DEFICIENT EXPERIENCE-DEPENDENT PLASTICITY IN THE VISUAL CORTEX OF ARC NULL MICE

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Cortina McCurry

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Signature of Author .....

Cortina L. McCurry
Department of Brain and Cognitive Sciences
May 19th, 2009

Certified by.....

Mriganka Sur, Ph.D.
Newton Professor in Neuroscience
Thesis Supervisor

Accepted by.....

Earl Miller, Ph.D.
Picower Professor of Neuroscience
Chairman, Department Committee for Graduate Students

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ABSTRACT

Within the visual cortex a vast assortment of molecules work in concert to sharpen and refine neuronal circuits throughout development. With the advent of genetic mouse models it is now possible to probe the individual contributions of single molecules implicated in this process.

The Arc (activity-regulated cytoskeletal associated) gene is an effector immediate early gene that has been suggested to play a critical role in synaptic plasticity. The goal of this thesis is to understand the workings of Arc within the visual cortex. Specifically, we ask how genetic deletion of Arc influences plasticity, and how visual response properties differ between cells types containing, and not containing Arc. To elucidate a role for Arc in visual cortical plasticity we took advantage of knockin mice expressing GFP in place of Arc protein (referred to as KO mice for simplicity). We combined intrinsic signal imaging, visually evoked potentials, and two-photon *in vivo* calcium imaging to assess plasticity in juvenile and adult wild-type (WT), heterozygote, and KO mice.

We find that plasticity is disrupted in the visual cortex of Arc KO mice in the absence of obvious deficits at the level of basal response properties. In addition, this work has revealed that: 1) Arc is necessary for the establishment of normal ocular dominance during development and critical for deprived eye depression in the visual cortex of juvenile animals 2) Loss of Arc impairs AMPA receptor internalization in visual cortex- a necessary requirement for synaptic weakening after lid suture. 3) Open eye potentiation fails to occur after extended deprivation in the absence of Arc 4) Arc is required for stimulus response potentiation in juvenile animals. 5) Arc is not required for the synaptic scaling up of response suggesting a specific role in Hebbian plasticity. 6) Single cell analysis within the binocular zone of Arc-GFP homozygotes reveals that the distribution of Arc lacking GFP-positive cells does not display a contralateral-bias as compared to controls, and the majority of Arc-lacking GFP-positive cells receive equal input from each eye, suggesting that Arc is critical for synaptic weakening during development. Together, these experiments illustrate the essential role for Arc in experience-dependent plasticity within the visual system.

Thesis supervisor: Mriganka Sur, Ph.D., FRS
Title: Department Head and Newton Professor in Neuroscience

ORGANIZATION OF THESIS

For those eager readers venturing into the depths of this thesis, I include the following roadmap: This thesis is organized into five chapters. First, an introduction to experience-dependent plasticity within the visual system is provided to orient the reader to the history and current challenges within the field. Second, I provide two sections outlining experiments designed to assay plasticity in juvenile and adult Arc KO mice. In these experiments, intrinsic signal imaging and VEPs (in collaboration with Jason Shepherd in the Bear lab) are used to probe how functional loss of Arc affects visual cortex plasticity at different points in development. In the third section, I present experiments using in vivo two-photon calcium imaging to investigate visual response properties in identified cell types within Arc-GFP heterozygous and homozygous mice. To our knowledge, we provide the first in vivo two-photon functional imaging experiments assaying visual response properties in genetically identified knockout cells. Each of the three experimental sections contained within this thesis is organized into a brief introduction, followed by a section outlining methods and results, and concluding with a detailed discussion. The final chapter of my thesis provides a careful discussion of the overarching implications of this work and how it contributes to our current understanding of visual cortex plasticity.

TABLE OF CONTENTS

CHAPTER 1

CURRENT MECHANISMS AND MOLECULES PROPOSED TO UNDERLIE EXPERIENCE-DEPENDENT PLASTICITY

INTRODUCTION

Neurons are exquisitely sensitive to changes in sensory input. Even subtle alterations in activity can result in profound changes in receptive field properties within neural circuits. Monocular deprivation is the predominant model system for probing how sensory experience alters visual circuitry. In this chapter I will review literature illustrating the fundamental role of activity in normal visual cortex development, provide an overview of the current mechanisms and molecules thought to mediate plasticity, and delineate how Arc can be situated within the current framework of experience-dependent plasticity.

Hubel and Wiesel: Plasticity in higher mammals

"Our very first recording from a kitten began badly, with nothing but electrical artifacts during the first penetration....Even with the patience of a couple of oysters we could end up yearning for the day to end, regardless of whether or not it was successful."

David Hubel 2005

Throughout development, changes in experience shape and mold neural networks via a process referred to as experience-dependent plasticity. Even prior to eye-opening spontaneous activity arising from the retina guides and refines cortical connectivity (Meister et al., 1991). Modifications of neural circuitry within the brain are dependent upon both the form of activity being relayed and the target brain region. While these changes are most pronounced early in development, various forms of synaptic plasticity operate throughout life to refine and optimize neural circuitry in the face of changing external demands. Much of our current understanding of the visual system rests upon experiments conducted by Hubel and Wiesel in the 1960's (Hubel and

Wiesel, 1962, 1963). As the quote above indicates, these experiments were not easy. In their memoir, Hubel and Wiesel describe how trying many of the experiments could be, and how often long hours resulted in failure (Hubel and Wiesel, 2005). However, their curiosity and collaboration provided them with enough energy to persevere and the discoveries from their early experiments transformed the state of visual neuroscience.

While numerous researchers since this time have contributed substantially to the canon of knowledge regarding visual system physiology, it was in these seminal experiments that we first learned that visual inputs from thalamus into V1 of cats were segregated into eye-specific ocular dominance columns. These early experiments were exciting specifically because they revealed that normal experience is critical for refinement of the visual pathway and that sensory deprivation in early development can significantly alter the structure and function of these columns within the visual cortex. This phenomenon was termed ocular dominance plasticity.

Using the cat and monkey visual cortex as an experimental system, they discovered that visual deprivation resulted in a surprising degeneration of connections. In the case of monocular deprivation, the percentage of cells being driven by the deprived eye was significantly reduced and the size of ocular dominance columns being served by the deprived eye decreased. The decrease in size of deprived ocular dominance columns indicated that reduction of activity through the deprived eye pathway led to a loss of afferent projections to the visual cortex. Interestingly, they found a parallel expansion of ocular dominance columns receiving input from the nondeprived open eye and a concomitant strengthening of inputs being driven by this eye (Figure 1A-C). The competitive nature of this shift was revealed with experiments where animals were binocularly deprived. While most cortical cells remained responsive to the nondeprived eye following monocular deprivation, in the case of binocular deprivation many cells remained responsive to both eyes. This result provided evidence that it is the relative balance in drive between inputs that determines the plastic changes

that take place. Most strikingly, they discovered that this weakening and strengthening could only occur at a very specific time early in development and often led to an irreversible reduction of visually driven activity through the deprived eye within the visual cortex.

Beginning at three weeks of age, monocular lid suture causes a shift in ocular dominance, with maximal sensitivity to this procedure being reached at four to six weeks. After this point, there is a decrease in degree to which the shift can be achieved. Attempting the same manipulation in adult cats approximately 1 year old is unsuccessful in causing any effect, regardless of the length of deprivation, suggesting that outside of the sensitive period the visual cortex is insensitive to changes in visual experience. Along the same vein, an additional discovery revealed that visual experience is critical for normal closure of the critical period. Cats raised in complete darkness have an extended critical period (Cynader, 1980; Mower et al., 1981). In these animals, monocular deprivation by lid suture results in an increase in the representation of the nondeprived eye well past the end of the critical period in normally reared cats. Explanations for the developmental decline in plasticity will be discussed in detail in subsequent sections.

While knowledge of physiological and anatomical effects of reduced afferent activity has facilitated insights into visual cortex function, elucidating the molecular underpinnings of activity driven changes has become the Holy Grail of many of today's studies of experience-dependent plasticity. The advent of the mouse as a model system has greatly expedited progress towards this goal.

The mouse as a model system for cortical plasticity

The mouse is extremely amenable to genetic manipulation, and with the increase in knockin, knockout, and transgenic mouse lines it is possible to investigate the

contribution of both single molecules (Sawtell et al., 2003; Wang et al., 2006) and cell types (Gandhi et al., 2008; Sohya et al., 2007) to changes occurring in response to manipulations of experience. Many of the current theories for ocular dominance plasticity such as Hebbian plasticity (Cruikshank and Weinberger, 1996), Bienenstock-Cooper-Munro (BCM) (Bienenstock et al., 1982) and homeostatic plasticity (Turrigiano et al., 1998), have been tested both in vivo and in vitro by taking advantage of genetic mouse models where molecules that may be involved in these mechanisms are genetically manipulated. Below, I present a number of experiments that rely on genetic techniques to test the function of specific molecules or pathways implicated in experience-dependent plasticity.

The mouse visual cortex provides a simple model system for studying the mechanisms underlying experience-dependent plasticity. While the visual cortex of the mouse does not contain ocular dominance columns, there exists a monocular region receiving input from the contralateral eye and a smaller binocular segment receiving input from both the contralateral and ipsilateral eyes (Figure 2). Within this binocular region there exists a contralateral bias similar to that found in other species. Monocular closure of the dominant eye results in a shift in ocular dominance towards the nondeprived eye which mimics the affect found at the level of ocular dominance columns in higher mammals such as monkeys, cats, and ferrets. The majority of work described within this thesis regarding experience-dependent plasticity will be based upon studies conducted within the mouse visual cortex.

The visual cortex of the mouse is a highly laminar structure, and this organization can be used to inform our understanding of the sites of plasticity. Visual input from the thalamus projects to layer 4 of the visual cortex. From here, the supragranular layers 1, 2, and 3, serve as an integrative center for gathering data and send it out to deeper layers in addition to other cortical areas. Layers 5 and 6 form the infragranular layers, and are the primary output layers of visual cortex, projecting to

both cortical and subcortical areas. In the mouse visual cortex, layer 2/3 and 4 serve as the primary sites of ocular dominance plasticity. Interestingly, they are also the layers expressing the highest levels of Arc protein (Figure 3), the primary plasticity molecule being investigated in this thesis. As mentioned above, there exists a sensitive period for sensory manipulations within the mouse. This occurs between postnatal days 24-32 (P24-P32). The developmental expression of Arc correlates well with maturation of visual cortex plasticity (Figure 4). The mRNA of Arc can first be detected in rats at postnatal day 8 (P8) (Lyford et al., 1995). Arc expression is critically dependent upon activity (Figure 5). After eye opening (~P13), Arc expression increases drastically and reaches a peak at P21. Arc protein follows a similar pattern of expression, though more gradual, showing an initial increase after eye opening and slowly reaching a peak between P28-P30. This developmental progression suggests that Arc may be important during the increased period of synaptogenesis and pruning that occurs after eye opening in visual cortex.

Hebbian plasticity, the BCM theory (metaplasticity), and homeostasis have all been proposed to explain the activity-dependent mechanisms underlying how experience so drastically impacts cortical circuits. Of these the most familiar may be that of Donald Hebb. Hebb proposed that a basic mechanism for synaptic learning would be such that repeated firing by a presynaptic neuron of its postsynaptic target would result in robust potentiation (Hebb, 1949). Hebb's postulate states that, "When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased". Often this quote is simply referenced by the mnemonic "neurons that fire together wire together". This principle states that temporal correlation of electrical activity between afferent fibers and that of the postsynaptic neuron receiving these fibers, results in a strengthening or maintenance of the connection. In the case where temporal correlation does not exist, afferent

projections will be weakened and lose their hold on the cell. Activity-dependent potentiation (LTP) and depression (LTD) are thought to underlie this process (Malenka and Bear, 2004). Studies conducted in vivo suggest that LTP and LTD may explain the experience-dependent plasticity that occurs within the visual cortex after monocular deprivation (Crozier et al., 2007; Heynen et al., 2003; Kirkwood et al., 1996). Ocular dominance shifts are commonly explained by an initial reduction of deprived-eye responses followed by a delayed strengthening of nondeprived eye responses. LTD-like mechanisms are proposed to mediate the initial depression of the deprived eye response. Indeed experiments conducted in both mice and rats show that lid suture mimics the changes found with LTD and that similar molecules are recruited (Heynen et al., 2003). While LTP has been hypothesized to mediate open eye potentiation, a specific role in ocular dominance plasticity has not been discovered. However, stimulus response potentiation (SRP), a persistent form of response enhancement detected with visually evoked potentials, is a mechanistic correlate of LTP known to operate in mice (Frenkel et al., 2006). In this form of learning, repeated presentation of gratings of a single orientation over a period of days results in a significant potentiation of response to that orientation. The amplitude of response to an orthogonal orientation is comparable to the initial response. However, repeated exposure to a novel orientation can also result in a potentiation of response amplitude. While this potentiation can take place in both juveniles and adults the absolute magnitude of SRP is reduced in adult mice. Modifications in response to the initial repeated orientation are extremely persistent and last over several days. Interestingly, SRP is NMDAR-dependent and requires AMPAR trafficking, as does LTP, and open eye potentiation (Frenkel et al., 2006). In order to probe the interaction between SRP and ocular dominance plasticity Frenkel et. al induced SRP in two groups of WT animals for a period of 6 days. After this point, the first group of mice was returned to their home cages, and the second group was deprived of one eye for an additional 7 days to investigate open eye potentiation.

The response magnitude remained elevated in the first group of mice, revealing the persistent nature of SRP, however in the second group an additional potentiation of the open eye response was seen suggesting that activity through the intact open eye is enabled by monocular deprivation.

Homeostatic mechanisms may also contribute to ocular dominance plasticity. In response to silencing activity with TTX, a compensatory increase in firing referred to as synaptic scaling occurs within visual cortex (Turrigiano and Nelson, 2004). This form of scaling is multiplicative, meaning that it acts globally at all synapses, and it is thought to work in tangent with Hebbian mechanisms of NMDA receptor-dependent LTP and LTD to stabilize neural networks and prevent runaway excitation or quiescence. Recent evidence suggests that homeostatic mechanisms may modify the threshold for synaptic plasticity, and play a role in ocular dominance plasticity.

Mrisic-Flogel et al used two-photon functional calcium imaging to examine the ocular dominance distribution of individual cells before and after a period of monocular deprivation (Mrsic-Flogel et al., 2007) (Figure 6). They found that the relative change in ocular dominance that occurred in a given cell depended on the amount of visual drive received from each eye. As such, a weakening of deprived eye response only took place in those cells receiving input from the open eye. This weakening occurred rapidly within a period of only 2-3 days of monocular deprivation followed by a slower potentiation of the open eye response after 3 days of deprivation. These findings align with a Hebbian framework of synaptic competition, and suggest that the mismatch between activity through the open eye and that of the sutured eye may lead to decorrelated activity and thus synaptic weakening. However, in the case of those cells receiving input predominantly from the deprived eye there was an increase in responsiveness that the authors attributed to a homeostatic mechanism of synaptic scaling. In support of this conclusion, they also found that neuronal responsiveness increased in monocular visual cortex after deprivation of the contralateral eye. In addition, binocular deprivation

resulted in an increase in response to both eyes within the binocular zone of visual cortex, suggesting that indeed reduced drive through the closed eyelids is sufficient to trigger homeostatic mechanisms for maintaining firing rates. One major aspect of their experimental design that makes interpreting their findings difficult is that they were unable to perform chronic imaging in mice. Because they were imaging across animals it is not possible to specifically assess the contribution from each eye and the subsequent changes that occur after monocular deprivation. However, further evidence that homeostatic response compensation is likely to be an important component of OD plasticity comes from experiments using mice lacking tumor necrosis factor alpha (TNF-alpha), a cytokine derived from glia and implicated in homeostatic synaptic scaling. While depression of the deprived eye response occurs similarly to that found in WT animals after lid suture, TNF-alpha mutant mice have reduced ocular dominance plasticity due to a deficit in open-eye response potentiation(Kaneko et al., 2006).

An additional theory frequently drawn upon to explain ocular dominance shifts is the BCM theory. The BCM theory postulates a flexible modification threshold that regulates the strength and direction of plasticity based upon the history of post-synaptic activity (metaplasticity) (Bienenstock et al., 1982). For example, decreasing overall cortical activity by dark rearing shifts the modification threshold such that LTD is no longer preferred and LTP is easily elicited (Czepita et al., 1994). As a case in point, mice that have been injected with tetrodotoxin (TTX) do not display deprived eye depression, however open eye potentiation is facilitated (Frenkel and Bear, 2004).

A number of studies have implicated the NMDA pathway in ocular dominance plasticity (Fagiolini et al., 2003; Kleinschmidt et al., 1987; Roberts et al., 1998). NMDA receptors are heteromeric ion channels, composed of NR1 and NR2 subunit proteins. Blocking NMDA receptors reduces the effects of monocular deprivation. This has been shown by pharmacological blockade (Kleinschmidt et al., 1987), reduction of the NR1 subunit using antisense oligonucleotides (Roberts et al., 1998), and most recently genetic

knockout of the NR2A subunit (Cho et al., 2009). NMDA receptors that are composed of the NR1 and NR2B subunits tend to have slower kinetics with currents on the order of ~250ms. By contrast, incorporation of the NR2A subunits speeds up the kinetics five-fold. As such the specific subunit configuration confers distinct properties on the NMDA receptor and due to subtle differences in calcium flow (Vicini et al., 1998). Interestingly, NMDA receptors are developmentally regulated and after birth there is a gradual decline in the presence of the NR2B subunit, and an increase in NR2A that correlates with the peak and decline of the critical period for plasticity (Chen et al., 2000). Genetic removal of the NR2A subunit leads to deficient synaptic weakening after monocular lid suture, and a precocious increase in open eye potentiation proposed to be due to a decrease in the NR2A / NR2B ratio (Cho et al.,2009). Conversely, increases in the NR2A / NR2B ratio are thought to raise the threshold for LTP and the gradual developmental switch in NR2B and NR2A receptor subunits may be related to closure of the critical period.

The start and closure of the critical period have also been suggested to be dependent upon levels of inhibition (Hensch and Fagiolini, 2005). Mice lacking GAD65, one of two isoforms critical for the synthesis of gamma amino-butyric acid (GABA), show no shift in ocular dominance following brief monocular deprivation (Hensch et al., 1998a) possibly due to a disruption in LTD (Choi et al., 2002). Amazingly, infusion of the GABA-agonist diazepam can restore ocular dominance plasticity in both juveniles and adults, suggesting that an appropriate level of inhibition is critical for normal plasticity to occur. In addition, by modulating inhibition earlier in development a precocious critical period can be elicited. Normal WT mice injected with diazepam just after eye opening show a shift in ocular dominance after lid suture (Fagiolini and Hensch, 2000). Going along with this, over expression of brain-derived neurotrophic factor (BDNF), a molecule known to facilitate the maturation of inhibition, also speeds up the time course of ocular dominance plasticity. In addition, visual acuity in BDNF-over expressing mice

matures more rapidly than in normal WT animals (Hanover et al., 1999; Huang et al., 1999). Clearly, these studies suggest that the overall balance of inhibition within the cortex is critical for plasticity.

Another study showed that subtle alterations in the amount of inhibition had a significant influence on whether plasticity could occur at all. Too much and too little inhibition could equally delay initiation of the critical period. These subtle changes in inhibition may be mediated by fast-spiking basket cells synapsing on pyramidal cells containing the GABA A receptor (Fagiolini et al., 2004). Infusing the GABA synthesis inhibitor (MPA) or the GABA A receptor antagonist (picrotoxin) reduced inhibition, and restored ocular dominance plasticity in adult rats (Berardi et al., 2004). What might be the role of inhibition during the sensitive period? One suggestion is that a specific level of inhibition may be necessary to facilitate the subtle changes in activity between the two eyes that underlie the competitive affects of monocular deprivation. In an in vitro preparation the precise timing of activity was shown to be able to determine the precise direction of plasticity (Bi and Poo, 1998; Markram et al., 1997). While most studies probing ocular dominance plasticity have focused on changes occurring at the level of excitatory cells, the data mentioned above suggest that plasticity in inhibitory cells may be of equal importance. In experiments discussed in Chapter 4, we use fluorescently labeled mice combined with two-photon functional imaging to dissect out how alterations in activity may differentially influence excitatory and inhibitory cells.

In adult mice, the affects of deprivation are qualitatively different from critical period mice. In the case of ocular dominance plasticity, while the adult visual cortex is susceptible to alterations in activity (Tagawa et al., 2005) the time course of the effect is much slower and the extent of plasticity reduced. For example, brief deprivation no longer elicits an affect on the deprived eye response (Sawtell et al., 2003). By contrast, extended deprivation results in a minor decrease in deprived eye response and a larger potentiation of the open eye response that leads to a saturating shift in ocular dominance

(Sato and Stryker, 2008). While a slight depression of the deprived eye response has been shown with both VEPs (Frenkel et al., 2006) and intrinsic signal optical imaging (Sato and Stryker, 2008) in adult mice, most studies agree that this depression is transient and that open eye response potentiation dominates the ocular dominance shift at this point in development. Similar to young animals, there is a dependence upon NMDAR however it is possible that synaptic scaling may also play a greater role at this time point than in younger animals.

Interestingly, many of the molecules shown to be important for ocular dominance plasticity lie upstream of Arc and signal through NMDA receptors. Of particular interest is the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK). The MAPK pathway plays a pivotal role in a number of forms of synaptic plasticity such as hippocampal learning (Atkins et al., 1998), and the promotion of genes required for consolidation of memory. Inhibition of this pathway suppresses the induction of LTP and prevents the shift in ocular dominance towards the nondeprived eye (Di Cristo et al., 2001) In addition, the cAMP/protein kinase A (PKA) signaling cascade is crucial for experience-dependent plasticity in a wide variety of species and appears to be required for normal ocular dominance plasticity. In the visual cortex, inhibiting the PKA subunits RIBeta, RIalpha, RIIbeta, and RIIalpha results in disrupted LTP, LTD, and ocular dominance plasticity (Beaver et al., 2001; Liu et al., 2003). Interestingly, in visual cortex, specific deletion of the RIBeta subunit of PKA has no significant effect on ocular dominance plasticity, although LTP and LTD are severely disrupted (Hensch et al., 1998b).

RIIBeta is the predominant PKA subunit within the neocortex. Mice lacking the RIIbeta regulatory subunit of PKA show deficits in LTD (Fischer et al., 2004). In the hippocampus of RIIbeta -/- mice (P10-P14), NMDAR-dependent LTP is disrupted while LTD remains intact at the Schafer collateral-CA1 synapse. Mechanisms taking place at this synapse have been suggested to be similar to those within layers 2/3 and 4 of visual

cortex. Interestingly, just a few days later at a time point that coincides with the critical period for visual cortex plasticity, there is a developmental switch and LTP remains intact whereas LTD is completely abolished (Yang et al., 2009). Targeted deletion of the RIIbeta subunit of PKA blocks the shift in ocular dominance that occurs after monocular deprivation during the critical period (Fischer et al., 2004). While LTP is normal, LTD occurring within the visual cortex is completely disrupted. These results suggest that ODP plasticity may be primarily mediated by the RIIBeta isoform of PKA. Interestingly, at the synapse, RIIBeta is localized near and interacts with AMPA receptors. In hippocampus, RIIBeta has been shown to promote phosphorylation of the Ser845 regulatory site of GluR1 (Colledge et al., 2000). Changes in phosphorylation of this site have been suggested to mediate LTD via changes in AMPA receptor trafficking (Ehlers, 2000). Internalization of AMPA receptors may mediate the depression of deprived eye response occurring after lid suture (reviewed in detail below). Because RIIBeta -/- mice lack a mechanism for dephosphorylation it has been suggested that these mice exist in a chronic LTD like state, which occludes further LTD, and is manifested as a disruption in ocular dominance plasticity.

A role for Arc in experience-dependent plasticity

Interestingly, the induction of Arc is dependent on both PKA and MAPK/ERK activity (Waltereit et al., 2001) suggesting that this downstream molecule may also play a key role in ocular dominance plasticity. Arc is an ideal molecule for mediating the changes that occur in response to differential activity, specifically ocular dominance plasticity. One, its developmental expression follows that of the sensitive period for changes, two, its synaptic expression is regulated by and sensitive to changes in activity (Figure 5 and 7), and three, as our experiments show loss of Arc reduces visual cortex plasticity. Arc is a single copy gene that encodes a single protein. One of the most

fascinating aspects of the Arc gene is its ability to translocate to sites of recent plasticity. Arc mRNA accumulates in dendrites and at synaptic sites (Figure 7). Furthermore, Arc protein also accumulates and becomes enriched at sites of local synaptic activity suggesting that it is synthesized locally (Figure 8). Thus, one function for Arc may be to couple synaptic activity to protein synthesis-dependent synaptic plasticity. The involvement of Arc in plasticity was also suggested by two studies that found that patterns of activity that resulted in LTP also caused an increase in Arc at synaptic sites (Steward 1998 , Moga 2004). In the hippocampus, Arc expression was also found to be dependent on NMDA receptor activation (Steward and Worley 2001). Similarly, injecting WT mice with the NMDA receptor CPP prevents Arc induction in the visual cortex (Figure 9). As mentioned above, the NMDA receptor pathway is crucial for normal ocular dominance plasticity.

A recent study using GAD 65 KO mice provides further evidence for the importance of signaling through NMDA receptors (Kanold et al., 2009). These mice have a reduction in both GABA A and NR2A subunit levels. In addition, ocular dominance plasticity and LTD are completely disrupted (Choi 2007). Interestingly, application of diazepam rescues the deficit in NR2A subunit levels, leaving GABA A subunit levels unchanged, and restores both LTD and ocular dominance plasticity. Whether restoration of NMDA receptor function might enable normal ocular dominance plasticity due to Arc-mediated LTD-like mechanisms remains to be tested.

Arc is activated by both metabotropic glutamate (mGluR) and NMDA receptor activation. However, while mGluR activation triggers Arc translation within 5 mins in an elongation factor 2 -dependent manner (Waung et al., 2008), NMDAR activation triggers Arc on a longer time scale. This activation is under tight temporal and spatial control. Arc mRNA is a natural target for nonsense-mediated mRNA decay (NMD) by virtue of its two conserved 3'-UTR introns. NMD and other related translation-dependent mRNA decay mechanisms are thought to serve as critical brakes on protein

expression that contribute to the fine spatial-temporal control of Arc synthesis (Bramham et al., 2008). Interestingly, NMDA receptor activation enhances AMPA receptor endocytosis via a signaling mechanism required for the induction of LTD (Beattie et al., 2000) (Figure 10).

Two recent reports point to a role for Arc in regulating AMPA receptor trafficking. These studies reveal that the Arc protein interacts directly with endophilin and dynamin, two molecules involved in clathrin-mediated endocytosis to modulate the rate of AMPA receptor endocytosis at the synapse (Chowdhury et al., 2006) (Figure 11). The regulated trafficking of AMPA receptors is a key aspect of plasticity, specifically at the level of AMPA receptor endocytosis. In the visual cortex, changes in AMPA receptor subunit dephosphorylation and internalization are thought to underlie synaptic weakening. The depression in deprived eye response occurring after lid suture may be mediated by an LTD-like mechanism, as changes occurring after monocular deprivation in rat visual cortex mimic those found in response to LTD induction (Heynen et al., 2003). In addition, monocular deprivation for as little as 24 hours occludes further LTD. This result has also been replicated in the mouse (Crozier et al.,2007).

Regulation of AMPA receptor trafficking at the synapse places Arc in an ideal position to exert an influence on synaptic weight. Indeed, it has been suggested that Arc may serve as a homeostatic scaling molecule to "scale down" synapses in response to increased activity (Shepherd et al., 2006). Homeostatic mechanisms are rapidly emerging as a critical component of synaptic plasticity and possibly ocular dominance plasticity (see above). For example, in response to deprivation, neurons in slice culture display an increase in neuronal activity as measured by mEPSCs. The increase in mEPSC amplitude is not accompanied by a change in mEPSC frequency, suggesting a postsynaptic locus for the change. Accordingly, this same chronic inactivity has been shown to result in an increase in AMPA receptors at the membrane. Overexpression of Arc in cortical and hippocampal slice culture prevents this upregulation and leads to a

decrease in AMPA receptor mediated currents and a global loss in surface expression of AMPA receptors (Rial Verde et al., 2006). By contrast, Arc null cultures display an increase in surface AMPA receptors and AMPA receptor-mediated EPSCs. In hippocampal culture, TTX and bicuculline resulted in an increase and a decrease respectively in surface GluR1 in WT mice, however these same manipulations had no effect in Arc KO mice. Similarly, in cortical forebrain neurons, TTX treatment of WT neurons led to a significant increase in mEPSC amplitudes and a multiplicative shift in the cumulative distribution function (CDF) not seen with Arc KO neurons treated in the same manner. However, in response to bicuculline both WT and Arc KO showed a modest downregulation of AMPARs suggesting that mechanisms in addition to Arc may contribute to downregulation in cortical neurons (Shepherd et al.,2006).

One possible candidate is the protein kinase Polo-like kinase 2 (Plk2). Plk2 is induced by activity and contributes to synaptic weakening by degrading SPAR, a postsynaptic Rap GAP (GTPase-activating protein) and scaffolding protein found at synapses (Seeburg et al., 2008). After chronic elevation of activity, Plk2 is activated and facilitates the downward scaling of synaptic strength. Organotypic hippocampal slice cultures treated with RNAi targeted at Plk2 fail to downregulate membrane excitability in response to epileptiform activity elicited by GABA A receptor blockade (Seeburg and Sheng, 2008). However, the synaptic weakening mediated by Plk2 differs from that of Arc in that it does not appear to be multiplicative in nature. Thus, it may not contribute to global synaptic scaling in the same manner as Arc.

Surprisingly, in addition to a deficit in synaptic scaling down in response to high activity, the increase in surface expression found in Arc KO mice occludes further scaling up of synapses in response to activity blockade by TTX (Shepherd et al., 2006). This result suggests that synaptic scaling is completely disrupted in these mice.

Studies in rats which have been dark reared from eye opening provide evidence that this same homeostatic mechanism operates *in vivo* and take place throughout

development (Desai et al., 2002). This homeostatic plasticity operates such that decreases in activity result in a compensatory increase in mEPSC amplitude and AMPA receptor number. Conversely, increases in activity cause a concomitant decrease in quantal amplitude. Synaptic weakening is particularly relevant in the visual cortex after eye opening. A reduction in AMPA receptor mEPSC amplitude occurs between P14 and P18 in development (Figure 12) suggesting that the large increase in sensory and synaptic occurring after eye opening may trigger specific molecules that lead to a scaling down of synapses. Interestingly, the decline in mEPSC that occurs after eye opening parallels the increase in the expression of Arc protein. Because Arc has been shown to regulate AMPA receptor internalization it is possible that Arc may act during this time period to globally scale down all synapses in response to increased activity. Thus, the strongest inputs to a cell can activate Arc expression and reduce overall synaptic strength at the expense of weaker inputs. We hypothesize that this form of synaptic weakening may facilitate the sharpening of both orientation preference and ocular dominance. In support of this role for Arc, dark reared animals (in which Arc expression is low) do not show this same reduction in mini amplitude. In addition, binocularity and orientation selectivity remain in a deprived state in adult animals dark reared from birth (Fagiolini et al., 1994). Indeed, previous studies have shown that deprivation decreases basal levels of IEGs, while activity leads to a rapid increase in IEG expression. This supports the view that the decrease in AMPA receptor number and amplitude, in response to increased activity, may be directly due to higher levels of Arc protein expression after eye opening. Dark adapting mice for two days during the critical period causes an increase, or scaling up, of layer 2/3 AMPA receptor mEPSCs which mimics results found in culture using TTX (Goel and Lee, 2007). This increase may be mediated by TNF-alpha, a cytokine implicated in regulating AMPA receptor insertion, as studies in our lab have shown that TNF-alpha KOs do not scale up after deprivation. Interestingly,

as little as one day of exposure to light is sufficient to return AMPA mEPSCs to normal levels possibly mediated by Arc.

That Arc lies downstream of a number of plasticity molecules shown to play a role in ocular dominance plasticity, combined with its role in regulating AMPA receptor trafficking and the "scaling down" of synapses suggests that this molecule may be mechanistically situated at the intersection of a number of plasticity processes. In addition to playing a role in homeostatic plasticity, the Arc molecule is important for classical Hebbian forms of plasticity. Studies in the hippocampus have shown that after LTP inducing high frequency stimulation, continued Arc expression, during a critical window lasting between 2 and 4 hours, is necessary for local actin polymerization and consolidation of LTP (Soule et al., 2006). Injection of antisense oligodeoxynucleotides, which block synthesis of the Arc protein, into the hippocampus of adult mice revealed that inhibition of Arc expression impaired the maintenance, but not the induction of LTP. In a spatial water maze task, mice treated with the Arc antisense oligodeoxynucleotides had no deficits in short term memory or task acquisition, but consolidation was impaired (Guzowski et al., 2000). Similarly, experiments done in Arc KO mice show that loss of Arc results in a surprising 50% enhancement of early LTP, while consolidation is completely abolished. In addition, these mice exhibit deficits in LTD (Plath et al., 2006)(Figure 13). Tied together, these studies suggest that Arc may be intricately involved in the functional stabilization of synapses by interacting with the endocytic machinery to regulate AMPA receptor surface expression. The absence of Arc may result in a disruption in plasticity due to loss of a mechanism that limits total synaptic strength and ensures that neuronal transmission remains within an optimal operating range. Indeed, a recent study in hippocampal slice showed that saturation of synaptic strength leads to a shutdown of LTP (Roth-Alpermann et al., 2006). Whether disruptions in ocular dominance plasticity are due primarily to Hebbian or homeostatic deficits in Arc KOs remain to be seen, and may be difficult to untangle.

One caveat to many of the studies mentioned above is that the functions of Arc relevant to experience-dependent plasticity have been tested in either in vitro culture systems or acute slice. In addition, the majority of studies probe Arc function in the hippocampus and dentate gyrus. Aside from a 2006 study using Arc-GFP mice to examine orientation tuning in vivo (Wang et al., 2006), the function of Arc in visual cortical plasticity has been essentially neglected. The activity-dependence of Arc, its localization at the synapse, involvement in both Hebbian and homeostatic plasticity, and interactions with plasticity-related molecules, all suggest that Arc is intimately involved in key aspects of experience-dependent plasticity. To further explore Arc's role in the visual cortex in vivo, we took advantage of Arc null mice where the open reading frame of Arc has been replaced with GFP. In Chapters 2 and 3, I present experiments that examine how loss of Arc is disruptive to experience-dependent plasticity in both juveniles and adults. In Chapter 4, I combine visual identification of Arc containing neurons with functional two-photon imaging to probe how loss of Arc impacts both orientation tuning and ocular dominance in identified cell types. We find that Arc is critical for the sharpening of response properties within the visual cortex.

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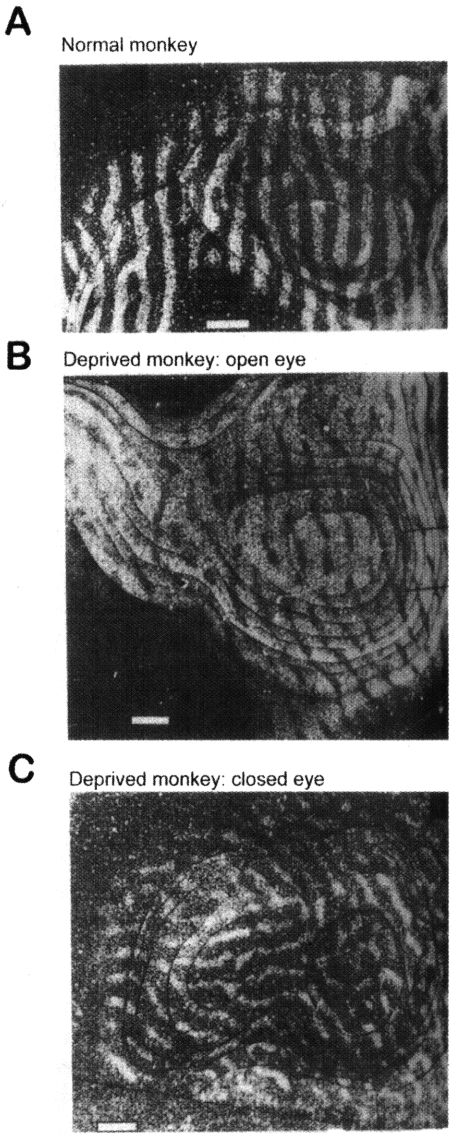


Figure 1. A. Ocular dominance columns in a normal adult monkey injected with a radio labeled tracer into one eye. The width of each column is the same for each eye. B. Image from a section of visual cortex similar to that in A from a monkey deprived of one eye from two weeks of age. Tracer was injected into the open eye. Columns serving the injected eye are shown in white. Notice the expansion of the open eye columns at the expense of the deprived eye columns (shown in black). C. Image from a section of visual cortex similar to that in A and B from a monkey deprived of one eye from two weeks of age. Tracer was injected into the deprived eye. Columns serving the deprived eye (white) are significantly reduced in size compared to those of the nondeprived eye columns. Adapted from Fundamentals of Neuroscience.

