Experience-induced *Arc/Arg3.1* primes CA1 pyramidal neurons for mGluR-dependent long-term synaptic depression

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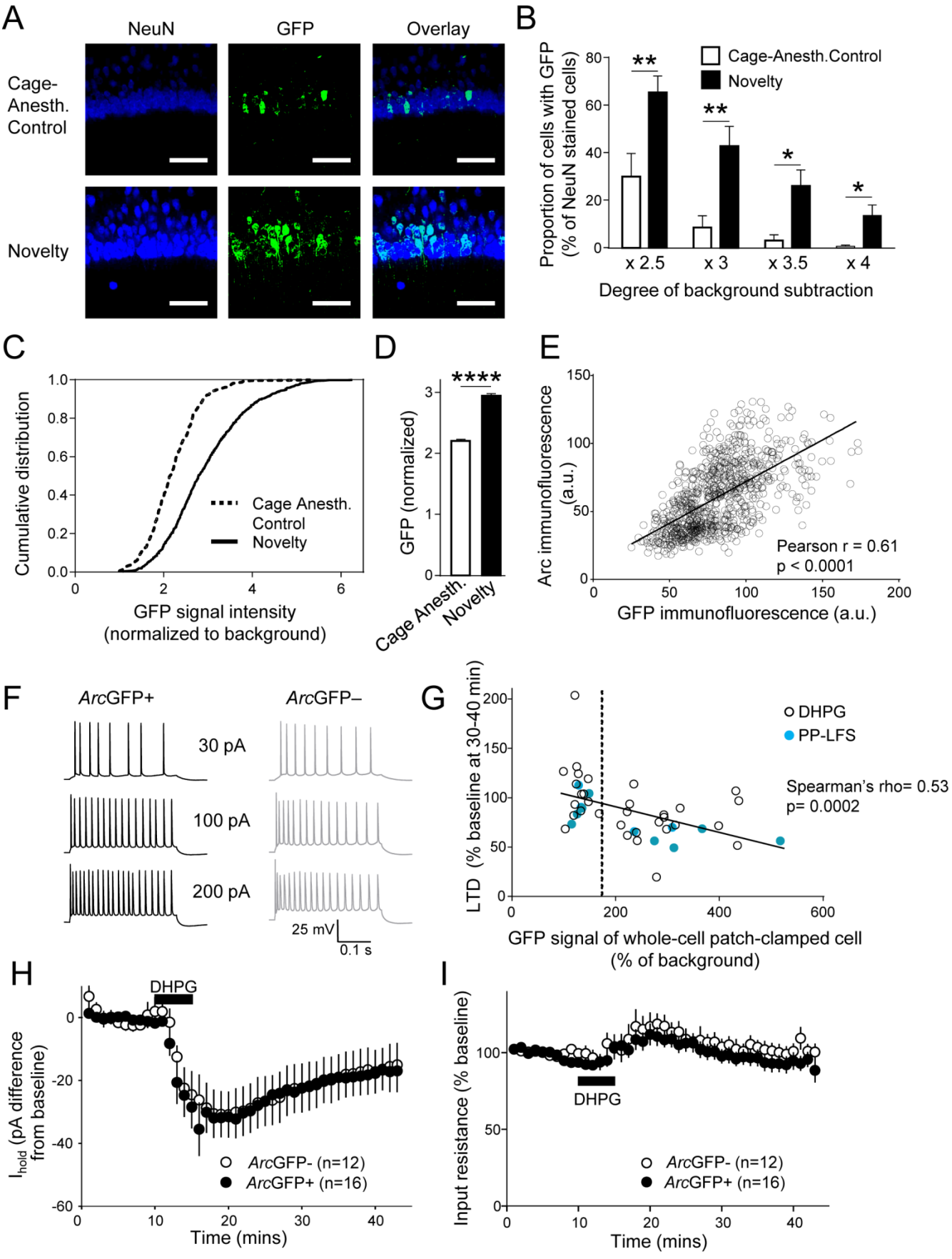
A
NeuN
GFP
Overlay
Cage-Anesth. Control
Novelty
B
Cage-Anesth.Control
Novelty
Proportion of cells with GFP (% of NeuN stained cells)
x 2.5
x 3
x 3.5
x 4
Degree of background subtraction
C
Cumulative distribution
Cage Anesth. Control
Novelty
GFP signal intensity (normalized to background)
D
GFP (normalized)
Cage Anesth.
Novelty
E
Arc immunofluorescence (a.u.)
Pearson r = 0.61
p < 0.0001
GFP immunofluorescence (a.u.)
F
ArcGFP+
ArcGFP–
30 pA
100 pA
200 pA
25 mV
0.1 s
G
DHPG
PP-LFS
Spearman's rho= 0.53
p= 0.0002
LTD (% baseline at 30-40 min)
GFP signal of whole-cell patch-clamped cell (% of background)
H
DHPG
Ihold (pA difference from baseline)
ArcGFP- (n=12)
ArcGFP+ (n=16)
Time (mins)
I
DHPG
Input resistance (% baseline)
ArcGFP- (n=12)
ArcGFP+ (n=16)
Time (mins)

