Morphogenesis is transcriptionally coupled to neurogenesis during peripheral olfactory organ development

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MS TITLE: Morphogenesis is transcriptionally coupled to neurogenesis during peripheral olfactory organ development

AUTHORS: Raphael Aguillon, Romain Madelaine, Harendra Guturu, Sandra Link, Pascale Dufourcq, Virginie Lecaudey, Gill Bejerano, Patrick Blader, and Julie Batut

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some criticisms and suggestions for improving your manuscript. If you are able to revise the manuscript along the lines suggested, I will be happy receive a revised version of the manuscript. Please also note that Development will normally permit only one round of major revision.

We are aware that you may have limited access to the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

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Reviewer 1

Advance summary and potential significance to field

see comments to the authors below

Comments for the author

Review Batut and colleagues

In their manuscript, Aguillon and colleagues describe how organ specification and organ assembly are linked using the olfactory placode formation in zebrafish as a model. They find that the proneural transcription factor neurog1 is required for correct assembly of the olfactory placode. They noticed that this defect resembles the olfactory assembly defect observed in cxcr4b mutants and speculate that neurog1 and cxcr4b might act in the same pathway. Consistent with this supposition, they find that the olfactory sensory neuron (OSN) assembly defects in neurog1 mutants and cxcr4b mutants are very similar, that cxcr4b expression in the OSNs requires neurog1, that expression of cxcr4b from the neurog1 promoter restores placode assembly, that neurog1 binds to an E-box motive in the promoter of cxcr4b, and that deletion of this E-box motive blocks expression of GFP in the olfactory placode from a cxcr4b transgene. This is a well-written manuscript and a beautiful study that sheds light on a mechanism of how organ specification and morphogenesis can be coupled. I only have a few suggestions/comments.

1. If neurog1 and cxcr4b act in the same pathway, double mutants should have the same phenotype as single mutants - maybe the authors would want to confirm this.
2. The authors have identified gRNAs that cut and delete the E-box in the cxcr4b promoter that is important for cxcr4b transcription in the OSNs and use these guides in transients. Could they make a stable line and confirm the prediction that deletion of this element (at the endogenous cxcr4b locus) recapitulates the neurog1 and cxcr4b phenotypes with respect to olfactory placode assembly.
3. The methods for cell tracking are very brief and should be expanded. It sounds like the cells were tracked in 3d in imaris, how is this converted to a 2d representation? Also, how exactly was the PCA done? The R scripts need to be supplied as sup data so that people can understand what was done. Also, the xyzt data for the tracked cells in the different genotypes should be provided as excel files and as sup data.

Minor:
1. "18 E-boxes clusters" should "read 18 E-box clusters"i

Reviewer 2

Advance summary and potential significance to field

In this manuscript the authors investigate how cell fate specification and morphogenetic movements in the olfactory placode of zebrafish are linked. Specifically, they show a role of the pro-neural transcription factor Neurog1 in regulating the movements of a subpopulation of olfactory neurons by controlling the expression of the chemokine receptor cxcr4b. Analysing cell movements they find that in the absence of Neurog1 anterior early neurons are delayed to converge to other neurons in the placode. This phenotype resembles cxcr4b mutants as well as mutants of its ligand and cxcr4b rescues Neurog1 loss of function. Finally, they show that Neurog1 directly binds to regulatory sequences upstream of cxcr4b and that this element is required for cxcr4b reporter activity. The link between cell fate and morphogenesis is not well established and the paper therefore makes some contribution to our understanding these processes.

Comments for the author

How cell fate specification and morphogenesis is coordinated is not well understood and the paper begins to address this question. Overall, the data are well presented and support the authors conclusion. However, there are a few points that need to be addressed.