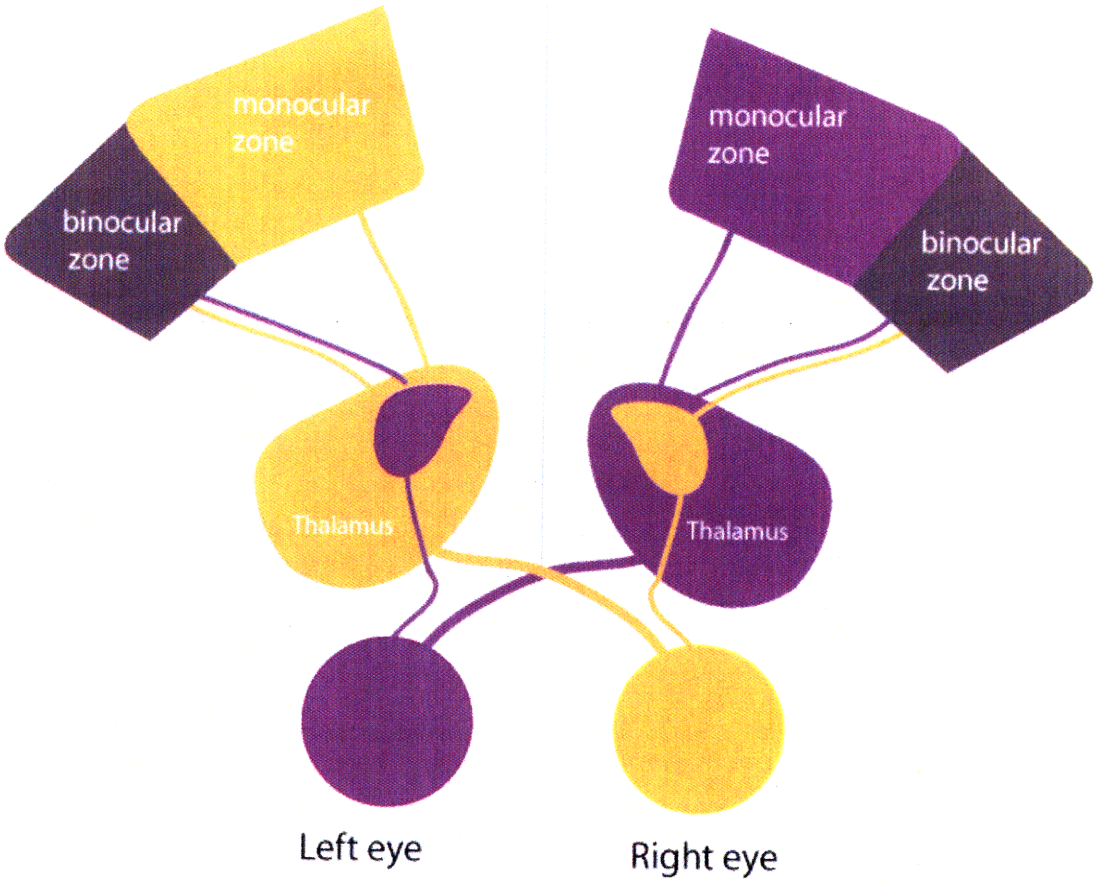


Figure 2. Schematic of the visual pathway in the mouse.

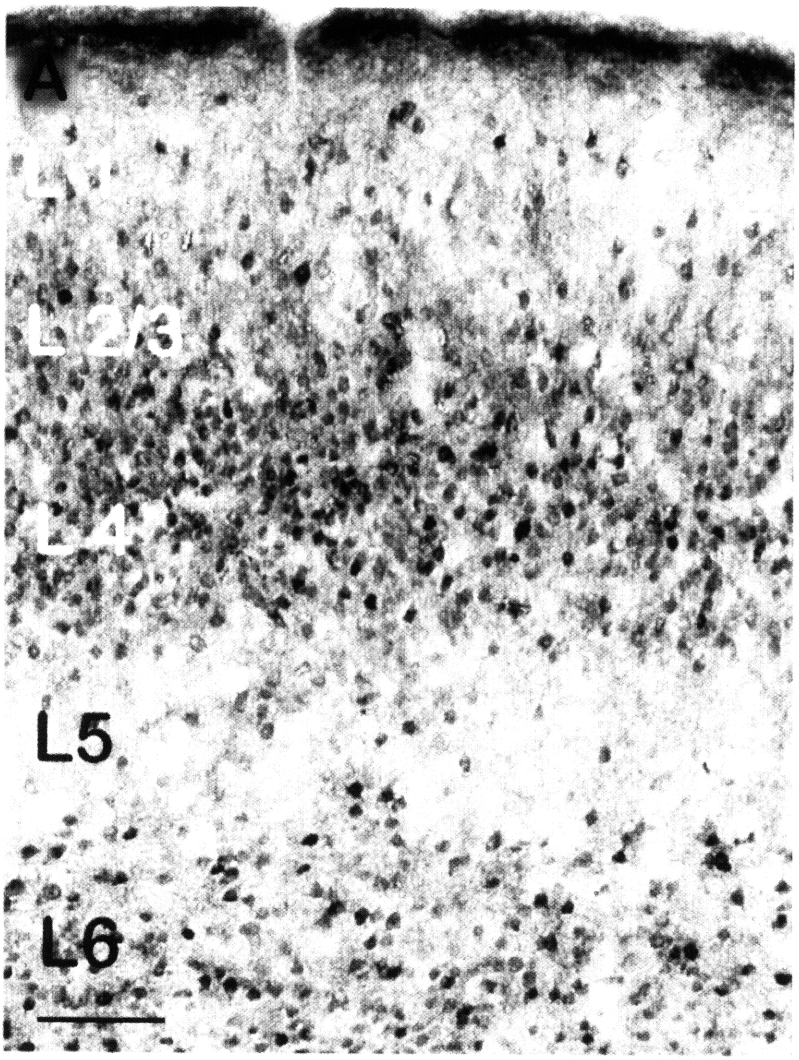


Figure 3. Laminar Arc protein expression in the visual cortex of a WT CP mouse dark adapted overnight and exposed to light. Arc expression can be seen in layers II/III, IV, and VI. Layers II/III and IV correspond to primary sites of ocular dominance plasticity in the mouse. *Scale bar= 50 μm*

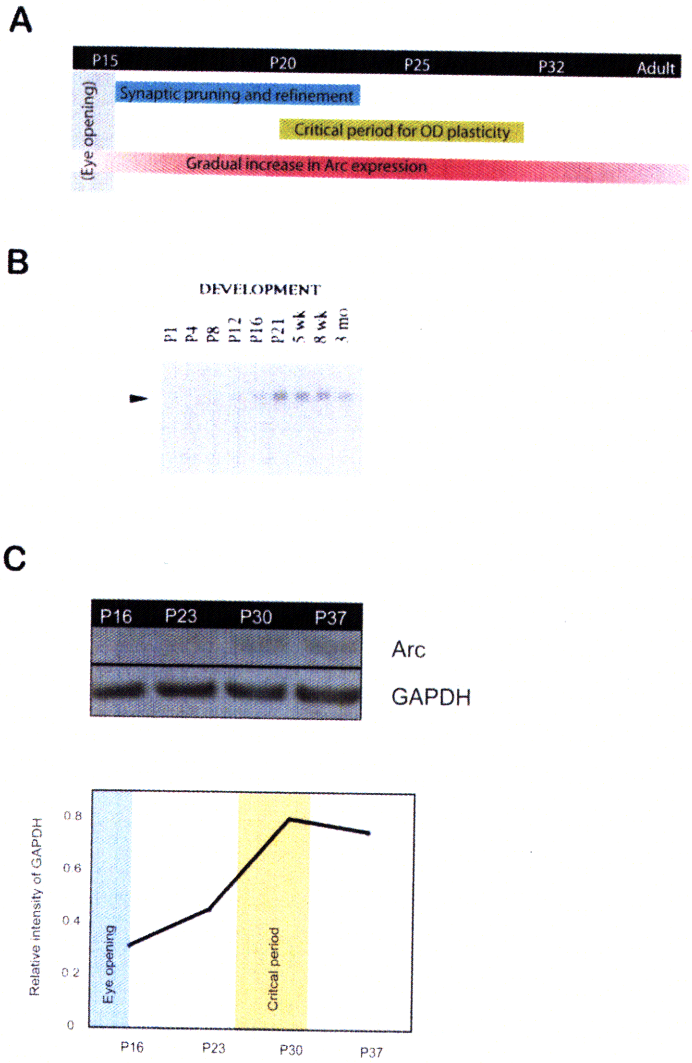
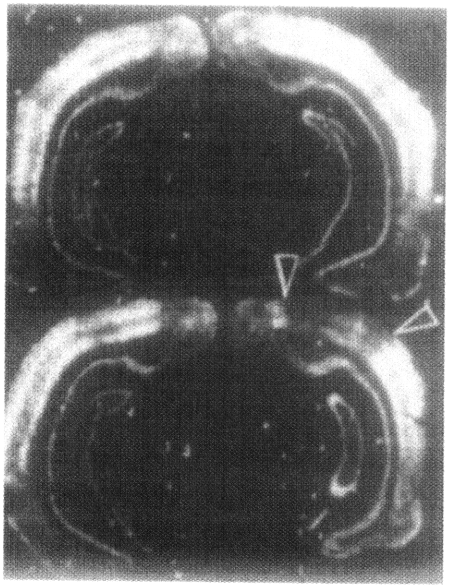


Figure 4. A. Developmental increase in Arc expression overlaps with the period of synaptic pruning/refinement and critical period plasticity. B. Peak of Arc mRNA expression falls within the peak of OD plasticity. Adapted from Lyford 1995. C. Arc protein expression shows a similar increase to that of Arc mRNA reaching a peak around P28.

A



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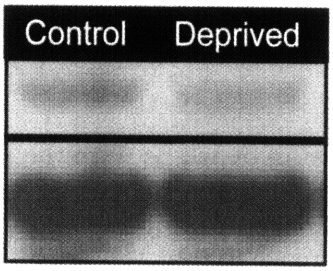


Figure 5. A. In situ hybridization illustrating Arc expression in visual cortex. Lower image is of a mouse monocularly injected with TTX. Arrows indicate the area of reduced Arc expression in the hemisphere contralateral to the injected eye. Adapted from Lyford 1995. B. Western blot of Arc expression in tissue from the nondeprived (control) and the deprived hemisphere of the visual cortex after lid suture.

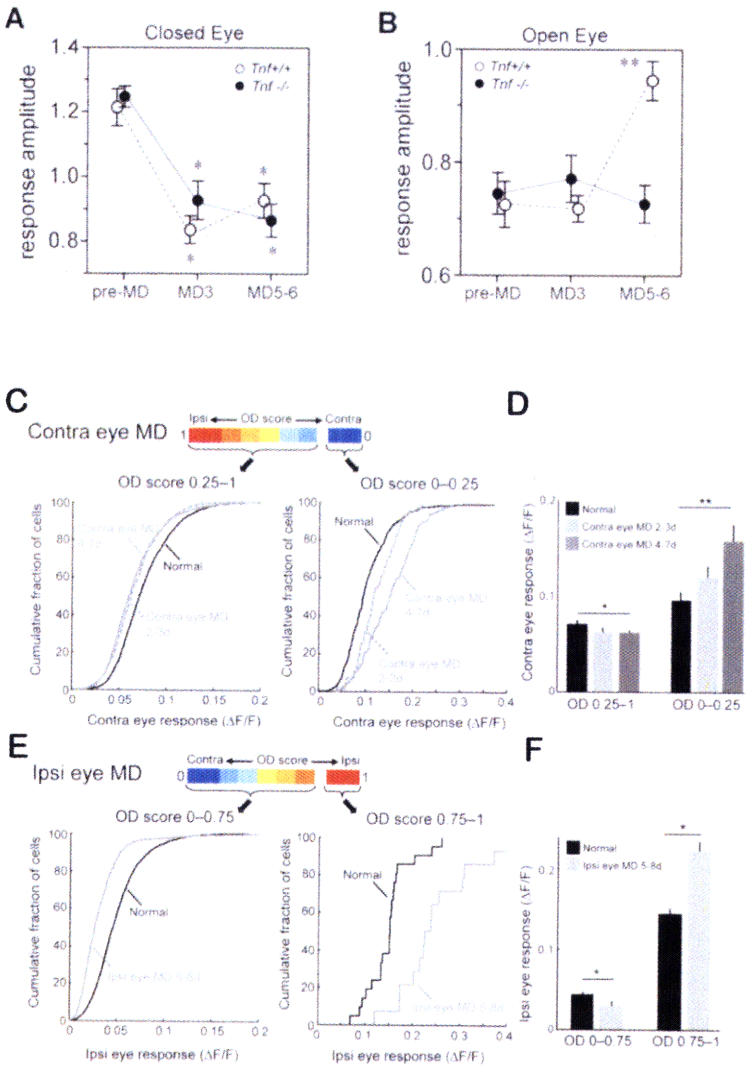
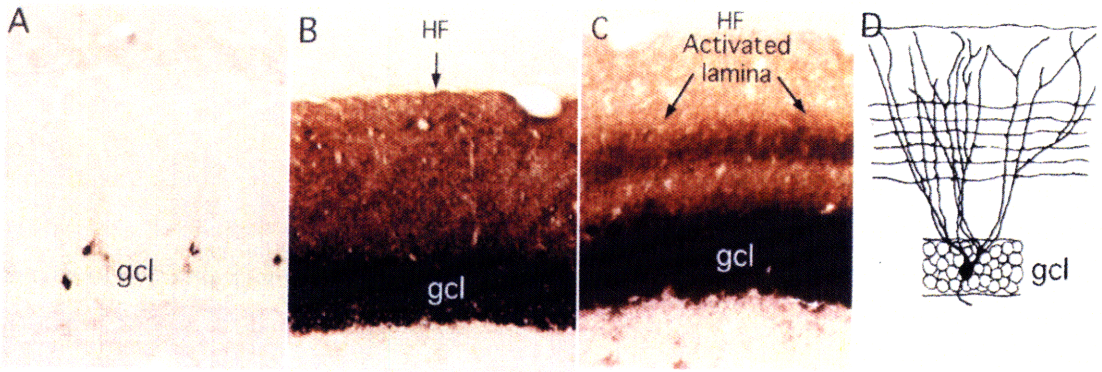


Figure 6. A. The initial response to brief deprivation is a depression of the deprived eye response. Arc is required for this initial depression. B. After a period of extended deprivation (5-7 days) there is a gradual potentiation of the open eye response strength. This has been attributed to scaling molecules such as TNF-alpha. TNF -/- mice do not show the same potentiation. Adapted from Kaneko 2008. C. Function calcium imaging experiments suggest that homeostatic mechanisms may operate in conjunction with Hebbian plasticity mechanisms. The response to deprivation depends upon the amount of input initially received from each eye prior to deprivation. There is a leftward shift in the distribution of those cells that are less contra (OD score of .25-1) prior to deprivation. Cells predominantly driven by the contralateral eye (0-.25) show a rightward shift. D. Response strength before and after deprivation for the range of OD scores. E. The initial eye-specific inputs also determine the direction of shift for ipsi eye MD. F.

Response strength before and after deprivation for the range of OD scores. Adapted from Mrsic-Flogel 2007.



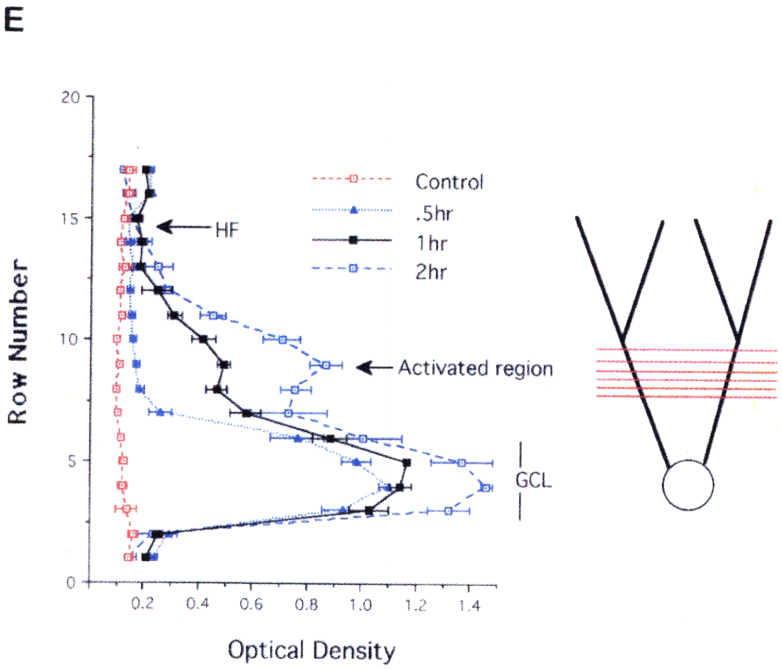


Figure 7. Arc mRNA is dynamically regulated by activity. A. Example of Arc labeling in the non-activated dentate gyrus. B. Global seizure induction results in Arc labeling throughout dentate gyrus. C. Targeted high-frequency stimulation of specific lamina results in a prominent band of labeling within the medial perforant path. D. Schematic illustration of the dendrites extending out from a dentate granule cell and the pattern of termination of medial perforant path projections. HF, hippocampal fissure; GCL, granule cell layer. E. Arc mRNA translocates from the nucleus to the activated region over a period of two hours. The increase in Arc labeling is specific to activated areas. There is a minimal increase in non-activated regions suggesting that Arc mRNA is captured by activated synapses. Adapted from Steward 2001.

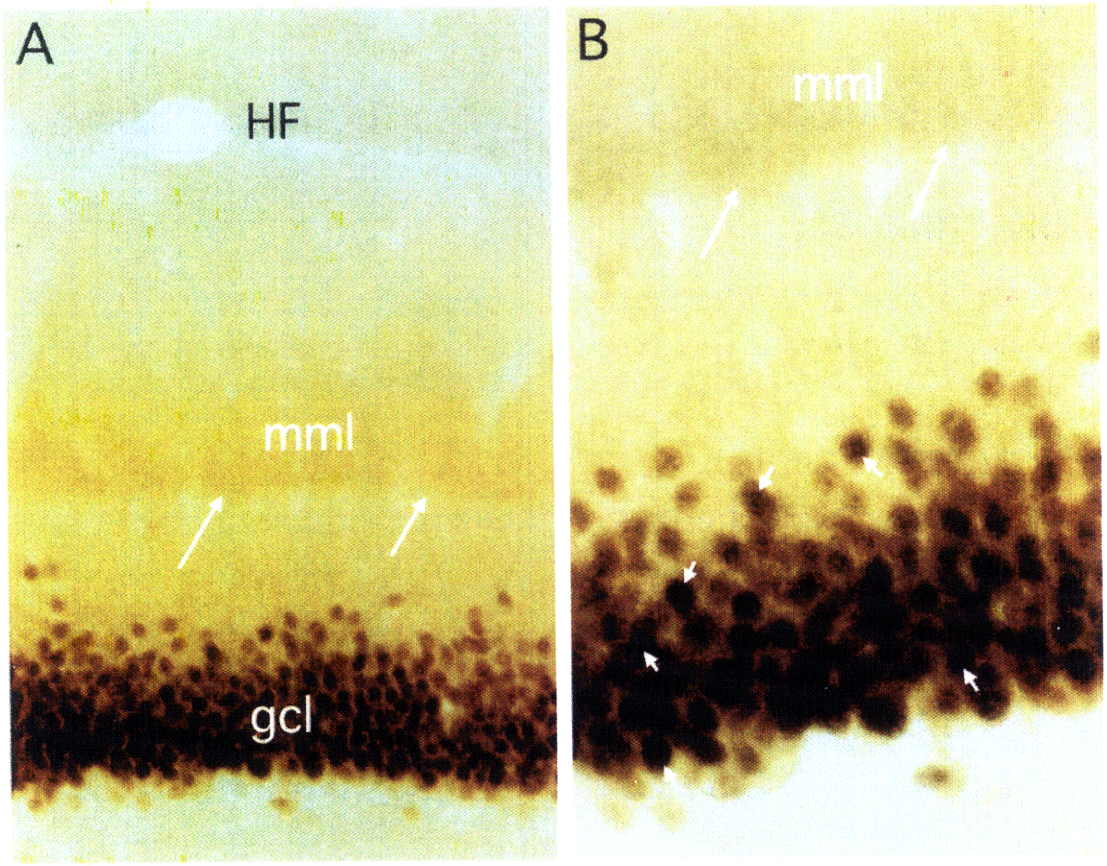


Figure 8. A. Arc protein expression follows a similar pattern of expression as that shown for Arc mRNA. B. Zoomed in image of A. Adapted from Steward 2001.

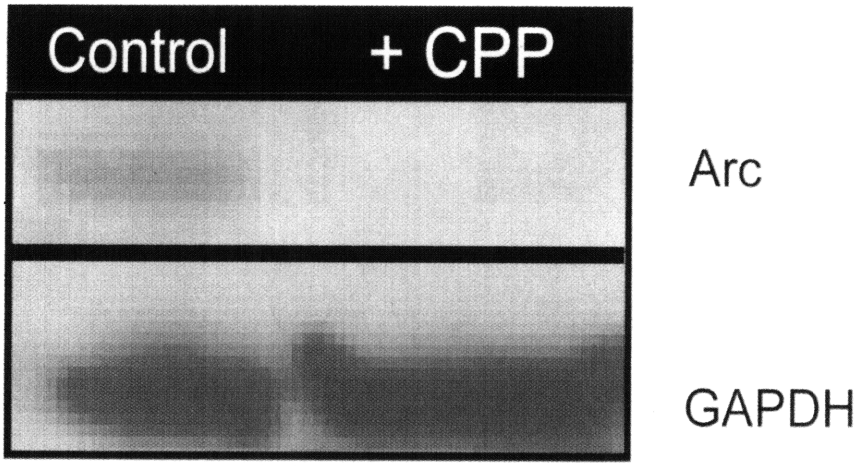


Figure 9. Arc expression is dependent upon NMDA receptor activation. Figure shows a Western blot of Arc expression in a WT critical period mouse deprived of light overnight and exposed to light for two hours (Control). A second mouse was deprived in a similar manner, but injected with the NMDA receptor antagonist CPP 30 mins before exposure to light (+CPP). Injection of CPP prevents Arc induction.

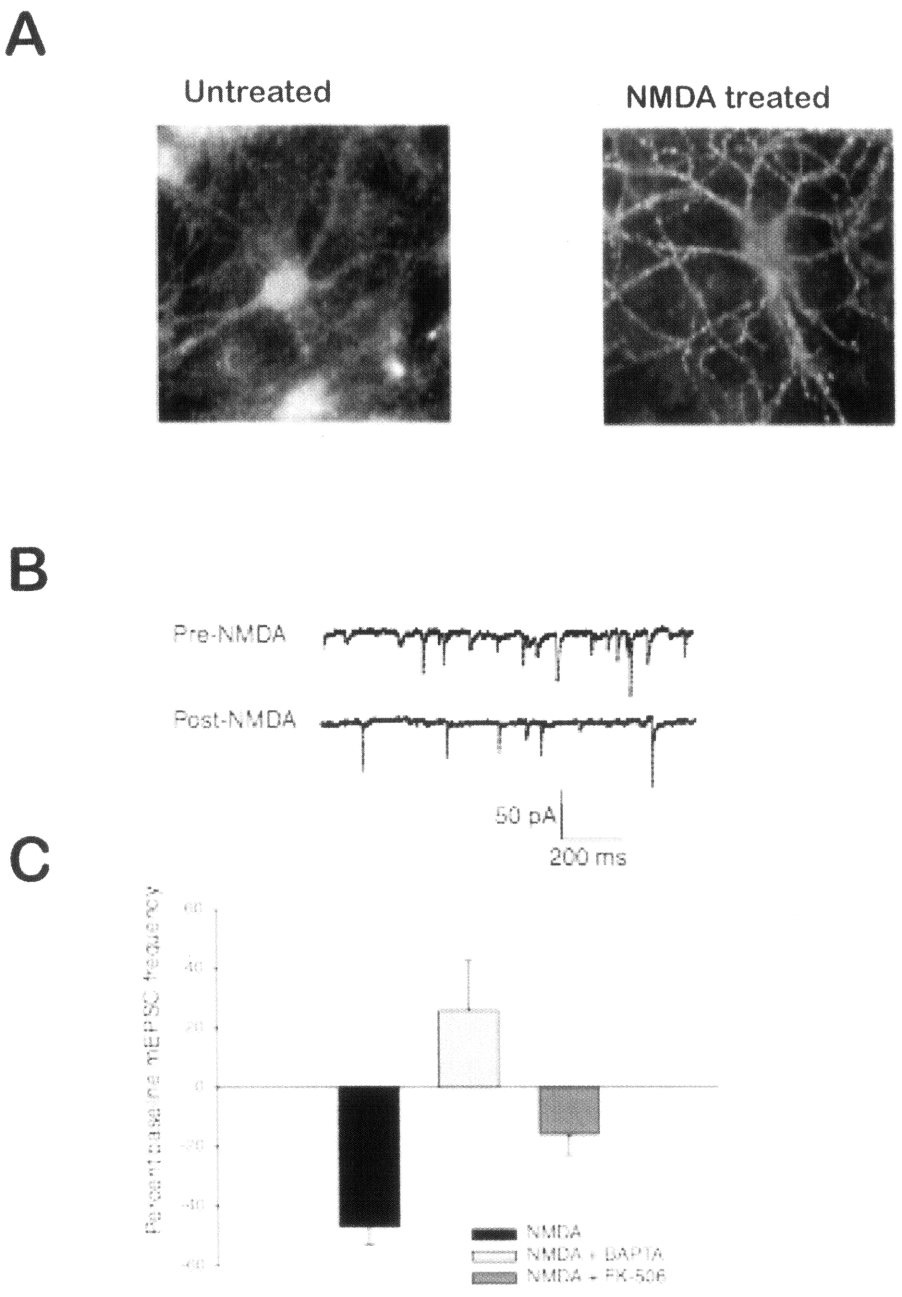


Figure 10. A. NMDA activation results in an internalization of AMPA receptors. B. There is a decrease in mEPSC frequency in cultured hippocampal neurons after NMDA application. C. Application of an NMDA receptor antagonist prevents this decrease in frequency. Adapted from Beattie 2000.

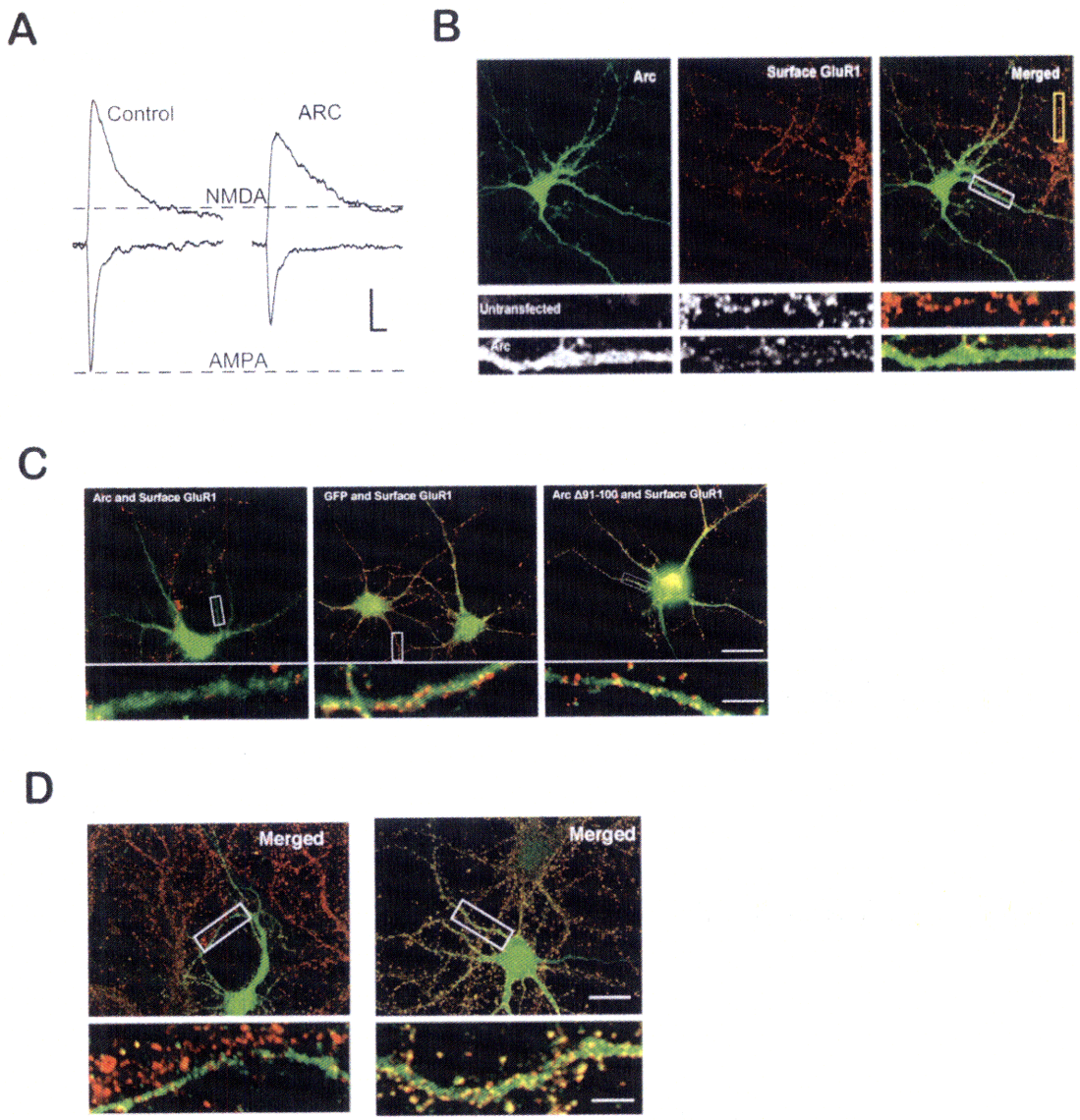


Figure 11. A. Overexpression of Arc reduced AMPA receptor currents. Adapted from Rial-Verde 2006. B. Similarly, overexpression of Arc results in an internalization of GluR1. Adapted from Shepherd 2006. C. Removal of the region of Arc that binds to endophilin (Δ91-100) abrogates Arc's ability to internalize AMPA receptors. D. A deletion mutant (Δ195-214) that does not bind dynamin has no affect on GluR1 surface expression. C,D Adapted from Chowdhury 2006.

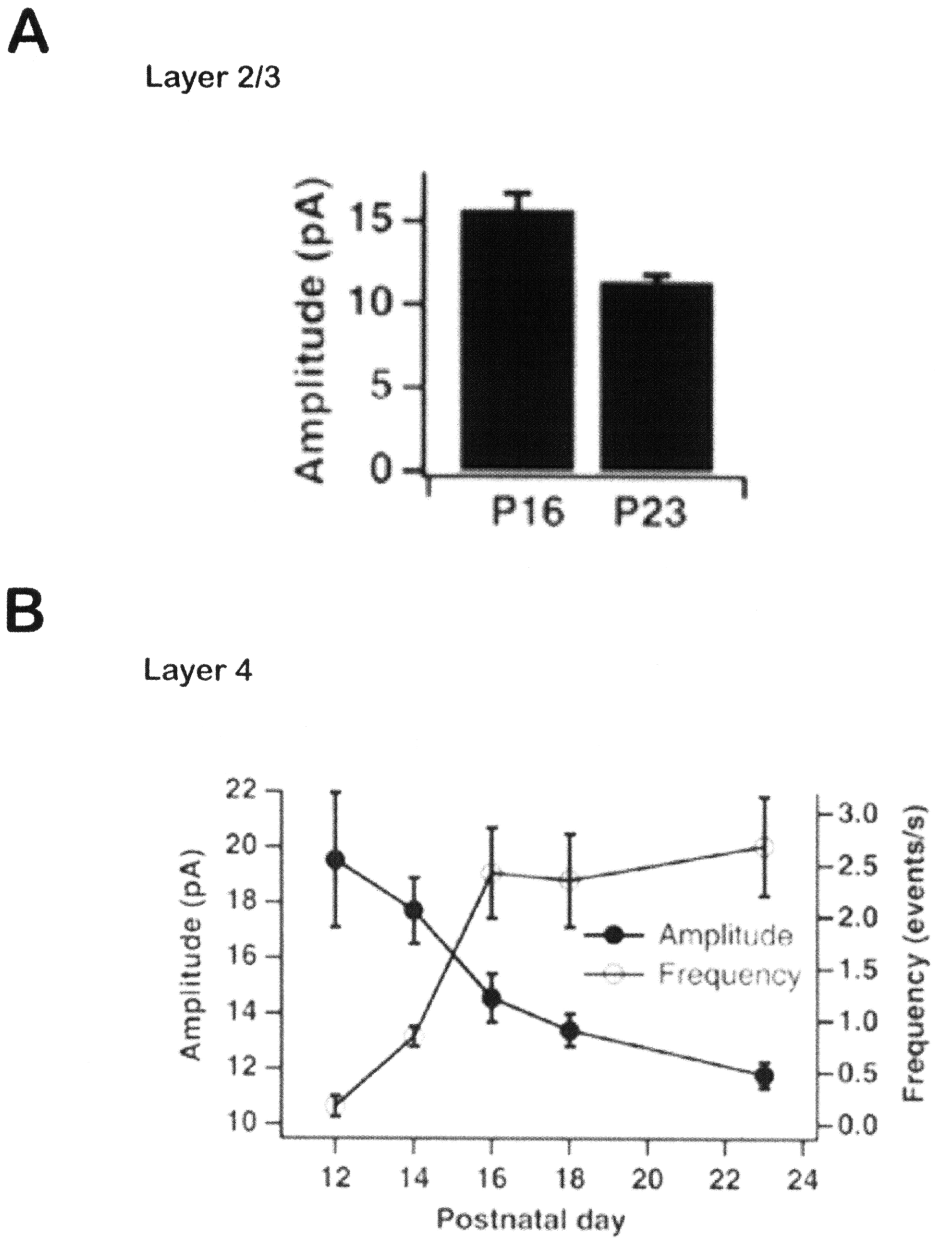


Figure 12. A. Developmental reduction in AMPA receptor mEPSC amplitude occurs within layer 2/3. B. There is a similar decrease seen in Layer 4 after eye opening. Adapted from Desai 2002.

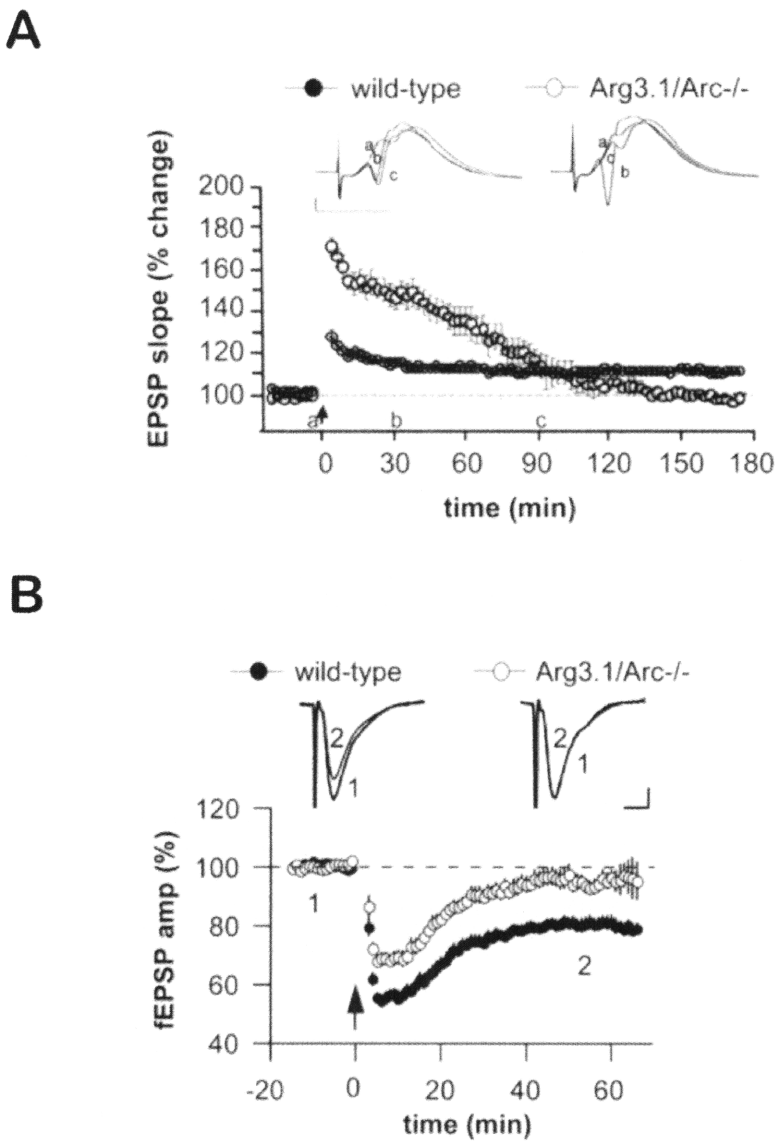


Figure 13. LTP at perforant path/granule cell synapses is disrupted in Arc KO mice. There is an enhancement in early LTP in the KO mice (~ 1.5 X WT) followed by a rapid decay to baseline. B. In addition, there is reduced LTD at Schaffer collateral/CA1 pyramidal cell synapses in Arc KO mice compared to WT. Adapted from Plath 2006.