Figure S1, Related to Figure 1. A-D) Brief novelty exposure enhances *Arc* induction in hippocampal CA1 neurons as measured by the *Arc*GFP reporter. **A-** *Representative images* of hippocampal area CA1 sections from *Arc*GFP-BAC mice stained for NeuN, a neuron soma marker, and GFP in a cage-anesthetized control mouse and a mouse exposed to 5 min novelty. The GFP immunofluorescence signal was thresholded at 4X above background fluorescence after immunohistochemical processing. For a representative image of GFP fluorescence of an acute slice involved in *in vitro* recordings, please see Fig 1B. Scale bar = 25 µm. **B-** *Novelty increases the % of GFP+ neurons*. After subtraction of 2.5 – 4 X background from the GFP immunofluorescence signal, the number of GFP+ cells were determined as a percentage of total NeuN+ neurons (n=6,10 slices for cage-anesthetized, novelty, * $p<0.05$, ** $p < 0.01$, unpaired student's t-test for each background subtraction). **C, D-** *Novelty increases GFP intensity in a GFP+ neuron*. Novelty (n=803 neurons, 10 slices) increases intensity of GFP immunofluorescence in GFP+ neurons (**** $p < 0.0001$, Two-sample Kolmogorov-Smirnov test) compaired to cage-anesthetized control mice (n=508 neurons, 6 slices). All cells with GFP signal greater than 1 x background were included in the analysis. **E) GFP and Arc protein immunofluorescence are correlated.** In novelty exposed ArcBAC-GFP mice, the intensities of GFP and Arc protein immunofluorescence (n=803 neurons, 10 slices) in the cell soma are highly correlated. **F**) **ArcGFP+ and ArcGFP- neurons have similar excitability.** Example traces of action potentials evoked by current injection of 30, 100, and 200 pA for ArcGFP+ (black) and ArcGFP– (grey) neurons in CA1 illustrate no difference in firing properties of ArcGFP+ and ArcGFP– neurons. **G) mGluR-LTD magnitude and *Arc*GFP intensity are correlated.** mGluR-LTD magnitude in individual CA1 neurons is plotted as a function of the GFP fluorescence intensity of that neuron. GFP fluorescence of CA1 neurons in acute brain slices was measured using confocal microscopy prior to obtaining a whole cell recording. GFP fluorescence is expressed as a percentage of background fluorescence (measured in a region most distant from CA1 cell body layer) of that slice. mGluR-LTD was measured by the percent change (normalized to baseline) in evoked EPSC amplitude or mEPSC frequency 30-40 min after DHPG (same cells as in Figures 1D,E,F; open circles) or from evoked EPSC experiments after PP-LFS (same cells as in Figure 1D,E; blue filled circles). The dotted line separates ArcGFP– (left) and ArcGFP+ (right) values. Spearman nonparametric correlation test was used to determine statistical significance (n = 45 cells). **H,I) DHPG causes similar changes in passive membrane properties of ArcGFP+ and ArcGFP- neurons.** DHPG application causes similar changes in holding current required to maintain membrane potential at -60 mV or input resistance in *Arc*GFP+ and *Arc*GFP– neurons from *Arc*GFP–BAC mice. Data taken from DHPG–LTD experiments in Figure 1D,F.

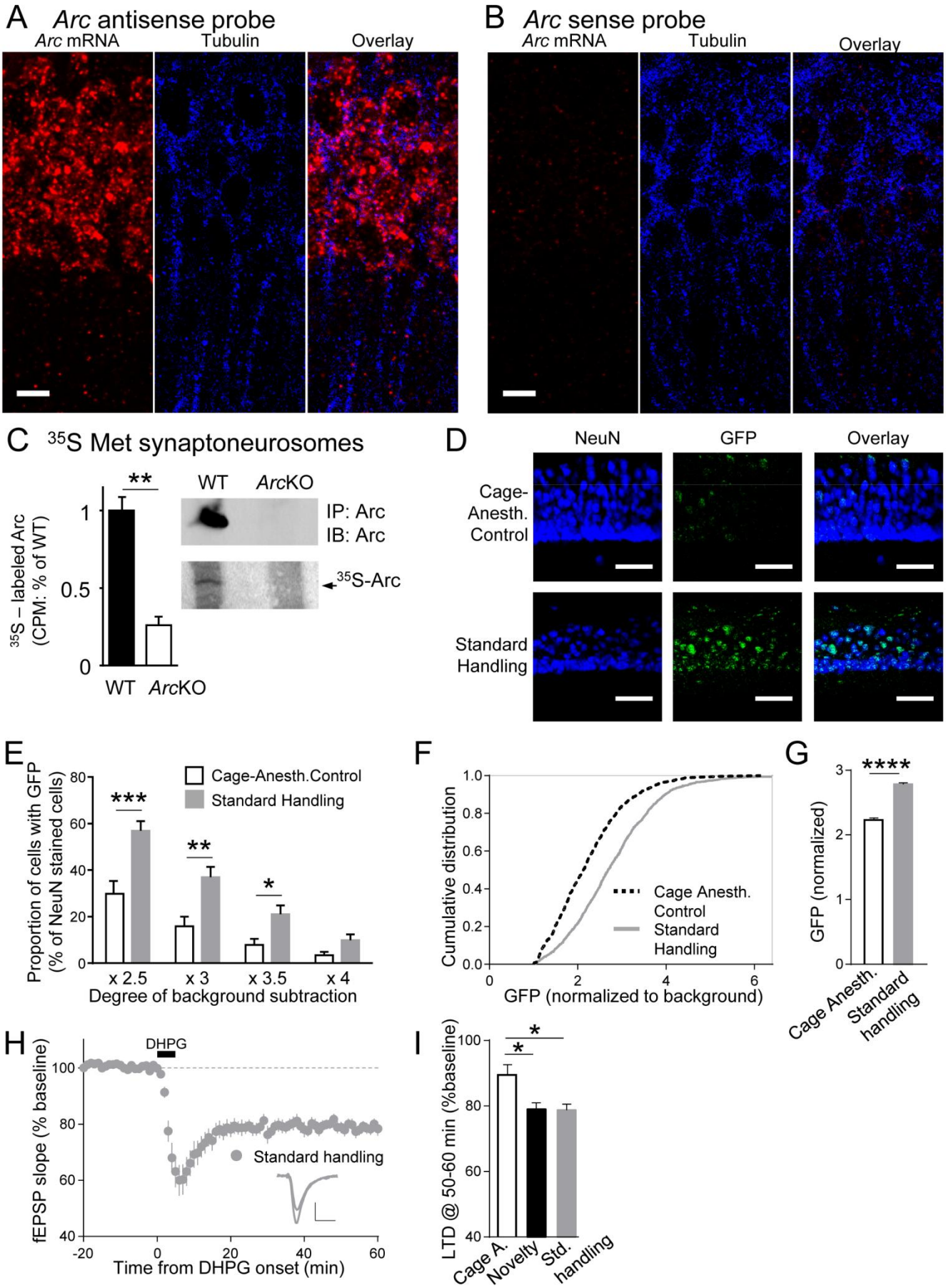
A Arc antisense probe
Arc mRNA
Tubulin
Overlay
B Arc sense probe
Arc mRNA
Tubulin
Overlay
C 35S Met synaptoneurosomes
**
WT
ArcKO
IP: Arc
IB: Arc
35S-Arc
35S – labeled Arc (CPM: % of WT)
1
0.5
0
WT
ArcKO
D
NeuN
GFP
Overlay
Cage-Anesth. Control
Standard Handling
E
Cage-Anesth.Control
Standard Handling
***
**
*
Proportion of cells with GFP (% of NeuN stained cells)
80
60
40
20
0
x 2.5
x 3
x 3.5
x 4
Degree of background subtraction
F
Cumulative distribution
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
GFP (normalized to background)
Cage Anesth. Control
Standard Handling
G
****
GFP (normalized)
3
2
1
0
Cage Anesth.
Standard handling
H
DHPG
fEPSP slope (% baseline)
100
80
60
40
Standard handling
-20
0
20
40
60
Time from DHPG onset (min)
I
*
*
LTD @ 50-60 min (%baseline)
100
80
60
40
Cage A.
Novelty
Std. handling

