J Physiol 586.18 (2008) pp 4385–4392

SYMPOSIUM REPORT

Comparisons of structural and functional abnormalities in mouse b-wave mutants

Maureen A. McCall[1] and Ronald G. Gregg[1,2]

Departments of [1] Ophthalmology & Visual Sciences and [2] Biochemistry & Molecular Biology, University of Louisville, Louisville, KY 40202, USA

In the most simplistic view, the retinal circuit can be divided into vertical excitatory pathways that use glutamate as their neurotransmitter and lateral inhibitory pathways in the outer and inner synaptic layers that modulate excitation via glycine and GABA. Within the vertical excitatory pathways, the visual signal is initiated in the rod, cone or both photoreceptors, depending on the adaptation state of the retina. This signal is transmitted to the rest of the retina through the bipolar cells, which can be subdivided based on: the photoreceptor that provides their input, their dendritic and axonal morphology, and the polarity of their response evoked by a luminance increment, e.g. depolarizing or hyperpolarizing responses. The polarity of this response is controlled by the type of glutamatergic postsynaptic receptor that is expressed on their dendritic terminals. Hyperpolarizing bipolar cells express AMPA/kainate receptors, whereas depolarizing bipolar cells (DBCs) express the metabotropic glutamate receptor 6 (Grm6). The electroretinogram (ERG) is a non-invasive method used to assess overall retinal function. The initiation of the visual signal in the photoreceptors is reflected in the ERG a-wave and the ensuing depolarization of DBCs in the b-wave. When there is failure of signal transmission from photoreceptors to DBCs or signalling within DBCs, the ERG a-wave is present, while the b-wave is absent or significantly reduced. This ERG phenotype has been found in the human population and is referred to as congenital stationary night blindness. Until recently, it had been assumed that the absence of a b-wave was indicative of a lack of signalling through the On pathway, leaving the Off pathway unaffected. Here we review recent findings that demonstrate that many mouse mutants share a no b-wave ERG phenotype but their retinal morphology and RGC responses differ significantly, suggesting very different effects of the underlying mutations on output from the DBCs to the rest of the retinal circuit.

(Received 7 July 2008; accepted after revision 21 July 2008; first published online 24 July 2008)
Corresponding author M. A. McCall: University of Louisville, Psychological & Brain Sciences, Life Sciences Building, Belknap Campus, University of Louisville, Louisville, KY 40292, USA. Email: mo.mccall@louisville.edu

Opening remarks

In the retina the visual signal is initiated when light interacts with opsin in the outer segments of the rod and cone photoreceptors. This signal causes a series of changes within the G-protein-mediated photoreceptor signalling pathway Calvert *et al*. 2006; Sarfare & Pittler, 2007). These include: a decrease in intracellular cGMP, closure of a cGMP-gated cation channel in the outer segments, an intensity-dependent hyperpolarization in membrane polarization and a concomitant decrease in glutamate release.

Like other CNS neurons, neurotransmitter (i.e. glutamate) release from photoreceptors depends on calcium influx, which in photoreceptors is mediated by L-type voltage-dependent calcium channels (L-VDCCs) (Ball & Gregg, 2002; Barnes & Kelly, 2002). The magnitude of the calcium influx, which depends on membrane polarization and stimulus intensity, creates a graded change in glutamate release (i.e. luminance increments reduce glutamate release) (Trifonov, 1968; Penn & Hagins, 1969; Baylor & Fuortes, 1970; Dowling & Ripps, 1973; Trifonov & Byzov, 1977). Photoreceptors contact two classes of BCs, hyperpolarizing bipolar cells (HBCs) and

This report was presented at *The Journal of Physiology* Symposium on *Retinal ganglion cells in model organisms: development, function and disease*, which took place in Fort Lauderdale, FL, USA, 26 April 2008. It was commissioned by the Editorial Board and reflects the views of the authors.

DOI: 10.1113/jphysiol.2008.159327

depolarizing bipolar cells (DBCs), named for the polarity of their response to a decrease in glutamate release (Saito & Kaneko, 1983; Attwell *et al.* 1987; Nawy & Jahr, 1991; Masu *et al.* 1995).

The activity of photoreceptors and DBCs has been assessed non-invasively in many species with the electroretinogram (ERG), a gross potential recorded at the corneal surface whose components reflect light-evoked retinal activity. The normal ERG waveform has two primary components, the a- and b-waves. The negative going a-wave represents the hyperpolarization of photoreceptors in response to a luminance increment (Robson & Frishman, 1998; Xu *et al.* 2003). Under dark-adapted conditions, the response of rod photoreceptors dominates the a-wave, while under light-adapted conditions this component reflects cone photoreceptor responses (note, in the mouse ERG the a-wave at light-adapted levels is absent). The b-wave reflects the depolarizing response of the DBCs and is dominated by rod or cone DBCs depending on the adaptation state of the retina (Robson & Frishman, 1998; Sharma *et al.* 2005). In addition to its use in human clinical assessments, the ERG also has been instrumental in identifying spontaneous animal mutants and characterizing genetically manipulated mice with defects in outer retinal signalling (Peachey & Ball, 2003). Numerous mutants with a-wave abnormalities have been identified and subsequent identification of the underlying gene defect has been important in defining proteins critical to the phototransduction cascade. Further, the ERG has identified a different abnormal phenotype: a normal a-wave but the absence or reduction of the b-wave. Human patients with this ERG phenotype have a disease referred to as congenital stationary night blindness (CSNB) (Miyake *et al.* 1986). The first spontaneous mouse mutant described with this functional phenotype was named *nob* for no b-wave (Pardue *et al.* 1998). Subsequently, additional no b-wave mutants were identified and the nomenclature extended using the chronology of their discovery (*nob, nob2, nob3, nob4*). Once the gene responsible for the phenotype was identified, the official nomenclature incorporated both the gene and allele name (phenotype) for each mutant (for example: Nyx^{nob} (Pardue *et al.* 1998), $Cacna1f^{nob2}$ (Chang *et al.* 2006), $Grm6^{nob3}$ (Maddox *et al*. 2008) and $Grm6^{nob4}$ (Pinto *et al.* 2007). In this review, we will refer to specific mouse mutants using this official nomenclature. When discussing no b-wave mutants in general terms we will use that terminology to differentiate them from *nob* mice (Nyx^{nob}). At times we will use the collective terminology *Grm6* mutants.

