Report

Current Biology

Coordinated Behavioral and Physiological Responses to a Social Signal Are Regulated by a Shared Neuronal Circuit

Highlights

- *C. elegans* male pheromone promotes hermaphrodite reproductive success
- Serotonin is required for hermaphrodites to respond to male pheromone
- The same neuronal circuit mediates behavioral and physiological responses
- Circuit sharing coordinates distinct phenomena occurring on different timescales

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In Brief

Aprison and Ruvinsky identify a neuronal circuit required for the germline of *C. elegans* hermaphrodites to respond to a male pheromone. The same circuit mediates reduced exploratory movement in response to the pheromone. Shared reliance on the same signaling mechanism coordinates multiple pheromone responses that promote reproductive success.

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Coordinated Behavioral and Physiological Responses to a Social Signal Are Regulated by a Shared Neuronal Circuit

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SUMMARY

Successful reproduction in animals requires orchestration of behavior and physiological processes. Pheromones can induce both "releaser" (behavioral) and "priming" (physiological) effects [1] in vertebrates [2, 3] and invertebrates [4, 5]. Therefore, understanding the mechanisms underlying pheromone responses could reveal how reproduction-related behaviors and physiology are coordinated. Here, we describe a neuronal circuit that couples the reproductive system and behavior in adult *Caenorhabditis elegans* hermaphrodites. We found that the response of the oogenic germline to the male pheromone requires serotonin signal from NSM and HSN neurons that acts via the *mod-1* receptor in AIY and RIF interneurons and is antagonized by pigment-dispersing factor (PDF). Surprisingly, the same neurons and pathways have been previously implicated in regulation of exploratory behavior in the absence of male-produced signals [6]. We demonstrate that male pheromone acts via this circuit in hermaphrodites to reduce exploration and decrease mating latency, thereby tuning multiple fitness-proximal processes. Our results demonstrate how a single circuit could coordinate behavioral and physiological responses to the environment, even those that unfold on different timescales. Our findings suggest the existence of a centralized regulatory mechanism that balances organismal resources between reproductive investment and somatic maintenance.

RESULTS

A number of small molecules called ascarosides perform pheromone functions in the nematode *Caenorhabditis elegans* [7–9]. The sexual identity of the pheromone blend released by live nematodes is determined by the ratio of concentrations of ascr#10, a male-enriched molecule [10], and ascr#3, its hermaphrodite-enriched counterpart [11]. As in other species, pheromones released by *C. elegans* males alter several aspects of reproductive physiology in the opposite sex. For example, ascr#10 affects the hermaphrodite germline—it improves sperm guidance [12] and increases the number of germline precursor cells (GPCs) [13] (Figure 1A). The GPC population is located in the distal gonad [14] and consists primarily of mitotically proliferating nuclei, although it also contains some nuclei in the early stages of meiosis [15]. Specific molecular pathways and cellular processes that are altered in the germline in response to male pheromones remain to be elucidated. It is clear, however, that the number of GPCs is highly consistent in animals of the same age, whereas exposure to ascr#10 increases the number of these cells in a variety of strains [11]. Because GPC counts could serve as a reliable indicator of pheromone effects on germline physiology, we leveraged this simple and readily quantifiable assay to dissect neuronal circuits that mediate pheromone effects on physiology of the hermaphrodite germline.

A Serotonergic Circuit Mediates Effects of ascr#10 on the Hermaphrodite Germline

Reasoning that neuromodulators are likely to be involved in coordinating multiple pheromone effects, we focused on serotonin, which is an attractive candidate because it regulates responses to the environment [16], including social signals. The key serotonin biosynthetic enzyme, tryptophan hydroxylase *tph-1* [17], is essential for the germline response to ascr#10 (Figures 1B and S1A). Loss of TPH-1 function selectively compromised response to this pheromone, but it did not alter the baseline number of GPCs (represented in figures by dots; Figures 1B, S1A, and S1B). Loss of TPH-1 did not affect co-cultured wild-type worms (Figure 1C), indicating that the defect was not likely to be due to aberrant pheromone production.

In *C. elegans* hermaphrodites, serotonin is synthesized in up to four bilateral pairs of neurons [17, 18], none of which are the chemosensory neurons implicated in response to ascr#10 [11]. Using strains in which the *tph-1* gene was deleted in individual neuron pairs, thus eliminating serotonin production [6], we found that TPH-1 function in NSM and HSN neurons was required for the germline response to ascr#10 (Figures 1B and S1A). To confirm the role of NSM and HSN neurons in regulating the germline response to ascr#10, we examined mutant strains in which development of these cells was affected. We found that *egl-1* mutants, in which HSNs undergo inappropriate cell death [19], and *ttx-3* mutants that compromise NSM development [20] did not respond to ascr#10 (Figures 1D and S1C).

To test whether exposure to ascr#10 upregulated expression of *tph-1*, as may be expected given that serotonin signaling from NSM and HSN neurons is required for the germline

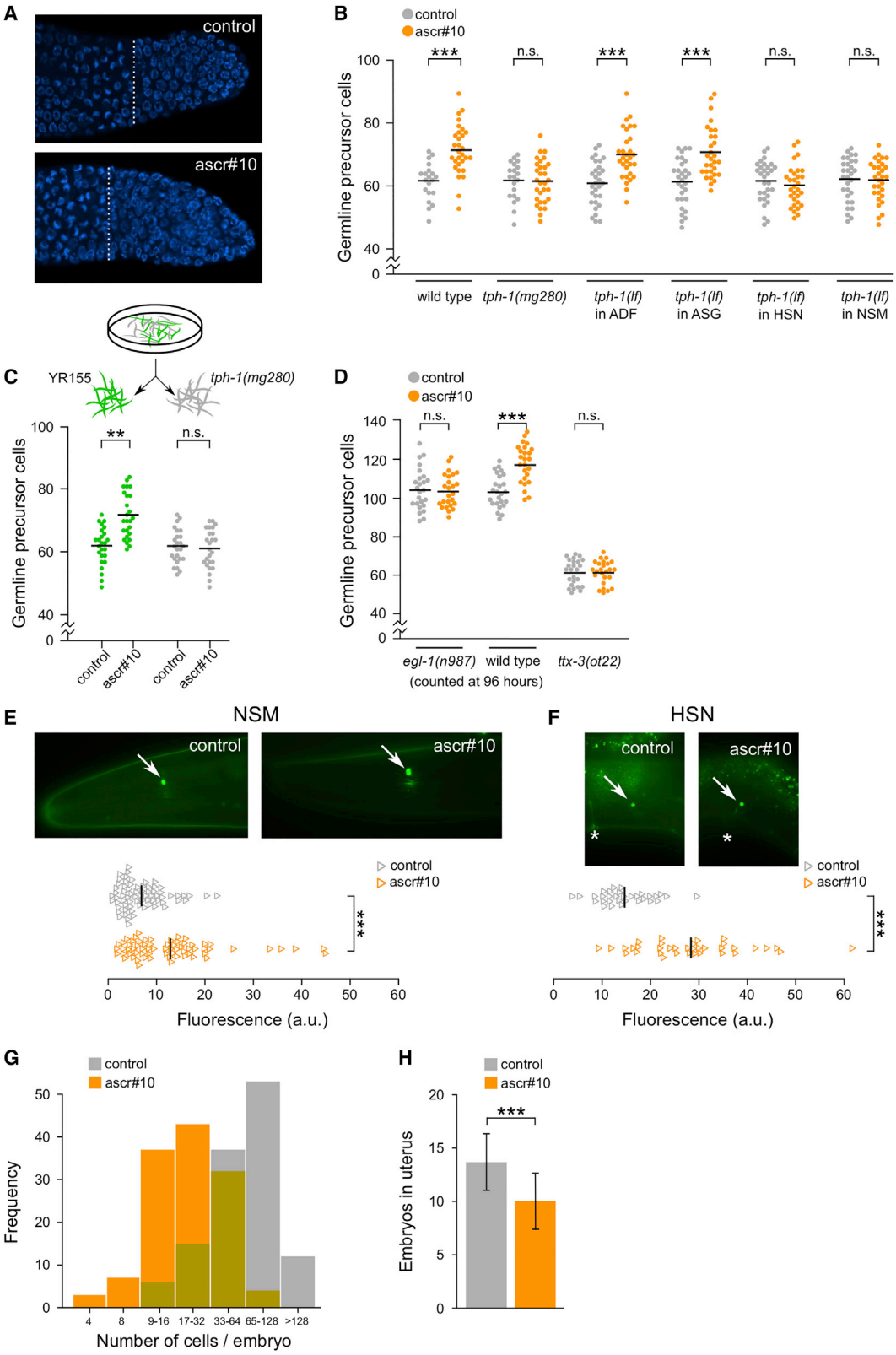

Figure 1. Serotonergic Signaling from NSM and HSN Neurons Is Required for the Hermaphrodite Germline Response to ascr#10

(A) Single confocal sections of extruded gonads stained with DAPI from Day 5 adult hermaphrodites (aged in the presence of ascr#10 or control). Germline precursor cells (GPCs) are defined as cells from the distal tip of the gonad to the distal edge of the transition zone (marked by the dotted line).

