ELSEVIER

Mechanisms of eye-specific visual circuit development

Andrew D Huberman

Eye-specific visual connections are a prominent model system for exploring how precise circuits develop in the CNS and, in particular, for addressing the role of neural activity in synapse elimination and axon refinement. Recent experiments have identified the features of spontaneous retinal activity that mediate eye-specific retinogeniculate segregation, the synaptic events associated with this process, and the importance of axon guidance cues for organizing the overall layout of eye-specific maps. The classic model of ocular dominance column development, in which spontaneous retinal activity plays a crucial role, has also gained new support. Although many outstanding questions remain, the mechanisms that instruct eye-specific circuit development are becoming clear.

Addresses
Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA

Corresponding author: Huberman, Andrew D (adh1@stanford.edu)

Current Opinion in Neurobiology 2007, **17**:73–80

This review comes from a themed issue on
Development
Edited by Ben Barres and Mu-Ming Poo

Available online 24th January 2007

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2007.01.005

Introduction

What mechanisms induce development of precise CNS circuitry? A classic model circuit for investigating this question is eye-specific visual connections; axons carrying visual information for the left and right eyes are segregated into non-overlapping domains in the lateral geniculate nucleus (LGN) and into ocular dominance columns (ODCs) in the visual cortex. The cellular events involved in eye-specific retinogeniculate refinement are well characterized: after an initial overlap phase, axons from the two eyes segregate by elaborating synapses and axon terminals in the same-eye territory and by eliminating synapses and axon terminals in opposite-eye territory [1,2]. Spontaneous retinal activity is known to be required for segregation to occur [3], but several important questions have remained unanswered: which features of spontaneous retinal activity are instructive for eye-specific segregation? What are the molecular mechanisms for eliminating weaker synapses and maintaining strong ones? What factors dictate the overall layout of eye-specific maps? Similar questions exist regarding ODC development as well. In the past decade, however, even the basic cellular events associated with ODC development have become controversial as several high profile studies [4,5] challenged the idea that ODCs are sculpted by early retinal activity. Here, I review recent advances in our understanding of retinogeniculate and ODC development, and I suggest experiments that could further elucidate how these circuits form.

The role of patterned retinal activity in eye-specific retinogeniculate segregation

Neural activity mediates development of eye-specific retinogeniculate projections through competitive interactions involving the relative levels of retinal ganglion cell (RGC) spiking in the two eyes [3,6]. Hebbian models predict, however, that correlated firing — and not simply activity levels per se — of neighboring RGCs is crucial for eye-specific segregation [7]. In theory, retinal waves [8] induce patterns of RGC spiking optimal for Hebbian-based axonal refinement but, surprisingly, the first experiments to perturb retinal waves did not observe any accompanying disruptions to eye-specific segregation in the LGN [9]. Moreover, some mutant mice with altered retinal waves exhibit severe eye-specific retinogeniculate defects [10], and yet pharmacologically inducing waves in these mutants does not rescue segregation [11]. Thus, to elucidate which aspects of spontaneous retinal activity instruct eye-specific segregation, Feller and colleagues [12••] undertook a systematic analysis of RGC spiking patterns in different strains of mutant mice, each with differently altered retinal waves but with varying degrees of eye-specific segregation in the LGN. They found that high frequency correlated bursting (>10 Hz) of neighboring RGCs is a key parameter required for eye-specific segregation. They also found that the precise time-window for RGC firing is not crucial for segregation, provided that the degree of correlated firing among closely spaced RGCs remains relatively higher than the degree of correlation among distant RGCs. Their results suggest why in previous experiments where retinal waves were disrupted, the anticipated defects in retinogeniculate refinement were not observed [6,9]. They also suggest which types of synaptic plasticity can (and cannot) drive segregation. For instance, because low frequency (~1 Hz) RGC firing is not related to segregation [12••], it is unlikely that typical forms of long-term depression (LTD) mediate synaptic elimination in this system. By contrast, the importance of high frequency RGC bursts suggests that bursts trigger plasticity mechanisms that both stabilize same-eye inputs and eliminate opposite-eye inputs onto single LGN cells (i.e. heterosynaptic depression). Through the use

