

## SUPPLEMENTAL MATERIAL

### Methods

#### *Subjects*

Rats were purchased (Taconic Farms; Germantown, NY or Charles River; Wilmington, MA) and bred to produce litters that were culled to 9-10 and weaned at 21-23 days. They were then housed 2-3/cage (standard-size, translucent plastic) with corn cob bedding. There was a 12 hr light cycle (onset, 7:00 a.m.), with food (Purina 5001; W.F. Fisher, Somerville, NJ) and water *ad libitum* at 66-74°F and 30-70% relative humidity.

#### *Immunocytochemistry*

In brief, animals were deeply anesthetized with urethane (2.5 g/kg, i.p.) and transcardially perfused with 0.9% NaCl for 3 min, followed by 4% paraformaldehyde (in 0.1 M TRIS buffer) for 5 min. The brain was removed, hemisected, and postfixed in 20 ml 4% paraformaldehyde at 4°C overnight. When blood was removed before perfusion it was removed from the ventricle. Sections (50 µm) were cut the next morning using a vibratome (TPI 1000; The Vibratome Co., St. Louis, MO) in 0.1M TRIS buffer and then transferred immediately to 0.1% Triton-X 100 in 0.1 M TRIS buffer (TRIS A). Free-floating sections were treated with 10% normal horse serum (Vector Laboratories, Burlingame, CA), incubated overnight in a goat polyclonal antibody to VEGF (1:1000; R & D Systems, Minneapolis, MN) that has been previously validated (Nicoletti et al., 2008), incubated with secondary antibody (biotinylated horse anti-goat IgG, 1:500; Vector), incubated in ABC (Vector Standard kit), and developed using diaminobenzidine (DAB) as a chromagen, enhanced with NiCl<sub>2</sub> (Scharfman et al., 2002; McCloskey et al., 2008). For the figures, the definition of CA1b and CA3b were based on descriptions by Lorente de No (Lorente de No, 1934).

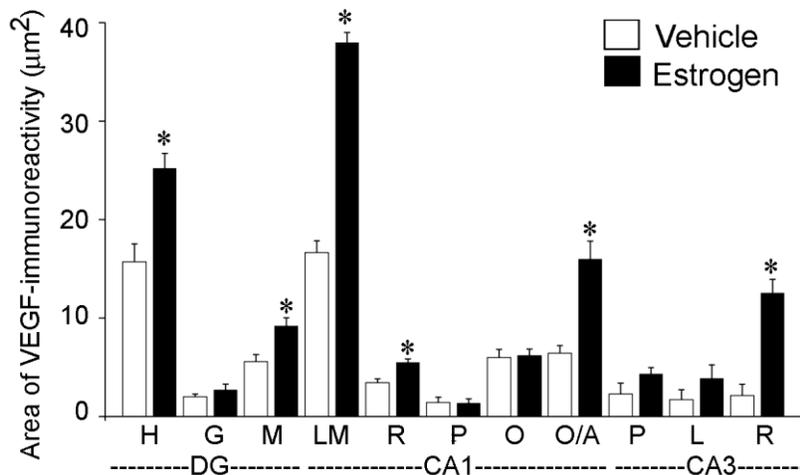
## References

Lorente de No R. (1934) Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *J Psychol Neurol Lpz* 46:113-177.

McCloskey DP, Hintz TM, Scharfman HE.(2008) Modulation of vascular endothelial growth factor (VEGF) expression in motor neurons and its electrophysiological effects. *Brain Res Bull.* 76:36-44.

