

# Loss of *Arc* renders the visual cortex impervious to the effects of sensory experience or deprivation

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**A myriad of mechanisms have been suggested to account for the full richness of visual cortical plasticity. We found that visual cortex lacking *Arc* is impervious to the effects of deprivation or experience. Using intrinsic signal imaging and chronic visually evoked potential recordings, we found that *Arc*<sup>-/-</sup> mice did not exhibit depression of deprived-eye responses or a shift in ocular dominance after brief monocular deprivation. Extended deprivation also failed to elicit a shift in ocular dominance or open-eye potentiation. Moreover, *Arc*<sup>-/-</sup> mice lacked stimulus-selective response potentiation. Although *Arc*<sup>-/-</sup> mice exhibited normal visual acuity, baseline ocular dominance was abnormal and resembled that observed after dark-rearing. These data suggest that *Arc* is required for the experience-dependent processes that normally establish and modify synaptic connections in visual cortex.**

Experience-dependent reorganization of eye-specific inputs during development is an important mechanism by which neuronal connectivity is established in the primary visual cortex (V1)<sup>1</sup>. Changes in neuronal activity lead to the strengthening or weakening of synapses, which are believed to initiate the structural remodeling of visual networks. During a period of heightened plasticity (postnatal day 25–32 (P25–32) in mice), V1 is exquisitely sensitive to changes in activity. Brief monocular deprivation results in notable functional and anatomical reorganization in the binocular zone of V1 as a result of a rapid weakening of the cortical response to the deprived eye and a shift in ocular dominance in favor of the nondeprived eye<sup>2</sup>. Extended periods of deprivation result in a compensatory strengthening of open-eye responses, suggesting that multiple molecular mechanisms mediate different phases of deprivation-induced plasticity in V1 (refs. 1,3–5).

The mechanisms underlying the changes induced by brief monocular deprivation are well studied. Early findings indicate that the initial cortical depression occurring after monocular deprivation is dependent on calcium signaling through NMDA receptors (NMDARs)<sup>6</sup>, appropriate levels of inhibition<sup>7</sup> and protein synthesis<sup>8</sup>. Recent evidence suggests that deprived-eye depression is induced by the loss of AMPA type glutamate receptors (AMPA) on the surface of cortical neurons via mechanisms similar to long-term synaptic depression (LTD)<sup>9–11</sup>. The regulated trafficking of these receptors is a major cellular mechanism underlying synaptic plasticity at excitatory synapses<sup>12</sup>. Reduction in surface expression of both GluR1 and GluR2 AMPAR subunits occurs after brief monocular deprivation<sup>9</sup>. Deprived eye depression occludes the induction of LTD in cortical slices<sup>9,10</sup> and the ocular dominance shift is prevented by manipulations that block AMPAR endocytosis<sup>13</sup>. The mechanisms underlying the strengthening of open-eye responses after longer periods of monocular deprivation

are less clear. The temporal separation of depression and strengthening suggests that these two phases are mediated by separate and distinct mechanisms and may operate independently. The loss of the dominant input as a result of deprivation may trigger metaplasticity or a homeostatic scaling of responses that results in a strengthening of the open eye<sup>4,5,14,15</sup>.

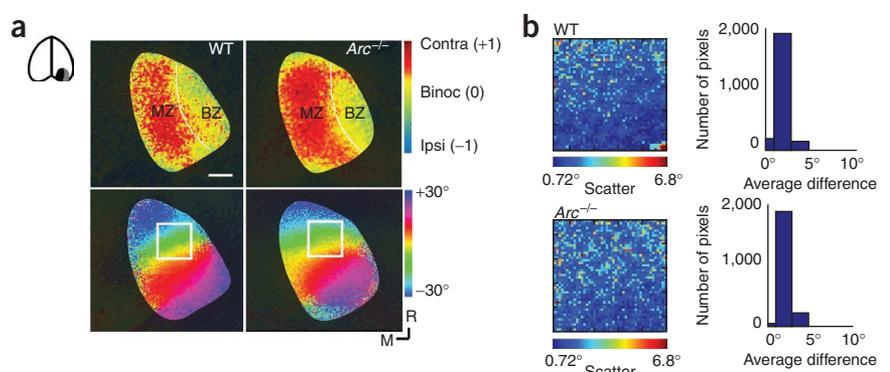
In addition to the effects of sensory deprivation, selective visual experience also elicits robust plasticity of responses in mouse V1. For example, selective exposure of mice to grating stimuli of one orientation causes a substantial increase in responsiveness to the experienced orientation, a phenomenon known as stimulus-selective response potentiation (SRP)<sup>16</sup>. SRP occurs in adults and juveniles, is specific to the stimulated eye, and develops over hours to days. Moreover, SRP depends on both NMDAR activation and AMPAR trafficking in the cortex, properties that are shared with long-term potentiation (LTP). Thus, SRP provides a framework for studying LTP-like processes in the intact brain, which are induced through normal experience rather than through artificial stimulation procedures.

The immediate early gene *Arc* (activity-regulated cytoskeletal associated protein), also known as *Arg3.1*, has been implicated in many forms of synaptic plasticity, including LTP<sup>17–19</sup>, LTD<sup>20,21</sup> and homeostatic scaling of AMPARs<sup>22,23</sup>. *Arc* gene expression and efficient *Arc* translation are dependent on NMDAR and group 1 mGluR activation<sup>23–25</sup>. These signaling pathways have been implicated in ocular dominance and many other forms of experience-dependent plasticity<sup>6,26–28</sup>, suggesting that *Arc* may act downstream of these receptors as an important effector molecule. In V1, *Arc* expression only occurs after eye opening and is activated by visual stimulation<sup>29,30</sup>. Moreover, *Arc* RNA induction is a reporter of ocular dominance plasticity in V1 (ref. 29). Taken together, these studies suggest that *Arc* is involved in experience-dependent plasticity in V1.

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**Figure 1** Loss of Arc does not affect V1 responsiveness and organization. (a) Intrinsic signal imaging of V1 (left) in wild-type and *Arc*<sup>-/-</sup> mice. Top, ocular dominance map of V1 in a wild-type mouse (WT, left) and an *Arc*<sup>-/-</sup> mouse (right). BZ, binocular zone; MZ, monocular zone. Scale illustrates binocularity index of pixels. Scale bar represents 500  $\mu$ m. V1 in *Arc*<sup>-/-</sup> mice was similar to that in wild-type mice in total area (wild type,  $n = 6$ , area =  $1.401 \pm 0.07$  mm<sup>2</sup>; *Arc*<sup>-/-</sup>,  $n = 10$ , area =  $1.270 \pm 0.15$  mm<sup>2</sup>;  $P > 0.5$ ,  $t$  test). Bottom, retinotopic organization of V1 in a wild-type mouse (left) and an *Arc*<sup>-/-</sup> mouse (right). Each image shows the mapping of elevation according to the scale bar on the right. (b) Scatter analysis of 50  $\times$  50 pixel area in white box in A for wild-type and *Arc*<sup>-/-</sup> mice. The receptive field center (phase) differences between sets of five adjacent pixels are shown in the histograms on the right. The precision of local mapping was comparable between wild-type and *Arc*<sup>-/-</sup> mice.



We investigated the role of Arc in experience-dependent plasticity *in vivo* using intrinsic signal optical imaging and visually evoked potentials (VEPs) to assess changes in cortical responses after manipulation of experience. We used *Arc*<sup>-/-</sup> mice in which *gfp* has been knocked-in to the *Arc* gene locus<sup>30</sup> to study how the loss of the Arc protein might influence two forms of experience-dependent plasticity: ocular dominance plasticity and SRP. Our findings suggest that, in the absence of Arc, synapses in V1 are rendered insensitive to the effects of both experience and deprivation.

