Report

Current Biology

Dynamic Regulation of Adult-Specific Functions of the Nervous System by Signaling from the Reproductive System

Highlights

- Male pheromone promotes reproductive behavior in *C. elegans* hermaphrodites
- Functional germline and ability to lay eggs are required for pheromone response
- Retrograde signal from the vulva continuously affirms ongoing reproduction
- Circuit ensures that only reproducing individuals behave in adult-specific manner

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In Brief

Aprison and Ruvinsky demonstrate that a retrograde signal from the vulva is required to permit adult-specific responses of *C. elegans* hermaphrodites to a male pheromone. The underlying circuit continuously apprises the nervous system regarding egg laying to match reproductive output with an appropriate behavioral repertoire.

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Report

Dynamic Regulation of Adult-Specific Functions of the Nervous System by Signaling from the Reproductive System

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SUMMARY

Unlike juveniles, adult animals engage in suites of behaviors related to the search for and selection of potential mates and mating, including appropriate responses to sex pheromones. As in other species [1], male sex pheromones modulate several behaviors and physiological processes in *C. elegans* hermaphrodites [2–5]. In particular, one of these small-molecule signals, an ascaroside ascr#10, causes reduced exploration, more avid mating, and improved reproductive performance (see the accompanying paper by Aprison and Ruvinsky in this issue of *Current Biology*) [6]. Here, we investigated the mechanism that restricts pheromone response to adult hermaphrodites. Unexpectedly, we found that attainment of developmental adulthood was not alone sufficient for the behavioral response to the pheromone. To modify exploratory behavior in response to male pheromone, adult hermaphrodites also require functional germline and egg-laying apparatus. We show that this dependence of behavior on the reproductive system is due to feedback from the vulva muscles that reports ongoing reproduction to the nervous system. Our results reveal an activity-dependent conduit by which the reproductive system continuously licenses adult behaviors, including appropriate responses to the pheromones of the opposite sex. More broadly, our results suggest that signals from peripheral organs may serve as an important component of assuring age-appropriate functions of the nervous system.

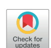
RESULTS AND DISCUSSION

Behavioral Response to Male Pheromone Is Restricted to Adult Hermaphrodites with a Functional Reproductive System

An ascaroside ascr#10 is a major component of the male sex pheromone blend in *C. elegans* [7]. Previous studies demonstrated that exposure to ascr#10 elicits several physiological responses in the germline of *C. elegans* hermaphrodites [2–4]. This molecule also alters hermaphrodite behavior—it substantially reduces exploratory movement and promotes behaviors that contribute to greater reproductive success, including faster engagement in copulation. A circuit that consists of serotonergic NSM and HSN neurons signaling via the *mod-1* receptor and of an opposing pigment dispersing factor (PDF) signaling is required to mediate the effects of asc#10 on both physiology and exploratory behavior [6].

In contrast to adult hermaphrodites, in which these pheromone responses were originally described, late larval (L4) hermaphrodites do not decrease exploratory movement in the presence of ascr#10 (Figure 1A). They also do not show decreased latency to engage in mating (Figure 1B), do not show increased expression of the serotonin-producing enzyme *tph-1* in NSM and HSN neurons (Figures 1C and 1D), and do not show the characteristic physiological response in the germline (Figure S1). A parsimonious interpretation of these results is that only sexually mature adult hermaphrodites are competent to respond to the ascr#10 pheromone.

Perhaps the most notable difference between late larvae and adults is the presence in the latter of a mature and functional reproductive system, including the germline. Suggestively, one of the two pairs of serotonergic neurons that are required for hermaphrodite response to ascr#10, HSNs, are located in the mid-body next to the vulva and are thought to be motor neurons that control egg laying [8]; their role in regulating exploratory behavior is somewhat puzzling [9]. We hypothesized that one possible role of HSNs may be to report on an aspect of the reproductive state that is relevant for exploratory behavior. To test this idea, we examined behavioral response to ascr#10 in animals with compromised reproduction. Four categories of self-sterile adult hermaphrodites did not reduce exploration in the presence of ascr#10—(1) mutants lacking germline (partially penetrant allele of *mes-1*) [10]; (2) mutants with severely reduced numbers of germline cells (conditional allele of *glp-1*) [11]; (3) mutants that produced only one type of gamete, either oocytes (*fog-2*) [12] or sperm (*fem-3* gain of function) [13], and thus no self-offspring; and (4) animals chemically sterilized with FUdR (Figures 2A and S2A). These germline defects, however, are unlikely to directly cause the inability to respond to ascr#10, because self-fertile but vulvaless [14] animals also did not reduce exploration in the presence of the pheromone (Figure 2B), whereas strains with even minimal egg laying showed behavioral response (Figures 2C and S2B). We therefore hypothesized that active egg laying was required to permit decreased exploration in the presence of ascr#10.



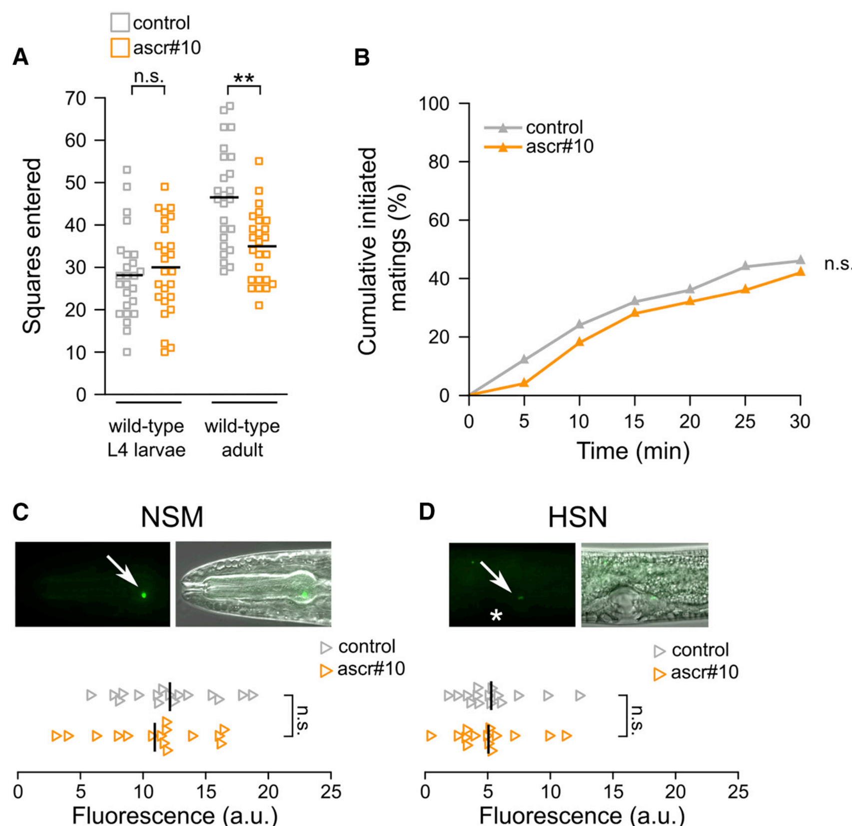


Figure 1. Larvae Do Not Respond to ascr#10

(A) Exploratory behavior of sexually immature L4 larval hermaphrodites compared to young adults.