The no b-wave ERG phenotype indicates that phototransduction is normal, but that synaptic transmission between photoreceptors and DBCs or signalling within DBCs is defective. Mouse (and human) mutants with this ERG phenotype fall into two groups that can be distinguished based on the severity of the b-wave loss: those with a complete loss of the b-wave (cCSNB) and those that retain a residual b-wave and oscillatory potentials, indicative of inner retinal activity (iCSNB) (Miyake *et al.* 1986). As the mutations were identified, it became evident that the iCSNB phenotype was caused by mutations in genes expressed presynaptically, in the photoreceptor terminals that control glutamate release. In contrast, cCSNB involved mutations in genes expressed postsynaptically, in the DBCs that control membrane depolarization. Below we discuss the results of analyses that have characterized retinal structure and function in several no b-wave mutant mouse models. We discuss how these results have advanced our understanding of: (1) the genes that control neurotransmitter release from photoreceptors and synaptogenesis in the outer plexiform layer (OPL); (2) the postsynaptic mechanisms that control DBC depolarization; and (3) the potential role of DBC output to the inner retinal circuit on the responses of retinal ganglion cells (RGCs).

Presynaptic no b-wave mouse mutants: Ca^{2+} influx through L-type VDCCs and the control of glutamate release from photoreceptors

Glutamate release from photoreceptors relies on Ca^{2+} influx through L-VDCCs. L-VDCCs are heteromultimeric proteins consisting of an α_1 subunit that forms the pore of the Ca^{2+} channel, and auxiliary β, $\alpha_2\delta$ and γ subunits that modulate the Ca^{2+} current, regulate channel activation and inactivation and, finally, control the proper assembly of the channel and its localization to the membrane (Catterall, 2000). Immunohistochemical data indicate that rod and cone photoreceptors express L-VDCCs that are comprised of α_{1F} and α_{1D} subunits in cones and α_{1F} only in rods (Morgans *et al.* 2005). While these two subunits result in channels with similar biophysical and pharmacological properties, their absence results in dramatically different functional outcomes. Knockout and mutant α_{1F} mice exhibit a significantly reduced ERG b-wave under both light- and dark-adapted conditions, consistent with this subunit's expression and function at both rod and cone photoreceptor terminals (Mansergh *et al.* 2005; Chang *et al.* 2006). Knockout α_{1D} mice have normal ERGs (Wu *et al.* 2007) and the dark-adapted ERG of the α_{1F}/α_{1D} double mutants shows no significant difference from the single α_{1F} mutant (McCall *et al.* 2008). Thus, the source of the residual ERG b-wave in α_{1F} mutant mice may involve other mechanisms, including expression of other L-VDCC α subunits. In addition to mutations in α_1 subunits, loss of either the β_2 or α_2/δ_4 L-VDDC subunit also disrupts the ERG b-wave (Ball *et al.* 2002; Wycisk *et al.* 2006*a*,*b*). Further, a similar ERG defect indicating disruption in synaptic transmission in the OPL, occurs in mice with mutations in: (1) a component of the ribbon synapse,

bassoon (Dick *et al.* 2003); (2) a molecule thought to modulate the L-VDCC, CaBP4 (Haeseleer *et al.* 2004); and (3) extracellular or intracellular matrix molecules (dystrophin; Rs1h) (Pillers *et al.* 1999; Johnson *et al.* 2006).

Postsynaptic no b-wave mouse mutants: defects in the Grm6 signalling cascade of DBCs

