective chaperones unless animals encountered the same threat (Fig. 1H), the neuronal learning circuitry appeared to confer, in some unknown way, a degree of specificity to the HSF-1 transcriptional response.

In *C. elegans* and other organisms, 5-HT signaling is known to mediate learning (*15, 21, 22, 26, 35-38*). We therefore tested whether the enhanced behavioral and transcriptional response to PA14 that occurred following the pre-exposure to PA14-derived odors required 5-HT. Indeed, compared to the 5 minutes needed for wild-type animals trained on PA14 odor to avoid PA14 lawns, animals that lacked functional tryptophan hydroxylase or *tph-1* (*39*), the rate limiting enzyme for 5-HT synthesis, took 1.5 hours to avoid PA14 after pre-exposure to the odor of PA14 (Fig. 2A and Fig. S2A). This was the case although, for reasons we do not understand, *tph-1* control animals avoided PA14 lawns earlier than wild-type control animals in our assay (Fig. 2A and Fig. S2A). In addition, the loss of 5-HT specifically abolished the enhanced activation of HSF-1 upon pre-exposure to the odor of PA14, while not being required for HSF-1 activation on PA14 *per se*: this was observed by the fact that *tph-1* mutant animals did induce *hsp70* (F44E5.4/F44E5.5) expression when exposed to PA14 lawns as assessed by qRT-PCR. However *tph-1* null animals pre-exposed to OP50 or PA14 odors both expressed similar levels of *hsp70* (F44E5.4/F44E5.5) mRNA upon subsequent encounter with PA14 lawns (Fig. 2B), and there was no increase in *hsp70* expression based on prior olfactory experience. Since 5-HT is synthesized only in neurons in *C. elegans*

(*35, 39*), we tested whether 5-HT-dependent olfactory learning was preferentially activating HSF-1 in neurons. This was not the case. Single molecule fluorescent *in situ* hybridization (smFISH) used to detect *hsp70* (F44E5.4/F44E5.5) mRNA across the entire organism indicated that exposure to PA14 induced F44E5.4/F44E5.5 mRNA in all tissue types including neurons, intestine and germline cells, and mRNA expression was enhanced in all these tissues in wild-type animals when trained on PA14 odor (Figs.2C-F and Fig. S2B). Consistent with the whole animal qRT-PCR data, *tph-1* mutant animals also induced *hsp70* (F44E5.4/F44E5.5) mRNA when exposed to PA14 but the induction of mRNA in *tph-1* mutant animals remained the same irrespective of prior olfactory training and was similar to that in control wild-type animals mock trained on OP50 (Figs.2C-F and Fig. S2B). Taken together, these studies showed that both the enhanced avoidance behavior as well as enhanced HSF1-dependent chaperone levels were mediated by the 5-HT-dependent learning circuitry.

How might olfactory learning enhance HSF-1 transcriptional activity? To answer this, we examined whether olfactory training modified any of the steps known to accompany HSF-1 activation. HSF1-dependent transcription of *hsp* genes is a multistep process that varies to some extent between species (*1, 4, 40-45*). In mammalian cells, HSF1-dependent *hsp* expression involves conversion of the HSF-1 monomers to trimers, increased phosphorylation and other post-translational modifications, acquisition of competence to bind heat shock elements (HSEs) on promoters of *hsp* genes and the recruitment of HSF-1 to these HSE elements in a manner that is dependent on the chromatin landscape and transcriptional machinery. We characterized these steps for the transcriptional activation of HSF-1 in *C. elegans* (Figs. S3 and Figs. S4A, B). Consistent with its role as an essential gene in development (*46*), *C. elegans* HSF-1 is a nuclear protein (*7, 45, 47*) which appears to be trimerized (Fig. S3C) and phosphorylated (Fig. S3D) even at ambient temperatures, as detected by an antibody against endogenous *C. elegans* HSF-1 (Fig. S3A, B). Electrophoretic mobility shift assays (EMSA) indicated that in accordance with its trimerization capability at ambient temperatures, *C. elegans* HSF-1, like yeast HSF-1 (*43*), can bind DNA containing canonical *C. elegans* heat shock elements (HSEs) *in vitro* (Figs. S3E, F). However, in

agreement with the lack of expression of inducible *hsp* genes at ambient temperatures in the absence of stress, *C. elegans* HSF-1 does not constitutively bind the *hsp70* promoter region *in vivo* as assayed by chromatin immunoprecipitation (ChIP) (Fig. S4A). The gross phosphorylation levels, trimerization or the ability of *C. elegans* HSF-1 to bind HSE-containing DNA *in vitro* does not significantly change with stress-induced transcriptional activation (Figs. S3C-F). Nevertheless transcriptional activation of *C. elegans* HSF-1 upon heat shock caused it to bind *hsp70* (F44E5.4/F44E5.5) *in vivo,* as seen by a five fold enrichment of the *hsp70* (F44E5.4/F44E5.5) region as assayed by ChIP (Fig. S4A). In addition, exposure to stress such as elevated temperatures (Fig. S4B), and PA14 lawns (Fig. 3A) caused the relocalization of HSF-1 into punctate nuclear bodies (*7, 45, 47*), that were approximately 0.4µm in diameter and formed either as single HSF-1 nuclear bodies or as clusters.

