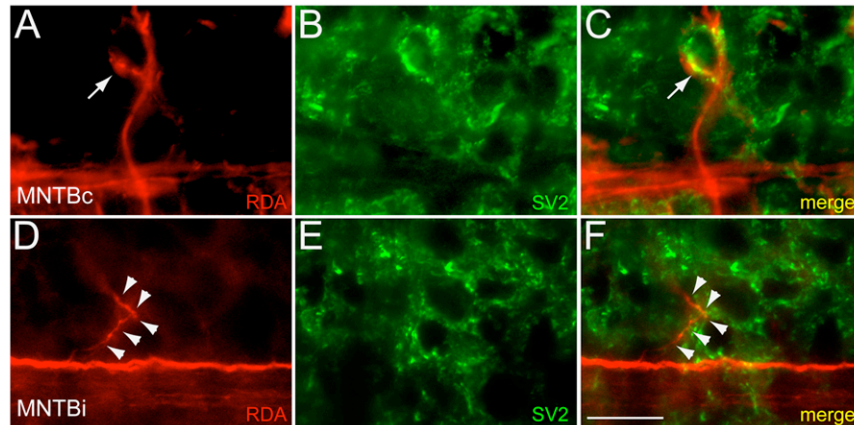
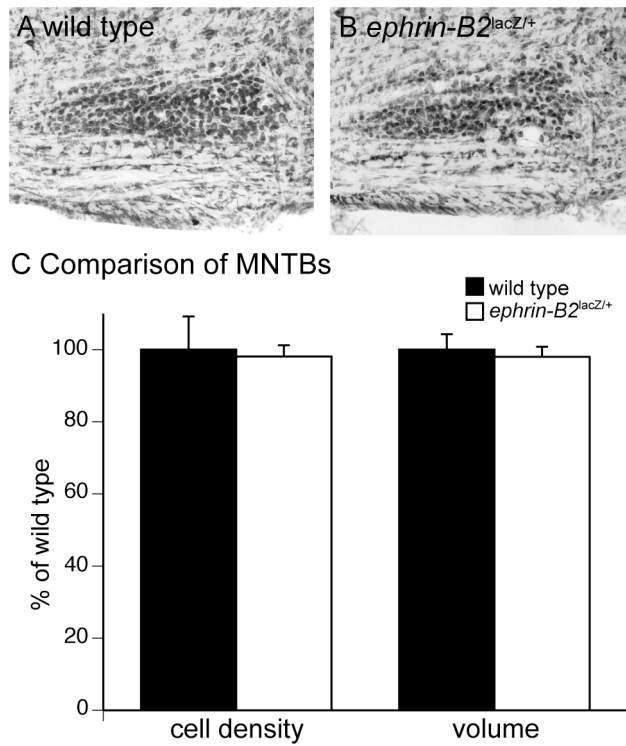


Supplemental Figure 1. Ephrin-B2 is not expressed in a mediolateral gradient in postnatal MNTB. At P4 (top panel) and P12 (bottom panel), optical density measurements of ephrin-B2 expression were compared along the mediolateral axis of MNTB. A linear regression revealed no gradient of expression across this axis of MNTB, at these two early developmental timepoints.

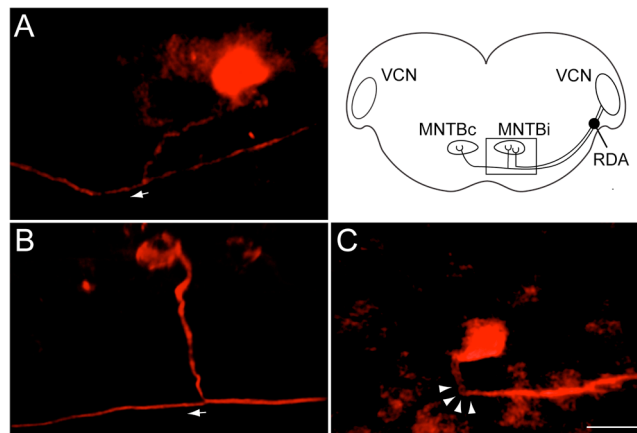


Supplemental Figure 2. Terminations in MNTBc but not MNTBi are

immunopositive for SV2. *A.* RDA labeling in MNTBc of a P3 wild type reveals an immature calyceal termination (arrow). *B.* Expression of SV2 in the same section of MNTBc as *A.* *C.* Merge of panels *A* and *B* reveals that the immature calyceal termination in MNTBc is immunopositive for SV2 (arrow). *D.* RDA labeling in MNTBi of a P3 wild type mouse reveals an ipsilateral branch (arrowheads). *E.* Expression of SV2 in the same section of MNTBi as in *D.* *F.* Merge of panels *D* and *E* shows that the RDA-labeled branch is not immunopositive for SV2 (arrowheads). Scale bar in *F* also applies to *A-E*, and indicates 20 μm .



Supplemental Figure 3. Wild type and *ephrin-B2*^{lacZ/+} brainstem morphology are similar. **A.** Thionin-stained tissue showing MNTB morphology in a P12 wild type mouse. **B.** Thionin-stained tissue showing MNTB morphology in a P12 *ephrin-B2*^{lacZ/+} mouse. **C.** MNTB cell density and volume in *ephrin-B2*^{lacZ/+} mice (white) as a proportion of wild type (black) mean values reveals no significant difference in these mutants.



Supplemental Figure 4. Individual axon labeling in mutant mice indicates origins of ipsilateral calyces. Focal labeling of individually resolved axons was performed at P6-28 in *ephrin-B2*^{lacZ/+} mice. Schematic diagram indicates dye placement and orientation of images. **A.** Calyx in MNTBi arises from an axon traveling horizontally towards the midline (arrow). **B.** Another example of a calyx in MNTBi arising from an axon projecting towards the midline (arrow). **C.** Calyx in MNTBi arises from an axon projection from the ipsilateral VCN. Arrowheads indicate the lack of axon projection toward the midline. Scale bar in C also applies to A-B and indicates 10 μm .