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Nob Mice Wave Goodbye to Eye-Specific Segregation

Spontaneous retinal activity is necessary to establish and maintain eye-specific projections to the LGN, but whether the spatial and temporal structure of this activity is important remains unclear. A new study by Demas et al. in the current issue of *Neuron* shows that when the frequency of spontaneous retinal waves is increased and waves abnormally persist past eye opening, eye-specific projections to the LGN desegregate. These results provide important new insight into the mechanisms that drive eye-specific refinement and stabilization.

The electrical activity of a developing neuron has a profound effect on the number and location of synapses it can form, but it is unclear how the pattern and timing of this activity are important. Eye-specific visual connections are a well-established model system for investigating how activity sculpts precise circuitry. In mammals, eye-specific retinogeniculate projections emerge from an initially imprecise state in which retinal ganglion cell (RGC) axons from the two eyes intermingle in the lateral geniculate nucleus (LGN), but subsequently segregate into eye-specific domains. This segregation occurs before the onset of sensory experience at a time when spontaneous "waves" propagate across the retina, inducing neighboring RGCs to fire synchronously. The temporal and spatial structure of retinal waves ensures that nearby RGCs are correlated in their firing and that RGCs located far apart or in different eyes do not fire together (Wong, 1999). Spontaneous retinal activity is necessary for both the segregation and maintenance of eye-specific inputs to the LGN (Penn et al., 1998, Chapman, 2000) and acts by driving a competitive axon-pruning process for synaptic target territory. If activity is eliminated or increased only in one eye, segregation still occurs, but the more active eye acquires more synaptic territory in the LGN at the expense of the less active eye (Penn et al., 1998; Stellwagen and Shatz, 2002).

Do the spatial and temporal features of retinal wave activity instruct eye-specific segregation or does activity simply play a permissive role? When the correlated firing of neighboring RGCs is diminished, as occurs in mice that lack the β2 subunit of the nicotinic acetylcholine receptor ($\beta 2\ nAChR^{-/-}$), retinotopic maps fail to refine in the LGN, superior colliculus, and visual cortex (McLaughlin et al., 2003; Grubb et al., 2003; Cang et al., 2005). $\beta 2\ nAChR^{-/-}$ mice also exhibit defects in eye-specific retinogeniculate segregation (Rossi et al., 2001), which has been attributed to a loss of high-frequency RGC bursting during wave activity (Torborg et al., 2005). Approximately 40% of RGCs, however, are silent in $\beta 2\ nAChR^{-/-}$ mice, whereas the rest of the RGCs exhibit significantly elevated spiking (McLaughlin et al., 2003). It is thus unclear whether the retinotopic and eye-specific defects observed in $\beta 2\ nAChR^{-/-}$ mice are due only to a loss of correlated RGC firing. The significantly elevated activity in some of the RGCs, for instance, might have enhanced their axon outgrowth and branching (Goldberg et al., 2002). Indeed, other studies report that if correlated RGC firing is disrupted but activity levels are kept stable, eye-specific retinogeniculate segregation occurs normally (Huberman et al., 2003). Those findings, combined with evidence that eye-specific layers can develop in the absence of binocular interactions (Williams et al., 1994), led to the hypothesis that axon guidance cues mediate eye-specific targeting in the LGN. However, in these experiments, some unknown (but nonetheless essential) features of spontaneous activity may have remained intact within the retina, thereby allowing eye-specific segregation to proceed. Recent findings demonstrate that axon guidance cues do indeed influence eye-specific pathfinding in the LGN (Huberman et al., 2005; Pfieffenberger et al., 2005), but rather than ruling out a role for activity, these results suggest a two-step model in which guidance cues mediate the initial targeting of retinogeniculate axons and then activity enhances their segregation. However, the features of activity that drive eye-specific segregation remain unclear.

The results of Demas et al. (2006) provide exciting new evidence that the pattern and timing of retinal waves actively maintain eye-specific retinogeniculate segregation. They examined no b-wave (*nob*) mutant mice that lack the b-wave component of the retinal ERG. The b-wave normally results from rod photoreceptor to ON-bipolar cell transmission. Demas et al. (2006) compared the spatiotemporal patterns of spontaneous RGC spiking in *nob* and wild-type (wt) mice by using multisite electrode array recordings in vitro and by recording directly from the optic nerves in vivo. During the stage when eye-specific segregation occurs from birth until postnatal day 12 (P12), spontaneous retinal activity is normal in *nob* mice. Mice open their eyes around P15, and waves normally dissapear shortly after that time. However, beginning at P15, and continuing

still weeks after eye opening, *nob* mice exhibit high-frequency retinal waves (Figure 1) (Demas et al., 2006). Does retinogeniculate segregation become abnormal when the structure and timing of wave activity is altered? Demas et al. (2006) show that eye-specific retinogeniculate segregation occurs on schedule in *nob* mice, but then around the time of eye opening (coincident with the onset of abnormal spontaneous retinal activity), axons from the two eyes desegregate in the LGN (Figure 1) (Demas et al., 2006). Although the *nob* mice have defects in visually mediated transmission, altered visual experience is not responsible for the desegregation, as dark rearing fails to prevent it. Rather, the abnormally frequent and persistent spontaneous retinal waves appear to destabilize eye-specific projections.