(legend continued on next page)

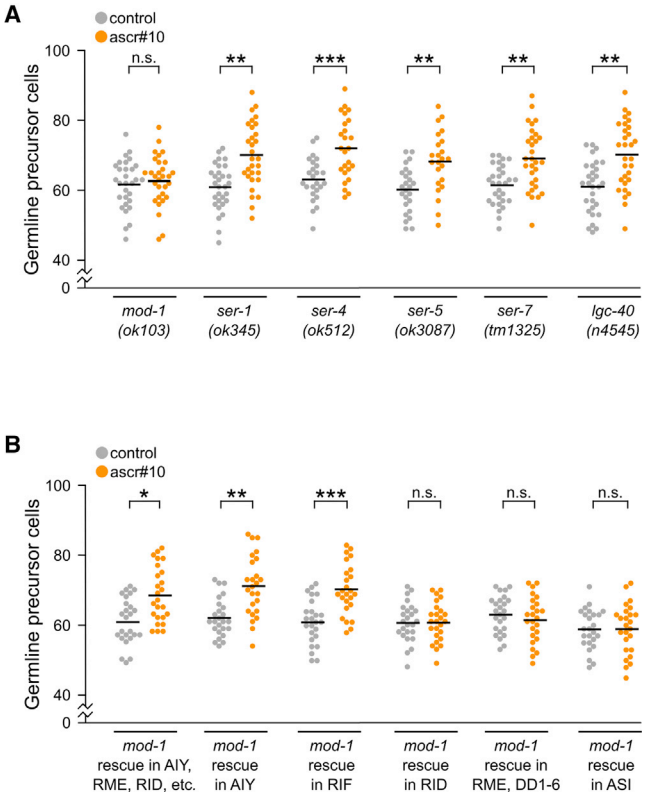

Figure 2. The Activity of a Serotonin Receptor *mod-1* Is Required for Hermaphrodite Germline Response to ascr#10

(A) Germline precursor cells following ascr#10 treatment in serotonin receptor mutants.

(B) Germline precursor cells following ascr#10 treatment in *mod-1* mutants expressing MOD-1 in subsets of the endogenous pattern.

Each dot represents one animal. *p < 0.05; **p < 0.01; ***p < 0.001. Black bars denote the mean.

See Figure S2 for additional related results and Data S1 for primary data and details of statistical analyses.

response to pheromone, we imaged reporters driven by *tph-1* regulatory elements. We observed ~1.5- to 2-fold increase in expression in NSM and HSN neurons (Figures 1E, 1F, and S1D). Although this increase is relatively modest, increasing the number of wild-type copies of the *tph-1* gene from two to three has previously been shown to alter *C. elegans* egg laying behavior [21]. Increased serotonergic signaling promotes egg laying, that is, it causes hermaphrodites to lay embryos sooner after fertilization and retain fewer embryos in the uterus [22]. Consistent with the observed increase of *tph-1* expression in the presence of ascr#10, we found that embryos laid on pheromone-conditioned plates were younger (Figure 1G) and that mothers retained fewer embryos (Figure 1H). Collectively, results presented above argue that ascr#10 potentiates serotonergic signaling from NSM and HSN neurons at least in part by increasing *tph-1* expression in these cells and that this potentiation is required for increasing the number of GPCs.

Six serotonin receptors have been annotated in the *C. elegans* genome [23]. Loss-of-function mutations in only one of them—an inhibitory serotonin-gated chloride channel *mod-1* [24]—abrogated the germline response to ascr#10 (Figures 2A and S2A). To identify the *mod-1*-expressing neurons that receive the serotonin signal released by NSMs and HSNs, we tested *mod-1* mutants in which MOD-1 function was restored in subsets of the overall expression pattern. We discovered that MOD-1 function in AIY or RIF interneurons was sufficient for the ascr#10 response (Figures 2B and S2B).

A Serotonergic Circuit that Controls Behavioral Response to ascr#10

The serotonergic circuit that consists of NSM and HSN neurons and the *mod-1* receptor has been previously shown to regulate exploratory behavior [6]. Freely moving *C. elegans* engage in roaming or dwelling, discrete locomotor states that persist for several minutes and are characterized by distinct patterns of directional movement, turns, etc. [6, 25, 26]. Serotonin signaling from NSMs and HSNs increases dwelling via the inhibitory *mod-1* receptor in AIY and RIF neurons [6], consistent with the role of AIY neurons in extending roaming episodes [27]. We were surprised by the implication of our findings—that essentially the same neurons and signaling pathways (see STAR Methods) appeared to be involved in regulating the germline response to male pheromone and a seemingly unrelated partitioning of exploratory behavior in the absence of exogenous pheromones. We therefore tested whether ascr#10 could alter how worms explore their environment.

To quantify exploratory behavior, we relied on an established assay—counting the number of squares over which a singled worm moved over a period of several hours [6, 28, 29]. ascr#10 upregulates *tph-1* expression in NSM and HSN neurons (Figures 1E and 1F), thus potentiating serotonergic signaling, which in turn reduces exploration [6]. Consistent with this logic, exploration (represented in figures by squares) was substantially reduced in the presence of ascr#10 in a manner dependent on *tph-1* function in both NSMs and HSNs (Figure 3B). Loss-of-function mutations in *mod-1* also precluded reduced exploration in the presence of the pheromone, but providing

(B) Germline precursor cells following ascr#10 treatment in *tph-1* mutants or animals with neuron-specific loss of TPH-1 function.

(C) Germline precursor cells in *tph-1* mutants and co-cultured wild-type animals.

(D) Germline precursor cells following ascr#10 treatment in mutants that affect HSN (*egl-1*) or NSM (*ttx-3*) development. The *egl-1* experiment was carried out at 96 h post-release from L1 arrest, because some nematodes had internal hatching. For this reason, a control at this time point is included. The *ttx-3* experiment was carried out at ~150 h (Day 5 adults), and its relevant control is shown in (B).

(E and F) Expression of *tph-1::YFP* (E) in NSM and (F) in HSN in adult hermaphrodites exposed to ascr#10. These hermaphrodites began egg laying during exposure to ascr#10. Arrows point to the cell body. Asterisks mark the position of the vulva. Anterior is to the left, and ventral is down. Quantification of fluorescence is shown below micrographs.

(G) Developmental stage (expressed as number of cells) of embryos laid by hermaphrodites exposed to ascr#10.

(H) Number of embryos retained in the uterus of hermaphrodites exposed to ascr#10; whiskers represent SD.

Each dot in (B), (C), and (D) represents one animal; each triangle in (E) and (F) represents one neuron. Black bars denote the mean. **p < 0.01; ***p < 0.001.

See Figure S1 for additional related results and Data S1 for primary data and details of statistical analyses.

