

Supplementary Figure 1. *A*, Images of rat retinal slice with the puffer pipette in different positions relative to the recorded RBC. *B*, IPSC amplitude decreased as the pipette was moved away from the IPL toward the ganglion cell layer. Gray region indicates the  $\pm 95\%$  confidence interval of the Gaussian fit (solid line; see Materials and Methods). In these experiments, the bath superfusion solution flowed in the same XY direction as the puff.

Supplementary Fig. 2. Diffusion of exogenous agents puff in the OPL and IPL. *A* Feedback IPSCs elicited by NMDA puffs (100  $\mu\text{M}$ , 75 ms) in a RBC ( $V_{\text{hold}} = 0 \text{ mV}$ ) delivered in the IPL (left), then the OPL (center), and then back to the IPL (right) before and after application of the NMDAR antagonist CPP (10 mM). *B*, Summary of 6 experiments showing that NMDA evoked IPSCs only when was puff applied in the IPL but not in the OPL.

Supplementary Fig. 3. The mGluR1 antagonist LY367385 does not affect glycine release evoked by synaptically released glutamate. *A*, Feedback IPSCs recorded in RBCs and elicited by CPPG puffs. *B*, Feedback IPSCs recorded in RBCs and elicited by kainate (KA) puffs. *C*, Feedforward EPSCs ( $V_{\text{hold}} = -60 \text{ mV}$ ) recorded in AII amacrine cells and elicited by CPPG puffs. *D*, Summarized effects of LY367385 on postsynaptic currents (PSCs) elicited by puffs of CPPG or kainate and recorded in RBCs or AII. Experiments were performed in the presence of GABAR antagonists (SR95531 and TPMPA).