(B) Latency of mating initiation with sexually immature L4 larval hermaphrodites.

(C and D) Expression of *tph-1::YFP* in (C) NSM and (D) HSN neurons in L4 larval hermaphrodites. Fluorescence and fluorescence overlaid with differential contrast images are shown. Arrows point to cell bodies. An asterisk marks the position of the developing vulva. Anterior is to the left, and ventral is down.

In (A), each square represents one animal; in (C) and (D), each triangle represents one imaged neuron. **$p < 0.01$. See Figure S1 for additional related results and Data S1 for primary data and details of statistical analyses.

The Role of Vulva Muscles in Reporting the Egg Laying State

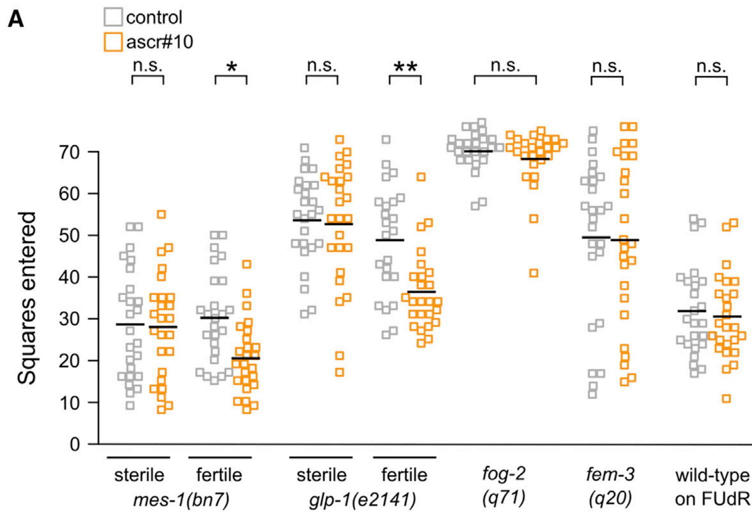
HSN neurons stimulate egg laying by exciting the vulva muscles [15]. Supporting the role of HSN neurons in behavioral response to ascr#10, we found that *egl-1(dm)* mutants in which HSNs undergo inappropriate cell death [16] did not reduce exploration in the presence of ascr#10 (Figure 2D). Animals in which HSNs were chemogenetically silenced using histamine-gated chloride channel (*HisCl*) [17] under control of an appropriate tissue-restricted promoter [18] explored more (Figure 2E), even though egg laying was not blocked completely (Figure S2C). This finding supports the idea that signaling from HSN neurons normally inhibits exploratory movement [6].

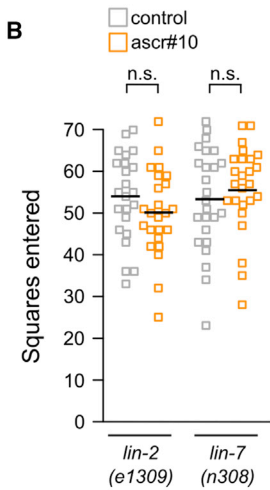
To further investigate the role of vulva muscle cells in behavioral response to ascr#10, we examined mutants—*egl-2*, *egl-19*, and *unc-103*—that alter muscle excitability. In all examined cases, we found lack of behavioral (Figures 3A, 3B, and S3A) and germline (Figures S3B and S3C) responses to acr#10. However, these genes are broadly expressed [19–21] and therefore likely have complex pleiotropic effects on behavior and physiology. For this reason, we focused on vm2 vulval muscles because they receive direct synaptic input from HSN neurons [22] and because there exists a mutant—*lin-12*(*wy750*)—in which outgrowth of postsynaptic vm2 muscle arms is disrupted [23], effectively severing HSN-vm2 synaptic communication. These animals showed neither behavioral (Figure 3C) nor germline (Figure S3D) response to ascr#10, although they continued to lay eggs. These results demonstrated that the act of egg laying per se is not alone sufficient to permit responses to ascr#10, which additionally require sensing the passage of embryos and communication between components of the egg-laying machinery.

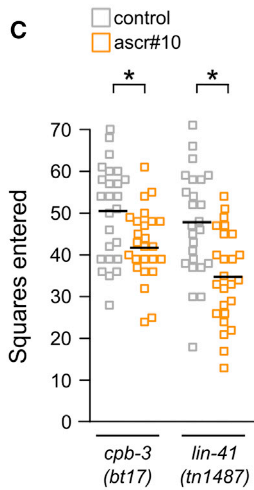
Because in *C. elegans* Notch signaling is known to be involved in multiple aspects of development and function of the nervous system [24–27], it is possible that *lin-12*(*wy750*) has defects beyond HSN-vm2 communication. For this reason, we also tested whether chemogenetic silencing of vm2 muscles [18] alters response to ascr#10. We found that hermaphrodites with silenced vm2s did not lay eggs (Figure S3E), did not reduce exploratory behavior in the presence of ascr#10 (Figure 3D), and did not respond in the germline (Figure S3F). Notably, silencing of vm2 muscles had an effect on the upregulation of *tph-1* expression in response to ascr#10. Unlike in hermaphrodites with functional vulva muscles that upregulated *tph-1* expression in both NSM and HSN neurons, animals with silenced vm2s showed increased *tph-1* expression only in NSM, but not in HSN neurons (Figures 3E, 3F, S3G, and S3H). We concluded that functional vm2 muscles synaptically connected to HSN neurons are required for appropriate ascr#10 response. We expect future work to elucidate which aspect of vm2 function needs to be communicated to HSNs, as well as the specific signal(s) that mediate this communication, to allow adult-like hermaphrodite behavior in the presence of ascr#10.

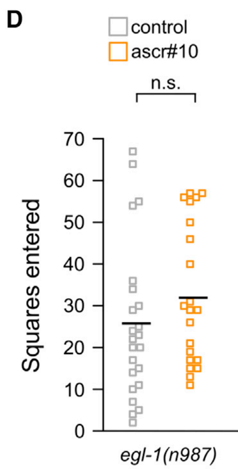
In the experiments shown in Figures 3D–3F, vm2 muscles were continuously silenced since before the age when the first eggs were supposed to be laid. It therefore remained formally possible that hermaphrodites could display appropriate reduction of exploratory behavior as long as they initiated egg laying. To test this hypothesis, we silenced vm2 muscles after the onset of egg laying and found that this treatment acutely eliminated the ability of hermaphrodites to alter exploratory behavior on ascr#10 (Figure 4A). This result demonstrated that continuous input from the reproductive system is required to permit adult-like behavior.