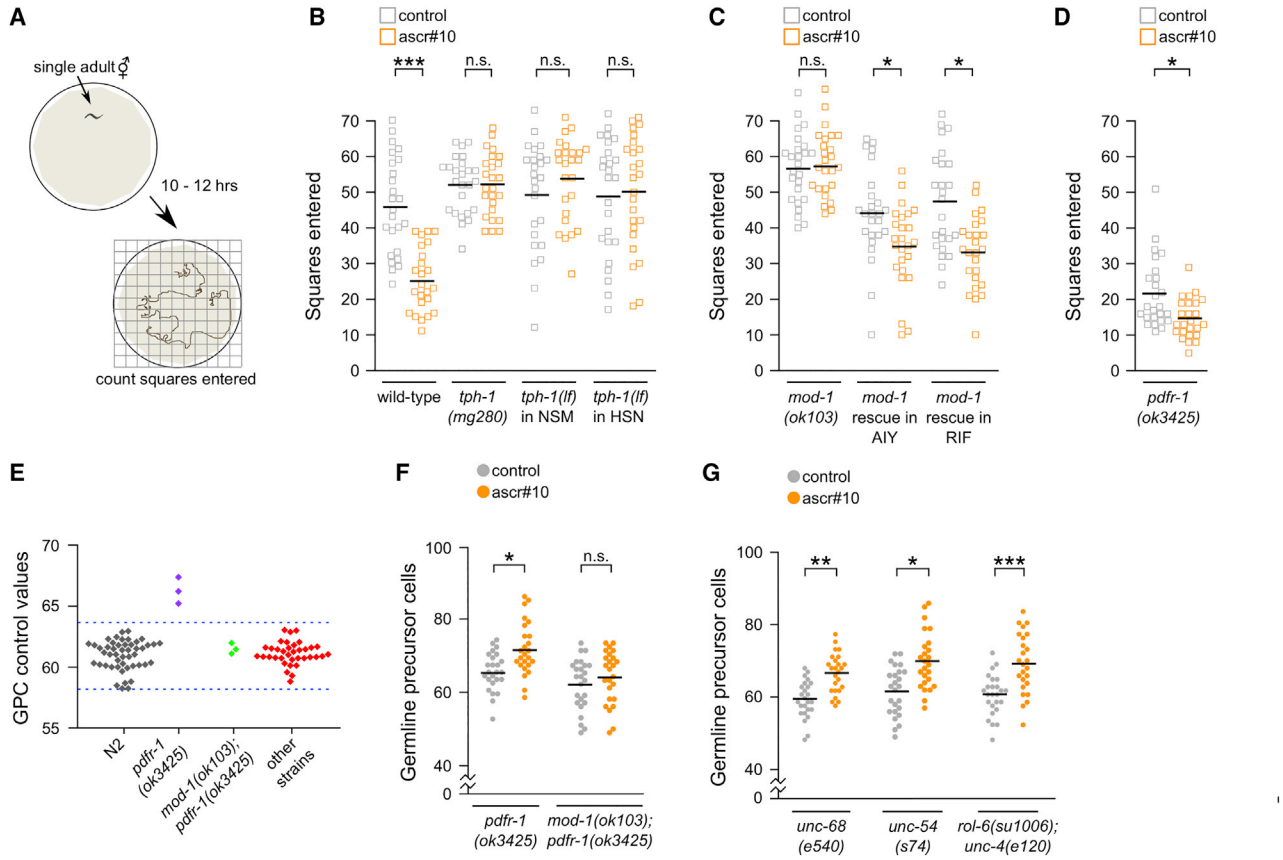

Figure 3. The Circuit that Alters Exploratory Behavior in Response to ascr#10

(A) Schematic protocol for assaying exploratory behavior (after [6]).

(B) Exploratory behavior of *tph-1*mutant and strains with cell-specific loss of TPH-1.

(C) Exploratory behavior of *mod-1* mutant and strains with cell-specific rescue of this mutation.

(D) Exploratory behavior of *pdfr-1* mutant. Note, *pdfr-1* animals were allowed to explore for 24 h or twice the time allotted to other strains.

(E) Comparison of GPC count control values from experiments using N2 wild-type, *pdfr-1*, *mod-1;pdfr-1* mutants, and all other mutants tested in this study. Except for *pdfr-1*, baseline numbers of GPCs are remarkably consistent. Dashed lines delimit three SDs above and below the mean of all strains except *pdfr-1*. Observing any value above the dashed line by chance alone has a probability of ~0.0015 (see STAR Methods). Each diamond represents the average number of GPCs from a separate experiment with a given strain, not the number of GPCs in an individual animal, as elsewhere in this paper. This was done to effectively contrast the germline response to ascr#10 in one strain (*pdfr-1*) to that in several dozen other strains. Experiments with each strain involved matched controls, making this the most appropriate comparison.

(F) Germline precursor cells following ascr#10 treatment in *pdfr-1* and *pdfr-1;mod-1* mutants.

(G) Germline precursor cells following ascr#10 treatment in mutants with severely compromised locomotion.

In (B)–(D), (F), and (G), each dot or square represents one animal; in (E), each diamond represents a mean number of GPCs in a strain. Black bars denote the mean. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

See Figure S3 for additional related results and Data S1 for primary data and details of statistical analyses.

MOD-1 function in either AIY or RIF neurons was sufficient to restore the behavior (Figures 3C and S3A).

The dwelling-promoting activity of the NSM/HSN/*mod-1* circuit is opposed by the pigment-dispersing factor (PDF) signaling, which shortens dwelling and prolongs roaming episodes [6]. We found that, although loss of the PDF receptor, *pdfr-1*, caused (as expected) considerably reduced baseline movement, exposure to ascr#10 further decreased exploratory activity in these animals (Figure 3D). In the absence of ascr#10, *pdfr-1* mutants showed an increased number of GPCs compared to hermaphrodites of the wild-type N2 strain (Figure 3E). Exposure to male pheromone further increased the number of these cells (Figures 3F and S3B). Flavell et al. [6] reported that loss of *mod-1* suppressed gross defects of *pdfr-1* on exploratory movement. Similarly, we observed that, in *mod-1;pdfr-1* double mutants, the baseline number of GPCs was reduced to the wild-type level (Figures 3E and 3F), and the ability to respond to ascr#10 was lost (Figure 3F), as in *mod-1* mutants (Figure 2A), but unlike in *pdfr-1* mutants (Figure 3F). These results demonstrate that behavioral and germline responses of hermaphrodites to ascr#10 rely on the same neuronal circuit—serotonergic signaling from NSM and HSN neurons inhibits AIY and RIF interneurons via the *mod-1* receptor, and PDF signaling counters this activity. The effect of the active state of this circuit is to decrease hermaphrodite's exploratory behavior and to increase the number of GPCs in the presence of a male pheromone. The means by

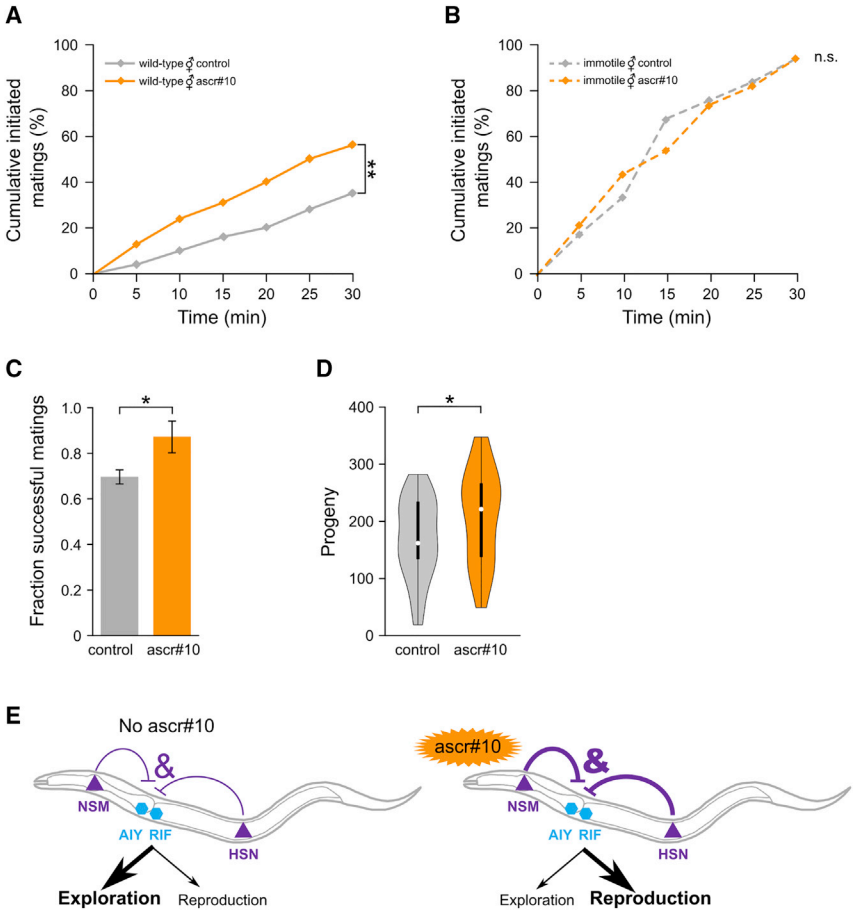

Figure 4. Effects of ascr#10 on Mating Behavior and Reproduction

(A and B) Latency of mating initiation with (A) wild-type or (B) essentially immotile *rol-6;unc-4* hermaphrodites.