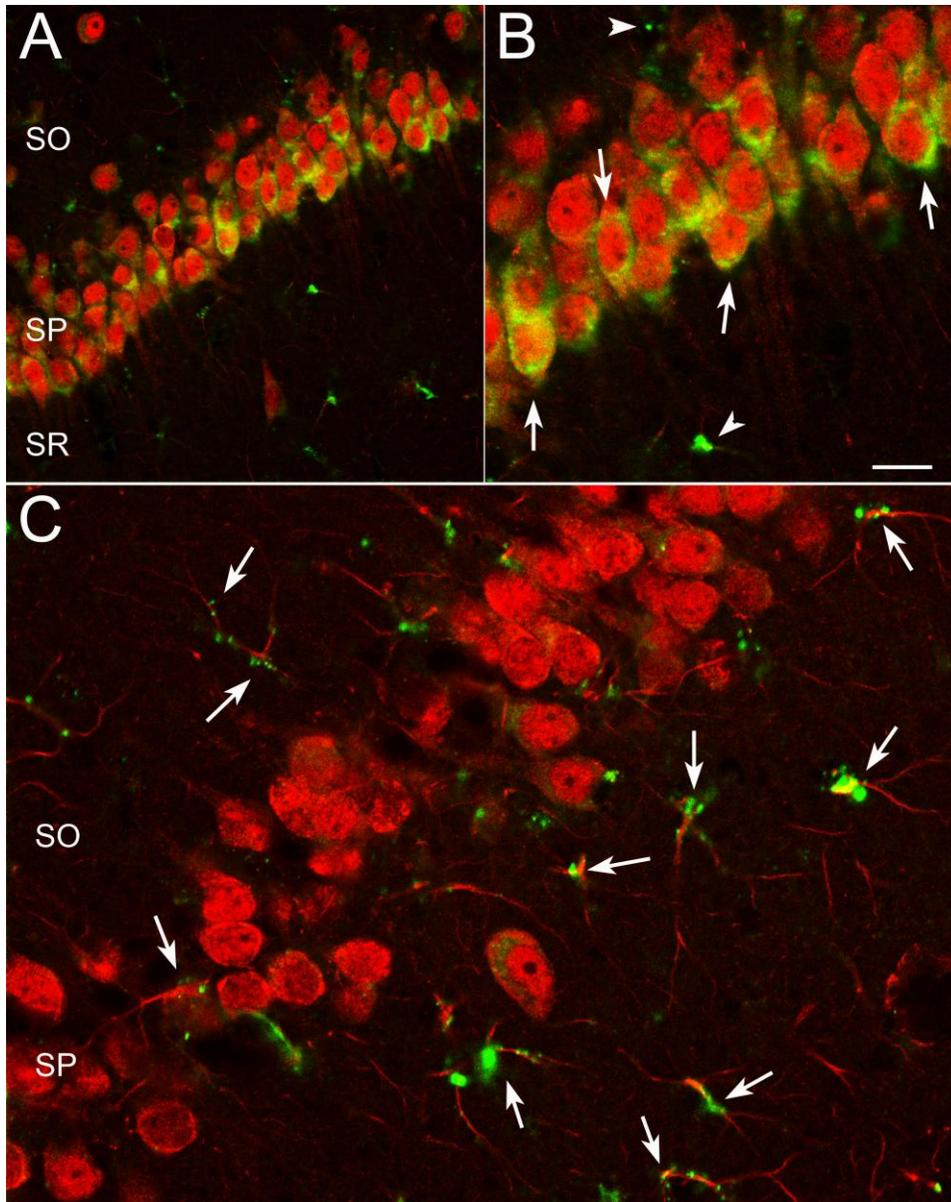
Nicoletti JN, Shah SK, McCloskey DP, Goodman JH, Elkady A, Atassi H, Hylton D, Rudge JS, Scharfman HE, Croll SD. (2008) Vascular endothelial growth factor is up-regulated after status epilepticus and protects against seizure-induced neuronal loss in hippocampus. *Neuroscience* 151:232-241.

Scharfman HE, Sollas AL, Smith KL, Jackson MB, Goodman JH.(2002) Structural and functional asymmetry in the normal and epileptic dentate gyrus. *J Comp Neurol.* 454:424-439.



**Supplemental Figure 1.**  $17\beta$ -estradiol increases the area of VEGF-immunoreactivity in the adult female rat hippocampus.

The area of VEGF-immunoreactivity that reached the threshold gray value for punctate staining was quantified for the same viewing frame that was used to count punctae. Results were similar to counted punctae (Figure 1) in that  $17\beta$ -estradiol-treated sections (n=9 rats) exhibited increased immunoreactivity relative to vehicle-treated control tissue (n=7 rats). Two-way ANOVA: effect of  $17\beta$ -estradiol vs. vehicle treatment,  $F= 50.365$  (d.f. 1,153)  $p<0.0001$ ; hippocampal region effect,  $F=66.649$  (d.f. 10, 153)  $p< 0.0001$ ; interaction between cycle stage and region effects,  $F=10.684$  (d.f. 10, 153)  $p <0.0001$ . Asterisks indicate that the effect of estradiol was significantly greater than vehicle ( $p<0.05$ ).