Previous studies demonstrated that afferent activity is necessary to maintain eye-specific segregation in the LGN (Chapman 2000). The LGN phenotype of *nob* mutants is reminiscent of these previous results. Interestingly, Demas et al. (2006) and Chapman (2000) describe similar asymmetries in the plasticity of contralateral-eye versus ipsilateral-eye projections. In both studies, the contralateral axons were found to be more stable. The results of Demas et al. (2006) are unique, however, because the pattern and timing of spontaneous retinal activity was altered as opposed to eliminated, in the *nob* mice, indicating that appropriately structured retinal activity may be necessary to maintain segregation. One important consideration is that the gene mutated in *nob* mice (*nyx*), which encodes the synaptic protein nyctalopin, is expressed by RGCs and LGN neurons as well as by retinal bipolar cells. Thus, in order to test whether the retinal wave phenotype of *nob* mice directly induces desegregation, Demas et al. (2006) made transgenic mice expressing YFP-tagged nyctalopin driven by the $GABA_c$ receptor $\rho 1$ subunit promoter and crossed those mice to *nob* mutants. Since the $GABA_c$ receptor $\rho 1$ promoter is found only in retinal bipolar cells, in the resulting mice, YFP:nyctalopin expression was rescued only in the outer plexiform layer of the retina where bipolar cells synapses reside. Remarkably, this was sufficient to restore normal spontaneous retinal activity and prevent desegregation (Figure 1) (Demas et al., 2006). By restricting genetic rescue only to the retina (and indeed to a single set of retinal synapses), this approach successfully avoided the caveats associated with brain-wide null mutations that have somewhat complicated the interpretation of previous studies that used mouse mutants to test the role of retinal activity in eye-specific development (Rossi et al., 2001; Torborg et al., 2005).

How might the persistence of retinal waves in *nob* mice lead to desegregation of newly refined eye-specific retinogeniculate projections? One possibility is that waves in *nob* mice occur at such high frequency and over such a long period of development, that RGCs from opposite eyes fire together repeatedly. Some LGN neurons still receive functional binocular inputs even after anatomical tracing indicates that eye-specific segregation is complete. Thus, the excessively frequent waves present in *nob* retinas may induce repeated correlated firing of these binocular inputs and thereby cause axon arbors situated in different eye-specific zones to gradually invade opposite-eye territory.

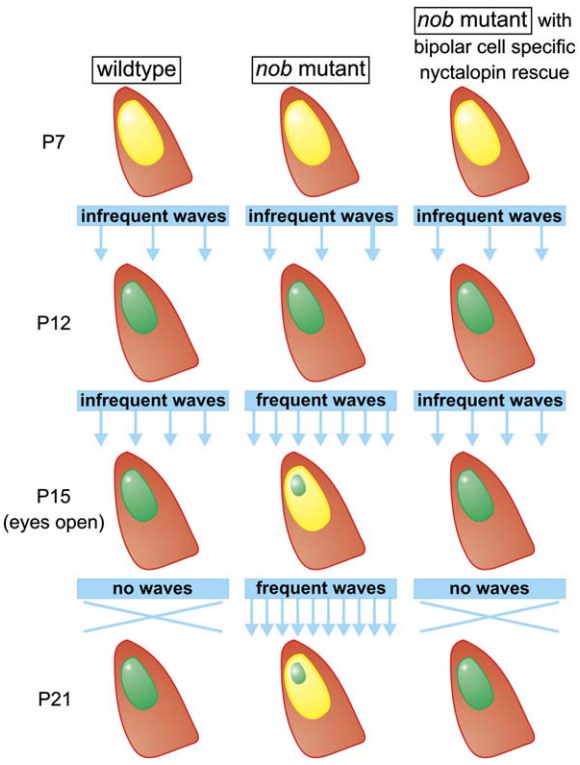

Figure 1. The Effects of the *nob* Mutation on the Development of Eye-Specific Projections to the Lateral Geniculate Nucleus

Red: axons from the contralateral eye. Green: axons from the ipsilateral eye. Yellow: overlapping axons from both eyes. The pattern of retinal wave activity at each age is indicated in the blue bars, and the arrows indicate the frequency of waves at each stage. In wild-type mice, axons from the two eyes segregate between P7 and P12 when waves are infrequent. Waves normally disappear around the time of eye opening (~P15) and axons from the two eyes remain segregated. In *nob* mice retinal waves and eye-specific segregation in the LGN are normal until P12, but then wave frequency increases and projections from the two eyes desegregate. Waves also continue past eye opening in *nob* mutants. *Nob* mice with nyctalopin rescued in retinal bipolar cells show normal wave activity at P15 and eye-specific segregation in the LGN is maintained. For details see Demas et al. (2006).

