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CHANGING COUPLING PATTERN OF THE ON–OFF DIRECTION-SELECTIVE GANGLION CELLS IN EARLY POSTNATAL MOUSE RETINA

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Abstract—In the adult rabbit and mouse retina, about 30% of the ON–OFF direction selective ganglion cells (DSGCs) are coupled via gap junctions. In early postnatal rabbit retinas, a greater proportion of morphological ON–OFF DSGCs shows coupling with a larger number of nearby somas. It is not clear whether the coupled ON–OFF DSGCs belong to the same subtype, or how coupling patterns change during development. In this study, we showed that in adult mouse retinas, all coupled ON–OFF DSGCs exhibited preferred directions (PDs) to superior, and this pattern emerged at postnatal day 15 (P15). At P13, the ON–OFF DSGCs with PDs to posterior were also coupled. Every ON–OFF DSGC in every subtype injected at P12 exhibited coupling. Therefore, a rapid decoupling process takes place in DSGCs around eye opening. Light deprivation delayed but did not halt the decoupling process. By using a transgenic mouse line in which green fluorescent protein (GFP) is selectively expressed in DSGCs with PDs to posterior and by performing *in situ* hybridization of cadherin-6, a marker for the DSGCs with PDs to superior and inferior, we showed that heterologous coupling existed between DSGCs with PDs to anterior and posterior till P12, but this heterologous coupling never spread to DSGCs positive for cadherin-6.

Key words: retinal ganglion cell, mouse, tracer coupling, gap junction, cadherin-6.

INTRODUCTION

Many types of neurons are connected via gap junctions in the mammalian retina. Interesting coupling patterns were first revealed when Neurobiotin was injected into different retinal neurons in the rabbit (Vaney, 1991). About 30% of ON–OFF direction-selective ganglion cells (DSGCs) injected exhibited gap junction coupling to neighboring DSGCs (Vaney, 1994). The gap junctions between DSGCs do not seem to conduct electric current under physiological conditions because the size of the receptive fields closely matched the size of the dendritic fields (Yang and Masland, 1992, 1994). Three lines of evidence suggest that the coupling between DSGCs is homologous. First, the coupled somas formed a regular mosaic and were mostly located outside the dendritic field of the injected cell. Second, in some strongly coupled cases where dendrites of the coupled cells can also be traced, they tiled the retina in a seamless fashion with very little overlap (Vaney, 1994). Third, electrophysiological recording demonstrated that neighboring DSGCs with extensively overlapping dendritic fields exhibited different preferred directions (PDs) (Amthor and Oyster, 1995).

More complex patterns emerged when the coupling was investigated in the early postnatal rabbit retina. Around postnatal day 3 (P3), over 90% of injected DSGCs exhibited coupling, and many surrounding DSGCs were coupled. At P5, the percentage of coupled DSGCs was reduced, and fewer cells were coupled and the coupling pattern appeared more regular. By P10, the number of coupled cells was further reduced to form a pattern similar to that observed in adults (DeBoer and Vaney, 2005). These results suggest that decoupling may reflect the process of DSGC maturation, and perhaps, the formation of functional circuitry.

ON–OFF DSGCs in the mouse retina are very similar to the rabbit counterparts in physiology, pharmacology, dendritic morphology and coupling patterns (Weng et al., 2005). It has been shown that DSGCs form gap junctions with neighboring cells using connexin45 (Schubert et al., 2005b; Volgyi et al., 2009; Pan et al., 2010). However, there is no direct evidence whether the coupled DSGCs exhibit the same preferred directions. If the coupling is homologous, which of the four subtypes (Oyster, 1968; Elstrott et al., 2008) is coupled? Although one coupled rabbit DSGC has been shown to exhibit the preferred direction to superior (Kanjhan and Vaney, 2008), it is not sufficient to answer these questions. Furthermore, in early development, is the coupling heterologous to begin with, or the coupling is always homologous?

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Abbreviations: A (Ant), anterior; Cdh6, cadherin-6; Col25α1, collagen25 alpha 1; DSGC, direction selective ganglion cell; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I (Inf), inferior; P (Post), posterior; PD, preferred direction; S (Sup), superior; SAC, starburst amacrine cell.

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We showed here that after P15, all coupled DSGCs in the mouse retina exhibited PDs to superior. At P13, the DSGCs with PDs to posterior also showed tracer coupling. At P12, every DSGC recorded and injected with Neurobiotin showed coupling. Dark-rearing appeared to postpone the decoupling process; nevertheless, complete decoupling was still achieved by P30. We also showed that heterologous coupling can be seen early in development between subtypes exhibiting PDs to anterior and posterior but the heterologous coupling never spread into DSGCs exhibiting PDs to superior or inferior.

