THE EMBO JOURNAL

Manuscript EMBO-2010-76220

Food sensitizes C. elegans avoidance behaviors through acute dopamine signaling

Marina Ezcurra, Yoshinori Tanizawa, Peter Swoboda and William R Schafer

Corresponding author: William Schafer, mrc-lmb

Review timeline:

Submission date:	11 October 2010
Editorial Decision:	17 November 2010
Revision received:	21 December 2010
Additional Correspondence:	07 January 2011
Accepted:	07 January 2011

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 17 November 2010

Thank you for submitting your manuscript to the EMBO Journal. Your study has now been seen by three referees and their comments are provided below.

As you can see the referees find the analysis interesting and suitable for publication here pending adequate revisions. Referees #2 and 3 raise relative minor concerns. However referee #1 raises a number of relevant issues that should be resolved and they are detailed below. Referee #1 also indicates that the analysis concerning NPR-1 is not developed well enough and that additional experiments are needed to strengthen this part. This referee also suggests that, as this part is not essential for the main conclusions that you could consider removing the data from the paper. I am in agreement with the referee, if you have data on hand or can generate data to strengthen this part then add to the manuscript if not then I would also suggest removing it. We can discuss this issue further if that is helpful. Given all the available input, I would like to ask you to submit a suitably revised manuscript for our consideration. When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

Review of Ezcurra et al. "Food sensitizes C. elegans ..."
An important goal of molecular neurobiology is to trace how environmental inputs alter the function of neuronal circuits, and consequently modify an animal's behavioral repertoire. In this study, the authors address this general question using C. elegans sensory responses to food as a model. The authors find that food sensitizes repellent responses and avoidance behaviors mediated by ASH neurons and that it does so through dopamine (DA) signaling. They show that food and exogenous DA enhance avoidance behavior and ASH calcium response to several repellents. The food effect depends upon the functional integrity of sensory cilia in DA neurons, and the expression of DOP-4 DA receptors in ASH. Acute activation of DA neurons (with ChR2), leads to a transient enhancement of repellent avoidance responses. Finally, they show that NPR-1 neuropeptide receptors also regulate ASH sensory responses and may act downstream of the DA pathway.
Several prior studies suggested that the presence of food is detected by dopaminergic neurons, and that DA functions as an "ON food" signal. The present study does a very nice job of connecting food exposure to activation of dopaminergic neurons to increased responsiveness to soluble repellents. In general, I liked the study and believe that it will be of interest to a broad audience. However, some of the experiments are not fully convincing and the manuscript could be improved by the addition of a few more experiments and revisions (as detailed below).

The analysis of how NPR-1 alters ASH responses is less well developed, and is not entirely consistent with prior studies on NPR-1. If the authors wish to retain the NPR-1 analysis, more experiments are required. Alternatively, as the NPR-1 results are not essential for any of the main conclusions, the authors may prefer to remove these results for inclusion in a more complete subsequent analysis.

Specific comments:

1. I am a bit worried that the effects of exogenous DA may not accurately reflect the physiological function of Food evoked release of endogenous dopamine. In particular, 10 mM DA prolongs ASH calcium responses to CuCl2. Several results suggest that this may not be a physiological effect of DA: 1) Food exposure does not prolong ASH calcium responses to CuCl2; 2) Mutants lacking DA (i.e. cat-2) do not have a correspondingly shortened calcium response; 3) The prolonged ASH calcium response was not blocked in Dop-4 mutants. Currently, the text assumes that this is a physiological effect of DA, and that it is mediated by an unidentified DA receptor. It seems equally likely that this is a non-physiological effect of 10 mM DA, which could be mediated non-specifically by another class of receptors. Without further data, I would advise that the authors revise the text to indicate this possibility.

2. The authors show that 10 mM exogenous DA rescues the cat-2 mutant defects in ASH calcium and sensory responses. To avoid the potential for non-specific effects of exogenous DA (detailed above), a better control might be to rescue with L-DOPA instead.

3. A central conclusion of the paper is that exposure to food depolarizes the DA neurons, evoking DA release. This should be tested more directly by using a calcium probe to detect food induced calcium transients in the DA neurons.