Figure 1

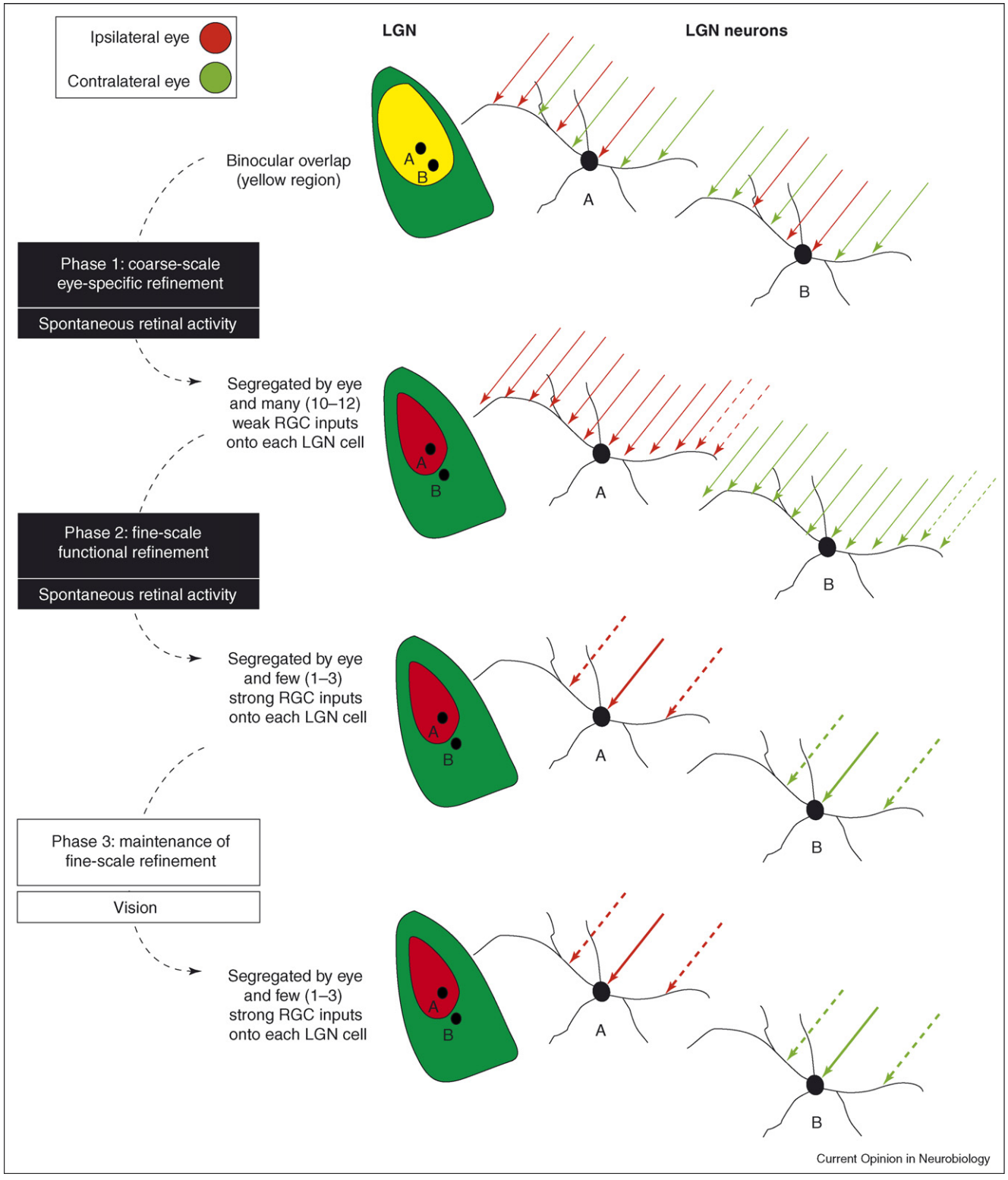


Diagram of the three major phases of synaptic refinement in the developing retinogeniculate pathway. The view of the entire mouse LGN is depicted on the left, and two LGN cells, labeled A or B, are shown at higher magnification to the right. RGC axonal inputs from the contralateral eye are depicted as the green region in the LGN and as green arrows projecting to LGN neurons A and B. RGC axons from the ipsilateral eye are depicted as the red regions of the LGN or as red arrows onto LGN neurons A and B. Phase 1: Early in development, axons from the two eyes overlap in the LGN (yellow). Cells A and B receive RGC inputs (arrows) from both eyes at this stage. Patterned spontaneous retinal activity

of various *in vitro* preparations (discussed below), such hypotheses can now be tested directly.

Maintenance of eye-specific retinogeniculate segregation

Spontaneous retinal waves persist for 1–2 weeks after eye-specific retinogeniculate segregation is completed [8], and if these post-segregation waves are blocked, retinogeniculate axons revert to an overlapping state [13]. Are waves merely permissive to maintain segregation or do particular patterns of RGC spiking actively stabilize eye-specific connections after they form? Demas *et al.* [14••] recently showed that so-called nob (no b-wave) mutant mice have abnormally frequent and persistent waves beginning just after eye-specific segregation is completed and axons from the two eyes desegregate in the LGN. Nob mutant retinas exhibit normal degrees of correlated RGC firing. Thus, their desegregation phenotype probably arises because waves occur so frequently and over such an extended period of development that RGCs residing at retinotopically matched positions in the two eyes fire simultaneously, thereby correlating the activity of residual binocular inputs onto single LGN cells [15] and triggering axonal growth leading to increased binocular overlap. This study [14••] along with the above-mentioned study from Feller and co-workers [12••] are the first to report a positive role for patterned retinal activity in eye-specific segregation. A major goal now is to determine the signals that translate spontaneous correlated RGC activity into structural and functional changes at retinogeniculate synapses, both during and after segregation.

