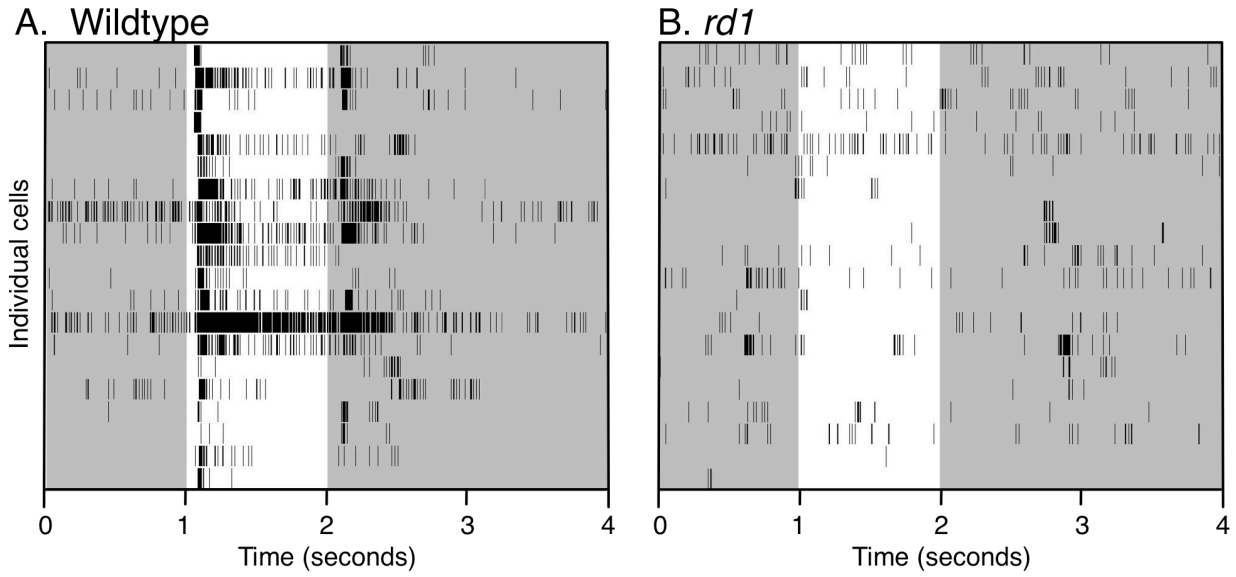
Light-evoked responses in wildtype and *rd1* mice.

Rods are absent and cones severely degenerated in B6 *rd1* retina at the age tested (P90) [1]. The early and severe photoreceptor loss is paralleled severe reduction in the electroretinogram, with no rod response and the cone response becoming unreliable by P16 [2]. Using multi-electrode recoding techniques, we have previously shown that light evoked responses are not apparent at the level of the ganglion cells in adult *rd1* mice [3]. This supports our premise that rod-cone generated responses should be effectively absent from the behavioral responses to light in this study, irrespective of the bipolar cell transmission pathway, and that behavioral responses in *rd1* mice reflect the intrinsic photosensitivity of the ipRGCs. **Figure S1** illustrates this point with data from subsequent recordings using the same methodology.