Synaptic transmission also requires the correct detection of the changes in the concentration of neurotransmitter in the synaptic cleft by the postsynaptic cells. In DBCs, glutamate binds to *Grm6* (Nakajima *et al.* 1993) and initiates a G-protein signalling cascade that ends with the closure of a non-specific cation conductance (Slaughter & Miller, 1981). The intermediate molecular components involved in this DBC signalling cascade remain relatively unresolved (see review by Snellman *et al.* 2008). Six postsynaptic no b-wave mutants have provided or verified the identity of four proteins critical to signal transduction in DBCs. Three disrupt *Grm6* itself: $Grm6^{Tm1Nak}$ (Masu *et al.* 1995); $Grm6^{nob4}$ (Pinto *et al.* 2007) and $Grm6^{nob3}$ (Maddox *et al.* 2008). In fact, it was the evaluation of the *Grm6* knockout mouse ($Grm6^{Tm1Nak}$) that verified that *Grm6* was the glutamate receptor responsible for DBC signalling. Two mutations disrupt G-proteins Gαo and Gβ5 ($Gnao1^{tm1Lbi}$ and $Gnb5^{tmCk}$) (Dhingra *et al.* 2000, 2002; Rao *et al.* 2007) and, again, it was the absence of a b-wave that provided convincing evidence that these G-protein subunits were intermediates in the DBC signalling cascade. The last mutation, Nyx^{nob}, disrupts the expression of nyctalopin, a protein whose function remains enigmatic (Gregg *et al.* 2003, 2007). Two lines of evidence place nyctalopin as an integral protein in the DBC signalling cascade. First, Nyx^{nob} DBCs do not respond to exogenous agonist (glutamate) application (Gregg *et al.* 2007). Second, expression of an enhanced yellow fluorescent protein (EYFP)–yctalopin fusion protein co-localizes with Grm6 on the DBC dendritic terminals, and more importantly, rescues the ERG b-wave, abnormalities in RGC responses and anatomical defects in their axon terminals in the dorsal Lateral Geniculate Nucleus (dLGN) (Demas *et al.* 2006; Gregg *et al.* 2007). While Gαo, Gβ5 and nyctalopin are now established elements within the DBC signalling cascade and cation channel gating, their exact positions within the cascade remain to be determined, as does the identity of the channel itself.

Pre- and postsynaptic no b-wave mutations lead to disparate morphological consequences

Early in postnatal development, the OPL thins due to disruption of its synaptic architecture in all presynaptic no b-ave mutants. In all cases, the synaptic ribbons in the rod photoreceptors are abnormal or absent and the dendrites of horizontal cells and the DBCs extend into the outer nuclear layer, and in some cases ectopic synapses appear to form with photoreceptors (Ball *et al.* 2002; Dick *et al.* 2003; Dick & Foroud, 2003; Chang *et al.* 2006). In contrast, retinal structure is normal at the light microscopic level in four postsynaptic mutants: $Grm6^{Tm1Nak}$ (Masu *et al.* 1995; Tagawa *et al.* 1999); $Grm6^{nob4}$ (Pinto *et al.* 2007); $Gnao1^{tm1Lbi}$ (Dhingra *et al.* 2000); and Nyx^{nob} (Pardue *et al.* 1998; Ball *et al.* 2003). In Nyx^{nob} and $Gnao1^{tm1Lbi}$ mice, the models studied at the EM level, both pre- and postsynaptic structures in the OPL, are normal (Dhingra *et al.* 2000; Pardue *et al.* 2001). There appears to be one exception to this general rule: abnormal OPL morphology has been observed in Gβ5 knockout mice, but this could result from altered expression of the long isoform of Gβ5 in photoreceptors rather than a change in the short isoform in DBCs (Rao *et al.* 2007).

Altered outer retinal neurotransmission results in disparate RGC phenotypes

The impact of outer retinal defects on retinal output in presynaptic no b-wave mutants has been explored by assessing the spontaneous and visually evoked response properties of their RGCs. Some very surprising results have been reported. *In vitro* assessments, using multi-electrode arrays, have been reported for $Grm6^{Tm1Nak}$, $Grm6^{nob4}$, Nyx^{nob} and $Grm6^{nob3}$ (Renteria *et al.* 2006; Demas *et al.* 2006; Pinto *et al.* 2007; Maddox *et al.* 2008). *In vivo*, RGC responses have been studied directly using extracellular recordings in the optic nerve for $Cacna1f^{nob2}$, $Grm6^{nob4}$, Nyx^{nob} and $Grm6^{nob3}$ (Chang *et al.* 2006; Demas *et al.* 2006; Pinto *et al.* 2007; Maddox *et al.* 2008). RGC output in $Grm6^{tm1Nak}$ and $Cacna1f^{tm1Tbh}$ has been studied by evaluating visually evoked responses in the superior colliculus (SC) (Sugihara *et al.* 1997; Mansergh *et al.* 2005). A broad generality can be made at this time; the response properties of RGCs in no b-wave mutants with presynaptic defects have only subtle defects, while RGCs in no b-wave mutants with postsynaptic defects are severely altered (Demas *et al.* 2006; Renteria *et al.* 2006; Chang *et al.* 2006; Pinto *et al.* 2007); Maddox *et al.* 2008; Heflin, McCall & Gregg, unpublished observations).

RGC responses in presynaptic no b-wave mutants

In vivo RGC response properties have been analysed for two presynaptic mutants, $Cacna1f^{nob2}$ (Chang *et al.* 2006) and $Cacn2b^{tm1Rgg}$ (McCall, Vessey & Gregg, unpublished observations) mice and their responses are similar to WT. As only preliminary data have been collected for

