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Visual responses in the lateral geniculate evoked by Cx36-independent rod pathways

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ABSTRACT

Emerging evidence indicates rods can communicate with retinal ganglion cells (RGCs) via pathways that do not involve gap-junctions. Here we investigated the significance of such pathways for central visual responses, using mice lacking a key gap junction protein ($Cx36^{-/-}$) and carrying a mutation that disrupts cone phototransduction ($Gnat2^{cpfl3}$). Electrophysiological recordings spanning the lateral geniculate revealed rod-mediated ON and OFF visual responses in virtually every cell from all major anatomical sub-compartments of this nucleus. Hence, we demonstrate that one or more classes of RGC receive input from Cx36-independent rod pathways and drive extensive ON and OFF responses across the visual thalamus.

1. Introduction

Rod bipolar cells are thought not to contact retinal ganglion cells (RGCs) directly, forcing rod signals to take a more circuitous route to reach these output neurons (Bloomfield & Dacheux, 2001; Sharpe & Stockman, 1999). Indeed, established views are that rods co-opt cone circuitry to communicate to the brain via two pathways with different sensitivities. The primary, highest sensitivity, rod pathway involves an excitatory chemical synapse between rod bipolar cells and AII amacrine cells which in turn couple to ON cone bipolar cells via gap junctions. A secondary, lower sensitivity pathway, involves gap junction connections between rods and cones themselves (Völgyi, Deans, Paul, & Bloomfield, 2004).

A critical component of both of these canonical rod pathways is gap-junction communication. These direct electrical connections between cells are formed by proteins of the connexin (Cx) family, of which Cx36 is particularly widely expressed in the mammalian retina. Cells expressing this protein include photoreceptors, cone bipolars and AII amacrine cells and, at least for these latter two retinal neurons, their coupling is dependent on its expression (Deans, Völgyi, Goodenough, Bloomfield, & Paul, 2002; Feigenspan, Teubner, Willecke, & Weiler, 2001; Mills, O'Brien, Li, O'Brien, & Massey, 2001). Moreover, consistent with the architecture of the established rod pathways, ON RGCs in $Cx36^{-/-}$ mice appear to completely lack rod-mediated signals (Deans et al., 2002).

However, over the past 10 years evidence has emerged for the existence of rod pathways that do not rely on gap-junctions. Hence, it is now known that some rods contact OFF cone bipolar cells directly (Hack, Peichl, & Brandstätter, 1999; Tsukamoto, Morigiwa, Ueda, & Sterling, 2001) and OFF RGCs in $Cx36^{-/-}$ mice receive rod signals (Völgyi et al., 2004). Even more recently, anatomical and functional evidence has emerged for synapses between rods and ON cone bipolar cells (Abd-El-Barr et al., 2009; Pang et al., 2010; Tsukamoto et al., 2007). While these data call into question received wisdom regarding the functional organization of the retina, the reported lack of rod ON signals in $Cx36^{-/-}$ RGCs raises the possibility that any such gap-junction independent rod pathway only impacts on relatively rare ganglion cell classes and/or is of relatively minor significance for central visual responses.

It is thus important to determine which, if any, RGCs receive input from these novel rod pathways. Several lines of evidence led us to suspect that intrinsically photosensitive, melanopsin expressing, retinal ganglion cells (mRGCs; reviewed in Bailes & Lucas, 2010), might receive input from 'atypical' rod pathways that did not rely on gap-junction communication; (1) through mRGCs (Göz et al., 2008; Güler et al., 2008; Hatori et al., 2008), rods drive mouse circadian response to light across a broad (>5 log unit) range which appears to involve multiple rod pathways with different sensitivities (Lall et al., 2010), (2) direct contacts between rod bipolar cells and mRGCs have been previously reported (Østergaard, Hannibal, & Fahrenkrug, 2007) and (3) these cells are rare (~2% of the total

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ganglion cell population; Baver, Pickard, Sollars, & Pickard, 2008; Hatori et al., 2008) and could easily have escaped detection in the original characterization of *Cx36*$^{-/-}$ mice.

To investigate the role of Cx36-independent rod pathways in mRGC-mediated responses we utilized *Cx36*$^{-/-}$;*Gnat2*^{cpfl3} mice. In addition to their lack of Cx36, these animals bear a mutation in the cone-specific transducin α-subunit which renders cones dysfunctional (Chang et al., 2006). Electroretinography (ERG) confirmed that ON bipolar cell responses in these animals were equivalent to wildtype for dim flashes but deficient with brighter flashes in the photopic range, consistent with a loss of cone function. We then went onto record light responses directly from the intergeniculate leaflet (IGL) and surrounding lateral geniculate nuclei (LGN) in these mice, a major target of mRGCs (Ecker et al., 2010). Surprisingly, we found responses to brief light increments and decrements in virtually all LGN neurons. These responses appeared at relatively low light levels and saturated in the low photopic range, consistent with a rod origin. Many of these cells also exhibited sustained responses characteristic of melanopsin phototransduction suggesting that Cx36-independent rod signals may indeed be routed by mRGCs. By contrast, a substantial proportion (~40%) of the cells from which we recorded these rod signals showed no evidence of melanopsin input indicating at least one (presumably rare) class of conventional RGC receives rod ON signals in the absence of Cx36. In summary these data add to the weight of evidence for tertiary rod ON and OFF pathways in the mammalian retina and suggest their influence on central visual targets is widespread.

2. Methods

2.1. Animals

All animal care was in accordance with institutional and Home Office (UK) regulations and the UK Animals Scientific Procedures, Act 1986. Animals were kept in a 12-h dark/light cycle environment at a temperature of 22 °C with food and water ad libitum.

