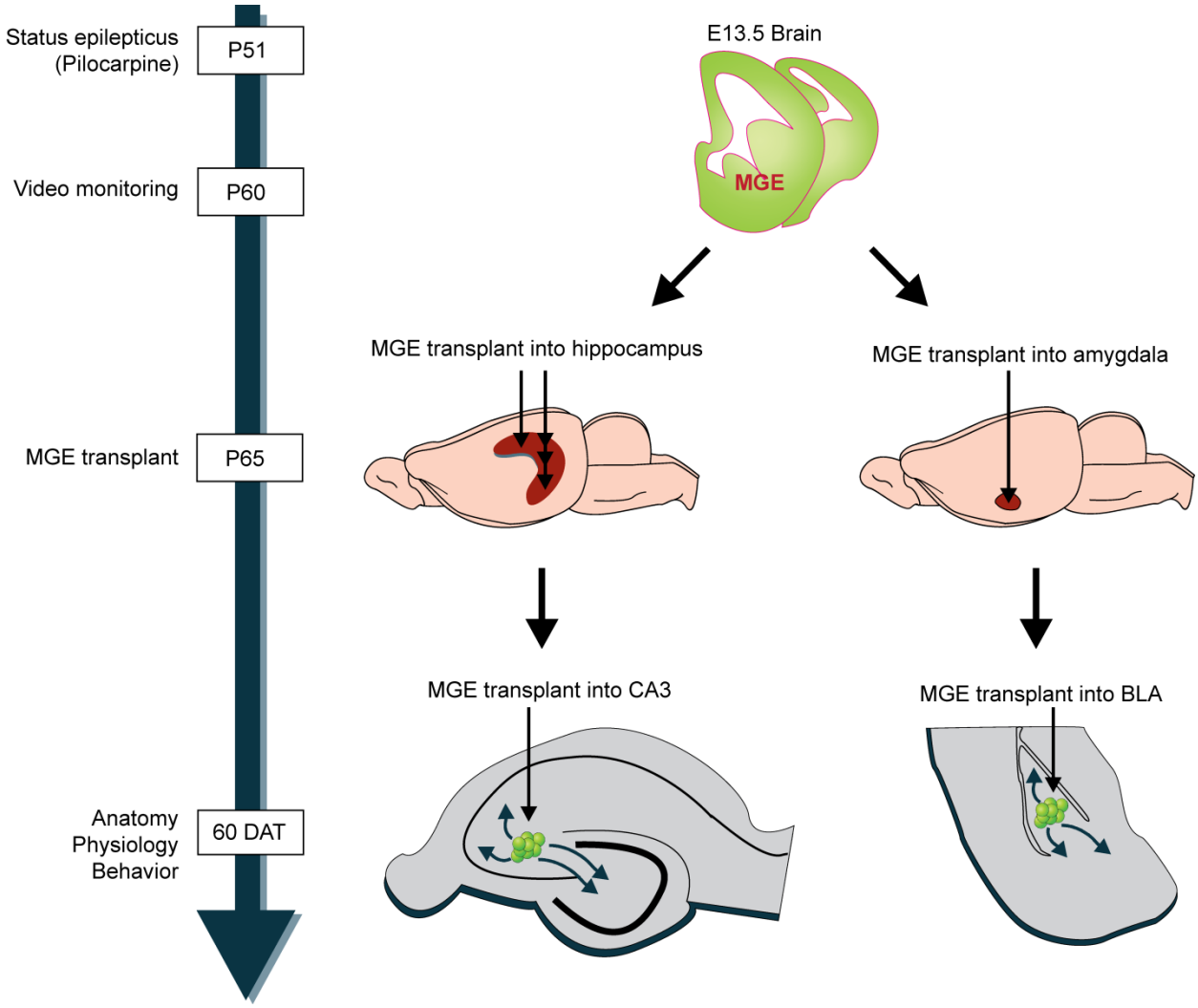
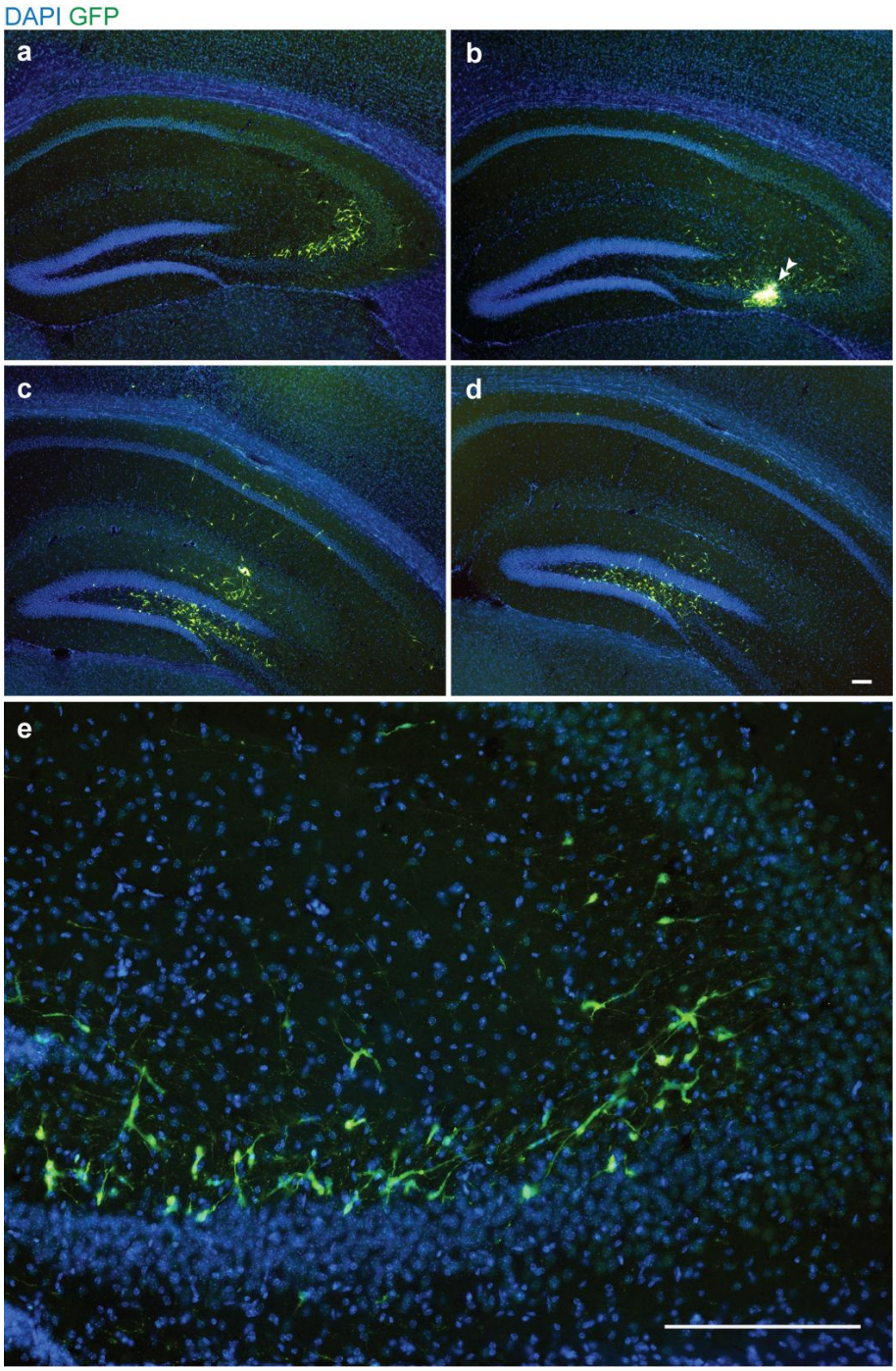
SUPPLEMENTARY FIGURES

GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior

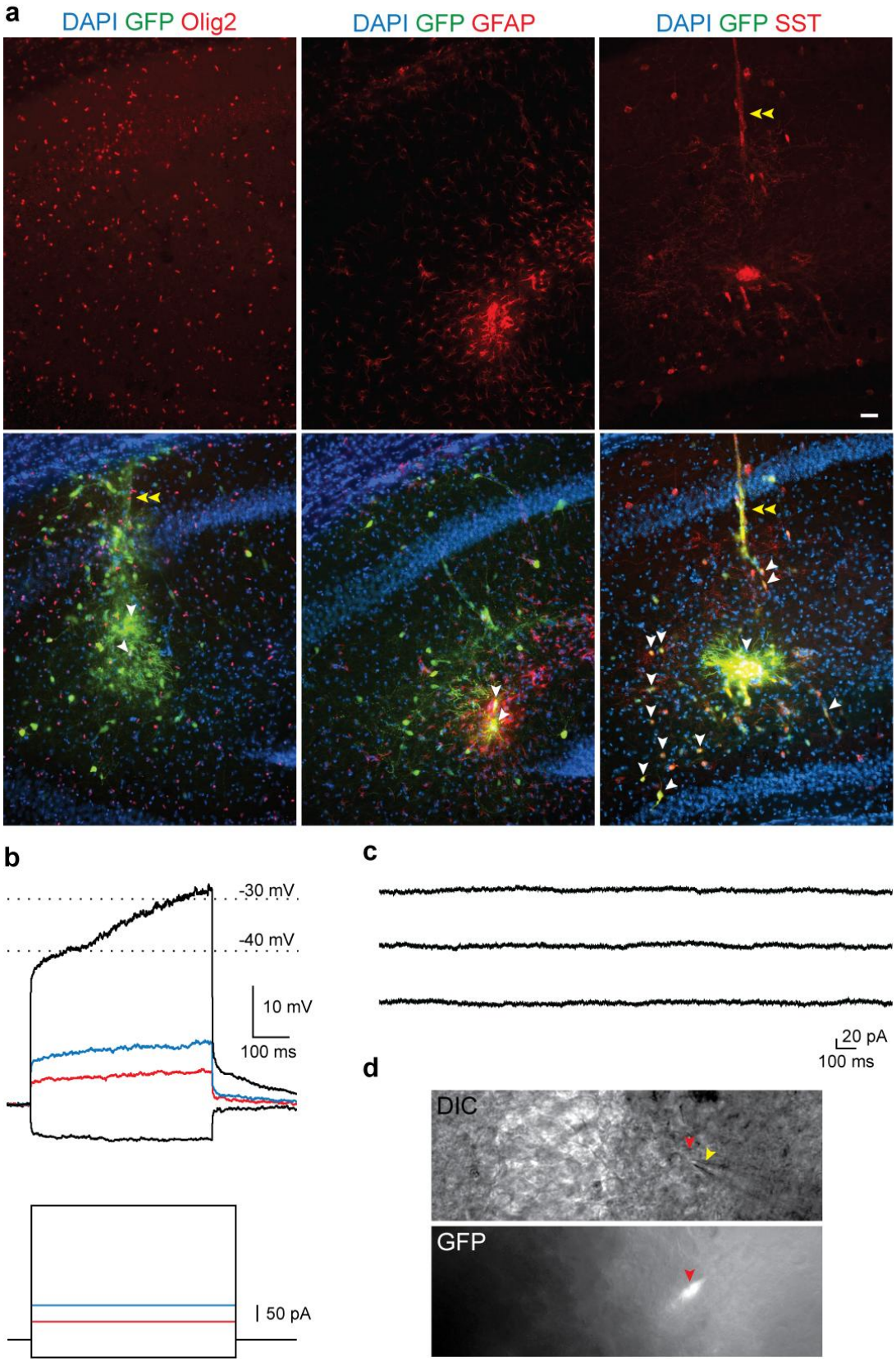
Robert F. Hunt, Kelly M. Girskis, John L. Rubenstein, Arturo Alvarez–Buylla, Scott C. Baraban