the *Cacnb2*^{tm1Rgg} mice, specific descriptions will focus on *Cacna1f*^{nob2} RGCs. At both light- and dark-adapted levels, *Cacna1f*^{nob2} RGCs have receptive fields (RFs) with centre/surround organization and can be classified as either On- or Off-centre with spot stimuli restricted within the RF (Chang *et al.* 2006). In *Cacna1f*^{nob2} On-centre RGCs, the most prominent defect under light-adapted conditions is a decrease in the spontaneous activity, as well as a compression of the dynamic range of their luminance contrast compared to WT. C*acna1f*^{nob2} Off-centre RGCs are indistinguishable from WT. Of interest is the reported loss of all visually evoked responses in the SC of the *Cacna1f*^{tm1Tbh} knockout mouse, implying the absence of visually evoked input from all RGC axons (Mansergh *et al.* 2005). Responses have not been evaluated in the SC of *Cacna1f*^{nob2} mice, but a lack of visually evoked activity seems unlikely given their robust RGC responses (Chang *et al.* 2006). In both mutants, the expression of the Cacna1f protein is eliminated, leaving the reason for the disparate result between the two alleles unclear. One potential explanation is that the two mutant mice are on different genetic backgrounds (SV129 for *Cacna1f*^{tm11Tbh} and C57Bl/6J for *Cacna1f*^{nob2}). Differences in phenotype resulting from background effects are not without precedence, and have been associated with human *CACNA1F* mutations (CSNB2: Bech-Hansen *et al.* 1998; cone dystrophy and Aland Island Eye Disease: Jalkanen *et al.* 2006, 2007). To directly address this difference, one of the mutants must be moved onto the other background strain by backcrossing for 8–10 generations.

The subtlety of the changes in the responses of *Cacna1f*^{nob2} and *Cacnb2*^{tm1Rgg} RGCs suggests that the view of an immutable L-VDCC subunit combination controlling glutamate release from photoreceptors may be too simplistic. Instead, these changes may reflect the presence of other subunits and/or subunit shuffling that might occur in the absence of expression of the primary subunit. Support for this hypothesis comes from the observation that there are differences in the characteristics of Ca^{2+} currents in mouse BCs (Berntson *et al.* 2003). It is possible that in the absence of the L-VDCC subunits that glutamate is released by a different mechanism and that the changes we observe in RGC responses result from the absence of Cacna1f expression in the inner plexiform layer (IPL) and concomitant alterations in synaptic function (Pan, 2001; Pan *et al.* 2001; Cui *et al.* 2003; Ma & Pan, 2003). What is required to begin to address these questions is a strategy in which L-VDCC subunit expression is selectively eliminated in either the OPL or the IPL.

RGC responses in postsynaptic no b-wave mutants

Of the six no b-wave mutants with defects in the DBC signalling cascade, RGC responses have been examined in all three *Grm6* mutants and in *Nyx*^{nob}. The *Gnao1*^{tm1Lbi} mutant survives for approximately 3 weeks after birth, complicating RGC analyses.

One change in the RGC response properties across all of these no b-wave mutants is consistent with their ERG phenotype. All have severely compromised signalling though the On pathway, although RGC assessment techniques and stimuli vary (Demas *et al.* 2006; Renteria *et al.* 2006; Pinto *et al.* 2007; Gregg *et al.* 2007; Maddox *et al.* 2008). Further, unlike recordings from WT RGCs, there are many spontaneously active RGCs that are unresponsive to visual stimuli in all of the postsynaptic no b-wave mutants.

Visually responsive RGCs within the *Grm6* mutants fall into two groups. In the first, a response is evoked at the onset of a full-field luminance increment. *In vivo*, these RGCs require bright full-field stimulation and have poorly defined RFs with no centre/surround organization. *In vitro*, some *Grm6* knockout On RGCs are observed with defined RF centres (Renteria *et al.* 2006). Regardless of recording technique or stimulus configuration, the response to the onset to the luminance increment in these RGCs is significantly delayed, and peak firing rates are significantly lower than WT RGCs or *Grm6* mutant Off-centre RGCs (Pinto *et al.* 2007; Maddox *et al.* 2008). In the second group of *Grm6* mutant RGCs, a response is evoked at the offset of a luminance increment. *In vivo,* these RGCs have robust responses and RFs with centre/surround organization and therefore can be referred to as Off-centre RGCs. In addition, the onset of a dark spot restricted within their RF centre elicits a robust excitatory response. The latency of the response onset of *Grm6*^{nob4} and *Grm6*^{nob3} Off-centre RGCs is the same as WT RGCs. While *Grm6*^{nob4} and *Grm6*^{nob3} RGCs share these general response characteristics, there are differences between the alleles.

When a full-field stimulus is used to characterize the response of WT RGCs, 51% of WT Off-centre RGCs maintain their RF centre response, responding only at luminance decrement (sign-conserving Off-centre RGCs). The other 49%, previously identified as Off-centre using spots restricted to their RF centre, alter their RF centre response and respond at both luminance increment and decrement (sign-altering Off-centre RGCs; (Sagdullaev & McCall, 2005). In addition, a small proportion of sign-altering Off-centre RGCs (31%) actually lose their Off component.

