CXCR4 and CXCL12 signaling regulates development of extrinsic innervation to the colorectum

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MS TITLE: CXCR4-CXCL12 signaling regulates development of extrinsic innervation to the hindgut

AUTHORS: Viktória Halasy, Emőke Szőcs, Ádám Soós, Tamás Kovács, Nóra Pecsenye-Fejszák, Ryo Hotta, Allan M. Goldstein and Nándor Nagy

I have now received all the referees' reports on the above manuscript. 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 raised some criticisms and recommend a revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee's comments, and we will look over this and provide further guidance.

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.

Reviewer 1

Advance summary and potential significance to field

This manuscript shows an interesting series of experiments that expand on the mechanisms involved in enteric nervous system (ENS) development, in particular shedding light over the role CXCR4/CXCL12 have in extrinsic innervation of the hindgut. To begin, they characterized the expression of CXCR4 during early chick development and found it to be expressed by vagal NCC at embryonic day 3 (E3). At later developmental time points, CXCR4 is expressed by the Nerve of Remak (NoR) and its neurites extending into the gut, however this expression in NoR is rapidly lost by E14. Through a clever transplant experiment, the authors characterized the contribution of NoR neurites innervating the developing ENS in the hindgut. Additionally, through the combination of in situ hybridization and immunofluorescence, the authors characterized the distribution of CXCR4 and its ligand CXCL12 in the hindgut and cloacal region of developing chick and found CXCR4 to be expressed by NoR and the pelvic ganglia but not in ENCCs migrating into the hindgut. On the other hand, the ligand CXCL12 was found in the NoR surrounding mesenchyme. Interestingly, the expression of this ligand is localized to the submucosal plexus and enteric ganglia when hindgut innervation is occurring.

Importantly, the authors further explored the role CXCR4/CXCL12 signaling plays in gut innervation by using a combination of hindgut explants in vitro and bead implantation in ovo of chick hindgut grafts along with the use of exogenous CXCL12 and a CXCR4 antagonist. With these experiments the authors confirm that CXCR4/CXCL12 signaling is critical for hindgut innervation from the NoR fibers.

Together these data increase our understanding of the cellular mechanism involved in the correct innervation of the hindgut, opening up new knowledge for the ENS development field that can help better understand defects, that have detrimental effects on human patients, and help develop novel therapeutics

Comments for the author

However, the following concerns should be addressed prior to publication:
1. In Figure 1.C,D,E the authors show merged images, having individual channels for CRXC4 and SOX10 can help make the claim clearer.
2. In Figure 4B', the boxed region does not contain all of the actual field of view shown in C.
3. Authors mention "...Some TUJ1+ nerve fibers may be of extrinsic origin from the GFP-negative NoR and some may come from GFP-expressing ENCCs in the hindgut that have differentiated to give rise to enteric ganglia...". Is there a way to distinguish the actual origin or percentage contribution of intrinsic vs extrinsic origin of the nerve fibers? And do intrinsic nerve fibers also respond to similar signals as extrinsic, or their innervation depends on a different pathway?
4. Fix several inconsistencies between main text, figure legends, and figure when showing the embryonic days. For example, in figure 4E-F" in the figure the authors have E10, while in figure legend it says E9.
5. Figure 5C, would benefit form zooming into the region interest (pelvic ganglia) instead of showing the full section, as it would free up space to show individual channels to see the actual expression for P75 and HU overlap.
6. Figure 6C'-C", label the structures in the zoomed in panels. Would make the image easier to interpret especially after the image has been rotated.
7. Authors mention "Cxcl12 did not promote ENCC migration from the intestine". A good experiment to complement and make this claim clearer would be to show a nuclear marker to quantify the cell number along the hindgut explants and compare cell numbers between the different culture conditions.
8. How would the authors explain the increase in Tuj1 staining in the NoR in Figure 9B stronger than Figure 9A.
Additionally, there seems to be a decrease in the complexity in the fiber network in Fig 9B compared to 9A. Is the DMEM incubation alone affecting the NoR and gut innervation?
9. In figure 10F-G authors need to show a representative image of the nerve fiber formation after implanting BSA-coated beads, to see if the technique itself is not affecting the nerve fiber size and numbers, which they represent in the graph but do not show visual proof of.

