

**Fig. S1: EphB1 expression in dLGN**

Coronal section at P4 after in situ hybridization against EphB1. EphB1 is not expressed in the dLGN, but is expressed in the vLGN.

**Fig. S2: Method for quantification of retinogeniculate projections**

On the merged image (a), the length of the dLGN and the extent of the ipsilateral projection are measured along the dorsoventral (DV) (yellow) and mediolateral (ML) (white) dLGN axes, tilted compared to the real DV and ML axes of the dLGN (compare with Fig. 2). The image of the ipsilateral projection (b) is thresholded at a fixed value (c) so that each pixel is either black (no signal) or white (signal). On the image of the contralateral projection (d), The dLGN region is outlined to restrict the measurement to this area and exclude the intergeniculate leaflet and optic tract. The contralateral image (d) is thresholded for every tenth value of grayscale from 0 to 255 (at 60 in e and 80 in f, for example). Comparing ipsilateral (c) and contralateral (e or f) thresholded images, the Metamorph software calculates the percentage of pixels with “ipsilateral” signal (white in c) that does not overlap with “contralateral” signal (and is thus black in e or f). The percentage of dLGN area occupied by ipsilateral axons is obtained by dividing the pixel area with signal corresponding to ipsilateral axons (white signal in c) by the total pixel area of the dLGN region delimited in d. (g, h) Schematic representation of two cases with identical percentage of dLGN territory occupied by ipsilateral terminations (green), but different distributions along the DV and ML axes with ipsilateral axons more scattered along the dLGN (h). Thus, these measurements allow quantitative assessment of the size,

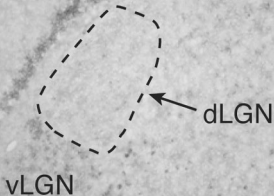
distribution and scattering of ipsilateral fibers in an manner independent from the segregation of ipsilateral axons.

**Figure S3: Segregation of ipsilateral projections in wild type and *EphBI*<sup>-/-</sup> mice after epibatidine treatment (on 20x magnification images).**

Segregation plot. Percentage of pixels containing only ipsilateral signal (no contralateral signal), as a function of the contralateral threshold (ipsilateral threshold is fixed).

Stars correspond to statistic significance between epibatidine and saline treated animals in wild-type mice and asterisks in *EphBI*<sup>-/-</sup> mice. Segregation of ipsilateral fibers is perturbed in the dLGN of both wild type and *EphBI*<sup>-/-</sup> mice after epibatidine treatment, with a stronger effect in *EphBI*<sup>-/-</sup> mice as saline treated *EphBI*<sup>-/-</sup> mice are already less segregated than saline treated wild-type mice. Error bars: SEM values. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

a



EphB1

