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Supplemental Information

Astrocytes Amplify Neuronal Dendritic Volume Transmission Stimulated by Norepinephrine

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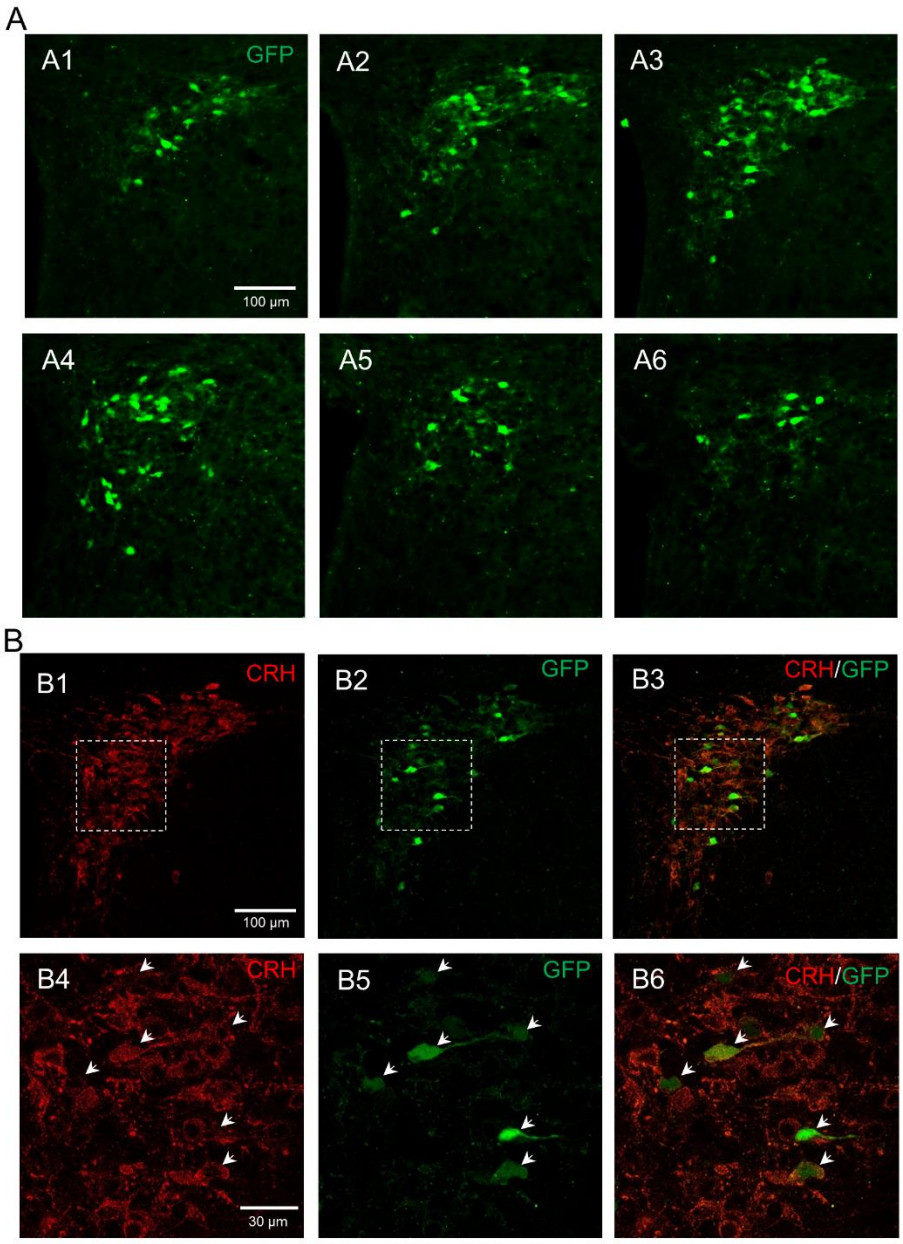

Figure S1. eGFP expression in CRH neurons in the CRH-eGFP mouse, Related to STAR Methods.

(A) Serial rostral-to-caudal (**A1-6**) expression of eGFP in sections of the PVN of a CRH-eGFP mouse.

(B) CRH immunofluorescence in a section from a CRH-eGFP mouse showing CRH immunolabeled neurons in the PVN (red, **B1**), CRH-eGFP-expressing neurons in the same section (green, **B2**), and the overlay of the two (**B3**). Lower panels (**B4-6**) are boxed region of the upper panels at higher magnification.