Synaptic changes associated with eye-specific segregation

In vitro recordings indicate that LGN neurons are binocularly innervated before, but not after, eye-specific segregation occurs [16] and that the strength of those connections can be modified by synaptic stimulation [17]. Until recently, however, little was known about the synaptic changes associated with eye-specific segregation. Guido and co-workers [15,18•] have developed a powerful *in vitro* preparation in which the optic nerves from either eye can be stimulated while single LGN neurons undergo intracellular recording. This allowed them to determine that, during the stage when axons from the two eyes overlap, single LGN neurons receive functional synaptic connections from ~4–6 RGCs in each eye. Then, as binocular segregation proceeds, the inputs from one eye onto a given cell are eliminated. Interestingly, even at the stage of maximal binocular convergence, one eye always exerts stronger synaptic drive onto a given LGN neuron than the other eye does. This suggests that there are differences in the number and/or distribution of synapses arising from each eye onto single LGN cells. Computational models predict that such biases could strongly impact the outcome of activity-dependent plasticity [19]. As has proven useful at the developing neuromuscular junction [20], visualizing the number and distribution of RGC synapses onto single LGN cells across development would be very useful for formulating models of how activity-based competition occurs in the CNS.

Coarse- versus fine-scale retinogeniculate axonal pruning

Immediately after eye-specific segregation is completed, each LGN cell receives weak inputs from ~10–12 RGCs. The number of RGC inputs onto each LGN cell then diminishes to just 1–3 inputs in the following weeks, and those few remaining inputs get much stronger (Figure 1) [21]. This fine-scale pruning of retinogeniculate inputs has important implications for the sharpening of receptive fields in the LGN [22]. A recent study by Hooks and Chen [23••] showed that fine-scale refinement is mediated by spontaneous retinal activity, after which visual experience plays a role in maintaining the few (1–3) strong connections that remain. Thus, there appear to be three phases of RGC afferent pruning in the LGN: a first phase of coarse-scale eye-specific segregation, a second phase of fine-scale functional refinement and maintenance of coarse-scale eye-specific segregation, and a third phase of visual-experience-dependent fine-scale maintenance (Figure 1). All three phases involve synapse elimination. The first two are driven by spontaneous retinal activity, but it still remains to be determined whether specific patterns of RGC activity are required for the fine-scale functional pruning to proceed normally. In any case, the plasticity mechanisms that drive axonal refinement at each stage are likely to be different from one another, because manipulations that prevent or disrupt eye-specific segregation do not alter fine-scale pruning (C Chen, personal communication) or visual receptive field refinement in the LGN [24,25]. Studies are now focused on discovering the particular types of synaptic plasticity that occur at each stage and identifying their associated molecular components that lead to synapse elimination and axonal remodelling.

Molecular mechanisms of eye-specific synaptic refinement

To begin to identify the proteins that translate activity-based competition into synaptic and axonal refinements, several groups have screened for mutant mice that exhibit

(Figure 1 Legend Continued) then mediates coarse-scale eye-specific segregation [3,12••]. Phase 2: After eye-specific segregation is completed, each LGN cells receives many weak RGC inputs (10–12) from one or the other eye. Spontaneous retinal activity then drives elimination of all but 1–3 RGC inputs onto each LGN cell, and the strength of the remaining RGC inputs increases (shown here as an increase in arrow size) [21,23••]. Phase 3: Visual experience maintains the few (1–3) strong RGC inputs from each eye onto each LGN cell [23••].

normal levels and patterns of spontaneous retinal activity and yet also exhibit defects eye-specific retinogeniculate refinement. Interestingly, among the few known mutants that have been shown to meet both these criteria, all are mutants lacking immune genes. These include the class I major histocompatibility complex (MHC) immune genes [26], the C-reactive-protein-like neuronal pentraxins [27], and components of the complement signalling pathway (B Stevens *et al.*, abstract 321.23/B61 Soc Neurosci Abstr, Atlanta GA, October 2006). Class I MHC and neuronal pentraxin gene expression is strongly regulated by neuronal activity levels *in vivo* [28,29], and pentraxins have been shown to regulate axonal outgrowth and neurotransmitter receptor clustering *in vitro* [29,30]. Attention is now focused on discovering the cellular and molecular mechanisms by which immune genes affect synapse turnover and axonal re-arrangements during eye-specific refinement. One hypothesis is that, similar to their role outside the CNS, immune proteins tag relatively less-active synapses for elimination by phagocytic cells such as microglia (B Stevens *et al.*, abstract 321.23/B61 Soc Neurosci Abstr, Atlanta GA, October 2006). This is an exciting new area of research that will no doubt have important implications for understanding activity-dependent development throughout the CNS.