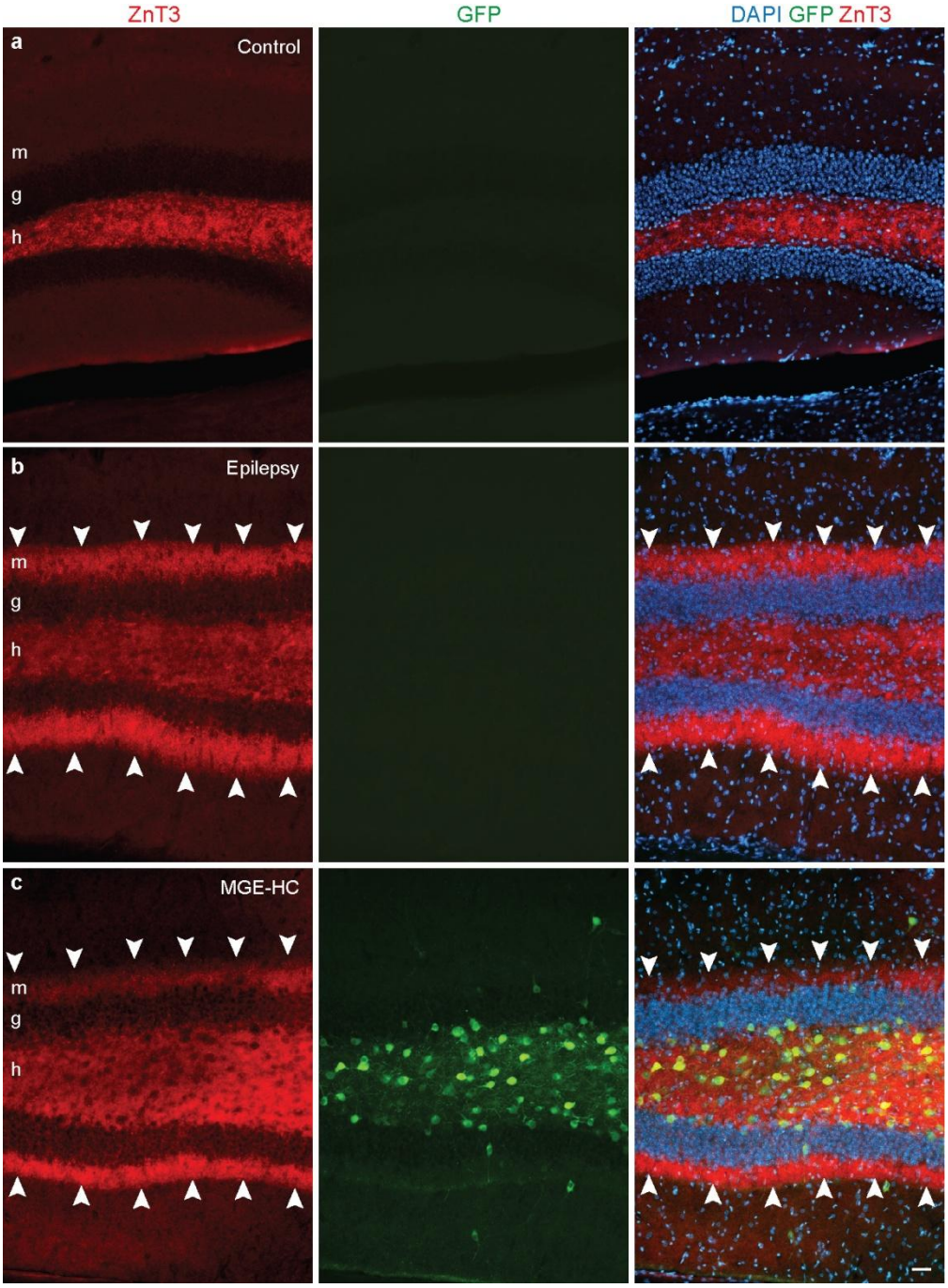
Supplementary Figure 1. Design of the cell transplantation procedure. An episode of status epilepticus (~2h of continuous seizures) was triggered in P51 adult mice by systemic injection of pilocarpine. Starting around P60, each mouse was video recorded and reviewed daily until at least one spontaneous convulsive seizure (Racine scale 3-5) was detected. Once a seizure was confirmed, mice were randomly assigned into untreated, vehicle-injected, or MGE treatment groups. For MGE transplantation, ventricular and subventricular regions of the MGE were taken from E13.5 β-actin:GFP mouse embryos and injected into either the CA3 subfield of the hippocampus or the basolateral amygdala. Most experiments were performed starting around 60 DAT.