## RESULTS

### Normal map organization and visual response in *Arc*<sup>-/-</sup> mice

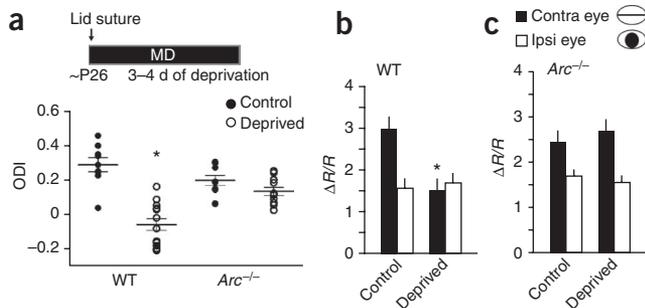
*Arc*<sup>-/-</sup> mice are viable and show no gross deficits in size or weight compared to wild-type mice<sup>17,30</sup>. Although previous reports have focused on Arc protein interactions in the hippocampus and dentate gyrus, few studies have examined Arc's function in cortex or *in vivo*. We examined the distribution of Arc protein expression in mouse V1 by immunofluorescence using an Arc-specific antibody. In V1, Arc did not colocalize with GFAP, which labels astrocytes, or with the inhibitory neuron marker GABA (Supplementary Fig. 1). This suggests that Arc protein is selectively expressed in excitatory neurons in V1, which is consistent with previous studies showing that Arc is predominately expressed in principal neurons that also express CaMKII<sup>31</sup>. Previous reports have found that Arc mRNA is regulated by physiological activity and is prominently expressed in V1 (refs. 24,29). As expected, no Arc expression was detected in *Arc*<sup>-/-</sup> tissue (Supplementary Fig. 1). In wild-type V1, Arc protein expression was detected in all cortical layers with the exception of layer 5, with the greatest expression being seen in layers 2/3 and 4, the predominant sites of ocular dominance plasticity (Supplementary Fig. 1).

We used intrinsic signal imaging to test whether the loss of Arc altered V1 responses and retinotopic organization<sup>32,33</sup>. Because previous studies implicated Arc protein in regulation of AMPARs, the major contributors to excitatory synaptic transmission, we asked whether the loss of Arc protein would influence the strength of response to visual stimulation in mouse V1. Mice were shown a periodic moving bar of light and cortical responses to contralateral and ipsilateral eye stimulation were assessed with optical imaging of intrinsic signals to create an ocular dominance map of V1 (see Online Methods). V1 in *Arc*<sup>-/-</sup> mice was similar to that in wild-type mice in terms of area and organization of binocular and monocular zones (Fig. 1a). To examine whether loss of Arc protein might effect retinotopic organization (Fig. 1a), we evaluated scatter in the retinotopic (phase)

maps (Fig. 1b). Map organization in *Arc*<sup>-/-</sup> mice was indistinguishable from that in wild-type mice (Supplementary Fig. 2). In addition, there was no significant difference ( $P > 0.5$ ) in the magnitude of response to binocular stimulation in V1 (Supplementary Fig. 2), nor were there differences in responses from the monocular zone of V1 (data not shown). These data indicate that a loss of Arc protein does not grossly disrupt the development of V1 organization. We assessed visual acuity in *Arc*<sup>-/-</sup> mice by measuring VEPs in response to sinusoidal gratings at various spatial frequencies, a well-established method of assessing visual function in mice<sup>27,34</sup>. There was no significant difference ( $P > 0.07$ ) between wild-type and *Arc*<sup>-/-</sup> mice in evoked responses at high spatial frequencies, regardless of whether responses were evoked binocularly or monocularly through either eye, suggesting that *Arc*<sup>-/-</sup> mice have normal visual acuity and responsiveness (Supplementary Fig. 3).

### Depression after brief monocular deprivation requires Arc

To determine how loss of Arc protein might influence cortical plasticity, we deprived mice of vision through one eye by suturing the eyelid closed for 3–4 d during the period of heightened plasticity in mice (P25–32). We then used intrinsic signal imaging to measure the cortical response to visual stimulation in the binocular zone of V1, contralateral to the deprived eye. As described above, stimuli were shown to each eye alternately, and we assessed the strength of response to contralateral or ipsilateral stimulation and calculated an ocular dominance index (ODI). This method has been shown to reliably detect the changes in ocular dominance that can be induced by monocular deprivation in wild-type animals<sup>35</sup>. Consistent with previous reports, wild-type mice showed a robust decrease in ODI after brief deprivation (Fig. 2a). By assessing the magnitude of response in deprived and nondeprived mice, we found that this shift appeared to be mediated by a diminished response to the deprived eye (Fig. 2b). In contrast, *Arc*<sup>-/-</sup> mice did not exhibit a change in ODI (Fig. 2a) and cortical responses to the deprived eye remained unchanged (Fig. 2c). These results indicate that Arc protein is required for the deprived-eye depression induced by brief monocular deprivation. In addition to intrinsic signal optical imaging, which mainly measures responses in superficial cortical layers, we used chronic VEP recordings to monitor changes in the strength of cortical responses in layer 4 before and after monocular deprivation<sup>27,34</sup>. Electrodes were implanted at a depth corresponding to layer 4 in V1 at P24–25. After habituation to the restraint apparatus, we recorded VEPs at P28 in fully awake, head-restrained mice in response to square



**Figure 2** Intrinsic signal imaging after monocular deprivation illustrates a requirement for Arc in deprived-eye depression after short-term monocular deprivation. **(a)** Top, monocular deprivation was initiated near the peak of the critical period for 3–4 d. Control mice were age-matched to deprived mice. Bottom, ODIs for individual mice are shown as circles. Horizontal bars represent group averages (wild type: control,  $n = 9$ ,  $ODI = 0.28 \pm 0.03$ ; deprived,  $n = 14$ ,  $ODI = -0.05 \pm 0.03$ ,  $P < 0.0001$ ,  $t$  test;  $Arc^{-/-}$ : control,  $n = 10$ ,  $ODI = 0.19 \pm 0.02$ ; deprived,  $n = 11$ ,  $ODI = 0.13 \pm 0.02$ ,  $P > 0.1$ ,  $t$  test). **(b)** Response magnitude in wild-type mice driven by the contralateral eye and ipsilateral eye, plotted as average  $\Delta R/R \times 10^{-3}$ . There was a depression in the contralateral eye response amplitude (control =  $2.9 \pm 0.27$ , deprived =  $1.62 \pm 0.23$ ,  $*P < 0.001$ ,  $t$  test). No change in the ipsilateral eye response was detected (control =  $1.56 \pm 0.21$ , deprived =  $1.68 \pm 0.19$ ,  $P > 0.8$ ,  $t$  test). **(c)** No change in contralateral response occurred in  $Arc^{-/-}$  mice after deprivation (control =  $2.25 \pm 0.28$ , deprived =  $2.5 \pm 0.26$ ,  $P > 0.2$ ,  $t$  test); similarly, no change in ipsilateral response was detected (control =  $1.35 \pm 0.23$ , deprived =  $1.64 \pm 0.19$ ,  $P > 0.2$ ,  $t$  test).  $\Delta R/R$  is the change in reflectance over baseline reflectance. Error bars represent s.e.m.