Olfactory pre-exposure to 2AA or PA14 odor did not enhance the ability of HSF-1 to bind DNA *in vitro* as assayed by EMSA (Figs. S3E, F), nor did it cause HSF-1 to bind *hsp70* promoter regions *in vivo* as seen by ChIP (Fig. S4A). However, exposure to 2AA or PA14 odor alone caused a significant fraction of HSF-1 to relocalize into the punctate nuclear bodies, similar to those seen upon exposure to PA14 lawns, even though exposure to the 2AA odor did not induce the transcription of *hsp* genes (Figs., 3A, B and 1H). The number of nuclei containing HSF-1 nuclear bodies following exposure of animals to the odor of 2AA varied between experiments, averaging 8.7% and ranging from 3 – 42% amongst the germline nuclei where HSF-1 was the easiest to visualize, and was visible in 71% of animals scored (n=24 animals and 707 nuclei). Compared to animals pre-exposed to the odor of PA14, only an average of 2.2% of germline nuclei ranging between 0-10 % (890 nuclei) exposed to the odor of OP50 showed any evidence of HSF-1 nuclear bodies. To understand how olfactory exposure may be enhancing the transcriptional ability of HSF-1 while not inducing DNA binding or transcription in itself, we investigated the location of the HSF-1 nuclear bodies using immunostaining. We hypothesized that since animals that were trained on PA14-derived odor did not induce *hsp70* gene expression unless they encountered PA14 lawns, olfactory signaling may be pre-emptively facilitating the association of HSF-1 with the transcriptional

machinery that could support transcription if the actual encounter with the threat were to occur. In *C. elegans,* the *hsp-16.2* promoter has been shown to localize to the nuclear pore complex (NPC) following heat shock (*48, 49*). However, although the HSF-1 nuclear bodies were occasionally in the vicinity of NPCs in germline nuclei, they did not co-localize with NPCs (Figs 3C, D). This was true of the heat shock-induced HSF-1 nuclear bodies that were involved in active transcription of *hsp* genes (n=145), the nuclear bodies that formed upon exposure to the 2AA odor in the absence of *hsp* transcription (n=180), and control animals exposed to the 'odor' of water (n=170). On the other hand, over half of the HSF-1 nuclear bodies (n=371) that were induced by olfactory exposure to 2AA odor co-localized with total RNA polymerase II (Pol II) (Figs. 3E, F). The number of HSF-1 nuclear bodies that co-localized with Pol II remained the same even upon heat shock while animals were actively involved in Pol II-dependent transcription of *hsp* genes (0.21 and 0.20 HSF-1 nuclear bodies/nucleus co-localized with Pol II in 2AA odor exposed animals, and upon heat shock respectively; Figs. 3E, F). Consistent with previous reports (*40, 50-52*), Pol II appeared to cluster in discrete nuclear regions even prior to 2AA exposure or heat shock (Fig. 3E), and the few HSF-1 nuclear bodies/nucleus present in control water-trained animals also co-localized with Pol II (0.09 HSF-1 nuclear bodies/nucleus co-localized with Pol II in control animals; Figs. 3E, F). However, more HSF-1 nuclear bodies formed following 2AA exposure or heat shock, and the formation of HSF-1 nuclear bodies did not appear to require Pol II: (RNAi)-induced knockdown of the large subunit of RNA polymerase II *ama-1* substantially decreased Pol II protein levels in oocyte nuclei (Fig. S4C) but did not interfere with the heat-shock induced formation of HSF-1 nuclear bodies in oocytes (Fig. S4D). We conclude from these studies that olfactory training on aversive signals such as 2AA was priming HSF-1 by pre-emptively concentrating it at nuclear loci in close proximity to RNA polymerase II. Although we do not yet understand the cellular mechanisms by which this occurred the ability of the serotonin-based learning circuitry to induce the co-localization of HSF-1 with Pol II in somatic nuclei could conceivably result in an enhanced transcriptional response upon actual encounter with the pathogen (*40, 41, 48, 50-52*).

Not only was HSF-1 activity enhanced by aversive olfactory stimuli, HSF-1 appeared to be required for the behavioral avoidance of PA14: downregulation of *hsf-1* through RNAi abrogated the behavioral plasticity observed upon exposure to PA14, and animals remained equally distributed between the PA14 and OP50 lawns (Fig. 4A and Fig. S5A). This was the case despite scoring the CI of *hsf-1* downregulated animals following 4-6 hours of being given the choice between PA14 and OP50, by which times, all wild-type animals raised on control RNAi, whether trained on PA14 or control-RNAi odors, had left the PA14 lawns (Fig. 4A, S5A). One mechanism by which HSF-1 can be activated in *C. elegans* is through the release of 5-HT from the ADF and NSM neurons in *C. elegans* (*7*). We therefore argued that the coincidence of 5-HT signaling, olfactory information and HSF-1 activation, could serve as a mechanism to flag a sensory stimulus as a threat, providing a basis for the coupling of the enhanced behavioral aversion with the enhanced transcriptional response seen in our experiments. To test if this was the case we co-incidentally activated 5-HT release using optogenetic methods, eliciting HSF-1 activation while animals were exposed to the odor of an attractive bacteria such as the *E. coli* HT115 (Fig. S5B). Animals whose serotonergic neurons were optogenetically stimulated (*7, 53*) in the presence of HT115 odor showed a significant aversion to HT115 instead of the normal attractive behavioral response (Figs. 4B, C). This aversion lasted for as long as 45 minutes following stimulation, and was displayed even when animals were given a choice between HT115 and PA14. Control animals that were mock stimulated by light were, as expected, attracted to HT115 and repelled by PA14. Thus, inducing 5-HT release during an olfactory stimulus appeared to be sufficient to associate olfactory information regarding odor with HSF-1 activation to trigger an aversive response of *C. elegans* to danger.