The proportion of sign-conserving versus sign-altering *Grm6*^{nob4} Off-centre RGCs is similar to WT (48 and 52% sign-conserving and -altering, respectively (Pinto *et al.* 2007). Among *Grm6*^{nob3} Off-centre RGCs, there are significantly fewer sign-altering cells (88 and 12% sign-conserving and -altering, respectively) compared to both *Grm6*^{nob} or WT (Maddox *et al.* 2008). Whenever an On response is present in sign-altering *Grm6* mutant Off-centre RGCs, the onset latency is significantly delayed

compared to either their Off response counterpart or to WT RGCs (Renteria *et al.* 2006; Pinto *et al.* 2007). These data indicate that there are subtle differences in the inner retina circuitry of *Grm6*^{nob3} and *Grm6*^{nob4} mice. Although the exact mechanism is currently unknown, it could be the consequence of an alteration in input between the On and Off pathways, a mechanism referred to as cross-talk, and for which there is mounting evidence (Zaghloul *et al.* 2003; Sagdullaev & McCall, 2005; Pang *et al.* 2007; Molnar & Werblin, 2007). If this were the case, one *Grm6* mutation might eliminate signalling through the On pathway, which would both eliminate On RGC responses and reduce On input to the Off pathway, which could produce results similar to *Grm6*^{nob3} (Maddox *et al.* 2008). Examination of the currents underlying the On responses in these mutants should help to resolve some of these questions.

While the ERG phenotypes of *Nyx*^{nob} and *Grm6* mutant mice are similar, some of their RGC response properties are very different. Notably, a novel phenotype is seen only in *Nyx*^{nob} RGCs. The spontaneous activity of *Nyx*^{nob} RGCs has a significant rhythmic bursting component with a fundamental frequency at about 4 Hz (Demas *et al.* 2006; Gregg *et al.* 2007). In addition, all *Nyx*^{nob} RGCs lack classic centre/surround RF organization (Helfin, McCall & Gregg, unpublished observations). Similar to *Grm6* mutants, there is an increase in the percentage of visually unresponsive *Nyx*^{nob} RGCs (Demas *et al.* 2006; Pinto *et al.* 2007; Gregg *et al.* 2007; Maddox *et al.* 2008). In addition, *in vivo*, all *Nyx*^{nob} RGCs require full-field stimulation and when On responses are evoked they have significant onset delays.

One hypothesis to account for the difference between *Grm6* mutant and *Nyx*^{nob} mutant RGC responses is that nyctalopin is expressed in both the OPL and the IPL (Bayley & Morgans, 2007), whereas *Grm6* is expressed only in the OPL. However, this explanation is inconsistent with the restoration of both a normal ERG b-wave and RGCs responses when a tagged nyctalopin fusion protein is expressed on the tips of *Nyx*^{nob} DBC dendrites (Demas *et al.* 2006; Gregg *et al.* 2007). Another hypothesis is that the state of the Grm6 gated cation channel differs between these mutants and creates the difference in downstream visual processing. In *Nyx*^{nob} DBCs, the cation channel is closed or absent (Gregg *et al.* 2007), and either condition may result in more hyperpolarized DBCs compared to WT. The state of the cation channel in *Grm6* mutant DBCs is unknown. However, taken together the state of the channel in *Nyx*^{nob} DBCs and the near-normal Off-centre RGC responses in *Grm6* mutants predicts that the channel is present and *Grm6* mutant DBCs have resting membrane potentials similar to WT. How a difference in the state of the cation channel translates into the vastly different RGC response properties in *Grm6* mutant and in *Nyx*^{nob} mutant mice is unknown and will require analyses of the DBC cation channels in all of these mutants.

A similar scenario could account for the allelic variance that we observe between *Grm6*^{nob3} and *Grm6*^{nob4} mutant RGCs. One *Grm6* mutation could result in the absence of the protein and in the other, its presence in an altered conformation. If this alters the resting membrane potential of the DBCs in the two *Grm6* mutants, then one mutation might create tonic glutamate release from DBC axon terminals and the other the absence of release. An absence of release would eliminate cross-talk between the parallel On and Off pathways, while tonic release could create persistent cross-talk and unusual signalling from the On to the Off pathway. This scenario is, however, very speculative. How the differences in the responses across RGCs in *Nyx*^{nob} and the various *Grm6* mutants arise await an understanding of the states of their cation channels and/or the function of nyctalopin in this pathway.

Concluding remarks

The results from the analyses of no b-wave mutants have advanced our understanding of retinal structure and function in several areas: (1) synaptic development in the OPL; (2) the state of the cation channel in DBCs; and (3) the impact of changes in DBC output on the responses of RGCs.

The presynaptic no b-wave mutants described here (*Cacna1f*^{nob2} and *Cacnb2*^{tm1Rgg}) share morphological abnormalities in common with those in several other mutants with disrupted control of glutamate release in the OPL (*bassoon, CaBP4, dystrophin, Rsh*, *Cacna2d4* (Ball *et al.* 2002; Dick *et al.* 2003; Haeseleer *et al.* 2004; Wycisk *et al.* 2006*a*,*b*). Synaptogenesis in the OPL of these mutants is markedly abnormal. There are few if any invaginating synapses formed and the dendrites of the DBCs and horizontal cells extend abnormally and may form ectopic synapses. In light of this morphological disruption it is remarkable that the impact on RGC responses is so subtle. This suggests that the primary circuitry responsible for centre/surround RF organization may reside in the inner retina, a conclusion reached using a knockout mouse lacking gap junctions between horizontal cells (Dedek *et al.* 2008). Further, the abnormal OPL synaptic organization, but normal RF centre/surround organization, may represent an important consideration in the restoration of function in retinas with retinitis pigmentosa (RP), as it would appear that restoration of normal ordered synaptic connections may not be required.

The differences across the postsynaptic no b-wave mutants described here (*Grm6*^{nob3}, *Grm6*^{nob4} and *Nyx*^{nob}), indicate that while the ERG is a powerful analytical tool, the impact of mutations that give rise to the same ERG defect may not be representative of the effect on retinal output. This is dramatically highlighted by the difference in RGC RF organization in mice with mutations in *Grm6* and *Nyx*.