wave-reversing sinusoidal gratings. We collected baseline recordings and then monocularly deprived mice for 3 d by lid suture. After opening the sutured eye, we gathered post-monocular deprivation recordings. Wild-type mice showed a robust ocular dominance shift (Fig. 3), whereas  $Arc^{-/-}$  mice did not exhibit a change in ocular dominance (Fig. 3). The shift in wild-type mice was the result of

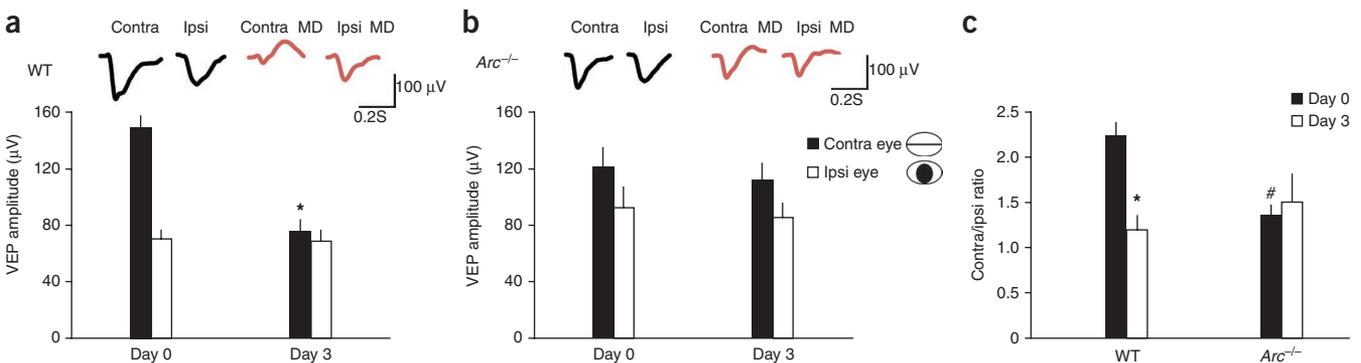
a significant depression ( $P \leq 0.0001$ ) in deprived-eye responses (Fig. 3a), which was not observed in  $Arc^{-/-}$  mice (Fig. 3b,c).

Monocular deprivation resulted in a marked loss of visual acuity in responses contralateral to the deprived eye while preserving acuity in the ipsilateral responses to the open eye in wild-type mice (Supplementary Fig. 4). In contrast,  $Arc^{-/-}$  mice did not exhibit changes in visual acuity after monocular deprivation (Supplementary Fig. 4), further supporting a role for Arc in ocular dominance plasticity.

Although Arc is expressed only in excitatory neurons, we examined the expression of several inhibitory markers that have been predictive of the state of functional inhibition in V1. Quantitative western blot analyses of VGAT, GAD65 and parvalbumin showed no difference between  $Arc^{-/-}$  and wild-type mice (Supplementary Fig. 5). In addition, no change in GABA expression was found, suggesting that gross changes in inhibition are unlikely to account for the plasticity phenotypes (Supplementary Fig. 5).

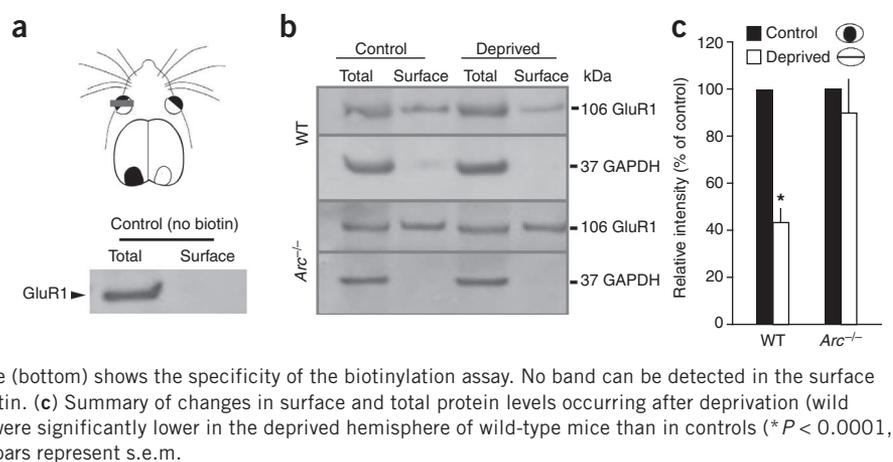
### Arc regulates AMPAR endocytosis in visual cortex

What causes the reduced deprived-eye depression in  $Arc^{-/-}$  mice? Manipulations of sensory activity are known to regulate synaptic AMPARs in the cortex. In response to as little as 24 h of monocular deprivation, AMPARs are rapidly internalized, decreasing the surface-to-total ratio, which mediates the depression in cortical responses from the deprived eye<sup>9</sup>. Recent experiments in cultured primary neurons have shown that Arc regulates AMPAR internalization via its interactions with the proteins dynamin and endophilin, two integral components of the clathrin-mediated endocytosis machinery<sup>36</sup>. High levels of Arc expression are found to accelerate the rate of AMPAR endocytosis, leading to decreased AMPAR surface expression, whereas loss of Arc reduces AMPAR endocytosis<sup>36</sup>. We thus hypothesized that loss of Arc protein might reduce the deprivation-induced removal of surface AMPARs and prevent the shift in ocular dominance. For these experiments, we focused on the GluR1 subunit, as previous work has shown that this subunit faithfully reports changes in AMPARs following LTD and ocular dominance plasticity in V1 (refs. 9,13). In addition, GluR1 shows high immunoreactivity



**Figure 3** Chronic VEP recordings show that  $Arc^{-/-}$  mice do not exhibit ocular dominance plasticity after short-term monocular deprivation. **(a)** Wild-type mice exhibited a significant depression in contralateral (deprived eye) responses ( $n = 11$ ; day 0 =  $149 \pm 8.8 \mu V$ , 3-d monocular deprivation =  $75.4 \pm 8.8 \mu V$ ,  $*P < 0.0001$ , paired  $t$  test). No significant change was observed in ipsilateral responses ( $n = 11$ ; day 0 =  $70.4 \pm 6.4 \mu V$ , 3-d monocular deprivation =  $68.8 \pm 8 \mu V$ ,  $P > 0.8$ , paired  $t$  test). Averaged waveforms across all mice are shown at top. MD, monocular deprivation. **(b)**  $Arc^{-/-}$  mice exhibited no changes in contralateral responses ( $n = 8$ ; day 0 =  $121 \pm 14.7 \mu V$ , 3-d monocular deprivation =  $111.3 \pm 13.5 \mu V$ ,  $P > 0.2$ , paired  $t$  test) or in ipsilateral responses ( $n = 8$ ; day 0 =  $92.5 \pm 15 \mu V$ , 3-d monocular deprivation =  $85.8 \pm 10.7 \mu V$ ,  $P > 0.7$ , paired  $t$  test). Averaged waveforms are shown at top. **(c)** Wild-type mice exhibited a significant shift in the contralateral to ipsilateral eye ratio ( $n = 11$ ; day 0 =  $2.2 \pm 0.16$ , 3-d monocular deprivation =  $1.2 \pm 0.16$ ,  $*P < 0.0001$ , paired  $t$  test), whereas  $Arc^{-/-}$  mice exhibited no significant shift in the contralateral to ipsilateral eye ratio ( $n = 8$ ; day 0 =  $1.4 \pm 0.12$ , 3-d monocular deprivation =  $1.5 \pm 0.33$ ,  $P > 0.8$ , paired  $t$  test).  $Arc^{-/-}$  mice exhibited a significantly smaller baseline contralateral to ipsilateral eye ratio than wild-type mice (wild type,  $n = 11$ , contralateral to ipsilateral eye ratio  $2.22 \pm 0.16$ ;  $Arc^{-/-}$ ,  $n = 8$ , contralateral to ipsilateral eye ratio  $1.37 \pm 0.12$ ,  $\#P < 0.001$ ,  $t$  test). Error bars represent s.e.m.

