Supporting Information

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SI Materials and Methods

Plasmids. GW1 full-length *Arc/Arg3.1* was constructed as follows: rArc/Arg3.1 coding sequence was amplified from pBSKII(+) rArc/Arg3.1 (1), and the 5′UTR and 3′UTRs were PCR amplified from oligo-dT-primed rat cDNA. Arc/Arg3.1 deletion constructs Arc/Arg3.1Δ91–100 and Arc/Arg3.1Δ195–214 were made with a QuikChange Site-Directed Mutagenesis Kit (Stratagene).

Cell Culture and Transfection. E20–21 rat hippocampi were dissected and treated with papain (10 units·ml^{-1}, 15 min; Worthington Biochemical) and then treated with a trypsin inhibitor (10 mg·ml^{-1}, 15 min; Sigma). After trituration, dissociated hippocampal neurons were plated on coverslips (2×10^5 cells/coverslip) coated with laminin (BD Biosciences) and poly-D-lysine (Chemicon). After 1 h plating, neurons were transferred into neurobasal medium with B27 (Gibco). Primary hippocampal cultures at 18–19 DIV were cotransfected with GW1-Arc/Arg3.1 and GW1-GFP with Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions, at a 1.5:1 molar ratio (1.5 μg of total DNA and 2 μL of lipid per 1.9 cm^2 well) to ensure that Arc constructs were expressed in all neurons expressing GFP. Nearly all neurons cotransfected with two different plasmids express both plasmids (2).

Immunohistochemistry. Tissue preparation and immunohistochemisty were performed as described (3, 4). Primary antibodies included rabbit anti-neuropeptide Y (1:8,000; ImmunoStar) and rabbit anti-calbindin (1:40,000; Swant). Primary antibodies were detected with biotinylated goat antirabbit (1:200; Vector Laboratories).

Drugs. Pentylenetetrazole (PTZ; Sigma) and kainate (Sigma) were dissolved in PBS at 5 and 3 mg/mL, respectively, and injected intraperitoneally at the doses indicated in *Results*.

Golgi–Cox Staining. To examine hippocampal morphology in Arc/Arg3.1 WT and mutant mice, brains from 3.5-mo-old animals were impregnated in Golgi solution with the FD Rapid Golgi staining kit (FD Neurotechnologies), according to the manufacturer's instructions. Coronal 100-μm sections were made on a cryostat, and spine morphology was examined after staining.

Quantitative Analysis of Brain Sections. Quantitative analysis of NPY and calbindin staining was performed as described (3, 5). Briefly, digitized images were obtained with a DEI-470 digital camera (Optronics) on a BX-60 micrscope (Olympus). Two coronal sections per mouse were selected. Integrated optical density was determined by BioQuant Image Analysis (R&M Biometrics) and averaged in two areas (0.04 mm^2 each) of each brain region. Relative NPY-IR levels were expressed as the ratio of integrated optical densities in the target region (mossy fibers and molecular layer of the dentate gyrus) and in the stratum radiatum of CA1 in the same section. No changes were detected in the stratum radiatum. The mean ratio of WT mice was defined as 1.0.

Seizure Severity Score. After PTZ administration, each mouse was placed in a new cage and its behavior was video recorded for 20 min. An investigator blinded to the genotype and treatment of the mice quantified the time course and severity of seizures. Seizure severity scores were: 0 = normal exploratory behavior; 1 = immobility; 2 = generalized spasm, tremble, or twitch; 3 = tail extension/forelimb clonus; 4 = generalized clonic activity; 5 = bouncing or running seizures; 6 = full tonic extension.

Chronic EEG Recordings. Adult (aged 7–9 mo) *Arc/Arg3.1*$^{-/-}$, *Arc/Arg3.1*$^{+/-}$, and *Arc/Arg3.1*$^{+/+}$ WT littermate mice were implanted for chronic video-EEG monitoring. Mice were anesthetized with Avertin (1.25% tribromoethanol/amyl alcohol solution, injected i.p. at 0.02 mL/g). Teflon-coated silver wire electrodes (0.005-inch diameter) attached to a microminiature connector were implanted bilaterally into the subdural space over temporal, parietal, and occipital cortices. Digital EEG activity was monitored daily for up to 2 wk during prolonged overnight and random 2-h sample recordings (Harmonie software version 6.1c, Stellate Systems). Recordings of similar durations among genotypes were reviewed by an investigator unaware of the genotypes of the mice. A video camera was used to monitor behavior during EEG recording periods. All recordings were carried out at least 24 h after surgery on mice freely moving in the test cage.