**Supplemental Figure 2.** VEGF and NeuN label hippocampal neurons after seizures.

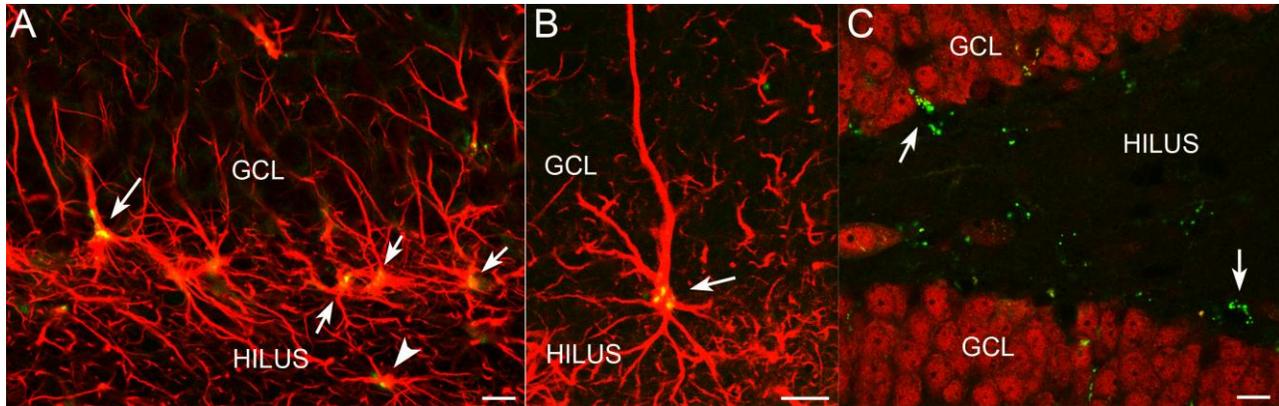
A-B. VEGF immunofluorescence (green; arrows) in an adult male rat that had generalized seizures. The rat was injected with the chemoconvulsant pilocarpine (380 mg/kg, s.c.) 24 hrs before perfusion-fixation, and exhibited status epilepticus (generalized continuous seizures) within 60 minutes (for detailed methods from a previous study, see Scharfman et al., 2002). After 1 hr it was treated with the anticonvulsant diazepam and it was perfusion-fixed the next day. Neuronal nuclei were labeled using an antibody to a neuronal nuclear protein, NeuN (red).

Four of the double-labeled neurons are marked by arrows and VEGF immunofluorescent punctae are marked by arrowheads. SO= stratum oriens; SP= stratum pyramidale; SR= stratum radiatum.

Calibration (in B) = 60  $\mu\text{m}$  for A, 30  $\mu\text{m}$  for B.

C. VEGF immunofluorescence (green) and NeuN labeling (red) in another section from a different male rat which had status epilepticus 24 hrs before perfusion-fixation. Punctate VEGF-immunolabeling is adjacent to processes that are labeled with NeuN (yellow). This contrasts to the naïve condition, where there is preferential astrocytic VEGF-immunoreactivity (Figure 1), and this pattern appears to 'switch' to primarily neuronal immunoreactivity after seizures.