**Figure 4** Arc is required for the decrease in surface AMPARs after short-term monocular deprivation. (a) Schematic of mouse brain showing the segments of V1 dissected for biochemical analysis. Because V1 is dominated by contralateral eye responses, cortex contralateral to the deprived eye was termed 'deprived' and cortex ipsilateral to the deprived eye was termed 'control'. (b) Example immunoblots of total and biotinylated surface proteins in the visual cortex of *Arc*<sup>-/-</sup> and wild-type mice. Full blots are presented in **Supplementary Figure 6**. GAPDH was used as an internal control to show that biotin specifically labeled surface proteins. In addition, a control image (bottom) shows the specificity of the biotinylation assay. No band can be detected in the surface lane of protein samples that were not exposed to biotin. (c) Summary of changes in surface and total protein levels occurring after deprivation (wild type,  $n = 5$ ; *Arc*<sup>-/-</sup>,  $n = 7$ ). Surface levels of GluR1 were significantly lower in the deprived hemisphere of wild-type mice than in controls ( $*P < 0.0001$ ,  $t$  test) but not in *Arc*<sup>-/-</sup> mice ( $P > 0.2$ ,  $t$  test). Error bars represent s.e.m.



in the middle and superficial layers of mouse V1 (ref. 37), which are key sites of ocular dominance plasticity.

We performed a biotinylation assay using acute slices to measure surface expression of AMPARs after monocular deprivation. Because Arc protein is primarily expressed in layers 2/3 and 4 of V1 (**Supplementary Fig. 1**), the deeper layers were microdissected out and discarded from both hemispheres. In wild-type mice, a significant decrease ( $P < 0.0001$ ) in the surface to total ratio of GluR1 could be detected in the deprived hemisphere (contralateral to the deprived eye; **Fig. 4a**) as compared with the nondeprived control hemisphere (**Fig. 4b,c**). Notably, *Arc*<sup>-/-</sup> mice showed no significant change ( $P > 0.2$ ) in the surface-to-total ratio of AMPARs in the deprived hemisphere (**Fig. 4b,c**). This result suggests that loss of Arc protein reduces AMPAR internalization and thus prevents the synaptic weakening that occurs in response to monocular deprivation.

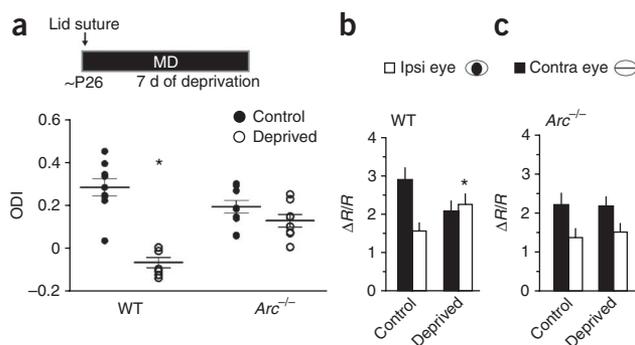
### Reduced open-eye potentiation in *Arc*<sup>-/-</sup> mice

The ocular dominance shift that occurred after long-term monocular deprivation occurs in two temporally distinct phases. In response to brief monocular deprivation, decorrelated input through the closed eye resulted in a Hebbian weakening of the deprived-eye response, which we found required Arc, whereas extended periods of deprivation resulted in potentiation of the open-eye response. It has been proposed that distinct cortical processes may mediate the two phases of ocular dominance plasticity, with Hebbian, LTD-like mechanisms mediating synaptic weakening and LTP or homeostatic scaling underlying open-eye response potentiation.

To address whether open-eye potentiation occurs in *Arc*<sup>-/-</sup> mice, we used intrinsic signal imaging to measure response magnitudes in mice deprived for 7 d. In response to deprivation, wild-type mice showed a significant shift ( $P < 0.0001$ ) in ODI (**Fig. 5a**). Consistent with previous reports, we found that this shift was mediated by a significant increase ( $P < 0.05$ ) in open-eye responses (**Fig. 5b**). The increase in open-eye response was accompanied by a decrease in the deprived-eye response (**Fig. 5b**). Notably, *Arc*<sup>-/-</sup> mice did not show a shift in ODI or significant ( $P > 0.6$ ) open-eye potentiation (**Fig. 5a,c**). Similar results were found with VEP recordings after 7 d of monocular deprivation. Wild-type mice exhibited a robust ocular dominance shift that was a result of both significant deprived-eye depression ( $P < 0.003$ ) and open-eye potentiation ( $P < 0.05$ ) (**Fig. 6**). In contrast, *Arc*<sup>-/-</sup> mice did not exhibit an ocular dominance shift or any significant changes in deprived-eye ( $P > 0.1$ ) or open-eye responses ( $P > 0.4$ ) (**Fig. 6**).

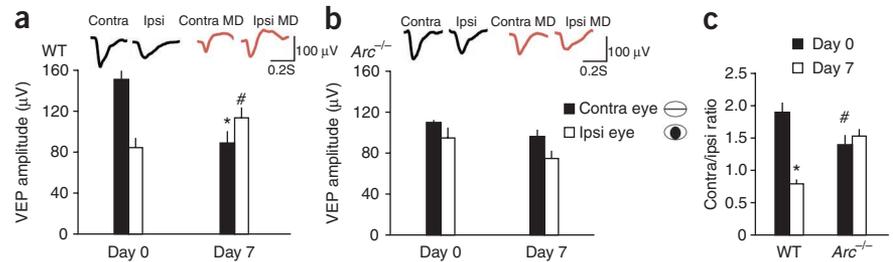
### Normal balance of eye-specific drive requires Arc

Layer 4 VEPs recorded in *Arc*<sup>-/-</sup> mice exhibited altered baseline contralateral to ipsilateral eye response ratios as compared with wild-type mice (**Figs. 3c and 6c**). After pooling baseline data from all VEP experiments, we found that there was a significant decrease in the contralateral to ipsilateral eye ratio of *Arc*<sup>-/-</sup> as compared with wild-type mice ( $P < 0.0001$ ; **Fig. 7a**). This was mostly because of a significant decrease ( $P < 0.006$ ) in contralateral responses (**Fig. 7b**). We hypothesized that establishing the contralateral to ipsilateral eye ratio in mice requires neuronal activity and visual experience. To test this, we dark-reared wild-type mice from birth and recorded baseline



**Figure 5** *Arc*<sup>-/-</sup> mice do not show a shift in ocular dominance after extended deprivation, as assessed by intrinsic signal imaging. (a) Top, monocular deprivation was initiated near the peak of the critical period for 7 d. Control mice were age-matched to deprived mice. ODIs for individual mice are shown as circles. Horizontal bars represent group averages (wild type: control,  $n = 9$ ,  $ODI = 0.28 \pm 0.03$ ; deprived,  $n = 7$ ,  $ODI = -0.063 \pm 0.02$ ,  $*P < 0.0001$ ; *Arc*<sup>-/-</sup>: control,  $n = 10$ ,  $ODI = 0.19 \pm 0.02$ ; deprived,  $n = 8$ ,  $ODI = 0.13 \pm 0.02$ ,  $P = 0.17$ ). (b) Response magnitude in wild-type mice driven by the contralateral eye and ipsilateral eye, plotted as average  $\Delta R/R \times 10^{-3}$ . Some depression was observed in the contralateral eye response amplitude, although it was not significant (control =  $2.9 \pm 0.27$ , deprived =  $2.1 \pm 0.23$ ,  $P > 0.05$ ). Lid suture resulted in an increase in the ipsilateral eye response (control =  $1.56 \pm 0.21$ , deprived =  $2.49 \pm 0.17$ ,  $*P < 0.05$ ). (c) No change in contralateral response occurred in *Arc*<sup>-/-</sup> mice after deprivation (control =  $2.25 \pm 0.28$ , deprived =  $2.2 \pm 0.21$ ,  $P > 0.6$ ); similarly, no change was detected in ipsilateral response (control =  $1.35 \pm 0.23$ , deprived =  $1.5 \pm 0.21$ ,  $P > 0.6$ ).  $\Delta R/R$  is the change in reflectance over baseline reflectance. Error bars represent s.e.m. Statistical analyses for **a-c** conducted using one-way ANOVA with Bonferroni correction.