Ephrin-As pattern the layout of eye-specific maps

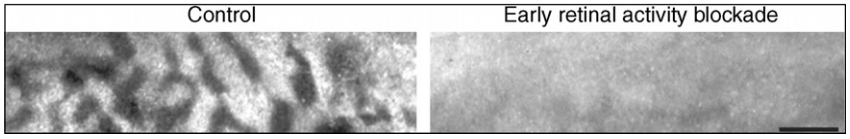
A salient aspect of eye-specific connectivity is that axons from the contralateral and ipsilateral eye terminate in stereotyped locations within the LGN. This regularity cannot easily be explained by activity-dependent mechanisms. What regulates the overall layout of eye-specific maps? One clue is that 'eye-specific' projections do not correspond to whether a given RGC axon arises from the contralateral or ipsilateral eye but, instead, to whether it arises from the nasal or temporal portion of the retina [31]. Ephrin-As and their receptors, EphAs, are known to regulate topographic mapping of RGC axons [32] and, recently, two studies demonstrated that ephrin-A–EphA interactions are also crucial for eye-specific retinogeniculate pathfinding. The first study found that, in mice lacking ephrin-A2, A3 and A5, eye-specific territories form but those territories are abnormally fractured and distributed throughout the LGN [33•]. The second study demonstrated that retinal overexpression of Eph-As induces eye-specific targeting errors in the LGN [34•]. Unlike in the developing spinal cord [35], activity does not appear to regulate ephrin expression in the developing retinogeniculate pathway [33•], nor do ephrins regulate RGC activity levels or patterns [34•]. Activity might regulate RGC pathfinding in response to ephrin-As through post-translational mechanisms, but this remains to be tested. In the meantime, the results of these two recent studies [33•,34•] indicate that activity and ephrin-As act in parallel to construct eye-specific maps: activity segregates axons from the two eyes, and ephrin-As dictate the shape, size and position of eye-specific territories.

It is noteworthy that, even in animals lacking all relevant ephrin-As, the pattern of eye-specific patches that form in the two LGNs are highly symmetric [33•]. This indicates that cues other than activity or ephrins-As play a role in patterning of eye-specific maps. Engrailed-2 can both attract nasal RGCs and repel temporal RGCs *in vitro* [36], making *engrailed-2* a good candidate to test in the context of eye-specific pathfinding. Also, a substantial degree of RGC axon segregation occurs within the optic tract [37]. Such pre-target sorting might influence the symmetry and layout of eye-specific retinogeniculate projections. Some of the factors that mediate pre-target-sorting have been identified in lower vertebrates [38]. The mammalian homologues of these factors can now be tested for a role in eye-specific mapping.

Development of ocular dominance columns

Just like eye-specific retinogeniculate projections, ODCs do not require visual experience to form [39]. But whether ODCs emerge through activity-dependent axonal refinement or, rather, through directed in-growth mediated by axon guidance cues [4,5] has been intensely debated. The controversy over how ODCs develop arises mostly because of differences in interpretation from various researchers rather than because of doubt about the validity of any particular findings. For instance, early studies that used monocular injections of transneuronal tracers to label retino-LGN–V1 axonal projections, observed continuous label in V1 (indicating lack of ODCs) of young animals, and alternating label in V1 (indicating the presence of ODCs) of older animals [40]. This led the authors of those studies to conclude that ODCs emerge from an initially overlapping and imprecise state [40]. However, the tendency for transneuronal tracers to 'spill-over' in the LGN of young animals caused other researchers [4,5] to challenge those conclusions. Moreover, these recent studies [4,5] showed that direct focal injections of anterograde tracers into single eye-specific layers in the LGN give rise to patchy labelling in V1, even when such tracing is carried out at very early stages of thalamocortical development. Whether the patchy labelling in V1 truly represents ODCs, however, is subject to interpretation as well. Given the difficulty of unequivocally visualizing ODCs in immature animals, the pruning versus targeted in-growth debate still awaits resolution. The advent of transneuronal tracers that are not subject to problems related to spillover in young animals ought to someday resolve this controversy but, regardless, optical imaging studies have conclusively shown that ODCs are present earlier in development than can be detected by transneuronal tracing from the eye [41], thereby indicating that prior experiments describing a key role for retinal activity

Figure 2



Blocking early spontaneous retinal activity permanently alters patterning of ODCs [43••]. Photomicrographs of the pattern of transneuronal (retina-LGN–visual cortex) proline label from one eye in V1. Left panel: ODCs (periodic dark-light variation in the pattern of proline label) are readily seen in V1 of adult ferrets that were injected with control solution in both eyes for the first ten postnatal days of life (P1–P10). Right panel: no ODCs (only continuous bright proline label) are present in V1 of adult ferrets that were injected with an activity blocker (1mM epibatidine) in both eyes P1–P10. The equivalent portion of V1 is shown in the two micrographs. Scale = 1mm.

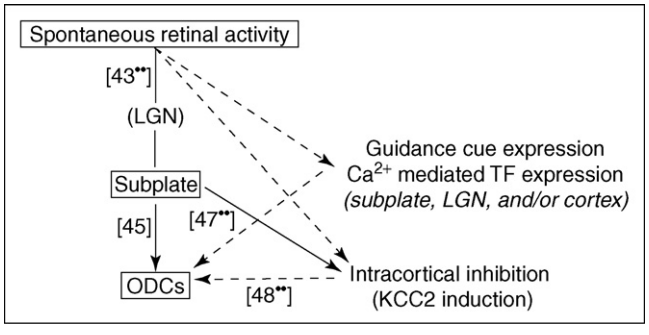
in ODC segregation [42] had actually disrupted retinal activity *after* ODCs already formed.

To clarify the role of activity in ODC development, Chapman and co-workers [43••] recently carried out experiments in which they pharmacologically blocked spontaneous retinal activity before ODCs normally form. The result of this early activity blockade is that ODCs never segregated normally (Figure 2). In addition, disrupting early spontaneous retinal activity permanently altered the refinement of binocular receptive fields in visual cortex. The results of this study are consistent with recent work from the Stryker laboratory [44••] showing a key role for patterned spontaneous retinal activity in retinotopic refinement in visual cortex, and they strongly support the traditional model of ODC development in which activity-mediated binocular competition plays an important role in segregating thalamocortical axons representing the two eyes. In future experiments, it would be useful to alter patterns of correlated RGC spiking while leaving retinal activity levels intact, to evaluate whether retinal waves are instructive or permissive for ODC development.