There appears to be a qualitative difference between the states of the nervous system before and after the onset of egg









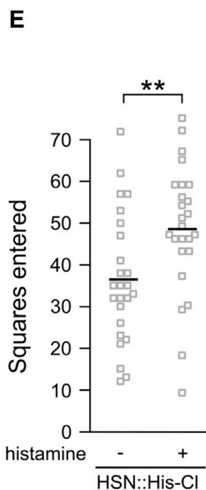


Figure 2. Functional Egg-Laying Apparatus Regulates Behavioral Response to ascr#10

(A) Exploratory behavior of hermaphrodites with compromised reproduction.

(B) Exploratory behavior of fertile but vulvaless animals.

(C) Exploratory behavior of fertile animals that have dramatically reduced brood sizes (~3 in *cpb-3* and ~2 in *lin-41* versus ~13 in N2).

(D) Exploratory behavior in mutant hermaphrodites lacking HSN neurons.

(E) Exploratory behavior in adult hermaphrodites in which HSN has been chemogenetically silenced. Each square represents one animal.

*$p < 0.05$; **$p < 0.01$. See Figure S2 for additional related results and Data S1 for primary data and details of statistical analyses.

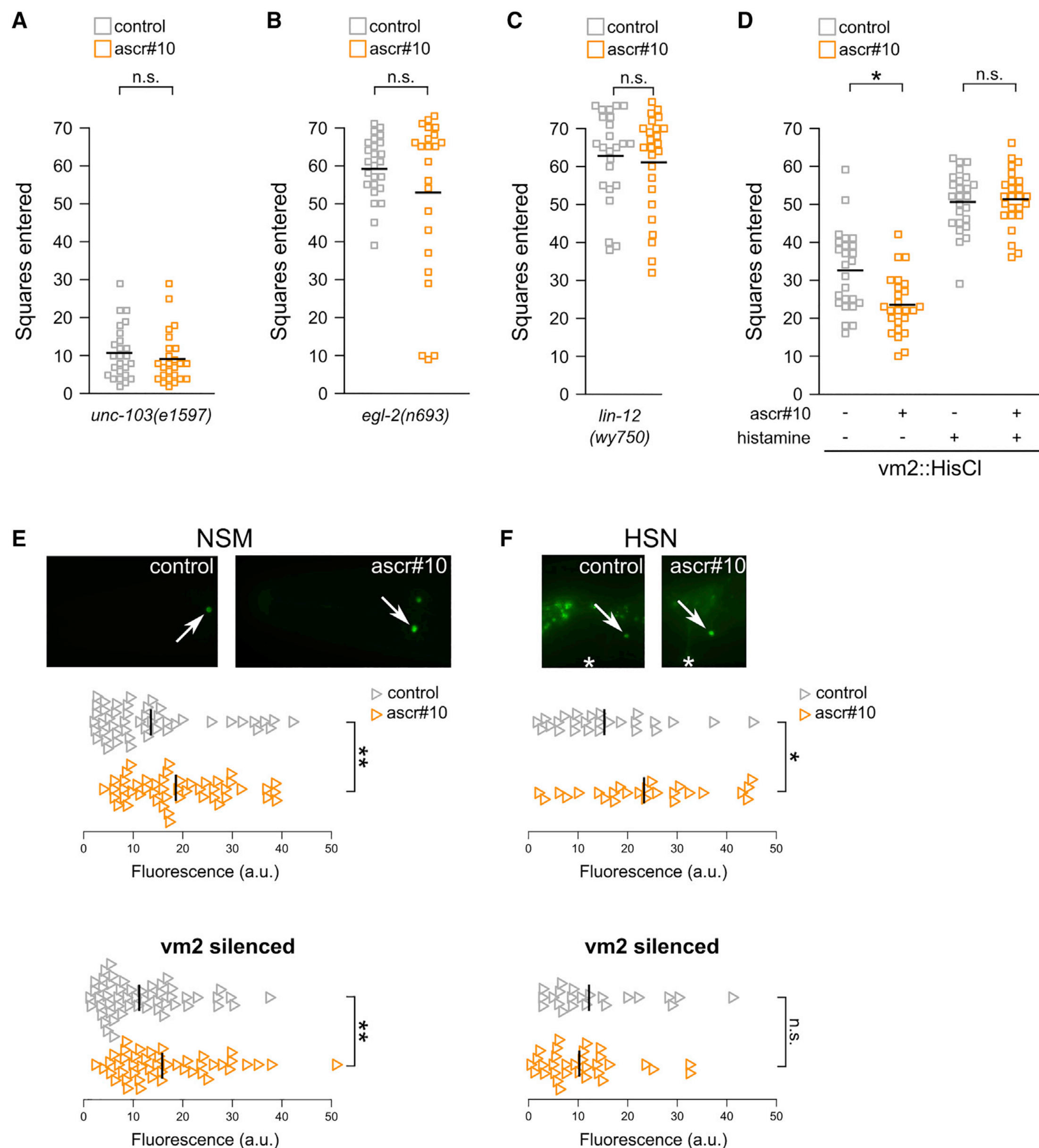


Figure 3. The Role of vm2 Vulva Muscles in Regulating Behavioral Response to ascr#10

(A and B) Exploratory behavior in (A) *unc-103* and (B) *egl-2* mutant hermaphrodites that have defects in muscle excitability.

(C) Exploratory behavior in a mutant hermaphrodite in which vm2 vulva muscles do not synapse with HSN neurons.

(D) Exploratory behavior in hermaphrodites with functional or silenced vm2 vulva muscles. In this experiment, silencing commenced prior to initiation of egg laying. Comparison of the two gray data series demonstrates that silencing of vm2 muscles increases exploratory movement.

(E and F) Expression of *tph-1::YFP* in (E) NSM and (F) HSN neurons in adults with functional or silenced vm2 vulva muscles. Arrows mark cell bodies. Asterisks mark the location of the vulva. Anterior is to the left, and ventral is down.

In (A)–(D), each square represents one animal. In (E) and (F), each triangle represents one neuron. *$p < 0.05$; **$p < 0.01$. See Figure S3 for additional related results and Data S1 for primary data and details of statistical analyses.

laying. For example, exposure to ascr#10 upregulates *tph-1* expression only in animals that have initiated reproduction, but not in adult animals of very similar age that are yet to lay their first egg (Figure 4B). In the absence of externally applied male pheromone, hermaphrodites also appear to display an inverse relationship between egg laying and exploratory movement (see below).

