Dentate gyrus morphogenesis is regulated by β-CATENIN function in hem-derived fimbrial glia

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DOI: 10.1242/dev.200953

Editor: Francois Guillemot

Review timeline
Original submission: 24 May 2022
Editorial decision: 5 July 2022
First revision received: 15 September 2022
Accepted: 22 September 2022

Original submission

First decision letter

MS ID#: DEVELOP/2022/200953

MS TITLE: Dentate gyrus morphogenesis is regulated by β-CATENIN function in hem-derived fimbrial glia.

AUTHORS: Arpan Parichha, Debarpita Datta, Varun Suresh, Mallika Chatterjee, Michael J. Holtzman, and Shubha Tole

I have now received the reports of three referees on your manuscript and I have reached a decision. The reports are appended below and 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, all the referees are enthusiastic about your work, but they also have significant criticisms and recommend a substantial 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 to receive a revised version of the manuscript. Your revised paper will be re-reviewed by 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

In their manuscript, Parichha investigate the role of ß-catenin in controlling the morphogenesis of the dentate gyrus. Using a conditional gene inactivation approach in mice, they show that ß-catenin deletion in the cortical hem and its derivatives, the Cajal-Retzius (CR) cells and the fimbrial glial scaffold, led to the formation of an ectopic protrusion from the ventricular surface of the hem. The glial scaffold of the fimbria was disorganized and migration of dentate gyrus progenitors along the dentate migratory stream was disturbed. Instead, ectopic glial projection as well as Tbr2+ progenitors, CR neurons and Prox1+ dentate gyrus granule cells were found in this protrusion. The authors also demonstrate differential gene expression and reduced N-Cadherin protein levels at adherens junctions in the cortical hem. Finally, conditional ß-catenin inactivation specifically in CR neurons did not cause a dentate gyrus phenotype. Taken together, these data suggest that ß-catenin mediated, altered gene expression and cell adhesion in glial scaffold cells might underlie the defective dentate gyrus formation.

The dentate gyrus as part of the hippocampal formation plays an important role in memory formation. It also is a site of adult neurogenesis being one of the few locations in the adult mammalian brain containing adult neural stem cells. Given these critical functions, it is important to investigate the currently poorly understood cellular and molecular processes that control dentate development. Hence, the manuscript by Parichha et al. address an important topic that will be great interest for the readers of Development.

Comments for the author

Overall, this manuscript makes an important contribution to understanding the development of the dentate gyrus. Their findings are supported by convincing, high quality images, but there are a few points the authors need to address to warrant publication in Development.

1. The authors claim that defects in the fimbrial glial scaffold are the cause of the dentate malformation in conditional ß-catenin mutants, but they do not consider much the ectopic protrusion which maybe the primary cause of the dentate phenotype. Ultimately, many, if not most, Tbr2+ progenitors, CR neurons and Prox1+ dentate gyrus granule cells are present in this structure, but what are the causes for its formation? Is it reduced cell adhesion or increased proliferation of cortical progenitors? Do similar protrusions occur in other mutants with ß-catenin conditional inactivation in the forebrain? The authors could also present a time course analysis investigating whether disruption of the glial scaffold precedes the formation of the protrusion or vice versa.
2. ß-catenin has important functions in controlling cell adhesion and canonical Wnt signalling mediated transcriptional regulation. It is difficult to distinguish between these roles, but the authors should shed more light on this issue. For example, they could investigate their mRNAseq data set which cellular processes and pathways are affected in the mutant. They should also confirm the altered expression of the Wnt target genes by in situ hybridisation or qRT-PCR. Not only the localisation of N-Cadherin but also its mRNA expression might be affected. A better comparison with Wnt signalling mutants might also be helpful, since these mutants appear to have a milder phenotype lacking the ectopic protrusion.
3. The authors should at least discuss the possibility that gene expression changes in the cortical hem may alter hem signalling and may thereby affect the specification of progenitors in the dentate neuroepithelium. This misspecification could indirectly lead to migration defects of these progenitors and/or of specified dentate granule cells.
4. The manuscript would benefit from a better structure. The data showing the successful inactivation of ß-catenin should be presented before the description of the phenotype, whereas the paragraph on the transcriptional/cell adhesion roles would be better suited for the end of the paper.