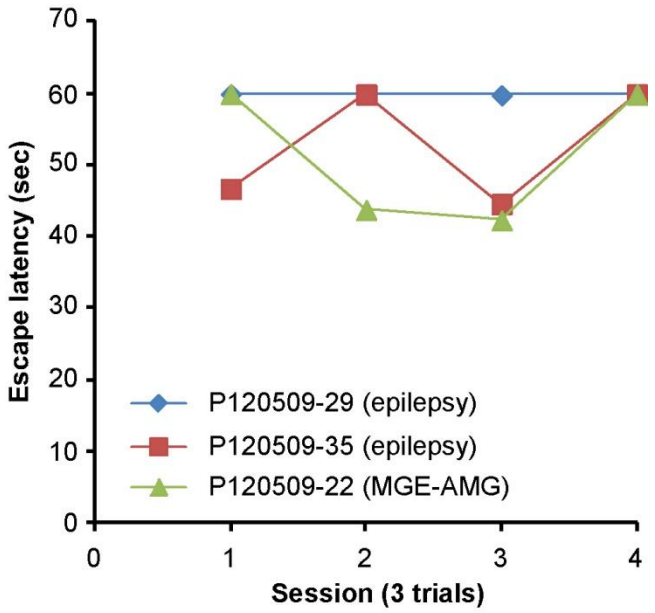
Supplementary Figure 2. Distribution of MGE-derived cells 7 DAT into adult hippocampus. a-d. Serial sections (300 µm apart) of the hippocampus of an adult P67 control recipient mouse labeled for transplanted MGE cells (green) and DAPI (blue). White double arrowhead in **b** indicates the injection site. **e.** Higher magnification of a different section from the same animal shows MGE cells with migratory morphologies positioned along stratum lucidum in CA3. Scale bars: 100 µm (**a-d**), 200 µm (**e**).


a
DAPI GFP Olig2
DAPI GFP GFAP
DAPI GFP SST
b
-30 mV
-40 mV
10 mV
100 ms
50 pA
c
20 pA
100 ms
d
DIC
GFP

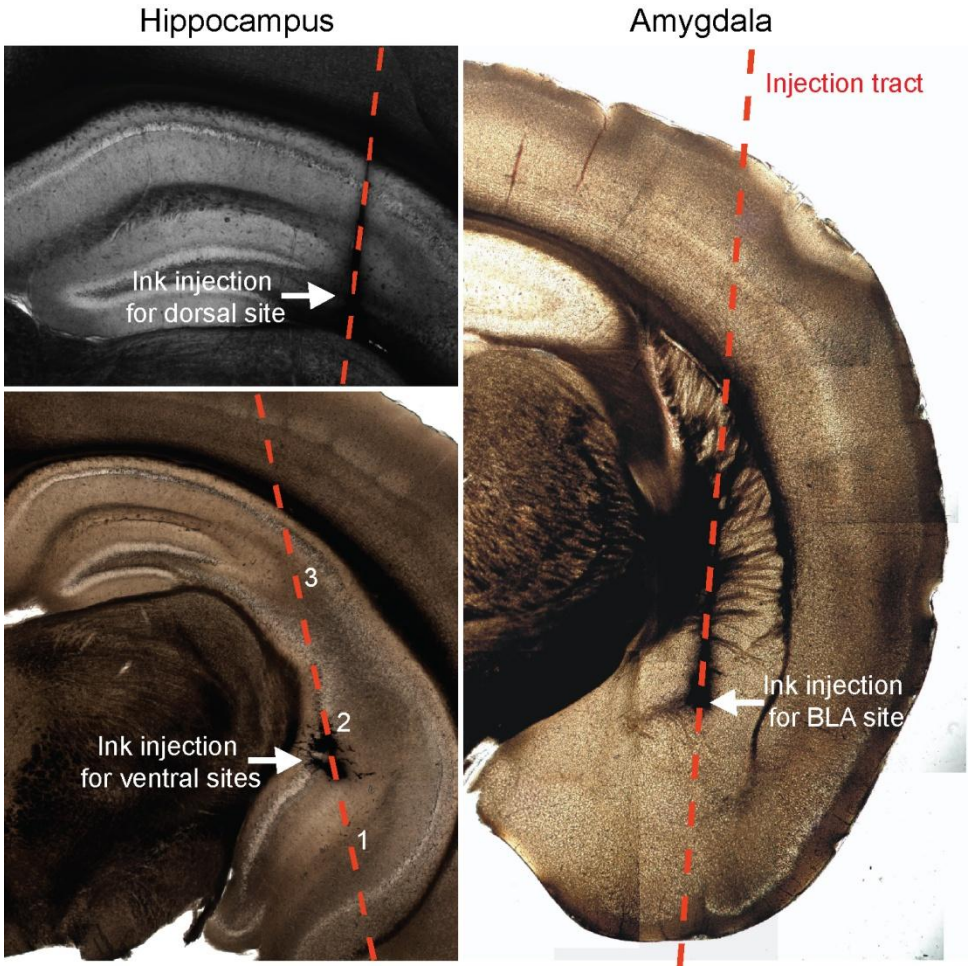
Supplementary Figure 3. MGE cell injections generate a small number of glia at the transplantation site. a. Examples of transplantation sites into CA3 of the hippocampus from three different animals (60 DAT) show that a small number of GFP cells colabel for Olig2 or GFAP. Note that even at the injection site, many GFP-labeled cells still coexpress SST. White arrowheads indicate colabeled cells. Yellow double arrowheads indicate injection tracts. Scale bar: 100 μm. **b.** To confirm the presence of a small number of glial cells around the injection, we obtained whole-cell patch-clamp recordings from 8 cells with glia-like morphologies at the injection site. These cells had relatively hyperpolarized resting potentials (-77.6 ± 4.2 mV), fast decay time constants (12.0 ± 2.9 ms), and did not fire action potentials upon depolarization. Shown are example current-clamp responses of a transplanted cell (61 DAT) to the indicated square wave current pulses (bottom) from a holding potential near -70mV. Note that strong depolarization did not evoke action potentials in this cell. **c.** Voltage-clamp recording (Vh = -70mV) from the same cell shown in **b** shows that this cell also did not receive excitatory synaptic input. **d.** DIC and epifluorescent images of the cell shown in **b** and **c** during recording. Red arrows indicate the GFP-labeled cell; yellow arrow indicates the tip of the recording pipette.