An Activity-Dependent Mechanism that Licenses Adult-like Functions of the Nervous System

Adult animals display behavioral repertoires that facilitate reproduction. These most notably include mating-related performance but also foraging and food preferences [29]. Here, we presented evidence that only adult *C. elegans* hermaphrodites respond to male sex pheromone ascr#10 by altering exploratory

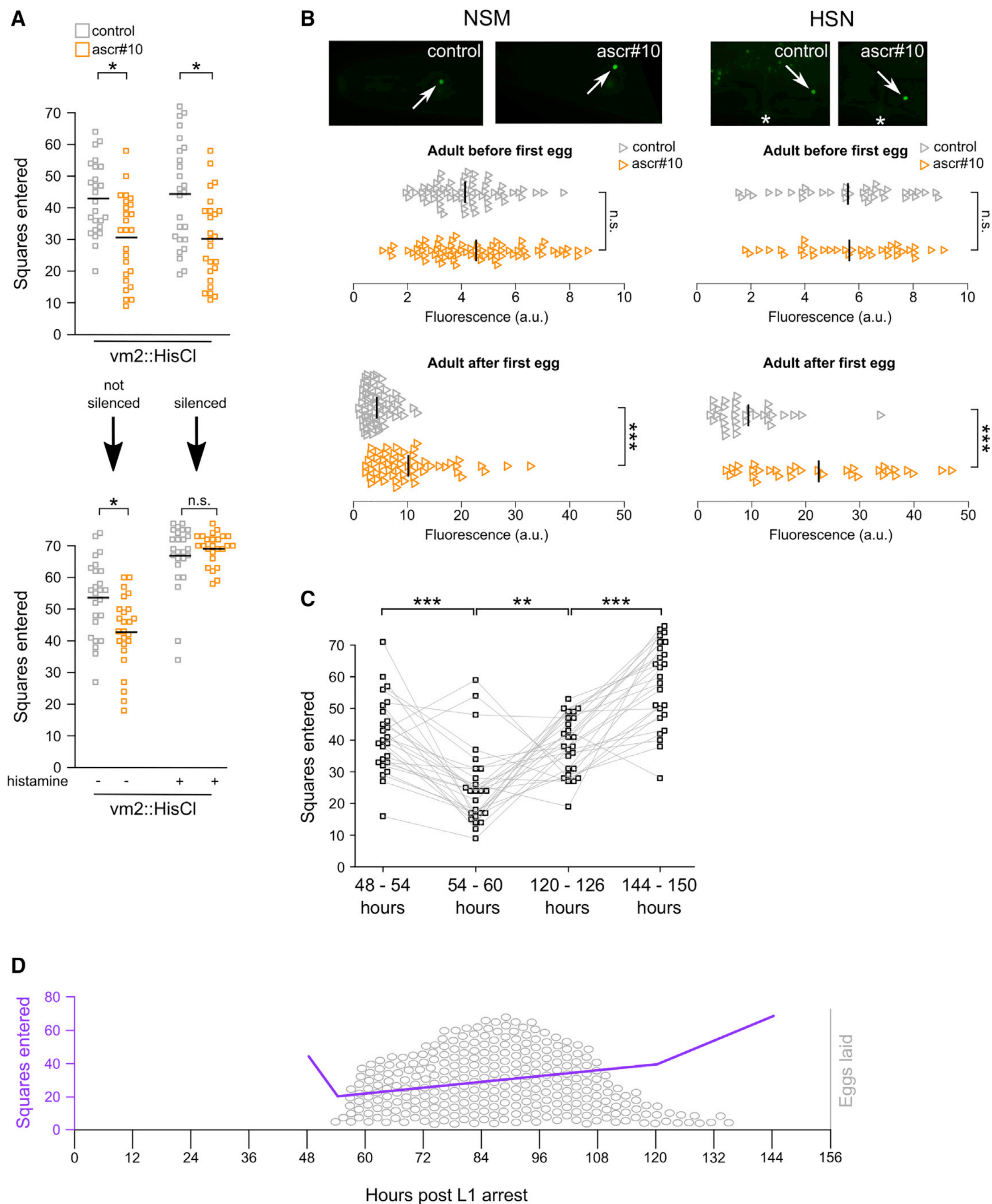


Figure 4. The Role of Active Reproductive State in Regulating Exploratory Behavior

(A) Exploratory behavior in hermaphrodites before and after chemogenetic silencing of vm2 vulva muscles. Note: top of this panel shows exploratory behavior in hermaphrodites after they have laid their first egg. The experiment in top portion of the panel was carried out on animals that were between 48 and 60 h after release from L1 arrest. In the bottom, the same animals between 60 and 72 h are shown. Comparing gray data series between animals before and after vm2 silencing (right portion of the panel) demonstrates that silencing of vm2 increases exploratory movement.

(B) Expression of *tph-1::YFP* in NSM and HSN neurons in adults exposed to ascr#10 before they have laid their first egg (48–54 h after release from L1 arrest) and after they have laid their first egg (54–60 h after release from L1 arrest). Arrows point to the cell body. Asterisks mark the location of the vulva. Anterior is to the left, and ventral is down.

(C) Exploratory behavior of individual hermaphrodites during four episodes (~6 h each) that encompass key events across the reproductive span. First and second episodes were carried out immediately before and immediately after the onset of egg laying, respectively. In the third time window, hermaphrodites had few remaining self-sperm. In the fourth, they exhausted supplies of self-sperm. The same animals were tested across these four time windows—gray traces connect exploratory movements of the same animal. Age is expressed as hours after release from L1 arrest.

(legend continued on next page)

behavior and aspects of germline physiology, whereas larvae and pre-reproductive adults do not. In the companion paper [6], we showed that hermaphrodite responses to ascr#10 facilitate reproductive success.

Progression of sexual maturation in the nervous system constitutes a developmental program that is regulated by several genes in the heterochronic pathway [30, 31]. Our findings reveal an additional layer of regulation because simply reaching adulthood is not sufficient for hermaphrodites to respond to male sex pheromone. At least three categories of adults are not capable of altering exploratory behavior in the presence of ascr#10—sterile individuals, those fertile but unable to lay eggs (due to obstruction of the vulva), or egg-laying adults with defects in HSN neurons or connectivity between HSN and vm2 muscles. We propose that, in all three of these cases, the underlying cause of behavioral defects is the lack of signal from vm2 via HSN to other neurons (including AIY and RIF; see [6]) that egg laying, this most adult of behaviors, is taking place.