CHAPTER TWO

DEFICIENT PLASTICITY IN THE VISUAL CORTEX OF JUVENILE ARC KO MICE

ABSTRACT

Visual experience can profoundly modify cortical function. The molecular processes underlying these changes are poorly understood, but are thought to occur via mechanisms similar to those mediating synaptic plasticity. Here we show that Arc, which has previously been shown to play a critical role in various forms of synaptic plasticity, is essential for experience-dependent plasticity in mouse visual cortex. Using intrinsic signal imaging and visual evoked potential recordings (VEPs) we find that Arc knock-out (KO) mice do 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 KO mice lack stimulus-selective response potentiation (SRP), a form of experience-dependent cortical plasticity that exhibits properties similar to long-term potentiation (LTP). Although Arc KO mice exhibit normal visual acuity, baseline ocular dominance was abnormal. A similar phenotype was observed after dark rearing wild-type (WT) mice from birth, suggesting that Arc and experience-dependent processes are required for the normal establishment of ocular dominance in mouse 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). Changes in neuronal activity lead to a strengthening or weakening of synapses, which is believed to facilitate the structural remodeling of visual networks. During a period of heightened plasticity (P23-P32 in mice), the visual cortex is exquisitely sensitive to changes in activity. During this period, even brief monocular deprivation (MD) results in striking functional and anatomical reorganization within the binocular zone of V1 (Antonini et al., 1999) due to an initial and rapid weakening of the cortical response to the deprived eye and a shift in ocular dominance in favor of the nondeprived eye (Gordon and Stryker, 1996). Interestingly, after an extended period of deprivation, a compensatory strengthening of the open eye response takes place, suggesting that multiple molecular mechanisms may mediate different phases of deprivation-induced plasticity in V1 (Frenkel and Bear, 2004; Kaneko et al., 2006; Mrsic-Flogel et al., 2007).

The mechanisms underlying the response to brief deprivation have been well studied. Early studies indicated that the initial cortical depression occurring in response to deprivation is dependent upon calcium signaling through NMDA receptors (Bear et al., 1990), appropriate levels of inhibition (Hensch et al., 1998), and protein synthesis (Taha et al., 2002). In addition, weakening of the deprived eye response may require alterations in AMPA type glutamate receptors (AMPAR), which mediate fast excitatory transmission within the cortex. Indeed, reductions in surface expression of both GluR1 and GluR2 AMPAR subunits occur after brief MD (Allen et al., 2003; Heynen et al., 2003), possibly due to the induction of Hebbian LTD-like mechanisms (Allen et al., 2003; Crozier et al., 2007; Heynen et al., 2003). The regulated trafficking of these receptors is a major cellular mechanism underlying synaptic plasticity at excitatory synapses (Malinow and Malenka, 2002; Shepherd and Huganir, 2007), and AMPA receptor phosphorylation and internalization may contribute to the loss of visual responsiveness observed after MD.

Due to competitive interactions between the two eyes, decreases in the deprived eye response results in a gradual strengthening of response to the open eye. The temporal separation of depression and strengthening has led to the suggestion that these two phases are mediated by separate and distinct mechanisms and may be independent of one another. It has been suggested that Hebbian competitive mechanisms initiated in response to brief deprivation may trigger a homeostatic scaling up of response that results in a strengthening of the open eye. In support of this, mice lacking tumor necrosis factor alpha (TNFa), a molecule critical for homeostatic synaptic scaling show normal deprived eye depression with a specific deficit in open eye potentiation, providing further evidence that these two processes may be mechanistically distinct. Because normal hippocampal LTP is found in these mice, it has been proposed that the lack of open eye potentiation is due to loss of a mechanism for synaptic scaling. However, it is unclear whether open eye potentiation occurs in the absence of an initial Hebbian depression after MD.

The immediate early gene Arc (activity-regulated cytoskeletal associated protein), also known as Arg 3.1, has recently been implicated in many forms of synaptic plasticity (Kaneko et al., 2006; Park et al., 2008; Shepherd et al., 2006; Tagawa et al., 2005; Wang et al., 2006; Waung et al., 2008), and is a prime molecular candidate to play a role in experience-dependent plasticity. Recent experiments in cultured primary neurons have revealed that Arc regulates AMPAR internalization via its interactions with the proteins dynamin and endophilin 2/3, two integral components of the clathrin-mediated endocytosis machinery (Chowdhury et al., 2006). High levels of Arc expression are found to accelerate the rate of AMPAR endocytosis, leading to decreased AMPAR surface expression (Shepherd et al., 2006), while loss of Arc reduces AMPAR endocytosis (Shepherd et al., 2006). Arc gene activation has been shown to be dependent upon activity through NMDA receptors (Lyford et al., 1995), and blocking NMDAR activation using MK-801 or CPP prevents efficient Arc induction (Bloomer et al., 2008). NMDAR dependent signaling pathways have been implicated in ocular dominance plasticity (Bear et al., 1990; Daw et al., 1999; Sawtell et al., 2003) suggesting that Arc may act downstream of these receptors to mediate plasticity by regulating AMPARs. We hypothesized that loss of a putative regulator of AMPA receptor trafficking would prevent the initial deprived eye depression that occurs in response to brief MD.

In the present study we took advantage of Arc knock-out (Arc KO) mice (Wang et al., 2006) to explore a role for Arc in visual cortical plasticity using intrinsic signal optical imaging and visually evoked potentials (VEPs) to assess changes in cortical responses after manipulation of experience.

METHODS

Animals

WT (C57/Bl6) and Arc KO mice (Wang et al., 2006) 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. 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 and the eyelid margins trimmed. The eye contralateral to the hemisphere being imaged was sutured using prolene sutures (Henry Schein) for 3-4 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), C-Fos (1:250, Santa Cruz), GFAP (1:500, Chemicon), GABA (1:500, Chemicon) and parvalbumin (1:500, Chemicon) was carried out on 30-40 μm thick coronal sections. Sections were analyzed using Image J software (http://rsbweb.nih.gov/ij/) and Photoshop CS3.

Western Blots

Animals (n=7, WT and Arc KO) 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 ug and run on a 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 (Heynen et al. 2003). V1 ipsilateral to the deprived eye was used as a within-animal control and comparison of surface 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 NaH2PO4, .5 mM CaCl2, 7 mM MgCl2). 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 NaH2PO4, 26 mM NaHCO3, 1 mM MgCl2, 2 mM CaCl2, 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 2X 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 on a 10% gel and transferred at 40 V for 80 mins.

Injection of cholera toxin subunit B

Mice were anesthetized with avertin (0.016 ml/g, i.p.). The schlera of each eye was pierced and vitreous fluid removed using a thin Hamilton syringe. Approximately 3 ul of CTB conjugated to either Alexa Fluor 488 or Alexafluor 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 (Tropea et al., 2006) .Stimulation consisted of a drifting bar (9°X72°) 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 (Kalatsky and Stryker, 2003). 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 (Smith and Trachtenberg, 2007). 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

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 (Frenkel and Bear, 2004).

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/m2.

RESULTS

Arc KO mice develop normal cortical map organization and visual responses

Activity-dependent induction of gene expression and protein synthesis is critical for normal neuronal development and function (Shepherd and Huganir, 2007). Arc mRNA expression can first be detected in visual cortex at eye opening (Lyford et al., 1995). In response to visual activity, Arc mRNA is rapidly induced and redistributed to active dendritic regions where local protein synthesis occurs (Steward et al., 1998; Waung et al., 2008). While Arc has been shown to be involved in aspects of learning and memory such as the consolidation of long-term potentiation (Guzowski et al., 2000), orientation tuning in visual cortex (Wang et al., 2006), and synaptic scaling in the hippocampus (Shepherd et al., 2006), no study has yet investigated how loss of a regulator of AMPAR trafficking might work *in vivo* to influence developmental plasticity in cortex.

To address this issue, we used Arc KO mice in which GFP has been knocked-in to the Arc gene locus (Wang et al., 2006) to study how loss of Arc protein might influence two forms of NMDA receptor dependent plasticity; ocular dominance plasticity and stimulus response potentiation (SRP). These animals are viable and show no gross deficits in size or weight compared to wild-type (WT) mice. While previous reports have focused on Arc protein interactions within the hippocampus and dentate gyrus, few studies have studied Arc in cortex. Therefore, we sought to investigate Arc expression within V1 of juvenile animals.

First, we examined the distribution of Arc protein expression in mouse visual cortex by immunofluorescence using an Arc specific antibody. In the visual cortex, Arc did not colocalize with a stain for GFAP, which labels astrocytes, or for the inhibitory neuron marker GABA (Figure S1A). This suggests that Arc protein is selectively expressed in excitatory neurons within V1.

Previous reports have shown that Arc mRNA is regulated by physiological activity and shows prominent expression in the visual cortex (Lyford et al., 1995; Tagawa et al., 2005). Within mouse 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 (Figure S1B). Protein was detected within cell bodies and dendrites of V1 pyramidal neurons. As expected, no Arc expression was detected in Arc KO tissue (Figure S1A).

We used intrinsic signal imaging to test whether loss of Arc altered visual cortex responses and retinotopic organization (Kalatsky and Stryker, 2003; Tropea et al., 2006). Because previous studies have implicated Arc protein in regulation of AMPA receptors, which are known to play a role in synaptic transmission, we asked whether loss of Arc protein would influence the strength of response to visual stimulation in mouse visual cortex. 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 KO mice was similar to that in WT mice in area and organization of binocular and monocular zones (Figure 1A). To examine whether loss of Arc protein might impact retinotopic organization (Figure 1A), we evaluated scatter within the retinotopic (phase) maps (Figure 1B). Map organization in Arc KO mice was indistinguishable from WT mice (Figure 1C). In addition, there was no significant difference in response magnitude within V1 in response to binocular stimulation (Figure 1D). These data demonstrate that loss of Arc protein does not grossly disrupt the development of normal visual cortex organization. We assessed visual acuity in Arc KO mice by measuring VEPs in response to sinusoidal gratings at various frequencies. There was no significant difference between WT and Arc KO responses at the highest detectable spatial frequency of 0.6 cycles per degree, suggesting that Arc KO mice have normal visual acuity (Figure 1E).

Arc protein is required for deprived-eye depression after short-term MD.

The initial cortical response to MD is a weakening of deprived eye inputs (Frenkel and Bear, 2004). Endocytosis of AMPA receptors is required for the initial depression of the deprived eye response that occurs after brief lid suture. 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 (P25-30). 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 MD in WT animals (Cang et al., 2005).

In keeping with previous reports, WT mice show a robust decrease in ODI after brief deprivation (Figure 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 (Figure 2B). By contrast, Arc KO mice did not exhibit a change in ODI (Figure 2A) and cortical responses to the deprived eye remained unchanged (Figure 2C). These results indicate that Arc protein is required for the deprived eye depression induced by brief MD.

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 MD (Porciatti et al., 1999; Sawtell et al., 2003). 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 gratings. We collected baseline recordings, and then monocularly deprived animals for 3 days by lid suture. After opening the sutured eye we gathered post MD recordings. WT mice show a robust ocular dominance shift (Figure 3A), but Arc KO mice do not exhibit a change in OD (Figure 3B). The shift in WT mice was due to a significant depression in deprived eye responses (Figure 3A), which was not observed in Arc KO mice (Figure 3B).

These findings prompted us to examine whether the anatomical substrates of ocular dominance plasticity remained unperturbed in Arc KO mice. During the pre-critical period, it has been shown that experience-dependent competition between the two eyes as early as eye opening is necessary for normal axonal refinement in central targets (Lu and Constantine-Paton, 2004; Pfeiffenberger et al., 2005; Smith and Trachtenberg, 2007). We used intraocular injection of CTB to

examine eye-specific segregation in the lateral geniculate nucleus; no gross changes in contralateral or ipsilateral inputs could be seen in Arc KO animals as compared to WT (Figure S2B).

Maintaining an appropriate balance of excitation and inhibition within the cortex is critical for normal experience-dependent plasticity. Indeed, previous work shows that alterations in inhibition may lead to altered plasticity in the visual cortex (Fagiolini and Hensch, 2000; Hensch et al., 1998), with a particular role for inhibition mediated by parvalbumin-positive interneurons (Fagiolini et al., 2004; Huang et al., 1999). In order to examine whether there were indirect effects on inhibition in Arc KO mice, we investigated the expression of several inhibitory markers within the visual cortex of Arc KO and WT animals. Westerns against VGAT, GAD65, and parvalbumin showed no difference between the two groups (Figure S2A-C). In addition, no change in GABA expression was found, suggesting that gross changes in inhibition are unlikely (Figure S2D).

Arc regulates AMPA receptor endocytosis in visual cortex

What might underlie the reduced deprived-eye depression in Arc KO mice? Manipulations of sensory activity are known to regulate synaptic AMPARs in the cortex (Goel et al., 2006). In response to as little as 24 hrs of MD, AMPARs are rapidly internalized, decreasing the surface to total ratio, which mediates the depression in cortical responses from the deprived eye (Heynen et al., 2003). In addition, cortical expression of a GluR2 c-terminus fragment that is known to block AMPAR endocytosis and LTD in vitro also blocks deprived-eye depression and the OD shift after short-term MD in vivo (Smith et al., *in press*). We hypothesized that loss of Arc protein might reduce the deprivation-induced removal of surface AMPARs, and thus prevent the shift in ocular dominance. For these experiments we decided to focus on the GluR1 subunit, as recent reports have indicated that GluR2 and GluR3 subunits are not required for activity-dependent endocytosis of AMPARs (Biou et al., 2008). In addition, GluR1 shows high immunoreactivity in the middle and superficial layers of mouse visual cortex (Kim et al., 2006), which are key sites of ocular dominance plasticity. To test the hypothesis we performed a biotinylation assay using acute slices in order to measure surface expression of AMPARs after MD. In this preparation, surface proteins are tagged with biotin and then pulled down using streptavidin beads that have a high affinity for the biotin molecule. Because Arc

protein is primarily expressed in layers 2/3 and 4 of visual cortex (Figure S1B), the deeper layers were microdissected out and discarded from both hemispheres. The surface receptors in the remaining tissue were then tagged with biotin and probed using a GluR1 specific antibody to quantify surface expression. In WT mice, a significant decrease in the surface/total ratio of GluR1 could be detected in the "deprived" hemisphere (contralateral to the deprived eye; Figure 4A) as compared to the "nondeprived" control hemisphere (Figure 4B and 4C). Strikingly, Arc KO mice showed no significant change in the surface/total ratio of AMPARs within the deprived hemisphere (Figure 4B and 4C). This result suggests that loss of Arc protein reduces AMPAR internalization and thus prevents the synaptic weakening that occurs in response to decreased activity.

Reduced open eye potentiation in Arc KO mice

The ocular dominance shift that occurs after long-term MD 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 involves Arc. Conversely, extended periods of deprivation result 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 KO mice, in the absence of response depression, 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 ocular dominance (Figure 5A). Consistent with previous reports, we found that this shift was mediated by a significant increase in open eye responses (Figure 5B). The increase in open eye response was accompanied by a slight decrease in the deprived eye response (Figure 5B). Strikingly, Arc KO mice did not show a shift in ODI or significant open eye potentiation (Figure 5C). Similar results were found with VEP recordings after 7 days MD. WT mice exhibited a robust OD shift that was due to both significant deprived eye depression and open eye potentiation (Figure 6A). In contrast, Arc KO mice did not exhibit an OD shift or any significant changes in deprived eye or open eye responses (Figure 6B).

Arc is required for stimulus-selective response potentiation

We have previously described an *in vivo* form of potentiation in mouse visual cortex, stimulus-selective response potentiation (SRP) that results from brief exposure to sinusoidal gratings of a specific orientation (Frenkel et al, 2006). Mechanistically, SRP exhibits hallmarks of LTP; it is NMDAR-dependent, is blocked by a GluR1 c-terminal peptide that has been shown to inhibit insertion of AMPARs at synapses, and it seems pathway specific. We also find that SRP expression requires protein synthesis as it is blocked by I.P. injections of cyclohexamide (data not shown). Since Arc KO mice exhibit a defect in open eye potentiation, we wondered whether SRP would also be disrupted due to a lack of Arc. Indeed, we find that Arc KO mice have a severe deficit in SRP (Figure 7) as compared to WT mice. This adds further weight to the idea that Arc is required for experience-dependent plasticity in the visual cortex.

Normal C/I ratio requires experience and is Arc-dependent

During the course of our MD studies we noticed that Arc KO mice seemed to have altered baseline C/I ratios. After pooling baseline data from our experiments we found that Arc KO mice have a significant decrease in C/I ratio as compared to WT mice (Figure 8A). This was mostly due to a significant decrease in contra responses (Figure 8B). We hypothesized that establishing the C/I ratio in mice requires neuronal activity or experience. To test this we dark reared 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 visual cortex (Wang et al, 2006). Dark reared mice exhibited a decrease in the C/I ratio due to a significant decrease in contra responses, similar to that observed in Arc KO mice (Figure 8A and B).

DISCUSSION

Multiple molecular mechanisms have been proposed to facilitate the experience-dependent changes that occur in visual cortex during development. Here, we investigated the role of Arc, a molecule that has been implicated in NMDA receptor dependent plasticity and AMPAR trafficking, in

visual cortex plasticity. We find that removal of Arc results in a profound deficit in experience-dependent plasticity. Our results show that loss of Arc protein leads to a reduced shift in ocular dominance, and impaired AMPA receptor internalization in response to lid suture, suggesting that Arc is crucial for the deprived eye depression that normally takes place after MD. In addition, both deprived eye depression and open eye potentiation fail to occur even after extended deprivation. We also find that Arc KO mice exhibit deficits in SRP. Strikingly, these deficits occur in the absence of major changes in visual response properties as Arc KO mice exhibit normal visual acuity, and retinotopic organization is similar to that found in WT mice. We do not observe any overt compensation in proteins specific for inhibitory synaptic transmission in Arc KO neurons. Since Arc is only expressed in excitatory cells in the visual cortex we believe that the phenotypes observed in Arc KO mice are not due to aberrant inhibition, although we cannot fully rule out this possibility.

Arc mediates MD-induced deprived eye depression

A number of studies provide evidence for competitive Hebbian mechanisms contributing to the decrease in deprived eye response (Crozier et al., 2007; Heynen et al., 2003; Rittenhouse et al., 2006). The shift in ocular dominance that occurs after brief visual deprivation serves as one of the most representative models of activity-dependent plasticity and has been shown to depend upon activation of NMDA receptors (Bear et al., 1990; Roberts et al., 1998; Sawtell et al., 2003). Indeed, removing or inhibiting components of the NMDA-dependent signaling pathway, such as MAPK, PKA, and CamKII-alpha, reduces the shift in ocular dominance seen after MD (Di Cristo et al., 2001; Rao and Daw, 2004; Taha et al., 2002). A recent study using GAD 65 KO mice provides further evidence for the importance of signaling through NMDA receptors. These mice have a reduction in both GABA A and NR2A subunit levels (Kanold et al., 2009). In addition, ocular dominance plasticity (Hensch et al., 1998; Kanold et al., 2009) and LTD are completely disrupted (Choi 2007). Interestingly, application of diazepam rescues the deficit in NR2A subunit levels, leaving GABA A subunit levels unchanged, and restores both LTD and ocular dominance plasticity. We find that Arc KO mice lack deprived eye depression, even after 7 days of deprivation. Since Arc transcription is also dependent upon activation of NMDA receptors, MAPK and PKA signaling cascades our data suggests that Arc is a downstream effector molecule for this pathway. Whether restoration of NMDA receptor function in GAD 65 KO

mice might enable normal ocular dominance plasticity due to Arc-mediated LTD-like mechanisms remains to be tested.

Arc may be required for NMDAR as well as mGluR dependent AMPA receptor removal. 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. As an important correlate of Arc function, in a mouse model of fragile X syndrome in which mGluR signaling is enhanced, Arc expression is increased and ocular dominance plasticity is significantly enhanced compared to WT mice (Park et al., 2008). In contrast, a 50% reduction of mGluR5 expression is sufficient to prevent the shift in ocular dominance, which normally occurs in response to lid suture (Dolen et al., 2007). These data support our finding that Arc, operating downstream of glutamate receptor activation, is required for deprived eye depression. Arc KO mice have significantly smaller contra responses and a decrease in C/I ratio (see discussion below). It is possible that Arc KO mice lack deprived eye depression because plasticity is occluded. We think this is unlikely because we do not see any overt differences in basal surface AMPAR expression compared with WT mice.

Arc is required for experience-dependent potentiation in visual cortex

In WT mice, we detected a robust potentiation of the open eye after 7 days of deprivation using both intrinsic signal imaging and VEPs. However, the open eye response fails to potentiate after an extended period of deprivation in the Arc KO mice. In addition, deprived eye response depression does not occur even after 7 days of monocular lid suture. It has been proposed that response depression and potentiation may be regulated by distinct mechanisms. Indeed, the strengthening of open eye response after longer periods of deprivation may rely upon homeostatic mechanisms such as synaptic scaling (Kaneko et al., 2006; Mrsic-Flogel et al., 2007). In support of this, mice lacking tumor necrosis factor alpha (TNF-alpha), a cytokine derived from glia and implicated in homeostatic synaptic scaling, have intact deprived eye depression after brief lid suture, however, there is reduced ocular dominance plasticity due to a deficit in open-eye response potentiation (Kaneko et al, 2008). An additional study found that MD resulted in a scaling up of response in cells within the binocular zone

receiving input predominantly from the deprived contralateral eye providing further evidence that homeostatic mechanisms may operate in concert with Hebbian plasticity (Mrsic-Flogel et al., 2007). However, in our current study, we find that both depression and potentiation are impaired in Arc KO mice suggesting that these two phases may not be completely separable. It is possible that an initial Hebbian depression in deprived eye responses mediated by Arc serves as a trigger for subsequent open eye potentiation. Alternatively, Arc may be directly required for the induction of open-eye potentiation. Consistent with this hypothesis, open eye potentiation was recently shown to require NMDAR function (Sato and Stryker, 2008). Moreover, we show that Arc is required for the expression of SRP, a form of experience-dependent plasticity that bears all the hallmarks of LTP.

Arc mediates activity-dependent establishment of normal C/I ratio in mice

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 (Fagiolini et al., 1994; Smith and Trachtenberg, 2007). In very young rats (P17-P19), there is a large number of binocular cells within the binocular zone of visual cortex (Fagiolini et al., 1994). However, by the peak of the critical period a contralateral bias has been established in cortex and continues throughout adulthood. This suggests that there may be an activity-dependent pruning of the weaker ipsilateral eye projections that occurs with development. Data from the visual cortex of dark reared adult rats- where Arc is very low- suggests that this may well be the case as these mice exhibit a greater percentage of binocular cells, compared to normally reared rats (Fagiolini et al., 1994). Using VEPs we find that Arc KO mice, and mice that have been dark reared from birth, show a significant reduction in the C/I ratio, reminiscent of the increased binocularity seen previously in dark reared rats (Fagiolini et al., 1994) . These data suggest that both experience and Arc are critical for the normal establishment of the C/I ratio. We believe the changes in ocular dominance in Arc KO 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 KO mice is not different from WT mice. However, it is also possible that Arc is required for the anatomical refinement of thalamocortical axonal arbors from the LGN.

One caveat of our study is that we use a full KO mouse that lacks Arc from birth. It is possible that Arc may affect the normal development of the visual cortex prior to any experience-dependent processes. However, Arc expression is virtually undetectable prior to eye opening in visual cortex (Tagawa et al., 2005; Wang et al., 2006), and its expression rapidly increases after eye opening during the period where experience-dependent changes take place. It is possible that Arc functions within the neuron to enhance 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 KO 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 C/I ratio. In line with a role for Arc in the sharpening of visual response properties, adult Arc KO mice show an increase in cells with low orientation specificity and broader tuning compared to heterozygous and WT mice (Wang et al., 2006).

In conclusion, we have found that Arc is critically involved in multiple forms of experience-dependent plasticity. Moreover, Arc is necessary for the establishment of the normal C/I ratio in mice. Together these experiments illustrate the essential role for Arc in experience-dependent plasticity within the visual system.

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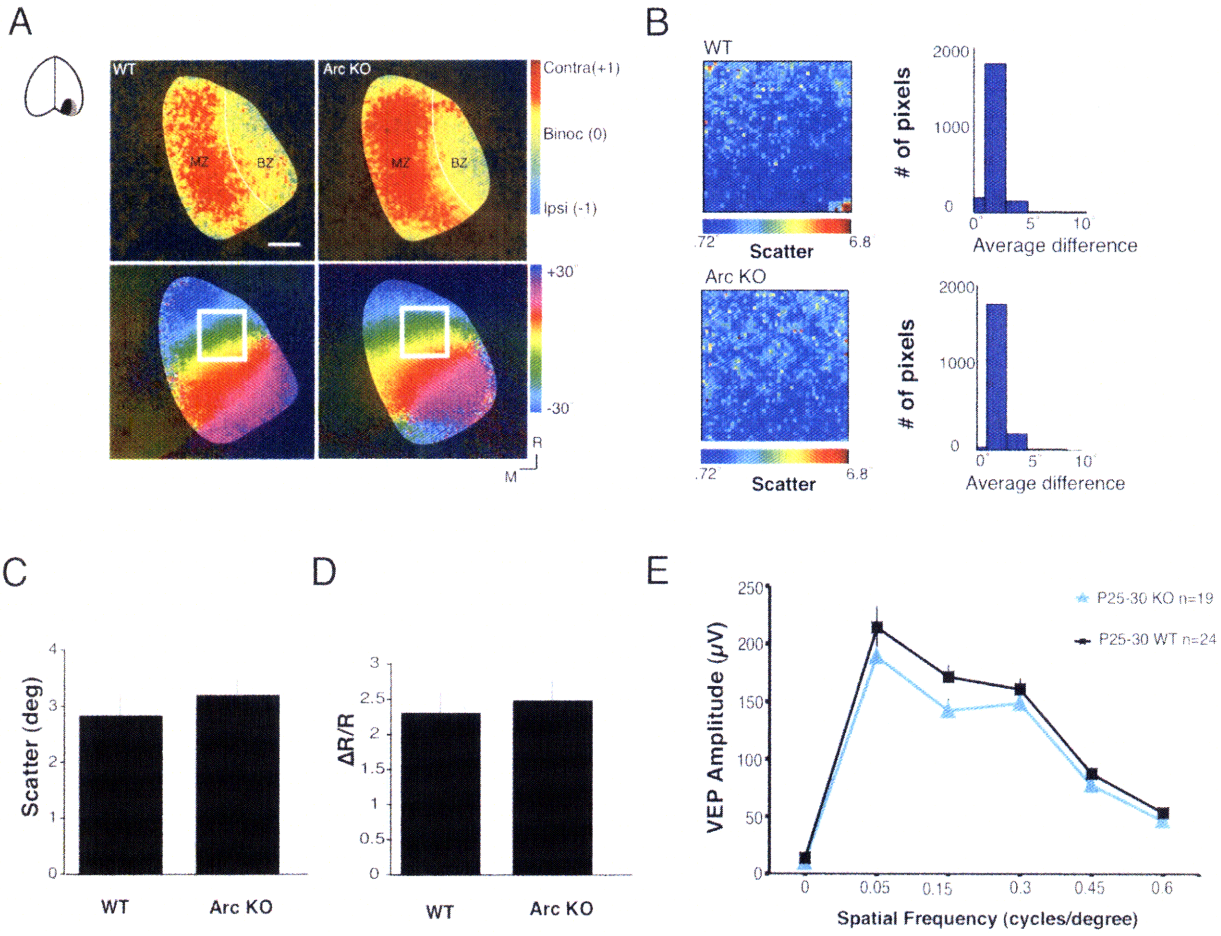
A
WT
Arc KO
MZ
BZ
Contra(+1)
Binoc (0)
Ipsi (-1)
+30
-30
R
M
B
WT
.72
Scatter
6.8
# of pixels
2000
1000
0
0
5
10
Average difference
Arc KO
.72
Scatter
6.8
# of pixels
2000
1000
0
0
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10
Average difference
C
Scatter (deg)
4
3
2
1
0
WT
Arc KO
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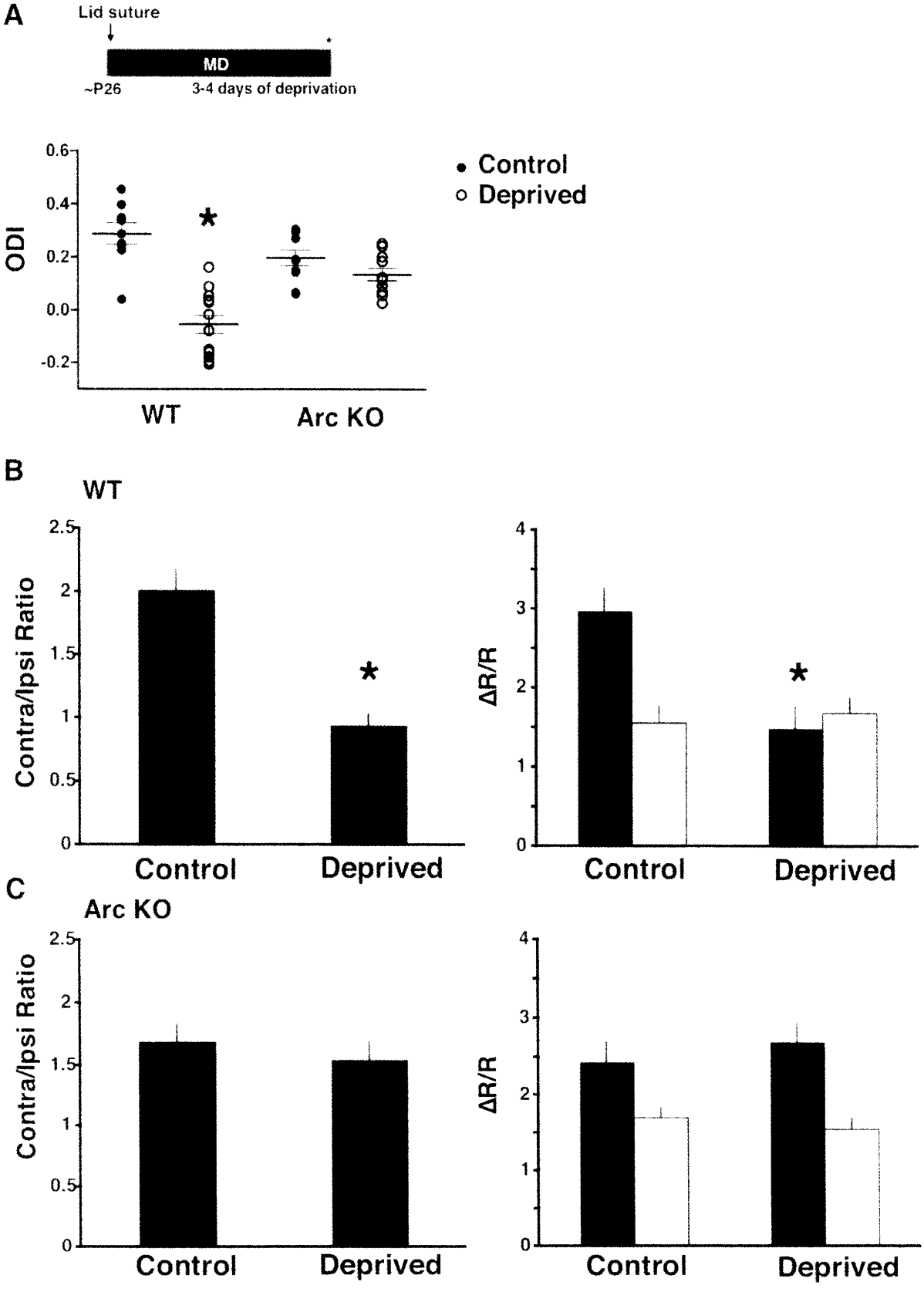
ΔR/R
3
2.5
2
1.5
1
0.5
0
WT
Arc KO
E
VEP Amplitude (μV)
250
200
150
100
50
0
P25-30 KO n=19
P25-30 WT n=24
0
0.05
0.15
0.3
0.45
0.6
Spatial Frequency (cycles/degree)

Figure 1. Loss of Arc does not affect visual cortex responsiveness and organization. (A) Intrinsic signal imaging of V1 (left inset) in WT and Arc KO mice. (Top) Ocular dominance map of V1, in a WT mouse (left) and an Arc KO mouse (right); MZ=monocular zone, BZ=binocular zone. Scale at right illustrates binocularity index of pixels. V1 in Arc KO mice is similar to that in WT mice in total area (WT n=6, area=1.401 ± .07 mm^2; Arc KO n=10, area=1.270 ± .15 mm^2; p>0.5, t-test). (Bottom) Retinotopic organization of V1 in a WT mouse (left), and an Arc KO mouse (right). Each image shows the mapping of elevation according to scale at top right. **(B)** Scatter analysis of 50X50 pixel area within white box in A. for WT and Arc KO **(C)** Analysis of scatter for total population, assessed as the average difference in receptive field center location between nearby pixels, indicates the maps are similar in Arc KO and WT mice (WT n=6; 2.85±.54 deg; Arc KO n=10; 3.2±.50 deg; p>0.8, t-test) Scale bar= 500 μm. **(D)** Response magnitude within the binocular zone, plotted as average ΔR/R x 10-3, in response to binocular stimulation, is similar in WT and Arc KO mice (WT n=6; ΔR/R=2.3±0.27; Arc KO n=10; ΔR/R=2.4±0.26; p>0.5, t-test). ΔR/R is the change in reflectance over baseline reflectance. Error bars represent standard error of the mean (SEM). **(E)** Visually evoked potentials were used to assess visual acuity in Arc KO and WT animals. No significant difference between the two groups could be detected at the highest spatial frequency tested (VEP amplitude at 0.6 cycle/deg, WT: 33.5 ± 3.5 μV, n = 22; Arc KO: 30.7 ± 6.4 μV, n = 19; p> 0.2, t-test).


A
Lid suture
MD
~P26
3-4 days of deprivation
ODI
0.6
0.4
0.2
0.0
-0.2
WT
Arc KO
Control
Deprived
B
WT
Contra/Ipsi Ratio
2.5
2
1.5
1
0.5
0
Control
Deprived
ΔR/R
4
3
2
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Control
Deprived
C
Arc KO
Contra/Ipsi Ratio
2.5
2
1.5
1
0.5
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Control
Deprived
ΔR/R
4
3
2
1
0
Control
Deprived

Figure 2. Intrinsic signal imaging after MD illustrates a requirement for Arc in deprived-eye depression after short-term MD (A)(Top) MD was initiated near the peak of the critical period for 3-4 days. Control animals 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, $p < .05$; Arc KO: control, n=10, ODI = 0.19±0.02; deprived, n=11, ODI = 0.13±0.02, $p>0.1$, Student's t-test). **(B)** (Left) A significant decrease in the contra/ipsi ratio is seen (control=2.019±.15, deprived=0.94±.09, $p<0.001$, Student's t-test). (Right) Response magnitude in WT animals driven by the contralateral eye (filled bars) and ipsilateral eye (open bars), plotted as average $\Delta R/R$ x 10-3. A depression in the contralateral eye response amplitude can be seen (control=2.9±.27, deprived= 1.62±.23, $p<0.01$, Student's t-test). No change in the ipsilateral eye response is detected (control=1.56±.21, deprived=1.68±.19, $p>0.8$, Student's t-test). **(C)** (Left) Ratio of contralateral eye response to ipsilateral response in Arc KO animals without and with MD of the contralateral eye. No change in contra/ipsi ratio is observed (control= 1.72±.14, deprived= 1.60±.13, $p>0.4$, Student's t-test). (Right) No change in contralateral (filled bar) response occurs in Arc KO animals after deprivation (control= 2.25±.28, deprived= 2.5±.26, $p>0.2$, Student's t-test); similarly, no change in ipsilateral (open bar) response is detected (control=1.35±.23, deprived=1.64±.19, $p>0.2$, Student's t-test). $\Delta R/R$ is the change in reflectance over baseline reflectance. Error bars represent SEM.