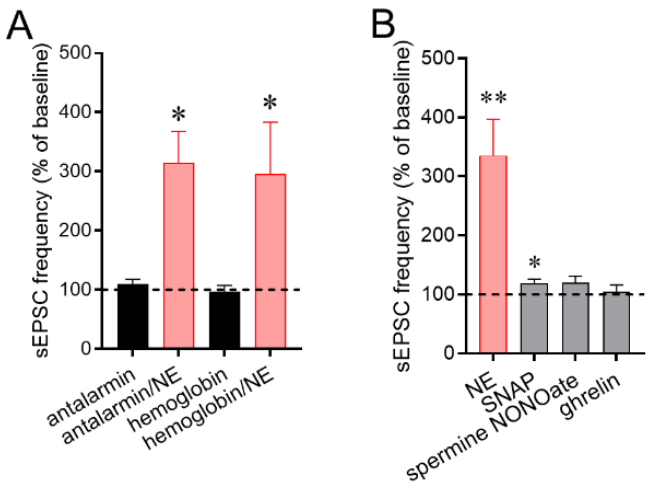

Figure S2. The retrograde messenger activated by norepinephrine is not CRH or NO, Related to Figure 4.

(A) The CRHR1 receptor antagonist, antalarmin, and a scavenger of extracellular NO, hemoglobin, had no effect on the NE-induced increase in sEPSC frequency (n = 5 and 6 cells, respectively).

(B) The NO donors (SNAP, spermine NONOate) failed to mimic the NE effect on sEPSC frequency (n = 8 and 5 cells, respectively), although SNAP caused a small increase in sEPSC frequency. Ghrelin had no effect on the sEPSC frequency (n = 10 cells). * $p < 0.05$; ** $p < 0.01$.

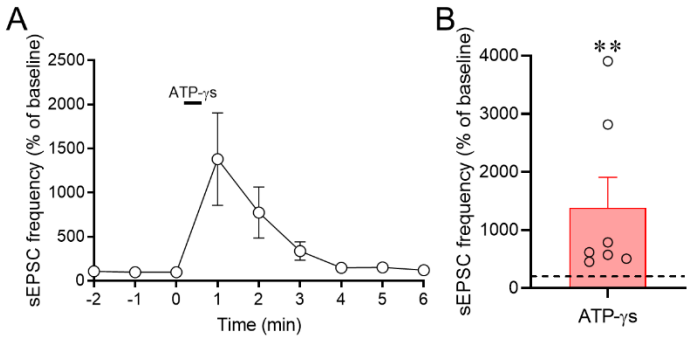

Figure S3. ATP mimicked the NE-induced facilitation of sEPSC frequency independent of activation of astrocytes, Related to Figure 4.

(A) Time plot of the increase in sEPSC frequency induced by the non-hydrolyzable ATP analog ATP-γs after a 2-h preincubation in ACSF containing FCA. The ATP-γs was puff applied focally for 30 sn (n = 7 cells).

(B) Scatter plot/bar graph of the mean ATP-γs-induced facilitation of sEPSC frequency (n = 7 cells). ** $p<0.01$.

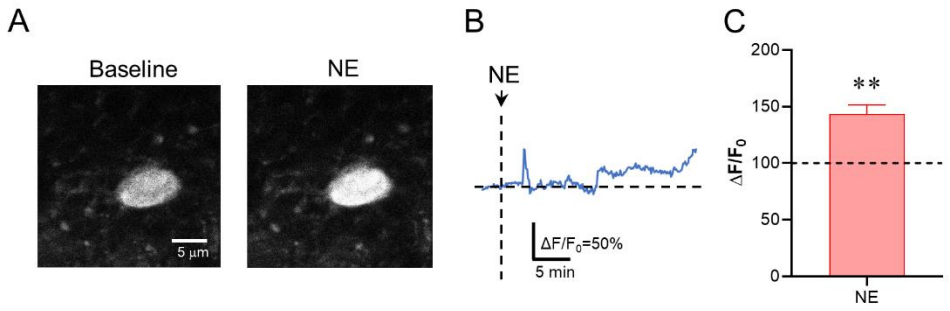

Figure S4. Norepinephrine-induced calcium response in astrocytes measured using two-photon microscopy, Related to Figure 4.

(A) Two-photon fluorescence images showing the glial calcium response to norepinephrine in an astrocyte in the PVN loaded with the glia-specific calcium indicator Rhod-2/AM.

(B) The normalized fluorescence intensity of the response to norepinephrine (NE) of the cell shown in A.