In summary, our data provide a mechanism whereby 5-HT release and HSF-1 activation cooperate to elicit behavioral avoidance and transcription of cytoprotective chaperones. Remarkably, 5-HT-dependent learning pre-emptively promotes HSF-1 concentration at nuclear regions close to RNA polymerase II required for the active transcription of *hsp* genes, potentially allowing animals to enhance chaperone expression in the event that they encountered the actual stressor (*40, 41, 48, 50-52*). The exact mechanism

by which 5-HT-dependent learning induces HSF-1 is localized to these regions and the nature of the genomic regions in the vicinity (*54, 55*) remain to be investigated. In *Drosophila* and mammalian cells, a fraction of RNA polymerase II is held paused at *hsp* loci until HSF-1 binding initiates transcription and the release of Pol II into the gene body (*40, 42, 51, 56, 57*). Nevertheless, HSF-1 binding alone is not the determining event for the release of Pol II pausing, as HSF-1 can bind *hsp70* loci without inducing transcription (*58*). We find in our studies too, that while aversive olfactory stimuli facilitate the formation of the HSF-1 nuclear bodies in the vicinity of Pol II, a second signal appears to be required for the actual transcription of *hsp* genes. This signal, we hypothesize, could involve the release of 5-HT upon subsequent encounter of the organism with the same stressor, and appears to confer the specificity of the transcriptional response. The rationale for the multistep activation of a fundamental cytoprotective stress response, such as the HSR and HSF1-dependent gene expression, remains poorly understood. Our data provide a context and a selective advantage for the existence of such a complex mechanism of activation. Organisms survive a range of environmental fluctuations and have evolved to colonize a vast diversity of environmental niches despite the sensitivity of protein-based biological processes to environmental perturbations. We believe that our data begin to address one mechanism through which such adaptation could occur.

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Acknowledgments

We would like to thank members of the V.P. laboratory for comments and discussion, and in particular Matthew Wheat for his assistance in gonad dissections. We would like to acknowledge Dr. Smolikove (University of Iowa) for reagents and advice, Dr. Yahr (University of Iowa) and Dr. Aballay (Duke University) for PA14 strains, Dr. Ruvinsky (Northwestern University, Chicago) for helpful suggestions and *Caenorhabditis* Genetics Center (funded by NIH Office of Research Infrastructure Programs (P40 OD010440) for worm and bacterial strains. F.K.O was supported by V.P's grants, a graduate student fellowship from the Developmental Studies Hybridoma Bank (DSHB) and a scholarship from Glenn/AFAR Foundation. V.P. is funded by the National Institutes of Health (NIH R01 AG 050653) and The Lawrence Ellison Medical Foundation (AG-NS-1056-13).