**Figure 6** *Arc*<sup>-/-</sup> mice exhibit no ocular dominance plasticity as assessed by chronic VEP recordings after long-term monocular deprivation. (a) Wild-type mice exhibited a significant depression in contralateral (deprived eye) responses ( $n = 7$ ; day 0 =  $152 \pm 9.2 \mu\text{V}$ , 7-d monocular deprivation =  $89.5 \pm 11.5 \mu\text{V}$ ,  $*P < 0.003$ , paired  $t$  test) and a significant potentiation in ipsilateral responses ( $n = 7$ ; day 0 =  $84.9 \pm 9.8 \mu\text{V}$ , 7-d monocular deprivation =  $114.2 \pm 10.1 \mu\text{V}$ ,  $\#P < 0.05$ , paired  $t$  test). Averaged waveforms are shown at top. (b) *Arc*<sup>-/-</sup> mice exhibited no changes in contralateral ( $n = 6$ ; day 0 =  $112 \pm 2.2 \mu\text{V}$ , 7-d monocular deprivation =  $100 \pm 6 \mu\text{V}$ ,  $P > 0.1$ , paired  $t$  test) or in ipsilateral responses ( $n = 8$ ; day 0 =  $96 \pm 8.6 \mu\text{V}$ , 3-d monocular deprivation =  $84 \pm 10 \mu\text{V}$ ,  $P > 0.4$ , paired  $t$  test). Averaged waveforms are shown at top. (c) Wild-type mice exhibited a significant shift in the contralateral to ipsilateral eye ratio ( $n = 7$ ; day 0 =  $1.9 \pm 0.14$ , 7-d monocular deprivation =  $0.8 \pm 0.06$ ,  $*P < 0.0001$ , paired  $t$  test), whereas the contralateral to ipsilateral eye ratio did not significantly shift in *Arc*<sup>-/-</sup> mice ( $n = 6$ ; day 0 =  $1.2 \pm 0.1$ , 7-d monocular deprivation =  $1.25 \pm 0.11$ ,  $P > 0.7$ , paired  $t$  test). *Arc*<sup>-/-</sup> mice had a significantly smaller baseline contralateral to ipsilateral eye ratio than wild-type mice (wild type  $n = 7$ , contralateral to ipsilateral eye ratio  $1.87 \pm 0.14$ ; *Arc*<sup>-/-</sup>  $n = 6$ , contralateral to ipsilateral eye ratio  $1.2 \pm 0.1$ ,  $\#P < 0.003$ ). Error bars represent s.e.m.



responses in P28–32 mice that had never been exposed to light. Dark rearing has previously been shown to markedly reduce *Arc* expression in V1 (ref. 30). Dark-reared mice exhibited a significant decrease in the contralateral to ipsilateral eye ratio ( $P < 0.0001$ ) as a result of significantly smaller contralateral responses ( $P < 0.0001$ ), similar to that observed in *Arc*<sup>-/-</sup> mice (Fig. 7a,b).

These findings prompted us to examine whether the anatomical organization of retinal input to the lateral geniculate nucleus (LGN) was normal in *Arc*<sup>-/-</sup> mice. During the pre-critical period, experience-dependent competition between the two eyes is necessary for normal axonal refinement in central targets<sup>38–40</sup>. We used intraocular injection of cholera toxin subunit B (CTB) to examine eye-specific segregation in the LGN; no gross changes in contralateral or ipsilateral inputs could be seen in *Arc*<sup>-/-</sup> mice as compared with wild types (Supplementary Fig. 7).

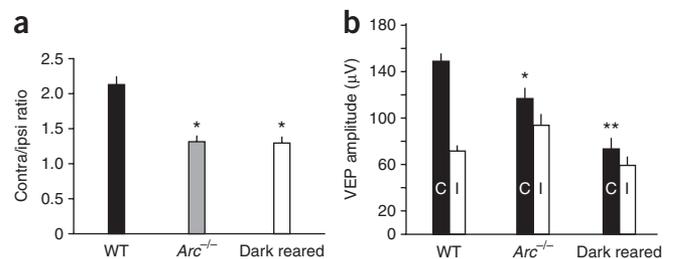
The altered baseline contralateral to ipsilateral eye ratio in *Arc*<sup>-/-</sup> mice raises the possibility that the observed absence of deprived-eye depression following monocular deprivation in the hemisphere contralateral to the deprived eye might arise because these inputs are already fully depressed. That is, the depression of deprived-eye responses after monocular deprivation might be occluded in *Arc*<sup>-/-</sup> mice. To address this possibility, we investigated the effect of monocular deprivation in the hemisphere ipsilateral to the deprived eye. The baseline ipsilateral responses are similar or slightly larger in *Arc*<sup>-/-</sup> mice, so any differences in deprivation-induced depression of *Arc*<sup>-/-</sup> responses are likely to be explained by an effect on the mechanisms of response depression rather than occlusion. We found that there was a significant increase ( $P < 0.05$ ) in the contralateral to ipsilateral eye ratio in the ipsilateral hemisphere after 7 d of monocular deprivation in wild-type mice, which was due to a significant decrease ( $P < 0.05$ ) in the ipsilateral (deprived eye) responses (Supplementary Fig. 8). However, *Arc*<sup>-/-</sup> mice showed no shift in contralateral to ipsilateral eye ratio or changes in ipsilateral responses. Moreover, we did not find any significant changes ( $P > 0.3$ ) in surface GluR1 between *Arc*<sup>-/-</sup> and wild-type V1 slices (Supplementary Fig. 9).

### Stimulus-selective response potentiation requires *Arc*

Another *in vivo* form of cortical response enhancement, SRP, results from brief exposure to sinusoidal gratings of a specific orientation<sup>16</sup>. Mechanistically, SRP exhibits hallmarks of LTP; it is NMDAR dependent and is blocked by a GluR1 C-terminal peptide, which inhibits insertion of AMPARs at synapses. As *Arc*<sup>-/-</sup> mice exhibit a defect in open-eye potentiation, we wondered whether SRP would also be disrupted by the lack of *Arc*. Indeed, we found that *Arc*<sup>-/-</sup> mice had a

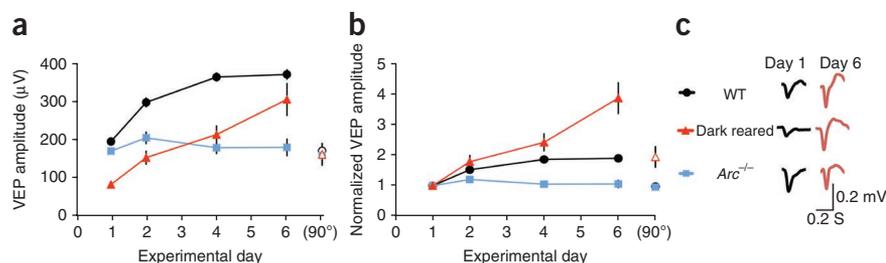
severe deficit in SRP (Fig. 8) as compared with wild-type mice. This adds further weight to the idea that *Arc* is required for multiple forms of experience-dependent plasticity in V1.