Morris Water Maze. Experimenters were blinded to the genotypes of the mice. Only male mice were used for behavioral assessment. The water maze consisted of a pool (122 cm in diameter) of opaque water (20 °C ± 1 °C) and a platform (14 cm in diameter) submerged 1.5 cm. Mice were first given four pretrainings (90 s/trial, day 0) in which they had to swim down a channel (15 × 122 cm) and mount a platform hidden 1.5 cm below the water surface at the middle of the channel. Hidden-platform training (days 1–5) consisted of 10 sessions (two sessions per day, 2 h apart), each with three trials. The platform location remained constant in the hidden-platform sessions, and the entry points were changed semirandomly between trials. The maximum trial time was 60 s. Mice that failed to find the platform were led to it and placed on it for 15 s. 24 and 72 h after the last hidden-platform training session, a probe trial was conducted by removing the platform and allowing mice to search in the pool for 60 s. For cued training sessions (days 9–11), the platform was marked with a visible mast, and the mice were trained to locate the platform over five sessions (two sessions per day for the first 2 d, 4 h apart; one session for the last day), each with two trials. The platform location was changed for each session. Time to reach the platform, time in target quadrant, platform crossings, path length, and swim speed were recorded with an EthoVision video tracking system (Noldus).

1. Lyford GL, et al. (1995) Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* 14:433–445.
2. Arrasate M, Finkbeiner S (2005) Automated microscope system for determining factors that predict neuronal fate. *Proc Natl Acad Sci USA* 102:3840–3845.
3. Kotloski R, Lynch M, Lauersdorf S, Sutula T (2002) Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. *Prog Brain Res* 135:95–110.
4. Palop JJ, et al. (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* 55:697–711.
5. Palop JJ, et al. (2005) Vulnerability of dentate granule cells to disruption of Arc expression in human amyloid precursor protein transgenic mice. *J Neurosci* 25:9686–9693.

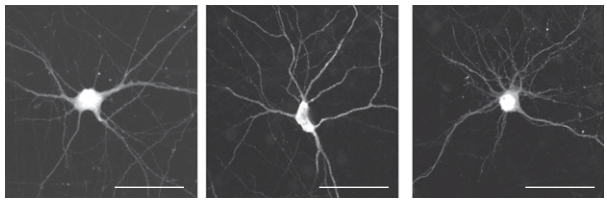

Fig. S1. Expression of Arc and mutant forms of Arc in neurons. WT Arc, and Arc mutants Δ91–100 and Δ195–214 are expressed in the cell body and dendrites of neurons. (Scale bar, 50 μm.)

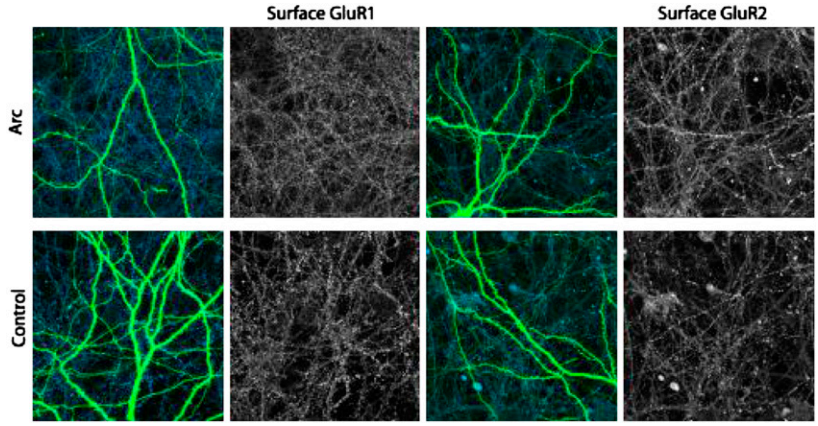

Fig. S2. Representative examples of surface GluR1 and GluR2 staining. Arc or a control vector and GFP were cotransfected into medium density primary hippocampal neurons at 18–19 DIV. At 36–48 h after transfection, the neurons were incubated with N-terminal GluR1 or GluR2 antibody for 45 min, fixed, and stained with the appropriate secondary antibody. Note that all neurons, including untransfected neurons, stain positively for surface GluR1; therefore, GluR1 puncta were analyzed by colocalization with a transfected spine.

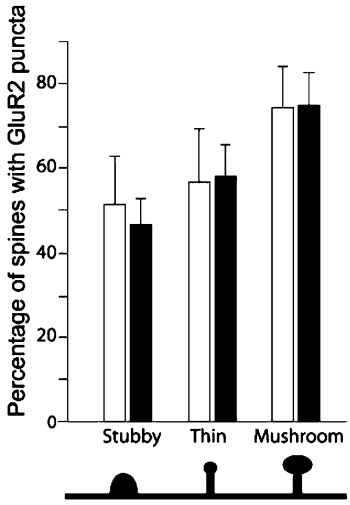

Fig. S3. Arc expression does not affect GluR2 surface expression at thin spines. At 18–19 DIV, hippocampal neurons were cotransfected with GFP and Arc or a control vector. At 36–48 h after transfection, neurons were incubated with a GluR2 N-terminal antibody for 45 min to stain surface GluR2. Arc expression did not alter surface staining of GluR2.