Figure S2, Related to Figure 2. **A-C) Specificity of reagents used to measure Arc mRNA and protein**. **A-** *Representative image* (40x) of a hippocampal CA1 section processed for FISH using digoxigenin-labeled riboprobe (antisense) demonstrates robust *Arc* mRNA expression after novelty. **B-** *Representative image* of a section after hybridization using the *Arc* sense probe shows little or no detectable signal in CA1 neurons after novelty. Scale bar = 10 µm. **C-** *Control experiment* demonstrating the specificity of the Arc immunoprecipation and ^{35}S Met incorporation into Arc for experiments in Figure 2F,G. Hippocampal synaptoneurosomes were prepared from wildtype (WT) or *Arc*GFP-KI$^{-/-}$ (*Arc*KO) mice and subjected to the ^{35}S Met incubation and Arc immunoprecipitation protocol as used in Figure 2F. **D,E) Standard handling procedures for slice physiology experiments induce *Arc*GFP and enhance mGluR-LTD.** Mice in the "standard handling" group display greater *Arc* induction than those in the cage-anesthetized group in hippocampal CA1 neurons as measured by the *Arc*GFP reporter. **D-** *Representative images* of hippocampal area CA1 sections from *Arc*GFP-BAC mice stained for NeuN, a neuron soma marker, and GFP in a cage-anesthetized mouse and a standard-handling mouse removed from its home cage, transported to the lab and anesthetized in the lab. The GFP immunofluorescence signal was thresholded at 3X above background fluorescence after immunohistochemical processing. Scale bar = 25 µm. **E-** *Quantified group data of % ArcGFP+ neurons* in standard handling and cage-anesthetized groups. After subtraction of 2.5 – 4 X background from the GFP immunofluorescence signal, the number of GFP+ cells were determined as a percentage of total NeuN+ neurons (n=10,17 slices for cage-anesthetized, standard-handling, * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$, unpaired student's t-test for each background subtraction). **F,G**) *GFP intensity in a GFP+ neuron* is greater for cells from the standard-handling group compared to those from cage-anesthetized mice (standard-handling=1515 neurons, 17 slices, cage-anesthetized=817 neurons,10 slices **** $p < 0.0001$, Two-sample Kolmogorov-Smirnov test). All cells with GFP signal greater than 1 x background were included in the analysis. **H)** For standard-handling mice, DHPG (100 µM; 5 min) induced LTD of population field (f) EPSPs measured with extracellular recordings (n=19 slices, 7 mice). Time course of average fEPSP slope (Avg±SEM) normalized to pre-DHPG baseline. **I)** Comparison of LTD magnitude (fEPSP slope; % baseline at 50-60 min after DHPG) in cage-anesthetized (n = 10 slices; 7 mice), novel object exploration (n = 20 slices; 8 mice) and standard-handling group (n = 19 slices; 7 mice) of mice (* $p<0.05$, Kruskal-Wallis test with Dunn's multiple comparisons test).