The idea of a feedback between components of the egg-laying machinery has been proposed previously [32]. HSN neurons serve as command motor neurons of egg laying by stimulating vm2 muscles [8], whereas vm2 muscles provide retrograde signal that modulates HSN activity [18]. Our results support this notion of a feedback because chemogenetic silencing of vm2 muscles prevented upregulation of *tph-1* expression in HSN, but not NSM, neurons (Figures 3E and 3F). HSN neurons are born in the embryo, and their axonal outgrowth and synaptogenesis occur during larval development [33], culminating in the acquisition of adult-specific activity patterns coincident with sexual maturity [18]. These facts are sufficient to explain the inability of juveniles and pre-reproductive adults to respond to ascr#10. Following the onset of adulthood, hermaphrodites that possess enough germline to produce offspring, unobstructed passage through the vulva, and functional HSN neurons and vm2 muscles initiate egg-laying cycles. In addition to stimulating egg laying, HSN and vm2 also continuously certify ongoing reproduction, thus licensing age-appropriate reproductive behaviors and physiology, each of which may have additional regulatory inputs. This model explains the previously puzzling [9] involvement of HSN neurons in exploratory behavior and stresses that signals from the reproductive system are continuously required to license adult-specific behaviors.

On the Physiological Relevance of Coordinating Reproductive and Exploratory Behaviors

Our results offer two lines of evidence suggesting an antagonistic relationship between reproduction and exploratory behavior. First, silencing of vm2 muscles in hermaphrodites, either before or after the onset of reproduction, resulted in cessation of egg laying and a concomitant increase in exploratory movement (Figures 3D and 4A). Second, given the relationship we established between egg laying and adult-specific behavioral response to ascr#10, it may be expected that hermaphrodites would alter ways in which they explore their habitat, depending on their reproductive status, even in the absence of external pheromones. To test this idea, we compared the extent of exploratory movement (over periods of ~6 h) by singled hermaphrodites just before versus just after the onset of egg laying. We found a notable decrease in movement in reproductively active individuals (Figure 4C). Consistent with the inverse relationship between reproduction and movement, animals at the end of the self-fertile period moved more than at the beginning of the reproductive phase and even more once self-sperm were exhausted (Figure 4C). We interpret these results as an indication of alternative states—one optimized for reproductive performance, the other focused on habitat exploration (Figure 4D). Organizing behavior in alternative states may be an efficient way of resolving the trade-off between somewhat mutually exclusive requirements on organismal performance imposed by reproduction and exploration [6].

A relationship between egg laying and locomotion in *C. elegans* has been noted previously. Hardaker et al. [34] have demonstrated that hermaphrodites tend to transiently increase (1) movement velocity in the immediate (<30 s) anticipation of egg laying episodes and (2) propensity for changing movement direction immediately (<60 s) following egg laying [34]. Both of these locomotory behaviors associated with reproduction require HSN neurons and serotonergic signaling [34], as do the responses we described here. It remains to be determined whether changes in movement seen on ~10-s timescales (described by Hardaker et al.) [34] are alone sufficient to account for the dramatically decreased exploration seen during the several days of the reproductive period (Figure 4D). In either case, the circuit described in Aprison and Ruvinsky [6] together with vm2 muscle cells appears to play an important role in shaping internal states that probabilistically control and ultimately coordinate locomotion and egg laying.

Finally, several elements of the circuit that regulates exploratory behavior are conserved among deeply diverged animal lineages [9, 35–38]. There are similarities between HSN neurons in *C. elegans* and raphe serotonergic neurons in mammals [39]. It is thus tempting to speculate that at least some aspects of the mechanism we described here may act in other species to provide activity-dependent input from the reproductive system in the regulation of adult-specific patterns of exploratory or other behaviors.

STAR★METHODS

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

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- LEAD CONTACT AND MATERIALS AVAILABILITY
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Conditioning plates with ascarosides
  - Exploration assays

(D) A model showing exploratory behavior superimposed on the egg laying schedule. Purple trace represents average performance from (C); egg laying data (represented as gray ovals) are from [28].

In (A) and (C), each square represents one animal. In (B), each triangle is one neuron. *p < 0.05; **p < 0.01; ***p < 0.001. See Figure S4 for additional related results and Data S1 for primary data and details of statistical analyses.

- Mating initiation assay
- Quantification of fluorescence
- Silencing HSN neurons and vulva muscles
- Staining and counting germline precursor cells
- Experiments involving FUdR
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - All experiments were compared against matched controls that were processed in parallel (Data S1)
- DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.10.011.

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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
5′Fluoro-2′-deoxyuridine	Sigma-Aldrich	Cat#F0503 CAS: 50-91-9
Experimental Models: Organisms/Strains		
C. elegans wild-type N2	Caenorhabditis Genetics Center	WB Strain N2
SS149 *mes-1(bn7)* X	Caenorhabditis Genetics Center	WB Cat# SS149, RRID:WB-STRAIN:SS149
CB4037 *glp-1(e2141)* III	Caenorhabditis Genetics Center	WB Cat# CB4037, RRID:WB-STRAIN:CB4037
CB4108 *fog-2(q71)* V	Caenorhabditis Genetics Center	WB Cat# CB4108, RRID:WB-STRAIN:CB4108
JK816 *fem-3(q20)* IV	Caenorhabditis Genetics Center	WB Cat# JK816, RRID:WB-STRAIN:JK816
JK1973 *fem-3(q96)* IV	Caenorhabditis Genetics Center	WB Cat# JK1973, RRID:WB-STRAIN:JK197
CB1309 *lin-2(e1309)* X	Caenorhabditis Genetics Center	WB Cat# CB1309, RRID:WB-STRAIN:CB1309
MT308 *lin-7(n308)* II	Caenorhabditis Genetics Center	WB Cat# MT308, RRID:WB-STRAIN:MT308
YM19 *cpb-3(bt17)* I	Caenorhabditis Genetics Center	WB Cat# YM19, RRID:WB-STRAIN:YM19
DG3784 *lin-41(tn1487)* I	Caenorhabditis Genetics Center	WB Cat# DG3784, RRID:WB-STRAIN:DG3784
DR730 *dpy-13(e184); ama-1(m118m238)* IV	Caenorhabditis Genetics Center	WB Cat# DR730, RRID:WB-STRAIN:DR730
MIA194 *lin-12(wy750)* III	Laboratory of K. Collins	N/A
MIA71 *keyIs19[ceh-24::HisCl::unc-54 3′ UTR lin-15(+)]; lite-1(ce314)lin-15B & lin-15A(n765)* X " = chemogenetic silencing of vm2 muscles"	Laboratory of K. Collins	N/A
MIA116 *keyIs21[egl-6p::HisCl::unc54 3′UTR + egl-6p::mCherry::unc-54 3′UTR + lin-15(+)]; lite-1(ce314) lin-15B & lin-15A(n765)* X " = chemogenetic silencing of HSNs"	Laboratory of K. Collins	N/A
MT2060 *egl-1(n987)* V	Caenorhabditis Genetics Center	WB Cat# MT2060, RRID:WB-STRAIN:MT2060
CB1597 *unc-103(e1597)* III	Caenorhabditis Genetics Center	WB Cat# CB1597, RRID:WB-STRAIN:CB1597
MT1444 *egl-2(n693)* V	Caenorhabditis Genetics Center	WB Cat# MT1444, RRID:WB-STRAIN:MT1444
MT1212 *egl-19(n582)* IV	Caenorhabditis Genetics Center	WB Cat# MT1212, RRID:WB-STRAIN:MT1212
DA695 *egl-19(ad695)* IV	Caenorhabditis Genetics Center	WB Cat# DA695, RRID:WB-STRAIN:DA695
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 MIA71, MIA116, and MIA194 were a gift from K. Collins (University of Miami). The remaining strains were obtained from the Caenorhabditis Genetics Center. Most strains were maintained at 20°C on OP50 under standard nematode growth conditions [40]. The following strains were maintained at 15°C: SS149 *mes-1(bn7),* CB4037 *glp-1(e2141),* JK816 *fem-3(q20),* JK1973 *fem-3(q96),* YM19 *cpb-3(bt17)*, DG3784 *lin-41(tn1487),* DR730 *dpy-13(e184); ama-1(m118m238).* Synchronized populations of larvae of all strains were prepared by hypochlorite treatment of gravid hermaphrodites [41]. 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 [42, 43]. Some strains were slightly delayed (2-3 hours) in their development and timing of experiments was adjusted to account for this delay. Strain DR730 *dpy-13(e184); ama-1(m118m238)* was delayed 24 hours. In addition, one strain, MIA194 *lin-12(wy750)*, developed normally but was delayed in the onset of egg-laying. This strain was singled to control or ascr#10 plates at 48 hours and transferred to fresh plates for the exploratory behavior experiment 12 hours later. 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.