It is possible that *Arc*<sup>-/-</sup> mice exhibit deficits in plasticity because maturation of the cortex is disrupted, as is the case in dark-reared mice. However, dark-reared mice exhibit robust ocular dominance plasticity even in adulthood<sup>41</sup>, which differs from the complete absence of ocular dominance plasticity observed in *Arc*<sup>-/-</sup> mice. To further compare plasticity in dark-reared and *Arc*<sup>-/-</sup> mice, we investigated SRP in mice dark-reared from birth. Dark-reared mice exhibited significantly smaller binocular VEPs at baseline than wild-type or *Arc*<sup>-/-</sup> VEPs (dark reared,  $83 \pm 9 \mu\text{V}$ ; wild type,  $195 \pm 10 \mu\text{V}$ ;  $P < 0.0001$ ,  $t$  test; *Arc*<sup>-/-</sup>,  $170 \pm 9 \mu\text{V}$ ,  $P < 0.0001$ ,  $t$  test; Fig. 8). However, dark-reared mice showed robust SRP (Fig. 8a), which is enhanced compared with wild-type mice when normalized to baseline values (Fig. 8b). Dark-reared mice were exposed to normal light-rearing conditions during the SRP experiment. VEPs resulting from exposure to the orthogonal (novel) orientation on day 6 of the experiment were significantly different ( $P < 0.03$ ) from baseline, suggesting that baseline VEPs recover close to light-reared mice levels after 5 d of light exposure. However, the VEPs resulting from the repeated orientation were significantly higher ( $P < 0.001$ ) than VEPs resulting from exposure to the orthogonal orientations, indicating that SRP still occurred.



**Figure 7** Dark-rearing wild-type mice from birth mimics the contralateral to ipsilateral ratio observed *Arc*<sup>-/-</sup> mice. (a) *Arc*<sup>-/-</sup> and dark-reared (DR) mice both had significant decreases in the contralateral to ipsilateral eye ratio in layer 4 VEPs as compared with wild-type mice (wild type:  $n = 16$ ,  $2.1 \pm 0.1$ ; *Arc*<sup>-/-</sup>:  $n = 16$ ,  $1.35 \pm 0.08$ ,  $*P < 0.0001$ ,  $t$  test; dark-reared:  $n = 11$ ,  $1.29 \pm 0.1$ ,  $*P < 0.0001$ ,  $t$  test). (b) The change in ocular dominance ratio in *Arc*<sup>-/-</sup> and dark-reared mice was mainly the result of a significant depression in contralateral (C) responses (wild type,  $146 \pm 6 \mu\text{V}$ ; *Arc*<sup>-/-</sup>,  $116 \pm 7 \mu\text{V}$ ,  $*P < 0.006$ ,  $t$  test; dark reared,  $74 \pm 9 \mu\text{V}$ ,  $**P < 0.0001$ ,  $t$  test), as ipsilateral responses (I) were not significantly different (wild type,  $72 \pm 5 \mu\text{V}$ ; *Arc*<sup>-/-</sup>,  $90 \pm 8 \mu\text{V}$ ,  $P > 0.07$ ,  $t$  test; dark reared,  $59 \pm 8 \mu\text{V}$ ,  $P > 0.2$ ,  $t$  test). Error bars represent s.e.m.

**Figure 8** *Arc*<sup>-/-</sup> mice lack stimulus-selective response potentiation (SRP), whereas dark-reared mice exhibit enhanced SRP in V1. (a) Wild-type mice exhibited large and sustained potentiation of binocular VEPs over many days of exposure to the same stimulus orientation ( $n = 11$ , day 1 =  $195 \pm 10 \mu\text{V}$ , day 6 =  $369 \pm 14 \mu\text{V}$ ,  $P < 0.0001$ , paired  $t$  test). Responses to a control orthogonal stimulus ( $90^\circ$ , open black circle) shown at day 6 were not significantly potentiated (day 6 ( $90^\circ$ ) =  $170 \pm 9 \mu\text{V}$ ,  $P > 0.09$ ,  $t$  test). Dark-reared mice had small VEPs at baseline, which became markedly potentiated after exposure to the same stimulus orientation ( $n = 12$ , day 1 =  $83 \pm 9 \mu\text{V}$ , day 6 =  $304 \pm 43 \mu\text{V}$ ,  $P < 0.001$ , paired  $t$  test). Responses to a control orthogonal stimulus ( $90^\circ$ , open red triangle) were significantly increased compared with baseline VEPs (day 6 ( $90^\circ$ ) =  $161 \pm 29 \mu\text{V}$ ,  $P < 0.03$ ,  $t$  test) but were also significantly smaller than the SRP orientation at day 6 ( $P < 0.04$ ). In contrast, we did not observe significant potentiation of responses to the same stimulus in *Arc*<sup>-/-</sup> mice ( $n = 16$ , day 1 =  $170 \pm 9 \mu\text{V}$ , day 6 =  $180 \pm 23 \mu\text{V}$ ,  $P > 0.7$ , paired  $t$  test). Responses to the control orthogonal stimulus ( $90^\circ$ , blue square) were also not significantly different from baseline (day 6 ( $90^\circ$ ) =  $159 \pm 12 \mu\text{V}$ ,  $P > 0.1$ ,  $t$  test), suggesting that there was no general decrease in responses over time. (b) VEPs normalized to baseline values indicated that there was a relative enhancement of potentiation as compared in dark-reared compared with light-reared mice, whereas *Arc*<sup>-/-</sup> mice had no relative potentiation of VEPs. (c) Average VEP waveforms at baseline (day 1) and after 5 d of repeated exposure to the same orientation (day 6).



Taken together, these data suggest that, even though *Arc*<sup>-/-</sup> and dark-reared mice share some similar cortical properties, the severe deficits in plasticity seem to be specific to the role of *Arc* in these processes, rather than a general defect in cortical maturation.

## DISCUSSION

Multiple molecular mechanisms have been proposed to mediate the experience-dependent changes that occur in V1 during development. Thus, it is notable that perturbation of a single effector gene that is not a critical neurotransmitter receptor is sufficient to render the visual cortex impervious to the effects of selective visual experience or deprivation. Our results indicate that loss of *Arc* protein leads to an absence of ocular dominance plasticity and impaired AMPAR internalization in response to brief monocular deprivation, suggesting that *Arc* is crucial for the deprived-eye depression that normally takes place after monocular deprivation. In addition, both deprived-eye depression and open-eye potentiation fail to occur, even after extended deprivation. We also found that *Arc*<sup>-/-</sup> mice had deficits in SRP. Notably, these deficits occurred in the absence of major changes in visual response properties, as *Arc*<sup>-/-</sup> mice exhibited normal visual acuity and retinotopic organization. We did not observe any overt compensation in proteins specific for inhibitory synaptic transmission in *Arc*<sup>-/-</sup> neurons. *Arc* is only expressed in excitatory cells in the visual cortex, suggesting that the phenotypes observed in *Arc*<sup>-/-</sup> mice are not the results of aberrant compensatory mechanisms of inhibition.