Fig. 1

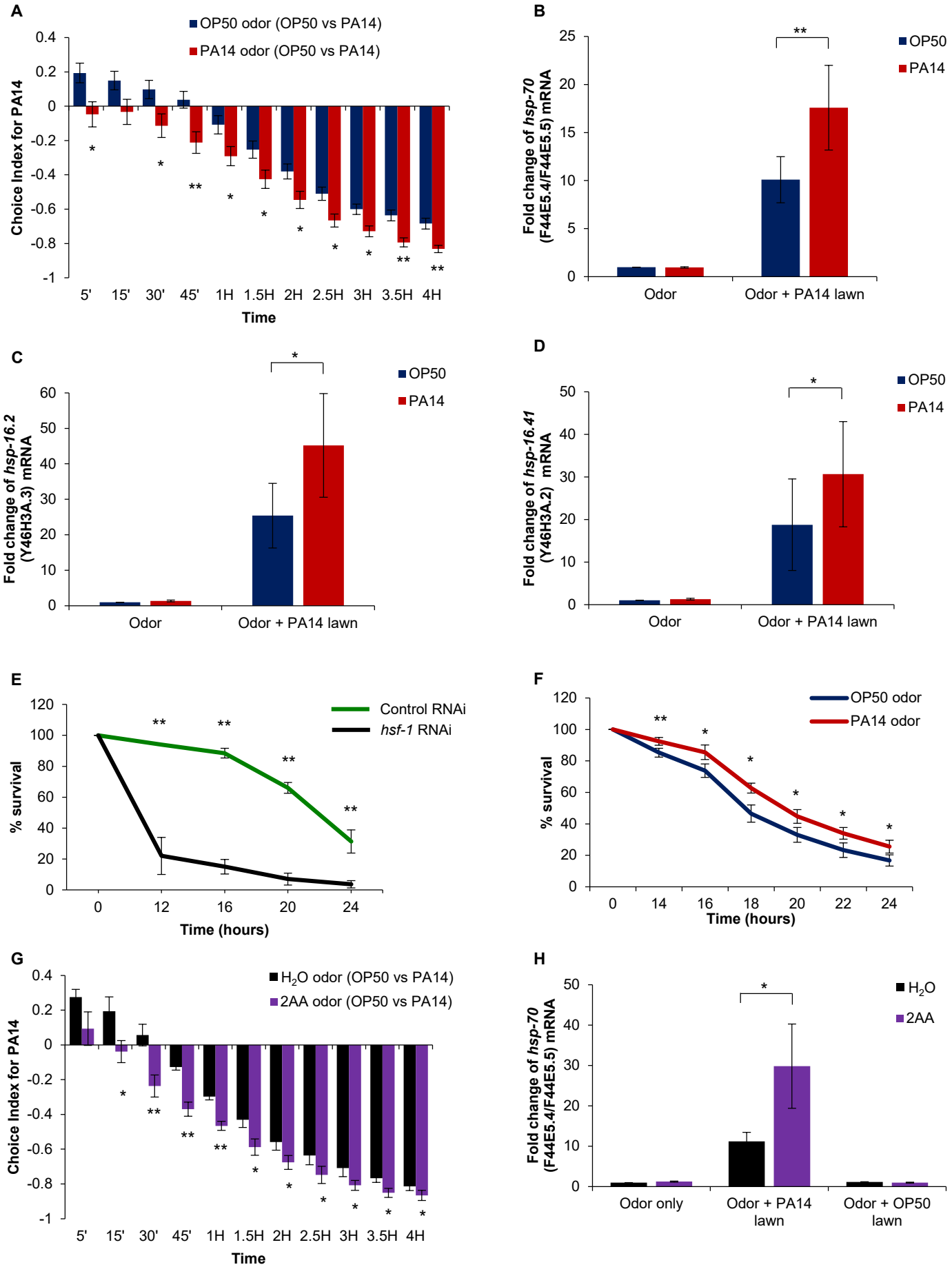
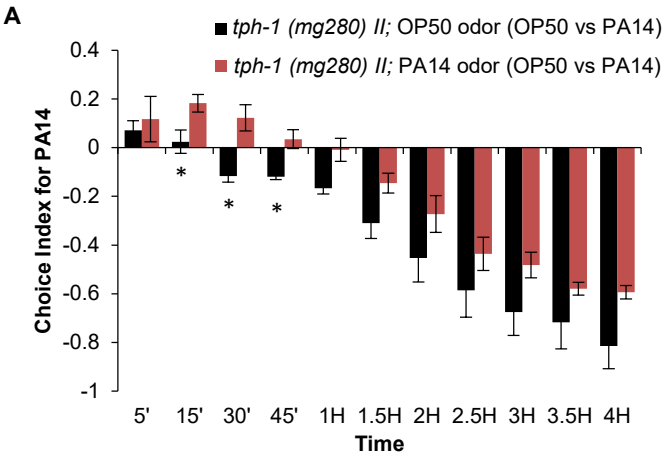
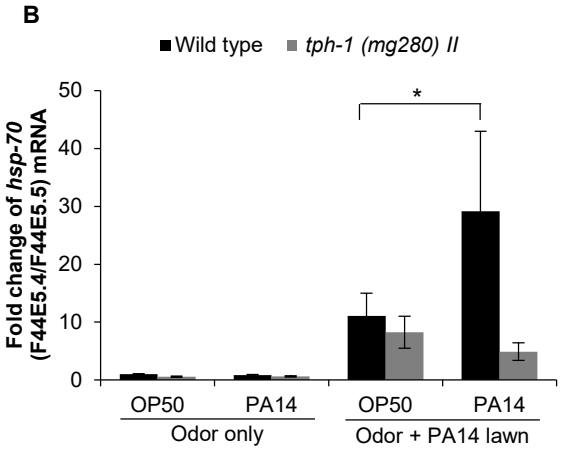

A
OP50 odor (OP50 vs PA14)
PA14 odor (OP50 vs PA14)
Choice Index for PA14
Time
5' 15' 30' 45' 1H 1.5H 2H 2.5H 3H 3.5H 4H
B
OP50
PA14
Fold change of hsp-70 (F44E5.4/F44E5.5) mRNA
Odor
Odor + PA14 lawn
C
OP50
PA14
Fold change of hsp-16.2 (Y46H3A.3) mRNA
Odor
Odor + PA14 lawn
D
OP50
PA14
Fold change of hsp-16.41 (Y46H3A.2) mRNA
Odor
Odor + PA14 lawn
E
Control RNAi
hsf-1 RNAi
% survival
Time (hours)
F
OP50 odor
PA14 odor
% survival
Time (hours)
G
H2O odor (OP50 vs PA14)
2AA odor (OP50 vs PA14)
Choice Index for PA14
Time
5' 15' 30' 45' 1H 1.5H 2H 2.5H 3H 3.5H 4H
H
H2O
2AA
Fold change of hsp-70 (F44E5.4/F44E5.5) mRNA
Odor only
Odor + PA14 lawn
Odor + OP50 lawn

