Critical period revisited: impact on vision
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Neural circuits are shaped by experience in early postnatal life. The permanent loss of visual acuity (amblyopia) and anatomical remodeling within primary visual cortex following monocular deprivation is a classic example of critical period development from mouse to man. Recent work in rodents reveals a residual subthreshold potentiation of open eye response throughout life. Resetting excitatory–inhibitory balance or removing molecular ‘brakes’ on structural plasticity may unmask the potential for recovery of function in adulthood. Novel pharmacological or environmental interventions now hold great therapeutic promise based on a deeper understanding of critical period mechanisms.

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Introduction
Much of our adult behavior reflects the neural circuits sculpted by experience during ‘sensitive’ or ‘critical periods’ (CP) in early postnatal life. At no other time does the surrounding environment so potently shape brain function [1–3]. Understanding how this plasticity waxes and wanes with age carries an impact far beyond neuroscience, including therapeutic approaches to developmental disorders or strategies for recovery from brain injury in adulthood [4,5].

For over 40 years the visual system has stood as the premier model of CP plasticity [6]. From human clinical cases to classical experiments in monkeys and cats, it is well known that the occlusion of one eye – monocular deprivation (MD) – only early in life yields a loss of visual acuity through the deprived eye that persists into adulthood (Supplementary Table 1). If left untreated, the ensuing amblyopia is permanent, a condition affecting 2–5% of the human population [7].

It should be noted that amblyopia is not a single abnormality caused only by deprivation, but is associated with several disorders of discordant visual input that occur in early childhood, such as strabismus (crossed eyes) and anisometropia (monocular defocus). Each type can be accounted for by the differential combination of reduced resolution and loss of binocularity [8]. Among them, deprivation amblyopia applies to most animal studies as a direct test of competition between inputs and shows the strongest correspondence between cortical ocular dominance (OD) and acuity loss [9].

Early MD produces a shift of neuronal spiking response in favor of the open eye, which is detected by single-unit electrophysiology from the primary visual cortex (V1) and followed by profound structural changes [6]. Importantly, these effects of MD have not been seen in adult animals [6]. Recovery from amblyopia caused by long-term MD initiated during the CP also does not occur readily in adulthood [6]. Strikingly, the duration of the CP (weeks to years) is proportional to the expected lifespan of the species [3], suggesting an evolutionarily conserved, robust and fundamental mechanism in neurobiology.

The rodent, especially the mouse, has now emerged as a valuable model system that allows genetic manipulation to dissect the molecular mechanisms of OD plasticity [2,10–13]. Rodents also exhibit a reduced behavioral acuity through the deprived eye during a CP matched to that measured by single-unit electrophysiology [14–17]. However, recent studies have reported residual cortical plasticity in adult rodents, prompting new questions about CP and adult plasticity [18,19]: What leads to the discrepancy with OD plasticity in other species? Are the same phenomena being measured? Is any change accompanying MD to be considered OD plasticity? What is the impact on vision?

To address these issues and to further extend their functional implications, here we re-examine the seemingly confusing reports of ‘adult OD plasticity’ in rodent visual cortex. On the basis of a characterization of differences to the CP, the impact on recovery from amblyopia will be discussed. We limit our review to classical deprivation amblyopia, not including other forms of adult plasticity such as perceptual learning [20] or retinal scotomas, which may share certain aspects of CP plasticity [19,21]

Adult OD plasticity in rodents?
We analyzed recent reports of OD plasticity in adult rodents along four criteria: measurement method, direc-
tion of plasticity, MD duration from age of onset, and anesthesia (see Supplementary Tables 2–6). Close inspection across studies rules out anesthetics as a factor, as no particular condition (e.g. urethane) can account for the differences between presence \[22,23,24*25,26\] or absence \[14,27,28,29*,30,31,32**\] of adult plasticity (Supplementary Table 6). For MD duration, while >3 days MD is certainly required for adult plasticity (unlike in the CP) \[22,33*\], there are numerous reports also showing little plasticity even after long-term adult MD \[14,16,17,27,34\] (Supplementary Table 5).

Age of MD onset varied widely across rodent studies (Supplementary Table 4). It is well known from higher mammals such as human, monkey, and cat that CP plasticity does not end abruptly but rather declines gradually \[35,36\]. For instance, single-unit recording outside layer 4 detects plasticity far later than within the input layer (Supplementary Table 1). Recent rodent work has largely reconfirmed these phenomena, including sub-granular changes lasting into adolescence \[24*,33*40\]. It may be more accurate to extend a declining, late phase of the mouse CP out to postnatal day P60 beyond the classical P35 \[15\]. Nevertheless, reports differ as to the degree of plasticity as late as three months of age, when rodents are considered fully mature (Supplementary Table 4).

