



Figure X: Inhibitory synapses terminate on the axon initial segment of LSO neurons. Coronal sections of tissue (20 μm) containing Lateral Superior Olive labeled for gephyrin (red), ankyrinG (cyan), synaptophysin1 (blue), and DAPI. Low power image (112x magnification) taken on a Zeiss Stereoscope (XX) showing a representative LSO (A), with approximate region imaged with SIM Microscopy show by the yellow box. (B) Post-SIM processing of image taken on a Zeiss LSM7100 for gephyrin (568 - red), ankyrinG (488 - cyan), and synaptophysin1 (blue). SIM processing was done using offline Zeiss SIM processing software, with a Weiner filter setting between -5.0 and -5.2, followed by deconvolution processing of individual channels using Metamorph. (C-D) Close-up of regions outlined from B, showing gephyrin puncta along the proximal AIS, double hashmarks indicate where AIS travels out of shown Z plane. AnkyrinG channel (488 - cyan) on (i) and off (ii) with dotted white guidelines added for clarity (added as reference, do not indicate location of signal). (E-H) Subsequent examples showing heterogeneity of gephyrin colocalization with the AIS. Imaging was targeted to the middle bend of the LSO, with 5/6 examples coming from LSO sections with similar morphologies across the rostral-caudal axis. All scale bars correspond to 5 μm , unless otherwise noted.