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1. Overall, it seems that there are less neurons in the Neurog1 mutant as seen when comparing movies S1 and S2; is it possible that changes in cell movement is due to smaller numbers of neurons?
2. The relationship of Neurog1 and cxcr4b expression is not clear;
presumably they are expressed in the same cells? They authors should show this.
3. It seems that cxcr4 is expressed along the entire anterior-posterior length of the olfactory placode, and Neuorg1 is expressed in all olfactory neurons. Why are only anterior neurons affected?
4. Please explain how anterior, medial and posterior EONs were defined.
5. It seems that only very few cells were analysed in the rescue experiment Fig. 3C; cell movements look quite different from controls suggesting that Cxcr4b is only part of the mechanism downstream of Neurog1.The authors should provide more numbers and more convincing data on the rescue, and also discuss the possibility of other mechanisms.
6. The authors should re-phrase the title and statements in the paper to be precise about their findings. They investigate the movements of olfactory neurons, but not olfactory placode morphogenesis. How do non-neural cells behave? Are they influenced by neuronal movements?

Minor comments:
The images in Fig 3A, B are very small; it would be nice to show high magnifications of the olfactory region to appreciate the phenotype.
I wonder if clustering would be better presented as heatmaps?

Reviewer 3

Advance summary and potential significance to field

The manuscript entitled “Morphogenesis is transcriptionally coupled to neurogenesis during peripheral olfactory organ development” is showing that loss of the neurogenic gene Neurog1 affects both neurogenesis and morphogenesis in zebrafish nasal placode. The study is well conducted and rigorous. It combines quantitative analysis of live-imaging data, with functional genetic manipulations such as rescue experiments ensuring robustness of claims and a “crispant” approach to determine the cis-regulatory elements involved in the neurog1-dependent expression of Cxcr4. There is however a fundamental question left unanswered: as the number of cells differentiating into nasal placode seems reduced in the neurog1 null (very obvious on their movies), the difference in movements described may be indirectly due to difference in behaviour in smaller populations. This key possibility would change the interpretation of the results and need to be addressed (see below).

Comments for the author

Major point:
- To assess whether the mutant has indeed consistently less cells in the initial placode at 12hpf, the authors need to quantify cell numbers in the mutant and in the rescued mutant. If it is indeed confirmed that the population is smaller in the mutant and that this reduction is rescuable, it could be the partial (or complete) cause of the lack of Cxcr4 expression and difference in cell behaviour, independently of loss of Neurog1. In this case the authors would need to ablate GFP+ placodal cells at 12hpf in wt and follow the cell behaviour in these ablated wildtype animals.

Minor points:
- Conservation argument is a bit weak; the authors should attenuate their statement in discussion and even more in the introduction.
- Tg(-8.4neurog1:gfp) labels the neural tube as well as the olfactory epithelium. It would help the reader to mention this and direct the attention of the reader to the olfactory epithelium. Highlighting or annotating the population of interest in the movies would increase the understanding.
- The difference between Movies S1 and S2 being not very pronounced, having them playing side by side would help comparison between wild-type and mutant situations.
- The reviewer is puzzled by the difference in number of cell tracks shown in all different movies.

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- PCA graphs are very difficult to understand for the reader. It is not clear how subtle differences are identified from the plot, or how the authors conclude that differences lie in their displacement along the AP axis. The conclusions drawn from the unsupervised clustering is a bit more intuitive but would benefit from clarification as well. A thorough explanation of how data are plotted and what information they give is crucial.
- Supplementary figure S1 C-D-E should go with S3 to ease reading of the article.

First revision

Author response to reviewers' comments

Our responses to the reviewers' comments and our changes to the manuscript are outlined here:

Reviewer 1:
In their manuscript, Aguillon and colleagues describe how organ specification and organ assembly are linked using the olfactory placode formation in zebrafish as a model. They find that the proneural transcription factor Neurog1 is required for correct assembly of the olfactory placode. They noticed that this defect resembles the olfactory assembly defect observed in cxcr4b mutants and speculate that Neurog1 and Cxcr4b might act in the same pathway. Consistent with this supposition, they find that the olfactory sensory neuron (OSN) assembly defects in neurog1 mutants and cxcr4b mutants are very similar, that cxcr4b expression in the OSNs requires Neurog1, that expression of cxcr4b from the neurog1 promoter restores placode assembly, that Neurog1 binds to an E-box motive in the promoter of cxcr4b, and that deletion of this E-box motive blocks expression of GFP in the olfactory placode from a cxcr4b transgene. This is a well-written manuscript and a beautiful study that sheds light on a mechanism of how organ specification and morphogenesis can be coupled. I only have a few suggestions/comments.