2.2. Electroretinography

2.2.1. Recording methodology

ERG responses were compared in *Cx36*$^{-/-}$;*Gnat2*^{cpfl3}, *Gnat1*$^{-/-}$;*Gnat2*^{cpfl3} and wild-type mice (aged 60–100 days). Experimentation was performed under dim red light (<2.4 $\log_{10}$ nW/cm^2, >650 nm), and mice were long-term dark adapted (>12 h) prior to recording. Mice were initially anaesthetized with intraperitoneal ketamine (70 mg/kg) and xylazine (7 mg/kg), which was maintained with an injection of subcutaneous ketamine (72 mg/ml) and xylazine (5 mg/ml).

Pupil dilation was achieved through application of mydriatics (tropicamide, 1%, and phenylephrine, 2.5%; Chauvin Pharmaceuticals, Essex, UK) to each eye. Hypromellose solution (0.5%; Alcon Laboratories Ltd., Herts, UK) was also applied to each eye to retain corneal moisture and to provide sufficient adherence of a contact lens electrode to the corneal surface. A silver wire bite bar provided head support and acted as a ground, and a needle reference electrode (Ambu® Neuroline) was inserted approximately 5 mm from the base of contralateral eye, sufficiently distal to exclude signal interference. Electrodes were connected to a Windows PC via a signal conditioner (Model 1902 Mark III, CED, Cambridge, UK) which differentially amplified (3000×) and filtered (band-pass filter cut-off 0.5–200 Hz) the signal, and a digitizer (Model 1401, CED). Throughout experimentation, core body temperature was maintained at ~37 °C via a custom-made hose coil connected to a water source at constant temperature. For 10 min prior to first recordings, electrode stability was monitored; electrodes displaying any baseline instability were rejected.

2.2.2. Scotopic ERGs

A xenon arc source (Cairn Research Ltd., Kent, UK) connected to a ganzfeld sphere provided white light flashes of equal intensity across the retina. To investigate the irradiance-response relationship of scotopic ERGs, light intensity was increased in a logarithmic scale using ND filters (Edmund Optics, York, UK) to provide corneal irradiances in the range of −4.8–3.2 $\log_{10}$ μW/cm. Light measurements were performed using a calibrated optical power meter (Macam Photometrics, Livingston, UK) and spectrophotometer (Ocean Optics, FL, USA). Calculated spectra were corrected according to photon energy and summed to determine the total photon flux at these intensities (which ranged between 4.6×10^8 and 4.6×10^{14} photons/cm^2/s). A series of 15 ms flashes were applied using an electrically controlled mechanical shutter (Cairn Research Ltd.). The inter-stimulus interval ranged from 1500 ms at dimmest intensities to 30 s at brightest intensities. The number of stimuli was reduced from 30 to 6 at increasing intensities, and an average trace was generated from recordings at each intensity. In addition, to assess the residual cone activity retained in young adult *Gnat2*^{cpfl3} mice, ERG responses of *Gnat1*$^{-/-}$;*Gnat2*^{cpfl3} mice were compared in response to white, 458 nm and 580 nm light flashes (10 nm bandwidth; 5.4×10^{12} and 1.6×10^{13} photons/cm^2/s respectively).

2.2.3. Photopic ERGs

Continuous bright white flashes (Grass Model PS33 Photic Stimulator, Astro-Med, Inc., RI, USA; fitted with a 400 nm high pass filter; 10 μs duration; peak corneal irradiance 3.3 $\log_{10}$ μW/cm^2) were applied at a frequency of 1.3 Hz against a rod-saturating, uniform white background light (metal halide source; 2.8 $\log_{10}$ μW/cm^2). This was maintained over a period of 20 min, and ERGs were recorded following each light pulse. Recordings were then grouped into sets of 25, and an average trace was generated for each set.

2.2.4. Data analysis

The amplitude of the a-wave (from the stable baseline prior to stimulus onset, to a-wave peak) and the b-wave (from a-wave peak to b-wave peak) were recorded for each dataset. Sigmoidal curves were fitted to all datasets ($y = V_{min} + (V_{max} - V_{min})/(1 + 10\text{^}((V_{50} - x)/\text{Slope}))$) using GraphPad Prism v.4 (GraphPad Software Inc., CA, USA). To compare datasets, *F*-tests were used to determine whether the best-fit parameters in the above relation differed significantly, i.e. whether curves were statistically distinguishable ($P < 0.05$).

2.3. In vivo neurophysiology

2.3.1. Surgical procedures

Adult male mice (80–120 days) were anaesthetized by i.p. injection of 30% (w/v) urethane (1.7 g/kg; Sigma, Dorset, UK) and placed in a stereotaxic apparatus (SR-15M; Narishige International Ltd., London, UK). Additional top up doses of anaesthetic (0.2 g/kg) were applied as required. Throughout the experiment the animal's temperature was maintained at 37 °C with a homeothermic blanket (Harvard Apparatus, Kent, UK). The skull surface was exposed and a small hole (~1 mm diam.) drilled 2.5 mm posterior and 2.3 mm lateral to the bregma. The pupil, contralateral to the craniotomy, was dilated with topical application of 1% (w/v) atropine sulphate (Sigma) and the cornea kept moist with mineral oil. A recording probe (A4X8-5 mm-50-200-413; Neuronexus, MI, USA) consisting of four shanks (spaced 200 μm), each with eight recordings sites (spaced 50 μm) was then positioned centrally on the exposed skull surface, perpendicular to the midline, and lowered to a