4. The ASH calcium trace in cat-2 mutants following 2 mM CuCl2 exposure seems longer on food. Is that the case? If so, how do the authors explain this? It would seem to suggest that food alters duration of calcium responses independently of DA.

5. The authors should include measures of response durations (and significance of any differences) for all calcium imaging experiments.

6. Is the prolonged ASH calcium trace evoked by 0.5 M glycerol on food (fig. 1c) blocked in dop-4 and cat-2 mutants?

7. Does photoactivation of DA neurons with (ChR2) alter ASH calcium responses to CuCl2?

8. Is the effect of photoactivation of DA neurons on locomotion (Fig. 5A) and ASH behaviors (B and C) eliminated in cat-2 and dop-4 mutants?

9. The authors argue that DA and 5HT don't generally alter ASH excitability because they do not alter the ASH calcium response to nose touch (fig. 2F and G). I would interpret these results more cautiously. Failure to observe a change in cameleon fluorescence following nose touch could arise for many technical reasons. Cameleon has intrinsically slow kinetics and could easily miss a transient response. Furthermore, the calcium transients detected in these experiments is in the ASH cell body, not in its axons. In fact, there is no proof that the somatic calcium detected in these experiments is relevant to the sensory evoked behavioral responses (see related comments #10 and 11). The authors should revise their discussion of these results to more accurately reflect these alternative explanations.

10. The authors should do a more careful analysis of the kinetics of ASH calcium responses and the corresponding behavioral responses (Figs. 3,7, and 8). This is an important issue because the slow somatic calcium transients recorded with the cameleon probe may not accurately reflect the endogenous calcium transients driving the ASH mediated sensory behaviors. In the current version of the manuscript, behavioral decline (during adaptation assays) is plotted over the course of 20 consecutive stimuli yet calcium traces are examined only after a chronic exposure to 10 mM CuCl2. Does the rate of adaptation of the ASH calcium responses match the decay in sensory behavioral responses?

11. Similarly, does the duration of ASH calcium responses to CuCl2 and glycerol quantitatively match the observed duration of sensory evoked backing?

12. The authors conclude that DOP-4 is not required for the slower rate of sensory adaptation on food (Fig. 3B). The data are not so clear. The change in adaptation on food is quantatively subtle (but apparently significant). The dop-4 mutant adaptation rate is intermediate between the cat-2 and wild type controls, and likely does not significantly differ from either. Based on these data, one cannot say much about whether DOP-4 is involved or not.

13. The authors contend that NPR-1 acts in ASH to regulate adaptation of calcium and sensory responses (Fig. 7). These results seem to conflict with a prior study from the Bargmann lab (Macosko, 2009), which showed that NPR-1 acts in RMG interneurons to regulate the sensitivity of a group of sensory neurons that form a gap junction network with RMG. To avoid confusing the literature, if the authors want to comment on NPR-1 function, they should address this issue. Does NPR-1 expression in RMG also restore the ASH sensory responses and behaviors (as Macosko showed for ASK responses)? Does inactivation of NPR-1 selectively in ASH (using a floxed allele and a CRE transgene) alter adaptation of ASH responses? Does NPR-1 act genetically downstream of DA because it acts in the downstream interneuron? Alternatively, I would not object to simply deleting the NPR-1 data.

14. The authors use cell specific RNAi to show that DOP-4 is required in ASH neurons. In general, it is difficult to determine site of action by RNAi. siRNAs are known to be amplified and spread between tissues. If you really want to test site of action, you should do a cell specific knockout using a floxed allele and a transgene expressing CRE recombinase in ASH.

Referee #2 (Remarks to the Author):

Review of "Food sensitizes C. elegans avoidance behaviors through acute dopamine signaling" by Ezcurra et al.

In this manuscript, the authors present data to show that several C. elegans behaviors, namely various aspects of avoidance of soluble repellents, are modulated by food availability and that this processes is dependent on dopaminergic signaling. Initiation of this signaling appears to be dependent on functional cilia, organelles enriched in sensory molecules, of dopaminergic neurons.