Reviewer 2

Advance summary and potential significance to field

Using conditional mutant mice, the authors have convincingly shown that beta-catenin plays an essential role within a specific set of glia in the developing cortical hem for the development of the dentate gyrus. The dorsomedial region of the telencephalon is important for the development of critical brain structures including the dentate gyrus and the choroid plexus. Part of this

structure, the cortical hem, has long been understood as critical for forebrain development and is believed to act primarily as a signalling centre, producing a complex cocktail of Wnts and BMPs that provide instructions to neighbouring cells. Despite the apparent anatomical simplicity of the region (at least when compared to other parts of the forebrain), elucidating the precise molecular pathways that govern development of the cortical midline region has proven challenging and many questions remain unanswered. This paper makes a specific contribution, by showing that the multifunctional molecule beta-catenin is required within fimbrial glia to permit correct migration of dentate granule neurons which arise from progenitors located adjacent to the hem to their final destination in the dentate gyrus.
This adds an interesting additional piece to the intriguing jigsaw puzzle of cortical midline development.

Comments for the author

I found this to be a clear, well written manuscript that presents interesting findings and that the authors' conclusions are supported by the data presented. I suggest only the following relatively minor changes mostly to help make the manuscript more easily understandable to a wider audience. Points are listed in the order in which they appear in the manuscript - Point 6 is the most important.
1. Line 44: state that Ai9 is an RFP-expressing cre-reporter strain (so reader doesn't have to look it up)
2. Figure 1 and accompanying text. Where is beta-catenin expressed in the midline at these stages? All cells or just some regions? It seems important to establish this first, before conducting cell type-specific experiments.
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4. As an aide to readers not already intimately familiar with the anatomy of this region, it would be helpful to mark the location of the marginal zone (MZ) on the images in panels 2A-D. In general, I think that labelling specific anatomical structures is helpful in these kinds of figures.
5. Line 118 and Figure S2. The RNAseq analysis is very interesting, but is hardly mentioned. Could the authors expand, to say more about the gene expression changes that they found, or relevant genes whose expression didn't change? It would also be good to see in situ or immuno confirmation of at least a few of the Wnt pathway components whose expression changed in the conditional mutants. This would give useful spatial information regarding gene expression levels, rather than just tissue-level quantitation.
6. I didn't really understand the N-cadherin expression analysis. It wasn't clear to me what the authors were trying to show with this data, exactly how it was measured or what the significance of the reported change in expression profile means (in the context of beta-catenin's two activities) – these should all be explained more clearly.
7. Line 143: The positions of the primary, secondary and tertiary matrices should be clearly indicated on the relevant panels in Figure 3.
In terms of potential future work, beyond the scope of the current paper, it would be very interesting to use the beta-catenin conditional mutant strain described by Valenta et al., 2011 (doi: 10.1101/gad.181289.111),
which retains beta-catenin's cell adhesion function but lacks transcriptional activity, to better distinguish between the adhesion and Wnt signalling activities of beta-catenin in the hem.

Reviewer 3

Advance summary and potential significance to field

Parichha et al. report disruption of b-catenin in the hem of mice. They find that this disruption leads to a disorganization of the glia scaffold, which in turn leads to an impairment in dentate migration. There are several comments that should be addressed:

Comments for the author

- Could the authors quantify the extent of the morphological phenotype on the scaffold? Perhaps quantification of the length and a measure of continuity of the scaffold would better reveal the extent of phenotypes. This might be best done at earlier stages, where it could ideally be done at a cellular level (E12 for example).

- Since b-catenin is expected to affect cell proliferation (for example Chenn and Walsh, 2002), it would be important to properly examine the proliferation in the LOF (perhaps with an EdU injection), especially in the context of the ectopic protrusions that the authors describe.
- Figure 2F. I don't find this graph as the most informative way to present these data. Is the total number of TRP73+ cells equal between the control and LOF?
Wouldn't it be better to calculate the # TRP73+ over DAPI and present the distribution of TRP73+ between MZ and outside in a stacked bar so that the numbers that the authors refer to in the text are actually visible on the graph in a clearer manner? The same is true for graphs in Figure 3.