(C) Fraction of 1-h matings between adult *fog-2(q71)* females and N2 males that yielded progeny; whiskers represent SD.

(D) Exposure of *fog-2(q71)* male-female pairs to ascr#10 during mating increased brood size. The violin plots show median (white dot) and 25th to 75th percentile (black bar).

(E) Model summarizing the circuit required for response to ascr#10 and its effects on exploration and reproduction.

*$p < 0.05$; **$p < 0.01$.

See Figure S4 for additional related results and Data S1 for primary data and details of statistical analyses.

which AIY and RIF signal downstream to modify behavior and germline physiology are not currently known but would be interesting to address in the future.

It is possible that reduced exploration in the presence of ascr#10 leads to increased number of GPCs because it diverts resources from movement to the germline. To test this hypothesis, we examined the germlines of mutants with severely impaired locomotion. We focused on mutants that had muscle-related defects and avoided those that broadly impaired nervous system functions (e.g., synaptic transmission). All tested mutants displayed at least some egg laying activity. The relevance of egg laying for pheromone response is addressed in detail in the accompanying paper (see the accompanying paper by Aprison and Ruvinsky in this issue of *Current Biology*) [30]. In all examined cases, both the baseline numbers of GPCs and the response to ascr#10 were indistinguishable from those in wild-type animals (Figures 3G and S3C; compare to Figure 1B), indicating that even the near lack of movement does not necessarily lead to alterations in the germline. Instead, the germline effects of ascr#10 likely reflect a persistent internal state imposed by the balance of the serotonin-PDF antagonism.

ascr#10 Improves Mating Efficiency and Promotes Reproductive Success

In search of a behavior affected by reduced exploration upon ascr#10 exposure, we considered mating because copulation in several *C. elegans* strains is notoriously inefficient in part due to hermaphrodites escaping from males [31]. We found that ascr#10 promoted faster initiation of copulation with vigorously moving (Figure 4A), but not immobile (Figure 4B) hermaphrodites, demonstrating that the effect was due to hermaphrodite response to pheromone, not improved male ability to locate mates. Male pheromone improved several other aspects of reproduction—a greater fraction of mating pairs produced offspring (Figure 4C) and successfully mated hermaphrodites produced larger broods (Figure 4D) and propagated faster in the presence of ascr#10 (Figure S4). These results underscore the importance of the male pheromone for reproductive fitness.

DISCUSSION

In a variety of species, sex pheromones influence reproductive traits, including mating-related behaviors and physiological responses that rely on changes in gene expression [1]. The male-enriched pheromone in *C. elegans*, ascr#10, modifies aspects of germline physiology in hermaphrodites, including improved sperm guidance [12] and increased number of GPCs in adults [13]. Here, we also demonstrated that, in the presence of the male pheromone ascr#10, hermaphrodites engage in less exploratory behavior. Reduced exploration can occur in response to other ascarosides [32] and likely reflects increased valuation of local resources relative to costs of leaving in search of a better food source elsewhere [33]. We found that reduced exploratory movement, reminiscent of the ability of ascr#10 to "retain" hermaphrodites [10], decreases the time required to initiate mating and contributes to increased reproductive success in this and possibly other ways. We draw four substantial conclusions.

First, our results show that, although behavioral and physiological effects of pheromones unfold on different timescales

(~1–10 min [6] versus ~10^3–10^4 min [13], respectively), they are coupled, at least in part, by the reliance on the same neuronal circuit. Alteration of multiple processes is a hallmark of neuromodulators like serotonin [34], and we found that serotonergic signaling from NSM and HSN neurons acts via *mod-1* receptor in AIY and RIF neurons to modulate internal states that manifest as reduced environmental exploration on shorter timescales and changes in the germline over longer periods of time.

Second, the serotonergic signals from NSM and HSN neurons, both of which are required, form an "AND" logic gate. The likely purpose of this circuit architecture is to integrate inputs that may be particularly salient for fine-tuning internal states. Given the role of NSM neurons in sensing food ingestion [35], they likely report on food availability, an important variable because exploratory behavior is determined by foraging strategies [36]. The peculiar involvement in exploratory behavior of HSN neurons that control egg laying has been noted previously [6]. Our results demonstrate that these neurons, in addition to serving as command motor neurons of egg laying [22], also mediate hermaphrodite's behavioral and germline responses to male pheromone. In the accompanying paper [30], we describe the role of HSN neurons in connecting the active reproductive status to the ability of hermaphrodites to respond to ascr#10.

Third, the serotonin/PDF circuit described here plays a major role in maintaining a balance along an axis that could be loosely termed "exploration versus reproduction" because exploratory and reproductive performances appear to be mutually antagonistic (Figure 4E). The circuit plays this role even in the absence of external pheromones, but exposure to ascr#10 leads to additional serotonergic signaling, further reducing exploration and promoting several reproductive traits, including mating behavior and germline physiology. We hypothesize that there exist additional exploration and reproduction-related traits and possibly other traits regulated in a coordinated manner by the same circuit. A plausible function of such centralized circuit would be to manage the various trade-offs inherent in optimizing reproductive versus exploratory performance, the latter involving foraging and thus possibly related to somatic maintenance. "A tangle" of trade-offs surrounds reproduction and somatic maintenance [37], including in humans [38], and the serotonin/PDF circuit discussed here could play an important role in regulating behavior and physiology in ways appropriate to organisms' internal state and external conditions.

Finally, the role of serotonin in modulating locomotor behavior has been documented in *C. elegans* [6], *Drosophila* [39], and mouse [40], implying conservation since the origin of Metazoa. The role of PDF appears to be similarly conserved, because related molecules promote activity in *C. elegans* [6, 41] as well as in insects and mammals [42]. These two modulators may comprise the core of the neuronal circuit that coordinates behavioral and physiological aspects of reproduction in animals. Of all *C. elegans* serotonergic neurons, HSNs have a gene expression profile that most resembles that of mouse raphe serotonergic neurons [43]. This discovery lends support to the tantalizing possibility that HSN and raphe neurons as well as their targets retain a signature of deep homology [44] inherited from a circuit that coupled reproductive behavior and physiology in an ancient metazoan ancestor that roamed and dwelt as far back as the Cambrian.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Quantification of fluorescence
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  - Architecture of the serotonergic circuit required for response to ascr#10
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- DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.10.012.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.Z.A. and I.R.; Methodology, E.Z.A. and I.R.; Validation, E.Z.A. and I.R.; Formal Analysis, E.Z.A. and I.R.; Investigation, E.Z.A.; Writing – Original Draft, E.Z.A. and I.R.; Writing – Review & Editing, E.Z.A. and I.R.; Funding Acquisition, I.R.; Supervision, I.R.