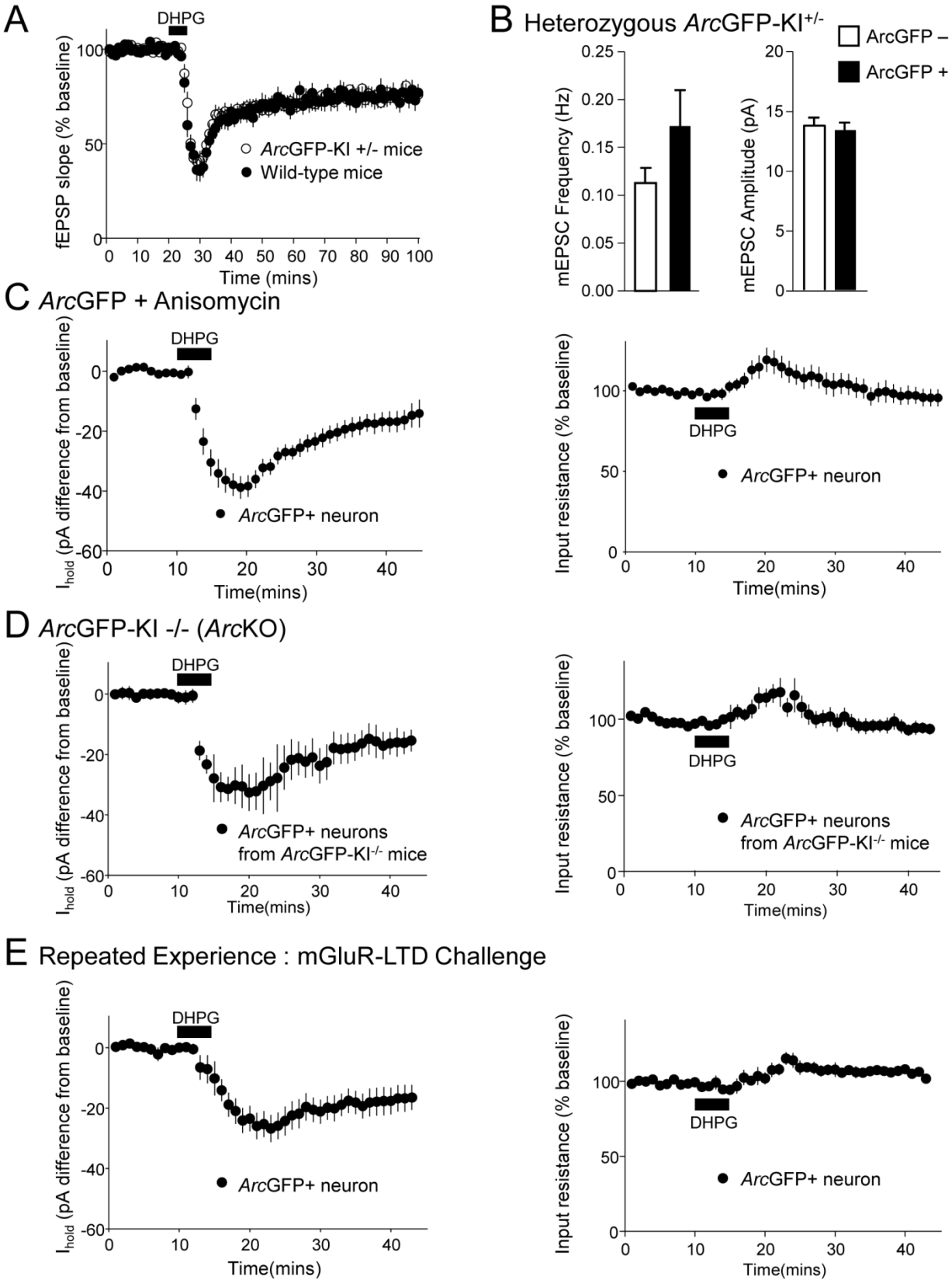

Figure S3, Related to Figure 3. A) mGluR-LTD magnitude is normal in Arc heterozygous *Arc*GFP-KI$^{+/-}$ mice. DHPG (100 µM; 5 min) induced LTD of population EPSPs measured with extracellular field potential

recordings was not different between heterozygous *Arc*GFP-KI$^{+/-}$ (n=15 slices, 8 mice) and WT littermate controls (n=14 slices, 7 mice). For this analysis, mice in both groups were not habituated to human handling and were anesthetized in the lab 3-7 minutes after removal from the home cage. **B) *Arc*GFP+ and *Arc*GFP– neurons from heterozygous *Arc*GFP-KI$^{+/-}$ mice had similar mEPSC frequencies.** There was no difference in mEPSC frequency (p = 0.11) and amplitude between *Arc*GFP+ and *Arc*GFP– neurons (n=8, independent consecutive recordings from neighbor *Arc*GFP+ and *Arc*GFP – cells in same acute slice) from novelty-exposed heterozygous *Arc*GFP-KI$^{+/-}$ mice. **C,D,E) Effects of DHPG on passive membrane properties of ArcGFP+ and ArcGFP- neurons used for LTD experiments in Figure 3. C-** In the presence of anisomycin, ArcGFP+ neurons (n=13) from novelty-exposed *Arc*GFP-BAC mice normally respond to DHPG application with acute changes in input resistance and holding current (required to maintain membrane potential at -60 mV), yet do not demonstrate LTD (Figure 3A). Data taken from DHPG–LTD experiments in Figure 3A. **D-** ArcGFP+ neurons (n=11) from novelty-exposed homozygous ArcGFP-KI$^{-/-}$ (*Arc*KO) mice respond to DHPG application with acute changes in input resistance and holding current, but do not express LTD (Figure 3C). Data taken from DHPG–LTD experiments in Figure 3C. **E-** After multiple repeated exposures to the same environment, ArcGFP+ neurons (n=8) respond to DHPG application with acute changes in input resistance and holding current, but do not express LTD (Figure 3E). Data taken from DHPG–LTD experiments in Figure 3E.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mice

