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

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Abstract

A myriad of mechanisms are suggested to account for the full richness of visual cortical plasticity. We report 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 find that *Arc*$^{-/-}$ mice do not exhibit depression of deprived eye responses or a shift in ocular dominance after brief monocular deprivation. Extended deprivation also fails to elicit a shift in ocular dominance or open eye potentiation. Moreover, *Arc*$^{-/-}$ mice lack stimulus–selective response potentiation. Although *Arc*$^{-/-}$ mice exhibit normal visual acuity, baseline ocular dominance is abnormal and resembles 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.

INTRODUCTION

Experience–dependent reorganization of eye–specific inputs during development is a major mechanism by which neuronal connectivity is established in the primary visual cortex (V1)[1]. Changes in neuronal activity lead to strengthening or weakening of synapses, which are believed to initiate the structural remodeling of visual networks. During a period of heightened plasticity (P25–P32 in mice), V1 is exquisitely sensitive to changes in activity. Brief monocular deprivation results in striking functional and anatomical reorganization within the binocular zone of V1 due to a rapid weakening of the cortical response to the deprived eye and a shift in ocular dominance in favor of the non-deprived eye2. 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 V11, 3–5.

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The mechanisms underlying the changes induced by brief monocular deprivation are well studied. Early findings indicated that the initial cortical depression occuring after monocular deprivation is dependent upon calcium signaling through NMDA receptors (NMDARs)6, appropriate levels of inhibition7, and protein synthesis8. Recent evidence suggests that deprived eye depression is induced by loss of AMPA type glutamate receptors (AMPARs) on the surface of cortical neurons via mechanisms similar to long–term synaptic depression (LTD) 9–11. The regulated trafficking of these receptors is a major cellular mechanism underlying synaptic plasticity at excitatory synapses12. Reduction in surface expression of both GluR1 and GluR2 AMPAR subunits occurs after brief monocular deprivation9. Deprived eye depression occludes the induction of LTD in cortical slices[9, 10], and the ocular dominance shift is prevented by manipulations that block AMPAR endocytosis[13]. 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 due to deprivation may trigger metaplasticity or a homeostatic scaling of responses that results in a strengthening of the open eye[4, 5, 14, 15].

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 called "stimulus–selective response potentiation" or SRP[16]. SRP occurs in adults and juveniles, is specific to the stimulated eye, and develops over hours to days. Moreover, SRP depends on both NMDA receptor activation and AMPA receptor trafficking in the cortex, properties that are shared with long–term potentiation (LTP). Thus, SRP provides a framework to study LTP–like processes in the intact brain, which are induced through normal experience rather than through artificial stimulation paradigms.

The immediate early gene Arc (activity–regulated cytoskeletal associated protein), also known as Arg 3.1, is implicated in many forms of synaptic plasticity, including LTP[17–19], LTD20, 21 and homeostatic scaling of AMPARs[22, 23]. Arc gene expression and efficient Arc translation are dependent on NMDAR and group 1 mGluR activation[23, 24, 25]. These signaling pathways are implicated in ocular dominance and many other forms of experience–dependent plasticity[6, 26–28], 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[29, 30]. Moreover, Arc RNA induction is a reporter of ocular dominance plasticity in V1[29]. Taken together, these studies suggest that Arc is a prime molecular candidate to play a role in experience–dependent plasticity in V1.

In the present study 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 $Arc^{-/-}$ mice in which GFP has been knocked–in to the Arc gene locus[30] to study how loss of 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 $Arc^{-/-}$ mice

$Arc^{-/-}$ mice are viable and show no gross deficits in size or weight compared to wild–type (WT) mice[17, 30]. While previous reports focused on Arc protein interactions within the hippocampus and dentate gyrus, few studies examined Arc's function in cortex or *in vivo*. We examined the distribution of Arc protein expression in mouse V1 by immunofluorescence using

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an Arc specific antibody. In V1, Arc did not colocalize with GFAP, which labels astrocytes, or with the inhibitory neuron marker GABA (Supplementary Fig. 1a). This suggests that Arc protein is selectively expressed in excitatory neurons within V1, which is consistent with previous studies showing that Arc is predominately expressed in principal neurons that also express CaMKII[31]. Previous reports show that Arc mRNA is regulated by physiological activity and shows prominent expression in V1[24, 29]. As expected, no Arc expression was detected in $Arc^{-/-}$ tissue (Supplementary Fig. 1a). Within WT V1, Arc protein expression was detected in all cortical layers with the exception of layer 5, with greatest expression being seen in layers 2/3 and 4, the predominant sites of ocular dominance plasticity (Supplementary Fig. 1b).

We used intrinsic signal imaging to test whether loss of Arc altered V1 responses and retinotopic organization[32, 33]. Because previous studies implicated Arc protein in regulation of AMPARs, the major contributors to excitatory synaptic transmission, we asked whether 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 Methods). V1 in $Arc^{-/-}$ mice was similar to that in WT mice in area and organization of binocular and monocular zones (Fig. 1a). To examine whether loss of Arc protein might impact retinotopic organization (Fig. 1a), we evaluated scatter within the retinotopic (phase) maps (Fig. 1b). Map organization in $Arc^{-/-}$ mice was indistinguishable from WT mice (Supplementary Fig. 2a). In addition, there was no significant difference in the magnitude of response to binocular stimulation in V1 (Supplementary Fig. 2b), nor were there differences in responses from the monocular zone of V1 (data not shown). These data demonstrate that loss of Arc protein does not grossly disrupt the development of V1 organization. We assessed visual acuity in $Arc^{-/-}$ mice by measuring VEPs in response to sinusoidal gratings at various spatial frequencies, a well established method of assessing visual function in mice[27, 34]. There was no significant difference between WT and $Arc^{-/-}$ mice in evoked responses at high spatial frequencies, regardless of whether responses were evoked binocularly or monocularly through either eye, suggesting that $Arc^{-/-}$ 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 days during the period of heightened plasticity in mice (P25–32). We then used intrinsic signal imaging to measure the cortical response to visual stimulation within the binocular zone of V1, contralateral to the deprived eye. As described above, stimuli were shown to each eye alternately, and the strength of response to contralateral or ipsilateral stimulation was assessed and an ocular dominance index (ODI) calculated. This method has been shown to reliably detect the changes in ocular dominance that can be induced by monocular deprivation in WT animals[35]. In keeping with previous reports, WT mice show a robust decrease in ODI after brief deprivation (Fig. 2a). By assessing the magnitude of response in deprived and nondeprived animals, this shift appeared to be mediated by a diminished response to the deprived eye (Fig. 2b). By contrast, $Arc^{-/-}$ 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 prior to and after monocular deprivation[27, 34]. Electrodes were implanted at a depth corresponding to layer 4 in V1 at P24–P25. After habituation to the restraint apparatus, VEPs were recorded at P28 in fully awake, head–restrained mice in response to square wave–reversing sinusoidal

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gratings. We collected baseline recordings, and then monocularly deprived animals for 3 days by lid suture. After opening the sutured eye we gathered post monocular deprivation recordings. WT mice showed a robust ocular dominance shift (Fig. 3a, c), whereas $Arc^{-/-}$ mice did not exhibit a change in ocular dominance (Fig. 3b, c). The shift in WT mice was due to a significant depression in deprived eye responses (Fig. 3a), which was not observed in $Arc^{-/-}$ mice (Fig. 3b).

Monocular deprivation resulted in a dramatic loss of visual acuity in responses contralateral to the deprived eye, while preserving acuity in the ipsilateral responses to the open eye in WT mice (Supplementary Fig 4a). In contrast, $Arc^{-/-}$ mice did not exhibit changes in visual acuity after monocular deprivation (Supplementary Fig 4b), 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 within V1. Quantitative western blot analyses of VGAT, GAD65, and parvalbumin showed no difference between $Arc^{-/-}$ and WT mice (Supplementary Fig. 5a–c). 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. 5d).