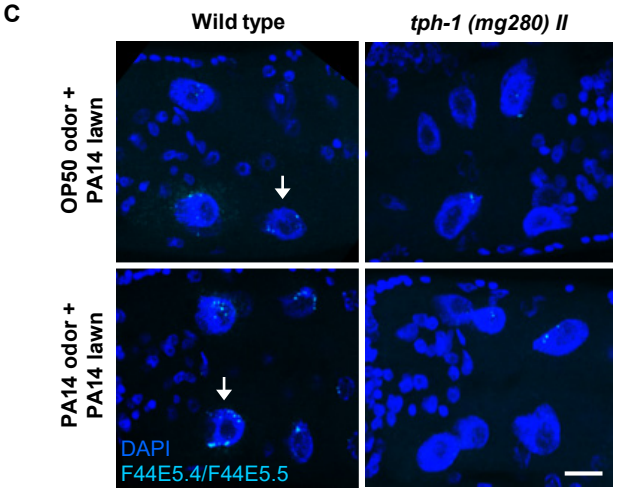
Fig. 1 – Olfactory learning enhances HSF-1 activation.

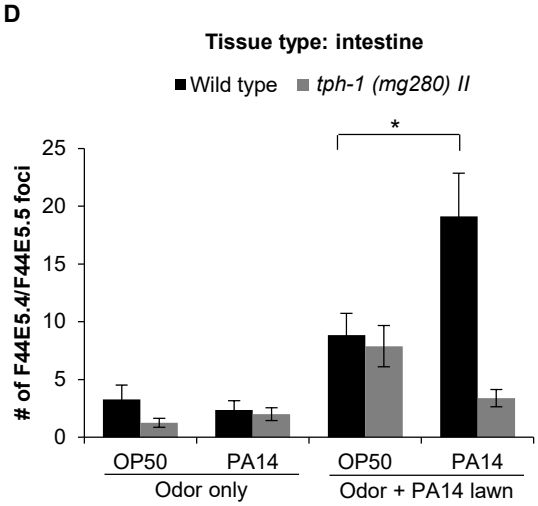
(A) Choice index for PA14 of wild-type animals pre-exposed to the odor of either OP50 or PA14. Preference was recorded at times indicated (x-axis). N = 16-17 experiments of 30 animals/condition. *p<0.05, **p<0.01; Student's t-test. **(B)-(D)** mRNA levels measured by qRT-PCR upon PA14 exposure calculated relative to wild-type animals pre-exposed to OP50 odor: (B) *hsp-70* (F44E5.4/F44E5.5), (C) *hsp-16.2* (Y46H3A.3) and (D) *hsp-16.41* (Y46H3A.2). Animals were treated as described (X-axis). N = (B) 37, (C) 9, and (D) 8 experiments of 30 animals/condition. *p<0.05, **p<0.01; Student's t-test for (B) and Wilcoxon sign test for (C-D). **(E)-(F)** Survival on PA14: (E) wild-type animals subjected to control or *hsf-1* RNAi knockdown and (F) wild-type animals pre-exposed to OP50 or PA14 odor. N = (E) 3 and (F) 4 experiments of 50 animals/condition. *p<0.05, **p<0.01; Student's t-test. **(G)** Choice index for PA14 of wild-type animals pre-exposed to the odor of either water or 2AA. Preference was recorded at times indicated (x-axis). N = 3-4 experiments of 30 animals/condition. *p<0.05, **p<0.01; Student's t-test. **(H)** mRNA levels of *hsp-70* (F44E5.4/F44E5.5) measured by qRT-PCR in wild-type animals pre-exposed to water or 2AA odor, and subsequently subjected to a PA14 or OP50 lawn. Values are relative to animals pre-exposed to the odor of water. N = 3-13 experiments of 30 animals/condition. *p<0.05; Wilcoxon sign test. Data represent means ± S.E.M. (A and G) Legends: pre-exposure conditions (choice).

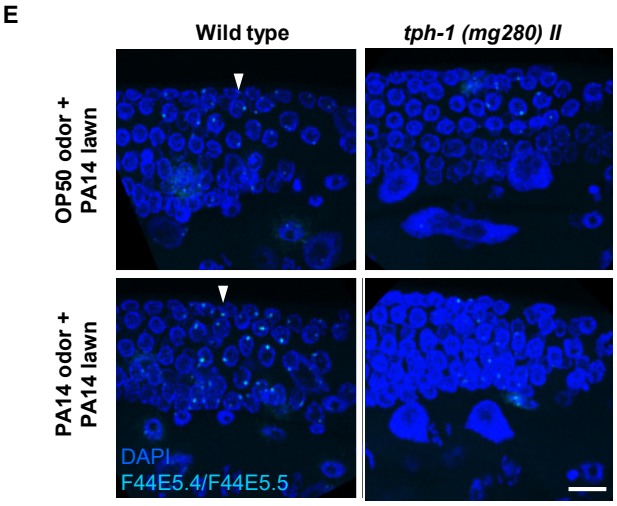
Fig. 2


A
tph-1 (mg280) II; OP50 odor (OP50 vs PA14)
tph-1 (mg280) II; PA14 odor (OP50 vs PA14)
Choice Index for PA14
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
*
*
*
5'
15'
30'
45'
1H
1.5H
2H
2.5H
3H
3.5H
4H
Time