1. If neurog1 and cxcr4b act in the same pathway, double mutants should have the same phenotype as single mutants - maybe the authors would want to confirm this.

We agree with the reviewer that it would have been informative to have this data but chose not to establish the necessary fish stocks. The data showing the similarity in the migration behaviour of olfactory progenitors in neurog1 versus ody/cxcr4b mutants can only be appreciated fully after extracting morphometric parameters from time-lapse confocal datasets. Getting these datasets for the single mutants was laborious given that only one of 4 embryos mounted is homozygous and we could not distinguish them from siblings prior to starting our acquisitions. As such, we felt that aiming for one of 16 in the double mutant context was too much to ask.

2. The authors have identified gRNAs that cut and delete the E-box in the cxcr4b promoter that is important for cxcr4b transcription in the OSNs and use these guides in transients. Could they make a stable line and confirm the prediction that deletion of this element (at the endogenous cxcr4b locus) recapitulates the neurog1 and cxcr4b phenotypes with respect to olfactory placode assembly.

The reviewer raises a frustrating point. It was our initial plan to delete the element at the endogenous locus but despite the apparent efficiency of our guide pair in transient, we were unable to identify stable carriers after screening well over one hundred injected F0s; for other projects in the lab we have had very good success creating stable deletions so the origin of our trouble with cxcr4b are not obvious. We also thought about looking at the expression of endogenous cxcr4b by in situ hybridisation after guide injection. Unfortunately, while determining whether a cell in the olfactory epithelium does not express cxcr4b at 24hpf might have been possible, its transcription becomes "independent" of Neurog1 around 18hpf. At stages where cxcr4b expression relies entirely on Neurog1 activity, say 15hpf, it is virtually impossible to identify cells that "should have" expressed cxcr4b given the less stereotypic shape of the structure at these earlier stages. Finally, to affect the expression of endogenous cxcr4 deletion of the element at both loci would

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have be required, which is not assured despite the apparent efficiency of our guide pair. Thus, while not completely satisfying we turned to the transient approach in the transgenic background.

3. The methods for cell tracking are very brief and should be expanded. It sounds like the cells were tracked in 3d in Imaris, how is this converted to a 2d representation? Also, how exactly was the PCA done? The R scripts need to be supplied as sup data so that people can understand what was done. Also, the xyzt data for the tracked cells in the different genotypes should be provided as excel files and as sup data.

We have added text to the Material and Method section that we hope will make things clearer. Cells were indeed tracked in 3d with the 2d representations of the figures being generated by R scripts that can now be accessed via GitHub (https://github.com/BladerLab/Aguillon_2020). As mentioned in the Materials and Methods, PCA and clustering were performed using the built-in R functions from the “FactoMineR” and “stats” packages, respectively. Finally, the raw data treated by our R scripts has also been deposited in GitHub as CSV files.

Minor:

1. "18 E-boxes clusters" should "read 18 E-box clusters"

We have made the change to the text.

Reviewer 2:
In this manuscript the authors investigate how cell fate specification and morphogenetic movements in the olfactory placode of zebrafish are linked. Specifically, they show a role of the pro-neural transcription factor Neurog1 in regulating the movements of a subpopulation of olfactory neurons by controlling the expression of the chemokine receptor Cxcr4b. Analysing cell movements they find that in the absence of Neurog1 anterior early neurons are delayed to converge to other neurons in the placode. This phenotype resembles cxcr4b mutants as well as mutants of its ligand and Cxcr4b rescues Neurog1 loss of function. Finally, they show that Neurog1 directly binds to regulatory sequences upstream of cxcr4b and that this element is required for cxcr4b reporter activity. The link between cell fate and morphogenesis is not well established and the paper therefore makes some contribution to our understanding these processes.