Reviewer 2

Advance summary and potential significance to field

The manuscript by Halasy et al. aims at providing a better understanding of the mechanisms involved in the extrinsic innervation of the gut during embryogenesis. Using chick embryo as a model, the authors hypothesized that CXCR4-CXCL12 signaling, a pathway previously reported as important in several developmental processes, might be implicated in regulating extrinsic innervation of the gut. This study provided convincing immunohistochemical and in situ hybridization data showing that CXCR4 receptor and its endogenous ligand CXCL12 were complementary in their distribution and well-positioned to regulate axon elongation from the sacral neural crest ganglion to the hindgut. Experiments based on chimeric tissue recombination and gut explant cultures strongly supported a role of CXCR4-CXCL12 signaling in promoting extrinsic axonal outgrowth to the gut. This study provides significant contribution to the under-studied mechanisms underlying gut extrinsic innervation. However, I have several concerns as described below.

Comments for the author

Major comments
1) In Fig. 2D,D',D'', CXCR4 seems to be expressed by P75-positive cells in the myenteric plexus while it is stated in the result description that no CXCR4 expression was associated with ENS cells. The authors might provide a higher magnification of the myenteric plexus to clarify this point.
2) In the grafting experiments of GFP+ vagal neural tube into WT chicken embryos (Fig. 4E,F), it is not clear why at least a fraction of TUJ1+ fibers are not also GFP+. One can expect that some of these TUJ1+ fibers originate from GFP+ ENCCs that have differentiated into enteric neurons and should then be GFP+.
3) Related to Fig. 8B, the authors claim that Cxcl12 did not promote ENCC migration from the intestine. This interpretation might be incorrect as P75 staining was not performed to visualize ENCCs. The fact that TUJ1+ neuronal cell bodies were detected in Fig 8B, the exit of ENCC or differentiated neurons from the intestine is greatly suggested. The authors should better characterize the cell bodies that have migrated outside the gut explant. It is possible that CXCR4-CXCL12 signaling might also be involved in ENCCs migration in this context.
4) The effects shown in Fig. 8 and Fig. 9 should be quantified, with the sample size and statistical analyses. The quantified parameters could be the length or area of extending neurites for Fig.8, the number/length of extrinsic nerve fibers for Fig. 9.
Also related to Fig. 9, the authors claim that 'TUJ1 immunostaining shows that the ENS is able to develop fully even in the presence of CXCR4 signaling inhibition' but the intrinsic neuronal fiber network seems to be affected by AMD3100 treatment, as it appears less dense than in control condition. Quantification of the number of Hu-positive enteric neurons and their neurite density should be performed.
5) For Fig. 10 F and 10 G, a picture of the control condition, hindgut with BSA beads, should be included with the same orientation as panels 10F/G.

Minor comments
1) Scale bars are missing in all the figures.
2) The arrowhead in Fig 1A'' pointing at CXCR4+/Sox10- intersomitic blood vessels is not well positioned.

Reviewer 3

Advance summary and potential significance to field

Halasy and colleagues present a really interesting study investigating the role of CXCR4-CXCL12 signalling in the extrinsic innervation of the colorectum. The authors observed CXCR4 expression in avian vagal neural crest cells and the nerve of Remak as well the pelvic ganglia of embryonic avians and mammals, and its cognate ligand CXCL12 in the hindgut mesenchyme and intrinsic ganglia. The authors found that perturbations of CXCR4/CXCL12 signalling in gut-NoR explants altered innervation of the prospective colorectum.
Understanding the guidance factors promoting innervation of the gut is important in the context of gut motility disorders such as Hirschsprung disease, where there is a failure of neural crest cells to migrate to the colon. In this study the authors highlight the important role of CXCR4-CXCL12 signalling.