Measurement method and direction of plasticity appear to be the most salient factors which distinguish CP from adult plasticity (Supplementary Tables 2 and 3). Functional changes can be subdivided into two categories on the basis of measurement methods: spike-related events and subthreshold (synaptic) events (Table 1). Spike-related signals from V1 (single-unit recording, flavoprotein imaging) accurately reflect the reduced acuity.
through the deprived eye during a CP (behavior, visual-evoked potential [VEP]). On the contrary, methods that detect primarily subthreshold changes (VEP amplitude Contra/Ipsi [C/I] ratio, immediate early gene [Arc/c-Fos] expression, and, hemodynamic imaging) reveal plasticity throughout life (Table 1; Figure 1).

Notably, adult plasticity in most cases is seen as an increase of subthreshold responses through the open eye. Spike-related output and acuity of the deprived eye is instead reduced during a limited CP (Table 1). A behavioral increase of open eye acuity measured by optokinetic head turning in mice is observed only in the monocular visual field [37]. The relevance of changes outside the binocular zone to OD plasticity is unclear. For example, MD fails to induce spine motility or pruning in the monocular zone of V1, where competition between the two eyes cannot occur [38,39].

Importantly, structural plasticity is mostly restricted to a CP (Table 1). Shortly (two days) after MD, the motility of spines is increased on apical dendrites of excitatory pyramidal neurons by an increase in tissue-type plasminogen activator (tPA)–plasmin proteolytic activity [39]. This motility reflects subthreshold changes, as two days of MD modestly alter single-unit recordings in mice [15,73]. Four days after MD, the number of spines is transiently and significantly pruned [38], followed by a retraction of thalamocortical axons. Axon terminals serving the open eye eventually expand as in other species and become fixed with age [40].

In adults, these structural changes on pyramidal cells become limited [38,39]. Non-pyramidal GABAergic interneurons instead display a dynamic remodeling of dendritic arbors even without MD, while pyramidal cells remain stable [41]. However, the interpretation needs caution, as in vivo imaging through a cranial window is associated with substantial glial activation after surgery, which does not happen when the skull is intact [42]. It will be interesting to determine whether interneurons specifically increase their structural dynamics upon MD at any age (Table 1).

What appears essential for vision is the proper communication of spike-related output from V1 to other areas involved in visual perception and behavior. Early visual deprivation is known to impact perceptual processes downstream from V1 in the extrastriate cortex [43], including holistic processing of objects and faces [44]. A CP for vision may then be defined more accurately by the presence of spike-related output (acuity) changes in V1 (Figure 1a). The use of VEP measurements has been somewhat misleading, as C/I ratio [22,23,25,45] is typically examined at the lowest spatial frequencies (0.04–0.14 cyc/deg) far from the acuity threshold and may not accurately reflect perception. Instead, VEP detection threshold correlates with psychophysical visual acuity in humans [46]. Following even a long-term MD of adult rats, even a long-term MD, no change in acuity by VEP is seen [14].

Surprisingly, while behavioral acuity clearly shows no loss [17], few reports in mice have directly examined VEP acuity in V1 after adult MD (Figure 1b). In one study [24*], little reduction is detected through the contralateral, deprived eye, despite a change in C/I ratio at low spatial frequencies. Although a change of acuity in the ipsilateral pathway is also reported, relatively low baseline values in the absence of MD (0.3 cyc/deg) compared to other studies (0.5–0.6 cyc/deg) [17,26,29*], compels further confirmation.
The meta-analysis of ‘adult plasticity’ in the rodent visual cortex thus reveals that proper binocular vision reflects changes in V1 spike output that become limited with age. Subthreshold plasticity may instead occur throughout life with little impact on acuity except during a CP (Figure 1a). Understanding why subthreshold changes do not influence vision beyond the CP may hold a therapeutic key to restoring spike output when recovery from amblyopia in adulthood is desired.

**Recovery of function in adulthood**

Impact on acuity is especially important if we want to understand the biological basis for recovery of function [4,5]. After long-term MD spanning the CP, reverse suture, or binocular experience alone is not potent [4,6,16,17]. Although slight behavioral gain after prolonged binocular vision (80 days) has been reported in rats, it is still far from complete [48].