Reviewer 2 Comments for the author
How cell fate specification and morphogenesis is coordinated is not well understood and the paper begins to address this question. Overall, the data are well presented and support the authors conclusion. However, there are a few points that need to be addressed.

1. Overall, it seems that there are less neurons in the Neurog1 mutant as seen when comparing movies S1 and S2; is it possible that changes in cell movement is due to smaller numbers of neurons?

There are indeed fewer neurons in the neurog1 mutant, a phenotype we described in detail some years ago now (Madelaine et al., Development, 2011). For two reasons, however, we do not think that this reduction plays a role in the defects we describe in the present manuscript. First, neurog1 mutants display an average of half the wildtype complement of olfactory neurons but the number varies considerably between embryos or even between placodes of the same embryo. Despite this, we have seen no correlation between neuronal numbers and the migratory behaviour of the neurons in the mutant background. Secondly, re-introducing cxcr4b in a neurog1 mutant background rescues the posterior migration of anterior cells despite a reduced number of neurons (see also the major point raised by Reviewer 3 below). This suggests that it is the lack of Cxcr4b guidance receptor and not the reduced number of EON that underlie the migration phenotype.

2. The relationship of Neurog1 and cxcr4b expression is not clear; presumably they are expressed in the same cells? They authors should show this.

As the Reviewer states in the point 3, globally cxcr4b and neurog1 appear to be expressed along the entire AP length of the developing olfactory plocode. Nonetheless, it is difficult to get finer grained appreciation of the expression patterns from the data in our original manuscript. We

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now provide data showing that from very early stages (14 hours post-fertilisation) cxcr4b expressing cells fall within the GFP+ placodal domain in embryos expressing the Tg(-8.4neurog1:GFP) transgene. There is considerable background in the epidermis flanking the developing placodes, probably due to amplification by the immunostaining against GFP. We have incorporated this new data into our resubmission as Figure S7.

3. It seems that cxcr4 is expressed along the entire anterior-posterior length of the olfactory placode, and Neurog1 is expressed in all olfactory neurons. Why are only anterior neurons affected?

We would like to point out that middle and posterior neurons are affected, and that this is mentioned in the manuscript. Nonetheless, the question remains as to why the anterior neurons are affected to a much greater degree in the absence of Neurog1, Cxcr4b or Cxcl12a than the other two populations. While we have no evidence yet to support them, we have two working hypotheses. The first is based on the architecture of the initial progenitor population and adhesion between cells within it. Briefly, olfactory progenitors are arranged in a horseshoe-shaped domain at the interface between the anterior neural plate and flanking non-neural ectoderm. Whereas the posterior-most cells on each side of this domain have no neighbours, cells at the anterior midline do. In wildtype embryos, signalling provided by Cxcr4b/Cxcl12a attracts cells to a central point on each side of the embryo and the force generated is sufficient to overcome the adhesion between cells at the anterior midline, thus separating the initial progenitors into left and right halves. In the absence of this attractive force, whereas cells at the anterior midline are not pulled apart as efficiently posterior cells are less constrained and migrate better; middle cells are already near their final position and are also less affected. Alternatively, it is possible that there is a default migration of the entire population towards the anterior and that Cxcr4b/Cxcl12a signalling overrides this in the anterior population, pulling it posteriorly against the default flow. Both hypotheses are supported in part by our data and it is quite possible that both are true to some extent.

4. Please explain how anterior, medial and posterior EONs were defined.

The three domains were defined as equal thirds of the initial progenitor domain along the antero-posterior axis. We chose this definition to permit comparison of our data with those of a previously published paper by Breau and colleagues (Nature Communication, 2017) that used the same approach.

5. It seems that only very few cells were analysed in the rescue experiment Fig. 3C; cell movements look quite different from controls suggesting that Cxcr4b is only part of the mechanism downstream of Neurog1. The authors should provide more numbers and more convincing data on the rescue, and also discuss the possibility of other mechanisms.