Humans with mutations in these various genes have clear deficits, particularly under low light conditions. However, these individuals do have functional vision. Assuming humans have the same variation in RGC output as observed across *Grm6*^{Tm1Nak}, *Grm6*^{nob3}, *Grm6*^{nob4} and *Nyx*^{nob} mouse mutants, this may represent significant plasticity within the visual system and an ability to construct relatively good spatial maps in the absence of anything resembling normal RF centre/surround organization. This result bodes well for therapies aimed at restoring light-mediated input to the retina in diseases such as RP and macular degeneration.

References

Attwell D, Tessier-Lavigne M, Wilson M & Mobbs P (1987). Bipolar cell membrane currents and signal processing in the axolotl retina. *Neurosci Res Suppl* **6**, S191–S204.

Ball SL & Gregg RG (2002). Using mutant mice to study the role of voltage-gated calcium channels in the retina. *Adv Exp Med Biol* **514**, 439–450.

Ball SL, Pardue MT, McCall MA, Gregg RG & Peachey NS (2003). Immunohistochemical analysis of the outer plexiform layer in the nob mouse shows no abnormalities. *Vis Neurosci* **20**, 267–272.

Ball SL, Powers PA, Shin HS, Morgans CW, Peachey NS & Gregg RG (2002). Role of the β_2 subunit of voltage-dependent calcium channels in the retinal outer plexiform layer. *Invest Ophthalmol Vis Sci* **43**, 1595–1603.

Barnes S & Kelly ME (2002). Calcium channels at the photoreceptor synapse. *Adv Exp Med Biol* **514**, 465–476.

Bayley PR & Morgans CW (2007). Rod bipolar cells and horizontal cells form displaced synaptic contacts with rods in the outer nuclear layer of the nob2 retina. *J Comp Neurol* **500**, 286–298.

Baylor DA & Fuortes MG (1970). Electrical responses of single cones in the retina of the turtle. *J Physiol* **207**, 77–92.

Bech-Hansen NT, Naylor MJ, Maybaum TA, Pearce WG, Koop B, Fishman GA, Mets M, Musarella MA & Boycott KM (1998). Loss-of-function mutations in a calcium-channel α1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nat Genet* **19**, 264–267.

Berntson A, Taylor WR & Morgans CW (2003). Molecular identity, synaptic localization, and physiology of calcium channels in retinal bipolar cells. *J Neurosci Res* **71**, 146–151.

Calvert PD, Strissel KJ, Schiesser WE, Pugh EN Jr & Arshavsky VY (2006). Light-driven translocation of signaling proteins in vertebrate photoreceptors. *Trends Cell Biol* **16**, 560–568.

Catterall WA (2000). From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* **26**, 13–25.

Chang B, Heckenlively JR, Bayley PR, Brecha NC, Davisson MT, Hawes NL, Hirano AA, Hurd RE, Ikeda A, Johnson BA, McCall MA, Morgans CW, Nusinowitz S, Peachey NS, Rice DS, Vessey KA & Gregg RG (2006). The nob2 mouse, a null mutation in Cacna1f: anatomical and functional abnormalities in the outer retina and their consequences on ganglion cell visual responses. *Vis Neurosci* **23**, 11–24.

Cui J, Ma YP, Lipton SA & Pan ZH (2003). Glycine receptors and glycinergic synaptic input at the axon terminals of mammalian retinal rod bipolar cells. *J Physiol* **553**, 895–909.

Dedek K, Pandarinath C, Alam NM, Wellershaus K, Schubert T, Willecke K, Prusky GT, Weiler R & Nirenberg S (2008). Ganglion cell adaptability: does the coupling of horizontal cells play a role? *PLoS ONE* **3**, e1714.

Demas J, Sagdullaev BT, Green E, Jaubert-Miazza L, McCall MA, Gregg RG, Wong RO & Guido W (2006). Failure to maintain eye-specific segregation in nob, a mutant with abnormally patterned retinal activity. *Neuron* **50**, 247–259.

Dhingra A, Jiang M, Wang TL, Lyubarsky A, Savchenko A, Bar-Yehuda T, Sterling P, Birnbaumer L & Vardi N (2002). Light response of retinal ON bipolar cells requires a specific splice variant of Gα_o. *J Neurosci* **22**, 4878–4884.

Dhingra A, Lyubarsky A, Jiang M, Pugh EN Jr, Birnbaumer L, Sterling P & Vardi N (2000). The light response of ON bipolar neurons requires Gα_o. *J Neurosci* **20**, 9053–9058.

Dick DM & Foroud T (2003). Candidate genes for alcohol dependence: a review of genetic evidence from human studies. *Alcohol Clin Exp Res* **27**, 868–879.

Dick O, tom Dieck S, Altrock WD, Ammermuller J, Weiler R, Garner CC, Gundelfinger ED & Brandstatter JH (2003). The presynaptic active zone protein bassoon is essential for photoreceptor ribbon synapse formation in the retina. *Neuron* **37**, 775–786.

Dowling JE & Ripps H (1973). Effect of magnesium on horizontal cell activity in the skate retina. *Nature* **242**, 101–103.