First revision

Author response to reviewers' comments

We thank the reviewers for their careful reading and thoughtful comments. Please find our pointwise responses below (Modified texts are in blue).

Reviewer 1
Reviewer 1 Advance Summary and Potential Significance to Field:
In their manuscript, Parichha investigate the role of -catenin in controlling the morphogenesis of the dentate gyrus. Using a conditional gene inactivation approach in mice, they show that -catenin deletion in the cortical hem and its derivatives, the Cajal-Retzius (CR) cells and the fimbrial glial scaffold, led to the formation of an ectopic protrusion from the ventricular surface of the hem. The glial scaffold of the fimbria was disorganized and migration of dentate gyrus progenitors along the dentate migratory stream was disturbed. Instead, ectopic glial projection as well as Tbr2+ progenitors, CR neurons and Prox1+ dentate gyrus granule cells were found in this protrusion. The authors also demonstrate differential gene expression and reduced N- Cadherin protein levels at adherens junctions in the cortical hem. Finally, conditional -catenin inactivation specifically in CR neurons did not cause a dentate gyrus phenotype. Taken together, these data suggest that -catenin mediated, altered gene expression and cell adhesion in glial scaffold cells might underlie the defective dentate gyrus formation.

The dentate gyrus as part of the hippocampal formation plays an important role in memory formation. It also is a site of adult neurogenesis being one of the few locations in the adult mammalian brain containing adult neural stem cells. Given these critical functions, it is important to investigate the currently poorly understood cellular and molecular processes that control dentate development. Hence, the manuscript by Parichha et al. address an important topic that will be great interest for the readers of Development.

Response: We thank the reviewer for his/ her appreciation of our study.

Reviewer 1 Comments for the Author:
Overall, this manuscript makes an important contribution to understanding the development of the dentate gyrus. Their findings are supported by convincing, high-quality images, but there are a few points the authors need to address to warrant publication in Development.

Reviewer's comment:
1. The authors claim that defects in the fimbrial glial scaffold are the cause of the dentate malformation in conditional b-catenin mutants, but they do not consider much the ectopic protrusion which maybe the primary cause of the dentate phenotype.
Response: The ectopic protrusion consists of primarily glial scaffold cells. We have rephrased the description to communicate this better **(Lines 70-73):** "In E12.5 Lmx1aCre::ß-Catenin LOF brains, the BLBP-positive fimbrial glial scaffold was no longer confined to the region of the hem as it was in the controls (Fig. 2B). Instead, BLBP+ cells formed an ectopic protrusion into the ventricle just above the choroid plexus (Fig. 2B-C, E)"

(Lines 216-223)
"In the absence of ß-CATENIN, the fimbrial glial scaffold is disorganized and forms an ectopic ventricular protrusion as early as E12.5, prior to the migration of Prox1+ dentate granule cells which begins at E14.5. Both, dentate granule cells and CR cells depend on the glial scaffold for their proper migration and both these cell types migrate into the protrusion instead (Fig. 4, Fig. S7, Supplementary movie 1). This disrupted migration does not improve with time, and the dentate gyrus fails to form. Therefore, the protrusion which is composed of disorganized glial scaffold cells in Lmx1aCre::ß-Catenin LOF brains is the major and proximate cause of the profoundly defective morphogenesis of the dentate gyrus."

Reviewer's comment:

Ultimately, many, if not most, Tbr2+ progenitors, CR neurons and Prox1+ dentate gyrus granule cells are present in this structure, but what are the causes for its formation? Is it reduced cell adhesion or increased proliferation of cortical progenitors?

Response: It is difficult to ascertain the molecular or mechanistic cause of an ectopic protrusion, but we expect it is linked to changes in cell adhesion, brought out in the altered distribution of N-CADHERIN and changes in ß-CATENIN mediated transcription (Fig. 2I-K, Fig. S3B & H, Fig. S5). It is also possible that ß-CATENIN mediated transcriptional change leads to alteration in factors that regulates fimbrial scaffold morphology, and this has resulted in the disorganized structure. We have rephrased the text to bring this out better.