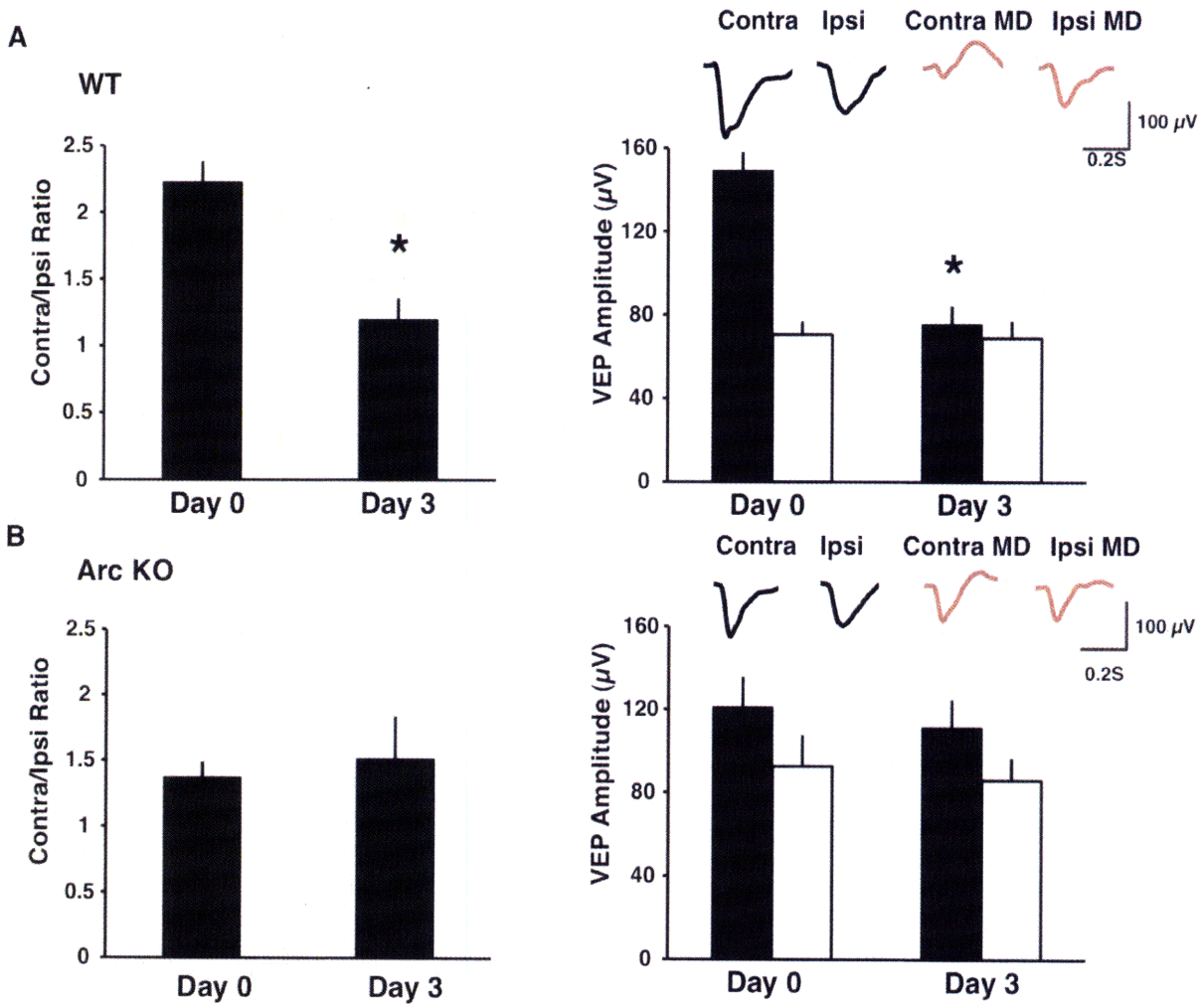


Figure 3. Chronic VEP recordings show that Arc KO mice do not exhibit ocular dominance plasticity after short-term MD. (A) (Left) WT mice exhibit a significant shift in the contralateral to ipsilateral eye ratio (n = 11; Day 0 = 2.2 ± 0.16, 3 Day MD = 1.2 ± 0.16, $p << 0.0001$, t-test). (Right) The change in ocular dominance ratio in WT mice is due to a significant depression in contralateral (deprived eye, C) responses (n = 11; Day 0 = 149 ± 8.8 μV, 3 Day MD = 75.4 ± 8.8 μV, $p << 0.0001$,

paired t-test). No significant change was observed in ipsilateral responses (open eye, I; n = 11; Day 0 = 70.4 ± 6.4 μV, 3 Day MD = 68.8 ± 8 μV, p>0.8, paired t-test). Averaged waveforms across all animals are shown at top. **(B)** (Left) Arc KO mice exhibit no significant shift in the contralateral to ipsilateral eye ratio (n = 8; Day 0 = 1.4 ± 0.12, 3 Day MD = 1.5 ± 0.33, p>0.8, paired t-test). (Right) Arc KO mice exhibit no changes in contralateral responses (C; n = 8; Day 0 = 121 ± 14.7 μV, 3 Day MD = 111.3 ± 13.5 μV, p>0.2, paired t-test) or in ipsilateral responses (I; n = 8; Day 0 = 92.5 ± 15 μV, 3 Day MD = 85.8 ± 10.7 μV, p>0.7, paired t-test). Averaged waveforms are shown at top. Error bars represent SEM.

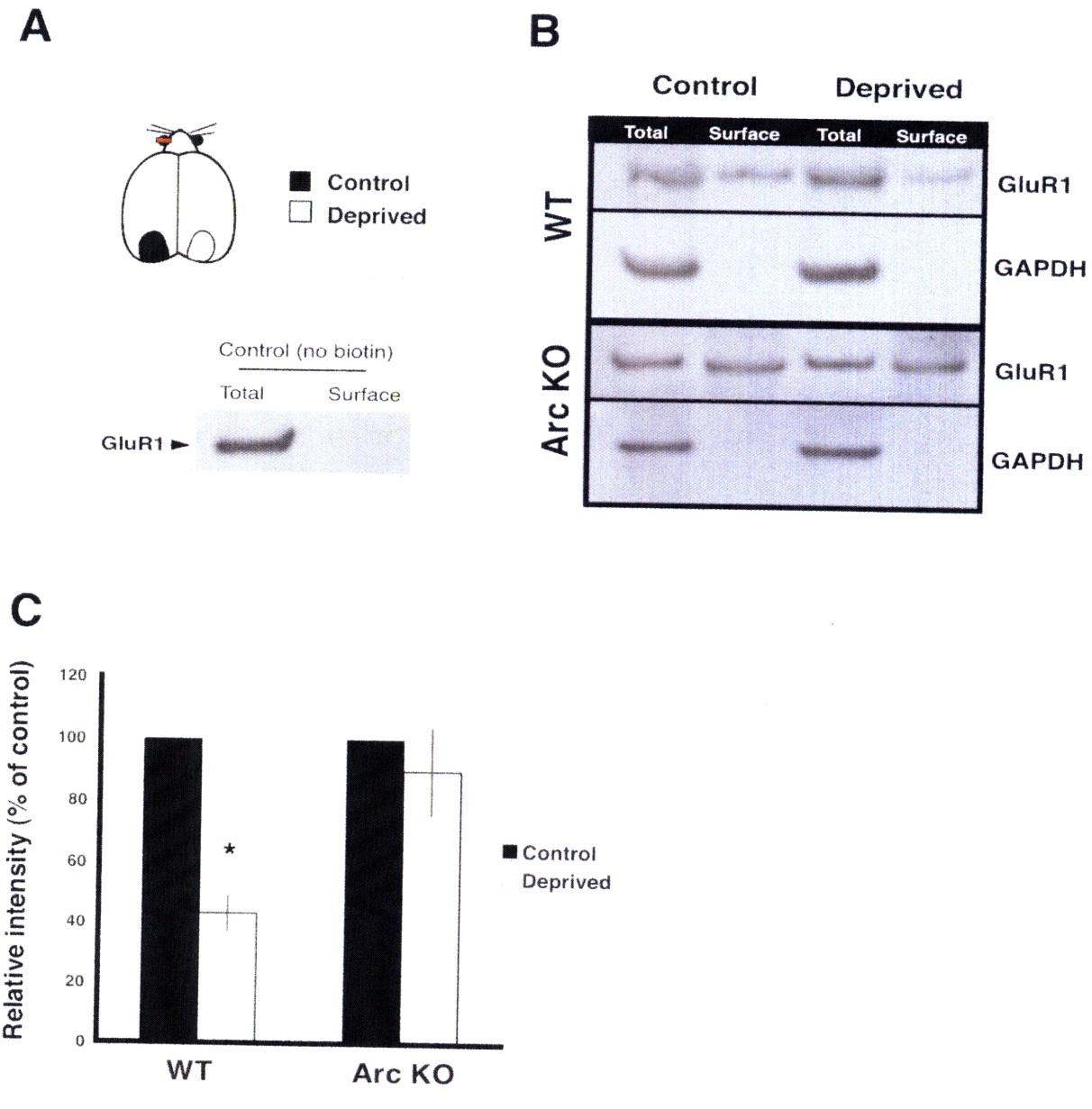
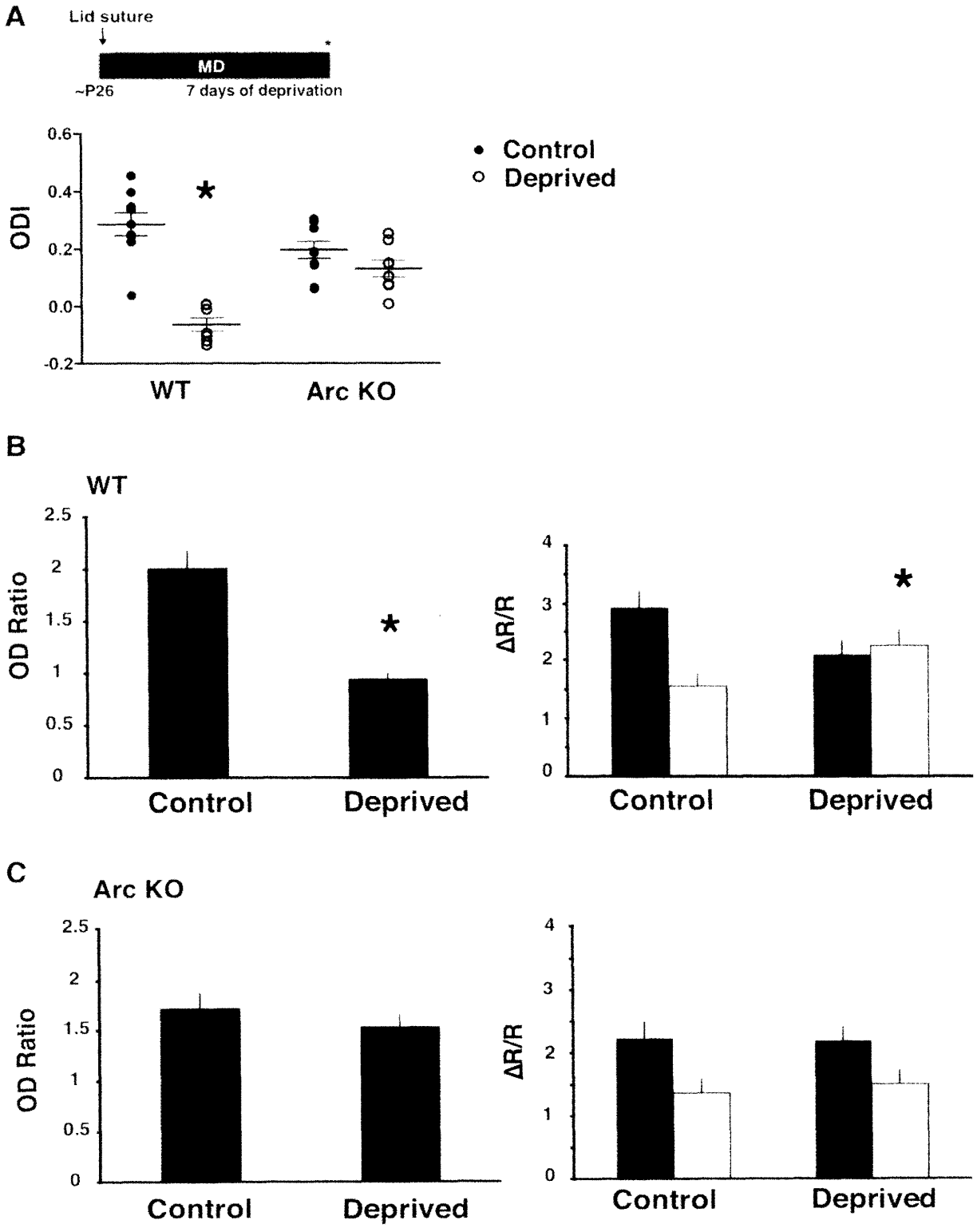

A
Control
Deprived
Control (no biotin)
Total
Surface
GluR1
B
Control
Deprived
Total
Surface
Total
Surface
WT
GluR1
GAPDH
Arc KO
GluR1
GAPDH
C
Relative intensity (% of control)
120
100
80
60
40
20
0
*
Control
Deprived
WT
Arc KO

Figure 4. Arc is required for the decrease in surface AMPARs after short-term MD. (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 KO and WT mice. 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 protein levels occurring after deprivation (WT, n=5; Arc, n=7). Surface levels of GluR1 were significantly lower in the deprived hemisphere of WT animals compared to control ($p<.0001$, Student's t-test), but not in Arc KO animals ($p >0.2$, Student's t-test). Error bars represent SEM.


A
Lid suture
MD
~P26
7 days of deprivation
ODI
0.6
0.4
0.2
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-0.2
*
WT
Arc KO
Control
Deprived
B
WT
OD Ratio
2.5
2
1.5
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Control
Deprived
ΔR/R
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Deprived
ΔR/R
4
3
2
1
0
Control
Deprived

Figure 5. Arc KO mice do not show a shift in ocular dominance after extended deprivation. **(A)**(Top) MD was initiated near the peak of the critical period for 3-4 days. Control animals 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±.03; deprived, n=7, ODI= -0.063±.02, $p<0.0001$; Arc KO: control, n=10, ODI = 0.19±.02; deprived, n=8, ODI = 0.13±.02, p=.17). **(B)** Ratio of contralateral eye response to ipsilateral response in WT animals, without and with MD of the contralateral eye. A significant decrease in the contra/ipsi ratio is seen (control=2.019±.15, deprived=0.95±.04, $p<0.0001$). (Right) Response magnitude in WT animals driven by the contralateral eye (filled bars) and ipsilateral eye (open bars), plotted as average $\Delta R/R$ x 10-3. A slight, albeit not significant, depression in the contralateral eye response amplitude can be seen (control=2.9±.27, deprived= 2.1±.23, $p>0.05$). Lid suture results in a increase in the ipsilateral eye response (control=1.56±.21, deprived=2.49±..17, $p<.05$). **(C)** (Left) Ratio of contralateral eye response to ipsilateral response in Arc KO animals without and with MD of the contralateral eye. No change in contra/ipsi ratio is observed (control= 1.72±.14, deprived= 1.540±.11, $p>.6$). (Right) No change in contralateral (filled bar) response occurs in Arc KO animals after deprivation (control= 2.25±.28, deprived= 2.2±.21, $p>0.6$); similarly, no change in ipsilateral (open bar) response is detected (control=1.35±.23, deprived=1.5±.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.

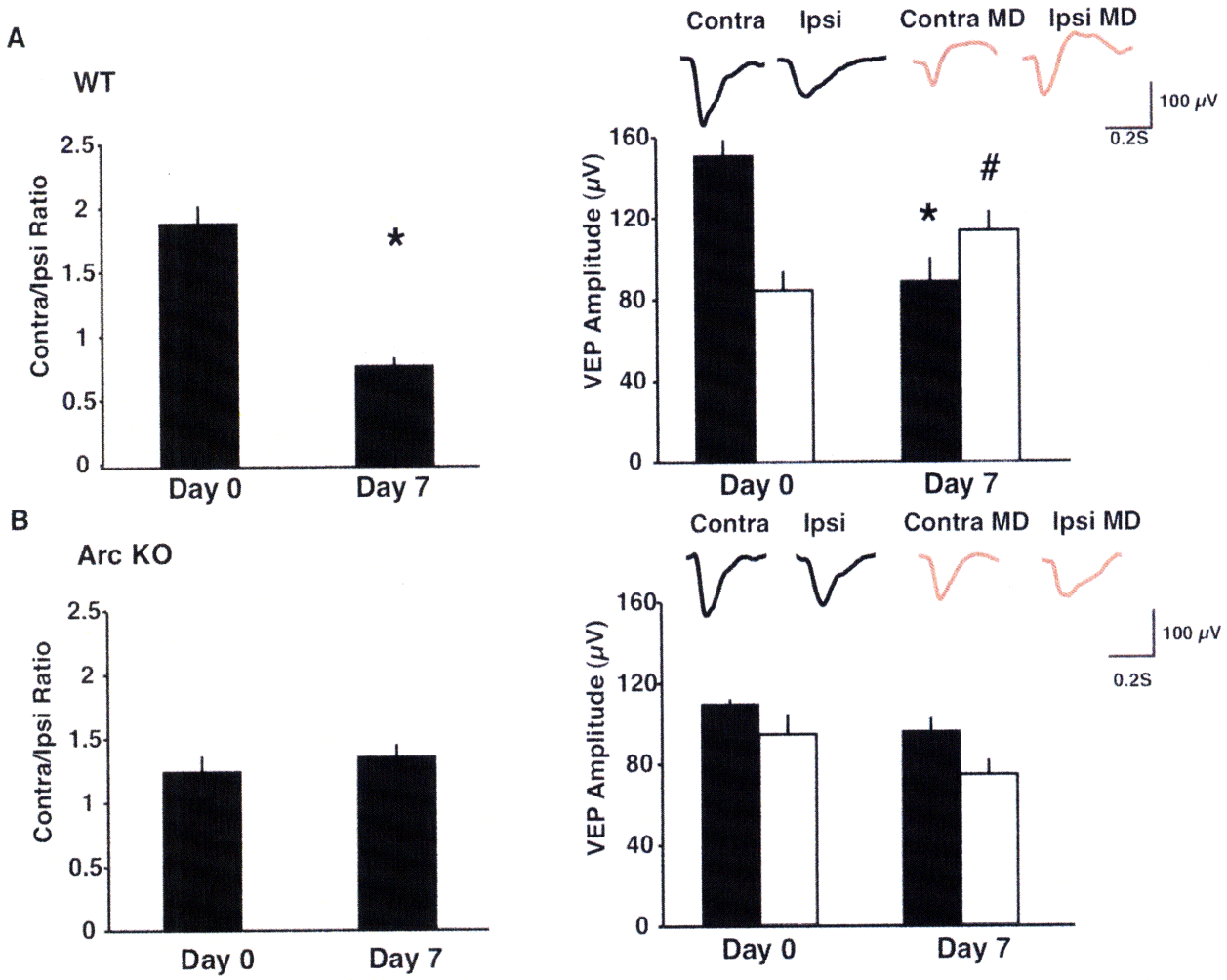


Figure 6. Arc KO mice exhibit no ocular dominance plasticity as assessed by chronic VEP after long-term MD. (A) (Left) WT mice exhibit a significant shift in the contralateral to ipsilateral eye ratio (n = 7; Day 0 = 1.9 ± 0.14, 7 Day MD = 0.8 ± 0.06, $p < 0.0001$, t-test). (Right) The change in ocular dominance ratio in WT mice is due to a significant depression in contralateral (deprived eye, C) responses (n = 7; Day 0 = 152 ± 9.2 μV, 7 Day MD = 89.5 ± 11.5 μV, $p < 0.003$, paired t-test) and a significant potentiation in ipsilateral responses (open eye, I; n = 7; Day 0 = 84.9 ± 9.8 μV, 7 Day MD = 114.2 ± 10.1 μV, $p>0.05$, paired t-test). Averaged waveforms are shown at top. **(B)** (Left) Arc KO mice exhibit no significant shift in the contralateral to ipsilateral eye ratio (n = 6; Day 0 = 1.2 ± 0.1, 7 Day

MD = 1.25 ± 0.11, p>0.7, paired t-test). (Right) Arc KO mice exhibit no changes in contralateral (C; n = 6; Day 0 = 112 ± 2.2 μV, 7 Day MD = 100 ± 6 μV, p>0.1, paired t-test) or in ipsilateral responses (I; n = 8; Day 0 = 96 ± 8.6 μV, 3 Day MD = 84 ± 10 μV, p>0.4, t-test). Averaged waveforms are shown at top. Error bars represent SEM.

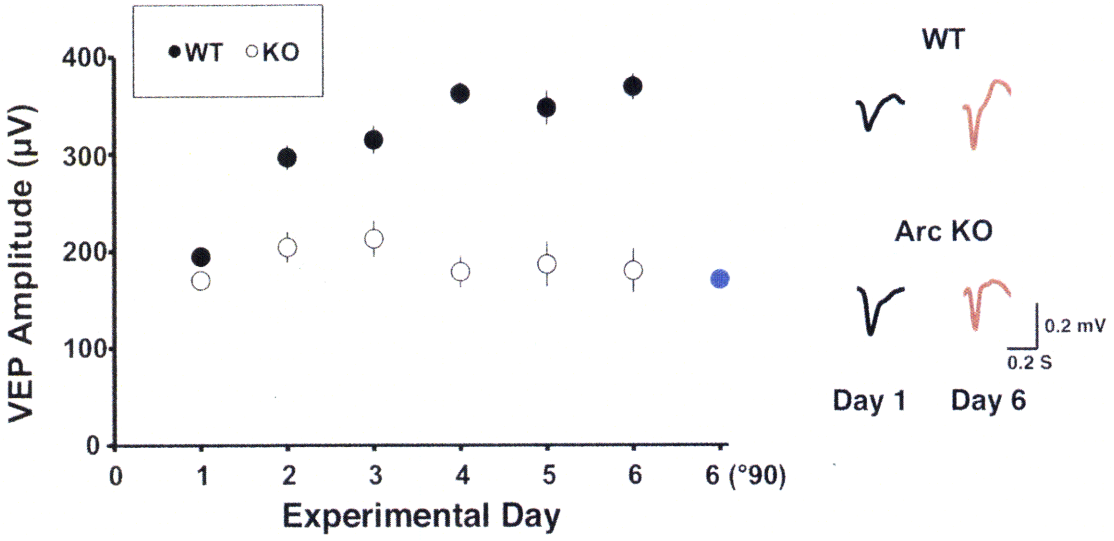


Figure 7. Arc KO mice do not exhibit stimulus-selective response potentiation (SRP) in visual cortex. WT mice exhibit large and sustained potentiation over many days of exposure to the same stimulus orientation (n=11). Responses to a control orthogonal stimulus (90°, blue circle) shown at day 6 were not significantly potentiated. Arc KO mice exhibit no significant potentiation of responses to the same stimulus (n-16). Responses to the control orthogonal stimulus (90°, open blue circle) were also not significantly different from baseline, suggesting no general decrease in responses over time.

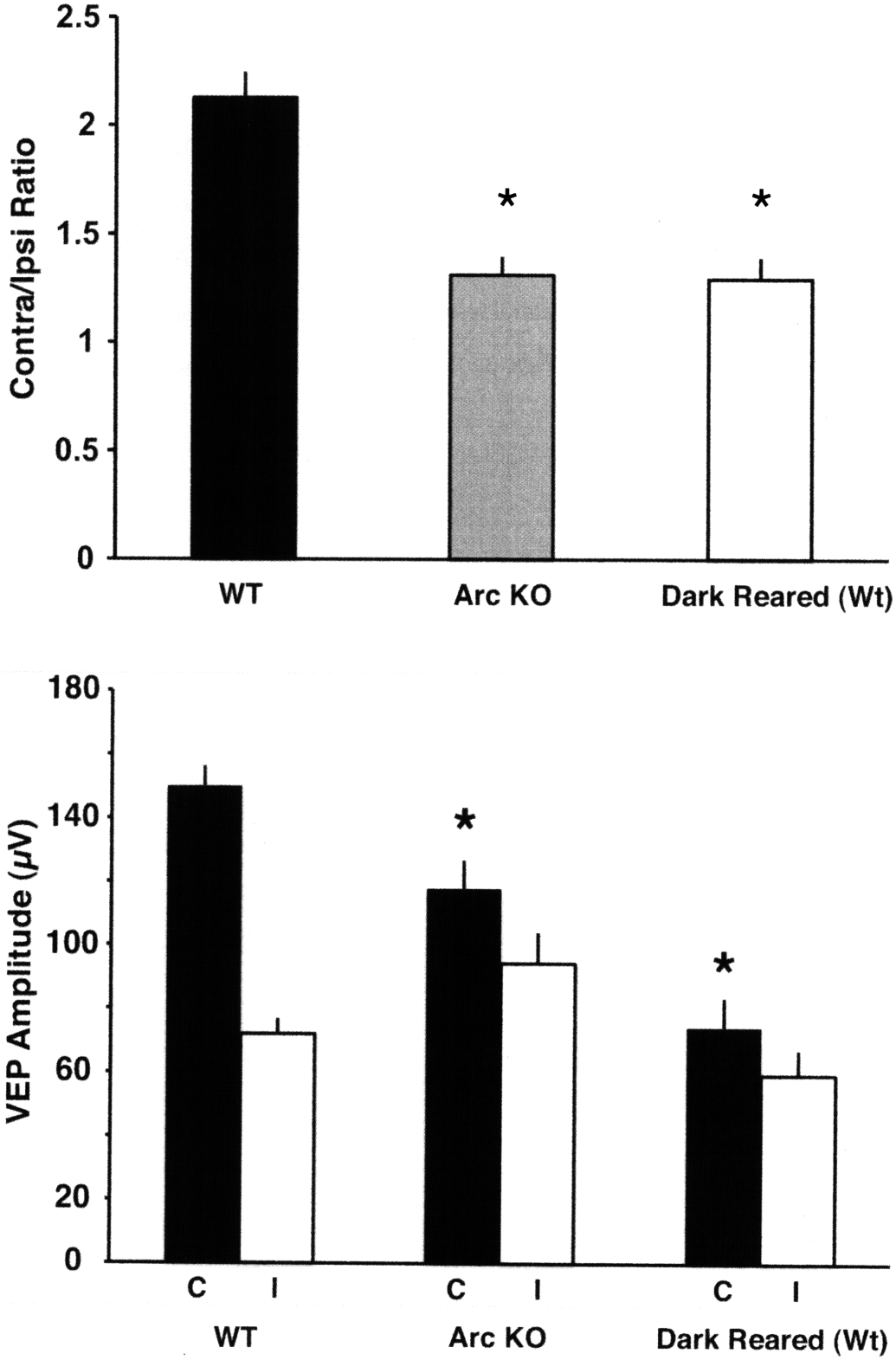
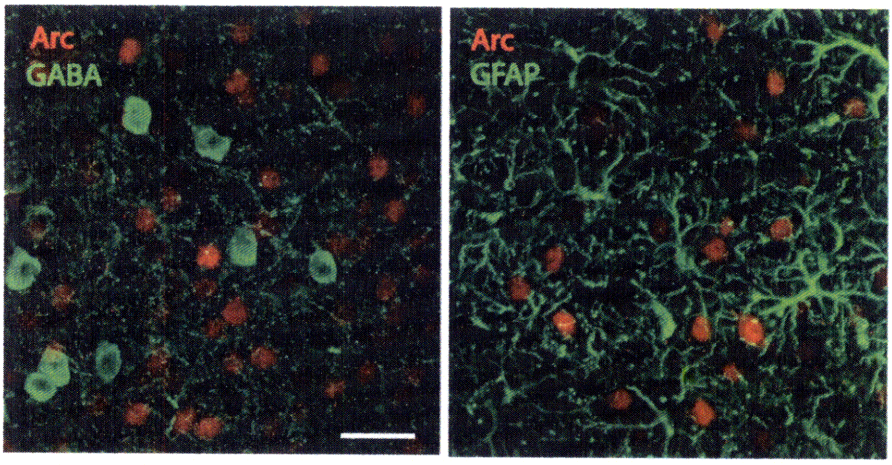

Contra/Ipsi Ratio
2.5
2
1.5
1
0.5
0
*
*
WT
Arc KO
Dark Reared (Wt)
VEP Amplitude (μV)
180
140
100
60
20
0
*
*
C
I
C
I
C
I
WT
Arc KO
Dark Reared (Wt)

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

SUPPLEMENTARY FIGURES

A



B

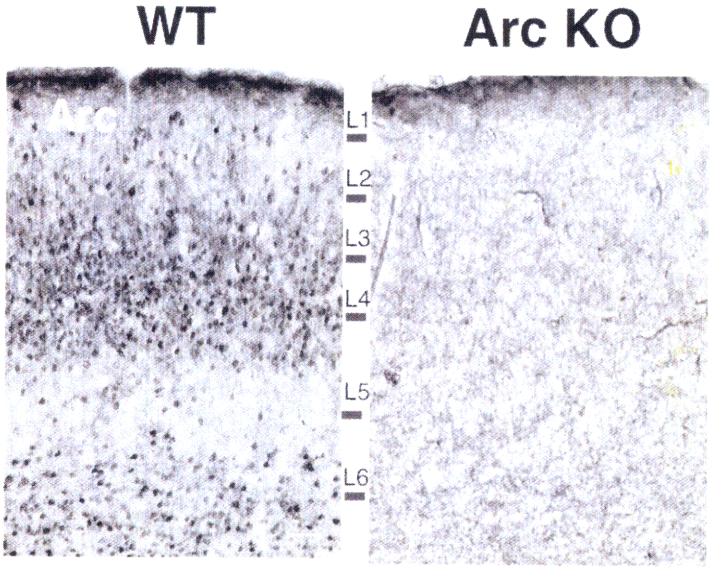


Figure S1. (A) Arc does not colocalize with GABA or GFAP. *Scale bar*=50 um. **(B)** Layer specific expression of Arc protein within the visual cortex of WT and Arc KO animals. Arc protein can be seen in layers 2-4, and 6. Only sparse labeling is detected within layer 5. As expected no Arc protein can be detected in mutant mice. *Scale bar* =50 um.

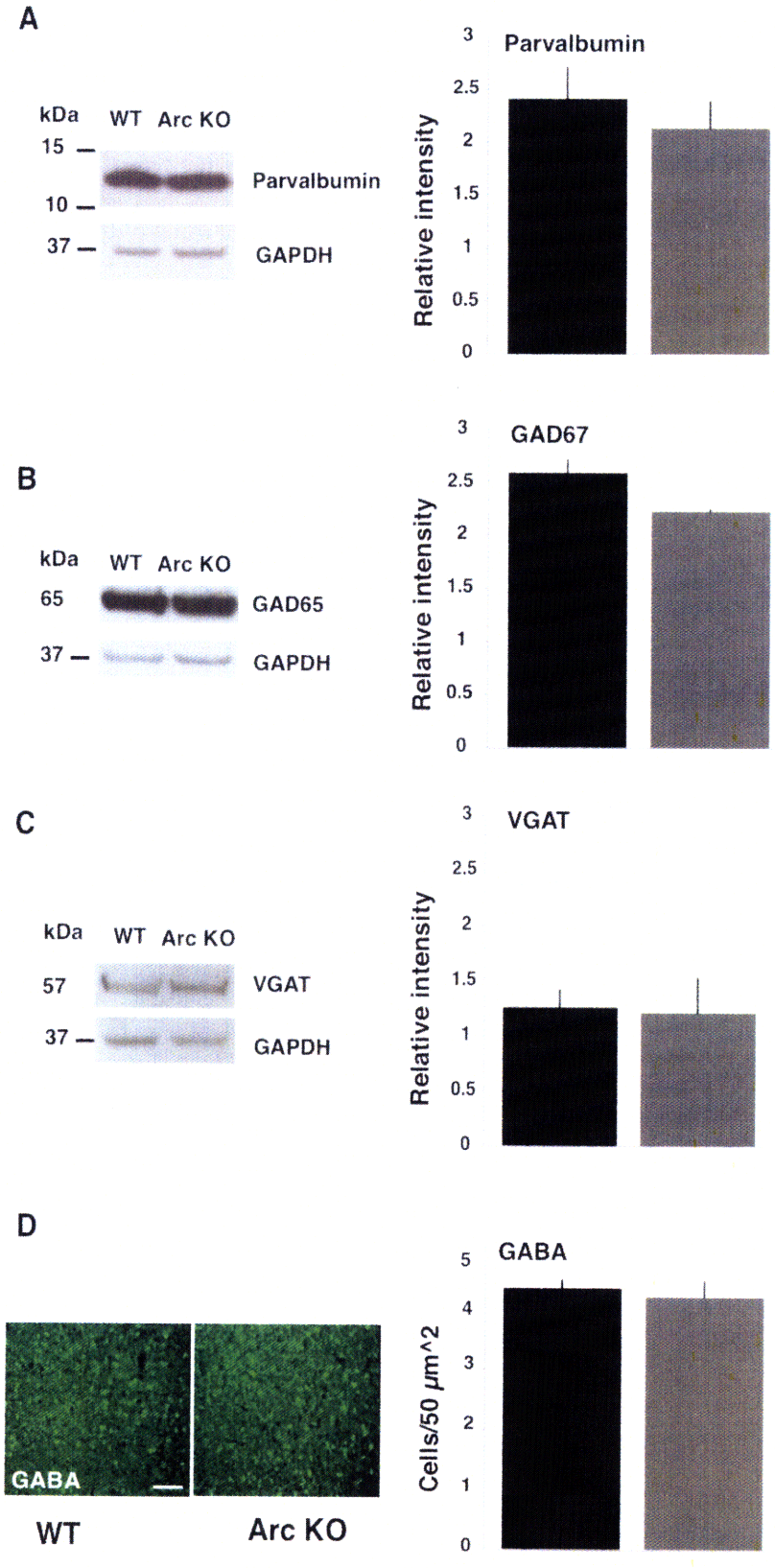

A
kDa
WT
Arc KO
15
10
37
Parvalbumin
GAPDH
Parvalbumin
Relative intensity
3
2.5
2
1.5
1
0.5
0
B
kDa
WT
Arc KO
65
37
GAD65
GAPDH
GAD67
Relative intensity
C
kDa
WT
Arc KO
57
37
VGAT
GAPDH
VGAT
Relative intensity
D
GABA
WT
Arc KO
GABA
Cells/50 μm^2
5
4
3
2
1
0

Figure S2. Quantitative Western blot analysis of inhibitory markers reveal no change in **(A)** parvalbumin ($p=0.54$, Student's t-test) **(B)** GAD65 ($p=0.18$, Student's t-test) **(C)** VGAT ($p=0.89$, Student's t-test) **(D)** or GABA within the visual cortex of WT (black bars) versus Arc KO (grey bars) animals (WT= 4.34±.22 cells/ 50 μm^2, Arc KO= 4.19±.12 cells/50 μm^2; $p>0.6$, Student's t-test). *Scale bar*= 50 μm.

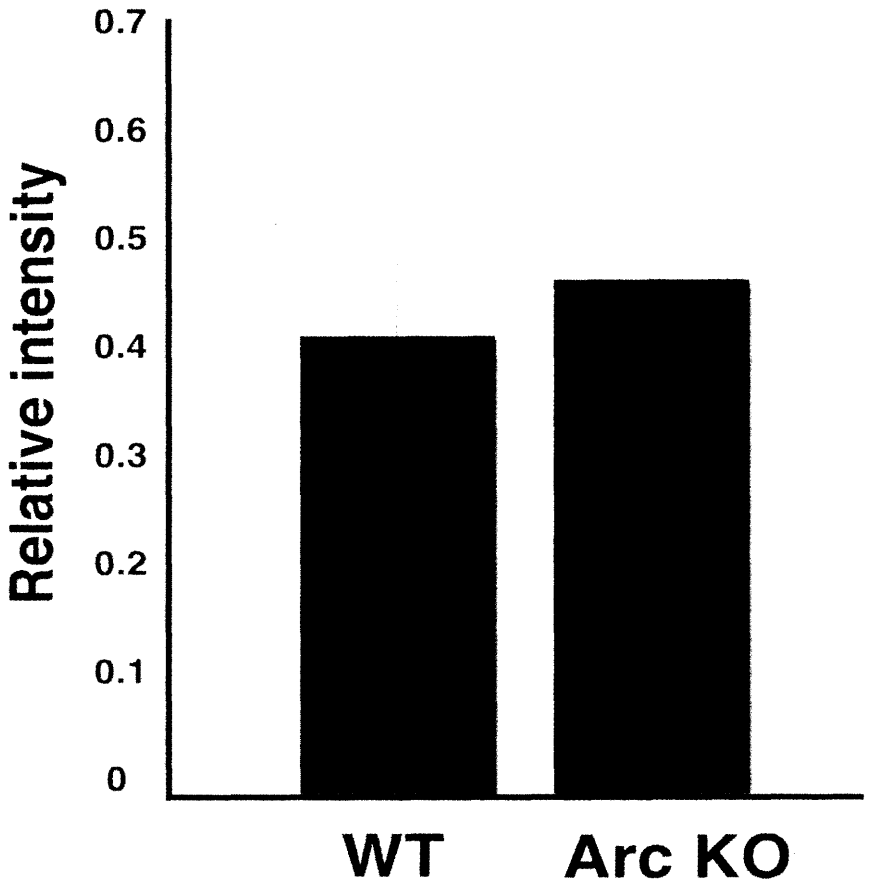


Figure S3. Surface expression of GluR1 in visual cortex of Arc KO critical period mice is not significantly different from WT (WT, n=5, Arc, n=7, p>.3, Student's t-test).

