Supporting Information

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SI Materials and Methods

Animals. Plasmid construction of pGL4.11-Arc7000-mCherry-Arc-UTRs was described previously (18). The plasmid was linearized and purified after removal of vector sequences. Tg mouse lines harboring the Arc promoter mCherry-Arc transgene (Arc-mCherry/Arc) were generated by microinjection of the purified DNA fragment into the pronucleus of fertilized C57BL/6 mouse eggs. Ge-nomic integration of the transgene and reporter expression were analyzed by PCR, Southern blotting, Western blotting, and histological assays. Among several lines that showed activity-dependent expression of mCherry-Arc, one line that exhibited high reporter expression in the neocortex was selected and used for this study. Genotypes were identified by PCR with the following specific primers: arcpromoter3 (5′-GAGCTGCCCACACTCGTAA-3′) and mcherry-tg3-2 (5′-TCAAATCAGGGGTCTGTGCG-3′). Requests for mice should be addressed directly to H.O. or H.B. Arc KO mice were obtained from Kuan Wang, NIH, Bethesda, and were previously described (22). Male C57BL/6 mice and female mcherry-Arc-Tg mice were bred together, yielding WT and Arc-Tg hemizygous (one allele of the transgene) littermates. Het erozygous Arc KO mice were bred togeterh to yield WT and KO offspring. Both male and female mice were used, and the exper imenter was blinded to genotype in all experiments. Male C57BL/6 mice (Charles River Laboratories) at the age of P22-25 were used for the Alzet pump implantation experiments. Male C57BL/6 mice (The Jackson Laboratory) at the age of P180 were used for lentiviral VEP experiments. All procedures were approved by the Institutional Animal Care and Use Committees of the Massachusetts Institute of Technology, the University of Utah, and The University of Tokyo Graduate School of Medicine, in conjunction with NIH guidelines.

IIHC. WT and Arc-Tg mice at P30 and P180 were dark-house)d for 24 h and either killed in the dark (“dark” condition) or exposed to light for 2 h and then killed (“light” condition). Brains were dissected out and fixed in 4% paraformaldehyde for 24 h, and then cryoprotected in 30% sucrose. Brains were sectioned on a cryostat at 30 μm and stored in cryoprotectant at −20 °C until needed. Sections containing visual cortex were blocked in 2% fish gelatin (Sigma–Aldrich)/0.1% Triton X-100 (Amresco) for 2 h, stained for Arc (custom-made antibody; ProteinTech) overnight at room temperature, and washed three times for 10 min each time in 1× PBS plus 2% fish gelatin. Slides were then incubated in secondary antibody (donkey anti-rabbit Alexa Fluor 488; Jackson ImmunoResearch) for 4 h and washed three times for 10 min each time in 1× PBS before being mounted on slides and covered-slipped in Fluoromount mounting media (Sigma–Aldrich). A 10-section (1,272 × 1,272-μm) z-stack (each plane 2 μm apart) of binocular V1 for each mouse was acquired using a 10x objective on a confocal microscope (FV1000; Olympus). Sections used from each mouse had the same coordinates relative to bregma (±3.39 mm) to control for location of binocular V1 and cortical thickness. A maximum intensity projection of the z-stack was created in ImageJ (NIH). Every maximum intensity projection image was thresholded to the same level, and the integrated density of Arc-positive cells in layer IV was measured. Layer IV was determined by measuring 250 μm down from the dorsal cortical surface and then applying a 200-μm-tall × 900-μm-wide region of interest to measure the integrated density of only layer IV neurons.

qRT–PCR. P30 and P180 WT and Arc-Tg mice were dark-housed for 24 h and then either killed in the dark (dark condition) or exposed to 2 h of light (light condition) before euthanasia. Brains were removed, visual cortices were isolated and homogenized in TRIzol Reagent (Thermo Fisher Scientific), and RNA was isolated following the manufacturer’s guidelines. Reverse transcription was performed using a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). qPCR was then performed using PowerUP SYBR Green Master Mix (Thermo Fisher Scientific). Analysis was performed by fitting the average of duplicate cycle threshold (Ct) values to a linear curve obtained from a serial dilution series for each primer set. Ct values for Arc from each cDNA sample, corresponding to RNA extracted from the visual cortex of one mouse, were normalized to GAPDH values run in parallel. Primers for amplification of murine Arc and GAPDH are listed below (5′→3′): Arc forward: GAG CAG ATC CCG GTG CTC T, Arc reverse: CCC CAC CAG CTA CAG AGA CA, and GAPDH forward: CAT GCC CTT CGG TGT TCC TA, and GAPDH reverse: GCC TGC TTC ACC ACC TTC TT.

VEP Recordings.