They show that dopaminerig signaling then acts, in part, through the DOP-4 receptor on the polymodal ASH neurons. Through imaging studies, they show that this signaling leads to increases in the magnitude and duration of responses of ASH neurons. Moreover, they show that food modulation of ASH adaptation is dependent on neuropeptide Y signaling to ASH neurons.

Overall this is an excellent paper on an interesting topic (deciphering the molecular and cellular bases of food modulation of animal behavior), based on tremendous amount of solid data (analyses of genetic mutants, functional rescue studies, imaging studies), and the manuscript's conclusions are well supported by data. I fully support publication pending a few, minor revisions.

1) Figure 2A: a number of pieces of evidence presented in the paper fail to show an involvement in serotonin signaling in the modulation of indicated behaviors. While 10 mM 5-HT is certainly sufficient to elicit responses, it will be good to provide a control to rule out trivial explanations such as having a bad batch of serotonin (although unlikely). Any serotonin-induced phenotype even if on another time scale will suffice.
2) Writing of a couple of sentences need to be just slightly toned down:
-Introduction: "The molecule perhaps most strongly implicated as a direct signal of food in C. elegans is dopamine". "most strongly" seems somewhat overblown given the literature on serotonin, octopamine, etc.
-Results section within the subheading "Modulation of avoidance behaviors requires dopaminergic sensory cilia". The last sentence "...indicating that dopamine signaling is directly activated by external sensory cues" . While the authors are likely correct, the use of the word "directly" is unwarranted in the absence of actual direct identification of what that the external signaling cue might be.

Referee #3 (Remarks to the Author):

This is an excellent study and I highly recommend publication in EMBO. I was particularly impressed that by the elegant approach the authors used to demonstrate, for the first time, that the modulation of ASH signaling requires the sensory cilia of the mechano-sensitive dopamine producing neurons. This has been assumed in the literature for years, but has never actually been shown.

I have only minor comments:

1) The authors state "When we restored the cilia of the ASH neurons alone using either the gpa-11 or the gpa-13 promoter...". This makes it sound like these two promoters are ASH-specific promoters. As is noted in the figure, these promoters express in multiple neurons, with ASH being the only neuron common to both promoters. This needs to be clarified since they actually mean that they restored the ASH neurons, but not the dopamine neurons.

2) Figure Legends: The "grey bars" (lines?) indicated are not seen in any of the figures. Do the authors mean the light grey boxes? If so, they do not show up on a black and white print out of the article, which would be problematic for most readers. The authors may need to increase the contrast, or use lines or something else that will show up better when printed out.

1st Revision - authors' response 21 December 2010

Referee #1 (Remarks to the Author):

Review of Ezcurra et al. "Food sensitizes C. elegans ..."
An important goal of molecular neurobiology is to trace how environmental inputs alter the function of neuronal circuits, and consequently modify an animal's behavioral repertoire. In this study, the authors address this general question using C. elegans sensory responses to food as a model. The authors find that food sensitizes repellent responses and avoidance behaviors mediated by ASH neurons and that it does so through dopamine (DA) signaling. They show that food and exogenous DA enhance avoidance behavior and ASH calcium response to several repellents. The food effect

depends upon the functional integrity of sensory cilia in DA neurons, and the expression of DOP-4 DA receptors in ASH. Acute activation of DA neurons (with ChR2), leads to a transient enhancement of repellent avoidance responses. Finally, they show that NPR-1 neuropeptide receptors also regulate ASH sensory responses and may act downstream of the DA pathway. Several prior studies suggested that the presence of food is detected by dopaminergic neurons, and that DA functions as an "ON food" signal. The present study does a very nice job of connecting food exposure to activation of dopaminergic neurons to increased responsiveness to soluble repellents. In general, I liked the study and believe that it will be of interest to a broad audience. However, some of the experiments are not fully convincing and the manuscript could be improved by the addition of a few more experiments and revisions (as detailed below).