B
Wild type
tph-1 (mg280) II
Fold change of hsp-70 (F44E5.4/F44E5.5) mRNA
50
40
30
20
10
0
*
OP50
PA14
OP50
PA14
Odor only
Odor + PA14 lawn


C
Wild type
tph-1 (mg280) II
OP50 odor + PA14 lawn
PA14 odor + PA14 lawn
DAPI
F44E5.4/F44E5.5


D
Tissue type: intestine
Wild type
tph-1 (mg280) II
# of F44E5.4/F44E5.5 foci
25
20
15
10
5
0
*
OP50
PA14
OP50
PA14
Odor only
Odor + PA14 lawn


E
Wild type
tph-1 (mg280) II
OP50 odor + PA14 lawn
PA14 odor + PA14 lawn
DAPI
F44E5.4/F44E5.5

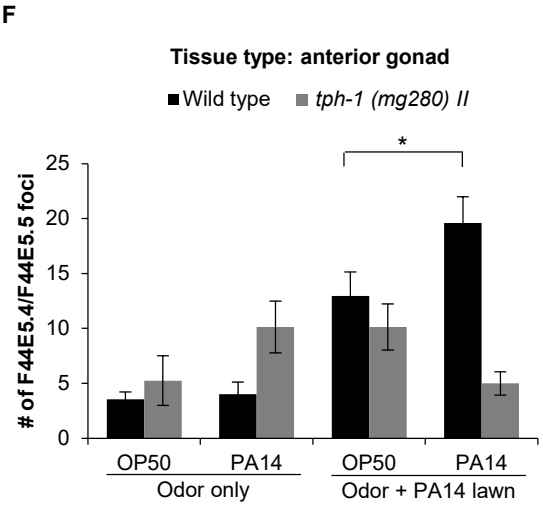

F
Tissue type: anterior gonad
Wild type
tph-1 (mg280) II
# of F44E5.4/F44E5.5 foci
25
20
15
10
5
0
*
OP50
PA14
OP50
PA14
Odor only
Odor + PA14 lawn

Fig. 2 – Serotonin is required for olfactory learning-mediated HSF-1 activation.

(A) Choice index for PA14 of *tph-1 (mg280) II* animals pre-exposed to the odor of either OP50 or PA14. Preference was recorded at times indicated (x-axis). N = 4 experiments of 30 animals /condition. *$p<0.05$; Student's t-test. **(B)** mRNA levels of *hsp-70* (F44E5.4/F44E5.5) measured by qRT-PCR upon PA14 exposure in wild-type and *tph-1 (mg280) II* animals pre-exposed to OP50 or PA14. Values are relative to wild-type animals pre-exposed to the odor of OP50. N = 8 experiments of 30 animals/condition. *$p<0.05$; Wilcoxon sign test. **(C) and (E)** smFISH confocal micrographs showing *hsp-70* (F44E5.4/F44E5.5) mRNA and DAPI in wild-type and *tph-1 (mg280) II* animals pre-exposed to OP50 or PA14, and subsequently subjected to a PA14 lawn. Images are of (C) intestinal nuclei (arrows) and (E) germline nuclei (arrowheads). Scalebars = 10µm. **(D) and (F)** Quantification of number of *hsp-70* (F44E5.4/F44E5.5) foci present in nuclei. N = 8-11 animals per condition; >200 nuclei/condition. *$p<0.05$; Student's t-test. (A, B, D and F) Data represent means ± S.E.M. (A) Legends: pre-exposure conditions (choice).