Arc regulates AMPAR endocytosis in visual cortex

What might underlie 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 hours of monocular deprivation, AMPARs are rapidly internalized, decreasing the surface to total ratio, which mediates the depression in cortical responses from the deprived eye[9]. Recent experiments in cultured primary neurons revealed that Arc regulates AMPAR internalization via its interactions with the proteins dynamin and endophilin, two integral components of the clathrin–mediated endocytosis machinery[36]. High levels of Arc expression are found to accelerate the rate of AMPAR endocytosis, leading to decreased AMPAR surface expression, while loss of Arc reduces AMPAR endocytosis[36]. 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[9, 13]. In addition, GluR1 shows high immunoreactivity in the middle and superficial layers of mouse V1[37], which are key sites of ocular dominance plasticity. We performed a biotinylation assay using acute slices in order 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. 1b), the deeper layers were microdissected out and discarded from both hemispheres. In WT mice, a significant decrease in the surface to total ratio of GluR1 could be detected in the “deprived” hemisphere (contralateral to the deprived eye; Fig. 4a) as compared to the “nondeprived” control hemisphere (Fig. 4b,c). Strikingly, $Arc^{-/-}$ mice showed no significant change in the surface to total ratio of AMPARs within 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 $Arc^{-/-}$ mice

The ocular dominance shift that occurs after long–term monocular deprivation occurs in two temporally distinct phases. In response to brief monocular deprivation, decorrelated input through the closed eye results in a Hebbian weakening of the deprived eye response, which we have shown requires Arc, whereas extended periods of deprivation result in potentiation of the open eye response. It has been proposed that distinct cortical processes may mediate the two

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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 $Arc^{-/-}$ mice we used intrinsic signal imaging to measure response magnitudes in mice deprived for 7 days. In response to deprivation, WT mice showed a significant shift in ODI (Fig. 5a). Consistent with previous reports, we found that this shift was mediated by a significant increase in open eye responses (Fig. 5b). The increase in open eye response was accompanied by a decrease in the deprived eye response (Fig. 5b). Strikingly, $Arc^{-/-}$ mice did not show a shift in ODI or significant open eye potentiation (Fig. 5a,c). Similar results were found with VEP recordings after 7 days of monocular deprivation. WT mice exhibited a robust ocular dominance shift that was due to both significant deprived eye depression and open eye potentiation (Fig. 6a, c). In contrast, $Arc^{-/-}$ mice did not exhibit an ocular dominance shift or any significant changes in deprived eye or open eye responses (Fig. 6b, c).

Normal balance of eye–specific drive requires Arc

Layer 4 VEPs recorded in $Arc^{-/-}$mice exhibited altered baseline contralateral to ipsilateral eye (C/I) response ratios as compared with WT mice (Fig. 3c, Fig. 6c). After pooling baseline data from all VEP experiments we confirmed that $Arc^{-/-}$ mice had a significant decrease in C/I ratio as compared to WT mice (Fig. 7a). This was mostly due to a significant decrease in contralateral responses (Fig. 7b). We hypothesized that establishing the C/I ratio in mice requires neuronal activity and visual experience. To test this, we dark–reared (DR) WT mice from birth and recorded baseline responses in P28–32 mice that had never been exposed to light. Dark-rearing has previously been shown to dramatically reduce Arc expression in V1[30]. DR mice exhibited a decrease in the C/I ratio due to significantly smaller contralateral responses, similar to that observed in $Arc^{-/-}$ 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 $Arc^{-/-}$ mice. During the pre–critical period, experience–dependent competition between the two eyes is necessary for normal axonal refinement in central targets[38–40]. 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 $Arc^{-/-}$ mice as compared to WT (Supplementary Fig. 7).

The altered baseline C/I ratio in $Arc^{-/-}$ 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 $Arc^{-/-}$ 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 $Arc^{-/-}$ mice, so any differences in deprivation–induced depression of $Arc^{-/-}$ 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 in the C/I ratio in the ipsilateral hemisphere after 7 days of monocular deprivation in WT mice, which was due to a significant decrease in the ipsilateral (deprived eye) responses (Supplementary Fig. 8). However, $Arc^{-/-}$ mice showed no shift in C/I ratio or changes in ipsilateral responses. Moreover, we did not find any significant changes in surface GluR1 between $Arc^{-/-}$ and WT V1 slices (Supplementary Fig. 9).

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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[16]. 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. Since $Arc^{-/-}$ mice exhibit a defect in open eye potentiation, we wondered whether SRP would also be disrupted due to a lack of Arc. Indeed, we found that $Arc^{-/-}$ mice had a severe deficit in SRP (Fig. 8) as compared to WT 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 $Arc^{-/-}$ mice exhibit deficits in plasticity because maturation of the cortex is disrupted, as is the case in DR mice. However, DR mice exhibit robust ocular dominance plasticity even in adulthood[41], which differs from the complete absence of ocular dominance plasticity observed in $Arc^{-/-}$ mice. To further compare plasticity in DR and $Arc^{-/-}$ mice we investigated SRP in mice dark–reared from birth. DR mice exhibit significantly smaller binocular VEPs at baseline than WT or $Arc^{-/-}$ VEPs (Fig. 8). However, DR mice show robust SRP (Fig. 8a), which is enhanced compared to WT mice when normalized to baseline values (Fig. 8b). DR 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 from baseline suggesting that baseline VEPs recover close to light–reared mice levels after 5 days of light exposure. However, the VEPs resulting from the repeated orientation were significantly higher than VEPs resulting from exposure to the orthogonal orientations, indicating that SRP still occurred. Taken together, these data suggest that even though $Arc^{-/-}$ and DR 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 remarkable 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 show 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 find that $Arc^{-/-}$ mice exhibit deficits in SRP. Strikingly, these deficits occur in the absence of major changes in visual response properties as $Arc^{-/-}$ mice exhibit normal visual acuity and retinotopic organization. We do not observe any overt compensation in proteins specific for inhibitory synaptic transmission in $Arc^{-/-}$ neurons. Arc is only expressed in excitatory cells in the visual cortex, suggesting that the phenotypes observed in $Arc^{-/-}$ mice are not due to 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[9, 11, 42]. 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*[6, 27, 43]. Indeed, removing or inhibiting components of the NMDAR–dependent signaling pathway, such as MAPK, PKA, and CAMKII, affects ocular dominance plasticity[8, 44]. Similar to mice with impaired NMDAR–mediated synaptic transmission[27, 45], we find that $Arc^{-/-}$ mice lack deprived eye depression, even after 7 days of deprivation. Since Arc transcription is also dependent upon activation of NMDARs, and MAPK and PKA signaling cascades[46], our data suggest that Arc

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is a critical downstream effector molecule for this pathway. Arc may be required for mGluR–dependent as well as NMDAR–dependent AMPAR removal[21]. 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[20, 21]. In this context it is interesting to note that similar to *Arc*$^{-/-}$ mice, mutant mice with a 50% reduction in mGluR5 expression also lack deprived–eye depression following 3 days of monocular deprivation[26]. 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 WT mice, a robust potentiation of open eye responses can be detected with both intrinsic signal imaging and VEPs after 7 days. However, open eye responses fail to potentiate after an extended period of deprivation in *Arc*$^{-/-}$ 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 upon homeostatic synaptic scaling3, 4. In support of this view, mice lacking tumor necrosis factor alpha (TNFa), a cytokine derived from glia and implicated in homeostatic synaptic scaling, show normal deprived eye depression but no open eye potentiation[4]. It has been proposed that the lack of open eye potentiation is due to loss of a mechanism for synaptic scaling because 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 depression45, 47. The current findings cannot distinguish among these alternative hypotheses since Arc is implicated in both LTP and homeostatic scaling. However, our ocular dominance plasticity data do 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 show that activity is critical for the sharpening and refinement of visual response properties such as ocular dominance and orientation tuning throughout development[48]. In very young rats (P17–P19) there is a large number of binocular cells within the binocular zone of V1[48]. However, by adolescence a contralateral bias has been established in cortex and continues throughout adulthood. This suggests that there may be an activity–dependent refinement of the C/I ratio. Data from V1 of dark–reared adult rats support this view as these rats exhibit a greater percentage of binocular cells compared to normally reared rats48. Using VEPs we find that *Arc*$^{-/-}$ mice and mice dark–reared from birth show a significant reduction in the C/I ratio, similar to that seen previously in dark–reared rats[48]. These data suggest that both experience and Arc are critical for the normal establishment of the C/I 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 times 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[49]. We believe the changes in ocular dominance in *Arc*$^{-/-}$ 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*$^{-/-}$ mice is not different from WT mice.