A pivotal role for the subplate in ODC development

How might early spontaneous retinal activity instruct ODC segregation? From P1–P10, eye-specific layers have not yet formed in the LGN [3]. Thus, activity-based information regarding whether a given thalamocortical axon will eventually carry visual signals for the left eye or the right eye can only arise through readout of spontaneous retinal activity. It is noteworthy that in the abovementioned study [43••] the retinal activity perturbation was restricted to the stage of development when LGN axons are innervating layer 4 and have collateral inputs to the subplate — a transient population of cells necessary for ODC segregation [45]. Spontaneous retinal activity is relayed via the LGN to the developing cortex [46•], and recent work from Shatz and co-workers [47••] demonstrated that an intact subplate is necessary for the development of intracortical inhibition in visual cortex. Intracortical inhibition, acting through highly specific circuitry, exerts a powerful influence on ODC patterning and plasticity [47••,48••,49•]. Thus, spontaneous retinal waves might excite subplate neurons (via thalamocortical axons) and thereby trigger maturation of those specific inhibitory circuits [49•], thereby driving ODC segregation (Figure 3). A testable prediction of this model is that blocking spontaneous retinal activity should prevent expression of KCC2 (the chloride transporter responsible for inhibitory transmission) in specific inhibitory cell types in visual cortex. Another testable idea is that spontaneous retinal activity regulates expression of calcium dependent transcription factors necessary for ODC refinement (Figure 3). A similar scenario has been demonstrated for development of whisker barrel maps in the somatosensory pathway [50]. It is also important to keep in mind that a role for axon guidance cues in ODC development has not yet been ruled out. ODCs are not present in mice and, unfortunately, transgenic ferrets, cats or monkeys do not yet exist, but through the use of *in vivo* gene transfer techniques such as electroporation [34•,51•], the role of axon guidance cues in ODC development can now be tested. It seems reasonable to expect

Figure 3



Schematic model of some of the known and hypothetical interactions between spontaneous retinal activity, the subplate, and ODC development. Solid arrows depict published findings, dashed arrows depict hypothetical interactions that are yet to be tested and/or published. An intact subplate is crucial for both ODC segregation [45] and maturation of inhibition in V1 [47••]. Inhibition in V1 is important for patterning ODCs [48••]. Blocking spontaneous retinal activity is crucial for ODC and binocular receptive field maturation (Figure 2) [43••]. In addition to affecting ODC segregation through activity-dependent correlation-based mechanisms, spontaneous retinal activity might drive expression of KCC2, transcription factors and/or guidance cues, necessary for ODC development.

that, as has now been thoroughly established for retinotopic [25,32,44••,51•] and eye-specific visual mapping [3,33•,34•], ODCs will develop through a combination of both activity-dependent [43••] and axon-guidance cue-based mechanisms.

Conclusions

Despite considerable debate in recent years, the field of eye-specific circuit development appears to be converging on a model in which the 'activity versus molecules' dichotomy is less prominent. Rather, a growing body of experimental data supports a model in which both patterned spontaneous retinal activity and axon guidance cues together contribute to the refinement of eye-specific circuits. As a general model for understanding CNS circuit development, it is my belief that the major challenges facing the field now are: to discover the precise types of synaptic plasticity that underlie retinogeniculate and ODC segregation; to identify the molecular mechanisms by which immune proteins modify synapses in response to activity; and to explore the role of axon guidance molecules and activity-dependent transcription factors in eye-specific circuit refinement.

Acknowledgements

AD Huberman was supported by a Helen Hay Whitney Postdoctoral Fellowship. I thank C Speer, C Chen and W Guido for critical reading of this manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sretavan D, Shatz CJ: **Prenatal development of individual retinogeniculate axons during the period of segregation**. *Nature* 1984, **308**:845-848.
2. Campbell G, Shatz CJ: **Synapses formed by identified retinogeniculate axons during the segregation of eye input**. *J Neurosci* 1992, **12**:1847-1858.
3. Penn AA, Riquelme PA, Feller MB, Shatz CJ: **Competition in retinogeniculate patterning driven by spontaneous activity**. *Science* 1998, **279**:2108-2112.
4. Crowley JC, Katz LC: **Development of ocular dominance columns in the absence of retinal input**. *Nat Neurosci* 1999, **2**:1125-1130.
5. Crowley JC, Katz LC: **Early development of ocular dominance columns**. *Science* 2000, **290**:1321-1324.
6. Stellwagen D, Shatz CJ: **An instructive role for retinal waves in the development of retinogeniculate connectivity**. *Neuron* 2002, **33**:357-367.
7. Shatz CJ: **Emergence of order in visual system development**. *Proc Natl Acad Sci USA* 1996, **93**:602-608.
8. Meister M, Wong RO, Baylor DA, Shatz CJ: **Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina**. *Science* 1991, **252**:939-943.
9. Huberman AD, Wang GY, Liets LC, Collins OA, Chapman B, Chalupa LM: **Eye-specific retinogeniculate segregation independent of normal neuronal activity**. *Science* 2003, **300**:994-998.
10. Rossi FM, Pizzorusso T, Porciatti V, Marubio LM, Maffei L, Changeux JP: **Requirement of the nicotinic acetylcholine receptor β2 subunit for the anatomical and functional development of the visual system**. *Proc Natl Acad Sci USA* 2001, **98**:6453-6458.
11. Torborg C, Wang CT, Muir-Robinson G, Feller MB: **L-type calcium channel agonist induces correlated depolarizations in mice lacking the beta2 subunit nAChRs**. *Vision Res* 2004, **44**:3347-3355.
12. •• Torborg CL, Hanson KA, Feller MB: **High frequency, synchronized bursting drives eye-specific segregation of retinogeniculate axons**. *Nat Neurosci* 2005, **8**:72-78.