(C) Mean normalized fluorescence intensity of the norepinephrine response in PVN astrocytes (n = 5 cells). Bath application of norepinephrine induced a significant increase in astrocytic calcium. ** $p < 0.05$.

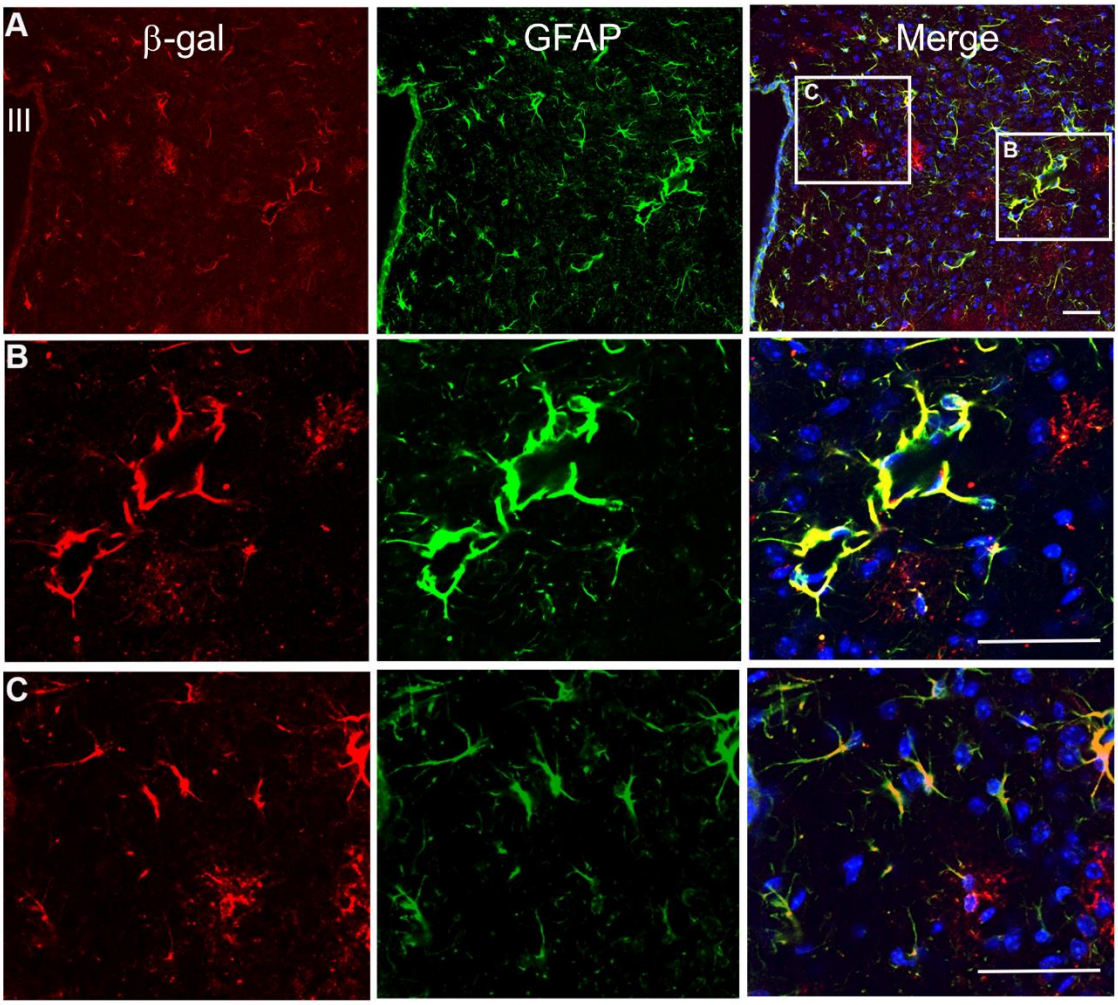

Figure S5. Immunohistochemical confirmation of dn-SNARE expression in PVN astrocytes, Related to Figure 4. Immunohistochemical labeling of β-galactosidase (β-Gal, red, left column) and glial fibrillary acidic protein (GFAP, green, middle column) in the PVN, and merge of the two with additional blue DAPI nuclear staining (Merge, right column). The dn-SNARE transgene co-expresses β-Gal, allowing the β-Gal immunolabeling to be used as a marker for dn-SNARE expression. GFAP immunostaining was used as an astrocytic marker.

(A) Low-magnification showing the whole PVN.

(B) High magnification of the region in A outlined by box B.

(C) High magnification of the region in A outlined by box C.

Scale bar = 50 μm in A-C.