One caveat of our study is that we use a germline knockout mouse lacking Arc from birth. It is possible that Arc may affect the normal development of V1 prior to any experience–dependent processes. However, Arc expression is virtually undetectable prior to eye opening in V1[29, 30], and its expression rapidly increases after eye opening during the period in which

experience–dependent changes take place. 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 $Arc^{-/-}$ and DR 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 C/I ratio. In line with a role for Arc in the sharpening of visual response properties, adult $Arc^{-/-}$ mice show an increase in cells with low orientation specificity and broader tuning compared to heterozygous and WT mice [30].

While dark–rearing mice induces similar effects to removing Arc^{-}, such as altered C/I ratio, dark–rearing has additional regressive effects such as a loss of orientation selectivity and acuity, and disrupted retinotopic maps[48] that are not observed in $Arc^{-/-}$ mice.[52]. In addition, dark–rearing also promotes subsequent plasticity upon light exposure, such as SRP and ocular dominance plasticity[44,50]. By contrast, $Arc^{-/-}$ mice appear impervious to the effects of experience and deprivation.

In conclusion, we have found that Arc is critically involved in multiple forms of experience dependent plasticity, including the establishment of the normal C/I ratio in mouse V1. Together these experiments illustrate 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

Animals

WT (C57/Bl6) and $Arc^{-/-}$ mice[30] on the same genetic background were used for all experiments (P25–P30). Mice were normally housed in cages under a 12 hour light–dark cycle, whereas dark–reared mice were reared in complete darkness. All experiments were performed under protocols approved by MIT's Animal Care and Use Committee and conformed to NIH guidelines.

Lid suture

Animals were anesthetized using Avertin (0.016 ml/g, i.p.) and the eyelid margins trimmed. The eye contralateral to the hemisphere being imaged was sutured using prolene sutures (Henry Schein) for 3–7 days. Animals were checked daily to ensure that the eye remained shut throughout the deprivation period.

Immunohistochemistry

Animals were transcardially perfused with saline followed by 4% paraformaldehyde (PFA). Brains were placed in 4% PFA overnight and cryoprotected in 20% sucrose. 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 μm thick coronal sections. Sections were analyzed using ImageJ software (http://rsbweb.nih.gov/ij/) and Photoshop CS3.

Western Blots

Animals were anesthetized gently with isofluorane prior to decapitation. 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, .1% SDS, protease inhibitor tablet (Roche 11836170001)). The homogenate was centrifuged (14,000 g for 5 mins) and the supernatant was removed and quantified. Samples were loaded at a concentration of 35 μg and run on a

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10% gel and transferred at 40 V for 80 mins. Antibodies used included GluR1 (1:500, Chemicon, rabbit), VGAT (1:250,Chemicon, rabbit), GAD65 (1:500, Chemicon, rabbit), and Parvalbumin (1:250 abcam, rabbit). Membranes were incubated in a secondary against the appropriate species for 2 hrs at room temperature. Membranes were developed using chemiluminescence (Amersham).

Biotinylation Assay

Acute slices (300 μm) were prepared from critical period animals deprived briefly by lid suture as described previously[9]. 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 animal was anesthetized using isofluorane 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_2PO_4, .5 mM $CaCl_2$, 7 mM $MgCl_2$). A vibratome was used to take 300 μm coronal sections containing the visual cortex. Slices were washed 3 times in ice–cold ACSF buffer (24 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM dextrose), prior to being incubated in 100 μM S–NHS–SS– biotin for 45 mins. After the incubation period the sections were washed two times in 100 μM 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 (14,000 g for 5 mins) and the supernatant removed. The protein concentration was determined and thirty percent 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 μl of streptavidin beads overnight at 4° C. The beads were centrifuged (3,500 g 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× loading buffer was added. The sample was boiled for 5 minutes, followed by centrifugation (7,000 g for 1 minute). Samples were run side by side, or processed in parallel, on a 10% gel, and transferred at 40 V for 80 mins.

Injection of cholera toxin subunit B (CTB)

Mice were anesthetized with Avertin (0.016 ml/g, by intraperitoneal injection). The sclera of each eye was pierced and a small quantity of vitreous fluid removed using a thin Hamilton syringe. Approximately 3 μl of CTB conjugated to either AlexaFluor 488 or 594 (Invitrogen) was injected.

Optical imaging of intrinsic signals

Animals were anesthetized with urethane (1.5 mg/kg) and chlorprothixene (0.2mg/mouse). Heart rate was monitored throughout the trial and only those animals 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 (630nm) to illuminate the cortex during visual stimulation, as previously described[33]. Stimulation consisted of a drifting bar (9° × 72°) moving continuously and periodically (9°/second) in an upward or downward direction. Frames were captured at a rate of 15 frames/second. Slow noise components were removed using a temporal high pass filter (135 frames) and the Fast Fourier Transform (FFT) component at the stimulus frequency ($9° \sec^{-1}$) was calculated pixel by pixel from the whole set of images [32]. The amplitude of the FFT component was used to measure the strength of visual drive for each eye. An ocular dominance index was calculated as ODI = (Rcontra − Ripsi)/ (Rcontra + Ripsi), 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

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[40]. We calculated the difference between the phase value of each pixel and the mean phase of its 5 nearest neighbors along with the standard deviation to get an index for map scatter.

VEP Recordings

All electrophysiological experiments were carried out blind to genotype and were generated by het × het matings.

Electrode Implantation

Mice were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine i.p., and a local anesthetic of 1% lidocaine hydrochloride 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, Bowdoinham, ME) were inserted 450 µm 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. Animals were monitored postoperatively for signs of infection or discomfort and were allowed at least 24 hr recovery before habituation to the restraint apparatus.

VEP Recording Procedure

VEP recordings were conducted in awake mice. Mice were habituated to the restraint apparatus prior to the first recording session. The animals were alert and still during recording. Visual stimuli were presented to left and right eyes randomly. A total of 100 to 400 stimuli were presented per condition. VEP amplitude was quantified by measuring trough to peak response amplitude, as described previously[5].

Visual Stimuli

Visual stimuli consisted of full–field sine wave gratings (0.05 cycles/deg) of varying contrast (0%–100%) generated by a VSG2/2 card (Cambridge Research System, Cheshire, UK) 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/m^2.