*Arc*GFP-BAC express a destabilized enhanced green fluorescent protein (d4EGFP) with a 4 hour half-life under the control of the Arc/Arg3.1 promoter on a BAC (Bacterial Artificial Chromosome) transgene (Grinevich et al., 2009). The destabilized GFP expression faithfully reports endogenous Arc induction in response to neuronal activity and experience with a decay time comparable to that of Arc (Grinevich et al., 2009). The *Arc*GFP-KI mice express a destabilized (2-h half-life) form of GFP (d2EGFP). The coding sequence for d2EGFP was knocked in and replaced the coding sequence of the endogenous Arc gene. Evidence that GFP is a reliable indicator of recent *Arc* induction in the *Arc*GFP-KI mice has been previously shown (Wang et al., 2006). Both *Arc*GFP-BAC and *Arc*GFP-KI mice were backcrossed for at least 3 generations onto C57/BL6J mice obtained from the UT Southwestern breeding core facility prior to experiments.

Reagents

(*RS*)-3,5-Dihydroxyphenylglycine (DHPG) was purchased from Tocris Bioscience (Minneapolis, MN), prepared as a 100X stock in distilled water, stored at -20 ºC and used within 10 days. Picrotoxin was purchased from Sigma-Aldrich (St. Louis, MO), and freshly dissolved in the artificial cerebrospinal fluid (ACSF) used during recording. DL-AP5 (DL-2-Amino-5-phosphonopentanoic acid) was purchased from Tocris Bioscience, prepared as a 10x stock in distilled water, stored at 4ºC and used within 7 days. Tetrodotoxin (TTX) was purchased from Enzo Life Sciences (Farmingdale, NY), prepared as a 1000X stock in distilled water, stored at -20 ºC, and used within 14 days. Anisomycin was purchased from Sigma-Aldrich and freshly dissolved in the ACSF used during recording.

Novel Experience

Novelty-exposed mice: All mice were handled daily for 4-5 days prior to the experiment to encourage familiarization and habituation to the experimenter and to handling procedures. The novel environment consisted of a 55x55x35 cm cardboard box containing randomly distributed wooden and plastic objects with unique shapes, sizes, colors and texture. The objects included *a) T-type ribbed yellow plastic tube* : SuperPet Fun-nel (SuperPet, California) 3.5'' long, 2'' wide, ribbed texture, translucent yellow, plastic T-tube with three openings, each opening of diameter 1.5'' *b) Coarse exterior wooden hut :* Small Gnawsome hut (Ware Manufacturing Inc, Arizona; model # 03883) A 5'' x 4'' x 3.25'' rectangular cuboid wooden hut with a coarse, unpolished texture. Entry to the hut was by an arch 2'' in height and 1.5'' in width. *c) T-type smooth white PVC pipe:* 3.5'' long, 1.5'' wide, smooth texture, opaque white, PVC T-tube with three openings, each opening of diameter 1.25''. *e) Smooth green plastic Igloo*: Itty Bitty Igloo (SuperPet, California; model # 60403) – 4'' x 4.5'' x 3'' igloo shaped smooth translucent green plastic structure. *E) Smooth exterior elevated wooden hut*: A 6'' x 4.5'' x 3'' wooden hut with a smooth finish and horizontal grooves cut into wood. Access to hut was via a

wooden ramp. 1.5'' diameter holes on all 4 sides of the hut allowed light illumination, exploration and exit of mouse from hut. After 5 minutes of a novel experience, the mouse was left undisturbed in a standard empty cage for 3 hours prior to anesthesia and acute slice preparation to allow newly induced *Arc* mRNA to reach the distal most dendritic regions (Steward and Worley, 2002; Wallace et al., 1998).

Cage-anesthetized mice: While preparing acute brain slices from wild-type mice, human handling and transfer of rodents is likely a novel experience and at least mildly stressful (Balcombe et al., 2004). Thirty seconds of a novel exposure is sufficient to induce maximal *Arc* induction in CA1 (Pevzner et al., 2012) and mild, novelty-related stress, such as putting a rodent in a new cage or box, or exposure to novel objects facilitates mGluR dependent LTD induction both *in vivo* and *in vitro* (Chaouloff et al., 2007; Niehusmann et al., 2010; Popkirov and Manahan-Vaughan, 2010). In these and related studies, protocols of prior habituation to humans or environments resulted in minimal (5–10% CA1 neurons) *Arc* induction (Guzowski et al., 1999) and decreased mGluR-LTD (Chaouloff et al., 2007; Niehusmann et al., 2010; Popkirov and Manahan-Vaughan, 2010). In agreement with these studies we find that the brief period of simply handling the mouse while transporting it to the lab in a new box significantly induces *Arc*GFP (Fig. S2D-G) and enhances mGluR-LTD (Fig. S2H,I). For cage-anesthetized control mice, : a) All mice were handled daily for 4-5 days prior to the experiment to encourage familiarization and habituation to the experimenter and to handling procedures and b) For minimal experimenter or environment triggered novel experience, deep anesthesia with Isoflurane was achieved within 10 seconds of removal from housing cages and mice were injected I.P. with Ketamine (125 mg/kg)/Xylazine (25 mg/kg) before transport to the lab for slice preparation.

"Standard-handling" protocol for mice: Mice in the standard-handling group were not habituated to human handling, and on the day of the experiment were removed from their home cage, transported to the lab and injected I.P. with Ketamine (125 mg/kg)/Xylazine (25 mg/kg) prior to slice preparation.