Fig. 3

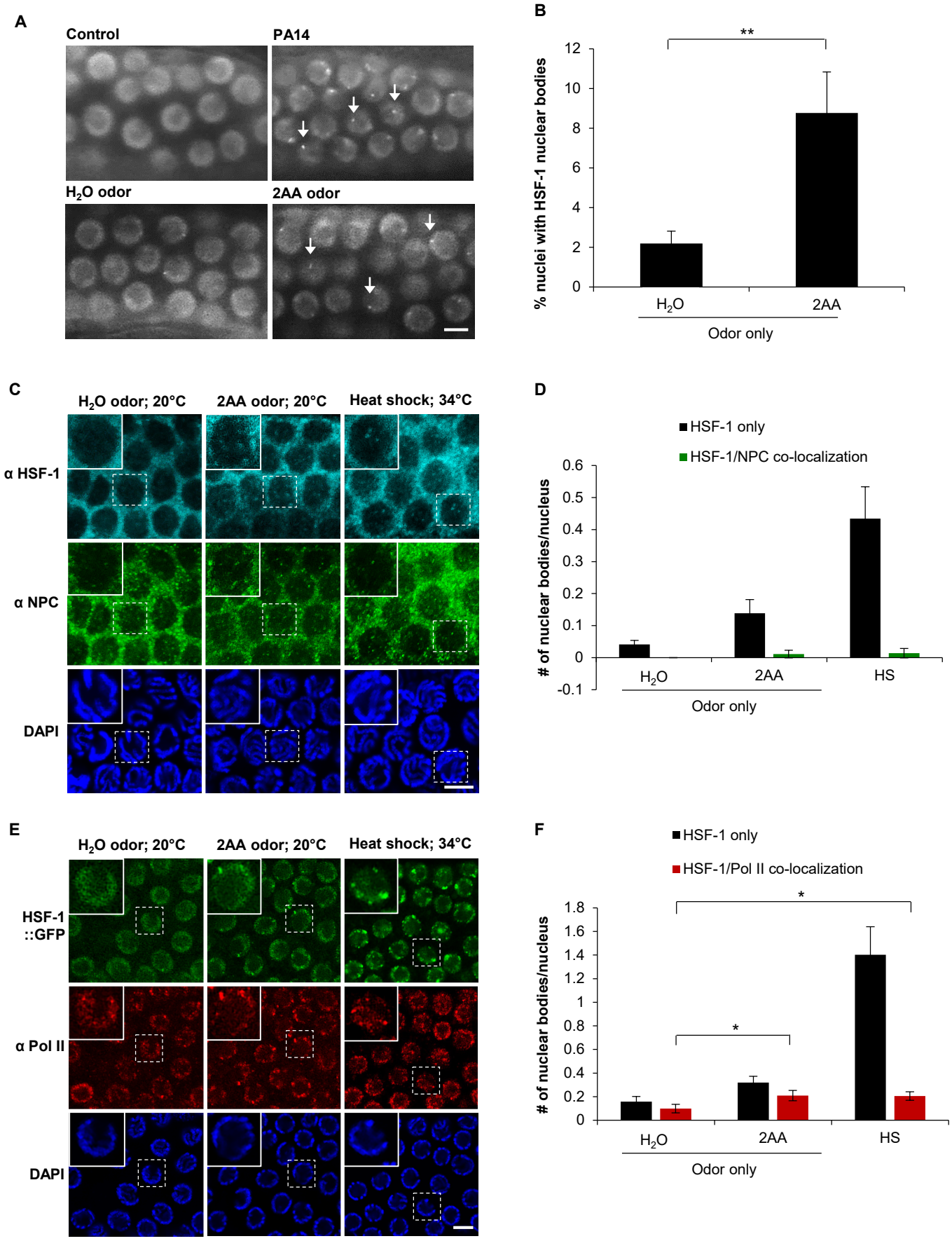

A
Control
PA14
H2O odor
2AA odor
B
**
% nuclei with HSF-1 nuclear bodies
12
10
8
6
4
2
0
H2O
2AA
Odor only
C
H2O odor; 20°C
2AA odor; 20°C
Heat shock; 34°C
α HSF-1
α NPC
DAPI
D
HSF-1 only
HSF-1/NPC co-localization
# of nuclear bodies/nucleus
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
H2O
2AA
HS
Odor only
E
H2O odor; 20°C
2AA odor; 20°C
Heat shock; 34°C
HSF-1 ::GFP
α Pol II
DAPI
F
HSF-1 only
HSF-1/Pol II co-localization
*
*
# of nuclear bodies/nucleus
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
H2O
2AA
HS
Odor only

Fig. 3 – Olfactory learning primes HSF-1 via increased association with RNA Polymerase II.

(A) HSF-1::GFP localization in germline nuclei: control animals at ambient temperature, animals on a PA14 lawn, animals pre-exposed to water-odor or animals pre-exposed to 2AA odor. Arrows indicate HSF-1 nuclear bodies. Scalebar = 5µm. **(B)** Quantification of HSF-1 nuclear bodies when animals are pre-exposed to water-odor or 2AA odor. N = 700-900 nuclei. **$p<0.01$; Student's t-test. **(C)** Immunofluorescence micrographs of HSF-1, Nuclear Pore Complex proteins (NPCs) and DAPI in germline nuclei of wild-type animals exposed to water-odor, 2AA odor and heat shock. Scalebar = 5µm. **(D)** Quantification of numbers of total HSF-1 nuclear bodies/nucleus and HSF-1 nuclear bodies/nucleus that co-localize with NPCs. N = 145-180 nuclei. No significant differences in co-localization; Student's t-test. **(E)** Immunofluorescence micrographs of HSF-1, RNA Pol II and DAPI in germline nuclei of dissected animals expressing HSF-1::GFP upon exposure to water-odor, 2AA odor and heat shock. Scalebar = 5µm. **(F)** Quantification of numbers of total HSF-1::GFP nuclear bodies/nucleus and HSF-1 nuclear bodies/nucleus that co-localize with RNA Pol II. N = 248-371 nuclei. *$p<0.05$; Student's t-test. (B, D and F) Data represent means ± S.E.M.