Comments for the author

Main points:
1. Several times in the results, expression levels are referred to.
pg 5: “CXCR4 was prominent on NCCs at the somite level, but its expression decreased as crest cells…”
Pg 6: “By E14 (HH40), CXCR4 expression was significantly reduced…”
Was expression quantified? Was intensity or threshold measured? Or was it simply observing reduced immunoreactivity? If the latter, the text should be changed to reflect this.
2. On page 7: “To characterize the immunophenotype of the P75+ cells further double-immunostaining was performed. Hu immunohistochemistry showed the presence of large number of neurons in the P75+ pelvic ganglia (Fig. 5C).”
If a further characterisation was performed, I would expect to see more markers examined other than Hu. e.g. noradrenergic neurons (Tyrosine Hydroxylase)
3. On page 11: “CXCR4high” and “CXCR4low”.
How was this determined? Was a threshold set? I would like to see some quantification.
4. In Figure 4: “After 7 days of CAM culture many chickGFP NoR-derived fibers enter the hindgut.”
If no quantification was performed, I would prefer something like
“immunoreactive fibres were observed” as opposed to “many.”
5. The chemical perturbation experiments are really nice, and inform the role of this signalling pathway in extrinsic colorectal innervation. Did the authors examine the ability of chickGFP NoR to innervate an aneural donor hindgut; in chimeric hindgut recombinants? This could provide some insights into patterns of extrinsic innervation seen in Hirschsprung patient aganglionic bowel.
Minor:
1. There are several statements made throughout the introduction and discussion.
e.g. “These enteric neural crest-derived cells (ENCCs) migrate and proliferate prior to differentiating into neuronal and glial subtypes.”
e.g. “Interestingly, HSCR is often associated with the presence of hypertrophic extrinsic nerve fibers in the aganglionic segment”
While these statements are well known to those in the field, it would still be nice to see relevant literature cited.
2. pg 4 “Disrupting CXCL12/CXCR4 signalling causes abnormal migration of cardiac neural crest cells?”
3. pg 14 “were obtained from the? Dr. Zoltán Jakus”
4. pg 16 “0.1% BSA” instead of “0,1% BSA”
5. Was PFA was used to fix tissue, how long was it used for?
6. In Figure 10 graph H, what is the scale/measurement of the Y-Axis.
7. Please add a scale bar to all figures.

First revision

Author response to reviewers' comments

Reviewer 1.

1. In Figure 1.C,D,E the authors show merged images, having individual channels for CRXC4 and SOX10 can help make the claim clearer.
Re: In response to this comment, we added 3 new figures (D'-D'") to Fig. 1, showing individual channels for CXCR4, SOX10 and DAPI.

2. In Figure 4B’, the boxed region does not contain all of the actual field of view shown in C.
Re: Thank you for the observation, the boxed region was extended to contain the whole field of view shown in image C.

3. Authors mention “...Some TUJ1+ nerve fibers may be of extrinsic origin from the GFP-negative NoR and some may come from GFP-expressing ENCCs in the hindgut that have differentiated to give rise to enteric ganglia...”. Is there a way to distinguish the actual origin or percentage contribution

of intrinsic vs extrinsic origin of the nerve fibers? And do intrinsic nerve fibers also respond to similar signals as extrinsic, or their innervation depends on a different pathway?

Re: We appreciate this important point. To distinguish the percentage contribution of intrinsic versus extrinsic origin we measured the number of GFP+/TUJ1+ nerve fibers at interplexus region and compared with GFP-/TUJ1+ fibers. Based on our results, we conclude that in case of GFP+ NoR transplanted CAM grafts the percentage results showed that TUJ1 immunoreactivity had a significantly higher positive rate in GFP- nerve fibers (68.03±9.00%) compared to GFP+ nerve fibers (31.96±9.00%) (Fig. 4D).
In response to the second question, we added a new Supplemental Fig 2, which show that CXCL12 alone is not chemoattractive for ENCCs. The role of GDNF to promote ENCC migration is known and demonstrated again here in Supplemental Fig 2B. This has been added to Results section.

4. Fix several inconsistencies between main text, figure legends, and figure when showing the embryonic days. For example, in figure 4E-F" in the figure the authors have E10, while in figure legend it says E9.
re: Thank you for the observation, we have corrected all inconsistencies.

5. Figure 5C, would benefit from zooming into the region interest (pelvic ganglia) instead of showing the full section, as it would free up space to show individual channels to see the actual expression for P75 and HU overlap.

re: We agree with the Reviewer's comment and in the revised version of the manuscript, we have included a new figure (Supplemental Fig 1) where we show the separate channels of double immunofluorescence staining performed with anti-HU, -neurofilament, -nNOS and, -P75 antibodies.

6. Figure 6C'-C", label the structures in the zoomed in panels. Would make the image easier to interpret, especially after the image has been rotated.

re: Thank you for the suggestion, structures were labeled as suggested.