Slice preparation

Acute hippocampal brain slices (400 µm) were prepared from 18- to 27-day-old mice as described previously (Huber et al., 2000; Volk et al., 2007) with some modifications. The NMDA receptor antagonist Ketamine (125 mg/kg) was used along with Xylazine (25 mg/kg) for anesthesia to prevent *Arc* induction during acute slice preparation (Lyford et al., 1995; Steward and Worley, 2001). Once anesthetized, mice were transcardially perfused with chilled (4°C) sucrose dissection buffer containing (in mM): 2.6 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 5 $MgCl_2$, 212 sucrose, and 10 dextrose aerated with 95% O_2/5% CO_2. The dissection buffer contained a high Mg^{2+}/Ca^{2+} (10:1) ratio to suppress synaptic transmission, neuronal activity and *Arc* induction during acute slice preparation. Hippocampi were dissected and transverse hippocampal slices were obtained on a Leica VT1200S slicer. For recordings involving measurement of evoked synaptic transmission, CA3 was cut off to avoid epileptogenic activity induced by DHPG. For whole-cell experiments, slices recovered for the

first 30 min at 35° C and for the next 30 min at room temperature in artificial CSF (ACSF). For local field potential recordings, slices recovered at and were maintained at 30° C. For both whole-cell and field potential experiments, ACSF contained (in mM) : 119 NaCl, 2.5 KCl, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 1 NaH2PO4 and 11 D-Glucose aerated with 95% O_2/5% CO_2 to pH 7.4, with one exception. For field potential recordings in Fig. S3A, ACSF contained (in mM) : 124 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 1.25 NaH2PO4 and 10 D-Glucose.

Electrophysiology

For whole cell recordings, one-three hours after slicing, slices were transferred to a recording chamber where they were submerged and perfused with ACSF containing the $GABA_A$ antagonist picrotoxin (0.025 mM) at 2.5-3 ml/min (30 ± 1° C). For PP-LFS (Paired-Pulse Low Frequency Stimulation) experiments, the NMDA receptor antagonist DL-AP5 (100 µM) was added to the ACSF to isolate the mGluR-dependent component of LTD (Huber et al., 2000; Volk et al., 2007). TTX (0.001 mM) was added to the ACSF for miniature EPSC (mEPSC) recordings. Whole-cell patch-clamp recordings were obtained from *Arc*GFP+ and *Arc*GFP– CA1 pyramidal neurons in acute hippocampal slices and visualized using IR-DIC optics and a Zeiss LSM 510 confocal microscope. Cells with GFP signal at or below background signal were considered *Arc*GFP− and cells with GFP signal at least twice that of background were considered *Arc*GFP+ (Fig. S1G). We attempted to record from the brightest ArcGFP+ neurons in an attempt to sample neurons that were activated by recent novel experience. Background fluorescence was determined as the average fluorescent signal in 3-5 circular 50 µM diameter regions without detectable GFP+ dendrites and most distant from CA1 cell body layer.

For evoked recordings, test stimuli (10-20 µA, 100 µs, monophasic current pulses) were delivered to Schaffer collateral axons ~50-100 µm from the recorded neurons using a platinum/iridium cluster electrode (FHC; catalog # CE2B55) every 20s to obtain a stable baseline of evoked EPSC amplitudes for at least 10 min prior to DHPG (100 µM; 5 min) or PP-LFS application. PP-LFS consisted of paired pulses (50 ms interstimulus interval) of low frequency (each pair repeated at 1Hz for 15 min) stimulation. Pipettes (5-7 MΩ) were filled with a 7.2 pH, 285 mOsm solution containing (in mM) 130 K-gluconate, 4 KCl, 2 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Na, 14 phosphocreatine-Tris, 10 sucrose. If needed, osmolarity was adjusted to 285 mOsm with water or sucrose. Cells were voltage clamped at –60 mV. Series and input resistance were measured in voltage clamp with a 800 ms, –10 mV step from a –60 mV holding potential (filtered at 30 kHz and sampled at 50 kHz) and monitored throughout the recording session. Cells were only included in the analysis if they had a series resistance < 25 MΩ that was stable throughout the experiment. mEPSCs were detected off-line using an automatic detection program (Minianalysis; Synaptosoft, Decatur, GA). A value greater than 3 times the RMS noise value was initially set as the detection threshold for the automatic program, followed by a second round of visual scrutiny and confirmation/exclusion of each program-detected mEPSC. The detection threshold was constant for the duration of each experiment. mEPSC frequency was calculated for a 10 min duration prior to

DHPG (baseline) and for a 10 min duration beginning 30 min after onset of DHPG application. Chemically induced mGluR-LTD was elicited by 50 µM DHPG for mEPSC experiments. To obtain reliable and robust LTD of evoked EPSCs and fEPSPs, we found we had to use 100 µM DHPG (5 min). Examination of action potential firing was done in CA1 slices independent of other experiments to avoid the possibility of neuronal firing activity inducing IEGs that could potentially act as a confound in our LTD experiments. Action potentials were elicited in response to 600-ms current steps of increasing amplitude.

For field potential recordings, one-three hours after slicing, slices were transferred to a recording chamber where they were submerged and perfused with ACSF at 2.5-3 ml/min (30 ± 1° C). Field potentials (fEPSPs) were evoked by stimulation of the Schaffer collateral pathway with a concentric bipolar tungsten electrode (FHC; catalog # CBBRC75). A glass pipette (1 MΩ) filled with ACSF was placed in the stratum radiatum of CA1 for recordings. Test stimuli were delivered every 30 s and a 20 min stable baseline was obtained at ~ 50% of the maximum FP amplitude.