(Lines 161-167)
"These results suggested that the transcriptional dysregulation of factors that control fimbrial scaffold morphogenesis and/or perturbed cell adhesion as a result of altered N-CADHERIN distribution may underlie the disruption of the fimbrial glial scaffold resulting in the ectopic protrusion seen in ß-Catenin LOF brains. A comparison with ß-Catenin LOF selective to its transcriptional role, leaving its cell adhesion intact (Valenta et al., 2011), would be a useful additional study in this context and may help to identify novel ß-CATENIN targets responsible for proper orientation and positioning of the fimbrial scaffold."

(Lines 223-227)
"While deficits in the organization of glia in the dentate region have been reported in some canonical Wnt pathway mutants such Lrp6 and Lef1 (Zhou et al., 2004), neither of these, nor any known mutant that lacks a component of adherens junctions (Zhang et al., 2013), displays such extreme disorganization as that seen in Lmx1aCre::ß-Catenin LOF brains."

We have investigated whether the ectopic blob is a result of increased proliferation of progenitors in the hem by performing immunostaining for well-established proliferation markers PHH3 & Ki67. We have included this data in Fig. S1F-I. In the text we have added this as "We examined whether this protrusion arose as a result of excessive proliferation of the ß-Catenin LOF hem progenitors. However, both PHH3 and Ki67 immunostaining revealed no apparent change in proliferation (Fig. S1F-I)" **(Lines 73-76)**

Reviewer's comment:
Do similar protrusions occur in other mutants with -catenin conditional inactivation in the forebrain?

Response: To our knowledge no other mutant displays such a pronounced disorganization of the fimbrial scaffold. We have included this discussion in (Lines 223-229)

"While deficits in the organization of glia in the dentate region have been reported in some canonical Wnt pathway mutants such Lrp6 and Lef1 (Zhou et al., 2004), neither of these, nor any known mutant that lacks a component of adherens junctions (Zhang et al., 2013), displays such extreme disorganization as that seen in Lmx1aCre::ß-Catenin LOF brains. These results highlight the function of a single factor, ß-CATENIN, as a key regulator of fimbrial scaffold organization, which ultimately controls the morphogenesis of a key hippocampal structure, the dentate gyrus."

Reviewer's comment:
The authors could also present a time course analysis investigating whether disruption of the glial scaffold precedes the formation of the protrusion or vice versa.

Response: We have added a new supplementary figure (Figure S2A) that contains a comprehensive timeline displaying the glial scaffold defect. The fimbrial scaffold defect is seen at E12.5 which precedes the initiation of the dentate migration which begins at E14.5. We have included this angle to the text.

(Lines 86-90)
"A comprehensive time-course panel from E11.5 to postnatal day (P)2 reveals misoriented fibers revealed by Glial Fibrillary Acidic Protein (GFAP) or BLBP labeling, in contrast to the well-organized alignment of these fibers in control brains (Fig. 2H; Fig. S2A). REELIN-positive cells were mislocalized within the protruded glial mass at all stages examined (Fig. 2G; Fig S2B)."

Reviewer's comment:

2. beta-catenin has important functions in controlling cell adhesion and canonical Wnt signalling mediated transcriptional regulation. It is difficult to distinguish between these roles, but the authors should shed more light on this issue. For example, they could investigate their mRNAseq data set which cellular processes and pathways are affected in the mutant. They should also confirm the altered expression of the Wnt target genes by in situ hybridisation or qRT-PCR.

Response: In Supplementary Figure S3, we present a detailed analysis of the RNAseq dataset.

a. We have plotted significantly upregulated or downregulated GO:BP terms, which reveal that both "Canonical Wnt signaling pathway" and "Positive regulation of cell junction assembly" are significantly downregulated upon loss of beta-catenin.
b. The top 50 differentially expressed genes (DEGs) are presented in a heatmap. This list includes several canonical Wnt signaling target genes that are down regulated in the mutant, indicating β-CATENIN mediated transcription has been altered.
c. Specific Wnt target genes *Sost, Wnt3, Fzd10, Runx2, Psck6, Dkk1, Notum,* and *Axin2* are significantly downregulated.
We have validated canonical Wnt target *Axin2* by in situ hybridization and LEF1 by immunohistochemistry and show that they are undetectable upon loss of β-CATENIN in hem.