The analysis of how NPR-1 alters ASH responses is less well developed, and is not entirely consistent with prior studies on NPR-1. If the authors wish to retain the NPR-1 analysis, more experiments are required. Alternatively, as the NPR-1 results are not essential for any of the main conclusions, the authors may prefer to remove these results for inclusion in a more complete subsequent analysis.

Specific comments:

1. I am a bit worried that the effects of exogenous DA may not accurately reflect the physiological function of Food evoked release of endogenous dopamine. In particular, 10 mM DA prolongs ASH calcium responses to CuCl2. Several results suggest that this may not be a physiological effect of DA: 1) Food exposure does not prolong ASH calcium responses to CuCl2; 2) Mutants lacking DA (i.e. cat-2) do not have a correspondingly shortened calcium response; 3) The prolonged ASH calcium response was not blocked in Dop-4 mutants. Currently, the text assumes that this is a physiological effect of DA, and that it is mediated by an unidentified DA receptor. It seems equally likely that this is a non-physiological effect of 10 mM DA, which could be mediated non-specifically by another class of receptors. Without further data, I would advise that the authors revise the text to indicate this possibility.

We have modified the text as suggested (p7, end of paragraph 1; p 13, end of paragraph 1).

2. The authors show that 10 mM exogenous DA rescues the cat-2 mutant defects in ASH calcium and sensory responses. To avoid the potential for non-specific effects of exogenous DA (detailed above), a better control might be to rescue with L-DOPA instead.

Although L-DOPA is used to rescue dopamine deficits in mammals because it crosses the blood-brain barrier, in *C. elegans* it is standard (and less problematic) to rescue with dopamine hydrochloride. To function as a dopaminergic agonist in *C. elegans*, L-DOPA would need to be taken up by dopaminergic neurons, converted to dopamine and then rereleased; presumably the majority of L-DOPA would remain unconverted in the body cavity and have a high potential for non-specific, non-DA-related effects. Consistent with this possibility, a meeting abstract (Goshima et al., WBPaper00011916) reported effects of L-DOPA on locomotion that appear to be dopamine-independent. Thus, we think it appropriate to use exogenous DA treatment for these experiments, while noting potential caveats (see above) in the revised text.

3. A central conclusion of the paper is that exposure to food depolarizes the DA neurons, evoking DA release. This should be tested more directly by using a calcium probe to detect food induced calcium transients in the DA neurons.

We tested if food gives rise to calcium transients in the dopaminergic CEP neurons by using our perfusion system to stimulate with bacterial food. We observed increases in the YFP/CFP ratio, consistent with a direct sensation of food. However, since the bacteria themselves are fluorescent we were not able to detect reciprocal YFP/CFP intensity changes that would make us confident these were real calcium responses. We have therefore not included these data in the paper.

(Note: for the imaging experiments in the constant presence of food, bacterial autofluorescence only affected the baseline ratio, and ratio changes on stimulus presentation could be clearly detected. Note that an increased baseline ratio would tend to reduce the apparent ratio change, so the increased responses on food are if anything underestimated as a result of this effect).

4. The ASH calcium trace in cat-2 mutants following 2 mM CuCl2 exposure seems longer on food. Is that the case? If so, how do the authors explain this? It would seem to suggest that food alters duration of calcium responses independently of DA.

The duration of the *cat-2* responses to copper (and glycerol) do not appear to be significantly longer on food. We calculated the area under the ratio curve (see below) for *cat-2* on and off food and found no significant difference; these data are in the new Supplemental Figure 1.

5. The authors should include measures of response durations (and significance of any differences) for all calcium imaging experiments.

It is difficult to measure response duration per se because the decay of the ratio signal is affected by both the dynamics of the calcium decrease and the relatively slow off-kinetics of the cameleon indicator (half time > 1s). However, we can indirectly asses the response duration by measuring the area under the ratio curves, which integrates response magnitude and duration. We computed this for all genotypes, and the data are presented in the Supplemental Figure.