EXPERIMENTAL PROCEDURES

Whole-mount retina preparation

C57BL/6N mice, aged P12, 13, 15 and adulthood (> P30), reared with normal visual experience (12 h light/dark cycle), or completely deprived of light from birth to the day of experiment, were used in this study. A transgenic line in which Enhanced GFP is expressed under the control of Dopamine Receptor D4 promoter (DRD4-EGFP) (Huberman et al., 2009) aged P3, 8 and 12 were also used. The transgenic mice were obtained via *in vitro* fertilization (sperms from DRD4-EGFP transgenic mice, eggs from FVB/NJ mice) and subsequent crossing into a C57BL/6N mice background. Detailed information about DRD4-EGFP mice and links to endogenous DRD4 gene expression pattern are available at http://www.mmrrc.org/strains/231/0231.html (see Gong et al., 2003). Formal approval to conduct animal experiments described in the article had been obtained from the Institute of Biophysics, Chinese Academy of Sciences. Use and handling of animals were strictly in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

Animals were dark adapted for at least 1 h, deeply anaesthetized with an i.p. injection of a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg), and decapitated. For P3 and P8 mice, diethyl was used as an anesthetic. A scald was made on the temporal side of the cornea via a scorching needle and the eyes immediately enucleated under very dim red light. A cut into the scald from posterior to anterior was made on the retina to mark the orientation. The retina was carefully dissected from the pigment epithelium in Ames' medium equilibrated with 95% O_2 and 5% CO_2, and flat-mounted, ganglion cell layer up, on a coverslip coated with 0.1% poly-L-lysine (P6282-5MG, Sigma–Aldrich, St. Louis, MO). Sometimes the retina was attached to a piece of black membrane filter (AABP02500, Millipore, Billerica, MA) with a 2 mm diameter hole to allow adequate infrared illumination and visual stimulation onto the retina during the prolonged electrophysiological recording. The whole-mount retina was then transferred into a recording chamber (0.5 ml in volume) on the fixed stage of an upright microscope (DMLFSA, Leica) equipped with epifluorescence and a 40× water-immersion objective (Leica HCX APO L 40×/0.80 W U-V-I) configured for differential interference contrast (DIC). The preparation was continuously superfused with oxygenated bicarbonate-buffered Ames' medium at 35 °C.

Patch clamp recording

Micropipettes were manufactured from thick-walled borosilicate filament glass tubing (1.5 mm outer diameter, 0.86 mm inner diameter; Sutter Instruments, San Rafael, CA) using a Flaming–Brown P97 puller (Sutter, Inc.). Under infrared illumination and visual control using a cooled CCD camera (CoolSNAP HQ, Photometrics, Atlanta, GA), a pipette was advanced to the retina using an MP 285 micromanipulator (Sutter, Inc.), and the inner limiting membrane was dissected to expose the somas of several retinal ganglion cells (RGCs). RGCs within a radius of about 0.5 mm from the optic disk were targeted (to avoid distortion of PDs of ON–OFF DSGCs caused by flat-mounting of the retina) and their spike activities recorded in the loose-patch mode with a pipette ($5 \sim 8\ M\Omega$) filled with Ames' medium. Using a flashing spot and a moving bar, the ON–OFF DSGCs could be identified, their receptive fields mapped and the preferred-null axes determined. The formulation of intracellular (pipette) solution used was as follows, in mM: 120 potassium gluconate, 5 NaCl, 10 KCl, 1 $MgCl_2$, 1 EGTA, 10 HEPES, 2 adenosine triphosphate (ATP), and 0.5 guanosine-5'-triphosphate (GTP), adjusted to pH 7.2 with 1 M KOH. For voltage clamp recording, 120 mM cesium methanosulfonate and 10 mM CsCl were used in substitution of potassium gluconate and KCl, and 5 mM lidocaine N-ethyl bromide (QX314-Br, L5783, Sigma–Aldrich) was included; pH was adjusted to 7.2 with 1 M CsOH. To reveal coupling pattern of the recorded ON–OFF DSGCs, 0.4% Neurobiotin (SP-1120, Vector Laboratory, Burlingame, CA) was added in the pipette solution and was infused into the cell for at least 20 min during whole cell recording. Sometimes 0.1% Lucifer Yellow (L-0258, Sigma–Aldrich) was also included in the pipette solution for studying the dendritic morphology during recording. Data acquired from the Axopatch 200B amplifier (Molecular Device, Sunnyvale, CA) were low-pass filtered at 2 kHz, digitized simultaneously with an A/D converter (Digidata 1322A, Molecular Device), and stored on a personal computer. Offline data analysis was done using Clampfit (Molecular Device), Matlab 2006b (The MathWorks, Inc., Natick, MA), and plotted with OriginPro 7.5 (MicroCal Software Inc., Northampton, MA).

Light stimulation

Stimuli were generated using a program written in VC++ and Directx 8 Software Development Kit (SDK), displayed on a monitor (Sony E230) and focused onto the retina through a microscope condenser. Two types of light stimuli were generated: (1) A spot of 25–1000 μm in diameter flashed on for 0.5–5 s, was used to determine the size of the receptive field and response polarity, (2) A rectangle of 100 × 500 μm moving parallel to its long axis in one of 12 directions with 30° intervals at speeds from 300 to 750 μm/s over a 1500 × 1500 μm area was