Not only the localisation of N-Cadherin but also its mRNA expression might be affected. A better comparison with Wnt signalling mutants might also be helpful, since these mutants appear to have a milder phenotype lacking the ectopic protrusion.

Response: From the RNA-seq data we have analyzed the expression levels of NCAD and found that it did not change significantly. We have added this in the supplementary Fig. S5F & G. We have expanded our description of N-CADHERIN protein localization and included a sentence about mRNA levels in:

(Lines 156-167)
"In β-Catenin LOF brains, there was a marked flattening of the peak intensity at cell boundaries, indicating a diffused distribution of N-CADHERIN, and suggesting altered cell-cell adhesion, although the mRNA levels of *Cdh2,* which encodes N-CADHERIN, appear unaltered (Fig. 2K, Fig. S5F and G). These results suggested that the transcriptional dysregulation of factors that control fimbrial scaffold morphogenesis and/or perturbed cell adhesion as a result of altered N- CADHERIN distribution may underlie the disruption of the fimbrial glial scaffold resulting in the ectopic protrusion seen in β-Catenin LOF brains. A comparison with β-Catenin LOF selective to its transcriptional role, leaving its cell adhesion intact (Valenta et al., 2011), would be a useful additional study in this context and may help to identify novel β-CATENIN targets responsible for proper orientation and positioning of the fimbrial scaffold."

(Lines 223-229)
"While deficits in the organization of glia in the dentate region have been reported in some canonical Wnt pathway mutants such Lrp6 and Lef1 (Zhou et al., 2004), neither of these, nor any known mutant that lacks a component of adherens junctions (Zhang et al., 2013), displays such extreme disorganization as that seen in Lmx1aCre::β-Catenin LOF brains. These results highlight the function of a single factor, β-CATENIN, as a key regulator of fimbrial scaffold organization, which ultimately controls the morphogenesis of a key hippocampal structure, the dentate gyrus."

Reviewer's comment:
3. The authors should at least discuss the possibility that gene expression changes in the cortical hem may alter hem signalling and may thereby affect the specification of progenitors in the dentate neuroepithelium. This misspecification could indirectly lead to migration defects of these progenitors and/or of specified dentate granule cells.

Response: Thank you for this point. We have included this analysis in a new panel (Figure S6D, H) that gave an interesting contrast in the TBR2+ and PROX1+ population which we have included in the results.

(Lines 194-201)
"The TBR2+ intermediate progenitor population in the 2ry and 3ry matrix of β-Catenin LOF brains was similar to that in controls (Figure S6D. However, the total number of PROX1 cells in the β-Catenin LOF brain was significantly less than that in controls (Figure 3G; Supplementary Figure S6H), suggesting that reduced numbers of DG progenitors were specified, or their proliferation/ survival was affected. DG specification requires Wnt signaling from the hem (Lee et al., 2000). While it appears that DG cell fate was specified at least in terms of PROX1, the underlying basis of the reduction in PROX1+ cells is an important angle for further studies focused on the mechanism of specification of DG cells."

Reviewer's comment 4: The manuscript would benefit from a better structure. The data showing the successful inactivation of -catenin should be presented before the description of the phenotype, whereas the paragraph on the transcriptional/cell adhesion roles would be better suited for the end of the paper.

Response: We agree with the first point, and we have moved the relevant panels from Supplementary Figure 1 to the main Figure 1 (Fig 1E-G). We think that the two different roles of β-CATENIN are better discussed after the first description of the mutant phenotype in Figure 2 because it is useful for the reader to have this dual function and the back of his/her mind when presented with subsequent analyses of Cajal-Retzius cells and dentate migration disruption.