6. Is the prolonged ASH calcium trace evoked by 0.5 M glycerol on food (fig. 1c) blocked in dop-4 and cat-2 mutants?

We tested this, and found that *cat-2* animals do not have significantly prolonged glycerol-evoked calcium transients on food (Figure 2F, Supplemental Figure1), whereas *dop-4* animals do (Figure 7D, Supplemental Figure 1).

7. Does photoactivation of DA neurons with (ChR2) alter ASH calcium responses to CuCl2?

Because the excitation wavelengths of ChR2 and yellow cameleon overlap, we were unable to do these experiments. (A method to surmount this problem with spinning disc confocal microscopy has been described by Guo et al, but we do not have access to such a microscope).

8. Is the effect of photoactivation of DA neurons on locomotion (Fig. 5A) and ASH behaviors (B and C) eliminated in cat-2 and dop-4 mutants?

This good suggestion. We have crossed the *dat-1::ChR2* array into *cat-2* and *dop-4* backgrounds and analyzed the effects of these genes on locomotion and escape behaviors. We found that *dat-1::ChR2* photoactivation caused neither slowing nor enhancement of repellent avoidance in a *cat-2* background, indicating that both behavioral effects are mediated by dopamine. In contrast, *dop-4* affected avoidance but not slowing, suggesting its effects are at least somewhat specific to ASH. These data have been added to Figure 6.

9. The authors argue that DA and 5HT don't generally alter ASH excitability because they do not alter the ASH calcium response to nose touch (fig. 2F and G). I would interpret these results more cautiously. Failure to observe a change in cameleon fluorescence following nose touch could arise for many technical reasons. Cameleon has intrinsically slow kinetics and could easily miss a transient response. Furthermore, the calcium transients detected in these experiments is in the ASH cell body, not in its axons. In fact, there is no proof that the somatic calcium detected in these experiments is relevant to the sensory evoked behavioral responses (see related comments #10 and 11). The authors should revise their discussion of these results to more accurately reflect these alternative explanations.

We agree that the modality-specificity of the food/dopamine effects on ASH calcium responses does not rule out the possibility of effects on processes like synaptic transmission or axonal excitability. To address the possibility that food and dopamine affect processes downstream of chemosensation, we generated an ASH-specific ChR2 line and tested the effects of food and dopamine on escape responses evoked by photoactivation of ASH (Results, page 7 paragraph 2; new Figure 3). We find that food and dopamine do not alter the magnitude or the frequency of ChR2-evoked escape responses, suggesting that dopamine's effects are most likely at the level of the sensory response.

10. The authors should do a more careful analysis of the kinetics of ASH calcium responses and the

corresponding behavioral responses (Figs. 3,7, and 8). This is an important issue because the slow somatic calcium transients recorded with the cameleon probe may not accurately reflect the endogenous calcium transients driving the ASH mediated sensory behaviors. In the current version of the manuscript, behavioral decline (during adaptation assays) is plotted over the course of 20 consecutive stimuli yet calcium traces are examined only after a chronic exposure to 10 mM CuCl2. Does the rate of adaptation of the ASH calcium responses match the decay in sensory behavioral responses?

We have added new behavioral adaptation experiments using a prolonged exposure protocol that more closely matches the procedure used for calcium imaging (Results p 9, paragraph 1; Figure 4C, D). This method was based on one used in a previous paper (Hilliard et al, 2002) in which animals were incubated in a solution of copper chloride, allowed to recover, then tested for escape responses using the drop test. Specifically, animals were incubated in copper chloride for 15 sec and then given a 2 min recovery time before testing; this matches the time courses in the imaging experiments.

We found that for wildtype animals, pre-exposure to copper leads to the avoidance index decreasing from 0.9 (pre-exposure) to 0.2 (post-exposure) in the absence of food, and from 0.9 (pre-exposure) to 0.5 (post-exposure) in the presence of food (Figure 4C, D). This is similar to the results we get from calcium imaging, showing that calcium responses parallel behavioral responses. The quantitative difference in the results could reflect the fact that the behavioral assays are performed on dry plates, and the imaging in a buffer perfusion. We have observed that humidity of the plates and the air has an effect on behavioral avoidance and adaptation.