Gregg RG, Kamermans M, Klooster J, Lukasiewicz PD, Peachey NS, Vessey KA & McCall MA (2007). Nyctalopin expression in retinal bipolar cells restores visual function in a mouse model of complete X-linked congenital stationary night blindness. *J Neurophysiol* **98**, 3023–3033.

Gregg RG, Mukhopadhyay S, Candille SI, Ball SL, Pardue MT, McCall MA & Peachey NS (2003). Identification of the gene and the mutation responsible for the mouse nob phenotype. *Invest Ophthalmol Vis Sci* **44**, 378–384.

Haeseleer F, Imanishi Y, Maeda T, Possin DE, Maeda A, Lee A, Rieke F & Palczewski K (2004). Essential role of Ca^{2+}-binding protein 4, a Cav1.4 channel regulator, in photoreceptor synaptic function. *Nat Neurosci* **7**, 1079–1087.

Jalkanen R, Bech-Hansen NT, Tobias R, Sankila EM, Mantyjarvi M, Forsius H, de la Chapelle A & Alitalo T (2007). A novel CACNA1F gene mutation causes Aland Island eye disease. *Invest Ophthalmol Vis Sci* **48**, 2498–2502.

Jalkanen R, Mantyjarvi M, Tobias R, Isosomppi J, Sankila EM, Alitalo T & Bech-Hansen NT (2006). X linked cone-rod dystrophy, CORDX3, is caused by a mutation in the CACNA1F gene. *J Med Genet* **43**, 699–704.

Johnson BA, Ikeda S, Pinto LH & Ikeda A (2006). Reduced synaptic vesicle density and aberrant synaptic localization caused by a splice site mutation in the Rs1h gene. *Vis Neurosci* **23**, 887–898.

Ma YP & Pan ZH (2003). Spontaneous regenerative activity in mammalian retinal bipolar cells: roles of multiple subtypes of voltage-dependent Ca^{2+} channels. *Vis Neurosci* **20**, 131–139.

Maddox DM, Vessey KA, Invergo BM, Cantrell DR, Inayat S, Troy JB, Balannik V, Hicks Wl, Hawes NL, Byers S, Smith RS, Hurd R, Howel D, Gregg RG, Chang B, Naggert JN, Pinto LH, Nishina PM & McCall MA (2008). *Nob3*(*Grm6*^{nob3}), A spontaneously arising allele of *Grm6* demonstrates allelic variance in retinal ganglion cell visual responses? *J Physiol (in press)*.

McCall MA, Peachey NS & Gregg RG (2008). Neurotransmission in the mouse retina. In *Eye, Retina, and Visual System of the mouse,* ed. Chalupa LM & Williams RW, pp. 175–188. MIT Press, Cambridge MA, USA.

Mansergh F, Orton NC, Vessey JP, Lalonde MR, Stell WK, Tremblay F, Barnes S, Rancourt DE & Bech-Hansen NT (2005). Mutation of the calcium channel gene Cacna1f disrupts calcium signaling, synaptic transmission and cellular organization in mouse retina. *Hum Mol Genet* **14**, 3035–3046.

Masu M, Iwakabe H, Tagawa Y, Miyoshi T, Yamashita J, Fukuda Y, Sasaki H, Hiroi K, Nakamura Y, Shigemoto R, Takada M, Nakamura K, Nakao K, Katsuki M & Nakanishi S (1995). Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. *Cell* **80**, 757–765.

Miyake Y, Yagasaki K, Horiguchi M, Kawase Y & Kanda T (1986). Congenital stationary night blindness with negative electroretinogram. A new classification. *Arch Ophthalmol* **104**, 1013–1020.

Molnar A & Werblin F (2007). Inhibitory feedback shapes bipolar cell responses in the rabbit retina. *J Neurophysiol* **98**, 3423–3435.

Morgans CW, Bayley PR, Oesch NW, Ren G, Akileswaran L & Taylor WR (2005). Photoreceptor calcium channels: insight from night blindness. *Vis Neurosci* **22**, 561–568.

Nakajima Y, Iwakabe H, Akazawa C, Nawa H, Shigemoto R, Mizuno N & Nakanishi S (1993). Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J Biol Chem* **268**, 11868–11873.

Nawy S & Jahr CE (1991). cGMP-gated conductance in retinal bipolar cells is suppressed by the photoreceptor transmitter. *Neuron* **7**, 677–683.

Pan ZH (2001). Voltage-activated Ca^{2+} channels and ionotropic GABA receptors localized at axon terminals of mammalian retinal bipolar cells. *Vis Neurosci* **18**, 279–288.

Pan ZH, Hu HJ, Perring P & Andrade R (2001). T-type Ca^{2+} channels mediate neurotransmitter release in retinal bipolar cells. *Neuron* **32**, 89–98.

Pang JJ, Gao F & Wu SM (2007). Cross-talk between ON and OFF channels in the salamander retina: indirect bipolar cell inputs to ON-OFF ganglion cells. *Vision Res* **47**, 384–392.

Pardue MT, Ball SL, Mukhopadhyay S, Candile SI, McCall MA, Gregg RG & Peachey NS (2001). *nob*: A mouse model of *CSNB*1. In *New Insights Into Retinal Degenerative Diseases,* ed. Anderson RE, Lavail MM & Hollyfield JG, pp. 319–328. Kluwer Academic/Plenum Publishers, New York, USA.