Exploration assays

The exploration behavior of hermaphrodites was measured using the assay described in Flavell et al. [9]. 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 6-16 hours at 20°C. 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. *mes-1(bn7)* is a partially-penetrant allele that results in ~50% of animals lacking the germline at 20°C. The number of GPCs in the gonads of unaffected fertile *mes-1* hermaphrodites are similar to those of N2. *glp-1(e2141)* hermaphrodites were shifted from 15°C to the non-permissive temperature (25°C) at the end of the L1 larval stage, which prevented the normal proliferation of germline cells. YM19, DG3784, and DR730 had dramatically reduced brood sizes – they produced ~2-3 offspring during the ~12 hours of exploratory behavior assays, compared to > 13 in the wild-type N2 strain. In the experiment displayed in Figure 4C, exploratory movement was measured in a cohort of hermaphrodites at 48-54 h (immediately before the onset of reproduction), at 54-60 h (immediately after the onset of reproduction), at 120-126 h (when most animals had very few remaining self-sperm), and at 144-150 h (when hermaphrodites exhausted self-sperm and were thus self-sterile). In all cases, reproductive status of each individual was verified by visual inspection. Age represents hours post release from L1 arrest. Animals were moved to fresh plates at the onset of each experimental episode, allowing us to trace exploratory performance of individual animals across the entirety of their reproductive span.

Mating initiation assay

The ability of male/hermaphrodite pairs to initiate mating was determined using an assay described in Fagan et al. [44]. 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 L4 hermaphrodite (~41 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. Plates were checked every 5 minutes for signs of mating initiation – the placement of the ventral side of the male tail against the hermaphrodite [45]. 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.

Quantification of fluorescence

Thirty L4 (~38 hours post release from L1 arrest in Figures 1C and 1D) or young adult hermaphrodites (Figures 3E, 3F, and 4B) 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 1 and 4, and a hybrid between OH12495 and MIA71 in Figure 3.

Silencing HSN neurons and vulva muscles

To reversibly silence HSN neurons and vm2 muscles, we used a transgenic strain expressing histamine-gated chloride channel (*HisCl*) [17] under control of an appropriate tissue-restricted promoter, as reported previously [18]. Experiments were carried out as previously described [17]. Histamine was added for a final concentration of 10mM to the NGM plates when they were poured.

The strain MIA116 is developmentally delayed by about 24 hours. Transgenic worms were placed on either control or histamine-containing plates at 48 hours post release from L1 arrest (that is, prior to onset of egg-laying) and transferred to fresh control or histamine-containing plates for exploratory behavior testing from (71 to 80 hours post release from L1 arrest) or at ~60 hours (that is, soon after the onset of egg-laying).

Staining and counting germline precursor cells

Hermaphrodites were aged as above in small populations of 30 per plate and stained with DAPI (4′,6-diamidino-2-phenylindole) as described previously [3] using a variation of the protocol by Pepper et al. [46]. 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. [47]. In addition to mitotic nuclei, this population contains some nuclei in the early stages of meiosis [48]. In all animals, GPCs in only one of the two gonad arms were counted. In four cases, GPCs were counted earlier than on Day 5 (~150 hours) – in *lin-12(wy750)* and *egl-2(n693)* at 120 hours, in *unc-103(e1597)* at 96 hours, and in vm2::HisCl with silenced vm2 muscles at 72 hours due to increased internal hatching. In the experiment shown in Figure S1, exposure to ascr#10 commenced at the L1 stage and continued through the end of the L4 stage; GPC were counted in these animals.

Experiments involving FUdR

35mm plates were prepared using NGM to which 5-fluoro-2′-deoxyuridine (Sigma) was added to yield a final concentration of 400 μM as in Mitchell et al. [49]. 25 of these plates were conditioned with ascr#10 as above and 25 were kept as control plates. An overnight culture of OP50 was concentrated 20X and applied to these plates as a lawn. At about 46 hours post release from L1 arrest, 60 hermaphrodites in the L4.8 stage of vulval development as described by Mok et al. [50] were transferred to four additional plates containing FUdR. They were monitored during molting and all appeared to molt normally. At the 48-hour stage, worms were singled to the prepared lawn plates for the exploratory behavior experiment. Reduced exploration on FUdR has been noted previously [49].

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 1A and 1C). 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 Figure 1B relied on a log rank test. To evaluate significance of changes in exploratory behavior, as shown in Figure 4C, we conservatively asked whether a given animal explored less (when comparing 48-54 h window to 54-60 h window) or more (for the other two comparisons) than in the immediately previous sampled episode. We used a binomial test on the resultant categorical data. Numbers of worms, trials, *p-value*s, and statistical tests applied are specified in Data S1.