A number of studies provide evidence for competitive Hebbian mechanisms contributing to the decrease in deprived-eye responses after monocular deprivation<sup>9,11,42</sup>. The shift in ocular dominance that occurs after brief visual deprivation serves as one of the most representative models of activity and NMDAR-dependent plasticity *in vivo*<sup>6,27,43</sup>. Indeed, removing or inhibiting components of the NMDAR-dependent signaling pathway, such as MAPK, PKA and CAMKII, affects ocular dominance plasticity<sup>8,44</sup>. Similar to mice with impaired NMDAR-mediated synaptic transmission<sup>27,45</sup>, we found that *Arc*<sup>-/-</sup> mice lacked deprived-eye depression, even after 7 d of deprivation. Because *Arc* transcription is also dependent on activation of NMDARs, and MAPK and PKA signaling cascades<sup>46</sup>, our data suggest that *Arc* is a critical downstream effector molecule for this pathway. *Arc* may be required for both mGluR-dependent and NMDAR-dependent AMPAR removal<sup>21</sup>. In hippocampal cultures, mGluR-induced decreases in AMPARs are prevented in the absence of

*Arc* protein, whereas overexpression of *Arc* mimics mGluR-induced decreases in AMPAR surface expression<sup>20,21</sup>. In this context, it should be noted that, similar to *Arc*<sup>-/-</sup> mice, mutant mice with a 50% reduction in mGluR5 expression also lack deprived-eye depression following 3 d of monocular deprivation<sup>26</sup>. Therefore, *Arc* may be a critical component of a final common pathway by which appropriate activation of either NMDARs or mGluRs triggers synaptic depression and loss of visual responsiveness.

In wild-type mice, a robust potentiation of open-eye responses can be detected with both intrinsic signal imaging and VEPs after 7 d. However, open-eye responses fail to potentiate after an extended period of deprivation in *Arc*<sup>-/-</sup> mice. Two processes are proposed to account for the delayed open-eye potentiation. One proposal is that the strengthening of open-eye responses after longer periods of deprivation relies on homeostatic synaptic scaling<sup>3,4</sup>. In support of this view, mice lacking tumor necrosis factor alpha, a cytokine derived from glia and implicated in homeostatic synaptic scaling, have normal deprived-eye depression, but no open-eye potentiation<sup>4</sup>. It has been proposed that the lack of open-eye potentiation is a result of the loss of a mechanism for synaptic scaling, as normal LTP is found in these mice. Alternatively, visual deprivation and the consequent reduction in cortical activity may cause a metaplastic adjustment of the properties of NMDAR-dependent LTP that enables open-eye potentiation. In support of this view, open-eye potentiation is selectively prevented by NMDAR blockade initiated after the initial deprived-eye depression<sup>45,47</sup>. Our findings cannot distinguish between these alternative hypotheses, as *Arc* is implicated in both LTP and homeostatic scaling. However, our ocular dominance plasticity data support the hypothesis that *Arc* is a critical mediator of NMDAR-dependent synaptic plasticity, regardless of the valence of the change. The finding that *Arc* is required for the expression of SRP, a form of experience-dependent plasticity that bears all the hallmarks of LTP, further strengthens this conclusion. Thus, our data suggest that *Arc* is required for bidirectional, experience-dependent synaptic plasticity in mouse V1 *in vivo*.

Numerous studies have shown that activity is critical for the sharpening and refinement of visual response properties such as ocular dominance and orientation tuning throughout development<sup>48</sup>. In very young rats (P17–19), there are a large number of binocular cells in the binocular zone of V1 (ref. 48). By adolescence, however, a contralateral bias has been established in cortex that continues throughout adulthood. This suggests that there may be an activity-dependent refinement of the contralateral to ipsilateral eye ratio. Data from V1 of

dark-reared adult rats support this view, as these rats exhibit a greater percentage of binocular cells compared with normally reared rats<sup>48</sup>. Using VEPs, we found that *Arc*<sup>-/-</sup> mice and mice dark reared from birth had a significant reduction ( $P << 0.0001$ ) in the contralateral to ipsilateral eye ratio, similar to that seen previously in dark-reared rats<sup>48</sup>. These data suggest that both experience and *Arc* are critical for the normal establishment of the contralateral to ipsilateral eye ratio. Input from retinal ganglion cells to the LGN is roughly 9:1 in favor of the contralateral projections, but the volume of the binocular segment of the dorsal LGN occupied by contralateral retinogeniculate inputs is only 2.4-fold larger than the volume occupied by ipsilateral inputs, which can be accounted for by a three-to-one convergence of contra inputs to LGN neurons<sup>49</sup>. We believe that the changes in ocular dominance in *Arc*<sup>-/-</sup> mice occur at the level of the cortex, as *Arc* is not present in the thalamus at any age and we have shown that eye-specific segregation in the thalamus of *Arc*<sup>-/-</sup> mice is not different from wild-type mice.

One caveat of our study is that we used a germline knockout mouse lacking *Arc* from birth. It is possible that *Arc* may affect the normal development of V1 before any experience-dependent processes. However, *Arc* expression is virtually undetectable before eye opening in V1 (refs. 29,30) and its expression rapidly increases after eye opening, during the period in which experience-dependent changes occur. *Arc* may contribute to the refinement of response properties by the removal or reduction of weaker inputs and the potentiation of stronger inputs. This would result in a sharpening of overall receptive field properties throughout development. In both *Arc*<sup>-/-</sup> and dark-reared mice, the loss of a putative mechanism for synaptic refinement may retard the emergence of mature response properties. In the case of ocular dominance, this would manifest in an increase in binocular cells and a reduction of the contralateral to ipsilateral eye ratio. Consistent with a role for *Arc* in the sharpening of visual response properties, adult *Arc*<sup>-/-</sup> mice show an increase in cells with low orientation specificity and broader tuning compared with heterozygous and wild-type mice<sup>30</sup>.

Although dark rearing mice induces effects that are similar to removing *Arc*, such as altered contralateral to ipsilateral eye ratio, dark rearing has additional regressive effects, such as a loss of orientation selectivity and acuity, and disrupted retinotopic maps<sup>48</sup> that are not observed in *Arc*<sup>-/-</sup> mice. In addition, dark rearing also promotes subsequent plasticity on light exposure, such as SRP and ocular dominance plasticity<sup>44,50</sup>. In contrast, *Arc*<sup>-/-</sup> mice appear impervious to the effects of experience and deprivation.

In conclusion, we found that *Arc* is important for multiple forms of experience-dependent plasticity, including the establishment of the normal contralateral to ipsilateral eye ratio in mouse V1. These data indicate that *Arc* is a critical component of the molecular machinery that leads to lasting modifications of V1 in response to changes in the qualities of sensory experience.

## METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/natureneuroscience/>.

Note: Supplementary information is available on the Nature Neuroscience website.

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## AUTHOR CONTRIBUTIONS

C.L.M. and J.D.S. conducted experiments and data analysis and wrote the manuscript. D.T. assisted with optical imaging experiments. K.H.W. provided the *Arc*<sup>-/-</sup> mouse line. M.S. and M.F.B. helped design experiments and supervised the project.

## COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## ONLINE METHODS

**Animals.** Wild-type (C57/Bl6) and *Arc*<sup>-/-</sup> mice<sup>30</sup> on the same genetic background were used for all experiments (P25–30). Mice were normally housed in cages under a 12-h light-dark cycle, whereas dark-reared mice were reared in complete darkness. All experiments were performed under protocols approved by the Massachusetts Institute of Technology's Animal Care and Use Committee and conformed to US National Institutes of Health guidelines.