Fig. 4

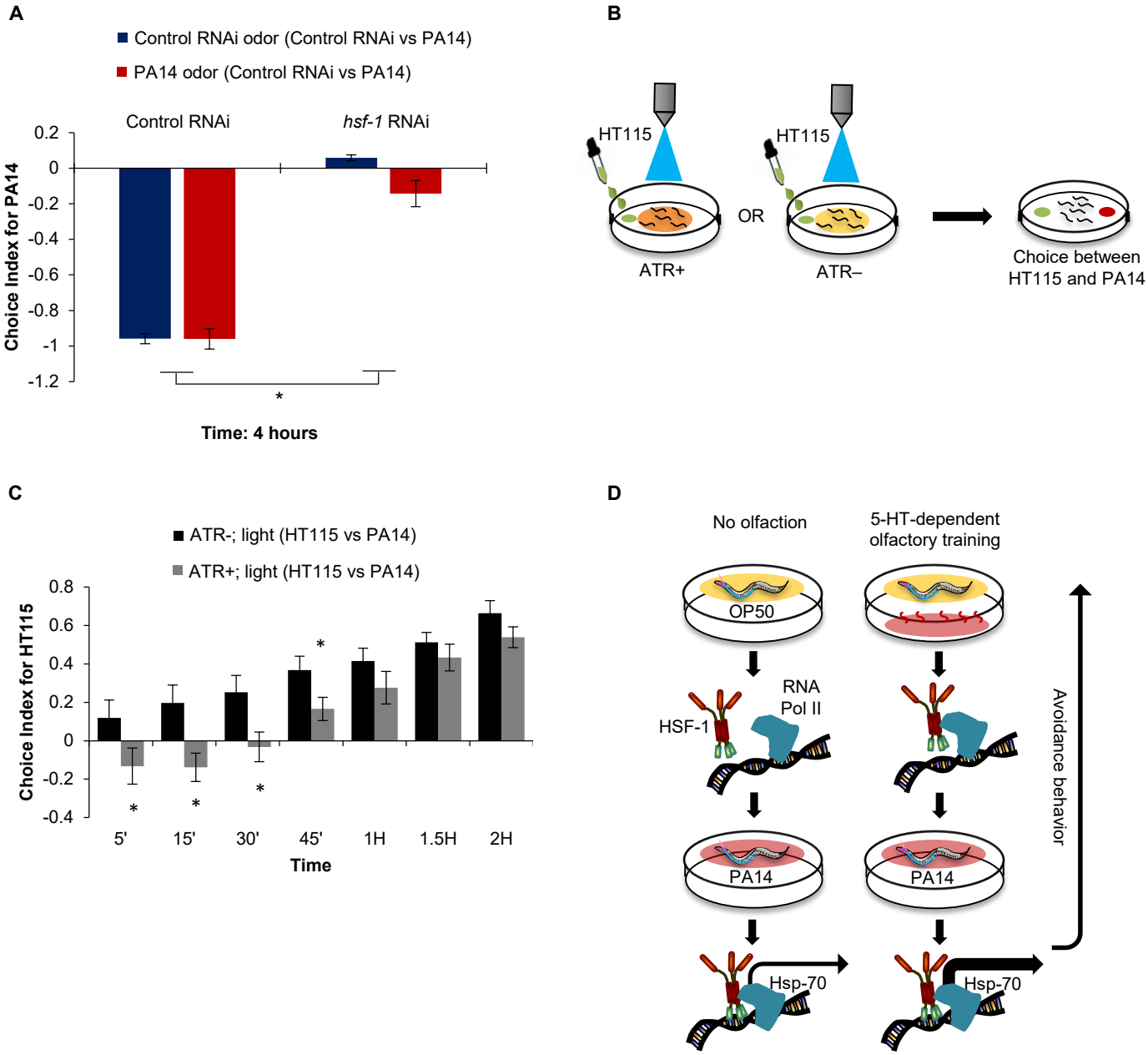

A
Control RNAi odor (Control RNAi vs PA14)
PA14 odor (Control RNAi vs PA14)
Control RNAi
hsf-1 RNAi
Choice Index for PA14
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
*
Time: 4 hours
B
HT115
HT115
OR
ATR+
ATR–
Choice between HT115 and PA14
C
ATR-; light (HT115 vs PA14)
ATR+; light (HT115 vs PA14)
Choice Index for HT115
0.8
0.6
0.4
0.2
0
-0.2
-0.4
5'
15'
30'
45'
1H
1.5H
2H
Time
D
No olfaction
5-HT-dependent olfactory training
OP50
RNA Pol II
HSF-1
PA14
PA14
Hsp-70
Hsp-70
Avoidance behavior

Fig. 4 - 5-HT signaling couples olfactory information with HSF-1 activation to mark sensory stimuli as threats.

(A) Choice index for PA14 of wild-type animals subjected to control or *hsf-1* RNAi knockdown pre-exposed to the odor of either control RNAi bacteria or PA14. Preference was recorded at time indicated (x-axis). N = 6 experiments of 30 animals/condition. *$p<0.05$, Student's t-test. **(B)** Schematic of olfactory pre-exposure to HT115 in conjunction with optogenetic excitation of serotonergic neurons, followed by behavioral choice assay. ATR+ indicates the presence of all-*trans* retinal required for the light-induced excitation of channelrhodopsin (expressed in the serotonergic neurons) necessary to release 5-HT. ATR– indicates control-mock excited animals. **(C)** Choice index for HT115 in animals +/- ATR following optogenetic excitation of serotonergic neurons. Light-induced excitation was coupled with HT115 odor. N = 7 experiments of 10 animals/condition. *$p<0.05$; Student's t-test. **(D)** Model: 5-HT-dependent olfactory learning facilitates the association between RNA Pol II and HSF-1, resulting in enhanced avoidance behavior as well as enhanced transcription of HSF-1 targets in a stressor-specific manner. (A and C) Data represent means ± S.E.M. (A and C) Legends: pre-exposure conditions (choice).