Statistical analysis

Statistical analysis of experiments, to assess significance, were conducted using student's t–test or ANOVA (one and two way) and with Bonferroni correction as required. Specific tests used for each experiment are stated in the figure legends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Tropea D, Van Wart A, Sur M. Molecular mechanisms of experience-dependent plasticity in visual cortex. Phil. Trans. R. Soc 2009;364:341–355.
2. Gordon JA, Stryker MP. Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J Neurosci 1996;16:3274–3286. [PubMed: 8627365]
3. Mrsic-Flogel TD, et al. Homeostatic regulation of eye-specific responses in visual cortex during ocular dominance plasticity. Neuron 2007;54:961–972. [PubMed: 17582335]
4. Kaneko M, Stellwagen D, Malenka M, Stryker MP. Reduced response to monocular deprivation in TNF-alpha knockout mouse suggests that homeostatic synaptic scaling contributes to rapidly activity-dependent plasticity in developing visual cortex. Neuron 2006;58:673–680. [PubMed: 18549780]
5. Frenkel MY, Bear MF. How monocular deprivation shifts ocular dominance in visual cortex of young mice. Neuron 2004;44:917–923. [PubMed: 15603735]
6. Bear MF, Kleinschmidt A, Gu QA, Singer W. Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J. Neurosci 1990;10:909–925. [PubMed: 1969466]
7. Hensch TK, et al. Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science 1998;282:1504–1508. [PubMed: 9822384]
8. Taha S, Hanover JL, Silva AJ, Stryker MP. Autophosphorylation of alphaCaMKII is required for ocular dominance plasticity. Neuron 2002;36:483–491. [PubMed: 12408850]
9. Heynen AJ, et al. Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nat Neurosci 2003;6:854–862. [PubMed: 12886226]
10. Allen CB, Celikel T, Feldman DE. Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. Nat Neurosci 2003;6:291–299. [PubMed: 12577061]
11. Crozier RA, Wang Y, Liu CH, Bear MF. Deprivation-induced synaptic depression by distinct mechanisms in different layers of mouse visual cortex. Proc Natl Acad Sci USA 2007;104:1383–1388. [PubMed: 17227847]
12. Shepherd JD, Huganir RL. The cell biology of synaptic plasticity: AMPA receptor trafficking. Annual Review of Cell and Developmental Biology 2007;23:613–643.
13. Yoon BJ, Smith GB, Heynen AJ, Neve RL, Bear MF. Essential role for a long-term depression mechanism in ocular dominance plasticity. Proc Natl Acad Sci U S A 2009;106:9860–9865. [PubMed: 19470483]
14. Goel A, Lee HK. Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. J Neurosci 2007;27:6692–6700. [PubMed: 17581956]
15. Desai NS, Cudmore RH, Nelson SB, Turrigiano GG. Critical periods for experience-dependent synaptic scaling in visual cortex. Nat Neurosci 2002;5:783–789. [PubMed: 12080341]
16. Frenkel MY, et al. Instructive effect of visual experience in mouse visual cortex. Neuron 2006;51:339–349. [PubMed: 16880128]
17. Plath N, et al. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron 2006;52:437–444. [PubMed: 17088210]
18. Messaoudi E, et al. Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus in vivo. J. Neurosci 2007;27:10445–10455. [PubMed: 17898216]
19. Guzowski JF, et al. Inhibition of activity-dependent Arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J. Neurosci 2000;20:3993–4001. [PubMed: 10818134]
20. Park S, et al. Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. Neuron 2008;59:70–83. [PubMed: 18614030]
21. Waung MW, Pfeiffer BE, Nosyreva ED, Ronesi JA, Huber KM. Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron 2008;59:84–97. [PubMed: 18614031]
22. Shepherd JD, et al. Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. Neuron 2006;52:475–484. [PubMed: 17088213]

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23. Rial Verde EM, Lee-Osbourne J, Worley PF, Malinow R, Cline HT. Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron 2006;52:461–474. [PubMed: 17088212]
24. Lyford GL, et al. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 1995;14:433–445. [PubMed: 7857651]
25. Steward O, Worley PF. Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation. Cell 2001;30:227–240.
26. Dolen G, et al. Correction of fragile X syndrome in mice. Neuron 2007;56:955–962. [PubMed: 18093519]
27. Sawtell NB, et al. NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron 2003;38:977–985. [PubMed: 12818182]
28. Daw NW, Reid SN, Beaver CJ. Development and function of metabotropic glutamate receptors in cat visual cortex. J Neurobiol 1999;41:102–107. [PubMed: 10504197]
29. Tagawa Y, Kanold PO, Majdan M, Shatz CJ. Multiple periods of functional ocular dominance plasticity in mouse visual cortex. Nat Neurosci 2005;8:380–388. [PubMed: 15723060]
30. Wang KH, et al. In vivo two-photon imaging reveals a role of arc in enhancing orientation specificity in visual cortex. Cell 2006;126:389–402. [PubMed: 16873068]
31. Vazdarjanova A, et al. Spatial exploration induces Arc, a plasticity-related immediate-early gene, only in calcium/calmodulin-dependent protein kinase II-positive principal excitatory and inhibitory neurons of the rat forebrain. The Journal of Comparative Neurology 2006;498:317–329. [PubMed: 16871537]
32. Kalatsky VA, Stryker MP. New paradigm for optical imaging: temporally encoded maps of intrinsic signal. Neuron 2003;38:529–545. [PubMed: 12765606]
33. Tropea D, et al. Gene expression changes and molecular pathways mediating activity-dependent plasticity in visual cortex. Nat Neurosci 2006;9:660–668. [PubMed: 16633343]
34. Porciatti V, Pizzorusso T, Maffei L. The visual physiology of the wild type mouse determined with pattern VEPs. Vision Res 1999;39:3071–3081. [PubMed: 10664805]
35. Cang J, Kalatsky VA, Ouml, Wel S, Stryker MP. Optical imaging of the intrinsic signal as a measure of cortical plasticity in the mouse. Visual Neuroscience 2005;22:685–691. [PubMed: 16332279]
36. Chowdhury S, et al. Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 2006;52:445–459. [PubMed: 17088211]
37. Kim TJ, Ye EA, Jeon CJ. Distribution of AMPA glutamate receptor GluR1 subunitimmunoreactive neurons and their co-localization with calcium-binding proteins and GABA in the mouse visual cortex. Mol Cells 2006;21:34–41. [PubMed: 16511345]
38. Lu W, Constantine-Paton M. Eye opening rapidly induces synaptic potentiation and refinement. Neuron 2004;43:237–249. [PubMed: 15260959]
39. Pfeiffenberger C, et al. Ephrin-As and neural activity are required for eye-specific patterning during retinogeniculate mapping. Nat Neurosci 2005;8:1022–1027. [PubMed: 16025107]
40. Smith SL, Trachtenberg JT. Experience-dependent binocular competition in the visual cortex begins at eye opening. Nat Neurosci 2007;10:370–375. [PubMed: 17293862]
41. Gianfranceschi L, et al. Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. Proc Natl Acad Sci U S A 2003;100:12486–12491. [PubMed: 14514885]
42. Rittenhouse CD, et al. Stimulus for rapid ocular dominance plasticity in visual cortex. J Neurophysiol 2006;95:2947–2950. [PubMed: 16481452]
43. Roberts EB, Meredith MA, Ramoa AS. Suppression of NMDA receptor function using antisense DNA blocks ocular dominance plasticity while preserving visual responses. J Neurophysiol 1998;80:1021–1032. [PubMed: 9744918]
44. Di Cristo G, et al. Requirement of ERK activation for visual cortical plasticity. Science 2001;292:2337–2340. [PubMed: 11423664]
45. Cho K, Khibnik L, Philpot BD. The ratio of NR2A/B NMDA receptor subunits determines the qualities of ocular dominance plasticity in visual cortex. Proc Natl Acad Sci USA 2009;106:5377–5382. [PubMed: 19276107]

46. Waltereit R, et al. Arg3.1/Arc mRNA induction by Ca2+ and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. J. Neurosci 2001;21:5484–5493. [PubMed: 11466419]

47. Sato M, Stryker MP. Distinctive features of adult ocular dominance plasticity. J. Neurosci 2008;28:10278–10286. [PubMed: 18842887]

48. Fagiolini M, Pizzorusso T, Berardi N, Domenici L, Maffei L. Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. Vision Res 1994;34:709–720. [PubMed: 8160387]

49. Coleman JE, Law K, Bear MF. Anatomical origins of ocular dominance in mouse primary visual cortex. Neuroscience 2009;161:561–571. [PubMed: 19327388]

50. Cynader M. Prolonged sensitivity to monocular deprivation in dark-reared cats. Journal of Neurophysiology 1980;43:1026–1040. [PubMed: 7359175]