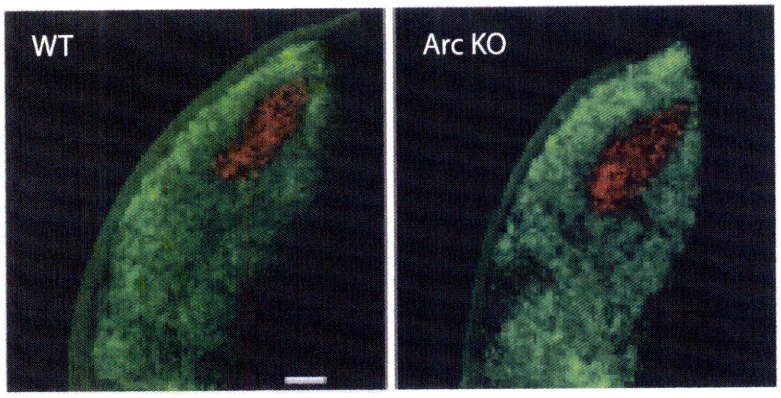


Figure S4. Tracing of the retinofugal pathway with cholera toxin subunit B conjugated to Alexa Fluor488 and 594, reveals normal eye specific segregation within the thalamus of Arc KO animals compared to control. Contralateral projections are labeled green, ipsilateral projections are labeled red. *Scale bar*=100 μm. Error bars represent SEM.

CHAPTER 3

DISRUPTED PLASTICITY IN ARC KO ADULT MICE

ABSTRACT

Sensory input is believed to be essential for the functional refinement of neuronal circuits in the mammalian visual system. Mice dark reared from birth display broader orientation tuning and a reduced ocular dominance ratio compared to normally reared mice. In addition, dark reared mice have a reduction in plasticity at the normal peak of sensitivity, and the critical period is shifted to adulthood. Interestingly, the cortical state of Arc KOs shares many similarities with that of dark reared mice i.e. reduced orientation selectivity, binocularity, and impaired juvenile plasticity. To determine whether plasticity is reinstated in Arc KO mice in adulthood we monocularly deprived WT and Arc KO adult mice (>P60) for 4-7 days. In keeping with the literature, we find that brief deprivation has no effect on the magnitude of eye-specific response in the visual cortex of WT mice. Similarly, ocular dominance remained at control levels in briefly deprived Arc KO adult mice. In response to 7 days of deprivation, a slight potentiation of open eye strength occurred in WT mice. However, reduced potentiation occurred in Arc KO mice. Thus, we find Arc protein is necessary for the expression of plasticity even in adulthood.

INTRODUCTION

Numerous experiments have demonstrated the remarkable ability of the brain to adapt to altered sensory experience. This plasticity is particularly salient in mice during a critical period early in development (P26-P32). Interestingly, the closure of the critical period is not as defined as previously thought, and there is now evidence to suggest that the adult visual cortex has the capacity to undergo significant plasticity. This plasticity has been detected using visually evoked immediate early gene expression (Pham et al., 2004; Tagawa et al., 2005), single-unit recordings (Gordon and Stryker, 1996), VEPs (Pham et al., 2004; Sawtell et al., 2003) and intrinsic signal imaging (Sato and Stryker, 2008).

However, the extent and quality of plasticity in adult mice differs significantly from that found in juvenile animals (Hofer et al., 2006). In juvenile mice ocular dominance plasticity occurs in two distinct stages 1) an initial depression of the deprived eye response after a brief period of lid suture followed by 2) an increase in open eye strength with extended deprivation. The initial phase is believed to be due to an LTD-like mechanism, whereas the open eye potentiation may rely upon an LTP-like strengthening (Frenkel et al., 2006) or synaptic scaling (Kaneko et al., 2006; Mrsic-Flogel et al., 2007). By contrast brief deprivation is ineffective in eliciting a significant depression of the deprived eye response in adult animals (Sato and Stryker, 2008; Sawtell et al., 2003). In addition, it is only with extended deprivation that a shift in ocular dominance occurs. This shift is mediated primarily by a potentiation of the open eye response.

The impact of monocular deprivation at various stages of development may be related to alterations in the overall cortical state. For instance, over development there is a maturation of inhibition (Fagiolini and Hensch, 2000; Hanover et al., 1999; Huang et

al., 1999) a down-regulation of CREB-mediated gene expression (Pham et al., 1999), and changes at the level of myelin (McGee et al., 2005), that may influence the plastic response. In addition, microarray studies indicate that there are distinct sets of visually regulated gene expression across the lifespan of the animal (Majdan and Shatz, 2006; Tropea et al., 2006). Many genes are only activated when monocular deprivation takes place during the critical period (Tropea et al., 2006). Thus the cortical response to deprivation may be drastically different depending upon the assortment of molecules present at each stage of development. In addition, impairments of plasticity in early development due to loss of a particular molecule may not be predictive of their impact in adulthood (Glazewski et al., 1996).

In Chapter 1 we showed that Arc KO juveniles fail to express normal ocular dominance plasticity after deprivation. In addition, another form of NMDA receptor dependent plasticity, stimulus response potentiation, is also impaired. Arc KO juvenile mice show a reduced contra/ipsi ratio that is similar to that found in dark reared animals suggesting that Arc is necessary for proper maturation of receptive field properties in visual cortex. However, little is known about visual cortex plasticity in adult Arc KO mice.

At present there has been only one study examining visual cortical response properties in adult knockout mice (Wang et al., 2006). These experiments revealed that Arc adult animals have reduced orientation selectivity compared to both Arc heterozygotes and WT mice. Because Arc protein has been implicated in regulating the internalization of AMPA receptors (Chowdhury et al., 2006; Rial Verde et al., 2006; Shepherd et al., 2006), this deficit is most likely due to a failure to remove weaker nonspecific inputs- resulting in a broadening of the tuning curve in the absence of Arc. The broadening of tuning found in Arc KO adults is highly reminiscent of that found in dark reared mice. Similar to Arc KOs, mice reared in darkness from birth -and where

Arc protein is low due to a blockade of activity- show extremely poor orientation selectivity compared normally reared mice (Fagiolini et al., 1994).

Along this same line, mice that have been dark reared from birth remain in a plastic state and the visual cortex remains sensitive to monocular deprivation even in adulthood (Fagiolini et al., 1994). More specifically, dark rearing appears to shift the entire critical period such that plasticity is reduced in these mice at the normal peak of sensitivity and increased at later ages. This result has been shown in cats (Mower et al., 1981) and mice (Iwai et al., 2003). Whether plasticity in Arc KO mice follows a similar progression in unknown.

In order to further probe the effect of loss of Arc protein later in development we assayed visual cortex plasticity in Arc adult mice using monocular deprivation. We were curious whether plasticity might be reinstated in adulthood. Thus, we deprived WT and Arc KO mice for brief and extended periods of time after P60 and examined ocular dominance plasticity. We find that plasticity remains impaired in the visual cortex of Arc null mice even in adulthood.

METHODS

Animals

WT (C57/Bl6) and Arc KO mice (Wang et al., 2006) on the same genetic background were used for all experiments (>P60). Mice were normally housed in cages under a 12 hour light-dark cycle. 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 (20μl/g) and the eyelid margins trimmed. The eye contralateral to the hemisphere being imaged was sutured using prolene sutures (Henry Schein) for 3-4 days. Animals were checked daily to ensure that the eye remained shut throughout the deprivation period.

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 (Tropea et al., 2006) Stimulation consisted of a drifting bar (9°X72°) 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 (Kalatsky and Stryker, 2003). 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.

RESULTS

The activity-dependent modulation of gene expression in response to changes in sensory input is a critical aspect of experience-dependent plasticity. The immediate-early gene Arc is extremely sensitive to such changes in input. Interestingly, Arc expression peaks during the critical period after which point it plateaus into adulthood (Chapter 1 of this thesis). Our earlier experiments showed that loss of Arc abolished plasticity during the peak of the critical period (Chapter 2). However, the influence of a gene in juvenile plasticity is often not predictive of its affect at later ages (Glazewski et al., 1996). Thus, it is possible that loss of Arc protein may not result in a similar deficit in adult mice. A further possibility is that the critical period for plasticity is merely shifted to a later point in development in Arc KO mice. Indeed, Arc KO mice share many similarities to mice that have been dark reared from birth and where plasticity only occurs later in development (Fagiolini et al., 1994; Iwai et al., 2003).

In adults, brief (3-4 day) monocular deprivation does not induce an ocular dominance shift (Gordon and Stryker, 1996; Sawtell et al., 2003). However, He et al found that ocular dominance plasticity occurs within the adult visual cortex when monocular deprivation is preceded by a period of dark rearing. In this study, a shift in ocular dominance could be detected in dark-reared WT mice after only 4 days of deprivation. This shift is mediated by a robust depression of the deprived eye response, similar to what is seen in juvenile animals, and a simultaneous potentiation of the open eye (He et al., 2006). To examine whether Arc KO mice show accelerated plasticity compared to WT mice, adult mice (>P60) were deprived briefly of vision through one eye and the hemisphere contralateral to this eye imaged to assess changes in response. In accordance with the literature, adult WT mice did not show a shift in ocular dominance at this time point (Figure 1B and C). In addition, the magnitude of eye-

specific response did not change (Figure 2A). Surprisingly, there was no significant shift in ocular dominance in Arc KO adult mice either (Figure 1B and D), ruling out the possibility that the immature cortical state of Arc KO mice might facilitate greater plasticity in adult mice. Both the deprived and open eye response remained unchanged (Figure 2B).

Interestingly, in Arc juvenile mice, plasticity is abolished in response to both brief and extended periods of deprivation. To test whether a longer deprivation period might drive plasticity in Arc KO adult mice, we extended the deprivation period to 7 days. In adult mice, extended deprivation is due to a delayed strengthening of the open eye response. This potentiation is thought to underlie adult ocular dominance shifts as very little deprived eye depression occurs at this stage (Sato and Stryker, 2008; Sawtell et al., 2003).

Both WT and Arc KO adult mice were deprived of one eye for 7 days and ocular dominance plasticity tested. An alpha value of .10 was used to assess significance and a one-way ANOVA performed using the same controls as those from the brief deprivation experiments. WT mice that were deprived for an extended period of deprivation show a significant shift in ocular dominance (Figure 3A and B). However, a significant shift did not occur in Arc KO mice (Figure 3A and C). In WT mice, the shift in ocular dominance was mediated by a weakly significant (Bonferroni correction, n.s.) increase in the open eye response strength (Figure 4A). We did not detect this potentiation after four days of deprivation, suggesting that the process occurs much more slowly than in juvenile mice. Interestingly, in the Arc KO mice, there was a trend towards an increase in the open eye response; however, this increase did not reach significance (Figure 4B). We were also curious whether a depression of the deprived eye response might be detected after 7 days in WT mice. There was no significant change in the deprived eye response compared to control (WT, control: $\Delta R/R = 2.6$, deprived: $\Delta R/R = 2.7$, $p = .99$, one-way ANOVA, $\Delta R/R \times 10^{-3}$), suggesting that in adult WT mice the shift in ocular dominance is

mediated purely by open eye potentiation. A decline in LTP occurs with age, and while present in young mice does not occur in the visual cortex of adults. Stimulus response potentiation (SRP) shares many features with LTP, and occurs in both juveniles and adult mice suggesting that mechanisms for response strengthening are present in mice (Frenkel et al., 2006). In addition, it will be of interest to determine whether open eye potentiation in the adult visual cortex is mediated by other mechanisms such as synaptic scaling (Kaneko et al., 2006). Interestingly, in Arc KO mice there was a small trend towards an increase in the deprived eye response that paralleled that seen for the open eye, however this did not reach significance (Figure 4B).

DISCUSSION

In this study we have examined ocular dominance plasticity in WT and Arc KO adult mice. We find that brief deprivation is ineffective in eliciting a shift in ocular dominance in either WT or Arc KO mice. In addition, while ocular dominance does shift in adult WT mice, there is a reduced shift in Arc KOs revealing that loss of Arc protein impairs experience dependent plasticity in both juvenile and adult mice.

However, the deficit in Arc KO juveniles appears to be much more extreme than that found in the adult mice suggesting that compensatory mechanisms may occur to a greater degree in adult KOs. In Arc KO juveniles, there is no deprived eye response depression after brief deprivation. In adult animals this depression is not expected to occur and was not seen in WT or Arc KOs. Thus, as expected there was no shift in ocular dominance in WT or Arc KO adults after brief deprivation. However, after extended deprivation, while there was no significant shift in ocular dominance in Arc KO adults, as found in WT adults, there was a slight trend towards a decrease.

Evidence from the literature has shown that extended deprivation causes open eye potentiation in both juveniles and adults. Indeed, in WT juveniles we find a

significant potentiation of the open eye response strength after deprivation. In juvenile Arc KOs this does not occur (see Chapter 2). However, in WT adult mice, we found only a weakly significant potentiation of the open eye response that appeared to underlie the significant shift in ODI in these mice. While not significant, there was a trend towards potentiation in Arc KO adults suggesting that some level of plasticity may take place. Interestingly, this trend towards potentiation also occurred for the deprived eye suggesting that in Arc KO adults other mechanisms not requiring Arc may mediate some plasticity.

It is interesting that the deficit in plasticity is much more extreme in Arc juvenile mice. Studies of plasticity in the somatosensory system have provided convincing evidence that molecules critical for plasticity in juveniles may not be important for adult plasticity and vice versa. Barrel cortex plasticity can be tested in a similar manner to that of the visual cortex; removal of vibrissae results in the depression of deprived cortical vibrissa response and a potentiation of the spared vibrissa response. Similar to the visual cortex this plasticity is greatest in juvenile mice, however adult plasticity does occur. In adult mice lacking CamKII-alpha, barrel cortex plasticity is severely impaired (Glazewski et al., 1996). Whereas the response to vibrissa removal is extremely significant in WT adults, homozygous adult mice do not show a shift in the vibrissa dominance index, and the response to the deprived vibrissa remains high. In addition, a potentiation and expansion of the spared vibrissa does not occur. Conversely, in juvenile mutant mice, barrels form normally, and there is no difference between receptive field properties of CamKII-alpha mutant mice and WT mice. In addition, plasticity is completely intact and potentiation of the spared whisker response and depression of the deprived whisker response take place normally.

What might underlie the trend towards potentiation in the Arc KO adult mice? Homeostatic synaptic scaling is a form of activity-dependent plasticity where the strength of individual synapses onto a cell are globally increased or decreased in

response to a reduction or an increase in activity, respectively. Interestingly, recent studies suggest that in addition to Hebbian forms of plasticity, these homeostatic mechanisms may underlie ocular dominance plasticity and facilitate the scaling up of response seen with longer periods of deprivation (Kaneko et al., 2006; Mrsic-Flogel et al., 2007). Indeed, synaptic scaling has been shown to operate in adult mice, however the mechanism for scaling appears to differ from that found in younger animals in that it may not be multiplicative in nature (Goel and Lee, 2007). As mentioned previously in Chapter 2, although a severe deficit in ocular dominance plasticity is present in juvenile Arc KO mice the synaptic scaling up of response after four days of deprivation remains intact (Supplementary figure). Whether intact synaptic scaling is responsible for the trend towards a potentiation of response strength in adult Arc KO mice is unknown. However the fact that there is a trend towards potentiation of the deprived and nondeprived eye, and that they appear to occur to the same degree suggests a scaling related mechanism. It will be interesting to examine whether scaling contributes to adult plasticity in future experiments.

Interestingly, in the adult hippocampus, plasticity is severely impaired in the absence of Arc (Plath et al., 2006). Arc knockout mice have an extreme deficit in both LTP and LTD. Knockout mice tested on a spatial learning task showed normal acquisition of the task, however a deficit appeared during the late acquisition phase where mice were trained to learn a new platform location. In addition, knockout mice tested on a fear conditioning task were able to form a short lasting association between a tone and a shock, however testing 24hrs later revealed a deficit in fear-related memory as measured by reduced freezing in response to the tone or a return to the initial training environment. WT mice, by contrast, showed significant freezing in response to the tone and placement in the initial context after 24hrs.

It is clear from our findings that plasticity in Arc KO mice remains significantly reduced compared to WT mice even in adulthood. However, the deficit in plasticity is

abated compared to younger mice. This result suggests, Arc may be most essential during the peak of the critical period for ocular dominance plasticity. At this point Arc expression is at its highest. After ~4 weeks of age there is a slight decline of Arc expression into adulthood. Therefore, in may be that loss Arc has the greatest impact and results in a more pronounced deficit in plasticity at early ages within the visual cortex.

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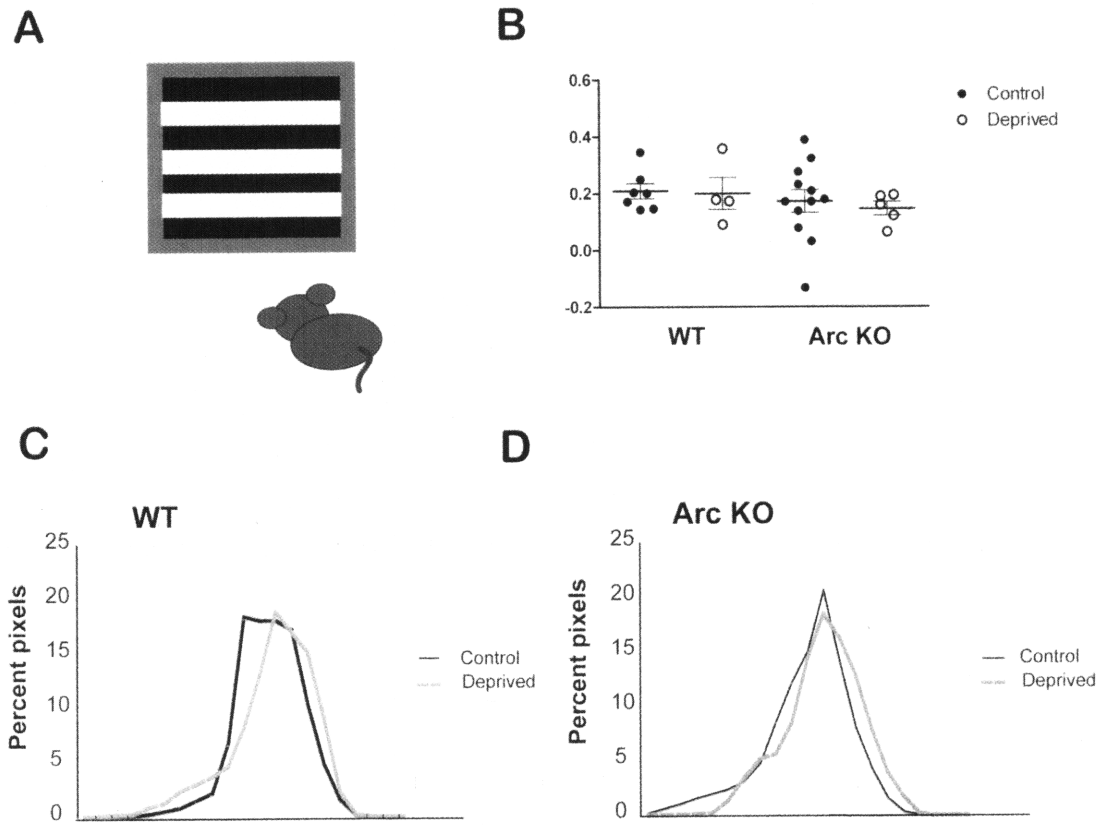


Figure 1. A. Schematic of the optical imaging setup. Control and deprived adult WT and Arc KO mice were placed ~20 cm away from a screen and shown drifting bars in either elevation or azimuth. B. ODIs for individual mice are shown as circles. Closed circles depict control mice, open circles deprived mice. Horizontal bars represent group averages. Four days of monocular lid suture were not sufficient to elicit a shift in ocular dominance in WT or Arc KO adult mice, (WT, control: n=7, ODI=.20±.02, deprived: n=4, ODI=.19±.05, p>.64, Student's t-test; Arc, control: n=12, ODI=.17±.03, deprived: n=5, ODI=.15±.02, p >.70, Student's t-test). Error bars represent SEM. C, Pixel distributions from binocular V1 of WT mice, and D. Arc KO mice. Each ODI distribution is derived as the mean of the population response over hundreds of pixels in each animal, plotted as the percent of pixels at each ODI.

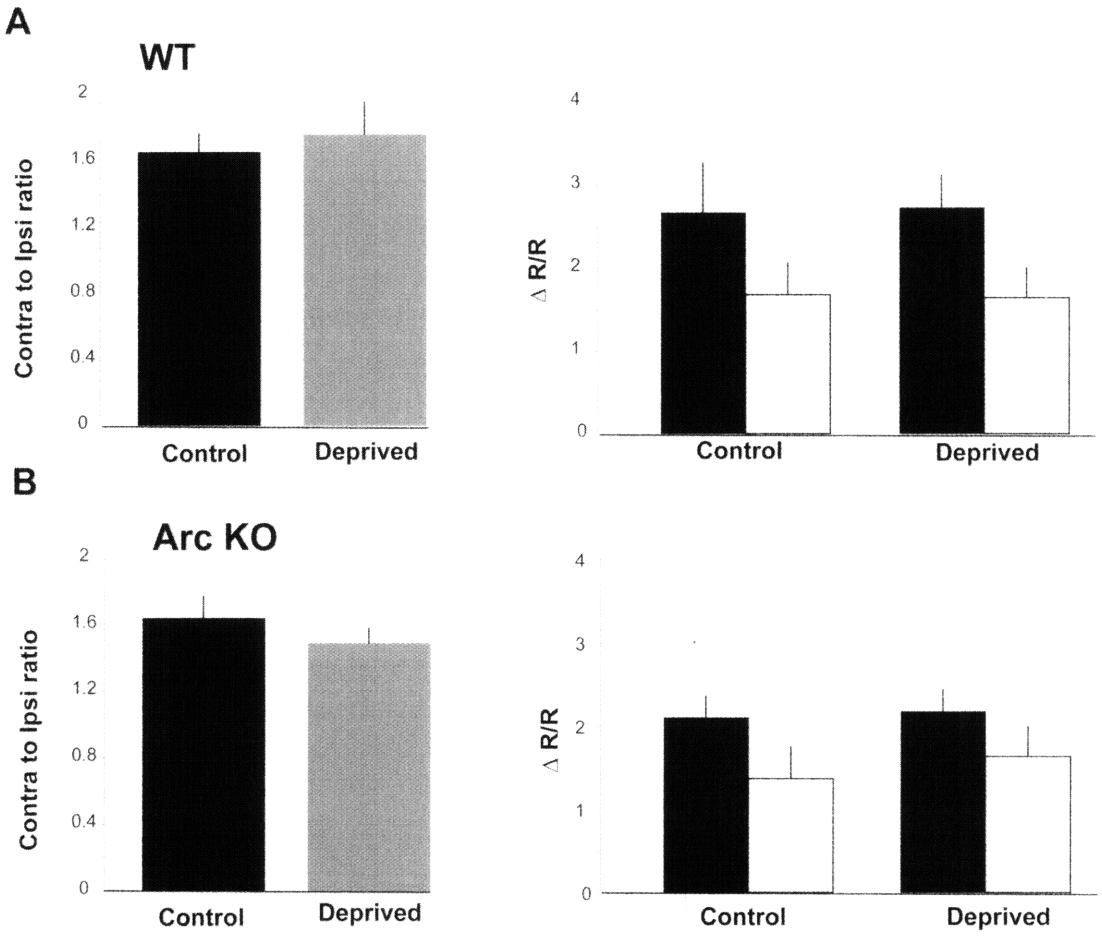


Figure 2. A. (Left) Ratio of contralateral eye response to ipsilateral response in WT animals, without and with MD of the contralateral eye. No change in the contra/ipsi ratio is seen (control=1.65±.10, deprived=1.75±.19, p>.3, Student's t-test). (Right) Response magnitude in WT animals driven by the contralateral eye (filled bars) and ipsilateral eye (open bars), plotted as average $\Delta R/R \times 10^{-3}$. Deprived eye depression does not occur in WT adult mice (control=2.6±.58, deprived= 2.7±.38, p > .6, Student's t-test). No change in the ipsilateral eye response is detected (control=1.6±.38, deprived=1.6±.35, p>0.8, Student's t-test). B. (Left) Ratio of contralateral eye response to ipsilateral response in Arc KO adult animals without and with MD of the contralateral eye. No change in contra/ipsi ratio is observed (control= 1.64±.13, deprived= 1.49±0.08, p>0.5, Student's t-test). (Right) No change in contralateral (filled bar) response occurs in Arc KO animals after deprivation (control= 2.1±.38, deprived= 2.1±.35, p>0.9, Student's t-test); similarly, no change in ipsilateral (open bar) response is detected (control=1.31±.24, deprived=1.61±.24, p>0.7, Student's t-test). All error bars represent SEM. $\Delta R/R$ is the change in reflectance over baseline reflectance.

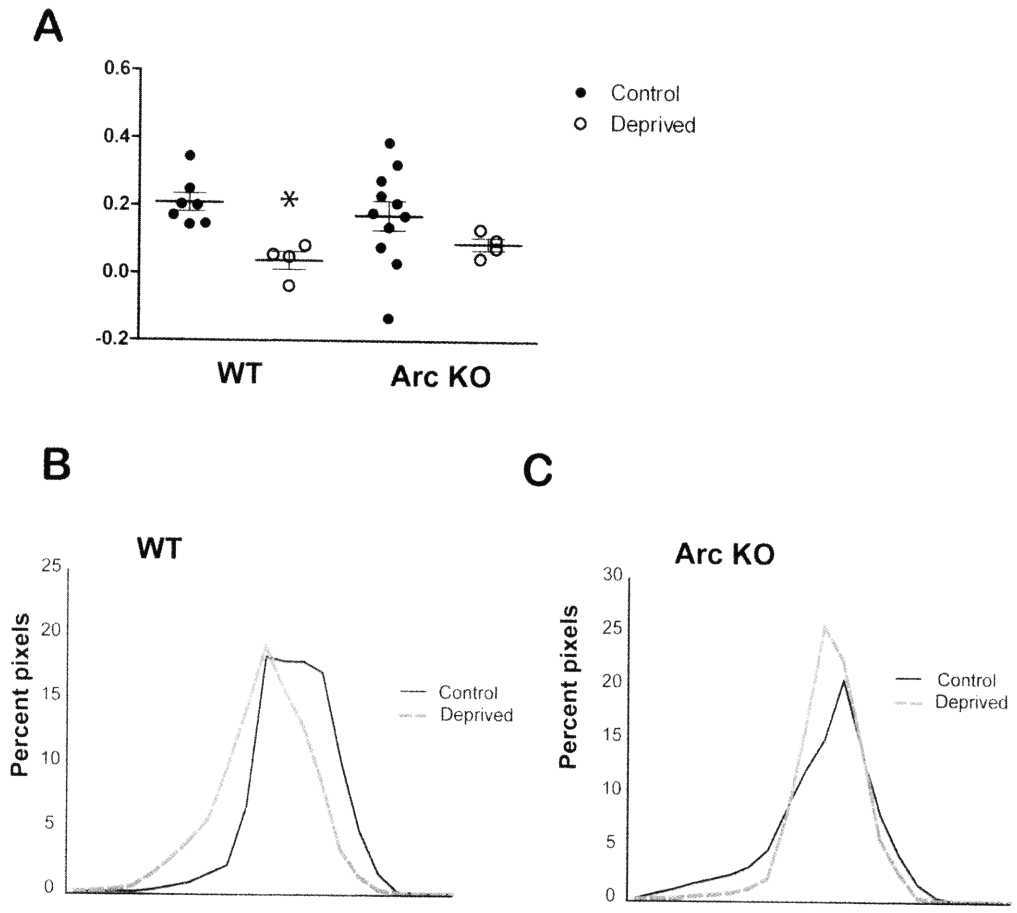


Figure 3. A. ODIs for individual mice are shown as circles. Closed circles depict control mice, open circles deprived mice. Horizontal bars represent group averages. Seven days of monocular lid suture resulted in a significant shift in ocular dominance in WT mice (control: n=7, ODI=.20±.02, deprived: n=4, ODI=.03±.01, p=.01). No significant shift occurred in Arc KO mice (control: n=12, ODI=.17±.03, deprived: n=4, ODI=.08±.02, p=.3747). Error bars represent SEM. B, C, Pixel distributions from binocular V1 of individual animals, as indicated at right. Each animal's ODI is derived as the mean of the population response over hundreds of pixels in each animal, plotted as the percent of pixels at each ODI. Statistical analyses in A) conducted using one-way ANOVA with Bonferroni correction.

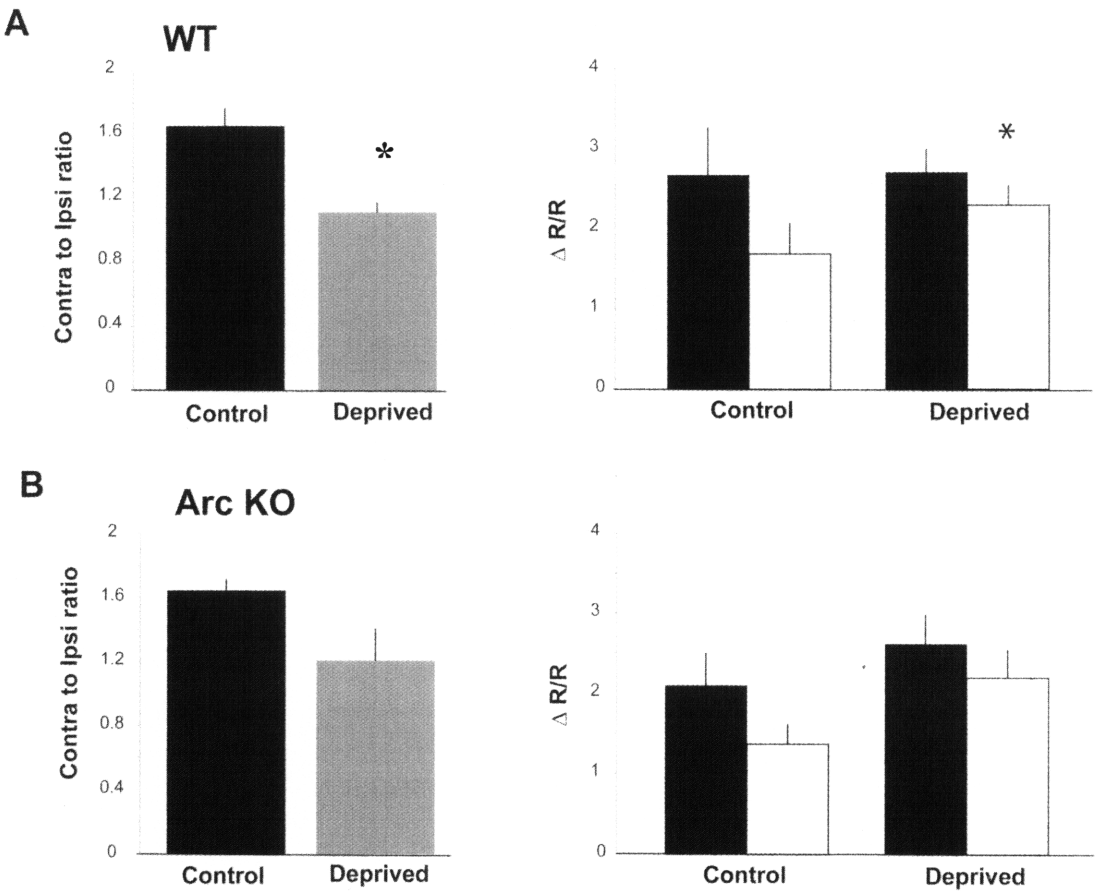
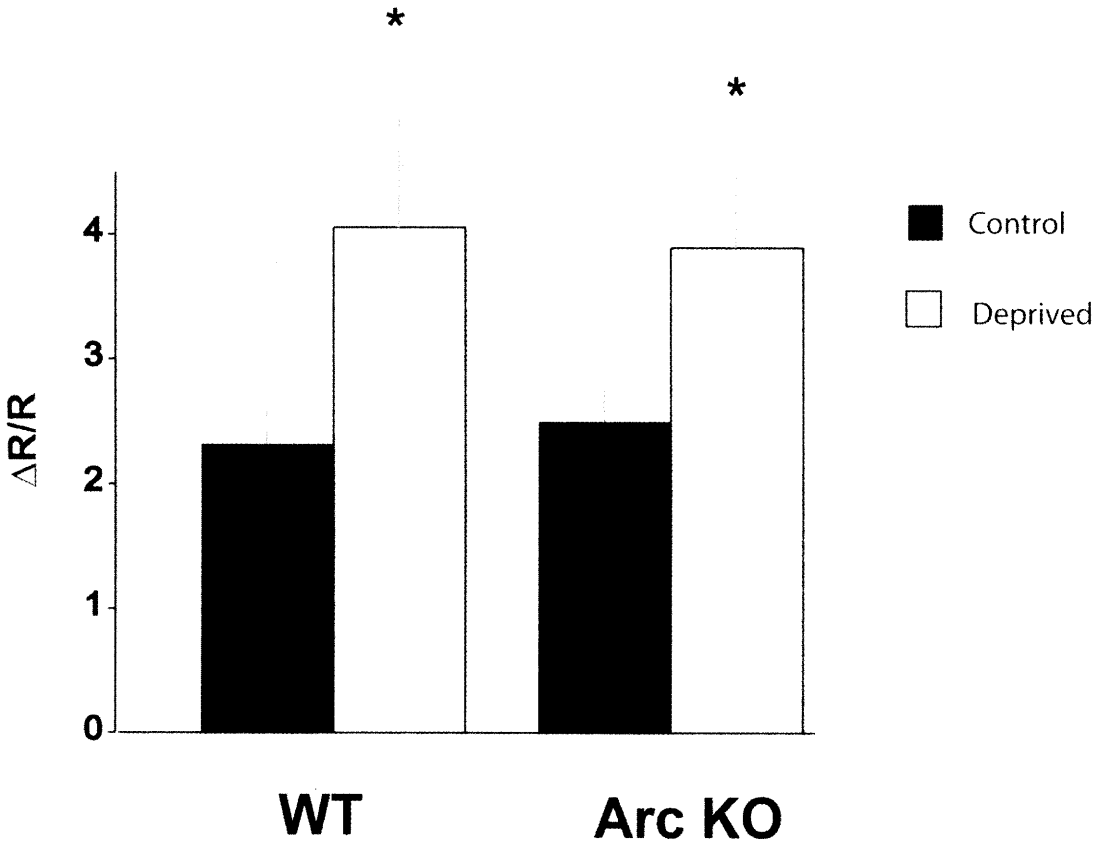


Figure 4. A. (Left) Ratio of contralateral eye response to ipsilateral response in WT animals, without and with MD of the contralateral eye. A significant change in the contra/ipsi ratio is seen after 7 days of monocular deprivation (control=1.65±.10, deprived=1.1±.05, p=.014). (Right) Response magnitude in WT animals driven by the contralateral eye (filled bars) and ipsilateral eye (open bars), plotted as average $\Delta R/R \times 10^{-3}$. Deprived eye depression does not occur in WT adult mice deprived for 7 days (control=2.6±.58, deprived= 2.7±.28, p=.99). However a weakly significant potentiation of the open eye response is detected (control=1.6±.38, deprived=2.2±.25, p=.07). B. (Left) Ratio of contralateral eye response to ipsilateral response in Arc KO adult animals without and with MD of the contralateral eye. No significant change in contra/ipsi ratio is observed (control= 1.64±.13, deprived= 1.21±0.06, p=.15). (Right) No significant change in contralateral (filled bar) response occurs in Arc KO animals after deprivation (control= 2.1±.38, deprived= 2.6±.35, p=.74); similarly, no change in ipsilateral (open bar) response is detected (control=1.31±.24, deprived=2.1±.35, p=.21). All error bars represent SEM. $\Delta R/R$ is the change in reflectance over baseline reflectance. Statistical

analyses in A) and B) conducted using one-way ANOVA (alpha=.10) with Bonferroni correction.



Supplementary Figure. Synaptic scaling up intact in Arc KO mice. WT and Arc KO animals were dark adapted for 4 days and reexposed to light. Intrinsic signal imaging was used to assess whether responses scaled up. Both WT and Arc KO mice display a significant increase in response (WT: control, n=6, $\Delta R/R = 2.3 \pm 0.27$; deprived, n=3, $\Delta R/R = .40$, p=.05, Student's t-test; Arc KO: control, n=10, $\Delta R/R = 2.4 \pm 0.31$; deprived, n=5, ODI = 3.89 ± 0.65 p=.02, Student's t-test). Error bars represent standard error of the mean.

CHAPTER 4

IN VIVO TWO-PHOTON FUNCTIONAL IMAGING OF IDENTIFIED SUBTYPES AND KNOCKOUT CELLS IN THE VISUAL CORTEX OF ARC-GFP MICE

ABSTRACT

Experience is essential for the sharpening of both eye-specific and orientation preference within visual cortex. A major question in neuroscience is how excitatory glutamatergic and inhibitory GABA-ergic cells uniquely contribute to these response properties, and how their responses differ during plasticity. Recent advances in the development of genetic mouse models provide an opportunity for more detailed inquiry into the individual cell types composing cortical circuits in mouse visual cortex. While techniques such as intrinsic signal imaging and visually evoked potentials serve as powerful tools for studying the overall population response of neural circuits, they lack single-cell resolution. In the case of single-cell recordings, although individual units can be isolated a major flaw is the inability to identify the exact cell type being recorded.