Supplementary Figure 4. Mossy fiber sprouting is present in pilocarpine-treated mice that received MGE cell grafts into the hippocampus. Sections of the dentate gyrus are labeled for ZnT3 (mossy fiber terminals, red), GFP-labeled cells (green), and DAPI (blue) in **a**, an age-matched control mouse; **b**, vehicle-injected epileptic mouse; and **c**, 60 DAT into the hippocampus of a mouse that received bilateral MGE cell grafts. White arrowheads indicate mossy fiber sprouting into the inner molecular layer. Abbreviations: m, molecular layer; g, granule cell layer; h, hilus. Scale bar: 50 μm.



Supplementary Figure 5. Mice excluded from water maze analysis. In the visible platform component of a Morris water maze test, two mice in the "epilepsy" group and one mouse that received MGE cells into amygdala (MGE-AMG) did not actively search for the platform and showed a significant deficit in visible platform training, defined as an average latency (mean of all trials in sessions 3 and 4) greater than the average escape latency plus two SD in naïve controls (28). These animals were excluded from further analysis.



Supplementary Figure 6. Preliminary dye injections. Injection coordinates were verified in a series of preliminary dye and/or cell injection studies into control and epileptic mice. For hippocampal injections, the polymorph region of CA3was chosen due to the relatively large size of this area and our observation that cell injections into this region did not produce any obvious tissue damage. Numbers correspond to the three locations along the hippocampal axis that received cell grafts. For amygdalar injections, the basolateral nuclei (BLA) were targeted in all mice.