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The rat retina has five types of ganglion-cell photoreceptors

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ABSTRACT

Intrinsically photosensitive retinal ganglion cells (ipRGCs) are inner retinal photoreceptors that mediate non-image-forming visual functions, e.g. pupillary constriction, regulation of pineal melatonin release, and circadian photoentrainment. Five types of ipRGCs were recently discovered in mouse, but whether they exist in other mammals remained unknown. We report that the rat also has five types of ipRGCs, whose morphologies match those of mouse ipRGCs; this is the first demonstration of all five cell types in a non-mouse species. Through immunostaining and λ_{max} measurements, we showed that melanopsin is likely the photopigment of all rat ipRGCs. The various cell types exhibited diverse spontaneous spike rates, with the M1 type spiking the least and M4 spiking the most, just like we had observed for their mouse counterparts. Also similar to mouse, all ipRGCs in rat generated not only sluggish intrinsic photoresponses but also fast, synaptically driven ones. However, we noticed two significant differences between these species. First, whereas we learned previously that all mouse ipRGCs had equally sustained synaptic light responses, rat M1 cells' synaptic photoresponses were far more transient than those of M2–M5. Since M1 cells provide all input to the circadian clock, this rat-versus-mouse discrepancy could explain the difference in photoentrainment threshold between mouse and other species. Second, rat ipRGCs' melanopsin-based spiking photoresponses could be classified into three varieties, but only two were discerned for mouse ipRGCs. This correlation of spiking photoresponses with cell types will help researchers classify ipRGCs in multielectrode-array (MEA) spike recordings.

1. Introduction

Intrinsically photosensitive retinal ganglion cells (ipRGCs) are mammalian retinal output neurons that contain the photopigment melanopsin (Provencio et al., 1998) and function as photoreceptors. They mediate non-image-forming photoresponses such as pupil reflexes, entrainment of circadian rhythms to light/dark cycles, and regulation of pineal melatonin secretion. IpRGCs were discovered by David Berson and colleagues (Berson et al., 2002) who labeled them in rats through retrograde transport of dyes injected into the suprachiasmatic nucleus (SCN), site of the circadian clock. All retrolabeled ipRGCs shared a common morphology, suggesting they constitute a single cell type. Specifically, their somas were intermediate in size among neurons in the ganglion cell layer (GCL), and their long, tortuous dendrites branched sparsely and terminated in the OFF sublamina of the inner plexiform layer (IPL) (Berson et al., 2002). Another study of SCN-projecting rat ipRGCs also described them as having sparse and tortuous dendrites, though three dendritic stratification patterns were seen: ON, OFF, and ON/OFF (Warren et al., 2003). However, all cells' dendrites appeared to project toward the OFF sublamina, suggesting they ultimately

Abbreviations: ChAT, choline acetyltransferase; D-AP5, D-2-amino-5-phosphonovalerate; DNQX, 6,7-dinitroquinoxaline-2,3-dione; GCL, ganglion cell layer; GFP, green fluorescent protein; IPL, inner plexiform layer; ipRGC, intrinsically photosensitive retinal ganglion cell; L-AP4, L(+)-2-amino-4-phosphonobutyrate; LGN, lateral geniculate nucleus; MEA, multielectrode array; ND, neutral density; PACAP, pituitary adenylate cyclase-activating polypeptide; PBS, phosphate buffered saline; RBPMS, RNA-binding protein with multiple splicing; RGC, retinal ganglion cell; SCN, suprachiasmatic nucleus.

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terminated in that sublamina just like those described by Berson et al.

Subsequent studies revealed five morphological types of ipRGCs in mouse. The SCN-projecting type, with morphology virtually identical to that in rat, is named M1, whereas the new types are called M2 through M5 (Ecker et al., 2010; Viney et al., 2007). M2 cells have sparse, irregular dendrites confined to the ON sublamina of the IPL. M3 cells have both ON- and OFF-terminating dendrites. M4 cells have large somas and dense, radiate dendrites restricted to the ON sublamina, and seem identical to the previously described ON alpha cells (Estevez et al., 2012; Schmidt et al., 2014). M5's dendrites are also restricted to the ON sublamina, but they have a bushy appearance and cover a relatively narrow field (Ecker et al., 2010; Hu et al., 2013).

It is unknown whether other mammalian species also possess these five ipRGC types. Besides mouse, ipRGCs have been investigated most extensively in rat. Early rat studies observed melanopsin antibody staining only in the OFF sublamina, consistent with the existence of just the M1 type (Boudard et al., 2009; Hattar et al., 2002; Li et al., 2006; Ostergaard et al., 2007; Warren et al., 2006). Multielectrode-array (MEA) recordings of rat ipRGCs likewise failed to show obvious photoresponse heterogeneity (Weng et al., 2009). More recently, researchers began to notice melanopsin-immunopositive dendrites in the ON sublamina (Engelund et al., 2010; Hannibal et al., 2013; Ingham et al., 2009). By tracing the dendrites of individual melanopsin-immunopositive rat RGCs, a recent study detected M2 and M3 cells in addition to M1 cells (Esquiva et al., 2013). Pilot studies on rat retinas have also revealed rod/cone-independent light responses in M4-like ON alpha cells (Estevez et al., 2012). But M5 has not been found in rat, and M1 and M4 remain the only ipRGC types to have been unequivocally recorded in this species.