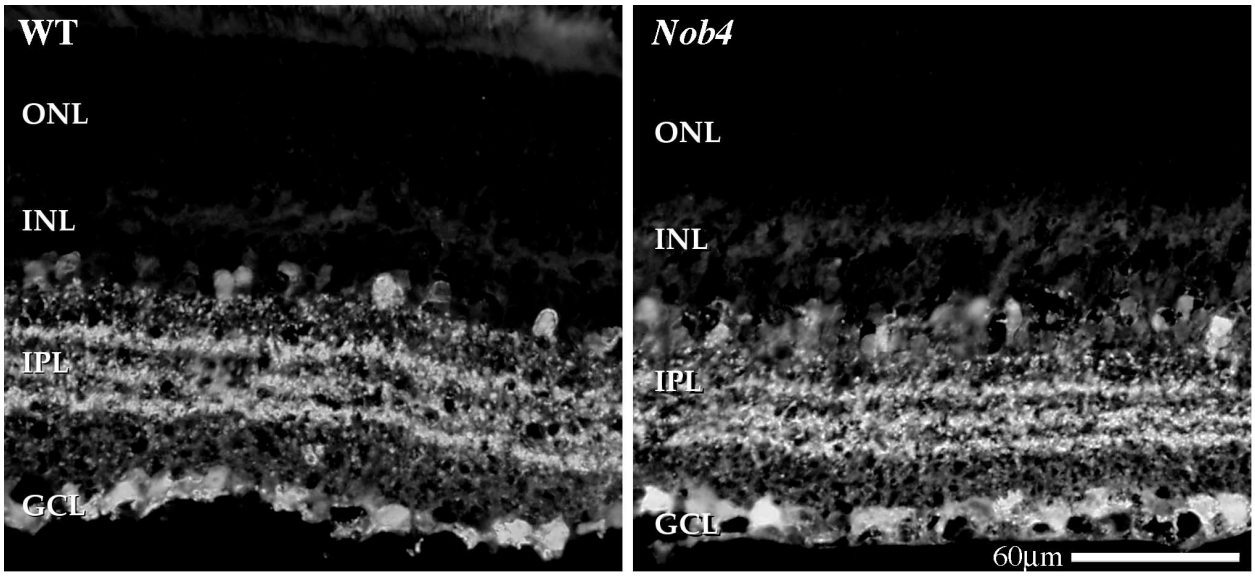
Figure S1. Light-evoked responses in wildtype and *rd1* retinas. Raster plots show activity for 20 representative ganglion cells from P28 **(A)** wildtype and **(B)** *rd1* retinas. Horizontal rows show spike activity of individual cells over a 4-second peri-stimulus period – each row showing a single cell. Vertical lines show the occurrence of individual spikes. Timing of the 1-second stimulus is shown by white background**.** At P28 in wildtype retinas, light-evoked changes in spike firing rate were recorded on most electrodes. By contrast, in *rd1*, of the 363 cells recorded at P28, there were no light-evoked responses identified. This is well before the age used in behavioral studies (P90). Some bursts of activity did coincide with changes in stimulus, but these were not distinguishable from random bursts in ongoing spontaneous firing.



Retinal structure in *Nob4* mice.

Extensive studies of retinal organization in mice lacking mGluR6 function suggested that synapses between rods-cones and ON-BCs form normally [4,5]. We recognize that absence of identifiable remodeling is not definitive, but observations do support the premise that the organization of the *Nob4* retina is normal or only subtly altered. This relative absence of remodeling of retinal pathways means that responses can be attributed to the absence of ON-BC input with reasonable confidence. A good example of the normal retinal lamination is apparent from our own light microscopy studies of the retinal organization of *Nob4* mice in **Figure S2**.

Figure S2. Calretinin immunolabeling. Representative sections show calretinin labeling in retinas from wildtype (left panel) and *Nob4* (right panel). Calretinin is expressed in a number of cell types of the retina. In the inner plexiform layer, calretinin labeling identifies specific sub-sets of amacrine cells. The layers of calretinin labeling stratify consistently in three distinct levels of the inner plexiform layer, and are therefore useful to assess the integrity of the fine structure of the inner retina. Calretinin labeling of inner retinal neuron cell bodies is similar between *Nob4* and wildtype mice, consistent with normal development of stratification in the inner retina of *Nob4* mice.