As we were sure that a similar number of cells had been analysed in the "Rescue" and "Control rescue" embryos as for the other conditions, we initially had trouble understanding why the Reviewer raised the question. Having taken a closer look at the graphics presented in Figure 3C, however, we are thankful that the point was raised as there is clearly a problem. We have found that the error stems from a bug in our initial R script for tracking. Briefly, instead of tracking cells from both placodes of 3 embryos, for 2 embryos of the "Rescue" condition we could only track cells of a single placode, and in one case a single cell from a single placode. To maintain the same numbers of cells analysed, we chose to track cells from a 4th embryo. Unfortunately, our initial R script for tracking was only designed to accept datasets from 3 embryos, the result being that some of the tracking data was not imported into the analysis. We have rectified the problem in the R script and reanalysed the datasets. The panels in Figure 3 now contain analysis of the complete datasets, which are accessible via GitHub (https://github.com/BladerLab/Aguillon_2020). While the PCA and clustering in Figure 3 have also changed, the results still support our conclusion that re-expressing Cxcr4b in neurog1 mutants rescues posterior migration of the anterior EON cohort.

Concerning the "quality" of the rescue, we agree that re-expressing Cxcr4b in neurog1 mutants does not result in completely wildtype migration behaviour. Nonetheless, while anterior EON initially migrated anteriorly in neurog1 mutants they migrate posteriorly in homozygous neurog1 mutant embryos carrying the rescue transgene. Furthermore, clustering indicates that rescued cells behave more like wildtype that mutant cells. We take these results to indicate that the migration phenotype has been rescued to a significant extent. We have modified the concluding

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sentence of the section to say that we cannot rule out that there are Cxcr4b-independent mechanisms involved in the migration. Nonetheless, we have chosen to maintain our conclusion that our rescue experiments and the similarity of the migration phenotype in neurog1 and cxcr4b mutant embryos suggest that Cxcr4b is the predominant downstream effector of Neurog1 during the early phase of olfactory cup morphogenesis.

We also reanalysed all other datasets using the new R script and found two other discrepancies with our initial analysis. The first concerns the total tracks for anterior, middle and posterior EON of cxcr4b mutant embryos (Figure 2A and S5A). The differences are limited to times after 18 hpf and do not affect the analysis that shows neurog1, cxcr4b and cxcl12a mutant EON cluster together. While we are not certain, we think that these differences may stem from analysis of a preliminary and incomplete dataset. The second concerns the mean tracks for the anterior EON population of cxcl12a mutants (Figure 2B and S5B). Somewhat surprisingly, while the total tracks, PCA and clustering for the cxcl12a mutant (Figure 2A,C,D and S5) are unchanged after reanalysis, the means were different from our initial analysis. Luckily, PCA and clustering of these data do not rely on the means but on the total dataset. We have changed the panels in Figures 2 and S5 where differences were found with our original submission.

Finally, trouble shooting and rewriting the R scripts was undertaken with the help of Marion Aguirrebengoa of the CBI bioinfomatics platform, BigA. As a consequence, we have added Marion to the authors of our revised manuscript because her help was primordial in identifying and solving the problem with our initial R script.

6. The authors should re-phrase the title and statements in the paper to be precise about their findings. They investigate the movements of olfactory neurons, but not olfactory placode morphogenesis. How do non-neural cells behave? Are they influenced by neuronal movements?

On this point, we disagree with the Reviewer. Morphogenesis describes processes underlying the generation of form. From 12 to 27hpf, placodal progenitors rearrange themselves from a rather loose horseshoe-shaped population to a relatively compact cup. While this change in form is driven by the migration of olfactory neural progenitors, it is a collective process and the form is affected when migration is impaired. As such, we believe that morphogenesis is the appropriate term for the overall process. We have, however, been carful to modified the text to use "morphogenesis" when talking about collective aspects of things and "migration" where talking about individual cellular behaviours.

Concerning non-neural cells, there are only olfactory neural progenitors and early-born neurons in the population at the stages we are studying. This said, we did look at cells in the overlying skin and their behaviours is not affected in any of the mutant contexts we analysed.

Minor comments:

-The images in Fig 3A, B are very small; it would be nice to show high magnifications of the olfactory region to appreciate the phenotype.

