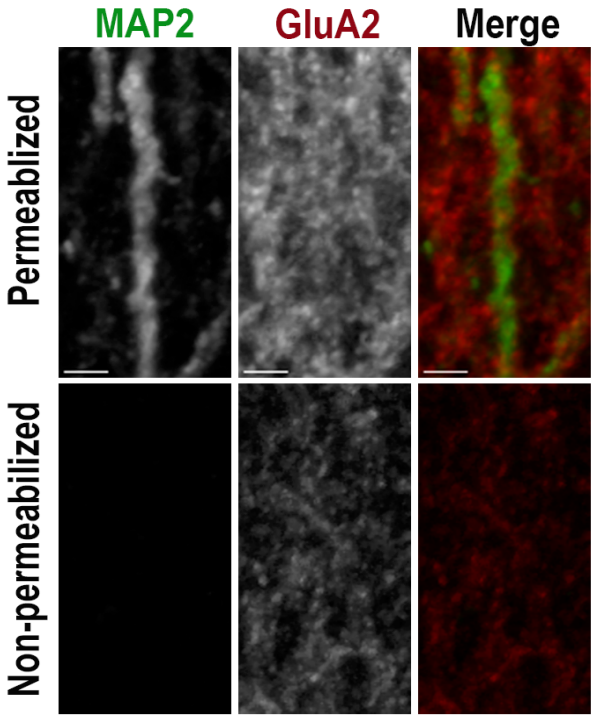
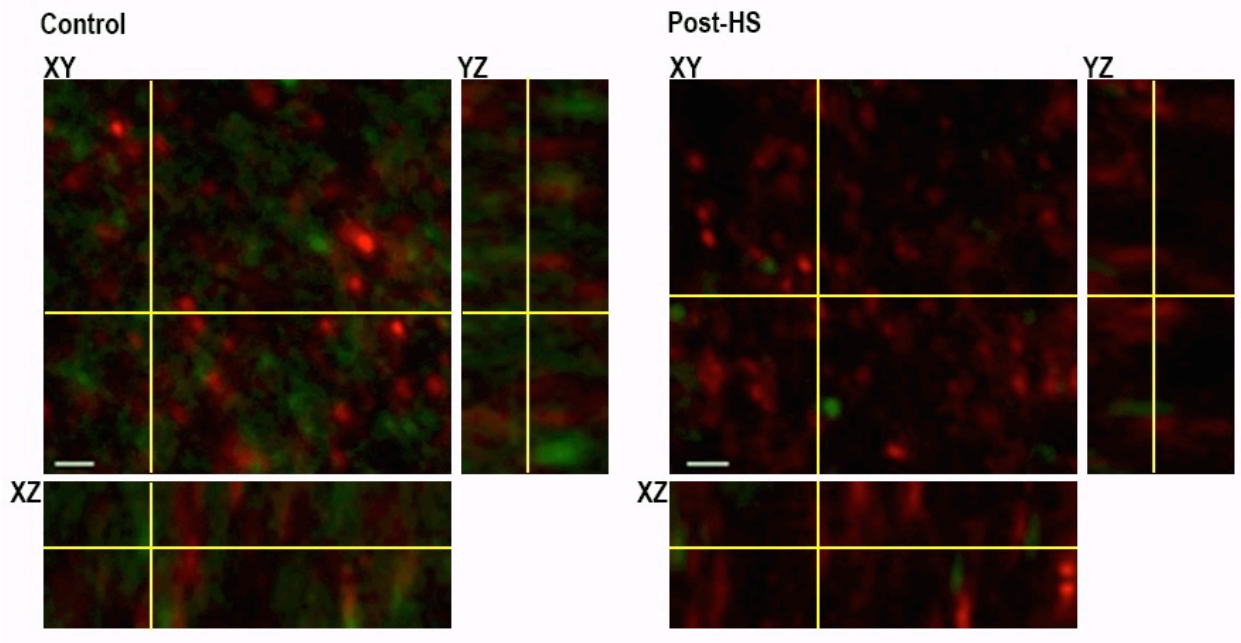
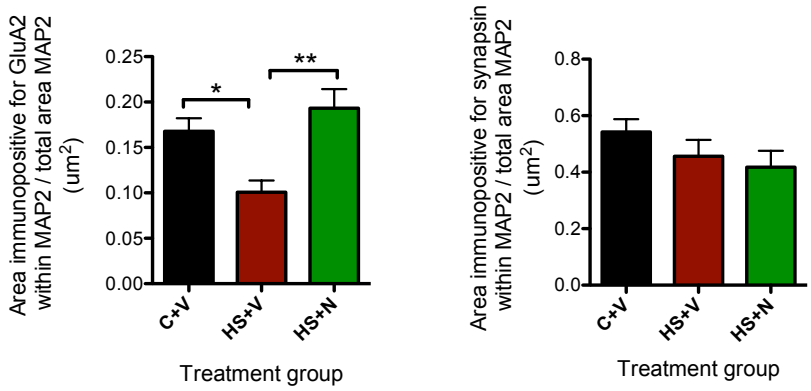