7. Authors mention "Cxcl12 did not promote ENCC migration from the intestine". A good experiment to complement and make this claim clearer would be to show a nuclear marker to quantify the cell number along the hindgut explants and compare cell numbers between the different culture conditions.

re: We appreciate the reviewer's advice. To quantify the cell numbers along the hindgut explants we have performed DAPI labeling combined with anti-SOX10 staining (n=5/culture conditions) and counted the SOX10+/DAPI+ cells in two 300x900 µm sized stripes/gut. Results were added as insets on Fig 8B and a new graph (see Fig8D,E).

8. How would the authors explain the increase in Tuj1 staining in the NoR in Figure 9B stronger than Figure 9A. Additionally, there seems to be a decrease in the complexity in the fiber network in Fig 9B compared to 9A. Is the DMEM incubation alone affecting the NoR and gut innervation?

re: We thank the reviewer for this observation. The weaker Tuj1 staining in Fig. 9A compared to Fig. 9B was due to improper microscopic settings. We repeated the wholemount immunostaining (n=5) and replaced Fig. 9A with a representative image.

9. In figure 10F-G authors need to show a representative image of the nerve fiber formation after implanting BSA-coated beads, to see if the technique itself is not affecting the nerve fiber size and numbers, which they represent in the graph but do not show visual proof of.

re: Thank you for this suggestion. We have provided the picture of control (BSA) bead transplanted hindgut for Fig. 10F.

Reviewer 2.

1) In Fig. 2D,D',D'', CXCR4 seems to be expressed by P75-positive cells in the myenteric plexus while it is stated in the result description that no CXCR4 expression was associated with ENS cells. The authors might provide a higher magnification of the myenteric plexus to clarify this point.

re: We agree with the reviewer's suggestion. To clarify this point we performed double immunostaining for anti-Hu, anti-CXCR4 and added Figure E, E', E" to Fig. 2. The Hu+ enteric neurons do not express CXCR4, however the extrinsic fiber network that reaches the enteric ganglia is CXCR4 positive.

2) In the grafting experiments of GFP+ vagal neural tube into WT chicken embryos (Fig. 4E,F), it is not clear why at least a fraction of TUJ1+ fibers are not also GFP+. One can expect that some of these TUJ1+ fibers originate from GFP+ ENCCs that have differentiated into enteric neurons and should then be GFP+.
re: Thank you for this comment and we apologize for the confusion. We have now clearly marked with arrowheads (Fig4G,G') the presence of GFP+/TUJ1+ nerve fibers in the interplexus region and added a new panel G" to show the GFP-/TUJ1+nerve fibers.
To distinguish the percentage contribution of intrinsic versus extrinsic origin we measured the number of GFP+/TUJ1+ nerve fibers at interplexus region and compared with GFP-/TUJ1+ fibers. Based on our results, we conclude that in case of GFP+ NoR transplanted CAM grafts the percentage results showed that TUJ1 immunoreactivity had a significantly higher positive rate in GFP- nerve fibers (68,03±9,00%) compared to GFP+ nerve fibers (31,96±9,00%) (Fig. 4D).

3) Related to Fig. 8B, the authors claim that Cxcl12 did not promote ENCC migration from the intestine. This interpretation might be incorrect as P75 staining was not performed to visualize ENCCs. The fact that TUJ1+ neuronal cell bodies were detected in Fig 8B, the exit of ENCC or differentiated neurons from the intestine is greatly suggested. The authors should better characterize the cell bodies that have migrated outside the gut explant. It is possible that CXCR4-CXCL12 signaling might also be involved in ENCCs migration in this context.

re: We appreciate this recommendation from the Reviewer. To characterize the effect of CXCL12 on the migration of nerve of Remak and ENCCs we have performed wholemount anti-SOX10 immunostaining of the gut explant and quantified the emigrated SOX10+ cells. Compared to the DMEM and AMD3100 treated cultures, where a small number of SOX10+ cells are seen migrating a short distance away from the gut, in case of the CXCL12 treated explants significantly higher SOX10+ cells migrated out from the hindgut and nerve of Remak and were always associated with the extending TUJ1+ neurites. We have added insets to Fig.8B and a new graph E to Fig8. In response to the second comment, we added a new Supplemental Fig 2, which show that CXCL12 alone is not chemoattractive for ENCCs. The role of GDNF to promote ENCC migration is known and demonstrated again here in Supplemental Fig 2B. This has been added to Results section.