We have overcome the shortcomings inherent in these techniques by using functional two-photon calcium imaging in Arc-GFP mice, which allows for single-cell resolution and genetic identification of cell type. Heterozygous and homozygous Arc-GFP mice, in which GFP expression is placed under the control of the Arc promoter, were used to explore 1) the individual contributions of putative inhibitory and excitatory cell types to visual cortical response properties and 2) assay how genetic deletion of the Arc gene in GFP expressing excitatory cells influences response properties of these cells and their nearby neighbors not expressing GFP (putative inhibitory cells). We find that both juvenile heterozygotes and homozygotes juvenile mice display broad orientation tuning during the critical period. By contrast, ocular dominance, known to mature prior to orientation selectivity, differs significantly between Arc heterozygote and homozygotes. In response to 5-6 days of monocular deprivation, both GFP-positive and –negative cells in Arc heterozygotes show a significant shift in ocular dominance. However, in the Arc homozygotes, GFP-positive cells fail to shift. Surprisingly, GFP-negative cells show a normal shift in ocular dominance suggesting that plasticity in putative inhibitory neurons can operate independently of excitatory cell plasticity. To our knowledge, we provide the first in vivo calcium imaging data from the visual cortex of genetically identified knockout cells at single–cell resolution.

INTRODUCTION

A number of methods such as intrinsic signal imaging, visually evoked potentials, single-unit recordings, and calcium imaging exist for mapping out the functional and spatial organization of cortical areas. Each of these techniques affords a number of advantages and disadvantages depending on the ultimate question being analyzed.

In Chapters 2 and 3, I described experiments using intrinsic signal imaging to assay plasticity in genetically modified mice. Intrinsic signal imaging is a powerful tool for visualizing brain activity and provides high spatial resolution and reduced invasiveness compared to more traditional techniques. By visualizing changes in intrinsic optical properties, such as the light reflectance of neural tissues in response to activity, it is possible to obtain a measure of functional response to stimulation over large cortical areas in the same animal over time. At wavelengths greater than 600 nm, active cortical regions have a higher absorption coefficient than inactive regions, due to an increase in deoxy-hemoglobin in the former case. This depletion of oxy-hemoglobin is followed by a local increase in blood flow and a subsequent decrease in deoxy-hemoglobin that lowers the amount of red light absorbed (Malonek et al., 1997). Thus, small changes in light reflectance, due to the oxygenation state of the cortex, provide an indirect readout of local neural activity. While intrinsic signal imaging provides a reliable measure of cortical response, the primary weakness of this technique is that it lacks resolution at the single-cell level.

The cerebral cortex consists of two primary cell types, neurons and astrocytes. At the level of neurons a further division can be made between excitatory glutamatergic cells and inhibitory GABAergic cells. A major question in neuroscience is how these cell types function individually and in concert to shape response properties such as binocularity and orientation preference within the visual cortex. Classically, isolation of individual cell responses has been accomplished with extracellular single-unit recording. However, single-unit recording is not

without its own flaws- primarily the inability to reliably separate out responses from identified subtypes of cells.

To get a better understanding of the specific role these cell types play in experience-dependent plasticity it is helpful to be able to directly visualize them in vivo and analyze their functional properties. Bulk loading of calcium dyes provides an opportunity to circumvent the low functional resolution of optical imaging of intrinsic signals and detect individual cell response within a population of cells. By pressure injecting a membrane permeant fluorescent calcium indicator such as Oregon Green BAPTA (OGB) into brain tissue within mouse visual cortex, it is possible to measure neuronal response properties such as ocular dominance and orientation tuning, among others. In addition, by combining this technique with the use of genetically labeled mouse lines expressing GFP (or other fluorescent markers) in specific cell types, and the infusion of membrane permeable dyes such as sulfarhodamine 101 (SR101), which labels only astrocytes, it is possible to overcome the inherent shortcomings of single-unit recordings mentioned above.

In previous experiments mentioned in this thesis, we used Arc-GFP homozygous mice to test how loss of Arc impacts ocular dominance plasticity. In these mice, the coding portion of GFP has replaced that for Arc protein, and is under the control of the Arc promoter. Thus, in homozygous mice, activity results in GFP being expressed in cells that would have contained Arc. In the case of Arc-GFP heterozygotes, these mice have one copy of Arc and one copy of GFP. Interestingly, this results in only a 20% decrease in Arc expression that does not affect neuronal response, and the overall physiology of Arc heterozygotes is comparable to WT (Wang et al., 2006). Because Arc is found specifically in CamKII-alpha excitatory neurons, and inhibitory interneurons in the cortex do not express CamKII-alpha (Liu and Jones, 1996), the majority of GFP-negative neurons in the visual cortex of Arc-GFP mice are putative inhibitory cells. Thus, it is possible to use these mice in combination with two-photon functional calcium imaging, to visualize excitatory neurons directly, and to differentiate their spiking activity from that of inhibitory cells.

Arc containing neurons account for ~96% of excitatory CamKII-alpha positive neurons. There is evidence that Arc may facilitate the sharpening of response properties such as orientation tuning and ocular dominance throughout development. Arc expression in the visual cortex is intimately linked with neuronal activity, and it is dynamically regulated at the level of both mRNA and protein (Lyford et al., 1995). Recent reports in culture (Chowdhury et al., 2006; Rial Verde et al., 2006; Waung et al., 2008), and in vivo (refer to Chapter 2 of this thesis) show that Arc is necessary for normal removal of AMPA receptors from the surface. This role is accomplished through interactions with dynamin and endophilin; key components of the clathrin-mediated endocytosis machinery (Chowdhury et al., 2006). It is thought that Arc facilitates the global removal of AMPA receptors in response to activity such that total synaptic strength is reduced. Indeed, Arc has been implicated in a form of synaptic scaling wherein prolonged changes in activity result in a global weakening of the strength of all of a neuron's synapses to stabilize firing (Shepherd et al., 2006). The major expression mechanism of synaptic scaling is changes in the postsynaptic accumulation of AMPA receptors (Turrigiano, 2008). Interestingly, the sharpening of orientation tuning is thought to be due to a weakening of nonpreferred inputs, and this weakening most likely occurs at the level of AMPA receptors, as these receptors are crucial for the formation of orientation maps (Yu et al., 2008). While excitatory cells display orientation preference prior to eye opening, this tuning is relatively broad and gradually sharpens in an activity-dependent manner throughout development. However, few studies have investigated orientation selectivity in juvenile mice at the peak of the critical period. Interestingly, the expression of Arc steadily increases around this time point, paralleling this refinement process, and hinting at a direct requirement for Arc. In support of this conclusion, in Chapter 2, we found, using intrinsic signal imaging and VEPs, that the normal contralateral bias found in WT animals is moderately reduced in Arc KO mice, suggesting that loss of a putative mechanism for synaptic weakening may facilitate the retention of weaker ipsilateral eye projections and result in more binocular cells. In line with this, adult Arc null mice expressing GFP show an increase in the number of GFP-positive cells

with low orientation specificity and also broader tuning compared to heterozygous and WT mice (Wang et al., 2006).

Inhibitory interneurons comprise 20-30% of cortical neurons. Within the cortex the dendritic axonal arbors of excitatory and inhibitory cells are extremely intermingled. However, connections between neighboring inhibitory and excitatory cells occur in a preferential manner such that inhibitory cells connect most with those excitatory cells providing them with reciprocal excitation, and only maintain weak connections onto other pyramidal cells (Yoshimura and Callaway, 2005). In general, the specific subtype of interneuron determines inhibitory-excitatory connections and this connectivity allows for inhibition to play a strong role in shaping the receptive field tuning properties of excitatory neurons. While there are local circuit rules that govern the direct connectivity of both pyramidal cells and interneurons within the superficial layers, there is common excitatory and inhibitory input that arises from layer 5. It has been shown that the excitatory and inhibitory balance determines normal information processing within the visual cortex. Inhibitory neurons are critical for maintaining this balance and act to refine ongoing and evoked cortical activity within the circuit. A number of studies demonstrate that changes at the level of inhibition can drastically impact response properties such as orientation tuning and ocular dominance (Chen et al., 2008; Mitchell et al., 2007; Sillito, 1975). In the case of orientation specificity, removal of inhibition (by iontophoretic application of the GABA antagonist bicuculline) results in a broadening of the tuning curve (Jirmann et al., 2008; Sillito, 1975, 1977). In a similar manner to Arc, inhibition is thought to act specifically to hinder responses to nonpreferred orientations, resulting in a sharpening of orientation tuning. This finding suggests that Arc and inhibition may be two sides of the same coin and function separately to fine-tune response properties within the brain.

In addition to influencing orientation tuning, maturation of this cell type may mediate the close of the critical period. Ocular dominance plasticity is disrupted in mice that lack GAD65, one of two major isoforms responsible for GABA (Hensch et al., 1998). In mice overexpressing BDNF, the GABAergic circuit matures early, accompanied by an early opening

and closing of the critical period (Hanover et al., 1999; Huang et al., 1999) Similarly, direct infusion of the homeoprotein Otx facilitates maturation of parvalbumin cells, resulting in a precocious critical period and an acceleration of critical period closure. In addition, loss of Otx2 prevents maturation of parvalbumin cells and ocular dominance plasticity does not occur. These experiments suggest that alteration of one cell type can influence overall cortical plasticity.

Cell specific responses to monocular deprivation have been reported in slice and most recently in vivo. In cortical slice, visual deprivation results in a potentiation in the circuit between fast-spiking inhibitory cells and pyramidal neurons (Maffei et al., 2006). In addition, a recent paper investigating how monocular deprivation influences inhibitory cells found that inhibitory cells have a dramatic delay in plasticity compared to excitatory cells. While two days of monocular deprivation are sufficient to induce an ocular dominance shift in excitatory cells, it is not until four days of deprivation that a shift occurs in inhibitory cells. This finding lends support to the theory that a subset of cells in the visual cortex may guide plasticity. Indeed, at the level of laminar processing, studies in the ferret have shown that plasticity within layer 2/3 precedes that of layer 4 (Trachtenberg et al., 2000). As shown in Chapter 1, loss of Arc results in deficits in both ocular dominance plasticity and SRP. Whether reduced plasticity in Arc lacking excitatory cells propagates throughout the visual circuit to other cell types remains to be seen.

In order to better understand the role of Arc at a single-cell level, and to study inhibitory contributions to plasticity, we decided to use in vivo calcium imaging of juvenile Arc-GFP mice to probe OD and orientation tuning at the peak of the critical period. This technique provides an excellent opportunity to test receptive field properties in identified cell types and to further explore how loss of a gene in identified cells affects processing in neighboring cells. Using this technique it is possible to assay visual response in four classes of OGB filled neurons: GFP- and SR101-negative cells (inhibitory), GFP-negative and SR101-positive cells (astrocytes), GFP-positive and SR101-negative cells (excitatory Arc-containing cells in heterozygotes), and GFP-positive and SR101-negative cells (excitatory Arc-lacking cells in homozygotes). While we

were interested in differentiating response properties in both excitatory and inhibitory neurons, we were also curious to see how genetic knockout of Arc and the resultant reduction of plasticity in excitatory cells affected overall plasticity in inhibitory cells.

We find that at the peak of the critical period, ocular dominance in both GFP-positive and –negative cells of Arc heterozygotes, and GFP-negative cells of Arc homozygotes, displays an adult contralateral bias. However, Arc lacking, GFP-positive cells do not show this contralateral bias and the majority of cells respond equally to both eyes. By contrast, we find that critical period orientation tuning is broad in both GFP-positive and –negative cells of Arc heterozygotes and homozygotes, suggesting that sharpening of orientation selectivity may occur at a slower rate compared to ocular dominance. In response to 5-6 days of monocular deprivation, both GFP-positive and –negative cells in Arc heterozygotes show a significant shift in ocular dominance. However, in the Arc homozygotes, GFP-positive, Arc-negative cells fail to shift. Surprisingly, GFP-negative, putative inhibitory cells show a normal shift in ocular dominance suggesting that plasticity in this cell type can operate independently of excitatory cell plasticity.

METHODS

Mice used

All experiments were completed according to established protocols and adhered to CAC guidelines. Arc homozygous (n=5) and Arc heterozygous (n=5) mice (postnatal day 26–32) were used for all experiments.

Lid suture

Mice were anesthetized with Avertin (20 μl/g). In addition, the analgesic Buprenex (1μl/g) was administered post surgery. The eyelid margins contralateral to the hemisphere being imaged were trimmed and the eye sutured shut using 2-3 horizontal mattress sutures. Mice were deprived for 5-6 days. Eyes were reopened on the day of surgery prior to craniotomy.

Dark adaption

Prior to experimentation, mice were dark-adapted for ~12 hours. Mice were then removed from the darkness and placed in a stimulation box and exposed to light (~250 lux) to drive GFP expression.

Immunohistochemistry

Sections were prepared from WT or Arc-GFP mice adapted in darkness overnight. Mice were exposed to light for 2 hrs as above. Mice were then perfused with saline and 4% PFA, and the brain removed and placed in 20% sucrose O/N. Brains were sectioned at 50 microns, and in the case of WT brains double-labelled using antibodies again Arc (Santa Cruz, Ms) and GFAP (Chemicon, Rb) or GABA (Chemicon, Rb). To examine colocalization between Arc and markers for inhibitory cell types, Arc-GFP mice were labeled with an antibody for GABA alone and a

secondary antibody tagged with Alexafluor 594. Colocalization was defined as those cells containing direct GFP fluorescence within the nucleus of GABA stained cells.

Anesthesia

Mice were anesthetized with a cocktail of Atropine (.001/g), Fentanyl (0.05 mg/kg), Midazolam (5.0 mg/kg), and Medetomidin (0.5 mg/kg) delivered i.p.. Mice were reinjected with a maintenance solution containing Atropine, Fentanyl and Medetomidin every hour s.c. In addition, all experiments were supplemented with isofluorane (approx .25%-.5%).

Surgery

A heating blanket was used to maintain body temperature at 38°C (monitored using a rectal probe). Eyes were protected and kept moist by a thin layer of eye ointment. Lidocaine was injected into the scalp over visual cortex and an incision made to expose the skull. The thin membrane over the skull was scraped away and the surface washed with saline. The skull was then affixed to a custom designed head chamber. A craniotomy was made over the binocular zone of visual cortex. This region was determined using stereotaxic coordinates (~3-3.3 mM lateral from midline). The skull was kept moist by bathing the surface with a solution of cold ACSF. A dremel drill was used to thin a rectangular area over the binocular zone and the bone was gently removed using sharp forceps. To facilitate matching of GFP and calcium dye loaded cells, Sulfarhodamine 101 (SR101) was placed on the cortex and allowed to be taken up for ~5 mins, and then gently rinsed away with warm ACSF. A thin layer of 2% agarose in ACSF was applied over the exposed region.

Injection of Oregon Green and Two-Photon Imaging

Pipettes were prepared using a pipette puller from Sutter Instruments (Model #P80). Oregon Green Bapta AM (OGB) was dissolved in 4ul of 20% DMSO and ~2 ul was loaded into the pipette for injection. Prior to insertion of the pipette, an optimal area within the binocular zone containing bright GFP positive cells was determined and the filled pipette lowered ~200 um below the cortical surface using fluorescent light to guide the injection. A z-stack of Arc-GFP positive neurons (excitation wavelength: 950 nm) was taken in order to align and identify cells after calcium imaging. A bolus injection of the dye was then made using a picospritzer (~1-5 psi for 2.5 minutes) such that a 300 um x 300 um area was filled. To ensure labeling of the binocular zone the dye was injected in the center of the stereotaxically determined binocular region.

The activity of OGB filled cells was imaged at an excitation wavelength of 950 nm (~35mW) using a custom-built microscope and a 20X water immersion objective and the response to visual stimulation analyzed. Imaged acquisition was conducted using freeware from the Svoboda lab.

Visual stimulation

For both orientation tuning and ocular dominance an episodic stimulus was used. In the case of orientation tuning, we stimulated the cortex using gratings which ranged from 0° to 359° in 20° steps. Single gratings were presented for four seconds on followed by a four second off period. A total of 18 orientations were presented. The orientation selectivity index (OSI) was calculated using the vector averaging method, and the preferred orientation was determined from the angle of the mean orientation vector (Dragoi et al., 2000).

For the ocular dominance study we presented random orientations ranging between 0° to 359° in 20° steps. The stimulus was presented with eight seconds on and eight seconds off, totally five repetitions of random gratings.

Data Analysis

A z-stack of GFP-positive cells was taken prior to injection of OGB. After injection, a z-stack was also taken of cells filled with OGB. This z-stack was taken so that calcium responses from GFP-positive and –negative cells could be determined post hoc. To facilitate matching, astrocytes contained within the GFP and OGB stacks were used as landmarks. In most cases, due to a slow infusion of OGB (see above), there were only minimal distortions in the location of cells before and after injection. All matching was done manually, and analyzed using Image J and Photoshop.

All images obtained during stimulation were analyzed using custom programs written in Matlab. In cases where movement artifact impaired analysis, image stabilization plugins in ImageJ were used to realign individual frames. We identified and circled cells manually to define the region of interest (ROI). Only cells with ΔF responses that were clearly distinguishable from the neuropil were chosen for subsequent analysis. Individual pixels enclosed within a cell's ROI were averaged to get a single time course. To determine the response amplitude, we subtracted both dark noise and background and then calculated the mean response across the stimulus period to determine $\Delta F/F$. We determined the baseline by taking the mean of the four frames immediately preceding stimulus onset.

RESULTS

GFP-positive cells in Arc-GFP mice serve as a marker for excitatory cell types

The visual cortex is home to two groups of neurons- excitatory and inhibitory cells. As mentioned above, each of these cell types plays a critical role in synaptic plasticity. A primary goal of current neuroscience research is to isolate the contributions of these cell types to overall neural activity. In support of this aim, a number of transgenic mouse lines are available which target specific subtypes of cells. One such line is the Arc-GFP knockin mouse (Wang et al., 2006). To identify excitatory neurons we used Arc-GFP mice in which GFP is under the control of the Arc promoter. The immediate early gene Arc serves as a reliable marker for excitatory neurons (Figure 1 A and B). Previous reports have shown that the mRNA of Arc does not colocalize with astrocytes or GABA-positive neurons in visual cortex (Figure 2) (Tagawa et al., 2005). However, one study examining Arc expression in the forebrain found that while Arc was found exclusively in non-GABAergic -CaMKII-positive hippocampal and neocortical neurons, some GAD65/67-positive neurons in these regions were observed to express Arc, but only after electroconvulsive seizure (Vazdarjanova et al., 2006).

To confirm the specificity of Arc protein to excitatory neurons, we first examined the visual cortex expression of Arc in combination with GFAP, a marker for astrocytes, and GABA, a marker for interneurons, using double immunofluorescent staining. We found no colocalization between Arc and either astrocytes or inhibitory cells (Figure 1A). In addition, we further confirmed that Arc and GABA do not overlap by examining colocalization of direct GFP fluorescence and GABA protein in Arc-GFP mice. As expected, no overlap was seen, confirming the specificity of Arc for excitatory cell types and proving the feasibility of using Arc-GFP expression to facilitate differentiation of cell types in vivo (Figure 1B).

Stimulating Arc expression and loading of OGB

In visual cortex there is a low basal level of Arc protein within the superficial layers. Thus caged Arc-GFP mice have very low GFP expression at any given time. In order to drive expression of GFP and visualize excitatory cells, mice were placed in a light sealed room overnight for ~18 hours prior to experimentation. Mice were then removed from the darkness and placed in a well-lit box and allowed to move around freely for ~ 15mins. After this point, mice were removed from the box and anesthetized. While the anesthesia took affect, mice received continuous visual stimulation. A craniotomy was then made over the visual cortex and a custom designed head plate was affixed to the mouse prior to being placed under the two-photon microscope for imaging (Figure 3A and B). In order to visualize astrocytes in addition to neurons, SR101 was placed on the surface of the cortex and allowed to be taken up for ~ 5 mins and then the cranial window was sealed completely with agarose. Mice were continuously stimulated throughout this process.

The binocular zone of mouse visual cortex receives input from both eyes. Because we were interested in assaying ocular dominance plasticity, in addition to orientation, all experiments were targeted to this region. The binocular zone of the visual cortex is readily identified using stereotaxic coordinates (~3mm from the midline). Using these coordinates we were able to reliably isolate the binocular zone in each experiment. Localization to the binocular zone was confirmed by the presence of a response to ipsilateral eye stimulation. After finding a region within the binocular zone relatively devoid of vasculature, we used two-photon imaging to examine the expression of Arc-GFP cells. We found, in agreement with a previous study (Wang et al., 2006), that there was no difference in GFP fluorescence in Arc homozygous or heterozygous mice. In some cases, it was necessary to adjust our location due to shadows from surrounding blood vessels, which reduced the optics and thus impaired visualization of GFP cells. Once an optimal imaging region was determined a pipette was lowered into the brain at a

depth of ~150 μm. The majority of our imaging took place between depths of 150-250 μm. This depth corresponds to layer 2/3 of visual cortex. In general we found that beyond a depth of ~250-300 μm light scattering impaired fluorescence detection.

One concern from our experiments was that the anesthesia might interfere with the expression of GFP. However, we found that by continuously stimulating the mice we could reliably see bright cells after 2 hrs. In addition, as mentioned above we saw no significant difference between overall GFP fluorescence in Arc homozygotes and heterozygotes as reported previously (Wang et al., 2006).

Because there is an overlap in the emission spectra of OGB and GFP, we relied upon post hoc matching of z-stacks taken before and after injection of OGB to determine the subtype of individual neurons. Cells that were positive for GFP prior to injection, and filled with OGB but lacking SR101 were classified as excitatory cells. Those cells that were negative for GFP, negative for SR101, and filled with OGB were classified as inhibitory cells. In our experiments this proved to be a reliable method for determining the cell type (Figure 3C). However, in early experiments we realized that rapid infusion of OGB often led to distortions of the surrounding tissue that impaired matching of cells taken prior to infusion. To overcome this, we reduced the infusion rate of OGB into the cortex. In addition to improving post hoc matching of the images, we found that slower infusion of OGB improved the overall cortical health of the mice and resulted in an improvement in spiking activity. In most experiments we were able to load a region of approximately 200 to 300 um^2. This resulted in labeling of approximately 50-100 cells/mouse.

Inhibitory neurons comprise between 20-30% of all neurons. We found that in most experiments 33-35% of neurons that took up OGB lacked GFP-expression-in a few animals this increased to ~40%. While this result is slightly higher than what is found within the literature, it suggests that while all GFP- positive cells are excitatory, there is a small population of cells that are GFP-negative which may also be excitatory and counted as inhibitory in our analysis.

Broad orientation tuning in GFP-positive and –negative cells of juvenile Arc heterozygotes and homozygotes

Using Arc-GFP heterozygote mice we asked whether orientation tuning was different in GFP-positive (putative excitatory cells) versus –negative neurons (putative inhibitory cells). We stimulated Arc heterozygous mice using gratings at eight different orientations (separated by 20° steps), and drifting in two different directions. We then measured changes in the fluorescence within individual cells in response to stimulation to readout the evoked calcium response (~1frame s^{-1}) (Figure 4A1 and A2). We found no significant difference in the distribution of orientation selectivity in GFP-negative cells and GFP-positive cells (Figure 5A; Kolmogorov-Smirnov test, $p>.2$). GFP-positive cells range from .493 (tuned) to .02 (poor selectivity). The mean OSI was .105 ± .008 (SEM). Similarly, we failed to find GFP-negative neurons with OSI values higher than .336, and the mean OSI was .100 ± .01 suggesting that during the critical period the majority of neurons within visual cortex are only mildly orientation selective (Figure 5B). It should be noted that a portion of the cells identified as GFP-negative may contain excitatory cells and it is possible that higher OSIs attributed to GFP-negative cells may actually be excitatory (see above). Those cells with OSI values less than .1 were considered to be poorly selective in our study. Interestingly, 62% and 46% of the cells found in GFP-negative and GFP-positive neurons respectively, of heterozygotes fell into the category. This percentage correlates with the percentage of neurons found to be broadly tuned in previous single unit studies (Hubener, 2003).

Next, we looked at orientation tuning in Arc homozygous mice (Figure 4B1 and B2). A previous report showed that Arc adult mice have reduced orientation selectivity compared to Arc heterozygous and WT mice. We wanted to see whether we could recapitulate this finding at a single cell level in critical period mice. Examples of typical responses from GFP-positive and –negative cells are shown in (Figure 4B1 and B2). We found that the distribution of OSIs did not differ between GFP-positive cells in the Arc homozygotes compared to the heterozygotes

(Figure 5A and B; Kolmogorov-Smirnov test, p>.1). The mean OSI in GFP-positive cells was .117 ± .008. OSI values ranged from .03-.305 (Figure 5C). As a further analysis, we examined orientation selectivity in GFP-negative cells in these mice. The range of OSIs found in the GFP-negative cells were similar to that found for GFP-positive cells (.03-.2182) with a mean of .104±.008 (Figure 5C). In addition, like the GFP-positive cells the overall distribution was shifted towards lower OSIs (Figure 5B; Kolmogorov-Smirnov test, p>.5). Overall, we detected no significant difference between GFP-positive and –negative cells in Arc heterozygote and homozygote mice (Figure 5C; one-way Anova, $F(3, 245) = .55$, $p = .64$). These findings suggest that differences in OSIs between Arc homozygotes and heterozygotes, in addition to subtypes of cells may not manifest until later in development.

Reduced contralateral bias in GFP-positive cells in Arc homozygotes

We next characterized the eye-specific response within the binocular zone of visual cortex. We took heterozygous and homozygous mice that were at the peak of the critical period and assayed ocularity in GFP-positive and GFP-negative cells. The ocular dominance score was calculated as IPSI/(IPSI+CONTRA), where a score of 0 indicates a predominantly contra response and a score of 1 indicates that a cells is exclusively driven by the ipsilateral eye. A value of .5 indicates that a cell is equally responsive to both contralateral and ipsilateral stimulation (Figure 6). We find that both GFP-positive and GFP-negative cells have similar distribution of ocular dominance in Arc heterozygote mice (Figure 7A and 7B; p>.4, Wilcoxon ranksum test) . While many cells within the binocular zone of heterozygous mice responded equally to both eyes, there was a significant contralateral bias. In addition, the average ocular dominance score agrees with that found in the literature using both intrinsic signal imaging and VEPs (Frenkel and Bear, 2004; Kalatsky and Stryker, 2003).

In our earlier study we found that Arc homozygotes had a trend towards more binocular ocular dominance compared to their wild-type counterparts (Chapter 2 of this thesis). We wanted to ask whether this also occurred at the level of single cells and if it was specific to

Arc lacking neurons. In agreement with our previous study, there was a significant shift in ocular dominance towards more binocular ocular dominance in GFP-positive cells within the Arc homozygotes (Figure 8A, $p<.01$, Wilcoxon ranksum test). However, GFP-negative cells in the Arc homozygote mice displayed a normal ocular dominance distribution as seen in heterozygotes with a trends towards a contralateral bias (Figure 8B).

Impaired ocular dominance shift in GFP-positive cells in Arc homozygotes

Next, we wanted to investigate how monocular deprivation might differentially affect plasticity in GFP-positive versus –negative cells. A recent study showed that inhibitory cell plasticity proceeds more slowly than excitatory cell plasticity, suggesting that inhibitory cell plasticity may occur in a follow the leader manner (Gandhi et al., 2008). Because inhibitory cell plasticity takes place more slowly than excitatory cells, we monocularly deprived Arc heterozygotes for 5-6 days. In agreement with a previous study (Gandhi et al., 2008) we find that there is a shift in both GFP-positive and –negative cells at this time point (Figure 9A,B,C and Supplemental Figure 1A and B). We then asked whether or not inhibitory plasticity could proceed in the absence of excitatory plasticity. As mentioned previously, Arc homozygotes display reduced plasticity compared to WT mice (Chapter 2 of this thesis). To determine whether there was a disruption of plasticity at the level of GFP-positive Arc lack cells compared to neighboring GFP-negative cells we monocularly deprived Arc homozygous mice. We were curious how inhibitory cells would behave in a circuit of Arc lacking cells with reduced plasticity and whether inhibitory plasticity would also be impaired.

We found Arc lacking GFP-positive cells fail to shift their ocular dominance score in response to monocular deprivation (Figure 10A, C and Supplemental Figure 1C). However, GFP-negative cells show a significant shift in ocular dominance (Figure 10B, C, and Supplemental Figure 1D). Thus, reduced plasticity in GFP-positive (putative excitatory) cells by removal of Arc protein is ineffective in preventing plasticity within GFP-negative (putative inhibitory) neurons.

Discussion

Experience is critical for the refinement of both orientation tuning and ocular dominance. In this study we have examined visual response properties in inhibitory (GFP-negative) and excitatory (GFP-positive) cells of Arc heterozygotes and homozygotes. While both GFP-positive and –negative cells of Arc heterozygotes and homozygotes display broad tuning within the critical period, we find a significant difference in ocular dominance for GFP-positive cells lacking Arc-protein.

It is conceivable that loss of a putative mechanism for synaptic weakening might have an impact on the eye-specific preference of individual cells. By assaying the ocular dominance for individual cells in both Arc heterozygotes and homozygotes we found that there is a greater predominance of purely binocular GFP-positive cells in Arc homozygotes. By contrast, a normal contralateral bias existed in GFP-positive cells in the Arc heterozygotes, and GFP-negative cells from both homozygote and heterozygote Arc mice. Our earlier report showed that dark reared mice mimic the cortical state of Arc KO mice (Chapter 2 of this thesis). These mice show a trend towards a lower ocular dominance compared to normally reared mice, indicating that activity is critical for the establishment of normal ocular dominance. In the case of Arc homozygotes, loss of a mechanism for synaptic weakening may impair the normal pruning of weaker ipsilateral inputs and lead to a more binocular ocular dominance score.

Furthermore, we assessed plasticity in Arc heterozygous and homozygous mice. As reported previously, monocular deprivation shifts ocular dominance in both GFP-positive and -negative cells (Gandhi et al., 2008; Mainardi et al., 2009). By contrast, we found that plasticity in GFP-positive cells of the Arc homozygotes is reduced compared to GFP-negative cells. This data supports our findings from Chapter 2. GFP-positive cells in the Arc homozygotes lack a mechanism for removal of AMPA receptors from the surface. This removal is thought to mediate the depression of the deprived eye response underlying shifts in ocular dominance. It is will be interesting to determine in future studies whether reduced plasticity in GFP-negative

cells affects the output of GFP-positive cells. Because inhibition plays such an important role in maintaining overall cortical activity, it is reasonable to expect that a reduction of inhibitory response might change cortical dynamics in some way. However, at present, it is difficult to compare changes in absolute response across animals due to differences in anesthesia, and the cortical state of each animal. Of additional interest would be to examine the contribution of individual subtypes of inhibitory cells in vivo. Inhibitory interneurons can be separated into three populations containing parvalbumin, calbindin, or calretinin (Defelipe et al., 1999). Double immunostaining using c-fos and parvalbumin, suggests that parvalbumin positive cells may have reduced ocular dominance plasticity after deprivation (Mainardi et al., 2009). In our hands, putative inhibitory cells show a ready shift after 5 days of deprivation. This may be due to contributions from other subtypes of inhibitory cells. In addition, as mentioned above ~5% of cells that are GFP-negative may actually be excitatory.

Arc expression in the visual cortex is critically dependent on NMDA receptor activity. Our calcium imaging data provide strong evidence that the NMDA receptor-signaling cascade is critical for the development of ocular dominance. In the mouse, there is a predominance of crossed (contralateral) optic nerve fibers compared to uncrossed (ipsilateral fibers). Despite this, in very young rats (P17-P19) there is a large number of binocular cells within the binocular zone of visual cortex (Fagiolini et al., 1994). However, by the peak of the critical period a contralateral bias has been established in cortex and continues throughout adulthood. This suggests that there may be an activity-dependent pruning of the weaker ipsilateral eye projections. Data from dark reared adult rats suggests that this may well be the case. Dark reared adult rats have a greater percentage of binocular cells compared to normally reared rats-similar to what we find for GFP-positive cells in the Arc homozygote mice.

Activity is also essential for the normal development of orientation tuning. Studies in adult ferrets have shown that the NMDA receptor signaling pathway is necessary for the developmental sharpening of orientation selectivity (Ramoa et al., 2001). However, our data suggest that orientation selectivity may mature at a slower rate than ocular dominance. In

support of this, experiments conducted in rats show that in very young rats, selectivity for orientation is virtually absent (Fagiolini et al., 1994). Gradually, over a period of weeks cells develop orientation specificity. At P30, the orientation selectivity of rats remains broad, however by P45 an adult sharpening of response has occurred. Interestingly, the removal of activity by dark rearing can also prevent this from occurring. For example, the visual cortex of P60 rats dark reared from birth resembles that of P19 rats. In line with this, a previous study examining orientation selectivity in adult Arc homozygotes found that these mice displayed broader orientation tuning compared to their WT and heterozygote counterparts. This result suggest that deficits in orientation selectivity between Arc homozygotes and heterozygotes may not be apparent until a later time point in development then the critical period.

Interestingly, Arc expression rapidly increases after eye opening and parallels the period during which experience-dependent changes take place. It is possible that Arc functions within the neuron to enhance response properties by the removal or reduction of weaker inputs. This would result in a sharpening of overall receptive field properties throughout development. In both Arc homozygotes, and dark reared mice, the absence of Arc may then result in a deficit in the emergence of mature response properties. In the case of ocular dominance this would manifest as more binocular cells, and for orientation selectivity broader orientation tuning.

As we have shown, Arc-GFP mice provide an excellent opportunity to differentiate the contributions of various subtypes of neurons to plasticity. In addition, a number of transgenic lines are becoming available that more specifically label subtypes of interneurons and astrocytes. Because of the wide variety of bright fluorescent markers available (e.g. RFP, tdTomato, and CFP to name a few), it will soon become possible to simultaneously image OGB and identify specific subtypes online by using a non-overlapping fluorescent marker; thus overcoming the limitation of post hoc matching of cells and facilitating more conclusive examination of cellular subtypes. Our findings for increased binocularity in GFP-positive cells of Arc homozygotes support the hypothesis that Arc may function to sharpen response properties throughout development by the removal or suppression of weaker inputs. In

addition, loss of Arc affects the plasticity of excitatory cells specifically, while leaving plasticity in inhibitory cells intact, suggesting a relative independence of plastic mechanisms operating in these neurons. Whether inhibitory cells possess an "Arc-equivalent" molecule remains to be seen. With the rapid pace of molecular genetics, combined with the ability to analyze the functional response of single cells, a more complete understanding of interactions between cellular subtypes and their individual contributions is in the near future.