Reviewer 2

Reviewer 2 Advance Summary and Potential Significance to Field:
Using conditional mutant mice, the authors have convincingly shown that beta-catenin plays an essential role within a specific set of glia in the developing cortical hem for the development of the dentate gyrus. The dorsomedial region of the telencephalon is important for the development of critical brain structures including the dentate gyrus and the choroid plexus. Part of this structure, the cortical hem, has long been understood as critical for forebrain development and is believed to act primarily as a signalling centre, producing a complex cocktail of Wnts and BMPs that provide instructions to neighbouring cells. Despite the apparent anatomical simplicity of the region (at least when compared to other parts of the forebrain), elucidating the precise molecular pathways that govern development of the cortical midline region has proven challenging and many questions remain unanswered. This paper makes a specific contribution, by showing that the multifunctional molecule beta-catenin is required within fimbrial glia to permit correct migration of dentate granule neurons which arise from progenitors located adjacent to the hem to their final destination in the dentate gyrus. This adds an interesting additional piece to the intriguing jigsaw puzzle of cortical midline development.

Reviewer 2 Comments for the Author:
I found this to be a clear, well written manuscript that presents interesting findings and that the authors' conclusions are supported by the data presented. I suggest only the following relatively minor changes, mostly to help make the manuscript more easily understandable to a wider audience. Points are listed in the order in which they appear in the manuscript - Point 6 is the most important.

Response: We thank the Reviewer for his/her comments. Our point-wise changes are detailed below.

1. *Line 44: state that Ai9 is an RFP-expressing cre-reporter strain (so reader doesn't have to look it up)*

Response: We have expanded on Ai9 reporter line in the text in **line 45.**

2. Figure 1 and accompanying text. Where is beta-catenin expressed in the midline at these stages? All cells or just some regions? It seems important to establish this first, before conducting cell type-specific experiments.

Response: We thank the reviewer for this point. Figure 1 has been modified to include ß-CATENIN immunohistochemistry. It is apparent that it appears to be present in all the cells of the hem at E12.5. This is consistent with the literature. We have modified the Results as follows:

(Lines 64-67).
"ß-Catenin (Ctnnb1) is expressed throughout the telencephalic midline neuroepithelium from E12.5 (Fig.1; Parichha et al., 2022, Kadowaki et al., 2007). An scRNA-seq study of E14.5 mouse cortex also identified *ß-Catenin* to be expressed in hem progenitors, CR cells, and choroid plexus epithelium (Loo et al., 2019, GSE123335)"

3. ***Line 69: 'these cells' should be 'most of these cells' - quantitation is provided later.***

We agree with the reviewer on this point and revised this to "many of these cells" **(line 79)**

4. As an aide to readers not already intimately familiar with the anatomy of this region, it would be helpful to mark the location of the marginal zone (MZ) on the images in panels 2A-D. In general, I think that labelling specific anatomical structures is helpful in these kinds of figures.

Response: Thank you for this suggestion, we have annotated our images such that the cartoon in 2A indicates the MZ region in which CR cells were scored in E. The legend has been modified to reflect this.

(Line 99)
"Cartoons of E12.5 sections indicating the marginal zone (red line, MZ) and the fimbria (green, Fi)"

5. Line 118 and Figure S2. The RNAseq analysis is very interesting, but is hardly mentioned. Could the authors expand, to say more about the gene expression changes that they found, or relevant genes whose expression didn't change? It would also be good to see in situ or immuno confirmation of at least a few of the Wnt pathway components whose expression changed in the conditional mutants. This would give useful spatial information regarding gene expression levels, rather than just tissue-level quantitation.

Response: We have expanded our analysis of the RNAseq data and added in situ hybridization and immunohistochemistry data in a new Supplementary Figure S3.

A. We have plotted significantly upregulated or downregulated GO:BP terms, which reveal that both "Canonical Wnt signaling pathway" and "Positive regulation of cell junction assembly" are significantly downregulated upon loss of beta-catenin.
B. The top 50 differentially expressed genes (DEGs) are presented in a heatmap (Fig. S3D). This list includes several Wnt signaling target genes that are down regulated in the mutant, indicating

ß-CATENIN mediated transcription has been altered.
C. Specific Wnt target genes *Sost, Wnt3, Fzd10, Runx2, Psck6, Dkk1, Notum,* and *Axin2* are significantly downregulated.
D. We have validated *Axin2* by in situ hybridization and LEF1 by immunohistochemistry and show that they are undetectable upon loss of ß-Catenin.
E. However, fimbrial scaffold markers such as GFAP, AQP4, ALDH1L1 are present and the corresponding *Gfap, Aqp4, Gfap, Fabp7 (Blbp)* mRNA does not change significantly upon loss of ß-CATENIN. Furthermore, known factors that regulate the fimbrial scaffold development such as SOX9, NFIA, NFIB appear unaltered in terms of protein or mRNA expression.