Here, we report that the rat has all five morphological types of ipRGCs. We further show that they are physiologically diverse, differing in spontaneous and light-evoked electrical activities. Finally, we compare and contrast their light-driven spiking responses with those of mouse ipRGCs.

2. Material and methods

2.1. Animals and eyecup preparation

All procedures were approved by the University Committee on Use and Care of Animals at the University of Michigan. Sprague Dawley rats of both genders were used, and ages ranged from 7 weeks to 4 months. Animals were housed in a 12hr-light/12hr–dark cycle, with all experiments conducted during the light phase.

Prior to each experiment, a rat was dark-adapted in a ventilated light-proof box overnight. Under dim red light, the animal was euthanized using carbon dioxide, then secondary euthanasia by bilateral pneumothorax or cervical dislocation. Both eyes were harvested and hemisected. After removal of the vitreous using forceps, the eyecups were transferred to room-temperature Ames' medium gassed with 95% O_2 5% CO_2. Each eyecup was cut into quadrants, which were kept in darkness for up to 9 h before being used for whole-cell recording.

2.2. Whole-cell recording, light stimulation, and chemicals

An eyecup quadrant was flattened with the scleral side down on the transparent bottom of a custom-made superfusion chamber and held down by a weighted net. The chamber was mounted on a fixed-stage upright microscope (Eclipse FN1; Nikon Instruments, Melville, NY). The bathing solution was Ames' medium gassed with 95% O_2 5% CO_2, which was maintained at 32 °C using a temperature controller (Warner Instruments, Hamden, CT) and fed into the superfusion chamber by a peristaltic pump at 2–3 mL/min. The preparation was kept in darkness except during stimulus light presentation.

The GCL was visualized through infrared transillumination and whole-cell current-clamp recording obtained from randomly selected somas using either a MultiClamp 700A or 700B amplifier (Molecular Devices; Sunnyvale, CA). Glass micropipettes with tip resistances 6–10 MΩ were pulled from thick-walled borosilicate tubings on a Narishige PC-10 puller (East Meadow, NY), and were filled with an intracellular solution containing (in mM): 120 K-gluconate; either 9 Neurobiotin-Cl (Vector Laboratories; Burlingame, CA) or 5 NaCl plus 4 KCl; 10 Hepes; 2 EGTA; 4 Mg-ATP; 0.3 Na-GTP; 7 Tris-phosphocreatine; 0.1% Lucifer Yellow; and KOH to set pH at 7.3. Using Molecular Devices CLAMPEX software, the liquid junction potential was calculated to be ~13 mV, which was taken into account in all recordings. PCLAMP 9 or 10 software (Molecular Devices) was used to acquire data. Signals were low-pass filtered at 2.4 kHz and sampled at 5 kHz. Series resistances ranged from 15 to 40 MΩ and were not compensated.

Stimuli were uniform full-field lights presented from below the superfusion chamber's transparent bottom, with intensity and wavelength adjusted using neutral density (ND) and narrowband filters respectively. Unless stated otherwise, stimulus wavelength was 480 nm. A PCLAMP-driven electromechanical shutter regulated stimulus timing. Because the experiments were performed on two rigs over >3 years using several sets of filters, stimulus intensities varied somewhat between the rigs and over time. When averaging photoresponses (Figs. 3, 4, and 5A–C), we pooled all the responses to stimuli produced using the same neutral density factor (e.g. ND2), and expressed light intensity as the midpoint of the intensity range ± half of that range. For example, "14.6 ± 0.1 log photons cm^{-2} s^{-1}" denotes 14.5 log photons cm^{-2} s^{-1} to 14.7 log photons cm^{-2} s^{-1}. Light intensities were measured using a UDT S370 radiometer (Gamma Scientific, San Diego, CA) with a rat eyecup covering the photodiode.

In the experiments examining melanopsin-based light responses, rod/cone signaling was blocked by 50 μM L(+)-2-amino-4-phosphonobutyrate (L-AP4, an agonist for group III metabotropic glutamate receptors), 40 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX, an AMPA/kainate receptor antagonist), and 25 μM D-2-amino-5-phosphonovalerate (D-AP5, an NMDA receptor antagonist). These glutamate analogs were purchased from Tocris (Minneapolis, MN). Unless stated otherwise, all other chemicals were purchased from Sigma (St Louis, MO).

2.3. Data analysis

The analyses shown in Figs. 3C and 4C–E were based on graded photoresponses, and measurements were made after the recordings had been low-pass filtered at 10 Hz. Photoresponse amplitude was measured relative to the pre-stimulus baseline. The latency to peak of a light response was measured relative to stimulus onset. In the experiment estimating the λ_{max} of intrinsic photoresponses, we assumed these responses were mediated by an opsin-based photopigment. Two to four sub-saturating intensities of 480-nm and 580-nm light steps with 10-sec duration were presented to each cell during rod/cone signaling block. Peak amplitudes of the responses were plotted versus stimulus intensities (Fig. 4E), and the lateral displacement between the 480-nm and 580-nm curves was measured at about 1/3 to half the maximal value of the 480-nm curve, where the two curves tended to be most parallel. Using the nomogram describing the spectral shape shared by all mammalian opsin-based photopigments (Lamb, 1995), the