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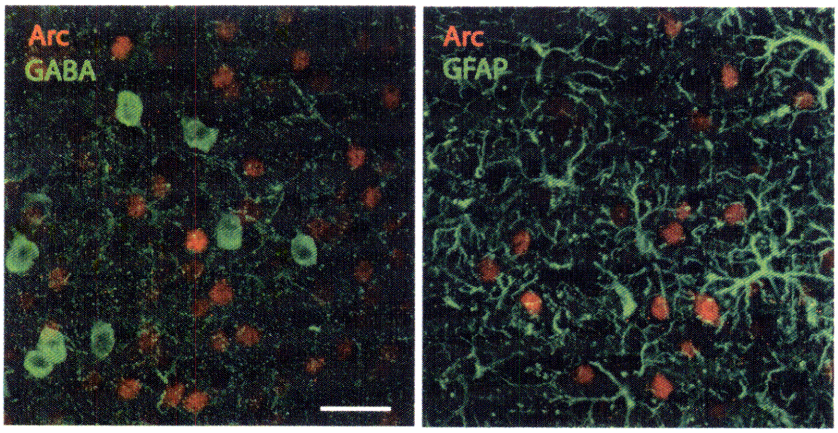
A



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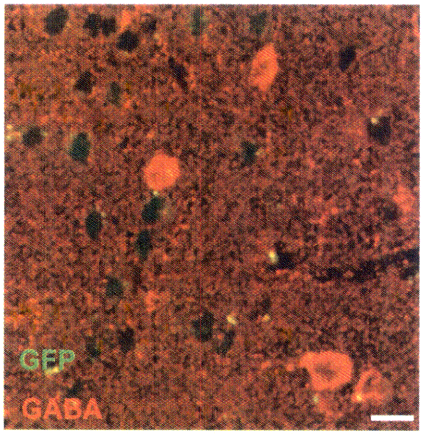

Figure 1. A. Sections from a WT CP mouse dark adapted overnight and exposed to light prior to being double-labeled with antibodies against Arc and GABA (specific for inhibitory cells), or Arc and GFAP (specific for astrocytes). No colocalization was detected suggesting that Arc is specific to excitatory cell types. *Scale bar= 50 μm.* B. Direct GFP fluorescence from an Arc-GFP mouse dark adapted overnight and exposed to light prior to sectioning and immunostaining using an antibody against GABA. No GFP fluorescence can be detected within the nucleus of GABA-positive cells. *Scale bar= 10 μm.*

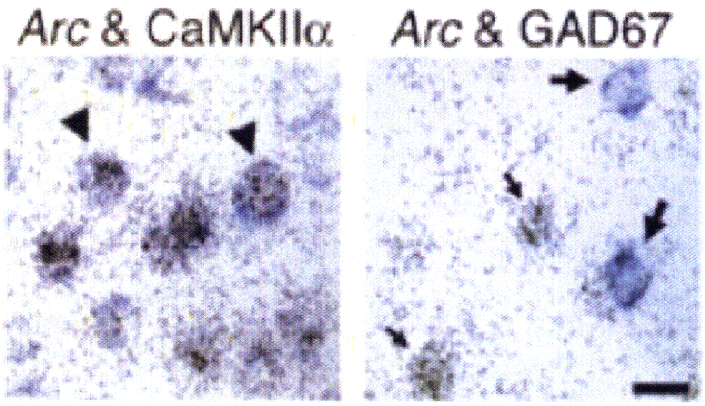

Figure 2. In situ hybridization using probes against mRNA for Arc, in addition to CamKII-alpha and GAD67, markers for excitatory and inhibitory subtypes respectively. Arc colocalizes with ~96% of all CamKII-alpha positive cells. No overlap was found between Arc and GAD67. Adapted from Tagawa et al 2005. *Scale bar=10um.*

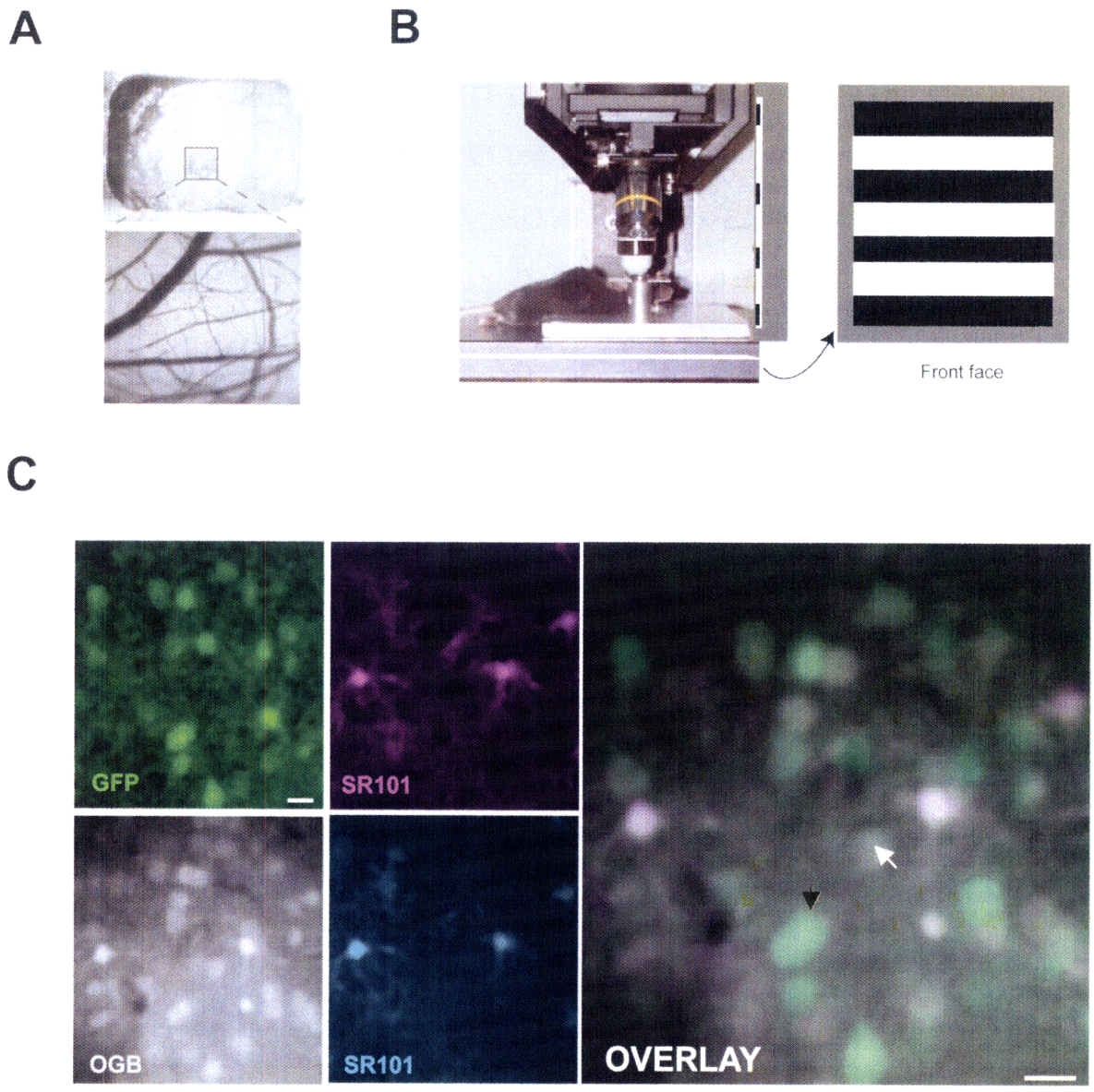

Figure 3. A. Image of a craniotomy centered over the visual cortex. The binocular zone was determined using known stereotaxic coordinates and verified by the presence of ipsilateral eye response to stimulation. B. Image of a mouse with head plate fixed and prepared for two-photon imaging (courtesy of Daniela Tropea). A screen was placed directly in front of the mouse and an episodic visual stimulus of 18 orientations was presented. C. (Top row) Images of GFP expression and (right) corresponding astrocyte labeling (SR101) prior to injection of OGB. (Bottom row) Image of filled cells after

injection of OGB and (right) corresponding astrocyte labeling. (Overlay) Images were aligned after the imaging session using astrocytes labeled before and after injection as landmarks. In the overlay image it is possible to see cells filled with OGB and GFP-negative (putative inhibitory cells, indicated by white arrow), in addition to cells filled with OGB and GFP-positive (putative excitatory cells, indicated by black arrow). *Scale bar=10um.*

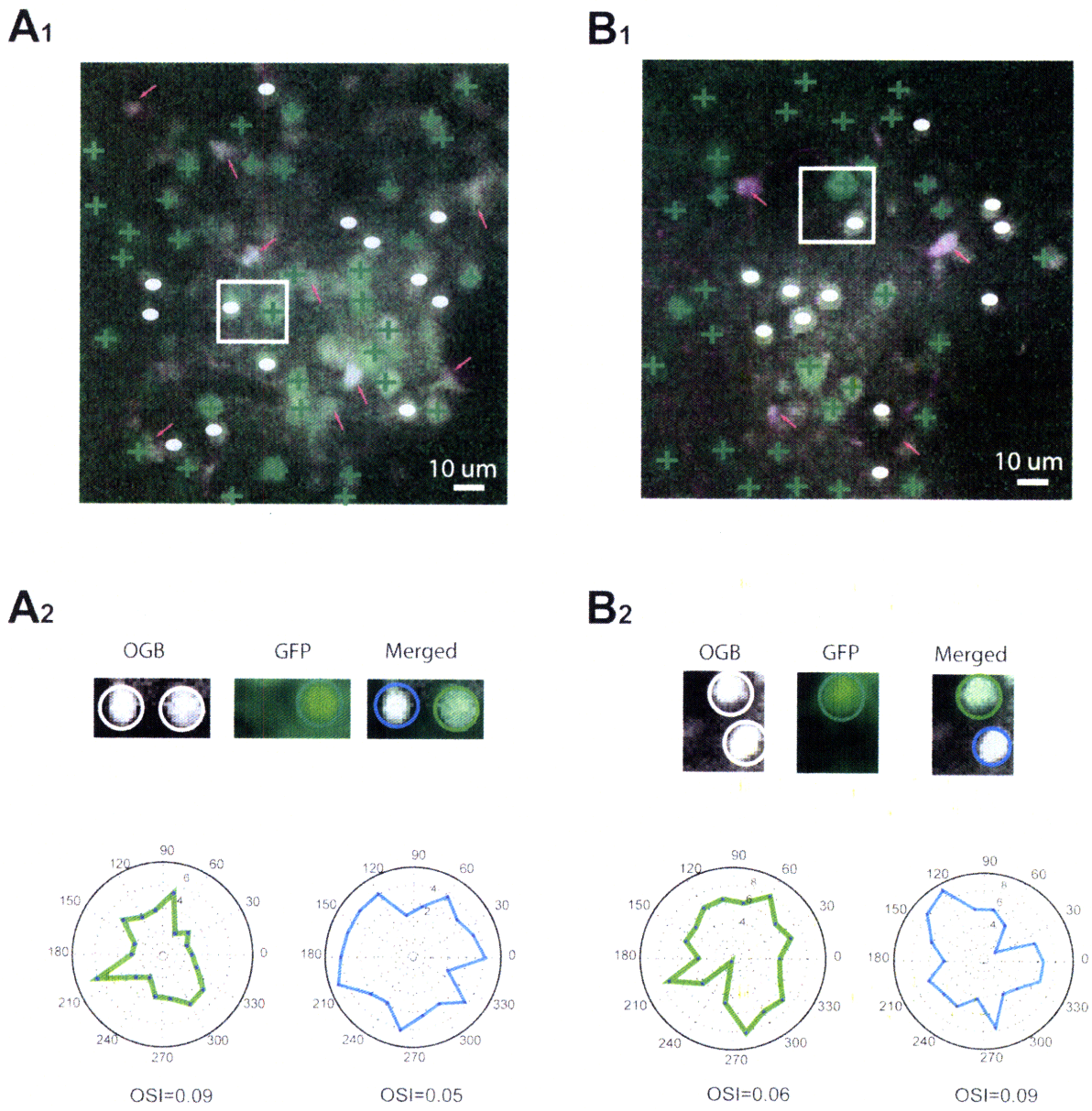

Figure 4. A1. Imaging plane in an Arc-heterozygous mouse in superficial layer 2/3 of visual cortex. (Green crosses= GFP-positive cells, White circles= GFP-negative cells, Pink arrows= Astrocytes). A2. (Top row) Zoomed in image of two OGB filled neurons, one GFP-positive (green circle) and the other GFP-negative (blue circle) contained within the white box in A1. (Bottom row) Polar plots of responses elicited after the presentation of gratings (0° to 360° at 20°steps) from the two cells. Juvenile mice display relatively broad tuning. The color of the trace corresponds to the response from the

neuron circled using the same color in the image above. B1. Imaging plane in an Arc-homozygous mouse at a similar point in layer 2/3. Cells are identified in the same manner as in A1. B2. Polar plot of response to presentation of gratings from two neurons contained within the white box in B1. Magnitude of response = $\Delta F/F \times 10^{-2}$.

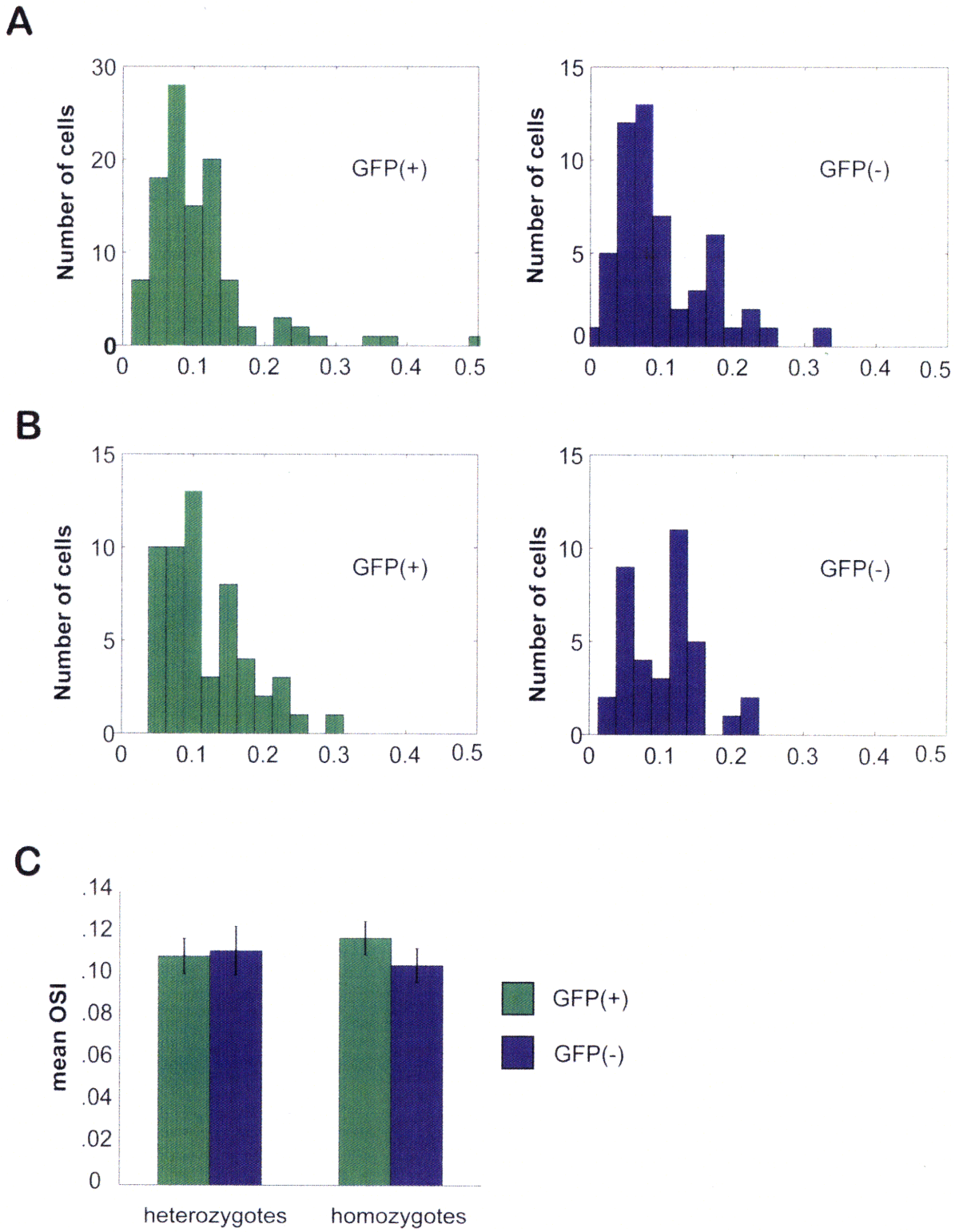

Figure 5. A. Distribution of OSIs from both GFP-positive and –negative responsive cells in juvenile Arc heterozygous mice. (n=3, GFP-positive n=106, GFP-negative n=54) B.

Distribution of OSIs from GFP-positive and –negative responsive cells in an Arc homozygote mouse. (n=2, GFP-positive n=55, GFP-negative n=37). C. In addition there was no significant difference between the mean OSI across groups (one-way Anova, $F(3, 245) = .55$, $p = .64$)

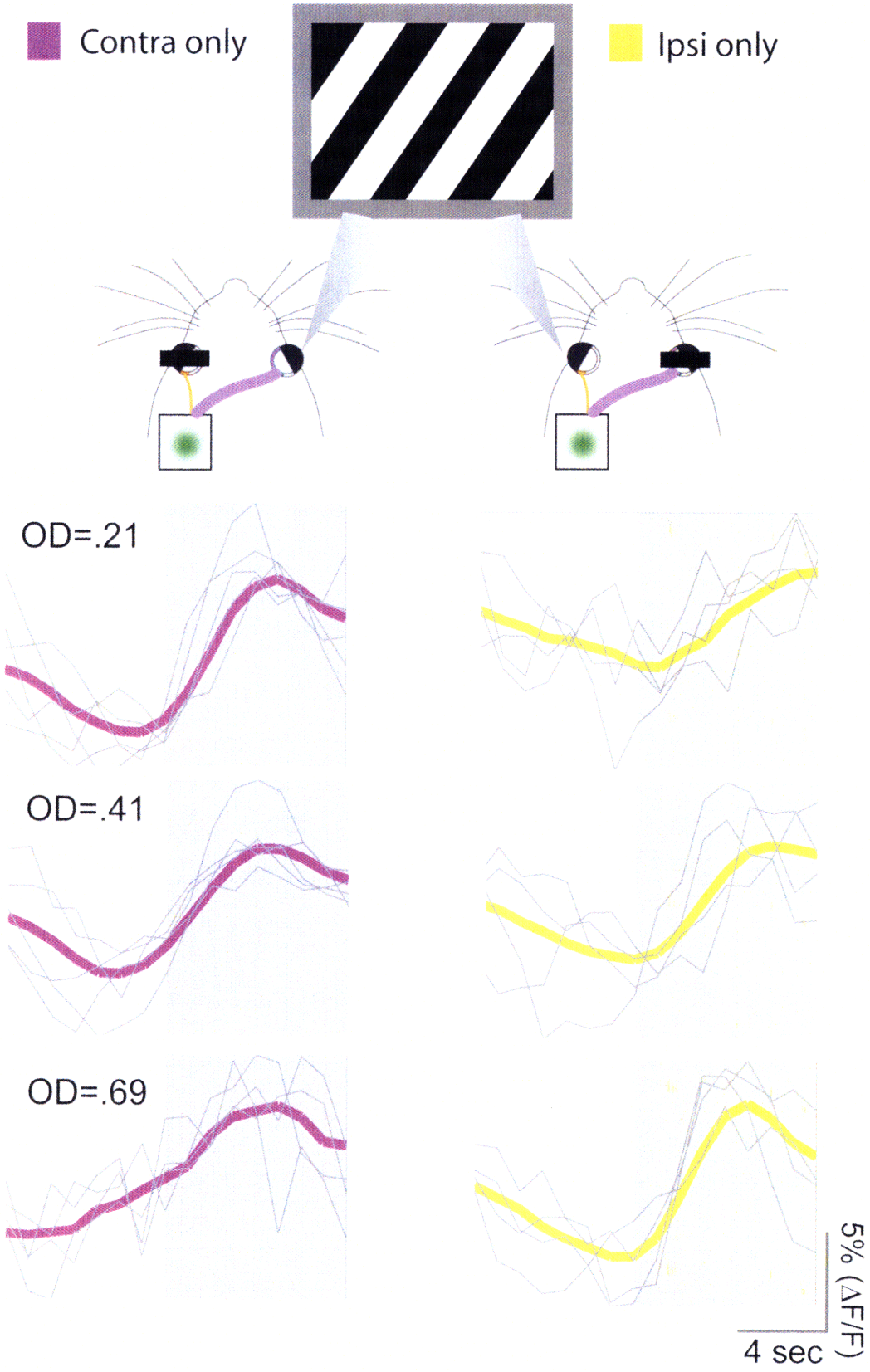
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Figure 6. A. Schematic depicting the detection of eye-specific response. The relative eye-specific drive was measured by stimulating each eye individually. B. Ocular dominance was calculated as IPSI/IPSI+CONTRA. A score of 0 equals a purely contralateral eye response and a score of 1 equals a purely ipsilateral response. A score of .5 indicates a purely binocular response. Sample traces underlying a range of OD scores are shown for the contralateral eye (purple) and the ipsilateral eye (yellow). Grey shaded area represents the period during which the cells were being stimulated.

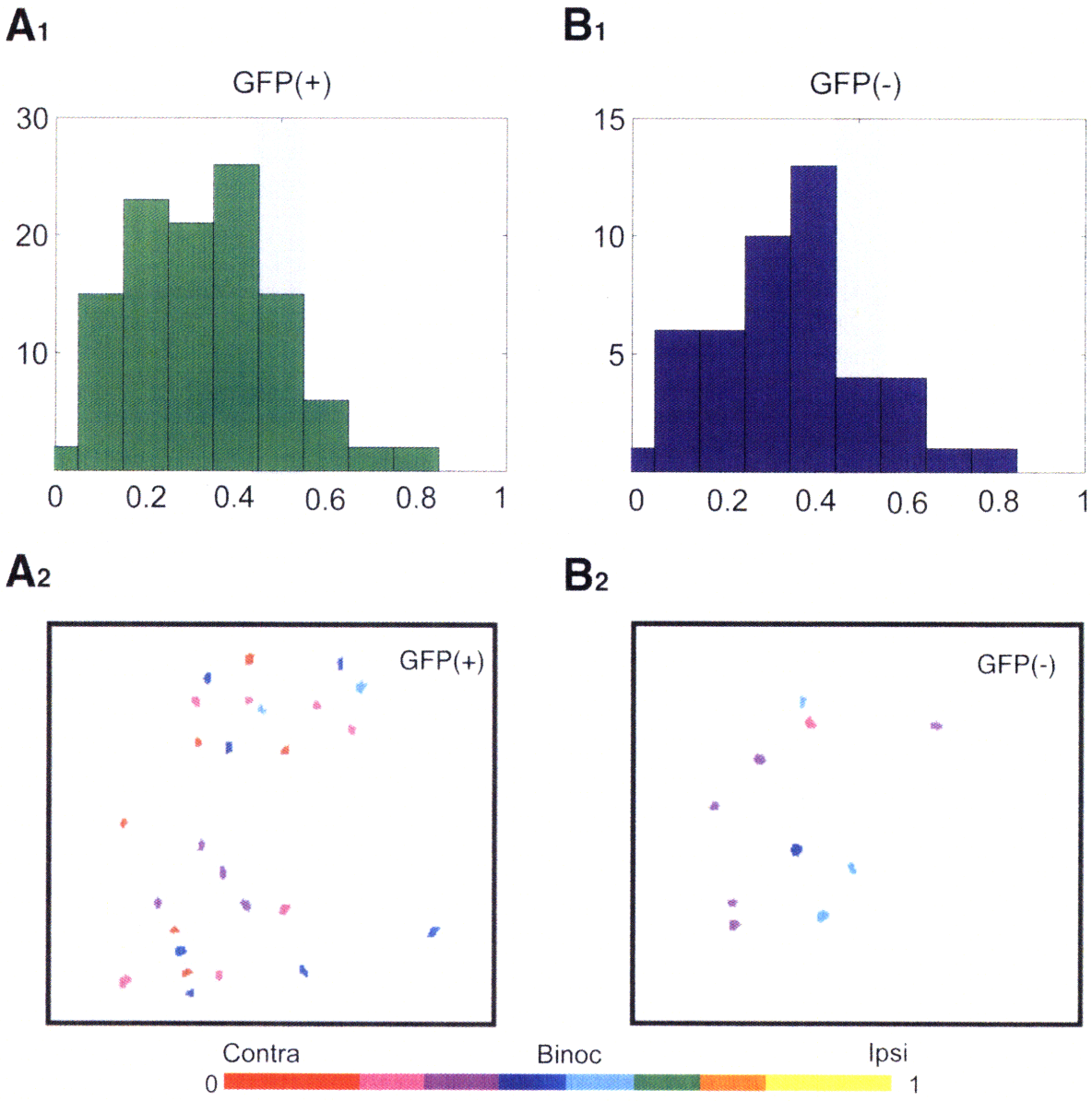


Figure 7. A1. Distribution of ocular dominance scores for 112 GFP-positive cells across 4 mice. A contralateral bias exists within the binocular zone. Grey shading indicates equal drive from both eyes. A2. Color-coded ocular dominance map of individual cells within layer 2/3 of visual cortex in an Arc-heterozygous mouse. Ocular dominance score calculated as in Figure 6. B1. Distribution of ocular dominance scores for 46 GFP-negative cells in 4 mice. Grey shading indicates equal drive from both eyes. B2. Color-

coded ocular dominance map of GFP-negative cells in an Arc-heterozygous mouse. Ocular dominance score calculated as in Figure 6.

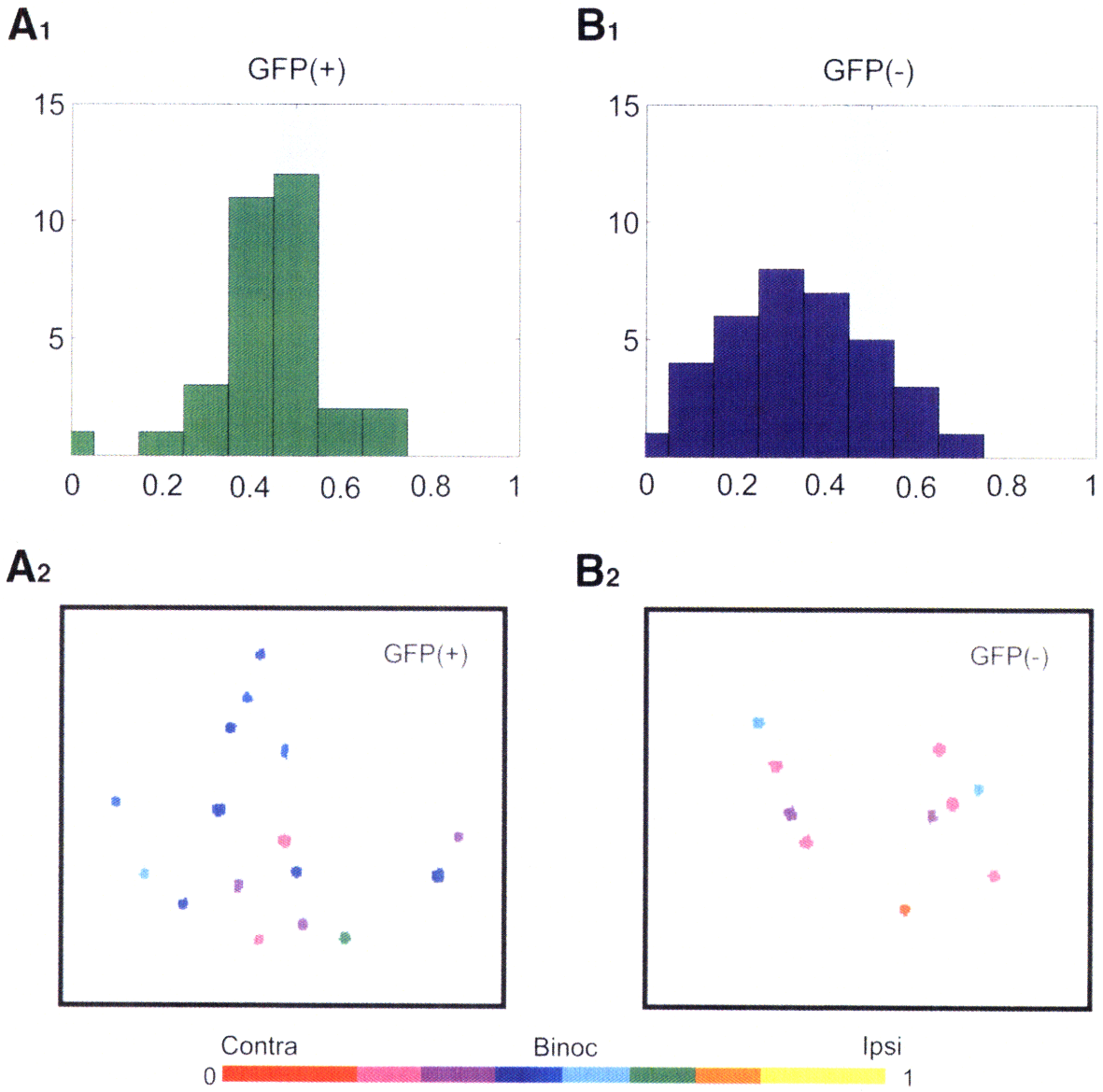


Figure 8. A1. Distribution of ocular dominance scores for 32 GFP-positive cells across 2 mice. A trend towards more binocular scores exists for GFP-positive cells within the binocular zone of Arc-homozygotes. Grey shading indicates equal drive from both eyes. A2. Color-coded ocular dominance map of individual cells within layer 2/3 of visual cortex in an Arc-homozygous mouse. Ocular dominance was calculated as in Figure 6. B1. Distribution of ocular dominance scores for 35 GFP-negative cells in 2 mice. A contralateral bias, similar to that found in heterozygote mice, can be seen in GFP-

negative cells contained within Arc homozygotes. Grey shading indicates equal drive from both eyes. B2. Color-coded ocular dominance map of GFP-negative cells in an Arc-heterozygous mouse. Ocular dominance score calculated as in Figure 6.

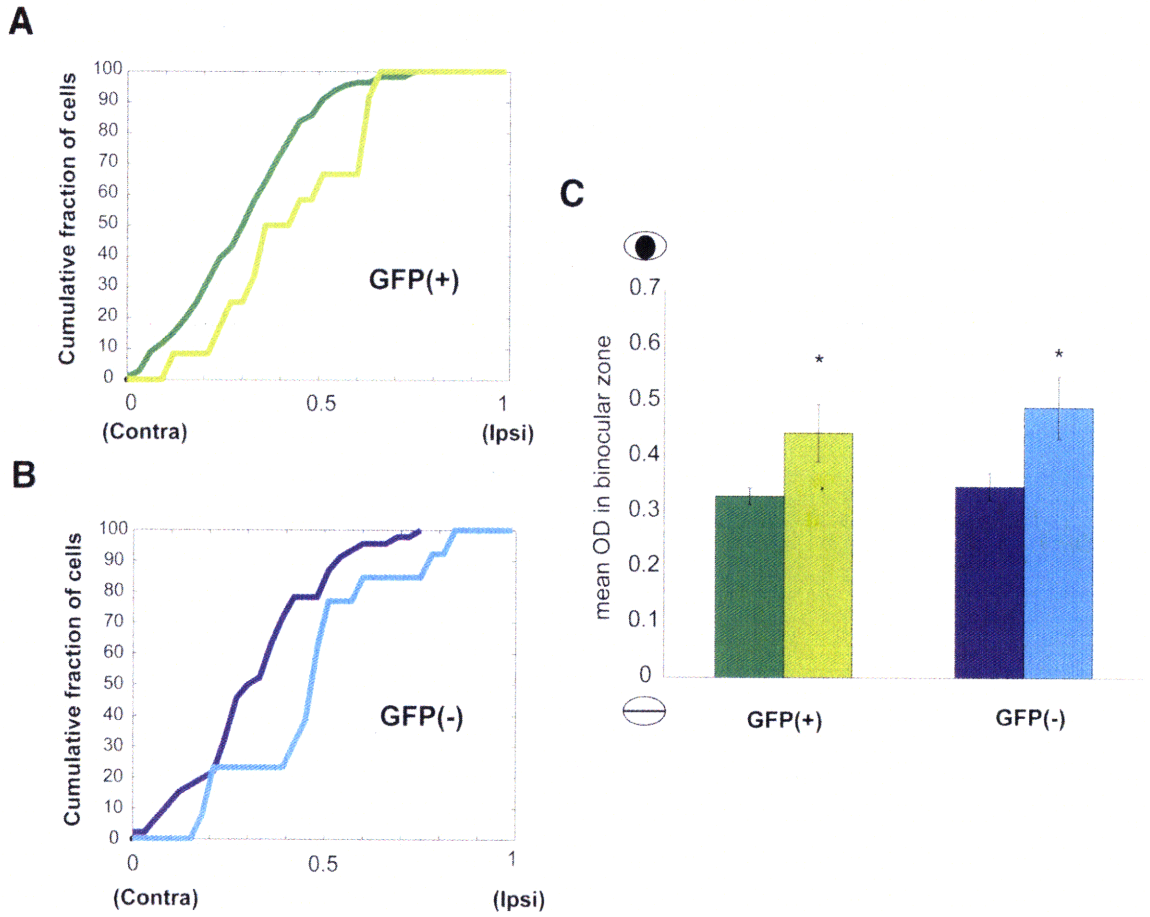


Figure 9. A1. Cumulative distribution of ocular dominance scores in control (dark green) and deprived (light green) Arc-heterozygous mice. A significant shift in ocular dominance scores towards lower values can be seen ($p<.05$, Wilcoxon ranksum test; n=4 mice, control=112 cells; n=1 mouse, deprived=12 cells). B. A significant shift in ocular dominance score can also be seen for the distribution of GFP-negative cells. Dark blue-control, light blue-deprived ($p<.05$, Wilcoxon ranksum test; n=4 mice, control=46 cells; n=1 mouse, deprived=13 cells). C. Mean ocular dominance score within the binocular zone of GFP(+) and GFP(-) cells shown in A and B for an Arc-heterozygous mice with and without monocular deprivation. A significant shift in the mean OD score after deprivation is detected for both GFP-positive ($p<.05$, Student's t-test; GFP-positive, No

MD= .32±.01, MD= .44±.05) and GFP-negative cells ($p<.05$, Student's t-test; GFP-negative, No MD=.34±.02, MD=.48±.05). Bars are color-coded as in A. and B.

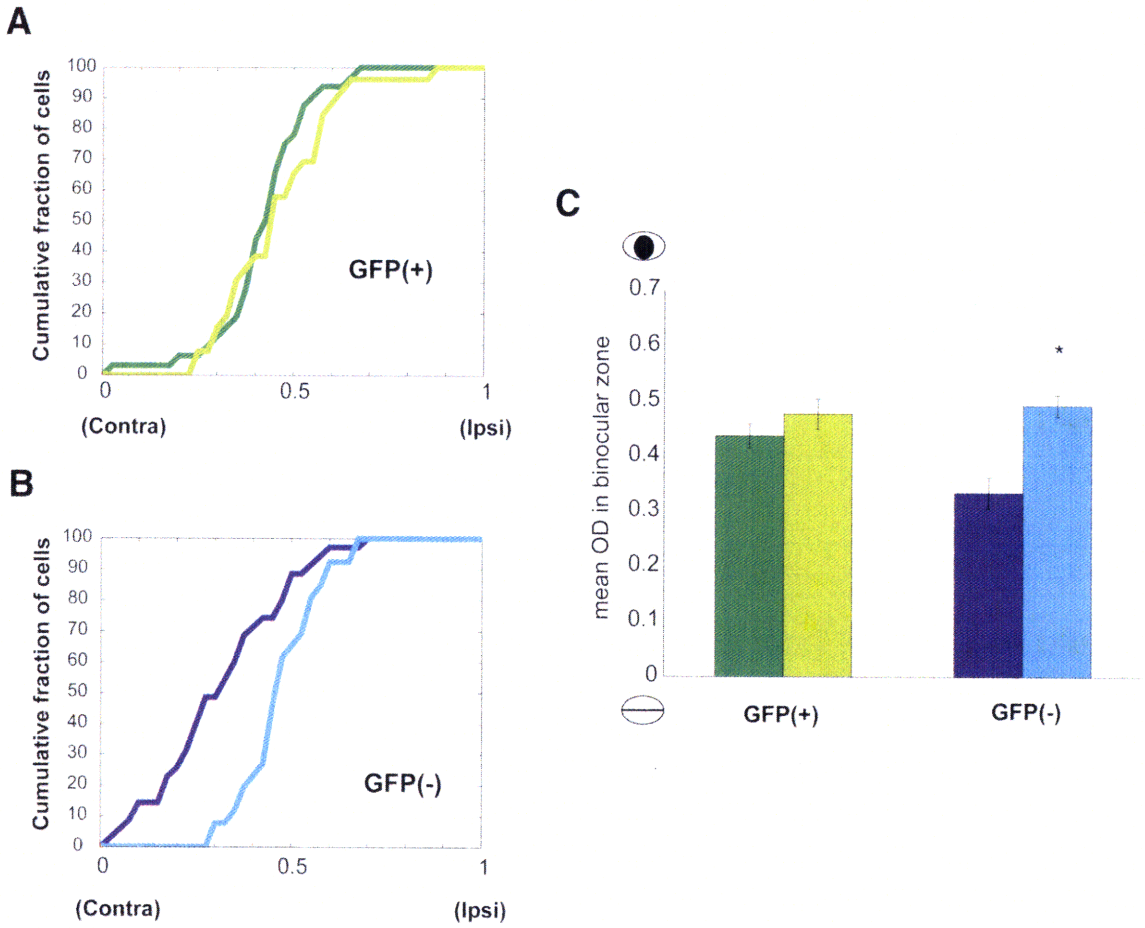
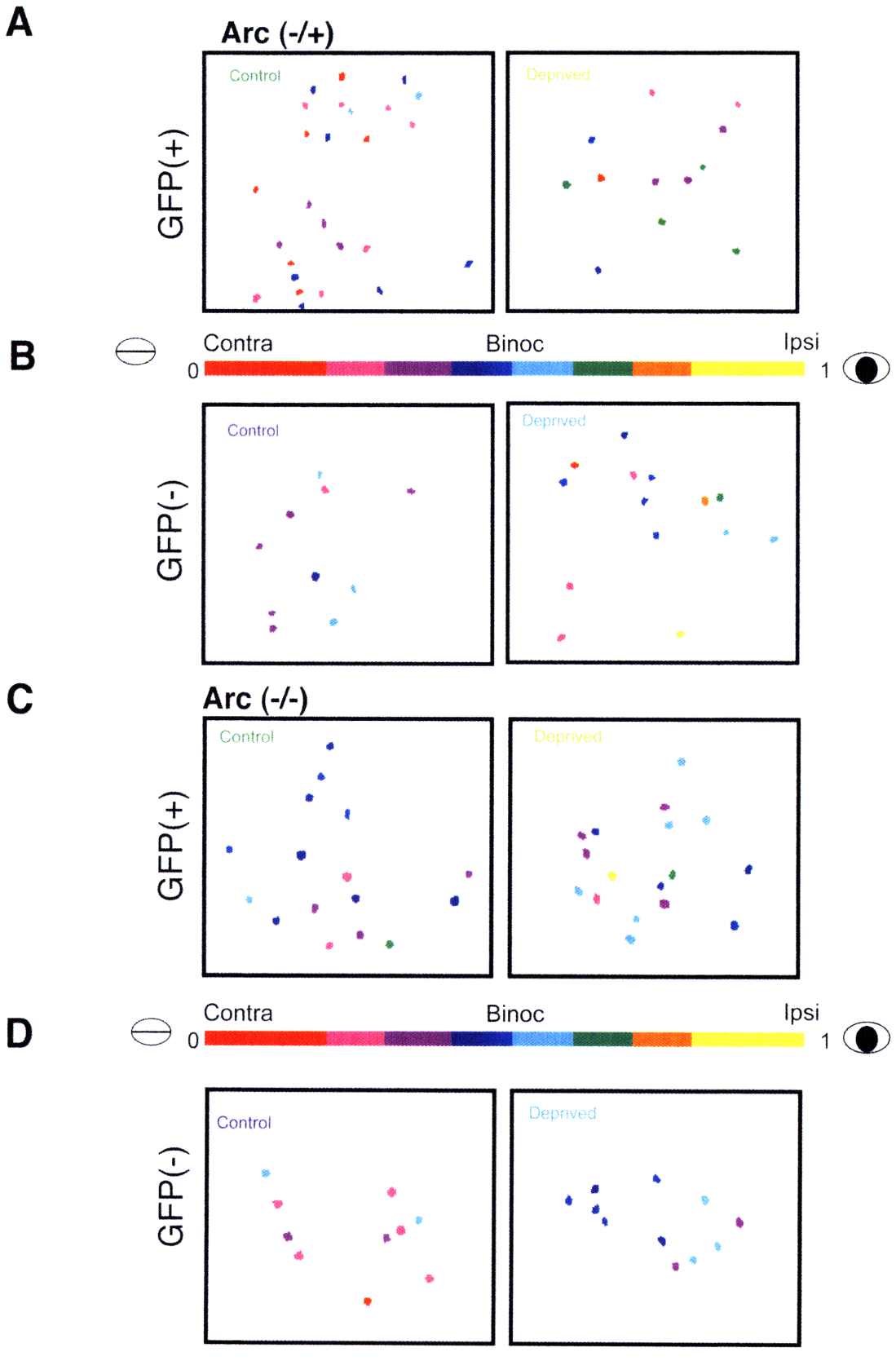


Figure 10. A. Cumulative distribution of ocular dominance scores in control (dark green) and deprived (light green) Arc-homozygous mice. A reduced shift in the ocular dominance score occurs in GFP-positive cells in Arc-homozygous mice. ($p>.45$, Wilcoxon ranksum test; ; n=2 mice, control=32 cells; n=3 mice, deprived=26 cells) B. However, a significant shift in ocular dominance score can be seen for GFP-negative cells. Dark blue- control, light blue-deprived ($p<.01$, Wilcoxon ranksum test; ; n=2 mice, control=35 cells; n=3 mice, deprived=26 cells). C. Mean ocular dominance score within the binocular zone of GFP(+) and GFP(-) cells in Arc-homozygous mice with and without monocular deprivation. No shift in the mean OD score is detected for GFP-positive cells after deprivation ($p>.25$, Student's t-test; GFP-positive, No MD= .43±.02,

MD= .47±.02) and GFP-negative cells ($p<.05$, Student's t-test; GFP-negative, No MD=.33±.02, MD=.49±.01). Bars are color-coded as in A. and B.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Supplementary Figure 1. Examples of color-coded ocular dominance maps of single cells within the binocular zone of visual cortex. A. GFP(+) and B. GFP(-) cells from a single plane within either a control or deprived Arc heterozygote mouse. C. and D. show examples of color-coded OD scores for both GFP(+) and GFP(-) cells, respectively, in Arc homozygotes with (control) and without (deprived) monocular deprivation.