DATA AND CODE AVAILABILITY

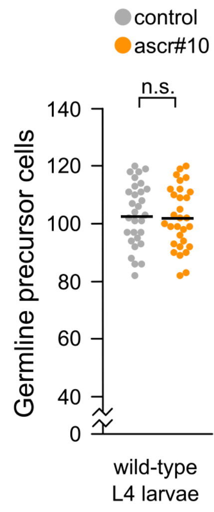
This study did not generate/analyze datasets.

Current Biology, Volume *29*

Supplemental Information

Dynamic Regulation of Adult-Specific Functions of the Nervous System by Signaling from the Reproductive System

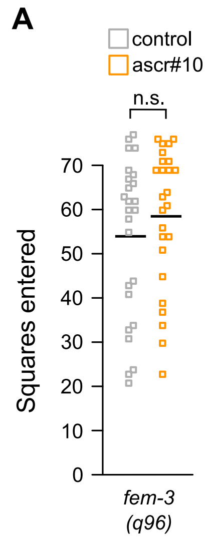
Erin Z. Aprison and Ilya Ruvinsky



control
ascr#10
n.s.
140
120
100
80
60
40
0
Germline precursor cells
wild-type
L4 larvae

Figure S1. Germlines of L4 larval hermaphrodites do not respond to ascr#10. Related to Figure 1.

Number of GPCs in L4 larval hermaphrodites on and off ascr#10. Each dot represents one animal. Black bars denote the mean. See Data S1 for primary data and details of statistical analyses.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n.s.
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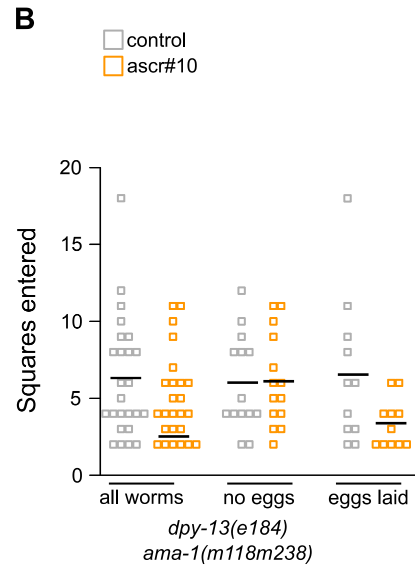
60
50
40
30
20
10
0
fem-3
(q96)


B
control
ascr#10
Squares entered
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15
10
5
0
all worms
no eggs
eggs laid
dpy-13(e184)
ama-1(m118m238)

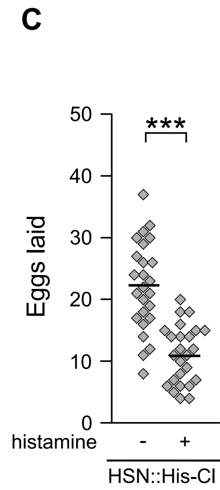
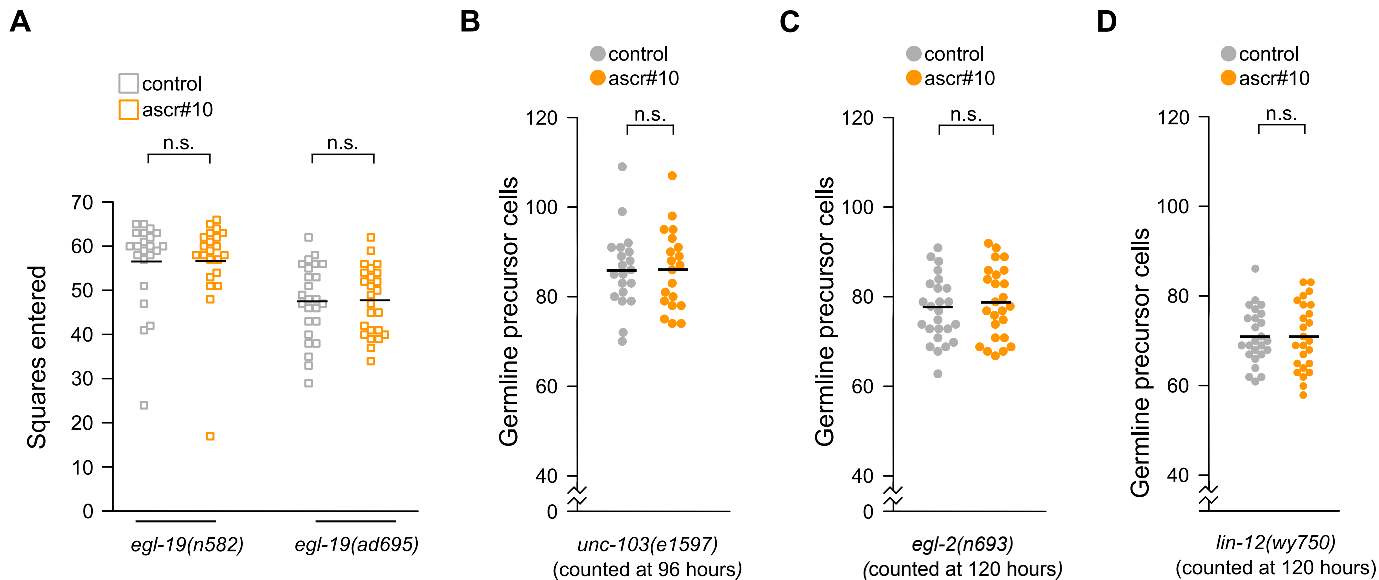

C
Eggs laid
50
40
30
20
10
0
***
histamine
-
+
HSN::His-Cl

Figure S2. Exploratory behavior in mutants with compromised reproduction. Related to Figure 2.