Immunocytochemistry, image acquisition and analysis

Acute hippocampal slices (400 µm) were prepared as for electrophysiology and submerged in a static submersion chamber containing ACSF + 0.001 mM TTX + 0.025 mM picrotoxin, aerated with 95% O_2/5% CO_2 to pH 7.4; Vehicle) with or without DHPG. Slices from the same mouse were randomly assigned to ACSF or DHPG groups and simultaneously processed and imaged. 5 minutes after DHPG or Vehicle treatment, acute slices were fixed overnight in 4% paraformaldehyde (in 0.1 M phosphate buffer). Fixed slices were washed thrice with PBS, each wash for 15 mins, then embedded in 3% agarose/PBS and re-sectioned on a Leica VT1000S vibratome to yield 50 µm thick sections for immunocytochemistry. Sections were blocked for 1 h at room temperature in PBS with 3% goat serum and 0.5% Triton-X. Primary antibodies were dissolved in blocking solution and applied to sections overnight at 4° C. For immunofluoresence of NeuN, Arc and GFP in soma, fixed sections were incubated in 1° anti-Arc (1:600, Synaptic Systems), 1° anti-NeuN (1:600, Millipore), and 1° anti-GFP (1:600, Aves Labs). Anti-NeuN was replaced with anti-βIII-tubulin (1:600, Abcam) for immunocytochemistry assay in dendrites. Primary antibodies were detected with subsequent application of AlexaFluor555 (AF555), AF488 or AF633 conjugated 2° antibodies to the appropriate species IgG (Molecular Probes). Each condition had at least two mice. To decrease variability in immunostaining across conditions, for every comparison, sections were incubated on the same day in independent wells but with the same stock solution of primary or secondary antibodies. A Zeiss LSM 510 Laser-scanning confocal microscope was used to take 1µm section images at a resolution of 20 pixels/µm^2. To assist the identification of source of dendrites (i.e. whether a dendrite originated in a *Arc*GFP+ or a *Arc*GFP– cell, 10 serial images (in a Z-stack, 1µm sections, lower resolution (1.3 pixels/µm^2) were taken above and below target high resolution image. The same laser and scanning settings were used for images within an experiment to allow for comparison across conditions. Fluorescence images were analyzed using Metamorph software (Molecular Devices). For analysis of immunofluorescence in the soma or dendrite, somatic or dendritic borders of *Arc*GFP+ and *Arc*GFP– cells

were traced (with the Trace Region tool) in the corresponding NeuN or tubulin immunostained image using Metamorph software. Cells with GFP signal at or below background signal were considered *Arc*GFP− and cells with GFP signal at least thrice that of background were considered *Arc*GFP+.The traced regions were copied to corresponding Arc and GFP images for analysis. Background fluorescence was determined as the average fluorescent signal in 3-5 circular regions (~20 µM diameter) without detectable GFP+ dendrites around a) basal dendritic region near the alveus and b) the distal most apical dendritic region. Images were set at a threshold value. For the soma analysis threshold values were set at 10x, 3x and 2.5x above background for NeuN, GFP and Arc, respectively. For soma analysis of Arc/NeuN in DHPG treatment conditions, data was collected from 2 mice for these conditions: ACSF=15 sections from 4 acute slices, DHPG= 12 sections from 4 acute slices. Slices from the same mouse were randomly assigned to ACSF (Vehicle) or DHPG groups and simultaneously processed and imaged. For counting ArcGFP+ cells in "cage-anesthetized control" versus "novelty" conditions (Fig. S1A,B) the border of every every NeuN+ region was traced and considered as a cell (Cage Anesthetized control=508 neurons from 6 slices, Novelty=803 neurons from 10 slices) , the traced borders were transfered to the corresponding GFP stained image, the average GFP signal intensity of each traced cell was extracted into Microsoft Excel, and a filter function 2.5 – 4 times background was employed to count all cells with a signal stronger than the filter . Threshold values for dendritic analysis (Fig. 2C,D) were set at 10x, 2x and 2x above background for tubulin, GFP and Arc, respectively. The Integrated Morphometric Analysis tool in Metamorph was used for quantification of fluorescence. Within each traced region (soma or dendrite) the area and intensity of each fluorescent patch (or object) above threshold was determined. The product of the area and intensity of each thresholded patch was used as the measure of fluorescence intensity for that patch. The fluorescence intensity of all patches within a traced region was summed and then divided by the area of the traced region to accommodate for differences in size of soma or dendrites. To quantify soma Arc levels between ArcGFP+ and ArcGFP- neurons in ACSF and DHPG conditions, Arc/NeuN immunofluorescence values were normalized to the average *Arc*GFP– soma Arc immunofluorescence value in the same image (Fig. 1A). To quantify the effects of DHPG on soma and dendritic Arc levels, Arc/tubulin values were normalized to the average *Arc*GFP– Arc/tubulin ratio in the Vehicle condition of that experiment. GFP levels were often dim or absent in the dendrites of *Arc*GFP+ neurons and therefore were not a reliable marker of dendrites from *Arc*GFP+ or *Arc*GFP– cell soma. Therefore, for GFP– dendrites, only dendrites that could be verified to be in continuation with an *Arc*GFP– soma in the confocal Z-stacks were labeled as *Arc*GFP– dendrites, leading to a lower but reliable number of GFP– dendrites analyzed for Arc protein. Apical dendrites were analysed 10 µm to 70 µm from the CA1 cell soma. The GFP intensity of dendrites analyzed from both Vehicle and DHPG conditions was similar: median (± SEM) GFP/tubulin ratio of GFP+ dendrites: ACSF=0.098 ± 0.05, DHPG=0.1277 ± 0.05, p=0.99, Mann-Whitney U Test. Dendritic analysis utilized: Vehicle=18 sections/10 acute slices from 5 mice; DHPG=11 sections/10 acute slices from 6 mice— 5 of which were similar to the Vehicle group; i.e. for experiments in each of the 5 mice common to both groups, slices from the same mouse were randomly assigned to Vehicle or DHPG groups and simultaneously processed and