DECLARATION OF INTERESTS

Some of the data reported here were used in a patent application 62/842,072.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
ascr#10	Laboratory of F. Schroeder	SMID ID:ascr#10; CAS: 1355681-08-1
Levamisole solution	Vector Labs	Cat#SP-5000
Vectashield mounting medium with DAPI	Vector Labs	Cat#H-1200
Experimental Models: Organisms/Strains		
C. elegans wild-type N2	Caenorhabditis Genetics Center	WB Strain N2
MT15434 *tph-1(mg280)* II	Caenorhabditis Genetics Center	WB Cat# MT15434, RRID:WB-STRAIN: MT15434
MT14984 *tph-1(n4622)* II	Caenorhabditis Genetics Center	WB Cat# MT14984, RRID:WB-STRAIN: MT14984
CX13571 *tph-1(mg280)* II*; kySi56 IV; kyEx4077[srh-142::nCre]* " = *tph-1(lf)* in ADF"	Laboratory of C. Bargmann	N/A
CX13572 *tph-1(mg280)* II*; kySi56 IV; kyEx4057[ceh-2::nCre]* " = *tph-1(lf)* in NSM"	Laboratory of C. Bargmann	N/A
CX13574 *tph-1(mg280)* II*; kySi56 IV; kyEx4081[ops-1::nCre]* " = *tph-1(lf)* in ASG"	Laboratory of C. Bargmann	N/A
CX13576 *tph-1(mg280)* II*; kySi56 IV; kyEx4107[egl-6::nCre]* " = *tph-1(lf)* in HSN"	Laboratory of C. Bargmann	N/A
CX15658 *tph-1(mg280)* II*; kySi56 IV; kyEx5262[ceh-2p::nCre, egl-6p::nCre]* " = *tph-1(lf)* in NSM and HSN"	Laboratory of C. Bargmann	N/A
CX13228 *tph-1(mg280)* II*; kySi56 IV = mosSCI (ChrIV) of loxP-tph-1genomic-loxP* " = control"	Laboratory of C. Bargmann	N/A
OH12495 *otIs517[tph-1(fosmid)::SL2::YFP::H2B + ttx-3::mCherry]*	Caenorhabditis Genetics Center	WB Cat# OH12495, RRID:WB-STRAIN: OH12495
GR1366 *mgIs42[tph-1::GFP + rol-6(su1006)]*	Caenorhabditis Genetics Center	WB Cat# GR1366, RRID:WB-STRAIN:GR1366
YR155 *Is[ric-19::GFP]*	This laboratory	N/A
MT9668 *mod-1(ok103)* V	Caenorhabditis Genetics Center	WB Cat# MT9668, RRID:WB-STRAIN:MT9668
MT9667 *mod-1(nr2043)* V	Laboratory of M. Koelle	N/A
DA1814 *ser-1(ok345)* X	Caenorhabditis Genetics Center	WB Cat# DA1814, RRID:WB-STRAIN:DA1814
AQ866 *ser-4(ok512)* III	Caenorhabditis Genetics Center	WB Cat# AQ866, RRID:WB-STRAIN:AQ866
RB2277 *ser-5(ok3087)* I	Caenorhabditis Genetics Center	WB Cat# RB2277, RRID:WB-STRAIN:RB2277
ser-5(tm2654) I	National BioResources Project	N/A
DA2100 *ser-7(tm1325)* X	Caenorhabditis Genetics Center	WB Cat# DA2100, RRID:WB-STRAIN:DA2100
MT14678 *lgc-40(n4545)* X	Caenorhabditis Genetics Center	WB Cat# MT14678, RRID:WB-STRAIN: MT14678
CX12905 *mod-1(ok103)* V*; kyEx3655[mod-1::mod-1::GFP; myo-3::mCherry]* " = *mod-1* rescue under endogenous promoter"	Laboratory of C. Bargmann	N/A
CX13106 *mod-1(ok103)* V*; kyEx3773[ttx-3::mod-1::GFP; myo-3::mCherry]* " = *mod-1* rescue in AIY"	Laboratory of C. Bargmann	N/A
CX13327 *mod-1(ok103)* V*; kyEx3924[exp-1::mod-1::GFP; myo-3::mCherry]* " = *mod-1* rescue in RID"	Laboratory of C. Bargmann	N/A
CX13482 *mod-1(ok103)* V*; kyEx 4052[unc-25::mod-1::GFP; myo-3::mCherry]* " = *mod-1* rescue in RME, DD1-6"	Laboratory of C. Bargmann	N/A
CX13500 *mod-1(ok103)* V*; kyEx4064[str-3::mod-1::GFP; myo-3::mCherry]* " = *mod-1* rescue in ASI"	Laboratory of C. Bargmann	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
CX14049 *mod-1(ok103)* V; *kyEx4352[odr-2b::inv(mod-1-sl2-GFP)]* " – crossed to CX13878 to produce *mod-1* rescue in RIF"	Laboratory of C. Bargmann	N/A
CX13878 *mod-1(ok103)* V; *kyEx4269[mod-1::nCre; myo-2::mCherry]* " – crossed to CX14049 to produce *mod-1* rescue in RIF"	Laboratory of C. Bargmann	N/A
CX14295 *pdfr-1(ok3425)* III	Caenorhabditis Genetics Center	WB Cat# CX14295, RRID:WB-STRAIN: CX14295
CX14391 *mod-1(ok103) V; pdfr-1(ok3425)* III	Laboratory of C. Bargmann	N/A
CB540 *unc-68(e540)* V	Caenorhabditis Genetics Center	WB Cat# CB540, RRID:WB-STRAIN:CB540
CB187 *rol-6(e187)* II	Caenorhabditis Genetics Center	WB Cat# CB187, RRID:WB-STRAIN:CB187
DR518 rol-6(su1006); unc-4(e120) II	Caenorhabditis Genetics Center	WB Cat#DR518,RRID:WB-STRAIN:DR518
CB4108 *fog-2(q71)* V	Caenorhabditis Genetics Center	WB Cat# CB4108, RRID:WB-STRAIN: CB4108
MT2060 *egl-1(n987)* V	Caenorhabditis Genetics Center	WB Cat# MT2060, RRID:WB-STRAIN: MT2060
OH161 *ttx-3(ot22)* X	Caenorhabditis Genetics Center	WB Cat# OH161, RRID:WB-STRAIN:OH161
FK134 *ttx-2(ks5)* X	Caenorhabditis Genetics Center	WB Cat# FK134, RRID:WB-STRAIN:FK134
BC347 *unc-54(s74)* I	Caenorhabditis Genetics Center	WB Cat# BC347, RRID:WB-STRAIN:BC347
HE33 *unc-95(su33)* I	Caenorhabditis Genetics Center	WB Cat# HE33, RRID:WB-STRAIN:HE33
Software and Algorithms		
ImageJ	NIH Image	https://rsbweb.nih.gov/ij/
R	The R Project for Statistical Computing	https://www.r-project.org

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Ilya Ruvinsky (ilya.ruvinsky@northwestern.edu). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The strains CX13571, CX13572, CX13574, CX13576, CX15658, CX13228, CX14391, CX12905, CX13106, CX13327, CX13482, CX13500, CX14049, and CX13878 were gifts from C. Bargmann and MT9667 from M. Koelle. *ser-5(tm2654)* was produced by the National BioResources Project. To generate YR155, ~1 kb upstream of *ric-19* was inserted (as a translational fusion incorporating the first several codons of *ric-19*) upstream of GFP in the FireLab vector PD95.75. The resulting plasmid was injected (at 50 ng/uL), together with a *pha-1* rescue construct (pBX at 2ng/uL) into *pha-1(e2123)*, as described in [45]. Rescued transgenic offspring were UV irradiated. Survivors that segregated the fluorescent marker in a Mendelian fashion were outcrossed to the N2 strain. This construct directs pan-neuronal expression allowing for easy detection of transgenic animals. The remaining strains were obtained from the Caenorhabditis Genetics Center. Strains were maintained at 20°C on OP50 under standard nematode growth conditions [46]. Synchronized populations of larvae of all strains were prepared by hypochlorite treatment of gravid hermaphrodites [47]. The liberated eggs were allowed to hatch overnight in M9 buffer. The following morning arrested L1 larvae were deposited onto lawn plates of OP50 at a density of 30-60 larvae per plate. 48 hours after release from larval arrest was designated as Day 1 of adulthood based on our previous experience staging N2 hermaphrodites [48, 49]. Some strains were slightly delayed (2-3 hours) in their development and timing of experiments was adjusted to account for this delay. On Day 1 of adulthood, hermaphrodites were transferred to either control plates or ascaroside treatment plates and housed in small populations of 30 worms per plate. Adult hermaphrodites were transferred every other day to fresh plates.

METHOD DETAILS

Conditioning plates with ascarosides

Synthetic ascaroside ascr#10 was provided by F. C. Schroeder (Cornell University). Concentrated solutions of ascarosides in ethanol were kept at −20°C. These stocks were diluted further with water and a total of 100 μL (for 60mm plates) or 50 μL (for 35mm plates) of ascaroside solution (for a total of 2.2 or 1.1 femtograms per plate, respectively) was applied to the surface of the agar and distributed

evenly with a glass rod. The plates were incubated at 20°C overnight to allow the ascaroside to absorb into the agar. The following day, the plates were seeded with a 20 μL spot (for experiments counting GPCs) or 50 μL evenly spread (for behavioral experiments) of 1:10 dilution of an overnight culture of OP50 and were incubated at 20°C for 24 hours. Control plates without ascarosides were prepared in the same manner, except 100 μL (or 50 μL) of water contained no synthetic ascaroside.