(A) Exploratory behavior of *fem-3(q96)*. This gain-of-function allele masculinizes the germline, resulting in exclusive sperm production. **(B)** Exploratory behavior of DR730 *dpy-13(e184); ama-1(m118m238)* hermaphrodites that have dramatically reduced brood size (<3 offspring in the course of this experiment vs. >13 in N2 animals). During the 12 hours of the experiment, only ~1/2 of these animals produced any offspring (in comparison, all N2 hermaphrodites of comparable age produce offspring). Separating DR730 hermaphrodites into those that did vs. those that did not have offspring, suggests that despite marginal mobility characterizing this strain, exposure to ascr#10 further reduced exploration in animals that have started to reproduce. **(C)** The number of eggs laid by hermaphrodites in which HSN has been silenced. These results are from the same animals in Figure 2E. In **A**, **B**, each square represents one animal. In **C**, each diamond represents the number of eggs laid by a single animal. Black bars denote the mean. *** $p<0.001$. See Data S1 for primary data and details of statistical analyses.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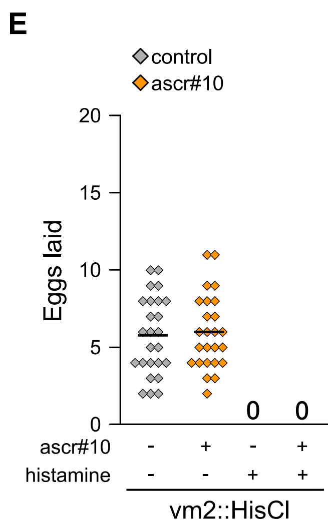
control
ascr#10
n.s.
n.s.
Squares entered
egl-19(n582)
egl-19(ad695)
B
control
ascr#10
n.s.
Germline precursor cells
unc-103(e1597)
(counted at 96 hours)
C
control
ascr#10
n.s.
Germline precursor cells
egl-2(n693)
(counted at 120 hours)
D
control
ascr#10
n.s.
Germline precursor cells
lin-12(wy750)
(counted at 120 hours)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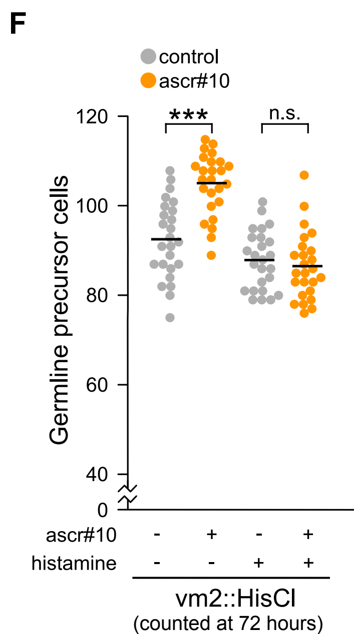
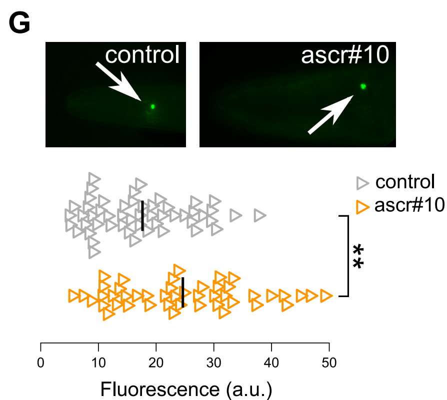
control
ascr#10
Eggs laid
0
0
ascr#10
histamine
vm2::HisCl


F
control
ascr#10
***
n.s.
Germline precursor cells
ascr#10
histamine
vm2::HisCl
(counted at 72 hours)


G
control
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control
ascr#10
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Fluorescence (a.u.)

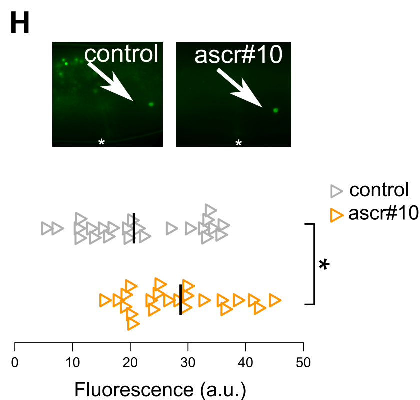
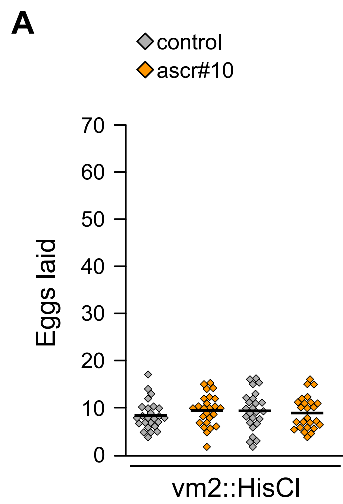

H
control
ascr#10
control
ascr#10
*
Fluorescence (a.u.)

Figure S3. Functional egg-laying apparatus regulates response to ascr#10. Related to Figure 3.

(A) Exploratory behavior in *egl-19(n582)* and *egl-19(ad695)* (egg-laying defective and egg-laying constitutive alleles, respectively). Neither strain responds to ascr#10. Number of GPCs in **(B)** *unc-103(e1597),* **(C)** *egl-2(n693),* and **(D)** *lin-12(wy750)*. These mutants were counted earlier because of increased egg retention and internal hatching. **(E)** Egg-laying in hermaphrodites in which vm2 vulval muscles were silenced soon after L4/adult molt, but prior to onset of egg-laying. These results are from the same animals in Figure 3D. **(F)** Number of GPCs in hermaphrodites with silenced vm2 vulval muscles on and off ascr#10. **(G)** Exposure to histamine does not alter expression of *tph-1::YFP* in response to ascr#10 in NSM or **(H)** HSN neurons in OH12495. Arrows point to the cell body. Anterior is to the left and ventral is down. Quantification of fluorescence is shown below micrographs. Each square (in **A**) and each dot (in **B**-**D** and **F**) represents one animal. In **E**, each diamond represents the number of eggs laid by one animal. In **G**, **H**, each triangle represents one neuron. 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.


A
control
ascr#10
Eggs laid
70
60
50
40
30
20
10
0
vm2::HisCl

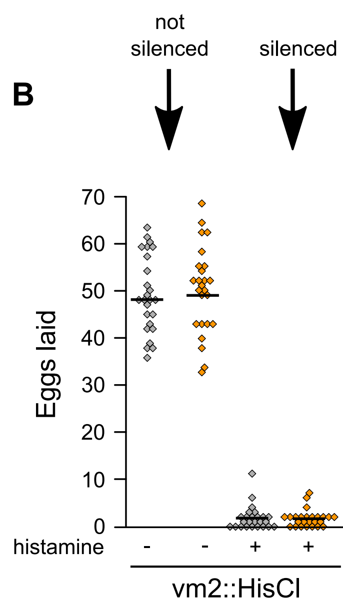

B
not
silenced
silenced
Eggs laid
70
60
50
40
30
20
10
0
histamine
-
-
+
+
vm2::HisCl

Figure S4. Consequences of chemogenetic silencing of vm2 vulva muscles. Related to Figure 4.

(A) Number of eggs laid by hermaphrodites with functional vm2 vulva muscles. **(B)** Number of eggs laid by the same hermaphrodites as in **A**, but with silenced vm2 vulva muscles. The experiment in **A** was carried out on animals that were between 48 and 60h after release from L1 arrest; in **B,** the same animals between 60 and 72h. Each diamond represents the number of eggs laid by one animal. Black bars denote the mean. See Data S1 for primary data and details of statistical analyses.