Electrode implantation. Mice were anesthetized with an i.p. injection of ketamine (50 mg/kg) and xylazine (10 mg/kg), and a local anesthetic of 1% lidocaine hydrochloride (0.1 mL) was injected over the scalp. Petrolatum ophthalmic ointment (Dechra Pharmaceuticals) was applied to protect the eyes. The scalp was sterilized by alternately applying Betadine and 70% alcohol. For purposes of head fixation, a post was fixed to the skull just anterior to bregma using cyanoacrylate and a further application of dental cement. Two small (<0.5 mm) burr holes were made in the skull overlying the binocular visual cortex (3 mm lateral to lambda), and tungsten microelectrodes (FHC) were inserted ~450 μm below the cortical surface along the dorsal-ventral stereotaxic axis, positioning the electrode tip in cortical layer IV. Reference electrodes were placed bilaterally in prefrontal cortex. Electrodes were secured in place using cyanoacrylate, and the entire exposed area of skull was covered with dental cement. Animals were maintained at ~37 °C throughout the procedure and recovery, and general condition and reflex signs were monitored closely. Mice were monitored postoperatively for signs of infection or discomfort, and were allowed at least 24 h for recovery before habituation to the restraint apparatus. All animals were killed after the last recording session and processed for histology to determine the precise location of the recording electrode and virus expression (if injected).

Recording procedure. VEP recordings were conducted in awake mice. Mice were habituated to the restraint apparatus before the first recording session. The mice were alert and still during recording. Visual stimuli were presented to left and right eyes randomly. A total of 200 stimuli were presented per condition. Visual stimuli consisted of full-field sine wave gratings (0.05 cycles per degree) of varying contrast (0–100%) generated by a VSG2/2 card (Cambridge Research Systems) and presented on a computer monitor suitably linearized by γ-correction. VEPs were elicited by horizontal, vertical, or oblique (45° or 135°) bars. The display was positioned 20 cm in front of the mouse and centered on the midline, thereby occupying 92° × 66° of the visual field. Mean luminance, determined by a photodiode placed in front of the computer screen, was 27 cd/m².

Analysis. VEP amplitude was quantified by measuring the trough-to-peak response amplitude of the average VEP waveform, as described previously (14). The fractional change in contra and ipsi
eye VEP amplitude was calculated as the VEP amplitude following MD divided by the VEP amplitude before MD, as described previously (20).

**Virus Production/Injection.**

**Virus production.** Kimberly Huber, University of Texas Southwestern Medical Center, Dallas, generously donated FUGW lentiviral plasmids for Ubq-GFP and Ubq-GFP-Arc. Lentiviral and packaging plasmids were transfected into HEK293 cells using FuGENE transfection reagent (Promega Corporation). Supernatant was collected 48–72 h posttransfection, spun at 3,000 × g at 4 °C, and then passed through a 0.45-μm filter. Filtered supernatant was then centrifuged at 75,000 × g to pellet the virus. After drying the pellet, virus was resuspended in ice-cold PBS and stored at −80 °C. The viral titer for each virus ranged from 8 to 9 × 10^8 genome copies per milliliter.

**Virus injection.** The experimenter was blinded to the viruses being injected before surgery. P180 WT mice (C57BL/6J) were used for these experiments. Virus injection was carried out during electrode implantation surgery (discussed above), after drilling of the burr hole over binocular visual cortex and before lowering the electrode. Injection was done using a Nanoject II Auto-Nanoliter Injector (Drummond Scientific). A pulled glass pipette was backfilled with mineral oil before being attached to the Nanoject II. Virus was then loaded through the tip of the pipette. The pipette tip was lowered ∼450 μm below the cortical surface and allowed to rest for 5 min. One microliter of virus was injected over 25 min. The pipette was left in place for 5 min before being withdrawn, and electrode implantation was completed as described above. To quantify relative Arc expression in GFP- and GFP-Arc–injected mice, a subset of mice did not receive electrode implantation surgery (discussed above), after drilling of the burr hole through the dura mater. Cannulae were fixed to the skull by applying instant adhesive (Loctite 454). The attached miniature slices of visual cortex were made in high-sucrose dissection buffer containing 87 mM NaCl, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 25 mM NaHCO₃, 0.5 mM CaCl₂, 7 mM MgCl₂, 75 mM sucrose, 10 mM dextrose, and 1.3 mM ascorbic acid. Slices were recovered for 15 min at 32 °C and then for at least 1 h at room temperature, in carbogenated artificial cerebrospinal fluid containing 124 mM NaCl, 5 mM KCl, 1.23 mM NaH₂PO₄, 26 mM NaHCO₃, 10 mM dextrose, 1 mM MgCl₂, and 2 mM CaCl₂ before recordings began.

**Recordings.** Extracellular field potential recordings were conducted using an interface chamber for Arc KO experiments and a submersion chamber for Arc-Tg slice experiments. A two-contact cluster microelectrode (FHC) was placed in white matter, a glass recording pipette was placed in cortical layer IV, and extracellular field potentials (40–50% of maximal response amplitude) were recorded. Baseline was collected at 0.03 Hz, and recordings were included only when the baseline drift was less than 5% over 30 min. LTD was induced by applying 900 pulses at 1 Hz for 15 min. The last 5 min of baseline and post-LFS recordings were averaged, and statistical comparisons were performed to determine the magnitude of LTD using two-tailed t tests.