Through careful quantitative analysis of RGC spiking patterns and LGN refinement in different lines of mutant mice, the authors determine which parameters of retinal waves are associated with eye-specific retinogeniculate segregation. This study provides strong support for Hebbian-based models of eye-specific segregation. It also suggests which types of synaptic plasticity are likely to be important for eye-specific refinement at developing retinogeniculate synapses.

13. Chapman B: **Necessity for afferent activity to maintain eye-specific segregation in ferret lateral geniculate nucleus**. *Science* 2000, **87**:479-482.
14. •• Demas J, Sagdullaev BT, Green E, Jaubert-Miazza L, McCall MA, Gregg RG, Wong RO, Guido W: **Failure to maintain eye-specific segregation in nob, a mutant with abnormally patterned retinal activity**. *Neuron* 2006, **50**:247-259.

A remarkable set of findings showing that, in nob (no b-wave) mutant mice, retinal waves and eye-specific segregation initially develop normally but then retinal wave frequency increases and axons from the two eyes desegregate in the LGN. This implies that, under normal conditions, wave frequency is tightly regulated to prevent spurious binocular correlations and thereby maintain eye-specific segregation in the LGN. A major strength of this study is that the authors carried out a transgenic synapse-specific rescue of nyctalopin, the protein absent in nob mice. This rescued both the retinal wave and LGN phenotype in nob mice. Along with the study by Torborg *et al*. [12••], this study is the first to demonstrate a positive role for specific patterns of spontaneous retinal activity in eye-specific visual circuit refinement.

15. Ziburkus J, Guido W: **Loss of binocular responses and reduced retinal convergence during the period of retinogeniculate axon segregation**. *J Neurophysiol* 2006, **96**:2775-2784.
16. Shatz CJ, Kirkwood PA: **Prenatal development of functional connections in the cat's retinogeniculate pathway**. *J Neurosci* 1984, **4**:1378-1397.
17. Mooney R, Madison DV, Shatz CJ: **Enhancement of transmission at the developing retinogeniculate synapse**. *Neuron* 1993, **10**:815-825.
18. • Jaubert-Miazza L, Green E, Lo FS, Bui K, Mills J, Guido W: **Structural and functional composition of the developing retinogeniculate pathway in the mouse**. *Vis Neurosci* 2005, **22**:661-676.

This study describes an *in vitro* explant preparation that allows either optic nerve to be stimulated while the intracellular responses of single LGN neurons are recorded. The results unequivocally show that the anatomical overlap of RGC axons in the LGN is accompanied by functional synaptic inputs from both eyes onto single LGN neurons and that, as axons from the two eyes segregate, functional synapses from one or the other eye onto a given LGN cell are eliminated. Careful quantification of the ontogeny of anatomical eye-specific segregation in the postnatal mouse LGN is also provided. Along with the study by Ziburkus and Guido [15], this analysis indicates that functional binocular convergence is present for a few days longer than anatomical tracing suggests.

19. Poirazi P, Brannon T, Mel BW: **Pyramidal neuron as a two-layer neural network**. *Neuron* 2003, **37**:989-999.
20. Kasthuri N, Lichtman JW: **The role of neuronal identity in synaptic competition**. *Nature* 2003, **424**:426-430.
21. Chen C, Regehr WG: **Developmental remodeling of the retinogeniculate synapse**. *Neuron* 2000, **28**:955-966.
22. Tavazoie SF, Reid RC: **Diverse receptive fields in the lateral geniculate nucleus during thalamocortical development**. *Nat Neurosci* 2000, **3**:608-616.

23. Hooks BM, Chen C: **Distinct roles for spontaneous and visual activity in remodeling of the retinogeniculate synapse**. *Neuron* 2006, **52**:281-291.

•• The authors employ an *in vitro* slice preparation in which single LGN cells are intracellularly recorded while RGC axons in the optic tract axons undergo graded stimulation. This allows them to estimate the number of RGC inputs onto each LGN cell. Previous work showed that, in the period immediately following eye-specific segregation, each LGN neuron receives weak input from 10–12 RGCs and that this number is eventually reduced to 1–3 strong RGC inputs. This study demonstrates that chronically blocking spontaneous retinal activity prevents this fine-scale functional retinogeniculate pruning. Visual experience then plays a role in maintenance of fine-scale pruning.