The figure has been modified as suggested by the Reviewer.

-I wonder if clustering would be better presented as heatmaps?

We spent some time choosing a strategy that would best convey the differences/similarities between the various genetic contexts. After testing various alternatives and showing them to colleagues, we finally settled on displaying the most pertinent PCA and where appropriate the clustering of the data in them. While the PCA are not straightforward to understand, we think that the clusters are relatively intuitive - as pointed out by Reviewer 3. We have added text to the Materials and Methods that we hope makes understanding the PCA simpler.

Reviewer 3:

The manuscript entitled “Morphogenesis is transcriptionally coupled to neurogenesis during peripheral olfactory organ development” is showing that loss of the neurogenic gene Neurog1

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affects both neurogenesis and morphogenesis in zebrafish nasal placode. The study is well conducted and rigorous. It combines quantitative analysis of live-imaging data, with functional genetic manipulations such as rescue experiments ensuring robustness of claims and a "crispant" approach to determine the cis-regulatory elements involved in the neurog1-dependent expression of Cxcr4. There is however a fundamental question left unanswered: as the number of cells differentiating into nasal placode seems reduced in the neurog1 null (very obvious on their movies), the difference in movements described may be indirectly due to difference in behaviour in smaller populations. This key possibility would change the interpretation of the results and need to be addressed (see below).

Reviewer 3 Comments for the author
Major point:
-To assess whether the mutant has indeed consistently less cells in the initial placode at 12hpf, the authors need to quantify cell numbers in the mutant and in the rescued mutant. If it is indeed confirmed that the population is smaller in the mutant and that this reduction is rescuable, it could be the partial (or complete) cause of the lack of Cxcr4 expression and difference in cell behaviour, independently of loss of Neurog1. In this case the authors would need to ablate GFP+ placodal cells at 12hpf in wt and follow the cell behaviour in these ablated wildtype animals.

Our global impression from the time-lapse data was that there is no significant difference in the size of the populations between the "Rescue" and "Control rescue" embryos. Nonetheless, we went back and quantified the number of EON in both conditions at 14 hours post-fertilisation, the earliest stage at which we can unequivocally determine if a cell is an EON. We find that the EON population is if anything smaller in the rescued than the neurog1 mutant context (neurog1-/-: 20.17 +/- 2.24 s.e.m versus neurog1-/-;Tg: 13.83 +/- 0.98 s.e.m) but the differences are not statistically significant. We have added this data to the text of the new version of our manuscript.
Minor points:
-Conservation argument is a bit weak; the authors should attenuate their statement in discussion and even more in the introduction.

We have modified the text in the Discussion paragraph and eliminated all reference to conservation in the introduction.

-Tg(-8.4neurog1:gfp) labels the neural tube as well as the olfactory epithelium. It would help the reader to mention this and direct the attention of the reader to the olfactory epithelium. Highlighting or annotating the population of interest in the movies would increase the understanding.

To simplify the presentation of the experimental system in Figure 1, we chose only to show schematics of the developing olfactory placodes/epithelium. As the Reviewer points out, however, the transgene we use is also expressed elsewhere during the developmental time window we study, a fact that is clearly evident in the movies. We agree that for readers who are new to studies of the zebrafish olfactory epithelium, this might cause confusion. With our revised submission, we now provide an annotated version of Movie S1 that we hope will resolve any problems readers might have.

-The difference between Movies S1 and S2 being not very pronounced, having them playing side by side would help comparison between wild-type and mutant situations.

As the reviewer points out, the differences between the two movies are not simple to appreciate while watching them separately. Indeed, even playing them side-by-side principally highlights differences in the final size of the olfactory epithelium in the two contexts rather than the migration behaviour of individual cells. While initially we thought it was important to show the "raw" data, it is clear that the differences can only be seen easily once the morphometric data from them has been extracted and compared. As such, and given that the new version of Movie S1 carries annotation as requested in the previous point, we have decided to remove Movie S2 from our revised submission.

-The reviewer is puzzled by the difference in number of cell tracks shown in all different movies.