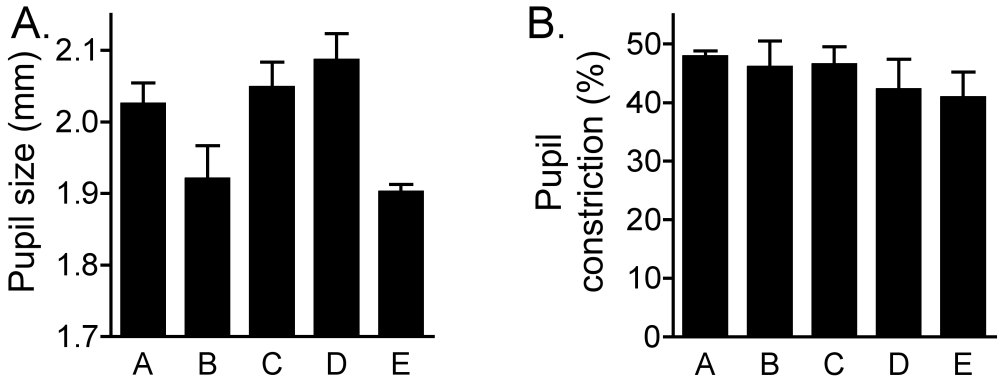
Control experiments of pupillometry under light dose ketamine/xylazine sedation.

Measuring the pupillary light reflex in mice requires either restraint or sedation, with each method having disadvantages. In restraint, pupillary light reflex amplitude is affected according to degree of anxiety or learned safety [6, 7]. This is a concern because the degree of anxiety between animals and between tests will vary, but also because anxiety related behaviors are elevated in retinal degeneration [8]. Therefore, even after pre-training or acclimation to restraint, the variability of stress with restraint, and with retinal disease will have an undefined affect on pupillary responses. Light sedation avoids the stress of restraint and allows multiple measures per session, but anesthetic agents do present complications. At a relatively high dose (100mg/kg ketamine with 10mg/kg of xylazine), ketamine/xylazine is known to induce moderate mydriasis (pupil dilation) and temporary cataracts in mice [9]. However, a number of studies have validated low dose ketamine and/or xylazine sedation in pupillometry [10-12].

To further validate light sedation with ketamine-xylazine as a suitable method for pupillometry recording, repeated tests were made in C57BL6 (wild-type) mice (*n* = 5). Animals were dark adapted for 2 to 4 hours, then given sub-cutaneous injection of 46mg/kg ketamine: 4.6mg/kg of xylazine. Ten minutes after injection, dark-adapted pupil size was recorded and the pupillary light reflex tested with a 10-second full-field stimulus at 19.8 $\mu Wcm^{-2}s^{-1}$. Trials were made in animals three times, each test being separated by 5 days of recovery. For analysis between tests, measures in an animal were paired and compared by Friedman test. Dark-adapted baseline was converted from a relative to an absolute measurement of pupil width using a ruler as scale in the plane of focus at the position of the mouse eye. Pupillary constriction amplitude was calculated as a percentage of dark-adapted baseline with 100% defined at a pupil size of 0.

At 46mg/kg ketamine: 4.6mg/kg of xylazine, mice were only sedated for 20 to 30 minutes but this was sufficient for testing. There was a difference between animals in dark-adapted baseline pupil size ($P = 0.008$), but not for a given animal between trials ($P = 0.52$) (**Figure S3**). Further, there was no difference in amplitude of the pupillary light reflex to a 19.8 $\mu Wcm^{-2}s^{-1}$ stimulus either between animals ($P = 0.21$) or between tests ($P = 0.37$). Finally, at this low dose, we have not observed temporary cataract formation in any animal even at >1 hour post-procedure, in this or other tests (*n* = 43).

Figure S3 - Effect of light ketamine/xylazine sedation on pupillary responses. Mean and SD of individual animals tested over three trials is shown for: **(A)** Dark-adapted pupil size, and **(B)** Percent pupil constriction to a 19.8 $\mu Wcm^{-2}s^{-1}$ stimulus.



The apparently minimal and predictable effect of sedation is an advantage when compared to the inevitably variable and undefined effect of anxiety with restraint [6, 7]. The absence of restraint anxiety is critical when comparing strains with retinal degeneration affecting anxiety related behaviors [8]. Therefore, low dose ketamine/xylazine appears well suited to comparison of pupillary light reflexes in mice on the same background and particularly where retinal dysfunction could affect baseline anxiety levels.

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