Staining and counting germline precursor cells

Adult hermaphrodites were aged as above in small populations of 30 per plate and stained with DAPI (4′,6-diamidino-2-phenylindole) as described previously [13] using a variation of the protocol by Pepper et al. [50]. Briefly, following washes in M9 and fixation with 95% ethanol, Day 5 adults were stained with Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA) and mounted on 2% agarose pads for visualization. We counted the number of nuclei in the proliferative zone, as defined by Crittenden et al. [14]. In all animals, GPCs in only one of the two gonad arms were counted. In three cases, GPCs were counted earlier than on Day 5 (~150 hours) – in *egl-1(n987)* and *unc-95(su33)* – counted at 96 hours and in *ser-5(tm2654),* counted at 120 hours due to increased internal hatching.

Cohabitation experiments

We wished to rule out a possibility that the primary cause of the lack of response to ascr#10 in some strains is production of aberrant pheromones. We performed "cohabitation" experiments, in which individuals from a mutant (*tph-1(mg280)* in Figure 1C or *pdfr-1(ok3425)* in Figure S3B) and a marked wild-type strain (YR155 *Is[ric-19::GFP]*) were housed together. 15 hermaphrodites of each strain (thirty total) were transferred to either control plates or plates conditioned with ascr#10. On Day 5 of adulthood, worms were separated using the GFP marker to identify the wild-type strain and the number of GPCs was counted.

Quantification of fluorescence

30 young adult hermaphrodites were transferred to either ascr#10 plates or control plates for 6 hours. After 6 hours of exposure, the hermaphrodites were transferred to 2% agarose pads and anesthetized with 12.5mM levamisole (Vector Laboratories, Burlingame, CA) for imaging. An exposure time was selected to ensure that the pixel intensity of the fluorescent signal was in the linear range. Images were acquired on a Leica DM5000B microscope using a Retiga 2000R camera and the corrected total cell fluorescence was measured in ImageJ (NIH). The average of these values is presented in arbitrary units. OH12495 *otIs517[tph-1(fosmid)::SL2::YFP::H2B + ttx-3::mCherry]* is shown in Figures 1E and 1F. Only OH12495 hermaphrodites that began egg-laying during their exposure to ascr#10 were included in Figures 1E and 1F. GR1366 *mgIs42[tph-1::GFP + rol-6(su1006)]* shown in Figure S1E include both worms that laid and those that did not during their exposure to ascr#10.

Embryo staging and number of embryos retained in the uterus

Twenty adult hermaphrodites (72 hours post release from L1 arrest) were allowed to lay eggs for one hour. At the end of the hour, the adults were transferred to another plate and dissolved in sodium hypochlorite solution. We counted fertilized eggs that remained after the adult hermaphrodite bodies were dissolved. The plates containing the eggs were put on ice while slides were prepared of the eggs. Preparation of slides and embryo staging was performed as described in [49]. Chilled M9 buffer was used to rinse the embryos off the plate into 1.5mL microcentrifuge tubes. The tubes were spun at 4°C for 1 minute at 0.5 x *g* and the excess supernatant was removed. The remaining volume (~10 μL) was deposited onto chilled slides prepared with agarose pads. Slides were kept on ice until they were examined by microscopy and the embryos were staged.

Exploration assays

The exploration behavior of hermaphrodites was measured using the assay described in Flavell et al. [6]. 35mm control or treatment plates were prepared with a uniformly seeded OP50 lawn. Day 1 adult hermaphrodites were singled to each plate and allowed to explore the plate for 10-16 hours at 20°C, except for *pdfr-1(ok3425)* hermaphrodites that were allowed to explore for 24 hours. After the exploratory period, the hermaphrodite was removed and the plate was laid over a grid of 86 squares. The number of squares entered by the worm tracks was counted. Each experiment was run in parallel with its matched control.

Mating initiation assay

The ability of male/hermaphrodite pairs to initiate mating was determined using an assay described in Fagan et al. [51]. ascr#10-conditioned and control 35mm plates were prepared as above. The evening before the assays were performed, small populations of N2 males at the L4 larval stage were segregated onto separate plates. Similarly, small populations of young adult hermaphrodites were kept on separate plates. The ascr#10 and control plates were seeded with a 20 μL drop of a 1:10 dilution of OP50 overnight culture. This amount yielded a bacterial spot ~7.5mm in diameter. To start the assay, a single adult hermaphrodite (72-78 hours post release from L1 arrest) was placed in the center of the bacterial spot and a single male was placed on the outer edge of the spot. In the few instances (10%–15%) when the hermaphrodite left the center of the bacterial spot before the male was placed on the plate, the male was positioned at a distance of ~1 radius of the bacterial spot away from the hermaphrodite. Plates were checked every 5 minutes for signs of mating initiation – the placement of the ventral side of the male tail against the hermaphrodite [52]. For every initiated mating, we noted the time since the start of the experiment and removed the plate from further consideration. Plates were monitored for up to 30 minutes.

Mated *fog-2* brood sizes and eating races

One hundred virgin CB4108 *fog-2(q71)* females were isolated from a synchronized population at the late L4 stage, approximately 44 hours post release from L1 arrest. These worms were housed on control plates in populations of 30 animals. After 12 hours on these plates, the virgin females were singled onto plates prepared in the same way as the mate finding assay above. A single *fog-2* female was positioned in the center of the 20 μL spot of OP50 and a *fog-2* male was added to the edge of the bacterial spot. Mating attempts were allowed to progress for 1 hour after which the male was removed. The mated *fog-2* females were transferred to fresh plates daily and progeny was counted. 34 of the 50 females singled onto control plates produced progeny. 41 of the 50 females singled onto ascr#10 plates produced progeny. This difference in mating success is significant ($p = 0.03$). Animals that exhibited internal hatching were censored from the counts (1 control worm, 3 ascr#10 worms). The protocol for eating races was modified from Hodgkin and Barnes [53] and began as in the *fog-2* brood size assay. Instead of counting progeny, plates were monitored every 12 hours for the consumption of the bacteria. Worms "finished" the race when all bacteria were consumed. 36 of the 50 females singled onto control plates successfully mated. 46 of the 50 females singled onto ascr#10 plates successfully mated. This difference in mating success is significant ($p = 7.8 \times 10^{-4}$). Females that did not succeed in mating produced no progeny and were therefore excluded from the analysis, thus the data presented in Figures 4D and S4 reflect only the offspring of productive matings.

Architecture of the serotonergic circuit required for response to ascr#10

The study that characterized the circuit responsible for generating roaming and dwelling states in *C. elegans*, identified three pairs of *mod-1*-expressing neurons as acting downstream of NSM and HSN neurons [6]. In that study, in addition to AIYs and RIFs, which we also found using the same strains as in the original report [6], ASI neurons were implicated because their ablation led to reduced exploration. However, restoring MOD-1 function in ASIs was not sufficient to rescue the *mod-1* defects we described in the present study – *mod-1(ok103); pASI::mod-1* animals were unable to respond to ascr#10 either behaviorally (Figure S3A) or in the germline (Figure 2B). For this reason, we favor a hypothesis that AIYs and RIFs are the primary neurons that rely on *mod-1* to regulate ascr#10 responses. We note that loss of ASI neurons abolishes the germline response to ascr#10 [11]. One possible reason for this requirement is the function of a TGF-beta-like ligand, DAF-7 [54], that is essential for the germline response to ascr#10 [11, 12].