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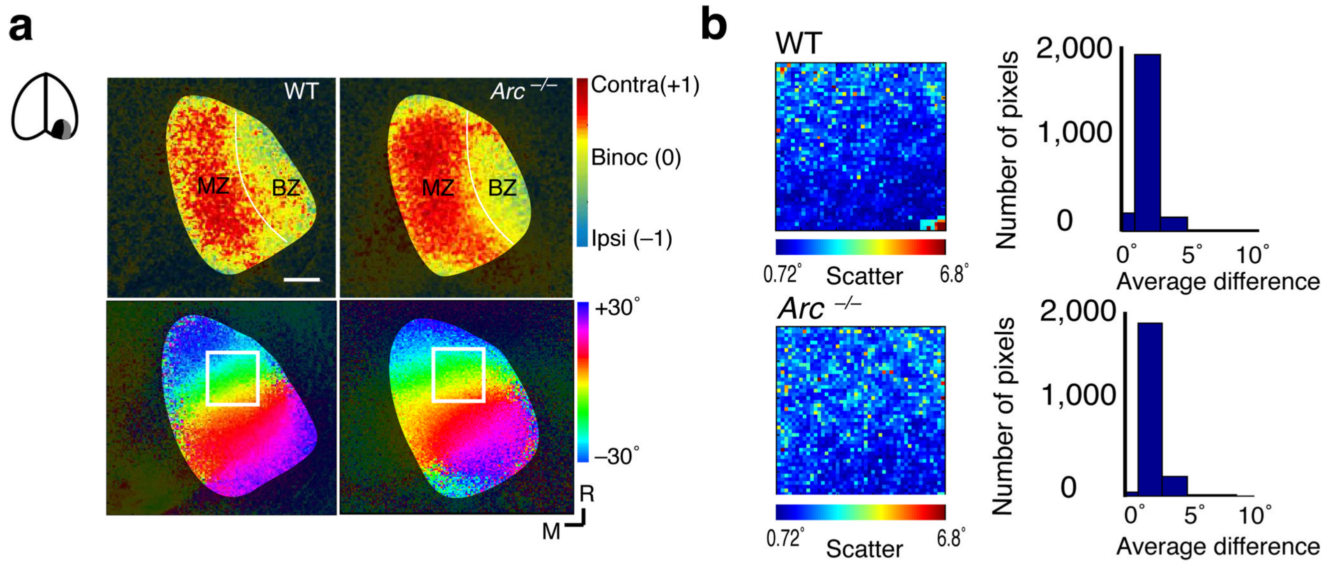

Figure 1.

Loss of Arc does not affect V1 responsiveness and organization. (**a**) Intrinsic signal imaging of V1 (left inset) in WT and *Arc*$^{-/-}$ mice. (Top) Ocular dominance map of V1, in a WT mouse (left) and an *Arc*$^{-/-}$ mouse (right); MZ=monocular zone, BZ=binocular zone. Scale at right illustrates binocularity index of pixels. Scale bar= 500 μm. V1 in *Arc*$^{-/-}$ mice is similar to that in WT mice in total area (WT n=6, area=1.401±0.07 mm^2; *Arc*$^{-/-}$ n=10, area=1.270±0.15 mm^2; p>0.5, t–test). (Bottom) Retinotopic organization of V1 in a WT mouse (left), and an *Arc*$^{-/-}$ mouse (right). Each image shows the mapping of elevation according to scale at top right. **(b)** Scatter analysis of 50×50 pixel area within white box in A, for WT and *Arc*$^{-/-}$ mice. The receptive field center (phase) difference between sets of 5 adjacent pixels is shown in histogram at right. The precision of local mapping is comparable between WT and *Arc*$^{-/-}$ mice.

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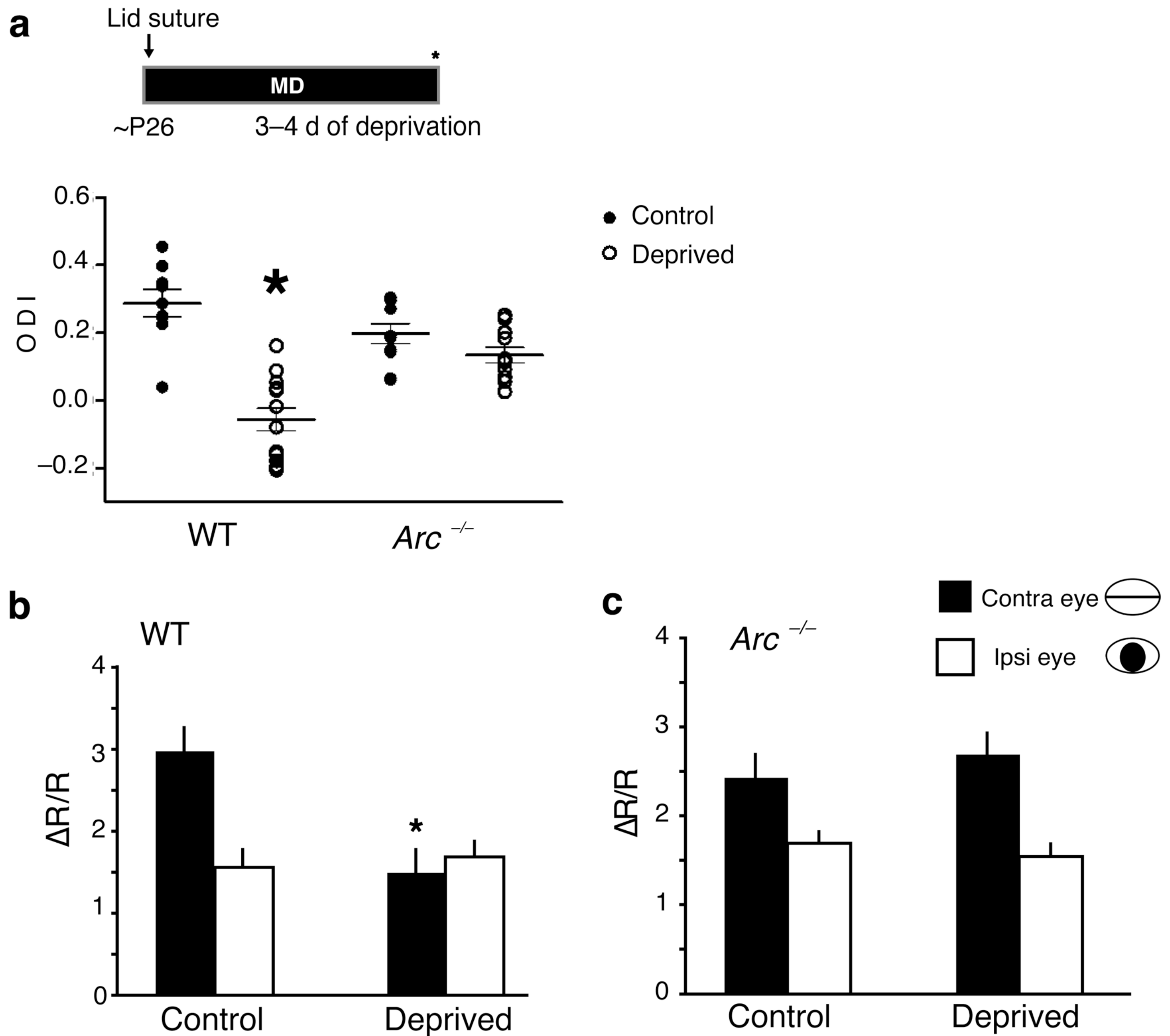

Figure 2.

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 days. Control mice were age–matched to deprived mice. (Bottom) ODIs for individual mice are shown as circles. Closed circles depict control mice, open circles deprived mice. Horizontal bars represent group averages. (WT: control, n=9, ODI=0.28±0.03; deprived, n=14, ODI=–0.05±0.03, p<0.0001, t–test *Arc*$^{-/-}$: control, n=10, ODI=0.19±0.02; deprived, n=11, ODI=0.13±0.02, p>0.1, t–test). **(b)** Response magnitude in WT mice driven by the contralateral eye (filled bars) and ipsilateral eye (open bars), plotted as average $\Delta R/R \times 10^{-3}$. A depression in the contralateral eye response amplitude can be seen (control=2.9±0.27, deprived=1.62±0.23, *p<0.001, t–test). No change in the ipsilateral eye response is detected (control=1.56±.21, deprived=1.68±.19, p>0.8, t–test). **(c)** No change in contralateral (filled bar) response occurs in *Arc*$^{-/-}$ mice after deprivation (control=2.25±0.28, deprived=2.5±0.26, p>0.2, t–test); similarly, no change in ipsilateral

(open bar) response is detected (control=1.35±0.23, deprived=1.64±0.19, $p>0.2$, t–test). ($\Delta R/R$ is the change in reflectance over baseline reflectance. Error bars represent SEM).