CHAPTER FIVE

CONCLUDING REMARKS

In conclusion, we find that Arc is critical for experience-dependent plasticity and the normal establishment of the C/I ratio. The data presented within this thesis provide the first evidence of Arc's involvement in mediating the cortical response to monocular deprivation. We also report that Arc is critical for SRP in visual cortex. It is clear from these experiments that Arc-GFP mice have proved useful for probing the contributions of Arc to experience-dependent plasticity at both a population and single-cell resolution. Using these mice we have been able to unveil previously unknown contributions of Arc to a number of processes operating within the visual cortex. However, a number of questions still remain.

Alternative genetic approaches to assaying Arc's function in vivo

While knockout mice provide useful tools for understanding how a particular molecule contributes to plasticity within specific regions of the brain, it would be ideal to have even more precise control over the point in development at which Arc is removed in order to more carefully examine its function and effects at the synapse. It is always an issue that knockout of a gene may result in compensatory changes within the animal that occlude or at times even magnify the deficit seen. Advances in genetic mouse models have produced methods that allow for the conditional knockout or tissue-specific targeting of a gene. With a conditional knockout it would be possible to probe Arc's function at a particular stage of development such as the peak of the critical period and examine how removal of Arc only during the period in which the eye is being deprived impacts plasticity. Similarly, Arc over-expressing mice also provide an opportunity to further investigate Arc's function in vivo. Increases in Arc protein might facilitate a precocious critical period or accelerate the shift in ocular dominance that occurs with lid suture.

In regard to single cell analysis of Arc function, Arc-GFP mice are clearly useful for the identification of specific cell types. However, Arc-GFP mice express a destabilized form of GFP (d2EGFP) where the half-life has been shortened to 2 hrs (Li et al., 1998), similar to the temporal profile of Arc protein. This, combined with the fact that the endogenous reporter is driving GFP expression, leads to a lower level of fluorescence than what might be seen otherwise. In addition, while two-photon imaging reduces the autofluorescence inherent in other microscopy techniques, the light scattering that occurs at deeper depths within the brain further occludes signal intensity. Thus, when examining GFP-expressing cells in deeper layer 2/3, even with strong stimulation it is possible that cells that appear to be GFP-negative may actually contain GFP, but, due to optics or overall expression, fluorescence may be extremely dim. While there are ways to optimize the signal intensity i.e. reducing scan speed, increasing laser power etc. a brighter fluorescent tag would be ideal.

One candidate transgenic line is the Arc-dVenus line (Eguchi and Yamaguchi, 2009). In these mice the Arc promoter is used to drive expression of a dVenus reporter. Importantly, a chimeric intron expressing a simian virus polyadenylation signal was used to increase the Arc promoter levels to 100-fold greater than endogenous Arc, thus increasing overall fluorescence intensity. Using this line it possible to visualize Arc-dVenus expression with epifluoresence even under low magnification, suggesting that with two-photon microscopy the cells would be remarkably bright. Interestingly, the authors report that Arc-dVenus mice do not display deficits in learning and memory, which would facilitate the use of this line for studies of plasticity using Arc solely as a marker for excitatory cells. In addition, it would be possible to cross these mice with other knockout lines to explore activity-dependent plasticity. However, while dVenus cells display laminar organization similar to that of Arc mRNA and protein in WT mice, one caveat of this study is that the authors fail to present evidence that dVenus fluorescent cells in visual cortex actually colocalize with endogenous Arc at a single cell

level. It would be necessary to confirm that dVenus-positive cells colocalize with Arc protein using immunofluorescence.

Probing synaptic scaling, LTD, and LTP in visual cortex of Arc KO mice

While the studies conducted within this thesis have all been in vivo, in vitro assays also provide an opportunity to further explore the role of Arc in both Hebbian and homeostatic mechanisms. Studies in hippocampal and forebrain culture have indicated that Arc is required for LTD, LTP, and synaptic scaling (Plath et al., 2006; Shepherd et al., 2006; Waung et al., 2008). As in the hippocampus, the forms of LTP and LTD operating within visual cortex are triggered by strong and weak activation of postsynaptic NMDA receptors. Indeed, we find in vivo that a number of processes thought to rely upon these mechanisms, such as deprived eye depression and open eye potentiation, are disrupted. Whether these processes are indeed disrupted at the level of a single synapse within the visual cortex remains to be seen.

As we have discussed throughout this thesis, increases and decreases in activity result in homeostatic changes at the level of AMPA receptors. For example, decreasing activity by dark-rearing mice results in an increase of AMPA receptor mEPSCs. Conversely, increased activity reduces mEPSCs globally (Seeburg and Sheng, 2008). Arc would be predicted to play a role in the synaptic weakening that occurs in response to increased activity such in the case of GABA blockade. It would be interesting to see whether loss of Arc would prevent this process within visual cortex. In addition, it would be possible to probe the contributions of Arc in a layer specific manner. Because the highest levels of Arc can be found within layers 2/3 and 4, and as Arc is not expressed with in layer 5 (Tagawa et al., 2005) it would be interesting to determine how response properties of cells in these layers differ from one another in the presence and absence of Arc.

An examination of the mechanistic contributions of Arc would be particularly relevant around eye opening. As mentioned previously, Arc is rapidly upregulated after eye opening (Lyford et al., 1995) and the gradual increase in Arc expression parallels the gradual decrease in AMPA receptor mEPSCs that takes place in layer 2/3 and 4 during the pre-critical period (Desai et al., 2002). One can easily hypothesize from the properties of Arc that it would play a critical role in this developmental refinement process.

Structural plasticity in Arc KO mice

In addition to functional changes such as synaptic strengthening, many cortical manipulations result in structural modification of dendritic processes (Engert and Bonhoeffer, 1999). Indeed, Arc protein has been shown to interact with the actin cytoskeleton, putting it in a prime position to not only influence synaptic strength but to play a role in structural remodeling of dendritic spines after NMDAR dependent LTP inducing activity. We have crossed Arc KO and WT mice into a GFP-S line that allows labeling of layer 2/3 neurons (Feng et al., 2000) (Figure 1). Changes in spine density and morphology are well correlated with synaptic plasticity (Oray et al., 2004). Indeed, after monocular deprivation there is a decrease in spine density in layer 2/3 of visual cortex (Mataga et al., 2004). It would be interesting to examine dendritic spine density in monocularly deprived Arc KO GFP-S mice and compare to structural changes in deprived WT GFP-S mice. It will be interesting to see whether there are both functional and structural deficits in Arc KOs compared to WT in response to reduced cortical drive.

In addition to examining spine density, it would be interesting to assay spine size and shape. Increased AMPA receptor surface expression in Arc KO mice may result in a greater number of large stubby and mushroom spines compared to WT mice. Whether these spines will have only the appearance of being stronger and mature spines, while being functionally immature, will be interesting to determine. Alternatively, it is equally possible that Arc lacking cells will have an increase in filopodia and thin spines.

Sustained synthesis of Arc protein is required for the expansion of the actin cytoskeleton which accompanies LTP suggesting that Arc synthesis may be crucial for stabilization of spine morphology after potentiating events (Soule et al., 2006). In Arc KO mice this stabilization may not occur. Arc expression is activated via BDNF-induced LTP (Ying et al., 2002), suggesting that Arc may work in concert with BDNF to stabilize newly formed synapses. BDNF is known to influence axonal arborization and most recently has been shown to exert an effect on postsynaptic neuronal connectivity as well by coordinating synapse formation between pre- and postsynaptic neurons. Microinjection of BDNF on tectal neurons in vivo results in increased axonal arborization and an additional increase in spine density on dendrites. Blocking NMDARs prevents this increase (Sanchez et al., 2006). Consistent with its hypothesized role in synaptic growth and reorganization, a recent study found that a number of the genes simultaneously co-upregulated with Arc in response to BDNF-induced LTP, are involved in synapse formation and maturation e.g. Narp and Neuritin (Wibrand et al., 2006). Neuritin, also known as the candidate plasticity gene 15 (CPG15), has previously been shown to promote dendritic and axonal arbor growth (Nedivi et al., 1998).

One form of perceptual learning found in mice that may depend on Arc is stimulus response potentiation (SRP), which occurs in mouse visual cortex (Sawtell 2006) and has been detected using visually evoked potentials. Interestingly, SRP is NMDAR-dependent and requires AMPAR trafficking. In Chapter 2, we have shown that Arc may be involved in this form of learning as SRP is completely disrupted in Arc KO mice. Using the GFP-S line it would also be possible to determine whether there is a structural correlate to SRP. As SRP is a mechanistic correlate of LTP, it is possible that induction of SRP would lead to enlargement or stabilization of dendritic spines. It would be interesting to examine structural changes in WT GFP-S and Arc KO GFP-S mice. In addition, because SRP is expressed in all layers it would also be feasible to use Arc KO GFP-S mice to examine differences in structural modifications occurring in those layers

normally containing Arc (layers 2/3 and 4) and compare them to layer 5 where Arc is not expressed. However, in the absence of Arc it is possible that structural correlates of SRP in layer 5 apical dendrites would also be impaired due to deficits in upstream neurons projecting to this layer.

A role for Arc in learning and adaptation paradigms

In addition, it will be interesting to examine whether Arc is involved in other forms of plasticity such as adaptation and perceptual learning at a single cell level using two-photon functional imaging. The mechanisms underlying SRP may be similar to those underlying adaptation. In this form of learning, repeated presentation of gratings of a single orientation result in a significant potentiation of response to that orientation. Modifications in response to the initial repeated orientation are extremely persistent and last over several days. However, adaptation operates over a much more rapid time period- seconds to minutes. Whether Arc dependent mechanisms also underlie phenomena such as adaptation remain to be seen.

Interaction with other candidate plasticity molecules

Intact operation of NMDA signaling pathways is critical for Hebbian plasticity to proceed normally (Kleinschmidt et al., 1987; Sawtell et al., 2003). NR2A KO mice have a deficit in deprived eye depression and a precocious potentiation of the ipsilateral eye response (Cho et al., 2009). It is unknown how an impairment of signaling through NMDA receptors due to loss of NR2A might influence activity dependent Arc induction. As Arc signaling is dependent upon NMDA receptors, it is tantalizing to predict that low levels of Arc in NR2A KO mice may mediate the disruption in deprived eye depression seen. However, the precocious potentiation of open eye response would not be predicted given that juvenile Arc KO mice do not show response potentiation after

extended MD. It would be useful to assay basal Arc expression in these mice to determine whether lower Arc levels are present in these mice compared to WT.

In addition, GAD65 KO mice have impaired ocular dominance plasticity and LTD (Choi et al., 2002; Kanold et al., 2009). Recent evidence suggests that this impairment may be due not only to changes at the level of inhibition, but also in levels of excitation. Application of diazepam rescues deficits in both LTD and ocular dominance plasticity, however this rescue is not due to enhancement of inhibition as previously thought as GABA A receptor levels remain unchanged after treatment. Instead, it appears that the restoration of plasticity is due to increased signaling through NMDA receptors. As Arc operates downstream of NMDA receptors, it is possible that a disruption of Arc induction occurs in GAD65 KO mice thus impairing LTD and ocular dominance plasticity. Along this line, a restoration of proper signaling through NMDA receptors by application of diazepam would also restore Arc function. Low levels of Arc in both the NR2A and GAD65 KO lines would confirm our findings that Arc is critical for ocular dominance plasticity and hone in on how upstream changes in inhibition and excitation may result in deficits in plasticity due to an impairment of the function at the level of a single molecule, Arc.

It is now apparent that both Hebbian and homeostatic processes operate hand in hand to mediate experience-dependent plasticity over various timescales. How Arc works in tandem with other molecules implicated in ocular dominance plasticity, such as the synaptic scaling molecule TNF-alpha, remains to be clarified. In addition, a comparison of orientation tuning in adult Arc KO and TNF-alpha mice would shed light on mechanisms underlying the sharpening of response to preferred and weakening of non-preferred inputs. Previous work suggests a role for Arc in the developmental weakening of non-preferred inputs that facilitates orientation selectivity. Whether TNF-alpha also plays a complementary role in the strengthening of the response of preferred inputs remains to be seen. In addition, it would be of interest to cross Arc KO mice with

TNF-alpha KO mice to determine how complete loss of synaptic scaling up and down might influence synaptic plasticity. Because synaptic scaling has been suggested to serve as a tool to prevent runaway excitation or depression as a result of Hebbian mechanisms, it is possible that all forms visual cortical plasticity would be completely impaired. However, due to the overlapping deficits of Arc KO and TNF-alpha mice, crossing these two lines would serve to eliminate both Hebbian and homeostatic scaling. Thus, it is not clear what findings might arise.

Conclusion

It is readily apparent from these experiments that the immediate early gene Arc is a critical player in synaptic physiology. Arc's involvement in plasticity and the developmental refinement of response properties provide only a small piece of the puzzle regarding the role of this molecule in experience-dependent plasticity. Our findings suggest the interesting possibility that disruption of Arc may also be involved in brain disorders such as autism and Alzheimer's disease as it lies downstream of a number of molecules that have been previously implicated in these disorders. In addition, while it is clear that Arc serves as a powerful regulator of AMPA receptor internalization, it is likely that Arc interacts with a number of other key molecules at the synapse. It will be interesting to see how the function of Arc, previously thought to serve merely as a marker for activity, is further elucidated through future experimentation.

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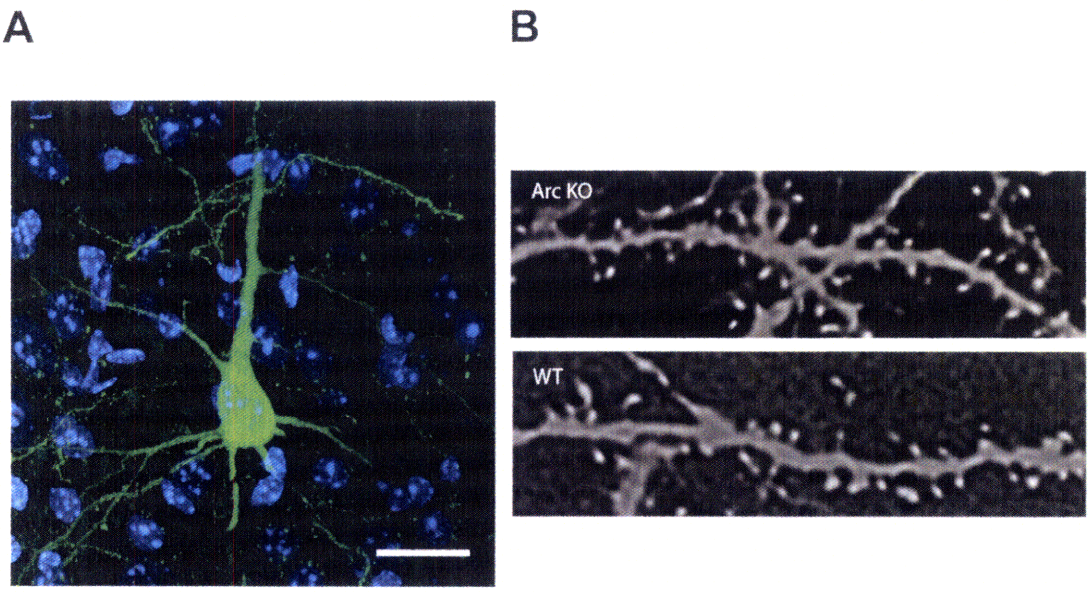

Figure 1. Fluorescent labeling of layer 2/3 cells in WT and Arc KO mice allows analysis of structural plasticity. A. Cell body and dendrites of a layer 2/3 cell in the visual cortex of a GFP-S mouse. Scale bar= 20 um. Image take at 63X B. Arc KO mice were crossed with GFP-S mice on a BL/6 background. Heterozygotes were bred and genotyped for Arc KO and positive GFP expression. Images depict primary branch of a GFP positive neuron in layer 2/3 of Arc KO/GFP-S (top) and WT/GFP-S (bottom).

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HO$)31'3$H'$Q RH,$)'2$->H((-'2-2$,H>-2?,-FH1'>,-)F-$D-Q$D-B$W H,X-2$Y1+D$D-$*S,1-IG)+H')Q$P)FX-+0)Q$

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I#%B'$7(!"&$#'*$"#;G%6(I7#8"#M'&*7(!"M@+$%&:;(0#M,(E757'#*6(A'N5;(0N7#K77&;() .(((((((F+MM7#(<===(

ZR)Q?)+-2$'2$FF-FF-2$D-$?+1Q1@HOR),H?F$H(S?+)+H')Q$)')Q@F1F$HO-Y),-$)>X)3-F$'2$,HR12-2$D--20)>X$H$

()')3-(-'+B$C,H2?>-2$(HQ->?Q,$2@')(1>F$H2-$(H2-Q1'3$F-O?Q-FFHOFFH,+-2$3Q FF@$(-+)Q$H(SHF1+H'F$F$2-)Q$

()+-,1)Q F$H,$-Y$,(@$->D'HQH31-FB(

!34F/A1041(E-A01-.(-HI-E2-4!-(((

F$+K7&$(*"&5+9$'&$;() 21(.%B7#5%$,(&%$%'$B7('&K(J#'K+'$7(F$+K7&$(3 8%*7 ((((((((((((((((((((((((((((((((((((((((((<==>?@#757&$(

/HQ(0H,)+-2$Y1+D$4 &^$J*M$H$->,?1+$)Q-'+-2$'2-,,-S,-F-'+-2$>1-'+F+F$'2$'31'--,FB$5FF1F+-2$1'$D-$2-R-QHS(-'+$)'2$

-N->?+H'HO'$''?)Q$)Q-NS-'F-$)12$/MG_ZTJZ$)(S?F$,-R1-Y$Y--X-'2B$5SS,HN1()+-Q@$%6'$HO$F+?2-'+F$>>-S+-2H

/MG_ZTJZ$,-$>>-S+-2H4 &^B$

!"M@+$7#(*"&5+9$'&$;() 21(&8"#M'&%B7(F7#B%*75('&K(17*6&"9':,(((((((((((((((((((0',(<==R?<==S(((((

C,HR12-2$H(S?+1'3$'2$->D'HQH3@$FFF+)'>-H4 &^$F+?2-'+F$)>?Q@$)'2$F+)OOBJ?12-2$H+D$NS-,1-'>-2$'2$HR1>-$

?F-,F$1'$,H?0Q-FDHH+1'3$H(S?+-,$FHO+Y),-$)'2$),2Y),-$,HQ-(F$)'2$2R1F-2$H'$SS,HS,1)+-$>+H'B(

A-0.-EFG2I04.(IE3JE0)(.-Q-A3I)-41(-HI-E2-4!-(

)21T) 7#%K%&0*'K7M,((F@#&:<==U(((
/H'>-1R-2HO'2$H,3)'1U-2$$-)(S?F$RF1-$NSHF1'3$)Q-'+-2$D3D$>DHHQ$F+?2-'+F$H$$,H(1'-'+$-F-),>D$Q0H,)+H,1-F$)+$
4&^B$*+?2-'+F$Y-,-$$,HR2-2$Y1HD$'$1'+,H2?>+H'$H$Q-$1'$$DE$$,H3,)(.$1R-'$D-$HSSH,+?'1H@$H$RF1-$Q0F.$'2$S-)X$
Y1HD$>?,,-'+$$,)2?)+-$F+?2-'+$0H?+$D-1,$-F-),>D$$FS1,-H()X-DF)$01)''?)Q$-R-'+E$$(

)21F+MM 7#E757'#*6(I#":#'M((F+MM 7#(<==S(
aH?'2-2$'2($)>1Q-)+-2$D-$1,F+$4*TC$HQ'+--,$E)@E$/HH,21')+-2$Y1HD$H>)Q$HH2$)'XF$'2$H'<$,HO1-F$H$F-'2$
)SS,HN1()+-Q$$6$RHQ'+--,F$H$F1-F$,H?'2$D-$PHF+H'$,-)E$ZR-'+HY'$''?)Q$H(SH'-'+HOF?((-,$$,H3,)(E$

!"&B7#:7(I#7B%7V W 77X7&K((<==R?@#757&$(
*-,R-2$F$$1)1FH'$0-+Y--'$D-$/H'R-,3-$CQ''1'3$-)($'2$D-$4&^$/),--,F$MO1>-E$M,3)'1U-2$1'OH,()+H'$F-FF1H'F.$Q-2$
0@$D-$/),--,F$MO1>-$'2$W,1H'3$/-'+-,$-)>D1'3$F+?2-'+FH-O1>1-'+Q$1'>H,SH,)+-$D-1,$-N+,)>?,,1>?Q,$'2$>)2-(1>$
-NS-,1-'>-F$1'+H$$H'>F-$'2$-,F?)F1R-$-,FH')Q$F+)+-(-'+E$(

L#'%(&K(!":&%%B7F*%7&*7(&$7#B%7V W 77X7&K((<==R?@#757&$(
*-,R-2H'D-$Q''1'3$H((1H+--$H,$''?)Q$2-S),+(-'+$$,)2?)+-$F+?2-'+$1'+,R1-Y$Y--X-'2E$/D)1,$H((1H+--$
,-FSH'F1Q-$H,$YH,X1'3$Y1HD$H>)Q$DH+-QF$H$H,3)'1U-$Y-QH(-$21''-,$'2$,)2?)+-$F+?2-'+$1'OH,()+H'$)'-QE$(

F%K&7,('&K(I'*%%*(1'YW "#X56"@(((<==R(
WH,X-2$H'$$-)($F$$,)2?)+-$F+?2-'+$-S-,-F-'+)+R-HH,3)'1U-$)N$YH,XFDHSE$-Q$-2$4&^$F+?2-'+F$'2$/)(0,123-$
,-F12-'+F$--21'3$FF1F+)'>-$Y1HD$)N$-+?,'FE$/-,+1O1-2$0@$D-$T*E$

J#'K+'$7E75%K7&*7(1+$"#;() 21F$+K7&$A%7(I#":#'M5(((((((((((((((((((((((((((((((((((((((<==Z((((((((
]1R-2$Y1HD$'2$F?S-,R1F-2$'2-,3,)2?)+-F$c)SS)$Q$D)$D-+);$1'$4&^$DH?F1'3E$HF+-,-2$$HF1H1R-$R1'3$-'R1,H'(-'+$
>HH,21')+-2$FH>1)Q$>+R1H1-F.$0?1Q$H((?'1H@$+(HFSD-,-$'2$-'>H?,)3-2$-,FH')Q$,HY+DE$

F$+K7&$(17'*67#;(J7'#(/@;(I6%/K7%6%;(I0((((((((((((((((((((((((((<==C((
E-F1'-2$'2$1(SQ-(-'+-2$H(S?+-,$F>1-'>-$?,,1>?Q($H,$2R)'>-2$'+D$'2$K+D$,)2-$F+?2-'+E$C,HR2-2$'$
1'+,H2?>+H'HW1'2HYF$'2$4)>1'+HFD$Q+H,(FE$(

24Q21-.(10AOF(

- 4-,121)'$>)2-(@.$"#$%&'(#)%"*%*+,'$%-(),"(,$/HHQ23-$/H,'-,$4 5.$a)Q$%66K$
- 4&^$D13D$>DHHQ$*+?21-F$C,H3,)(.$%/*#0,*1$2)"*3,$(,)4,-*#0,*5%$$&.$/)(0,123-.$4 5.$*S,1'3$%66"$
- 5/4 Z$1?'>D.$7,/)$)"8*#0,*1$2)".$/)(0,123-.$4 5.$*S,1'3$%66"$

0!12Q212-FTG343EF((
4&^$PQ>X$J,)2?)+-$*+?2-'+$FFH>1)+H'$/H</D)1,$PJ*5$+,-)F?,-,$J,)2?)+-$F+?2-'+$-S,-F-'+)+R-.$J*M$E1R-,F1H@$
&1H1)+R-.$4&^$P)Q$HH($E)'>-$*-)($4&^$E)'>-$D-)+-,$Z'F-(0Q-.$*-Q->+-2$H,$4>X1'F-@$E1F>HR-,@$W--X-'2.$P,123-$H$
P/J.$4 5T/$C,H3,)($*>DHQ,$)F-,$C,H3,)($*>DHQ,$1'>HQ$('1R-,F1H@$C,-F12-'+1)Q$*>DHQ,$C5$*S)>-$J,)'+$/H'FH,+1?($
QH)'$H?'2)+H'$>DHQ,$C,-F12-'+$HO$C-,OH,(1'3$,+F$*H>1-+@(

I/LA2!0123 4FT0LF1E0!1F(

C[$$E]@>X()'$*E$DH,'3$/E$]-)(@EE^,HS-)EW)+)')0-.E_)'$W),+$/E$4>/?,,@^{E}1)()(H,1$4E$*?,$J-'-$NS,-FF1H'$$$$$$$
S)+-,'$1'$RF?)Q$H,+-N$2?,1'3$D-$-,1+>)Q$-,1H2@$)S12$-3?Q+H'$HO$()+?,)+H'$'2$-R-,F)Q$0@$RF?)Q$2-S,1R)+H'$C5*$
%66K$$
<[ZEJ1)>H(-+1E^,HS-)EW1QH'$/E$4>/?,,@Ea?TEQ''-,@$4E$*?,$C),+1)Q$-R-,F)QHOT-+$*@'2,H(-<QX-$
F@(S+H(F$1'$4Z/C%$(?+)'+$(1-$C5*$%668$$
>[$/E$4>/?,,@E*D-SD-,2E^,HS-)EW)'3$4E$P-),$4E$*?,$T-2?>-2$-NS-,1-'>-<2-S-'2-'+QF+1>1@$1'$D-$RF?)Q$
>H,+-NHO,>$?Q$R-'1Q-$(1-$-?,H'$1'$F?0(1FFH'($$
Z[$/E$4>/?,,@E*D-SD-,2E^,HS-)EW)'3$4E$P-),$4E$*?,$T-2?>-2$H>?Q$2H(1')'>-$Q$F+1>1@$1'$D-$RF?)Q$>H,+-N$HO$
f?R-'1Q-$,>$?Q$(1-$*H>1-+@$H,$-?,HF>1-'>-$66K$$$
R[$/E$4>/?,,@E^,HS-)EW)'3$4E$*?,$$HQ-$H,$$>$1'$H'F+)1'1'3$2?Q$>H,$2H(1')'>-$Q$F+1>1@$*H>1-+@$H,$
G-?,HF>1-'>-$66"$$
ScMX)(H+H$T$$),)@)')$/E$4>/?,,@$$E$)@FD1$/)(c&$-3?Q+H'$HO$>+1'$$-@+HFX-Q+H'$1'$2-'2,1+>$FS1'-$H,()+H'$
)'2$F+)01Q1U)+H'$*H>1-+@$H,$-?,HF>1-'>-$66#$

Cortina McCurry

12 Inman Street, Cambridge, MA 02139 · (617)230-9534 · mccurry@mit.edu

EDUCATION

Massachusetts Institute of Technology, Cambridge, MA **2003-present**
PhD in Systems Neuroscience June 2009, GPA 4.8/5
Walle Nauta Award for Excellence in Graduate Teaching 2007- awarded to 3 students each term
National Eye Institute Training Grant recipient 2007-present
Norman Leventhal Presidential Fellow 2004-2007
MIT Presidential Scholar 2003-2004

Lincoln University, Oxford, PA **1999-2003**
Bachelor of Science, Chemistry, Math Minor
Graduated Summa Cum Laude
Coca-Cola Corporate Scholar- awarded to 50 students across the nation. Highly competitive

RESEARCH EXPERIENCE

Graduate Student, Brain and Cognitive Science Department, Cambridge MA **August 2003-present**
Discovered a critical role for the immediate early gene Arc in visual cortical plasticity.

Researcher, Lancaster University, Lancaster UK **Summer 2003**
Researched the role of amyloid beta protein deposits in the pathogenesis of Alzheimer's disease. Worked with physicists and molecular biologists to explore the utility of tools investigating mechanisms for cell death.

INDUSTRY AND GOVERNMENT EXPERIENCE

Intern, Coca Cola Company, Scientific and Regulatory Affairs, Atlanta, GA **Summer 2002**
Regulated new products developed in the EU, Mexico, Puerto Rico, and Argentina. Designed and implemented database tracking customs classifications and trade tariffs for import of Coca-cola concentrate in 100+ countries. Offered full time position with the company.

Intern, Coca Cola Company, Product Development, Atlanta GA **Summer 2001**
Reviewed development of new Coca-Cola products and investigated areas for product improvement. Researched the effect of aging on flavor and recommended procedures to increase shelf life. Worked with the Sprite/National Basketball Development League marketing team to design promotional toolkits for nationwide campaign.

Private Contractor, High Performance Computing, Army Research Labs, Aberdeen, MD **Summer 2000**
Evaluated and assessed the utility of various computational analysis software packages and provided feedback to management. Produced molecular dynamics code modeling usefulness of assorted glassy metal compositions as ideal materials for new army technologies.

CONSULTANT RELATED EXPERIENCE

Student consultant, MIT Diversity Initiative and Graduate Student Office **2003-present**
Collaborated with MIT GSO to recruit talented underrepresented scientists and engineers. Assisted in the development and execution of an annual all-expense paid CONVERGE campus preview weekend. Approximately 20% of students accepted to CONVERGE are accepted to MIT.

Computer consultant, MIT Information Services and Technology **May 2005-2006**
Provided computing and technology assistance to MIT students, faculty, and staff. Guided both experienced and novice users in troubleshooting computer software and hardware problems and advised on appropriate action.

LEADERSHIP AND PROGRAM DEVELOPMENT EXPERIENCE

MIT/Meridian Academy **Spring 2008**
Conceived of and organized a campus visit exposing talented high school students to prominent research laboratories at MIT. Students were provided with an introduction to life in a PhD program, given the opportunity to visit labs, and speak with current graduate student about their research. Aspire to make this a biannual event.

MIT Summer Research Program **Summer 2006**
Founded and facilitated the first MSRP Volunteer Day. Coordinated with local food banks and non-profits to send approximately 60 volunteers to sites around the Boston area. Event now an annual component of summer program.

Converge Preview Weekend **2005-present**
Served as a liaison between the Converge Planning Team and the MIT Careers Office. Organized information sessions, led by the Careers Office and Writing Center, teaching students to efficiently incorporate their extracurricular and academic experiences into a concise and persuasive personal statement.

Brain and Cognitive Science Interview Weekend **2005-present**
Served on the planning committee for annual department graduate student interview weekend. Chair committee responsible for working with local hotels to organize welcome dinner and graduate student information panel.

Sidney and Pacific Tax Workshop **2005**
Worked on a team as a graduate student representative to organize tax workshop. Helped MIT students and Cambridge residents needing assistance with tax returns. Certified by the IRS.

Graduate Residence Tutor, MIT Student Life Programs **2004**
Lived with and supervised undergraduates (Kappa Alpha Theta) in MIT housing. Fostered a positive living environment, coordinated social activities, built community atmosphere, and encouraged personal growth.

Student Teacher, Gear Up, Philadelphia, PA **2001**
Designed and implemented computer science curriculum for advanced 7th and 8th grade students. Provided an introduction to Windows and Macintosh platforms.

INVITED TALKS

- Meridian Academy, *Introduction to Neuroscience*, Coolidge Corner, MA, Fall 2008
- MIT High School Studies Program, *How the Brain Perceives the World*, Cambridge, MA, Spring 2007
- ACME Lunch, *Rewiring the Brain*, Cambridge, MA, Spring 2007

ACTIVITIES/HONORS

MIT Black Graduate Student Association Co-Chair, BGSA treasurer, Graduate student representative, GSO Diversity Initiative, MIT Ballroom Dance Team, MIT Dance Theater Ensemble, Selected for Mckinsey Discovery Weekend, Bridge to BCG, MARC Program Scholar, Laser Program Scholar, Lincoln University Presidential Scholar, PA Space Grant Consortium, Sloan Foundation Scholar, President of Performing Arts Society

PUBLICATIONS/ABSTRACTS

1. A. Lyckman, S. Horng, C. Leamy, D. Tropea, A. Watanabe, A. Van Wart, C. McCurry, T. Yamamori, M. Sur, Gene expression pattern in visual cortex during the critical period: rapid regulation of maturation and reversal by visual deprivation, PNAS 2008
2. E. Giacometti, D. Tropea, N. Wilson, C. McCurry, D. Fu, R. Flannery, M. Sur, Partial reversal of Rett Syndrome-like symptoms in MECP2 mutant mice, PNAS, 2009
3. C. McCurry, J. Shepherd, D. Tropea, K. Wang, M. Bear, M. Sur, Reduced experience-dependent plasticity in the visual cortex of Arc null juvenile mice, Neuron, (in submission)
4. C. McCurry, J.Shepherd, D. Tropea, K. Wang, M. Bear, M. Sur Reduced ocular dominance plasticity in the visual cortex of juvenile Arc null mice, Society for Neuroscience 2008
5. C. McCurry, D. Tropea, K. Wang, M. Sur A role for Arc in constraining adult ocular dominance plasticity, Society for Neuroscience 2007
6. K. Okamoto, R. Narayana, C. McCurry, Y. Hayashi. CamKII regulation of actin cytoskeleton in dendritic spine formation and stabilization, Society for Neuroscience 2005