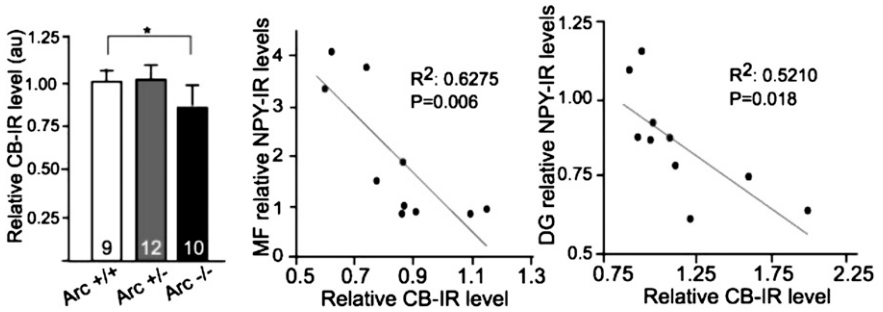

Fig. S4. $Arc^{-/-}$ mice show decreased levels of calbindin (CB) in dentate gyrus molecular layer. One-way ANOVA with post hoc Tukey test: $F_{(2,29)} = 5.606$; $^{*}P < 0.05$. Level of calbindin depletion correlates with NPY increases in mossy fibers and dentate gyrus of $Arc^{-/-}$ mice.

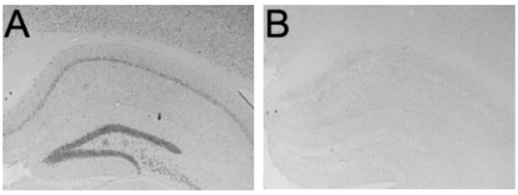

Fig. S5. Fos expression was highly induced 2 h after an unprovoked seizure in $Arc^{+/-}$ mouse (*A*); Minimal Fos expression was observed in an $Arc^{+/-}$ caged control (*B*).

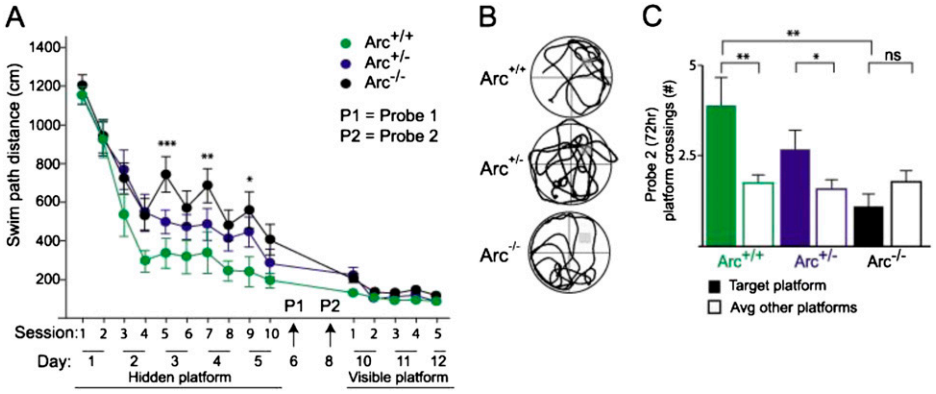

Fig. S6. Gene dose-dependent water maze deficits in $Arc^{+/-}$ and $Arc^{-/-}$ mice (n = 8–12 male mice/genotype, 5–7 mo of age). (*A*) Hidden platform learning curves differed by genotype (repeated-measures ANOVA: $P < 0.0001$). In Tukey–Kramer post hoc comparisons, $Arc^{+/+}$ mice differed from $Arc^{+/-}$ and $Arc^{-/-}$ mice ($P < 0.01$ and $P < 0.001$, respectively). In two-way ANOVA comparisons of individual sessions, $Arc^{+/+}$ mice differed from $Arc^{-/-}$ mice in sessions 5, 7, and 9 ($^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$). No differences in swim path were observed when the platform was visible. (*B* and *C*) Probe trial 72 h after hidden platform sessions. (*B*) Representative path tracings of 72-h probe trials. Circle depicts edge of water tank; gray square indicates approximate size and location of platform used during data acquisition. (*C*) Number of target platform crossings differed by genotype at the 72-h probe trial (one-way ANOVA of target crossings for $Arc^{+/+}$and $Arc^{-/-}$, $P < 0.01$). All genotypes except $Arc^{-/-}$ exhibited preference for the target location at 72-h probe trial ($^{**}P < 0.01$, $^{*}P < 0.05$).

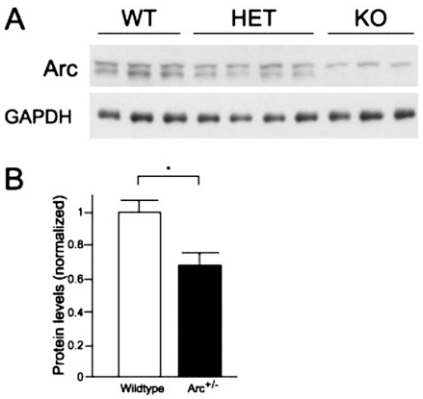

Fig. S7. Arc expression in Arc $^{+/-}$ mice. (*A*) Western blots of cortical lysates from $Arc^{+/+}$ (WT), $Arc^{+/-}$ (het), and $Arc^{-/-}$ (KO) mice. Each lane represents an individual mouse. (*B*) Quantification of signals, illustrating that heterozygotes express significantly less Arc (68 ± 7.2% of WT levels; t test, $P = 0.02$).