11. Similarly, does the duration of ASH calcium responses to CuCl2 and glycerol quantitatively match the observed duration of sensory evoked backing?

It is difficult to exactly compare the results of imaging experiments done on glued animals in liquid to behavioral experiments using drops of repellent on plates. Nonetheless, we observe that in imaging experiments the ratio signal in ASH begins falling immediately after the removal of even a short stimulus (see e.g. Figure 4), whereas reversal persist many seconds after the worm has crawled away from a drop of repellent. This suggests that the calcium transients in ASH are most likely of shorter duration than the reversals they evoke. This correlates with published observations by other labs (Faumont et al., 2006; Guo et al., 2009) that the transient activation of ASH leads to more persistent activation of the backward command neurons, which probably exhibit bistable activity states. Comments regarding this have been added to the text (Results, second paragraph).

12. The authors conclude that DOP-4 is not required for the slower rate of sensory adaptation on food (Fig. 3B). The data are not so clear. The change in adaptation on food is quantatively subtle (but apparently significant). The dop-4 mutant adaptation rate is intermediate between the cat-2 and wild type controls, and likely does not significantly differ from either. Based on these data, one cannot say much about whether DOP-4 is involved or not.

We retested adaptation in *cat-2* and *dop-4* animals using the pre-exposure protocol described above (new data in Figure 4C, D). We found that while *cat-2* adapts significantly more than wildtype in the presence of food, *dop-4* animals are not significantly different from wild-type. From this we conclude that *dop-4* is not necessary for food modulation of adaptation.

13. The authors contend that NPR-1 acts in ASH to regulate adaptation of calcium and sensory responses (Fig. 7). These results seem to conflict with a prior study from the Bargmann lab (Macosko, 2009), which showed that NPR-1 acts in RMG interneurons to regulate the sensitivity of a group of sensory neurons that form a gap junction network with RMG. To avoid confusing the literature, if the authors want to comment on NPR-1 function, they should address this issue. Does NPR-1 expression in RMG also restore the ASH sensory responses and behaviors (as Macosko showed for ASK responses)? Does inactivation of NPR-1 selectively in ASH (using a floxed allele and a CRE transgene) alter adaptation of ASH responses? Does NPR-1 act genetically downstream of DA because it acts in the downstream interneuron? Alternatively, I would not object to simply deleting the NPR-1 data.

We agree with the reviewer that there are many unanswered questions regarding the role of NPR-1

in avoidance behavior. While our rescue data argues for a cell-autonomous function for NPR-1 in ASH, it is reasonable to suppose NPR-1 could also influence ASH indirectly through RMG. Given the focus of this study on dopamine, it is probably outside the scope of this paper to thoroughly characterize the role of NPR-1 in ASH-mediated avoidance. Consequently, we have taken the reviewer's suggestion and removed this section, with the intention of publishing a more complete study at a later time.

14. The authors use cell specific RNAi to show that DOP-4 is required in ASH neurons. In general, it is difficult to determine site of action by RNAi. siRNAs are known to be amplified and spread between tissues. If you really want to test site of action, you should do a cell specific knockout using a floxed allele and a transgene expressing CRE recombinase in ASH.

We agree that cell-specific RNAi experiments are not as definitive as a cell-specific genetic deletion, but we don't think they are as problematic as the reviewer suggests. Although transgenic RNAi can in some cases spread between *C. elegans* tissues, it has never been reported to spread between neurons (presumably because neurons lack the SID-1 transporter, which is required for import, though not export, of RNAi triggers). Indeed, the original Esposito paper specifically looked for inter-neuronal spreading by targeting a pan-neuronal GFP reporter with cell-specific RNAi, and failed to detect effects on GFP expression outside the expression domains of the targeting promoters. Subsequent studies (e.g. Harris et al 2009, Chatzigeorgiou 2010) have also failed to detect evidence of RNAi spreading between neighboring neurons. Since we likewise failed to detect effects on ASH when *dop-4* dsRNA was expressed under non-ASH amphid promoters, we think it is unlikely that the modulation phenotypes caused by ASH-specific RNAi are the result of spreading.