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While rather hand-waving, there was some logic in our choice of how many tracks to present. Whereas we chose to annotate the full dataset extracted in the control to show the maximum data we retrieved from any single embryo, we only annotated one cell per mutant placode as we considered that there was no advantage to adding more. For the cxcr4b and cxcl12a mutants we only put a single cell for each placode for the anterior domain as these are the most affected population. For the rescue experiments, on the other hand, the choice was "aesthetic". The movie with the best orientation for the neurog1-/-;Tg condition was one in which we could only track one placode. As such, for consistency we chose to show one cell/one placode data for the corresponding rescue control context. If the Reviewer thinks it is necessary, we could harmonise the presentations to some extent.

-PCA graphs are very difficult to understand for the reader. It is not clear how subtle differences are identified from the plot, or how the authors conclude that differences lie in their displacement along the AP axis. The conclusions drawn from the unsupervised clustering is a bit more intuitive but would benefit from clarification as well. A thorough explanation of how data are plotted and what information they give is crucial.

PCA provides an unsupervised manner to analyse complex datasets. The problem with PCA graphics is that one feels that it should be possible to "see" the differences whereas the most important take home message concerns what the first and second principal components are of the analysis (PC1 and PC2) and their relative importance (% variance). Thus, while we agree completely with the reviewer that the clouds of points can confuse the reader, we felt bound to present the data in this manner as it provides a simple way to present the PC1 and PC2 variances, which are listed on the axis of the panels. In the new version of the manuscript we have added text to the Materials and Methods that we hope will help transmit these ideas more clearly.

Concerning the clustering, we agree that it is easier for the reader to interpret and provides a complementary way to visualise the data. We would like to note, however, that we asked that the data be clustered into 4 groups (k = 4) as the datasets compared in Figures 2 and 3 contain 4 genotypes, so it was supervised to some extent.

-Supplementary figure S1 C-D-E should go with S3 to ease reading of the article.

We have made the change suggested by the Reviewer.

Second decision letter

MS ID#: DEVELOP/2020/192971

MS TITLE: Morphogenesis is transcriptionally coupled to neurogenesis during peripheral olfactory organ development

AUTHORS: Raphael Aguillon, Romain Madelaine, Marion Aguirrebengoa, Harendra Guturu, Sandra Link, Pascale Dufourcq, Virginie Lecaudey, Gill Bejerano, Patrick Blader, and Julie Batut
ARTICLE TYPE: Research Report

I am happy to tell you that the referees are happy with your revisions and your manuscript has been accepted for publication in Development, pending our standard ethics checks. The referee reports on this version are appended below.

Reviewer 1

Advance summary and potential significance to field

This paper shows how cell specification and cell migration/morphogenesis are coupled. A proneural transcription factor induces cell fate and the guidance system to ensure organ assembly. This is a simple and nice example of how to link cell identity to cell behavior.

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Comments for the author

The authors have addressed all key concerns/suggestions of this reviewer.

Reviewer 2

Advance summary and potential significance to field

In this manuscript the authors investigate how cell fate specification and morphogenetic movements in the olfactory placode of zebrafish are linked.
Specifically, they show a role of the pro-neural transcription factor Neurog1 in regulating the movements of a subpopulation of olfactory neurons by controlling the expression of the chemokine receptor Cxcr4b. Analysing cell movements they find that in the absence of Neurog1 anterior early neurons are delayed to converge to other neurons in the placode. This phenotype resembles cxcr4b mutants as well as mutants of its ligand and Cxcr4b rescues Neurog1 loss of function.
Finally, they show that Neurog1 directly binds to regulatory sequences upstream of cxcr4b and that this element is required for cxcr4b reporter activity. The link between cell fate and morphogenesis is not well established and the paper therefore makes new contributions to our understanding these processes.

Comments for the author

The authors have addressed the points raised and improved the manuscript.

Reviewer 3

Advance summary and potential significance to field

I am now satisfied with the revised version of the paper.

Comments for the author

I am now satisfied with the revised version of the paper.

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