24. Huberman AD, Stellwagen D, Chapman B: **Decoupling eye-specific segregation from lamination in the lateral geniculate nucleus**. *J Neurosci* 2002, **22**:9419-9429.

25. Grubb MS, Rossi FM, Changuex JP, Thompson ID: **Abnormal functional organization in the dorsal lateral geniculate nucleus of mice lacking the beta 2 subunit of the nicotinic acetylcholine receptor**. *Neuron* 2003, **30**:1151-1172.

26. Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ: **Functional requirement for class I MHC in CNS development and plasticity**. *Science* 2000, **290**:2155-2159.

27. Bjartmar L, Huberman AD, Ullian EM, Renteria RC, Liu X, Xu W, Prezioso J, Susman MW, Stellwagen D, Stokes CC *et al.*: **Neuronal pentraxins mediate synaptic refinement in the developing visual system**. *J Neurosci* 2006, **26**:6269-6281.

28. Corriveau RA, Huh GS, Shatz CJ: **Regulation of class I MHC gene expression in the developing and mature CNS by neural activity**. *Neuron* 1998, **21**:505-520.

29. Tsui CC, Copeland NG, Gilbert DJ, Jenkins NA, Barnes C, Worley PF: **Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity**. *J Neurosci* 1996, **16**:2463-2478.

30. O'Brien RJ, Xu D, Petralia RS, Steward O, Huganir RL, Worley P: **Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp**. *Neuron* 1999, **23**:309-323.

31. Williams RW, Hogan D, Garraghty PE: **Target recognition and visual maps in the thalamus of achiasmatic dogs**. *Nature* 1994, **367**:637-639.

32. O'Leary DD, McLaughlin T: **Mechanisms of retinotopic map refinement: Ephs, ephrins, and spontaneous correlated retinal activity**. *Prog. Brain Res* 2005, **147**:43-65.

33. Pfieffenberger C, Cutforth T, Woods G, Yamada J, Renteria RC,
• Copenhagen DR, Flanagan JG, Feldheim DA: **Ephrin-As and neural activity are required for eye specific patterning during retinogeniculate mapping**. *Nat Neurosci* 2005, **8**:1022-1027.

This study demonstrates that, in mice lacking all of the ephrin-As normally expressed in the LGN during eye-specific segregation (ephrin-A2/3/5), patterning of retinogeniculate projections is severely perturbed: inputs from the two eyes still segregate, but the normal stereotyped shape, size and location of eye-specific domains is abolished. Altering retinal activity in ephrin knockouts leads to overlapping projections in the inappropriate areas of the LGN. Together with the study by Huberman *et al*. [34•], this study is one of the first to demonstrate a role for axon guidance cues in eye-specific pathfinding. It also shows that altering retinal activity does not alter EphA or ephrin-A mRNA expression.

34. Huberman AD, Murray KD, Warland DK, Feldheim DA,
• Chapman B: **Ephrin-As mediate targeting of eye-specific projections to the lateral geniculate nucleus**. *Nat Neurosci* 2005, **8**:1013-1021.

This study demonstrates that, after retinotopic mapping of RGC axons is completed, ephrin-As and their receptors are distributed in a manner sufficient to induce stereotyped patterning of eye-specific maps in the LGN. *In vivo* retinal electroporation of EphA receptors was then used to test the role of ephrin-A signalling in eye-specific pathfinding. Electroporated retinas overexpressing EphA3 or EphA5 exhibited normal levels and patterns of spontaneous retinal waves, and yet RGC axons still misprojected to the wrong eye-specific territory in the LGN. Along with the study by Pfieffenberger *et al*. [33•], this study is one of the first to demonstrate a role for axon guidance cues in eye-specific pathfinding.

35. Hanson MG, Landmesser LT: **Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules**. *Neuron* 2004, **43**:687-701.

36. Brunet I, Weinl C, Piper M, Trembleau A, Volovitch M, Harris W, Prochiantz A, Holt C: **The transcription factor Engrailed-2 guides retinal axons**. *Nature* 2005, **438**:94-98.

37. Plas DT, Lopez JE, Crair MC: **Pretarget sorting of retinocollicular axons in the mouse**. *J Comp Neurol* 2005, **491**:305-319.

38. Lee JS, von der Hardt S, Rusch MA, Stringer SE, Stickney HL, Talbot WS, Geisler R, Nusslein-Volhard C, Selleck SB, Chien CB, Roehl H: **Axon sorting in the optic tract requires HSPG synthesis by ext2 (dackel) and extl3 (boxer)**. *Neuron* 2004, **44**:947-960.

39. Horton JC, Hocking DR: **An adult-like pattern of ocular dominance columns in striate cortex of newborn monkeys prior to visual experience**. *J Neurosci* 1996, **16**:1791-1807.

40. LeVay S, Stryker MP, Shatz CJ: **Ocular dominance columns and their development in layer IV of the cat's visual cortex: A quantitative study**. *J Comp Neurol* 1978, **179**:223-244.

41. Crair MC, Horton JC, Antonini A, Stryker MP: **Emergence of ocular dominance columns in cat visual cortex by two weeks of age**. *J Comp Neurol* 2001, **430**:235-249.