Promisingly, recent rodent studies are beginning to uncover multiple strategies for better recovery (Figure 2a). One approach is the infusion of chondroitinase ABC (chABC) to degrade chondroitin sulphate proteoglycans (CSPGs), which typically condense into specialized extracellular matrix structures called perineuronal nets (PNNs) in the mature cortex. Adult chABC treatment coupled with reverse suture in amblyopic rats produces recovery of spike OD, behavioral and VEP acuity after long-term MD initiated during the CP (Figure 2b) [49]. Importantly, structural recovery of spine density is also observed after infusion, although the persistence of these anatomical effects is untested.

Strikingly, reverse suture coupled with environmental enrichment [50], chronic administration (one month) of the well-known antidepressant Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) [32], or 10-day dark exposure [28,51] also reveals functional recovery of acuity. However, in the latter case behavior is significantly less restored than VEP acuity (Figure 2b). Whether V1 spike output or structural changes also occur with these non-invasive approaches remains unknown. Similarly, another recent study in mice claims significant recovery from long-term MD when binocular vision is followed by reverse suture [52], but neither behavioral acuity nor persistence was examined.

Prior experience, such as an earlier OD shift, may interestingly set a sub-threshold scaffold for adult plasticity (as observed with chronic hemodynamic imaging in mice) [33]. Transient blockade of synaptic transmission during the CP with botulinum neurotoxin in one visual hemisphere of rats can extend OD plasticity into adulthood [53]. Surprisingly, OD plasticity is also observed in the opposite hemisphere, indicating that lack of callosal input during development is sufficient to unmask adult plasticity. These results further demonstrate the impact of CP development for resiliency in adulthood, as well as an important confound of individual history when assessing the true magnitude of adult recovery of vision in case reports from humans [54,55].

**Mechanism of reactivation**

We can draw a hypothesis for the mechanism of recovery on the basis of our current knowledge of the natural onset of OD plasticity. Recent gene expression studies reveal that distinct biochemical milieu characterize the juvenile and adult cortex [56,57]. Epigenetic changes in histone phosphorylation and acetylation may further control such gene expression [30].

The natural CP is a sequence of molecular events [10]. First, the transition from pre-CP to CP is triggered when a drastic adjustment of excitatory–inhibitory (E−I) balance reflects the late development of specific inhibitory cir-
Emerging strategies for recovery based on normal development. (a) Resetting E–I balance (red arrow): Transition from pre-CP to CP (dashed arrow) is now known to be triggered by a drastic adjustment of excitatory–inhibitory (E–I) balance when specific inhibitory circuits develop later than excitatory connections [10,34,58–61]. One strategy then is to reset this E–I balance to a pre-CP state to reactivate CP plasticity. chABC infusion, environmental enrichment, chronic Fluoxetine, and exposure to darkness yield this result. (b) Removing molecular ‘brakes’ (blue arrow): Downstream of the E–I trigger, structural reorganization ultimately consolidates plasticity (solid black arrow). Recently, several molecular ‘brakes’ have been identified which may inhibit neurite growth in adulthood. Chondroitin sulphate proteoglycans (CSPGs) condensed into perineuronal nets (PNNs) [27,49], myelin–inhibitors. Similarly, the Nogo receptor (NgR) on neurons binds several axonal growth inhibitors in myelin, such as Nogo–A/B, myelin–glycoprotein (MAG), or oligodendrocyte–myelin glycoprotein (OMgp) [63]. Genetic deletion of NgR, or its ligand Nogo-A/B in mice reveals continued spike plasticity well after the normal CP, suggesting their role as a brake for adult plasticity [64]. Both CSPGs and NgR were initially identified as factors responsible for regenerative failure after axonal injury in the adult central nervous system [63].

Stimulation of histone acetylation and active transcription by the histone deacytelase (HDAC) inhibitor, trichostatin A, induces a change in VEP C/I ratio when coupled with adult MD [30]. If spike response or acuity is altered as well, HDACs may qualify as an epigenetic brake [30]. Molecular brakes also need not be limited to adulthood. PirB, a major MHC1 (major histocompatibility complex class 1) receptor, limits plasticity at all ages. In mutant mice lacking functional PirB, 10 days of monocular enucleation from P19–P100 induces an expansion of Arc induction ipsilateral to the remaining eye [65]. In the pre-CP, subthreshold binocular interactions are evident by hemodynamic imaging [66], but the expression of polysialic acid (PSA) prevents premature OD shifts [67]. It will be interesting to determine how the removal of these molecular brakes – primarily found in the extracellular space – ultimately influences E–I balance and the recovery of vision.