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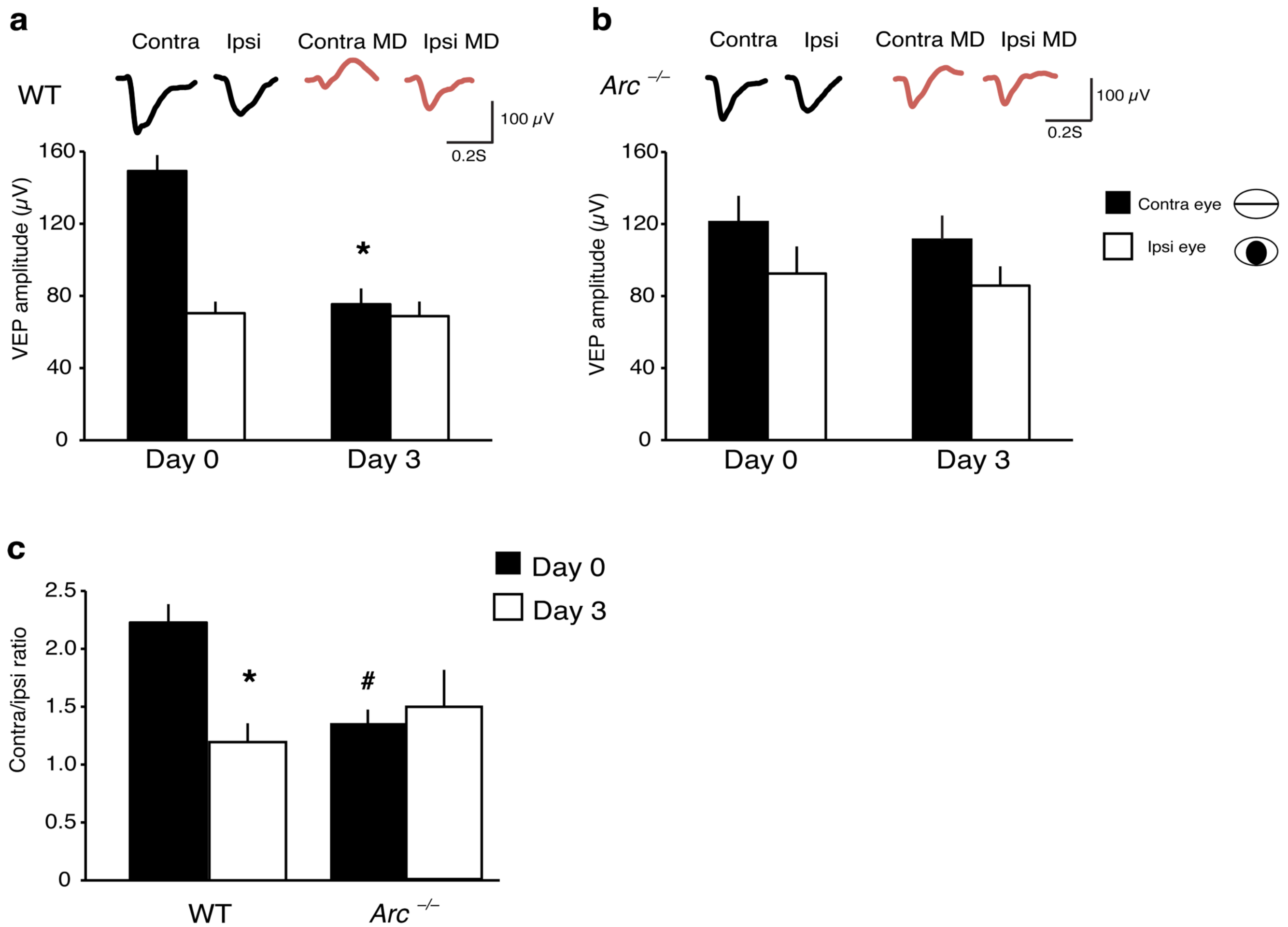

Figure 3.
Chronic VEP recordings show that *Arc*$^{-/-}$ mice do not exhibit ocular dominance plasticity after short–term monocular deprivation. **(a)** WT mice exhibit a significant depression in contralateral (deprived eye) responses (n=11; Day 0=149±8.8 μV, 3 Day monocular deprivation=75.4±8.8 μV,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

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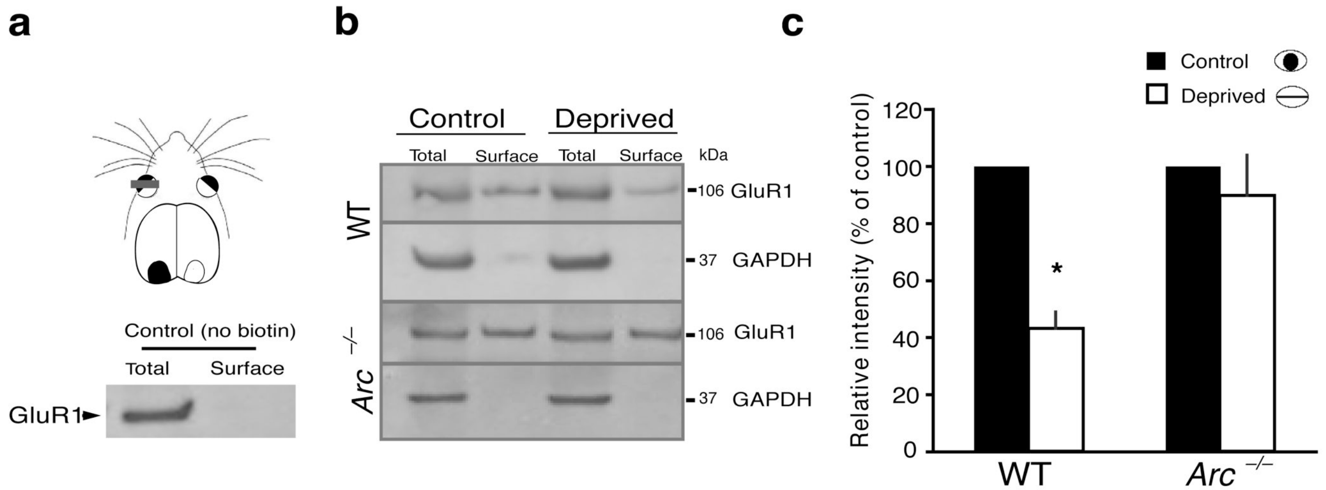

Figure 4.

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. Since V1 is dominated by contralateral eye responses, cortex contralateral to the deprived eye was termed "deprived" while cortex ipsilateral to the deprived eye was treated as "control". **(b)** Example immunoblots of total and biotinylated surface proteins in the visual cortex of $Arc^{-/-}$ and WT 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 sample not exposed to biotin. **(c)** Summary of changes in surface/total protein levels occurring after deprivation (WT, n=5; $Arc^{-/-}$, n=7). Surface levels of GluR1 were significantly lower in the deprived hemisphere of WT mice compared to control (*$p<.0001$, t–test), but not in $Arc^{-/-}$ animals ($p>0.2$, t–test). Error bars represent SEM.

