Review of: "Sonic hedgehog-dependent recruitment of GABAergic interneurons into the developing visual thalamus"

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The authors have used elegant conditional transgenic approaches to distinguish between the effects of Shh and neurosecretion on RGC-innervation dependent interneuron recruitment to the thalamus. They show that silencing RGC activity (Calb2-cre conditional induction of TeNT) results in reduced contra/ipsilateral segregation in the dLGN (which to some extent phenocopies the effects of tetrodotoxin-mediated RGC silencing) but no effect on interneurons or FGF15 expression in the target. Conversely, Shh inactivation (conditional Calb2-cre Shh inactivation) is associated with loss of Gad61 interneurons and reduced Fgf15 expression. The requirement for Hh signaling at the target is supported by data showing astrocyte expression of Hh signaling components and target genes. The major conclusion is that Shh release from RGCs but not activity is required for astrocyte FGF15 expression and interneuron recruitment. The manuscript is well written, and the phenotypic analyses are well done. I do think that there are instances where additional controls should be done to support the conclusions.

Things to address.

1. Fidelity of the Calb2-Cre system- the assumption here is that this cre driver will not impact Shh-dependent interneuron production. The authors should provide evidence that this is the case as this Cre driver is likely expressed in several regions of the CNS outside of the retina.

1. The efficiency Shh inactivation is investigated by qPCR for the Wt Shh transcript from whole retina. However, since this approach relies on recombination of two floxed alleles in post-mitotic RGCs, it could be inefficient, which in turn could explain why the Calb2-cre approach results in a milder phenotype than nestin-cre. The authors should characterize the timing of this Cre in RGC differentiation- is it active in all RGCs prior to target innervation? How efficient is Shh inactivation in RGCs in the retina- do all RGC exhibit loss of transcripts containing exon2 of the Shh gene (the floxed exon)?

1. How do the authors rule out that the phenotype of the conditional Shh ablation in RGCs is not a consequence of disrupting some other Shh-dependent function in RGCs.

1. The model is that Shh release from the RGC terminals is driving Hh signaling in astrocytes and Fgf15 induction, which

in turn recruits interneuron progenitors. The authors should show Hh signaling is differentially affected in the target in the conditional Shh mutants vs the TeNT induction model. The authors should also rule in/out altered astrocyte survival in the target to explain the reduction in FGF15. Are the astrocytes still there and not expressing Fgf15 or are they lost?

Additional suggestions for a mechanistically tight study:

1. Can the Shh loss of function phenotype on interneuron recruitment be rescued by FGF15 expression in astrocytes?

1. The authors indicate that Shh is an anterograde signal from RGC terminals at the target-this could be tested by experiments to compare the effects of wt vs transport deficient Shh variants on Hh signaling and interneurons at the target.