**Lid suture.** Mice were anesthetized using Avertin (0.016 ml g<sup>-1</sup>, intraperitoneal) and the eyelid margins trimmed. The eye contralateral to the hemisphere being imaged was sutured using prolene sutures (Henry Schein) for 3–7 d. Mice were checked daily to ensure that the eye remained shut throughout the deprivation period.

**Immunohistochemistry.** Mice were killed by transcardial perfusion with saline followed by 4% paraformaldehyde (wt/vol). Brains were placed in 4% paraformaldehyde overnight and cryoprotected in 20% sucrose (wt/vol). Immunohistochemistry for Arc protein (1:250, Santa Cruz, mouse), GFAP (1:500, Chemicon, rabbit) and GABA (1:500, Chemicon, rabbit) was carried out on 30–40- $\mu$ m-thick coronal sections. Sections were analyzed using ImageJ software (<http://rsbweb.nih.gov/ij/>) and Photoshop CS3.

**Western blots.** Mice were anesthetized with isoflurane before being decapitated. The visual cortex was dissected from both hemispheres and homogenized in a modified RIPA buffer (20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 1% Triton X-100 (vol/vol), 0.1% SDS (vol/vol), protease inhibitor tablet (Roche 11836170001)). The homogenate was centrifuged (14,000g for 5 min) and the supernatant was removed and quantified. Samples were loaded at a concentration of 35  $\mu$ g and run on a 10% gel and transferred at 40 V for 80 min. We used antibodies to GluR1 (1:500, Chemicon, rabbit), VGAT (1:250, Chemicon, rabbit), GAD65 (1:500, Chemicon, rabbit) and parvalbumin (1:250 abcam, rabbit). Membranes were incubated with secondary antibodies to the appropriate species for 2 h at room temperature (23  $\pm$  1  $^{\circ}$ C). Membranes were developed using chemiluminescence (Amersham).

**Biotinylation assay.** Acute slices (300  $\mu$ m) were prepared from critical period mice that were deprived briefly by lid suture as described previously<sup>9</sup>. V1 ipsilateral to the deprived eye was used as a within-animal control and comparison of surface GluR1 expression was made between the ipsilateral (nondeprived) and contralateral (deprived) hemispheres. The mouse was anesthetized using isoflurane and the brain rapidly dissected out and placed in ice-cold dissection buffer (75 mM sucrose, 10 mM dextrose, 87 mM NaCl, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.5 mM CaCl<sub>2</sub> and 7 mM MgCl<sub>2</sub>). A vibratome was used to take 300- $\mu$ m coronal sections containing the visual cortex. Slices were washed three times in ice-cold ACSF buffer (24 mM NaCl, 5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 26 mM NaHCO<sub>3</sub>, 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub> and 10 mM dextrose), before being incubated in 100  $\mu$ M S-NHS-SS-biotin for 45 min. After the incubation period, the sections were washed twice in 100  $\mu$ M lysine to quench the excess biotin. The superficial layers of the visual cortex were dissected out and homogenized in a modified RIPA buffer. The homogenate was centrifuged (14,000g for 5 min) and the supernatant removed. The protein concentration was determined and 30% of the supernatant was set aside for the total protein lane; ACSF was added to the remaining supernatant (for a total volume of 1 ml) and incubated with 40  $\mu$ l of streptavidin beads overnight at 4  $^{\circ}$ C. The beads were centrifuged (3,500g for 1 min) and the supernatant discarded. The beads were washed three times in a 1:1 cocktail of ACSF and modified RIPA buffer, after which 2 $\times$  loading buffer was added. The sample was boiled for 5 min, followed by centrifugation (7,000g for 1 min). Samples were run side by side or processed in parallel on a 10% gel and transferred at 40 V for 80 min.

**Injection of CTB.** Mice were anesthetized with Avertin (0.016 ml g<sup>-1</sup>, intraperitoneal injection). The sclera of each eye was pierced and a small quantity of

vitreous fluid removed using a thin Hamilton syringe. Approximately 3  $\mu$ l of CTB conjugated to either AlexaFluor 488 or 594 (Invitrogen) was injected.

**Optical imaging of intrinsic signals.** Mice were anesthetized with urethane (1.5 mg per kg of body weight) and chlorprothixene (0.2 mg per mouse). Heart rate was monitored throughout the trial and only those mice whose heart rate remained stable throughout the experiment were used. Intrinsic signal images were obtained using a CCD camera (Cascade 512B, Roper Scientific) and red filter (630 nm) to illuminate the cortex during visual stimulation, as previously described<sup>33</sup>. Stimulation consisted of a drifting bar (9 $^{\circ}$   $\times$  72 $^{\circ}$ ) moving continuously and periodically (9 $^{\circ}$  s<sup>-1</sup>) in an upward or downward direction. Frames were captured at a rate of 15 frames per s. Slow noise components were removed using a temporal high-pass filter (135 frames) and the fast Fourier transform component at the stimulus frequency (9 $^{\circ}$  s<sup>-1</sup>) was calculated pixel by pixel from the whole set of images<sup>32</sup>. The amplitude of the fast Fourier transform component was used to measure the strength of visual drive for each eye. ODI was calculated as  $(R_{\text{contra}} - R_{\text{ipsi}})/(R_{\text{contra}} + R_{\text{ipsi}})$ , where  $R$  refers to the response to each eye stimulated individually. Empirically defined correspondence between the strength of eye-specific drive and retinotopic organization of the cortex yielded the binocular zone as the top 40% of pixels responding to ipsilateral eye stimulation. To assess map organization, we calculated the phase scatter of the retinotopic maps<sup>40</sup>. We calculated the difference between the phase value of each pixel and the mean phase of its five nearest neighbors along with the s.d. to get an index for map scatter.

**VEP recordings.** All electrophysiological experiments were carried out blind to genotype and were generated by heterozygous  $\times$  heterozygous matings.

**Electrode implantation.** Mice were anesthetized with an intraperitoneal injection of 50 mg per kg ketamine and 10 (mg/kg) xylazine, and a local anesthetic of 1% lidocaine hydrochloride (mg ml<sup>-1</sup>) was injected over the scalp. 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 of lambda) and tungsten microelectrodes (FHC) were inserted 450  $\mu$ m below the cortical surface along the dorsal-ventral stereotaxic axis, positioning the electrode tip in layer 4. Reference electrodes were placed bilaterally in prefrontal cortex. Electrodes were secured in place using cyanoacrylate and the entire exposure was covered with dental cement. Mice were monitored postoperatively for signs of infection or discomfort and were allowed at least 24 h recovery before habituation to the restraint apparatus.

**VEP 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 100–400 stimuli were presented per condition. VEP amplitude was quantified by measuring trough to peak response amplitude, as described previously<sup>5</sup>.

**Visual stimuli.** 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 System) and presented on a computer monitor suitably linearized by  $\gamma$ -correction. VEPs were elicited by horizontal, vertical or oblique (45 $^{\circ}$  or 135 $^{\circ}$ ) bars. The display was positioned 20 cm in front of the mouse and centered on the midline, thereby occupying 92 $^{\circ}$   $\times$  66 $^{\circ}$  of the visual field. Mean luminance, determined by a photodiode placed in front of the computer screen, was 27 cd m<sup>-2</sup>.

**Statistical analysis.** Statistical analyses of experiments to assess significance were conducted using Student's  $t$  test or ANOVA (one and two way) and with Bonferroni correction as required.