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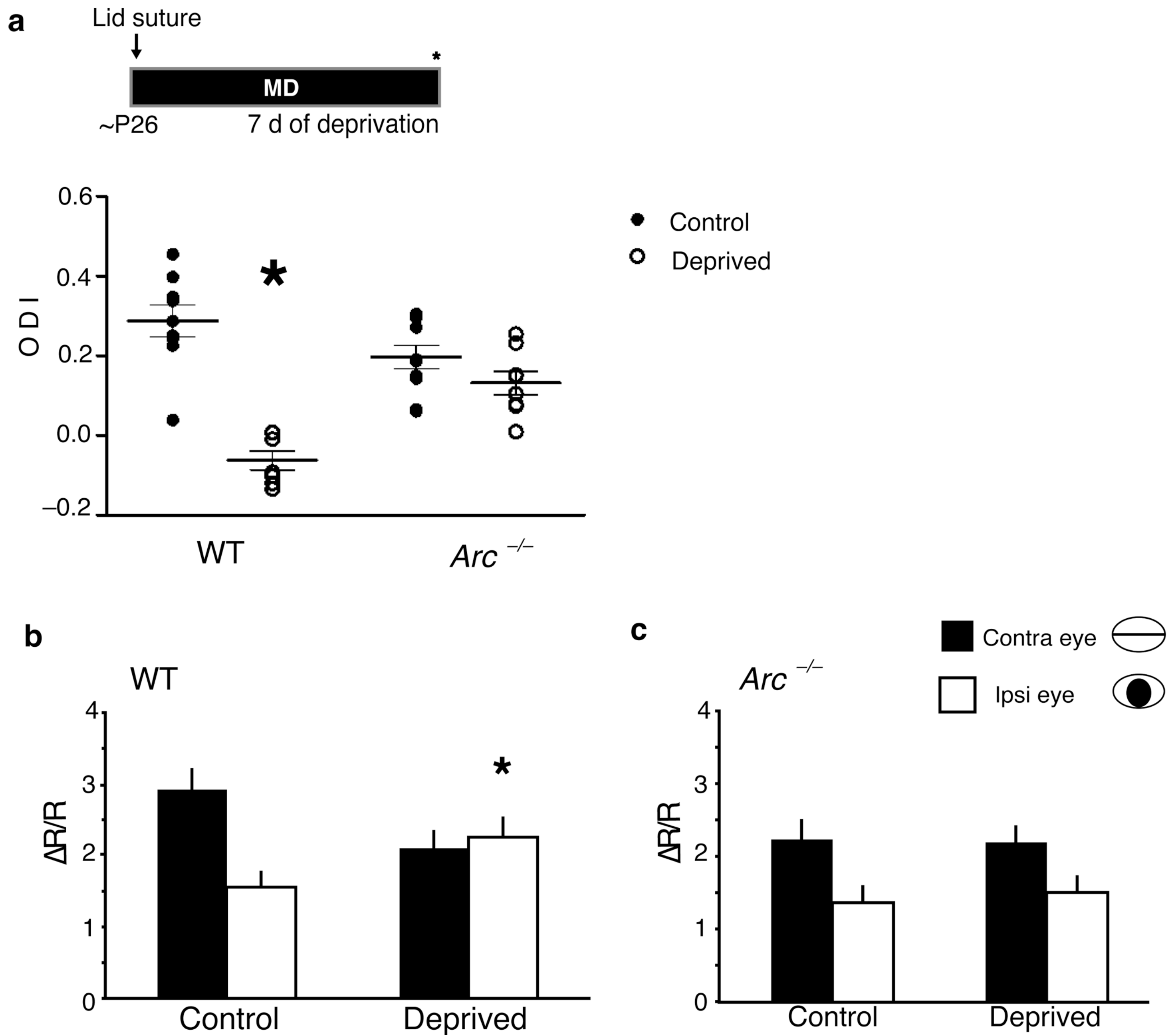

Figure 5.

Arc$^{-/-}$ 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 days. Control mice were age–matched to deprived mice. ODIs for individual mice are shown as circles. Closed circles depict control mice, open circles deprived mice. Horizontal bars represent group averages. (WT: control, n=9, ODI= 0.28±0.03; deprived, n=7, ODI=−0.063±0.02, p<0.0001; *Arc*$^{-/-}$: control, n=10, ODI=0.19±0.02; deprived, n=8, ODI=0.13±.02, p=0.17). **(b)** Response magnitude in WT mice driven by the contralateral eye (filled bars) and ipsilateral eye (open bars), plotted as average ΔR/R × 10−3. Some, albeit not significant, depression in the contralateral eye response amplitude can be seen (control=2.9 ±0.27, deprived= 2.1±0.23, p>0.05). Lid suture results in an increase in the ipsilateral eye response (control=1.56±0.21, deprived=2.49±0.17, *p<0.05). **(c)** No change in contralateral (filled bar) response occurs in *Arc*$^{-/-}$ animals after deprivation (control=2.25±0.28, deprived= 2.2±0.21, p>0.6); similarly, no change in ipsilateral (open bar) response is detected

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(control=1.35±0.23, deprived=1.5±0.21, $p>0.6$). ($\Delta R/R$ is the change in reflectance over baseline reflectance. Error bars represent SEM. Statistical analyses for a–c conducted using one–way ANOVA with Bonferroni correction).

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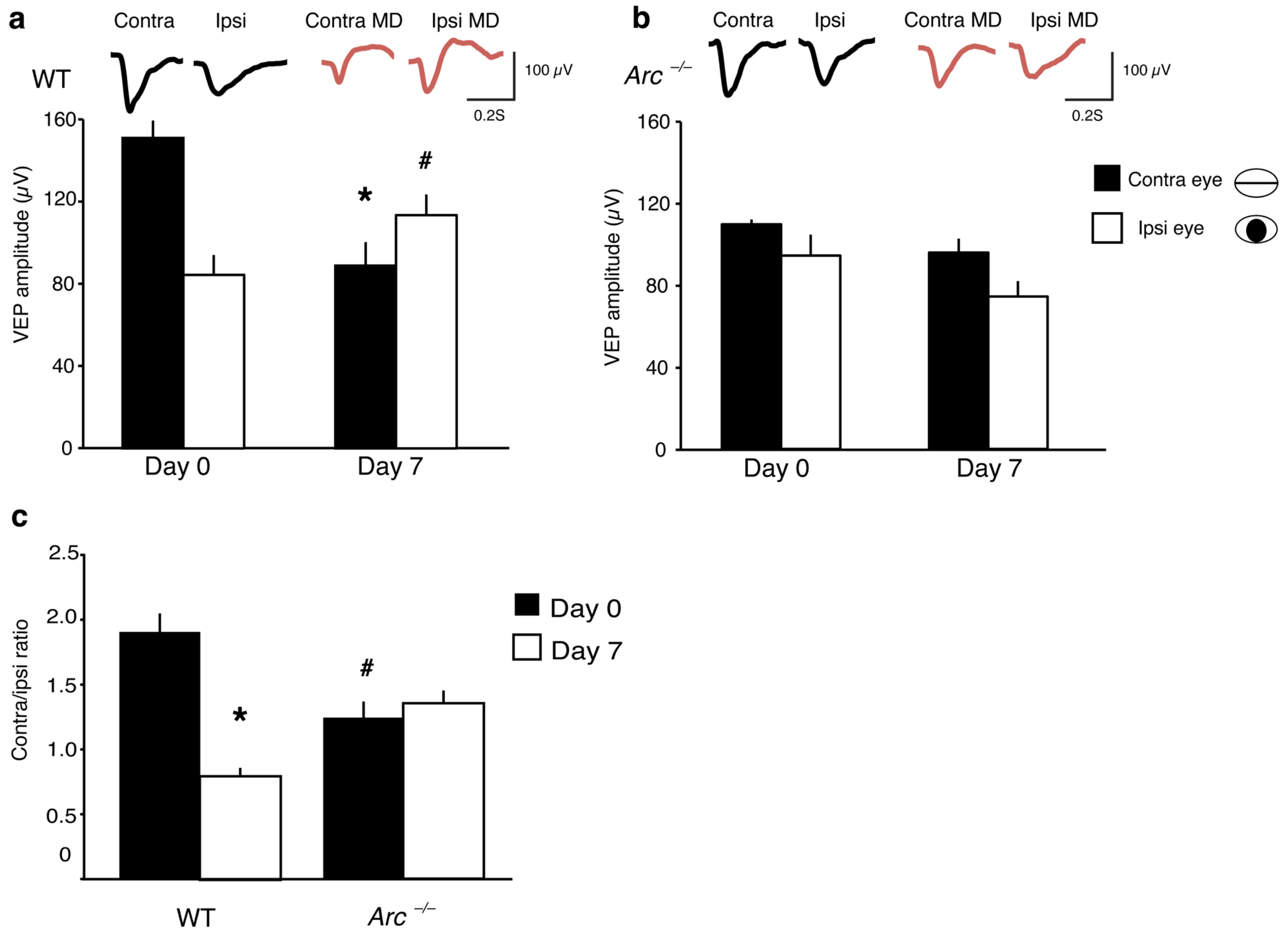

Figure 6.