So what do the new results of Demas et al. (2006) tell us about the role of activity in sculpting precise circuits in the normal developing brain? First, they strongly suggest that the normal cessation of retinal waves around the time of eye opening depends on functional maturation of photoreceptor to bipolar synapses. Waves shut off around the time of eye opening even if mice are raised in the dark, so understanding how this synaptic maturation turns off waves is an intriguing question for future investigation. Second, these new findings raise the question of whether the same features of activity that promote the stabilization and maintenance of newly refined retinogeniculate circuitry also promote segregation of initially intermingling axons early in development. Previous experiments show that elevating wave frequency and size in both eyes does not prevent eye-specific segregation (Stellwagen and Shatz, 2002). In those experiments, however, the frequency of waves was substantially lower than occurs in *nob* mice. The results of Demas et al. (2006) thus suggest that a threshold

level of wave frequency must occur in order for binocular inputs onto single LGN neurons to be coactive and stabilized. Lastly, their results underscore the notion that activity patterns continue to reinforce the precise structure of neural circuits after they form.

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Numbing the Senses: Role of TRPA1 in Mechanical and Cold Sensation

In this issue of *Neuron*, Kwan et al. demonstrate that TRPA1 is critical for the transduction of noxious cold and mechanical stimuli, as well as in mediating the activation of nociceptors by endogenous and natural irritants. Differences between the present report and a previous study indicate that further study is needed to reach a consensus on the role of TRPA1 in the transduction of mechanical and noxious cold stimuli.

The continuously intensive investigation of the Transient Receptor Potential (TRP) ion channel family has revealed their striking contribution to sensory biology. Across a diversity of vertebrate and invertebrate species, they are the molecular machinery required for the transduction of stimuli encompassing the five classical senses. Thermal stimuli within the perceptible range (from painfully hot to cold) activate a subset of TRP channels (dubbed thermoTRPs) from three diverse subfamilies (Patapoutian et al., 2003). In addition to their distinct thermal thresholds, all of the thermoTRPs are chemosensitive; they are activated specifically by endogenous, synthetic, and plant-derived molecules, most of which evoke cutaneous thermal and pain sensations.

A number of recent studies demonstrate unequivocally that similar to the noxious heat and capsaicin receptor TRPV1, cold-activated TRPA1 comprises a molecular site of integration of multiple pain producing stimuli, including pungent components derived from mustard oil, cinnamon oil and garlic, and the endogenous pro-algesic bradykinin (Bandell et al., 2004; Jordt et al., 2004). The issue of whether TRPA1 is activated by noxious cold, however, is not without controversy for two main reasons. First, calcium imaging and electrophysiogical studies of heterologously expressed TRPA1 failed to reproduce the finding that TRPA1 is activated by noxious cold temperatures, as first observed by Story et al. Second, the use of newly identified nonthermal TRPA1 agonists and variation in thermal/pharmacological response profiles of cultured sensory neurons began to reveal disparate observations among laboratories (Bandell et al., 2004; Jordt et al., 2004). Many of these observations were contrary to the initial hypothesis that the dual cold and heat sensitivity of a small subset of neurons could be explained by the expression of TRPA1 in a subset of TRPV1-positive neurons (Story et al., 2003).

While the field awaited knockout mice to resolve the "cold controversy," Corey et al. showed through a series of in vitro knockdown studies that TRPA1 was a promising candidate for the mechanosensitive transduction channel of hair cells in the inner ear (Corey et al., 2004). New and exciting questions arose as to whether mice deficient for TRPA1 would be impaired in their ability to hear as well as to sense cold and noxious chemical agents. Our anticipation is answered now with not one, but two independently generated lines of TRPA1 knockout (TRPA1$^{-/-}$) mice reported recently by Bautista et al. in *Cell* and by Kwan et al. (2006) in this issue of *Neuron*. However TRPA1 remains somewhat silent about its mysteries, not for lack of hearing in these mice, but rather stubborn inconsistencies regarding the role(s) of TRPA1 in somatosensation.

The widely accepted model for auditory mechanotransduction proposes that an ion channel gated by mechanical force is located at the tips of hair cell stereocilia (Gillespie et al., 2005). Mechanical forces are thought to be transmitted to the channel via an elastic structure (the "gating spring"). The very features of TRPA1, an ion channel with a predicted 16 N-terminal ankyrin repeats (a built in gating spring), made it an attractive candidtate as a potential mechanosensor. To this end, Corey et al. showed in late 2004 that TRPA1 mRNA is first expressed in the inner ear at the initiation of hair cell mechanotransduction in utero and that the protein is localized within stereocilia postnatally. They then utilized RNA interference (siRNA and morpholino) to knockdown mouse