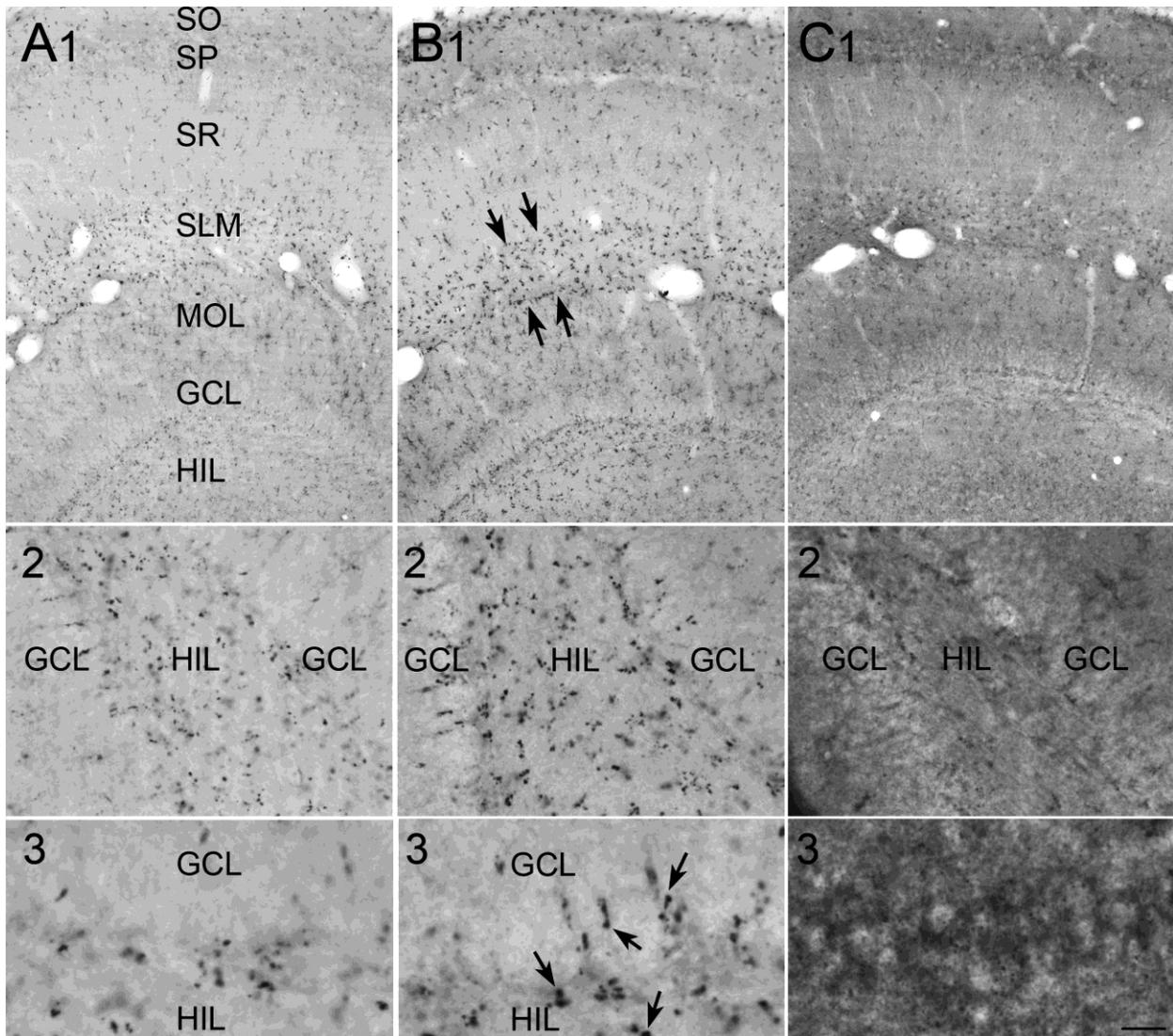
Calibration same as B.



**Supplemental Figure 3.** VEGF protein localized to GFAP-labeled astrocytes in the dentate gyrus.

A. VEGF (green) and GFAP (red) immunofluorescence in the dentate gyrus is shown. The tissue section was taken from a female rat that was perfused on proestrous morning. There are double-labeled cells (arrows) at the border of the granule cell layer (GCL) and hilus, and double-labeled cells in the hilus (arrowhead). At far left, the arrow points to a double-labeled cell with the morphology of radial glia-like progenitors, which are often pyramidal in shape and oriented with a large process ascending through the granule cell layer. Calibration = 20  $\mu\text{m}$ .

B. An example of a double-labeled cell with morphology similar to a radial glia-like progenitor in a section from a different animal. A large process is oriented perpendicular to the cell layer, extending towards the molecular layer. Calibration = 50  $\mu\text{m}$ .



**Supplemental Figure 4.** Loss of VEGF-immunoreactivity with time after sectioning.

A. A section is shown from a vehicle-treated Ovx rat that was stained using an antibody to VEGF immediately after sectioning the brain. In A1, the abbreviations are the same as in Figure 2. In A2, a higher power image of the dentate gyrus is shown, which is further expanded in A3.

B. VEGF immunoreactivity is shown from an estradiol-treated Ovx rat that was processed concurrently with the sections from the animal in part A. VEGF immunoreactivity is increased relative to A, especially in stratum lacunosum-moleculare (arrows) and is primarily punctate. The cell layers are weakly immunoreactive. The predominant immunolabeling is punctate or

globular. B2 and B3 are expansions of the dentate gyrus from the same section, analogous to A2 and A3. Arrows in B3 point to punctate staining.

C. VEGF immunoreactivity is shown for another estradiol-treated Ovx rat that was processed 1 day after sectioning instead of immediately. The reaction with chromagen (DAB) was prolonged because punctate immunoreactivity was weak. Prolonging incubation did not increase punctate staining, however. Instead, it resulted in higher background and immunoreactivity was evident in many layers, particularly pyramidal cell layers. C2 shows the dentate gyrus at higher magnification, and C3 is the CA1 pyramidal cell layer and adjacent strata at high magnification. Calibration (in C3) for A1, B1, C1 = 80  $\mu\text{m}$ ; A2, B2, C2 = 40  $\mu\text{m}$ ; A3, B3, C3 = 20  $\mu\text{m}$ .