$Arc^{-/-}$ mice exhibit no ocular dominance plasticity as assessed by chronic VEP recordings after long–term monocular deprivation. **(a)** WT mice exhibit a significant depression in contralateral (deprived eye) responses (n = 7; Day 0=152±9.2 μV, 7 Day monocular deprivation = 89.5±11.5 μV, *p<0.003, paired t–test) and a significant potentiation in ipsilateral responses (n = 7; Day 0=84.9±9.8 μV, 7 Day monocular deprivation=114.2±10.1 μV, #p<0.05, paired t–test). Averaged waveforms are shown at top. **(b)** $Arc^{-/-}$ mice exhibit no changes in contralateral (n=6; Day 0=112±2.2 μV, 7 Day monocular deprivation=100±6 μV, p>0.1, paired t–test) or in ipsilateral responses (n=8; Day 0=96±8.6 μV, 3 Day monocular deprivation=84 ±10 μV, p>0.4, paired t–test). Averaged waveforms are shown at top **(c)** WT mice exhibit a significant shift in the C/I ratio (n=7; Day 0=1.9±0.14, 7 Day monocular deprivation=0.8±0.06, *p < 0.0001, paired t–test), whereas $Arc^{-/-}$ mice exhibit no significant shift in the C/I ratio (n=6; Day 0=1.2±0.1, 7 Day monocular deprivation=1.25±0.11, p>0.7, paired t–test). $Arc^{-/-}$ mice exhibit a significantly smaller baseline C/I ratio than WT mice (WT n=7, C/I ratio 1.87 ±0.14; $Arc^{-/-}$ n=6, C/I ratio 1.2±0.1, #p<0.003) (Error bars represent SEM).

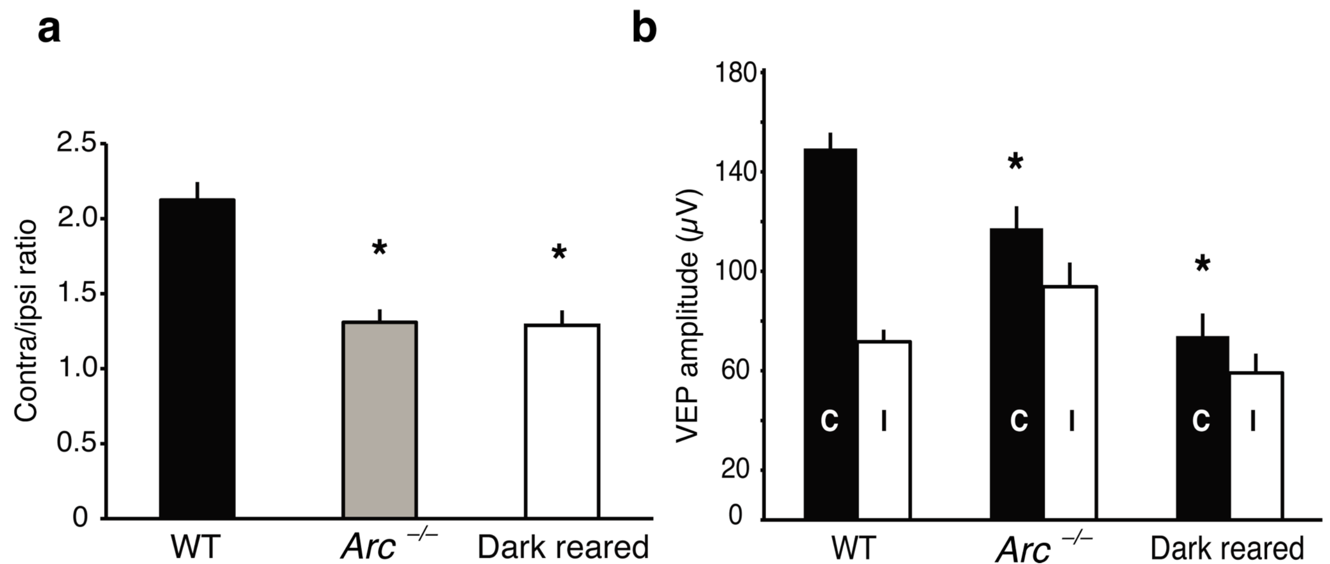

Figure 7.

Dark–rearing WT mice from birth mimics the contralateral to ipsilateral ratio observed *Arc*$^{-/-}$ mice. (**a**) *Arc*$^{-/-}$ and dark–reared (DR) mice exhibit a significant decrease in the C/I ratio in layer 4 VEPs as compared to WT mice (WT: n=16, 2.1 ±0.1; *Arc*$^{-/-}$: n=16, 1.35±0.08, *p<<0.0001, t–test; DR: n=11, 1.29±0.1, *p << 0.0001, t–test). (**b**) The change in ocular dominance ratio in *Arc*$^{-/-}$ and DR mice is mainly due to a significant depression in contralateral (C) responses (WT: 146±6 μV; *Arc*$^{-/-}$, 116±7 μV, *p<0.006, t–test; DR: 74 ±9 μV, *p<<0.0001, t–test) as ipsilateral responses (I) were not significantly different (WT: 72 ±5 μV; *Arc*$^{-/-}$, 90±8 μV, p>0.07, t–test; DR: 59±8 μV, p>0.2, t–test). (Error bars represent SEM).

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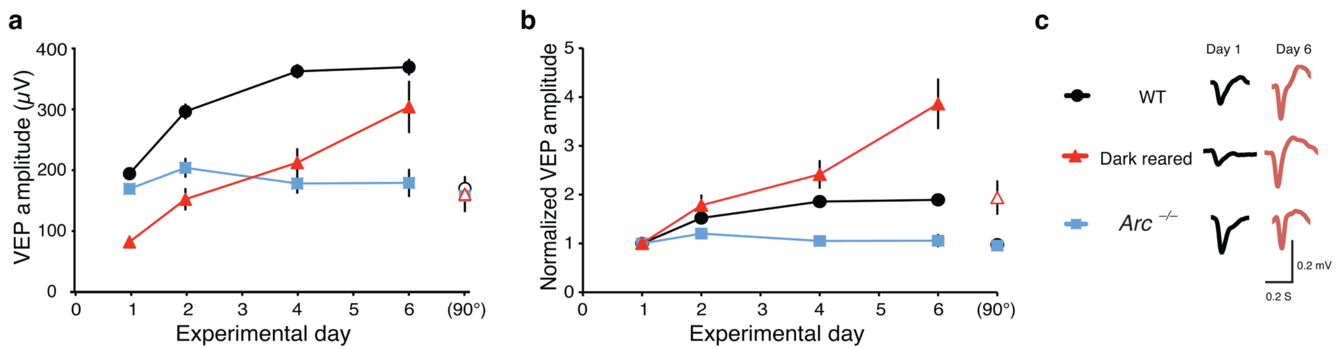

Figure 8.

Arc$^{-/-}$ mice lack stimulus–selective response potentiation (SRP) whereas dark–reared mice exhibit enhanced SRP in V1. **(a)** WT mice exhibit large and sustained potentiation of binocular VEPs over many days of exposure to the same stimulus orientation (n=11). Responses to a control orthogonal stimulus (90°, open black circle) shown at day 6 were not significantly potentiated. Dark–reared mice have small VEPs at baseline, which become dramatically potentiated after exposure to the same stimulus orientation (n=12). Responses to a control orthogonal stimulus (90°, open red triangle) are significantly increased compared with baseline VEPs but are also significantly smaller than the SRP orientation at day 6. In contrast, *Arc*$^{-/-}$ mice exhibit no significant potentiation of responses to the same stimulus (n=16). Responses to the control orthogonal stimulus (90°, blue square) were also not significantly different from baseline, suggesting no general decrease in responses over time. **(b)** VEPs normalized to baseline values show that dark–reared mice exhibit a relative enhancement of potentiation as compared to light–reared mice, while *Arc*$^{-/-}$ mice show no relative potentiation of VEPs. **(c)** Average VEP waveforms at baseline (day 1) and after 5 days of repeated exposure to the same orientation (day 6).

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