imaged. Analysis of dendritic *Arc* mRNA FISH was performed similar to that described for dendritic Arc protein immunofluorescence. Threshold values for dendritic *Arc* mRNA analysis (Fig. 2A) were set at 10x, 2x and 2x above background for tubulin, GFP and *Arc* mRNA, respectively. Apical dendrites were analysed 10 µm to 70 µm from the CA1 cell soma. The fluorescent values were normalized to the average *Arc*GFP– dendritic *Arc* mRNA immunofluorescence value in the same image. Only dendrites that could be verified to be in continuation with an *Arc*GFP– soma were labeled as *Arc*GFP– dendrites. Analysis involved 12 sections from 2 mice.

Fluorescent in situ hybridization with co-immunostaining

Digoxigenin-labeled Arc antisense and sense riboprobes (NCBI accession number NM_018790.2, nt 273-1369) were prepared by *in vitro* transcription using digoxigenin-labeled NTPs (Roche Applied Sciences). Coronal brain sections (20 µm) from *Arc*GFP-BAC mice were heated to 95°C in 0.01 M sodium citrate (3 times 5 min). After cooling, sections were treated with 0.5% Triton-X 100 in tris-buffered saline, and blocked for 1 hour in 0.1% Triton-X 100, 10% donkey serum in tris-buffered saline, followed by incubation with anti-GFP (chicken, 1:100, Aves) and anti-β-tubulin III (rabbit, 1:100, Sigma) antibodies in 0.1% Triton X-100 and 2% donkey serum in tris-buffered saline for 2 hours. Slices were washed and processed for Arc/Arg3.1-specific fluorescent in situ hybridization as described previously (Ivanova et al., 2011; Swanger et al., 2011) using Cy3-coupled tyramide amplification, with the following modification: following hybridization and stringency washes, Alexa488-coupled anti-chicken and Cy5-coupled anti-rabbit secondary antibodies were co-incubated with anti-digoxigenenin horse radish peroxidase-coupled antibody. Slices were imaged using a Nikon A1R confocal microscope at a resolution of 20 pixels/µm^2.

RNA extraction and quantitative real-time RT-PCR

Acute hippocampal slices (400 µm thick) were prepared from cage-anesthetized control or novelty-exposed mice as described for electrophysiology. From the slices, a rectangular block of CA1 dendritic tissue (str. radiatum) was obtained by a) a horizontal cut below and parallel to the lower border of CA1 cell bodies b) a horizontal cut at the hippocampal sulcus c) an orthogonal cut joining a) and c) and intersecting CA1 at the beginning of CA1's rectilinear architecture and d) an orthogonal cut joining a) and c) and intersecting CA1 at the end of CA1's rectilinear architecture. Because *Arc* mRNA is not expressed in glia or inhibitory neurons in CA1 (Vazdarjanova et al., 2006), the primary source of *Arc* mRNA in these tissue pieces should be from CA1 pyramidal neuron dendrites. RNA extraction and the reverse transcription reaction were conducted by using Cell-to-cDNA kit (Ambion) according to manufacturer's protocol. After obtaining the first strand cDNA, real-time PCR was conducted by using Fast SYBR Green Master Mix reagent (Applied Biosystems) in Applied Biosystems 7500 Fast Real-Time PCR System. Gene specific primers used for PCR reactions are as below: *Arc* 5'-AGCAGCAGACCTGACATCCT-3', 5'-GGCTTGTCTTCACCTTCAGC-3' ; *18S rRNA*, 5'-GTAACCCGTTGAACCCCATT-3' and 5'-CCATCCAATCGGTAGTAGCG-3'.

Synaptoneurosome preparation, ^{35}S-Met/Cys incorporation and immunoprecipitation of Arc

Synaptoneurosome preparation was conducted as previously described (Waung et al., 2008). In brief, whole hippocampi were dissected and the dentate gyrus was removed to enrich for CA3-CA1. CA3-CA1 enriched hippocampi were homogenized in buffer containing (in mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1.53 KH_2PO_4, 212.7 glucose, 1 DTT (pH 7.4), and protease inhibitor cocktail (Calbiochem). The homogenate was then passed through two 100 µm nylon filters and one 10 µm PVDF membrane (Millipore). ^{35}S-Met/Cys (100 µCi) with or without 100 µM R,S-DHPG were added into each sample for 15 minutes at 37°C followed by immunoprecipitation with anti-Arc antibody (Synaptic Systems). The immunoprecipitate was divided into two equal fractions, each run on a separate 8% SDS-PAGEs. For quantification, the ^{35}S-Arc band (55 kDa) from the first SDS-PAGE was cut out based on molecular weight and counted by a scintillation counter. The second SDS-PAGE was transferred onto a PVDF membrane and blotted for Arc to measure the total level of Arc protein that was immunoprecipitated. The membrane was then exposed to a PhosphorImager (Amersham Biosciences) for two months to visualize ^{35}S-labeled Arc protein bands. ^{35}S cpm values were normalized to total immunoprecipitated Arc obtained from the Arc western blot.

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