4) The effects shown in Fig. 8 and Fig. 9 should be quantified, with the sample size and statistical analyses. The quantified parameters could be the length or area of extending neurites for Fig.8, the number/length of extrinsic nerve fibers for Fig. 9.

re: We agree with the reviewer's suggestion. To quantify the effects shown in Figs 8 and 9 we have measured the number of SOX10+ cells emigrating from the explants, the length of extending TUJ1+ neurites, number of HU+ neurons and number of CN+ nerve fibers in the different culture conditions (n=5/culture condition), and we added the a new graph E and D to Fig8 and a new graph G to Fig9.

Also related to Fig. 9, the authors claim that 'TUJ1 immunostaining shows that the ENS is able to develop fully even in the presence of CXCR4 signaling inhibition' but the intrinsic neuronal fiber network seems to be affected by AMD3100 treatment, as it appears less dense than in control condition. Quantification of the number of Hu-positive enteric neurons and their neurite density should be performed.

re: To answer this question we performed double immunostaining for anti-Hu and anti-CN on the cross sections of catenary cultures. We quantified the number of HU+ cells, the TUJ1+ neurite

density and the number of CN+ neurites and added a new graph G to Fig. 9. We have also added a new paragraph (page 10) to the Results section to address this important comment.

5) For Fig. 10 F and 10 G, a picture of the control condition, hindgut with BSA beads, should be included with the same orientation as panels 10F/G.

re: Thank you for this suggestion. We have provided the picture of control (BSA) bead transplanted hindgut for Fig. 10.

Minor comments
1) Scale bars are missing in all the figures.

re: We appreciate this recommendation and the scale bars were added to all figures.

2) The arrowhead in Fig 1A'' pointing at CXCR4+/Sox10- intersomitic blood vessels is not well positioned.

re: Thank you for the observation, the arrowheads were repositioned.

Reviewer 3. Advance summary and potential significance to fieldReviewer 3. Comments for the authorMain points:
1. Several times in the results, expression levels are referred to.
Pg 5: "CXCR4 was prominent on NCCs at the somite level, but its expression decreased as crest cells…"
Pg 6: "By E14 (HH40), CXCR4 expression was significantly reduced…"
Was expression quantified? Was intensity or threshold measured? Or was it simply observing reduced immunoreactivity? If the latter, the text should be changed to reflect this.
re: Thank you for this suggestion. The Results section has been modified accordingly. See also Response to comment #3.

2. On page 7: "To characterize the immunophenotype of the P75+ cells further, double-immunostaining was performed. Hu immunohistochemistry showed the presence of large number of neurons in the P75+ pelvic ganglia (Fig. 5C)."If a further characterization was performed, I would expect to see more markers examined other than Hu. e.g. noradrenergic neurons (Tyrosine Hydroxylase).

re: We appreciate this valuable suggestion. According to previous reports, while the vagal neural crest cells are migrating rostrocaudally through the foregut and midgut less than 15% of the cells transiently express tyrosine hydroxylase (TH) (Young et al., 2002, J Neurosci), TH expressing vagal or sacral NCC-derived cells are never observed in the avian hindgut (Cochard et al., 1978; Young and Newgreen, 2000, Anat Rec).
Therefore, in order to further characterize the immunophenotype of P75+/CXCR4+ pelvic ganglia a new Supplemental Fig. 1 was added to show the results of neurofilament and nNOS expression.

3. On page 11: "CXCR4high" and "CXCR4low".
How was this determined? Was a threshold set? I would like to see some quantification.

re: Fluorescent images were analyzed in Image J, as previously described by Shihan et al (Biochem Biophys Rep. 2021 Mar; 25: 100916.). Three tissue sections from selected embryos were collected. For the immunofluorescence analysis, mean gray value were analyzed using Image J software. After converting the image to black and white with 8-bit type, the threshold of the image was adjusted (Image-Adjust-Threshold-Apply); then the area of the CXCR4+ vagal crest cells was measured and recorded (Analyze-Measure). The mean gray value was automatically calculated using image J. Quantification of the intensity values is shown in Figure 1B.