Pardue MT, McCall MA, LaVail MM, Gregg RG & Peachey NS (1998). A naturally occurring mouse model of X-linked congenital stationary night blindness. *Invest Ophthalmol Vis Sci* **39**, 2443–2449.

Peachey NS & Ball SL (2003). Electrophysiological analysis of visual function in mutant mice. *Doc Ophthalmol* **107**, 13–36.

Penn RD & Hagins WA (1969). Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature* **223**, 201–204.

Pillers DA, Fitzgerald KM, Duncan NM, Rash SM, White RA, Dwinnell SJ, Powell BR, Schnur RE, Ray PN, Cibis GW & Weleber RG (1999). Duchenne/Becker muscular dystrophy: correlation of phenotype by electroretinography with sites of dystrophin mutations. *Hum Genet* **105**, 2–9.

Pinto LH, Vitaterna MH, Shimomura K, Siepka SM, Balannik V, McDearmon EL, Omura C, Lumayag S, Invergo BM, Glawe B, Cantrell DR, Inayat S, Olvera MA, Vessey KA, McCall MA, Maddox D, Morgans CW, Young B, Pletcher MT, Mullins RF, Troy JB & Takahashi JS (2007). Generation, identification and functional characterization of the nob4 mutation of Grm6 in the mouse. *Vis Neurosci* **24**, 111–123.

Rao A, Dallman R, Henderson S & Chen CK (2007). Gβ5 is required for normal light responses and morphology of retinal ON-bipolar cells. *J Neurosci* **27**, 14199–14204.

Renteria RC, Tian N, Cang J, Nakanishi S, Stryker MP & Copenhagen DR (2006). Intrinsic ON responses of the retinal OFF pathway are suppressed by the ON pathway. *J Neurosci* **26**, 11857–11869.

Robson JG & Frishman LJ (1998). Dissecting the dark-adapted electroretinogram. *Doc Ophthalmol* **95**, 187–215.

Sagdullaev BT & McCall MA (2005). Stimulus size and intensity alter fundamental receptive-field properties of mouse retinal ganglion cells in vivo. *Vis Neurosci* **22**, 649–659.

Saito T & Kaneko A (1983). Ionic mechanisms underlying the responses of off-center bipolar cells in the carp retina. I. Studies on responses evoked by light. *J Gen Physiol* **81**, 589–601.

Sarfare S & Pittler SJ (2007). Focus on molecules: rod photoreceptor cGMP-gated cation channel. *Exp Eye Res* **85**, 173–174.

Sharma S, Ball SL & Peachey NS (2005). Pharmacological studies of the mouse cone electroretinogram. *Vis Neurosci* **22**, 631–636.

Slaughter MM & Miller RF (1981). 2-Amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Science* **211**, 182–185.

Snellman J, Kaur T, Shen Y & Nawy S (2008). Regulation of ON bipolar cell activity. *Prog Retin Eye Res* **27**, 450–463.

Sugihara H, Inoue T, Nakanishi S & Fukuda Y (1997). A late ON response remains in visual response of the mGluR6-deficient mouse. *Neurosci Lett* **233**, 137–140.

Tagawa Y, Sawai H, Ueda Y, Tauchi M & Nakanishi S (1999). Immunohistological studies of metabotropic glutamate receptor subtype 6-deficient mice show no abnormality of retinal cell organization and ganglion cell maturation. *J Neurosci* **19**, 2568–2579.

Trifonov YA (1968). Study of synaptic transmission between the photoreceptor and the horizontal cell using electrical stimulation of the retina. *Biofizika* **13**, 809–817.

Trifonov YA & Byzov AL (1977). The interaction in photoreceptor synapses revealed in experiments with polarization of horizontal cells. In *Vertebrate Photoreceptors*, ed. Barlow HB & Fatt P, pp. 250–263. Academic Press, London.

Wu J, Marmorstein AD, Striessnig J & Peachey NS (2007). Voltage-dependent calcium channel CaV1.3 subunits regulate the light peak of the electroretinogram. *J Neurophysiol* **97**, 3731–3735.

Wycisk KA, Budde B, Feil S, Skosyrski S, Buzzi F, Neidhardt J, Glaus E, Nurnberg P, Ruether K & Berger W (2006*a*). Structural and functional abnormalities of retinal ribbon synapses due to Cacna2d4 mutation. *Invest Ophthalmol Vis Sci* **47**, 3523–3530.

Wycisk KA, Zeitz C, Feil S, Wittmer M, Forster U, Neidhardt J, Wissinger B, Zrenner E, Wilke R, Kohl S & Berger W (2006*b*). Mutation in the auxiliary calcium-channel subunit CACNA2D4 causes autosomal recessive cone dystrophy. *Am J Hum Genet* **79**, 973–977.

Xu L, Ball SL, Alexander KR & Peachey NS (2003). Pharmacological analysis of the rat cone electroretinogram. *Vis Neurosci* **20**, 297–306.

Zaghloul KA, Boahen K & Demb JB (2003). Different circuits for ON and OFF retinal ganglion cells cause different contrast sensitivities. *J Neurosci* **23**, 2645–2654.

Acknowledgements

The authors would like to thank Drs B. T. Sagdullaev, G. L. Yarbrough and K. A. Vessey who contributed to the RGC studies discussed in this manuscript. This work was supported by National Eye Institute grants EY014701 (M.A.Mc.) and EY012354 (R.G.G.).