QUANTIFICATION AND STATISTICAL ANALYSIS

All experiments were compared against matched controls that were processed in parallel (Data S1). In the majority of instances, we used the Kolmogorov-Smirnov test to assess statistical significance of differences between treated and untreated animals (e.g., experiments like those shown in Figures 1B and 1E). This is a non-parametric alternative for comparing two samples that does not rely on the assumption of normal distribution of the underlying data. In several instances, our data did not appear to be normally distributed, justifying the choice. Analyses of data shown in Figures 4A, 4B, and S4 relied on a log rank test, in Figure 1G we used χ^2 test. The experiments that scored GPCs showed relatively low levels of variation, even though some of them were carried out months apart. Control values from 87 experiments (excluding those that were counted at times other than Day 5) were examined in Figure 3E. We compared 48 N2 wild-type control values from previous experiments, the control values from three experiments using *pdfr-1(lf)*, the control values from three experiments using the *mod-1(lf);pdfr-1(lf)* double mutant, and 36 control values from all the constructs and mutants (excluding *pdfr-1(lf)*) used in this study. The combined control values for all the experiments (except *pdfr-1(lf)*) had the average of 61.1 and the standard deviation of 1.09. $3\sigma \approx 5.4\%$ of the mean, demonstrating that the number of GPCs retained by Day 5 hermaphrodites is consistent even among different strains. Because only ~0.15% of values are expected to be three standard deviations higher than average by chance, we concluded that the observed values for *pdfr-1(lf)* were significantly higher than expected (Figure 3E). Numbers of worms, trials, p values, and statistical tests applied are specified in Data S1.

DATA AND CODE AVAILABILITY

This study did not generate/analyze datasets.

Current Biology, Volume *29*

Supplemental Information

Coordinated Behavioral and Physiological Responses to a Social Signal Are Regulated by a Shared Neuronal Circuit

Erin Z. Aprison and Ilya Ruvinsky

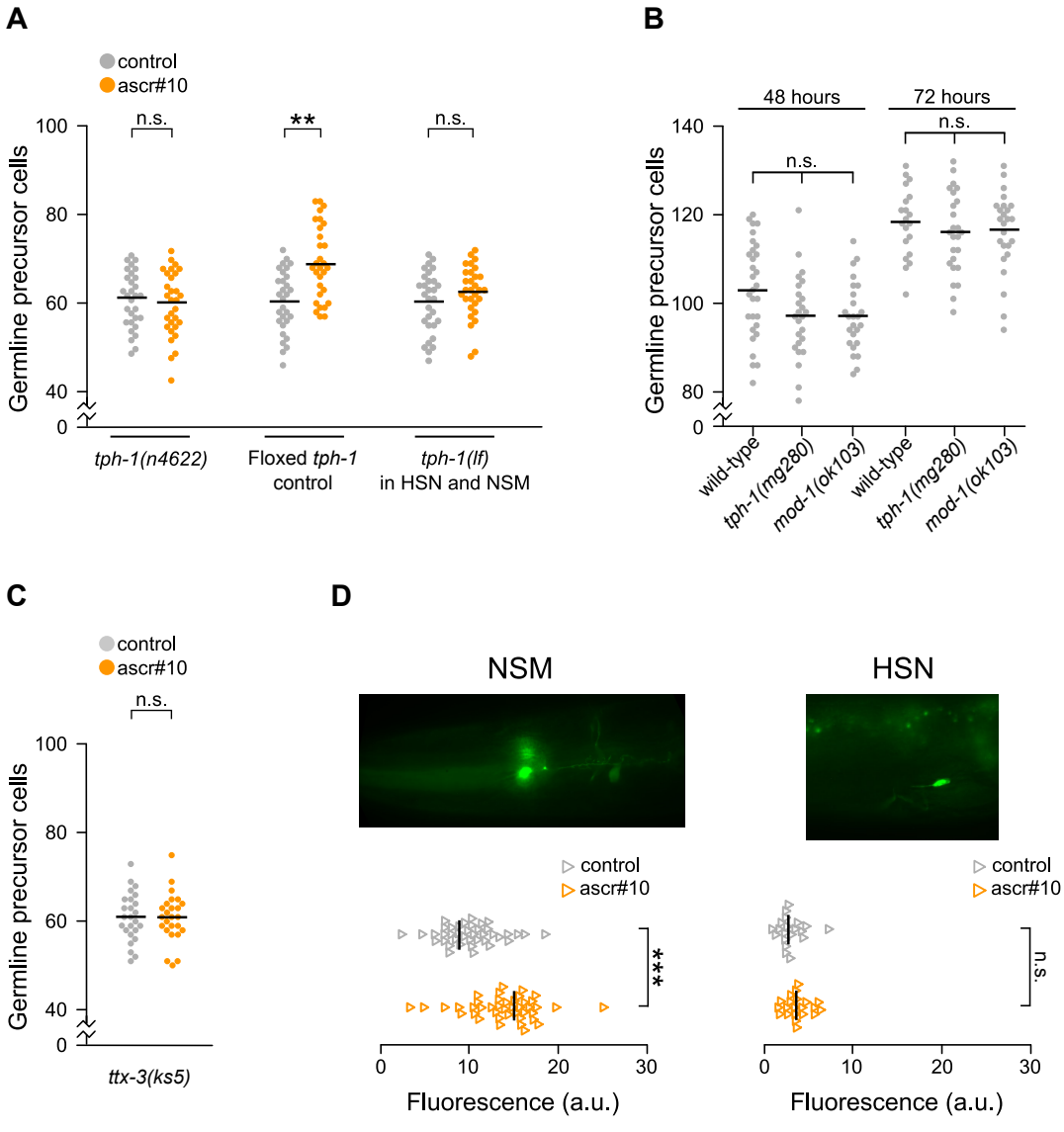
A
control
ascr#10
Germline precursor cells
100
80
60
40
0
n.s.
**
n.s.
tph-1(n4622)
Floxed tph-1
control
tph-1(lf)
in HSN and NSM
B
48 hours
72 hours
n.s.
n.s.
140
120
100
80
0
Germline precursor cells
wild-type
tph-1(mg280)
mod-1(ok103)
wild-type
tph-1(mg280)
mod-1(ok103)
C
control
ascr#10
n.s.
100
80
60
40
0
Germline precursor cells
ttx-3(ks5)
D
NSM
HSN
control
ascr#10
***
n.s.
0
10
20
30
Fluorescence (a.u.)

Figure S1. Serotonergic signaling from NSM and HSN neurons is required for the hermaphrodite germline response to ascr#10. Related to Figure 1.

(A) Germline precursor cells in a second *tph-1(lf)* allele, a strain (CX13228) carrying a single integrated copy of floxed *tph-1* locus and *tph-1(mg280)*, and a strain with *tph-1(lf)* in both HSN and NSM neurons (CX15658). **(B)** Germline precursor cells in wild-type, *tph-1(mg280)*, and *mod-1(ok103)* hermaphrodites at 48 and 72 hours post release from L1 arrest. **(C)** Germline precursor cells in a second *ttx-3(lf)* allele. **(D)** Expression of *tph-1::GFP* (GR1366) in NSM and HSN neurons. In this experiment, *tph-1::GFP* expression was measured at 54 hours post release from L1 arrest. Anterior is to the left and ventral is down. In **A**, **B, C** each dot represents one animal, in **D** each triangle represents one imaged neuron. Black bars denote the mean. ** $p<0.01$, *** $p<0.001$. See Data S1 for primary data and details of statistical analyses.

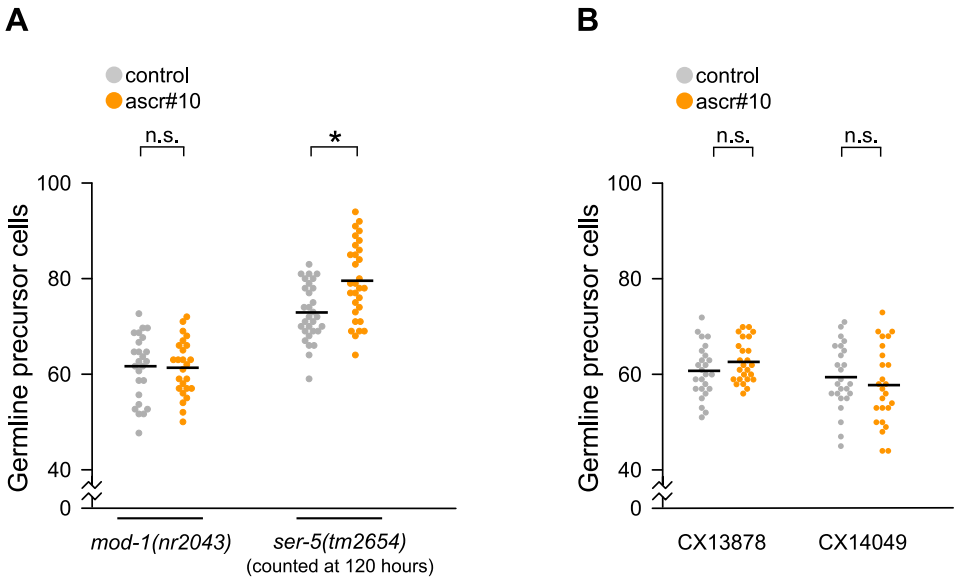
A
control
ascr#10
n.s.
*
Germline precursor cells
100
80
60
40
0
mod-1(nr2043)
ser-5(tm2654)
(counted at 120 hours)
B
control
ascr#10
n.s.
n.s.
Germline precursor cells
100
80
60
40
0
CX13878
CX14049

Figure S2. The activity of a serotonin receptor *mod-1* is required for hermaphrodite germline response to ascr#10. Related to Figure 2.