**Alzet pump implantation.** P22–25 mice were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg), i.p. and placed in a stereotaxic holder. Petrolatum ophthalmic ointment was applied to protect the eyes. Alzet micro-osmotic pumps (0.25 μL/h, no. 1002, Durect Corp) were filled with either saline or CHX (10 mg/mL; Sigma–Aldrich) and connected to brain infusion cannulae (Alzet brain infusion kit 3). The scalp was sterilized by alternately applying Betadine and 70% alcohol, and 0.1 mL of lidocaine (2%) was applied under the scalp before scalp incision. A midline incision (1–1.5 cm) was made in the scalp, and the skin and connective tissue were retracted. Following incision, a lidocaine (2%) and epinephrine (1:50,000) solution was applied to the periosteum to avoid excessive bleeding or discomfort. A small burr hole was made in the skull (0.1 mm anterior, 2.7 mm lateral to lambda) with a dental drill through which an infusion cannula was lowered (450 μm below the dura mater). Cannulae were fixed to the skull by applying instant adhesive (Loctite 454). The attached mini-pump was placed in an s.c. pocket at the nape of the neck. The rest of the exposed scalp was covered with dental cement to hold the cannula in place and prevent inflammation. Animals were maintained at −37 °C throughout the procedure and recovery, and general condition and reflex signs were monitored closely. Chemical infusion through the implanted pump continued for 5 d following surgery.
Fig. S1. Characterization of Arc-Tg mouse line. (A, Left) Arc-mCherry transgene mirrors endogenous Arc expression. In the hippocampus, basal expression of Arc-mCherry is low in both area CA1 and the dentate gyrus (DG). (Insets) Magnified CA1 and DG. (A, Right) Following electroconvulsive shock (ECS), levels of Arc-mCherry expression are dramatically increased in CA1 and DG, consistent with previous findings of ECS-induced induction of endogenous Arc. (Scale bar: 500 μm.) (B) Arc-mCherry transgene in the primary visual cortex reliably reports visual experience. (Left) P30 mouse dark-housed for 24 h (visual deprivation) has low levels of Arc-mCherry expression in all layers of visual cortex. (Right) P30 mouse dark-housed and then exposed to light for 2 h before euthanasia has increased levels of Arc-mCherry expression in the input layers of the visual cortex (layers II/III, IV, and VI). (C) Visually driven Arc-mCherry (mCh) expression is specific to visual areas and recapitulates endogenous (endo) Arc expression. Dark-housed mice (Light −) had little Arc-mCherry and endogenous Arc expression in visual cortex (Vis. Ctx) compared with light-exposed mice (Light +), as assessed by antibodies against mCherry (Anti-DsRed pAb) and Arc. This effect of light exposure was specific to the visual system, as both basal and light-induced Arc-mCherry and native Arc expression was equally low in the cerebellum (Clb).
Fig. S2. Validation of custom Arc antibody. (A) Side-by-side comparison of custom Arc antibody and commercially available Arc antibody (catalog no.156-003; Synaptic Systems). Custom and commercial Arc antibodies show comparable levels of staining in WT P30 mouse visual cortex (Upper Left and Center) and comparable levels of background in Arc KO P30 mouse visual cortex (Lower Left and Center). (Upper Right) WT tissue not stained with a primary antibody for comparison. (B) Western blot of visual cortex lysates from a P30 WT and Arc KO mouse stained with the custom Arc antibody. Arc is detected in WT, but not Arc KO, lysate.
**Fig. S3.** GFP-Arc lentivirus increases Arc expression in P180 WT visual cortex. P180 WT mice were injected unilaterally in the visual cortex with lentivirus expressing either GFP alone or GFP-Arc. (A) Representative images from visual cortex of a mouse injected with GFP lentivirus (Upper) and a mouse injected with GFP-Arc lentivirus (Lower). (Left and Middle) GFP expression from GFP and GFP-Arc lentiviruses (no antibody staining) is shown. (Right) IHC for Arc expression is shown. (B) Quantification of Arc expression from GFP- and GFP-Arc–injected mice (four GFP-injected, four GFP-Arc–injected). When images were set to a threshold determined by the maximum Arc expression in GFP-Arc–injected mice, there was no detectable Arc expression in layer IV of GFP-injected mice above background staining, as seen in noninjected P180 WT (Fig. 2). However, there was a significant increase in Arc expression in GFP-Arc–injected mice ($P = 0.02$, Student $t$ test). Data are normalized (norm.) to GFP and displayed as mean ± SEM.

**Fig. S4.** Model of Arc’s role in controlling the “juvenile” quality of OD plasticity. Various genetic, environmental, and pharmacological manipulations have been shown to promote juvenile-like plasticity in adult visual cortex. It has been proposed that these manipulations cause a general increase in the ratio of excitation (E) to inhibition (I). Here, we propose that the increase in E/I restores juvenile plasticity specifically by allowing the induction of activity-regulated genes such as Arc. Increased Arc expression promotes activity-dependent synaptic depression that underlies juvenile OD plasticity. *Significantly lower than contralateral eye at baseline.

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