4. In Figure 4: "After 7 days of CAM culture many chickGFP NoR-derived fibers enter the hindgut."
If no quantification was performed, I would prefer something like "immunoreactive fibres were observed" as opposed to "many."
Re: we appreciate this comment and performed more detailed analysis of TUJ1+ nerve fibers, as shown in Fig. 4D

5. The chemical perturbation experiments are really nice, and inform the role of this signalling pathway in extrinsic colorectal innervation. Did the authors examine the ability of chickGFP NoR to innervate an aneural donor hindgut; in chimeric hindgut recombinants? This could provide some insights into patterns of extrinsic innervation seen in Hirschsprung patient aganglionic bowel.
re: As illustrated in Fig. 10 B,C when segments of distal gut, excluding ceca, were cultured on the CAM for 8 days generated aganglionic hindguts. As shown in Fig. 10B.C, aganglionic grafts no HU+ neuronal or BFABP glial cells were found within the wall of the intestine. Despite the absence of intramural neurons, TUJ1 immunostaining was present in the hindgut+NoR grafts, representing neuronal processes emanating from the NoR. SOX10+ cells (Fig. 10D,E) were always associated to TUJ1+ nerve fibers.

Dissection of the preganglionic E5 hindgut alone, without midgut, nerve of Remak, cloaca, followed by 9 days on the CAM, leads to a well-developed graft, with normal development of smooth muscle, but no TUJ1+ nerve fibers or HU+ enteric neurons (Nagy et al., 2007, Developmental Dynamics).

Minor:
1. There are several statements made throughout the introduction and discussion.
e.g. “These enteric neural crest-derived cells (ENCCs) migrate and proliferate prior to differentiating into neuronal and glial subtypes.”
e.g. “Interestingly, HSCR is often associated with the presence of hypertrophic extrinsic nerve fibers in the aganglionic segment”
While these statements are well known to those in the field, it would still be nice to see relevant literature cited.
re: Thank you. We have added the relevant references as suggested.

2. pg 4 “Disrupting CXCL12/CXCR4 signalling causes abnormal migration of cardiac neural crest cells?”
re: Thank you for pointing this out, we added the word “cells’’ to the sentence.

3. pg 14 “were obtained from the? Dr. Zoltán Jakus”
re: We apologize for this author name and have corrected the source of animals.

4. pg 16 “0.1% BSA” instead of “0,1% BSA”
re: Corrected, many thanks.

5. Was PFA was used to fix tissue, how long was it used for?
re: All tissues were fixed overnight at 4oC in 4% PFA, we added this information to the Materials and Methods section.

6. In Figure 10 graph H, what is the scale/measurement of the Y-Axis.
re: Thank you for pointing out this omission. We measured the fiber density around the implanted microbeads. We added the relevant information to the graph.

7. Please add a scale bar to all figures.
re: Scale bars were added to all figures, and the figure legends were updated accordingly.

Second decision letter

MS ID#: DEVELOP/2022/201289

MS TITLE: CXCR4-CXCL12 signaling regulates development of extrinsic innervation to the colorectum

AUTHORS: Viktória Halasy, Emőke Szőcs, Ádám Soós, Tamás Kovács, Nóra Pecsenye-Fejszák, Ryo Hotta, Allan M. Goldstein and Nándor Nagy

ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

This manuscript shows an interesting series of experiments that expand on the mechanisms involved in enteric nervous system (ENS) development, in particular shedding light over the role CXCR4/CXCL12 have in extrinsic innervation of the hindgut in chick. CXCR4 is expressed by the Nerve of Remak (NoR) and its neurites extending into the gut, however this expression in NoR is rapidly lost by E14. On the other hand, the ligand CXCL12 was found in the NoR surrounding mesenchyme and the submucosal plexus and enteric ganglia when hindgut innervation is occurring. Importantly, the authors further explored the role CXCR4/CXCL12 signaling plays in gut innervation by using a combination of hindgut explants in vitro and bead implantation in ovo of chick hindgut grafts along with the use of exogenous CXCL12 and a CXCR4 antagonist.

With these experiments the authors confirm that CXCR4/CXCL12 signaling is critical for hindgut innervation from the NoR fibers. Together these data increase our understanding of the cellular mechanism involved in the correct innervation of the hindgut, opening up new knowledge for the ENS development field that can help better understand defects, that have detrimental effects on human patients, and help develop novel therapeutics.

Comments for the author

The authors have addressed all prior comment/concerns to satisfaction.