(A) Germline response to ascr#10 in strains carrying additional alleles of serotonin receptor genes. *ser-5(tm2654)* was counted at 120 hours (not ~150 hours as other strains) because of increased internal hatching. **(B)** Germline precursor cells in two parental strains (CX13878, CX14049) used to test whether expression of MOD-1 in RIF neurons could rescue the defect in *mod-1(ok103)*. To direct expression of MOD-1 in RIF (Figure 2B), two strains – CX14049 (*mod-1(ok103) V; kyEx4352[odr-2b::inv(mod-1-sl2-GFP)]*) and CX13878 (*mod-1(ok103) V; kyEx4269[mod-1::nCre; myo-2::mCherry]*) were crossed and the heterozygotes were tested. In **A, B** each dot represents one animal. Black bars denote the mean. * $p<0.05$. See Data S1 for primary data and details of statistical analyses.

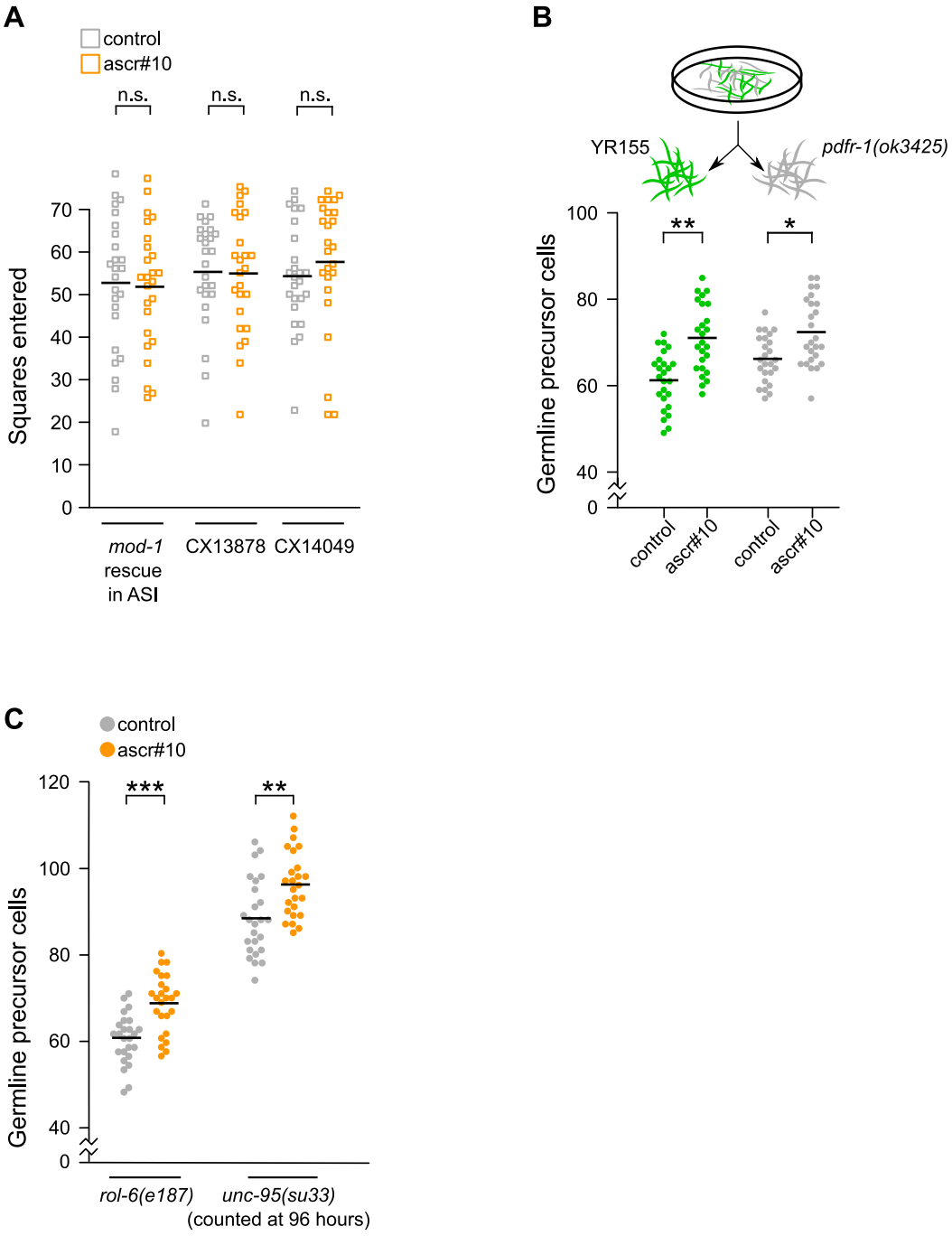
A
control
ascr#10
n.s.
n.s.
n.s.
Squares entered
70
60
50
40
30
20
10
0
mod-1
rescue
in ASI
CX13878
CX14049
B
YR155
pdfr-1(ok3425)
**
*
Germline precursor cells
100
80
60
40
0
control
ascr#10
control
ascr#10
C
control
ascr#10
***
**
Germline precursor cells
120
100
80
60
40
0
rol-6(e187)
unc-95(su33)
(counted at 96 hours)

Figure S3. The circuit that alters exploratory behavior in response to ascr#10. Related to Figure 3.

(A) Exploratory behavior of several strains used to test the effect of expression of MOD-1 in groups of neurons in *mod-1(lf)* mutants. Expression of MOD-1 in ASI neurons in *mod-1(ok103)* does not rescue the ability to reduce exploratory behavior in the presence of asc#10. Consistently, exposure of this strain to ascr#10 does not increase the number of GPCs (Figure 2B). Parental strains crossed to rescue MOD-1 function in RIF do not show rescue of exploratory behavior response to ascr#10. To rescue MOD-1 function in RIF, CX14049 *mod-1(ok103) V; kyEx4352[odr-2b::inv(mod-1-sl2-GFP)]* and CX13878 *mod-1(ok103) V; kyEx4269[mod-1::nCre; myo-2::mCherry]* were crossed and the heterozygotes were tested. **(B)** Germline precursor cells in *pdfr-1(lf)* hermaphrodites co-cultured with marked wild-type animals. **(C)** Germline precursor cells in additional strains with severely compromised locomotion. *unc-95(su33)* was counted at 96 hours because of increased internal hatching. Each square or dot represents one animal. Black bars denote the mean. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. See Data S1 for primary data and details of statistical analyses.

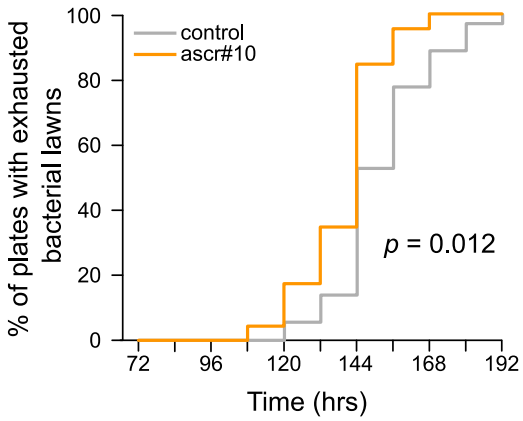
100
80
60
40
20
0
% of plates with exhausted bacterial lawns
control
ascr#10
p = 0.012
72
96
120
144
168
192
Time (hrs)

Figure S4. Faster propagation of *C. elegans* populations in the presence of ascr#10. Related to Figure 4.

Virgin *fog-2(q71)* females were mated for 1 hour with *fog-2* males and plates with successful matings were monitored every 12 hours until bacterial food was exhausted. See Data S1 for primary data and details of statistical analyses.