42. Stryker MP, Harris WA: **Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex**. *J Neurosci* 1986, **6**:2117-2133.

43. Huberman AD, Speer CM, Chapman B: **Spontaneous retinal**
•• **activity mediates development of ocular dominance columns and binocular receptive fields in v1**. *Neuron* 2006, **52**:247-254.

This study is the first to show that early spontaneous retinal activity is required for ODC segregation. Spontaneous retinal activity was pharmacologically silenced in both retinas of ferrets from postnatal day 1 (P1) until P10. This is before ODCs normally form and is the stage when LGN axons are in the subplate and innervating layer 4 of V1. Retino-LGN–V1 axons were then anatomically labelled when the ferrets reached adulthood. Remarkably, even though spontaneous retinal activity was normal in these animals from postnatal day 12 until adulthood [24], the early retinal activity block permanently disrupted ODC segregation and rendered the receptive fields of binocular neurons in V1 ~30 times larger than normal. Monocular receptive fields were not affected, indicating that retinal waves drive competitive interactions that refine binocular receptive fields in V1. Along with the study by Cang *et al*. [44••], this study provides evidence that early retinal waves are essential for thalamocortical refinement.

44. Cang J, Renteria RC, Kaneko M, Liu X, Copenhagen DR,
•• Stryker MP: **Development of precise maps in visual cortex requires patterned spontaneous activity in the retina**. *Neuron* 2005, **48**:797-809.

The authors demonstrate that, in mutant mice that lack retinal waves for the first week of postnatal development, geniculocortical connections fail to refine into a precise retinotopic map. Carefully timed enucleation experiments and analysis are used to rule out the possibility that the observed defects in the thalamocortical refinement are the indirect result of the altered retinogeniculate connectivity also present in these animals. Together with the study by Huberman *et al*. [43••], this study provides strong evidence that early retinal waves are essential for thalamocortical refinement.

45. Kanold PO, Kara P, Reid RC, Shatz CJ: **Role of subplate neurons in functional maturation of visual cortical columns**. *Science* 2003, **301**:521-525.

46. Hanganu IL, Ben-Ari Y, Khazipov R: **Retinal waves trigger**
• **spindle bursts in the neonatal rat visual cortex**. *J Neurosci* 2006, **26**:6728-6736.

This study provides the first direct evidence that spontaneous retinal waves induce spiking of visual cortical neurons early in development — an essential requirement for any model that posits a direct role of retinal activity in thalamocortical refinement. Waves induced spindle oscillations in V1. Spindle oscillations in particular might thus be important for maturation of thalamocortical or intracortical circuits.

47. Kanold PO, Shatz CJ: **Subplate neurons regulate maturation of**
•• **cortical inhibition and outcome of ocular dominance plasticity**. *Neuron* 2006, **51**:627-638.

The authors lesioned the subplate before ODCs normally segregate. Prior studies demonstrated that early subplate lesions of this sort prevent ODC segregation [45]. This study further demonstrates that, in the absence of the subplate, inhibitory circuits fail to mature in layer 4, thereby altering the direction of ODC plasticity in the subsequent crucial period. Given the importance of inhibition for ODC patterning [48••], maturation of inhibitory

circuits triggered by the subplate might also be important for the initial segregation of ODCs.

48. Hensch TK, Stryker MP: **Columnar architecture sculpted by**
•• **GABA circuits in developing cat visual cortex**. *Science* 2004, **303**:1678-1681.
The authors show that augmenting GABA in visual cortex alters the spacing and, at high doses, the segregation of ODCs. Although these GABA manipulations were initiated after ODCs are already beginning to be established [41], they provide compelling support for the idea that activity-dependent processes, and inhibition in particular, impact ODC refinement. Given other results showing the role of the subplate in maturation of inhibition and ODC segregation [45,47••] the findings in this study are likely to be of importance to mechanistic models of early ODC development as well.

49. Fagiolini M, Fritschy JM, Low K, Mohler H, Rudolph U, Hensch TK:
• **Specific GABAA circuits for visual cortical plasticity**. *Science* 2004, **303**:1619-1621.
This study reveals that GABA-mediated inhibition (probably arising from parvalbumin-containing inhibitory basket cells in V1) acts through alpha subunit-containing GABAA receptors (known to be present on the somas of V1 pyramidal neurons), to mediate physiological OD plasticity.

50. Ince-Dunn G, Hall BJ, Hu SC, Ripley B, Huganir RL, Olson JM, Tapscott SJ, Ghosh A: **Regulation of thalamocortical patterning and synaptic maturation by NeuroD2**. *Neuron* 2006, **49**:683-695.

51. Cang J, Kaneko M, Yamada J, Woods G, Stryker MP,
• Feldheim DA: **Ephrin-As guide the formation of functional maps in visual cortex**. *Neuron* 2005, **48**:577-589.
This paper reports retinotopic defects in the geniculocortical pathway of mice that lack various ephrin-A ligands. In addition, *in vivo* electroporation is used to overexpress ephrin-A5 directly in visual cortex of otherwise wild type mice. The results indicate that EphA–ephrin-A interactions in the developing thalamocortical pathway are important for visual mapping in V1.