Conclusion
Why do reports of ‘adult OD plasticity’ in rodents outnumber those of more traditional animal models? Certain aspects, such as gradual CP decline by layer, have long been known in higher mammals. Conversely, there is an absence of evidence rather than evidence of absence for adult OD plasticity in these other species. Applying recent methods for detecting subthreshold changes to cats and monkeys should establish whether vision in the nocturnal rodent is unusually plastic or relevant for clinical approaches to humans. Indeed, resetting E–I balance to mimic CP onset, or targeting molecular brakes that consolidate structural changes after the CP are promising strategies for functional recovery in adulthood. Amblyopia might then offer a more general model for understanding cognitive developmental disorders of similar etiology in early postnatal life, such as autism [68] or schizophrenia [69].

The seemingly disparate rescue reports above, such as chABC infusion, enriched environment, chronic Fluoxetine, and dark exposure, may all reset E–I balance to a pre-CP state. chABC breaks down the PNN that preferentially enwraps mature parvalbumin-positive basket cells, whose maturation triggers the endogenous CP [10]. Chronic Fluoxetine [32] and enriched environments [50] both reduce GABAergic transmission, and their rescue effect can be prevented by enhancing inhibition with diazepam. Adult dark exposure also decreases the level of GABA<sub>1</sub> receptors relative to excitatory AMPA receptors [28]. One possibility is that by resetting E–I balance, subthreshold changes can once again be translated into spike output changes, which normally occur when an optimal E–I balance is reached during the CP [61].

Another target for CP reactivation may be the structural reorganization, which ultimately consolidates plasticity. Recently, several molecular ‘brakes’ have been identified that are involved in this process. Enzymatic degradation of CSPGs by chABC in adult rat visual cortex may serve a dual purpose of E–I resetting and removal of neurite growth inhibitors. Similarly, the Nogo receptor (NgR) on neurons binds several axonal growth inhibitors in myelin, such as Nogo–A/B, myelin–glycoprotein (MAG), or oligodendrocyte–myelin glycoprotein (OMgp) [63]. Genetic deletion of NgR, or its ligand Nogo-A/B in mice reveals continued spike plasticity well after the normal CP, suggesting their role as a brake for adult plasticity [64]. Both CSPGs and NgR were initially identified as factors responsible for regenerative failure after axonal injury in the adult central nervous system [63].

Figure 3
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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cob.2008.05.009.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

31. Fagiolini M, Hensch TK: Barn owl tectum [1]. Dominance plasticity can be enhanced by a prior ocular dominance shift, Using hemodynamic imaging in mice, this work suggests that adult ocular dominance plasticity. Intrinsic flavoprotein imaging reflects direct (non-hemodynamic) changes in neuronal metabolism, which is coupled to spike discharge. No adult plasticity is detected.
35. Chronic administration (one month) of the common antidepressant Fluoxetine reactivates ocular dominance plasticity in adult rats and promotes recovery from amblyopia both electrophysiologically and behaviorally. These effects were accompanied by reduced inhibition and increased expression of brain-derived neurotrophic factor.
37. Using hemodynamic imaging in mice, this work suggests that adult ocular dominance plasticity can be enhanced by a prior ocular dominance shift, similar to the results of Knudsen exploring multisensory integration in the barn owl tectum [1].
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First demonstration of recovery from amblyopia in adult rats after long-term MD. Infusion of chondroitinases directly into V1 to disrupt ECM degradation promotes amblyopia recovery through a reduction of intracortical inhibition. Nat Neurosci 2007, 10:679-681.


Two non-invasive strategies for recovery from amblyopia. While environmental enrichment appears totally effective [50], dark exposure coupled with reverse suture or binocular experience in adults shows greater functional recovery of VEP acuity in V1 than for overall behavior [51]. Both interventions may be mediated by a reduction of GABAergic inhibition.


57. Tropea D, Kreiman G, Lyckman A, Mukherjee S, Yu H, Horig S, Sur M: Gene expression changes and molecular pathways mediating activity-dependent plasticity in visual cortex. Nat Neurosci 2006, 9:660-668. Two microarray studies [56,57] demonstrating distinct sets of visually-regulated gene expression across the lifespan. While some genes are activated by monocular enucleation (ME) at all ages [56], others are activated only when monocular vision occurs during the CP.