6. I didn't really understand the N-cadherin expression analysis. It wasn't clear to me what the authors were trying to show with this data, exactly how it was measured or what the significance of the reported change in expression profile means (in the context of beta-catenin's two activities) - these should all be explained more Clearly.

Response: We thank the Reviewer for pointing this out, and we have revised the phrasing in the text as below:

(Lines 149-159)

"To investigate the role of ß-CATENIN in adhesion, we examined the distribution of N- CADHERIN, a key member of cell-cell adherens junctions in neuroepithelium (Hirano and Takeichi, 2012), and enriched at the embryonic telencephalic midline (Kadowaki et al., 2007). We focused on the cell boundaries to assess the junctional integrity of the control and mutant hem (E13.5, Fig 2 I-K) and fimbrial glial cells (E16.5, Fig. S5). Hem and fimbrial glial cells were identified by co-immunolabeling for BLBP. In the control, N-CADHERIN distribution measured along a linear path traversing two neighboring cells reveals a sharp peak in intensity at the boundary between the cells. In ß-Catenin LOF brains, there was a marked flattening of the peak intensity at cell boundaries, indicating a diffused distribution of N-CADHERIN, and suggesting altered cell-cell adhesion, although the mRNA levels of *Cdh2*, which encodes N- CADHERIN, appear unaltered (Fig. 2K, Fig. S5F and G)."

A detailed description of the analysis is explained in the methods section as below "To quantify the NCAD distribution in Fig. 2K and Fig. S5D, E, a line (3.5um in length) was placed across the cell boundary when the BLBP channel was selected. 10 such ROIs were placed per brain and the intensity profiles were plotted from the NCAD channel using the "multiplot" option in the ROI manager. Every fourth slice of a Z stack was used for quantification."

7. Line 143: The positions of the primary, secondary and tertiary matrices should be clearly indicated on the relevant panels in Figure 3.

Now we have annotated the primary, secondary and tertiary matrices in Fig. 3. We thank the reviewer for suggesting this.

In terms of potential future work, beyond the scope of the current paper, it would be very interesting to use the ß-catenin conditional mutant strain described by Valenta et al., 2011 (doi:10.1101/gad.181289.111), which retains ß-catenin's cell adhesion function but lacks transcriptional activity, to better distinguish between the adhesion and Wnt signalling activities of beta-catenin in the hem.

Response: Although the *ß-Catenin* conditional mutant strain described by Valenta et al., 2011 would be a useful comparison in our study, unfortunately, we don't have this reagent at our disposal. We have discussed this limitation in the revised manuscript.

(Lines 164-167)
"A comparison with ß-Catenin LOF selective to its transcriptional role, leaving its cell adhesion intact (Valenta et al., 2011), would be a useful additional study in this context and may help to identify novel ß-CATENIN targets responsible for proper orientation and positioning of the fimbrial scaffold."

Reviewer 3

Reviewer 3 Advance Summary and Potential Significance to Field: Parichha et al. report disruption of *β-Catenin* in the hem of mice. They find that this disruption leads to a disorganization of the glia scaffold, which in turn leads to an impairment in dentate migration. There are several comments that should be addressed:

Reviewer 3 Comments for the Author:

Reviewer's comment : Could the authors quantify the extent of the morphological phenotype on the scaffold? Perhaps quantification of the length and a measure of continuity of the scaffold would better reveal the extent of phenotypes. This might be best done at earlier stages, where it could ideally be done at a cellular level (E12 for example).

Response: Quantifying the disorganization of the scaffold would indeed have been a good way to analyze the data. However, at E12.5, the stage in which the disorganization is first apparent and which is the likely cause of subsequent mis-migration (of Cajal-Retzius and dentate cells), BLBP staining reveals densely packed cells in control brains that do not yet have long processes. It is not possible to isolate individual cells and score their orientation. At E18.5 control fimbrial glia are extended and aligned and the β-Catenin LOF brains reveal marked disorganization. We have created a 3-D reconstruction in IMARIS and attached the movie as Supplementary Movie 1. This gives the reader a better appreciation of the extent of the disorganization.