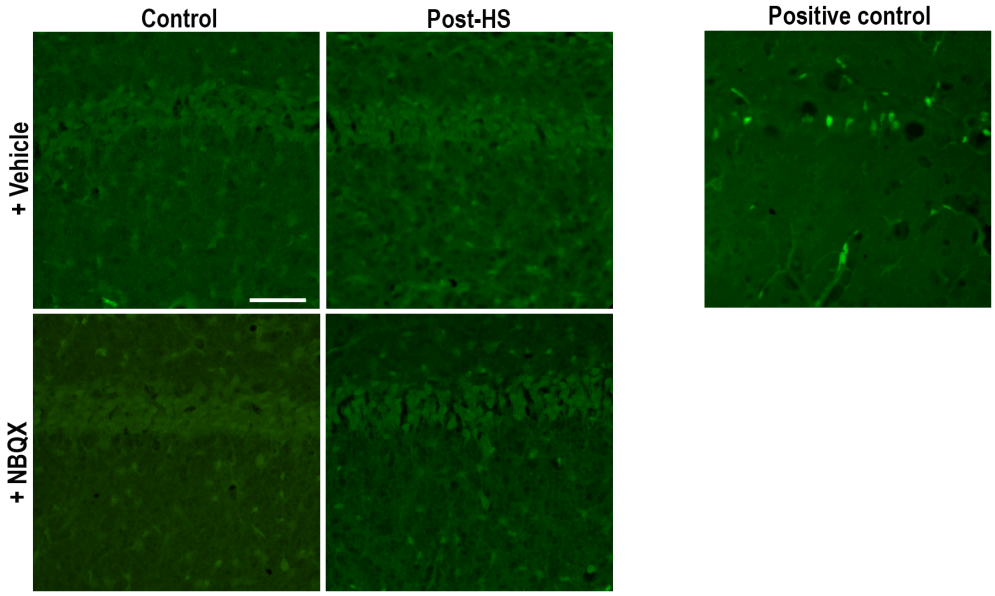
Supplemental Figure 1: GluA2 staining is specific to the extracellular domain when the membrane is not permeablized. To confirm that the staining methods used for GluA2 IHC in this study specifically labeled the extracellular domain of GluA2, we probed permeabilized (top) and non-permeablized (bottom) tissue with an antibody against extracellular GluA2 and an antibody against MAP2, a protein only found intracellularly. MAP2 staining only appeared in permeabilized tissue, and GluA2 shown much more staining in permeabilized tissue, indicating that the neuronal membranes are intact and GluA2 staining in non-permeabilized tissue is extracellular. Images are maximum projections of 3 consecutive z planes. Bar=2μm



Supplemental Figure 2: Example of increased GluA2/synapsin in multiple planes. A higher number of GluA2 puncta (green) associated with synapsin puncta (red) are visible in z-stacks from control P12 rats (left) as compared to 48-hour post-HS rats (right) in XY, YZ, and XY planes. Bar=1μm



Supplemental Figure 3: Total dendritic GluA2 surface expression decreases after HS, and is restored by NBQX treatment. In a subset of tissue used in the colocalization experiments described in Figure 4, the total area of dendritic GluA2 or synapsin expression was measured within a MAP2 (dendritic marker) mask. Using ImageJ, thresholded, binarized images of either the synapsin or the GluA2 channel were multiplied by the binary MAP2 mask to provide the area of synapsin or GluA2 within the MAP2 staining. This area was then divided by the total area of MAP2 staining in the analyzed field. The resulting data mirrored our synaptic colocalization results, showing a decrease in dendritic expression of GluA2 following HS (HS+V mean=0.10um±0.01; n=9 fields total from 3 rats) as compared to littermate controls (C+V mean=0.17±0.01; n=10 fields total from 3 rats), as well as prevention of the seizure-induced decrease in GluA2 by acute post-seizure treatment with NBQX (HS+N mean=0.19±0.02; n=7 fields total from 3 rats). Data were compared by ANOVA (p=0.001, F=8.9) with post-hoc Bonferroni's Multiple Comparison Test. Overall dendritic synapsin expression in the same fields is not significantly altered by HS, though there is a slight downward trend in HS rats (p=0.26 by ANOVA, F=1.5) regardless of treatment, which may in part explain why NBQX does not return synaptic GluA2 fully to control levels in Figure 4.



Supplemental Figure 4: Neither HS nor NBQX treatment caused acute cell death. Brain sections from rats of the same groups used for IHC were collected and stained using Fluoro-Jade B to label degenerating neurons. No positive staining was detected in any of the four groups, as compared to a positive control (right, adult mouse following seizures induced by kainic acid). Bar=50μm