Multielectrode Array Recordings Of Neural Activity Patterns In The Developing Retina Of The Cone Rod Homeobox Knockout (*Crx*-/-) Mouse

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Abstract
Using multielectrode array recordings from developing retinal ganglion cells, we show that in the dystrophic cone-rod homeobox (*Crx*) knockout retina where photoreceptors degenerate between 1 and 5 months postnatal, ganglion cells have developed strong compensatory physiological mechanisms to overcome the lack of visually-evoked activity. Indeed, they exhibit strong spontaneous bursting activity and slow oscillations. In addition, a large proportion of ganglion cells are intrinsically photosensitive, responding to blue light stimulation. These results show that the *Crx* retina is capable of developing compensatory mechanisms to counteract visual experience impairment during the critical wiring period of visual connections.

1 Introduction

The *Crx-/-* mouse is a model for retinal dystrophy. *Crx-/-* photoreceptors lack outer segments from the onset, resulting in complete absence of vision through the photoreceptor/bipolar/ganglion cell pathway from birth [1]. However, all other retinal layers appear intact at birth and start degenerating from one month postnatal, when wiring of visual connections have matured [2,3]. The degeneration process lasts 3-4 months, ultimately resulting in a retina lacking outer nuclear and plexiform layers whilst the inner retina remains mostly intact, with retinal ganglion cells (RGCs) still sending impulses to the visual centres of the brain via the optic nerve. As immature retinal neural activity, both in the form of spontaneous waves and early visual experience, is important for the development of visual connections, the *Crx* retina offers an interesting scenario to study early neural activity patterns in the absence of visual experience during the critical period for establishing visual connections.

2 Methods

We have used a 60 channel microelectrode array (MEA, 30 μm electrode diameter, 200 μm separation) (Multi Channel Systems MCS GmbH) to record firing patterns from the RGC layer of *Crx-/-* and wild-type (wt) retinas during the first postnatal month, before the onset of degeneration. Signals were acquired at 25 kHz without filtering. In addition to spontaneous activity, retinas were also stimulated with diffuse blue light (480 nm wavelength) to evaluate RGC intrinsic photosensitivity, a phenomenon normally encountered in ~3% of all murine RGCs (projecting to the circadian clock in the hypothalamus) [4,5]. Spikes were amplitude threshold-detected using MC_Rack to calculate total firing rates on each electrode. To evaluate global activity and propagation patterns, we have computed firing rates for each electrode as well as correlation indices as a function of distance between electrodes. To estimate local field responses we have also measured all intrinsic signal frequencies.

3 Results

Spontaneous waves of activity normally sweep across the RGC layer in wt mouse until postnatal day 14 (P14), after which they break down and disappear by P20 [6]. In the *Crx-/-* retina, propagating waves (Figure 1) disappear earlier, around P10, switching to strong random bursting.

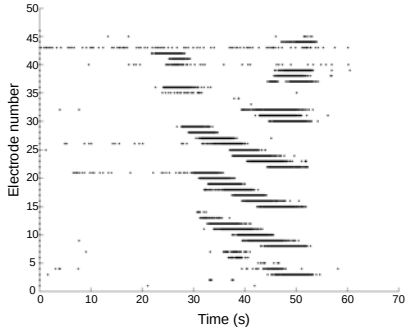

Figure 1: Raster plot of a spontaneous wave sweeping across the RGC layer of a P7 *Crx-/-* retina

Slow oscillations (~0.05Hz) (Figure 2), mediated by glutamatergic connections and intrinsic membrane

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properties, become conspicuous at P14, increasing in power over time. We were able to record such

Figure 2: bursting and oscillations recorded on one electrode from a P26 *Crx-/-* retina

oscillations up to 2 months postnatal, the oldest age studied (and well into the period of degeneration). We also found that correlation indices were 3 times higher in the *Crx-/-* retina than in wt for all ages investigated (Figure 3). Finally, we found a striking increase in responsiveness to blue light in *Crx-/-* retinas, with 12-93% of all electrodes on the array showing a significant response when compared with <4% in wt.

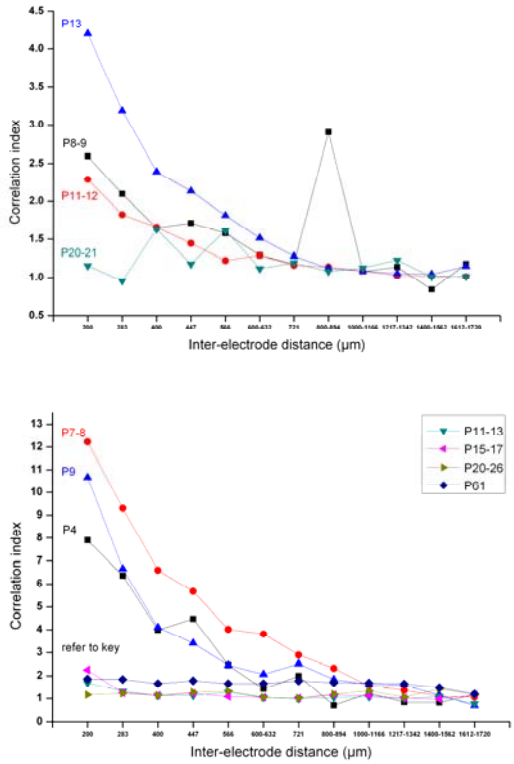

Figure 3: Correlation index plots as a function of distance between electrodes for *Crx-/-* versus wt retinas. Each data point represents average values for a certain age and

4. Conclusions

These results demonstrate that the *Crx-/-* retina is capable of developing compensatory mechanisms to counteract visual experience impairment during the critical wiring period of visual connections.

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