Reviewer's comment: Since β-catenin is expected to affect cell proliferation (for example Chenn and Walsh, 2002), it would be important to properly examine the proliferation in the LOF (perhaps with an EdU injection), especially in the context of the ectopic protrusions that the authors describe.

Response: This is a good point. In order to investigate the status of proliferation we have examined PH3 and Ki67 immunostaining and present a quantification in the Supplementary Figure S1F-I. We did not find increased proliferation, and this point has been included in the results.
(Lines 73-76).
"We examined whether this protrusion arose as a result of excessive proliferation of the β- Catenin LOF hem progenitors. However, both PHH3 and Ki67 immunostaining revealed no apparent change in proliferation (Fig. S1F-I)"

Reviewer's comment : Figure 2F. I don't find this graph the most informative way to present these data. Is the total number of TRP73+ cells equal between the control and LOF? Wouldn't it be better to calculate the # TRP73+ over DAPI and present the distribution of TRP73+ between MZ and outside in a stacked bar so that the numbers that the authors refer to in the text are actually visible on the graph in a clearer manner? The same is true for graphs in Figure 3.

Response: Thank you for suggesting this very useful representation, which we have included for TRP73+ cells in FigS1 C-E and for TBR2 & PROX1 in Fig. S6c & F. We also retained the violin plots in the main figures 2 and 3 because they present the distribution of the data better.

The total number of TRP73+ cells (MZ +Outside MZ) in control vs Lmx1aCre::β-Catenin LOF did not change significantly (Fig. S1D). However, the distribution of the cells changed significantly as is seen in the quantification (Fig. S1E).

Regarding the suggestion of counting the # TRP73+ over DAPI: This type of quantification would be useful if the phenotype involved a change in cell identity. However, our result indicates that the phenotype is one of disrupted migration, in which case the absolute numbers of cells are a more suitable quantification. We have maintained a fixed ROI (region of interest) and scored the TRP73+ cells within and outside this ROI for control and mutant.

Second decision letter

MS ID#: DEVELOP/2022/200953

MS TITLE: Dentate gyrus morphogenesis is regulated by Î²-CATENIN function in hem-derived fimbrial glia.

AUTHORS: Arpan Parichha, Debarpita Datta, Varun Suresh, Mallika Chatterjee, Michael J. Holtzman, and Shubha Tole
ARTICLE TYPE: Research Report

I am delighted 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

In their manuscript, Parichha investigate the role of ß-catenin in controlling the morphogenesis of the dentate gyrus. Using a conditional gene inactivation approach in mice, they show that ß-catenin deletion in the cortical hem and its derivatives, the Cajal-Retzius (CR) cells and the fimbrial glial scaffold, led to the formation of an ectopic protrusion from the ventricular surface of the hem. The glial scaffold of the fimbria was disorganized and migration of dentate gyrus progenitors along the dentate migratory stream was disturbed. Instead, ectopic glial projection as well as Tbr2+ progenitors, CR neurons and Prox1+ dentate gyrus granule cells were found in this protrusion. The authors also demonstrate differential gene expression and reduced N-Cadherin protein levels at adherens junctions in the cortical hem. Finally, conditional ß-catenin inactivation specifically in CR neurons did not cause a dentate gyrus phenotype. Taken together, these data suggest that ß-catenin mediated, altered gene expression and cell adhesion in glial scaffold cells might underlie the defective dentate gyrus formation.

The dentate gyrus as part of the hippocampal formation plays an important role in memory formation. It also is a site of adult neurogenesis being one of the few locations in the adult mammalian brain containing adult neural stem cells. Given these critical functions, it is important to investigate the currently poorly understood cellular and molecular processes that control dentate development. Hence, the manuscript by Parichha et al. address an important topic that will be great interest for the readers of Development.

Comments for the author

The authors have addressed my concerns and the manuscript has improved considerably. I highly recommend publication of this manuscript in Development.