In the revised version we have added an explanation of the caveats of cell-specific RNAi, and have softened our conclusions somewhat. In principle we could drop these experiments and rely on the cell-specific mutant rescue data as evidence for DOP-4 function in ASH, but our preference would be to retain them as additional evidence for cell autonomy.

Referee #2 (Remarks to the Author):

Review of "Food sensitizes C. elegans avoidance behaviors through acute dopamine signaling" by Ezcurra et al.

In this manuscript, the authors present data to show that several C. elegans behaviors, namely various aspects of avoidance of soluble repellents, are modulated by food availability and that this processes is dependent on dopaminergic signaling. Initiation of this signaling appears to be dependent on functional cilia, organelles enriched in sensory molecules, of dopaminergic neurons. They show that dopaminerig signaling then acts, in part, through the DOP-4 receptor on the polymodal ASH neurons. Through imaging studies, they show that this signaling leads to increases in the magnitude and duration of responses of ASH neurons. Moreover, they show that food modulation of ASH adaptation is dependent on neuropeptide Y signaling to ASH neurons.

Overall this is an excellent paper on an interesting topic (deciphering the molecular and cellular bases of food modulation of animal behavior), based on tremendous amount of solid data (analyses of genetic mutants, functional rescue studies, imaging studies), and the manuscript's conclusions are well supported by data. I fully support publication pending a few, minor revisions.

1) Figure 2A: a number of pieces of evidence presented in the paper fail to show an involvement in serotonin signaling in the modulation of indicated behaviors. While 10 mM 5-HT is certainly sufficient to elicit responses, it will be good to provide a control to rule out trivial explanations such as having a bad batch of serotonin (although unlikely). Any serotonin-induced phenotype even if on another time scale will suffice.

We tested the biological activity of the serotonin by looking for stimulation of fast pharyngeal pumping; this has been added to the methods section.

2) Writing of a couple of sentences need to be just slightly toned down:

-Introduction: "The molecule perhaps most strongly implicated as a direct signal of food in C. elegans is dopamine". "most strongly" seems somewhat overblown given the literature on serotonin, octopamine, etc.

This has been changed.

-Results section within the subheading "Modulation of avoidance behaviors requires dopaminergic sensory cilia". The last sentence "...indicating that dopamine signaling is directly activated by external sensory cues" . While the authors are likely correct, the use of the word "directly" is unwarranted in the absence of actual direct identification of what that the external signaling cue might be.

Referee #3 (Remarks to the Author):

This is an excellent study and I highly recommend publication in EMBO. I was particularly impressed that by the elegant approach the authors used to demonstrate, for the first time, that the modulation of ASH signaling requires the sensory cilia of the mechano-sensitive dopamine producing neurons. This has been assumed in the literature for years, but has never actually been shown.

I have only minor comments:

1) The authors state "When we restored the cilia of the ASH neurons alone using either the gpa-11 or the gpa-13 promoter...". This makes it sound like these two promoters are ASH-specific promoters. As is noted in the figure, these promoters express in multiple neurons, with ASH being the only neuron common to both promoters. This needs to be clarified since they actually mean that they restored the ASH neurons, but not the dopamine neurons.

We have changed the text as suggested.

2) Figure Legends: The "grey bars" (lines?) indicated are not seen in any of the figures. Do the authors mean the light grey boxes? If so, they do not show up on a black and white print out of the article, which would be problematic for most readers. The authors may need to increase the contrast, or use lines or something else that will show up better when printed out.

We have increased the contrast, and the “bars” are now properly described as boxes.

Additional Correspondence 07 January 2011

Thank you for submitting your revised manuscript to the EMBO Journal. I asked the original referee #1 to review the revised manuscript and I have now heard back from the referee. As you can see below referee #1 appreciates the introduced changes and supports publication here. I am therefore very pleased to proceed with the acceptance for publication here. You will receive the formal acceptance letter shortly.

Best wishes

Editor
The EMBO Journal

Referee #1 (Remarks to the Author):

The authors have addressed all concerns raised in the original review. I am happy to support publication of the revised manuscript in EMBO.

