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Connexin36 is Required for Gap Junctional Coupling of Most Ganglion Cell Subtypes in the Mouse Retina

Feng Pan^{1,2}, David L. Paul³, Stewart A. Bloomfield^{1,2}, and Béla Völgyi^{1,*}

¹Department of Ophthalmology, New York University School of Medicine, New York, New York 10016

²Department of Physiology and Neuroscience, New York University School of Medicine, New York, New York 10016

³Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

Abstract

Converging evidence indicates that electrical synaptic transmission via gap junctions plays a crucial role in signal processing in the retina. In particular, amacrine and ganglion cells express numerous gap junctions, resulting in extensive electrical networks in the proximal retina. Both connexin36 (Cx36) and connexin45 (Cx45) subunits are widely distributed in the inner plexiform layer (IPL) and therefore are likely contribute to gap junctions formed by a number of ganglion cell subtypes. In the present study, we used the gap junction-permeant tracer Neurobiotin to compare the coupling pattern of different ganglion cell subtypes in wild-type (WT) and Cx36 knockout (KO) mouse retinas. We found that homologous ganglion-to-ganglion cell coupling was lost for two subtypes after deletion of Cx36, whereas two other ganglion cell subtypes retained homologous coupling in the KO mouse. In contrast, deletion of Cx36 resulted in a partial or complete loss of ganglion-to-amacrine cell heterologous coupling in 9 of 10 ganglion cell populations studied. Overall, our results indicate that Cx36 is the predominant subunit of gap junctions in the proximal mouse retina, expressed by most ganglion cell subtypes, and thereby likely plays a major role in the concerted activity generated by electrical synapses.

Keywords

tracer coupling; gap junction; ganglion cell; amacrine cell; mouse

Electrical synaptic transmission via gap junctions is a prevalent mode of interneuronal communication in the central nervous system (for review see Meier and Dermietzel, 2006). In the retina, for example, gap junctions are formed by each of the five major neuronal types, resulting in a comprehensive network of low-resistance pathways for direct electrical signaling between cells. The conductance of many retinal gap junctions is regulated by light acting through neuromodulators such as dopamine and nitric oxide (Baldrige et al., 1998; Bloomfield et al., 1997; He et al., 2000; Mills and Massey, 1995; Weiler et al., 2000; Xin and Bloomfield, 1999; 2000). These neuromodulators activate a number of intracellular pathways resulting in the phosphorylation of gap junction connexin proteins and, in turn, changes in ionic conductance. Indeed, converging evidence indicates that electrical synaptic

transmission in the retina rivals the chemically mediated circuits in terms of complexity, function, and regulation (for review see Bloomfield and Völgyi, 2009).

Some 20 different connexin proteins have been found in mammalian tissue, which can assemble in various combinations to form different gap junctions. Because the regulatory properties of gap junctions strongly depend on the connexin composition, determining the cellular distribution of connexins is an important approach to understanding their functional roles. This is particularly true for the mouse, in which selected gap junctions can be disrupted by targeting various connexin genes for deletion. This technique has become an important tool by which to characterize the function of electrical synapses.

In the mouse retina, Cx50 and Cx57 are restricted to the outer plexiform layer (OPL; Hombach et al., 2004; Massey et al., 2003; O'Brien et al., 2006), whereas Cx45 and Cx36 are expressed abundantly in both synaptic layers (Dedek et al., 2006; Guldenagel et al., 2000; Petrasch-Parwez et al., 2004). Cx36 has been located at gap junctions of AII amacrine cells as well as junctions between rod and cone photoreceptors (Deans et al., 2002; Feigenspan et al., 2001, 2004; Guldenagel et al., 2001; Han and Massey, 2005; Lee et al., 2003; Lin et al., 2005; Maxeiner et al., 2005; Mills et al., 2001). We recently showed that 16 different ganglion cell subtypes express gap junctional coupling in the mouse retina (Völgyi et al., 2009). Considering the wide distribution of Cx36 and Cx45 in the IPL, it is likely that they are expressed by a number of ganglion cell subtypes. Indeed, Cx36 has been localized to the alpha ganglion cell subtype, whereas Cx45 is expressed by certain bistratified ganglion cells (Hidaka et al., 2004; Schubert et al., 2005a,b; Völgyi et al., 2005b). Still, the connexin composition of most ganglion cell gap junctions remains largely unknown.

In this study, we examined and compared the tracer coupling pattern of 15 ganglion cell subtypes in wild-type (WT) and Cx36 knockout (KO) mouse retinas. We found that, whereas deletion of Cx36 affected only one-half of the subtypes showing ganglion-to-ganglion cell coupling, it resulted in a dramatic reduction in nearly all ganglion-to-amacrine cell coupling. These results indicate that Cx36 is required for most heterologous coupling in the proximal retina. In contrast, our findings suggest that other connexin proteins, such as Cx45 (Schubert et al., 2005b), are also expressed at gap junctions that subserve homologous electrical coupling between neighboring ganglion cells.

MATERIALS AND METHODS

Preparation

Adult (P30–90) Cx36 KO mice with C57BL6/129SvEv hybrid background (Deans et al., 2001) were deeply anesthetized with an intraperitoneal injection of Nembutal (0.08 g/g body weight) and lidocaine hydrochloride (20 mg/ml) applied locally to the eyelids and surrounding tissue. A flattened retinal-scleral preparation developed for rabbit (Hu et al., 2000) was adopted and modified for the mouse. Briefly, the eye was removed under dim red illumination and hemisected anterior to the ora-serrata. Anterior optics and the vitreous humor were removed, and the resultant retina-eyecup was placed in a superfusion chamber. Several radial incisions were made peripherally, allowing the eyecup to be flattened. The chamber was then mounted in a light-tight Faraday cage and superfused with an oxygenated mammalian Ringer solution (pH 7.4, 32°C; Bloomfield and Miller, 1982). Surgery was carried out under red illumination, and retinas were dark adapted for 1 hour prior to experimentation. Animals were sacrificed immediately after enucleations. All animal procedures were in compliance with the NIH *Guide for the care and use of laboratory animals* and approved by the Institutional Animal Care and Use Committee at NYU School of Medicine. WT mice to which comparisons of tracer coupling of Cx36 KO ganglion cells

were made included both WT littermates of Cx36 KO mice and mice with C57BL6 background.

Neurobiotin injections

Ganglion cells were visualized by using transscleral illumination with infrared (IR) light and impaled with standard borosilicate glass microelectrodes (Sutter Instrument Co., Novato, CA). The use of IR light allowed us to visualize cells while maintaining retinas in a dark-adapted state. Electrodes were filled at their tips with 4% N-(2-amino-ethyl)-biotinamide hydrochloride (Neurobiotin; Vector Laboratories, Burlingame, CA), in 0.1 M Tris buffer (pH 7.6) and then back-filled with 4 M potassium chloride. Relatively large (>10 μ m in diameter) cell bodies were targeted with glass electrodes at random retinal locations to try and sample across the entire population of ganglion cell subtypes. Neurobiotin was iontophoresed into impaled neurons using a sinusoidal (3 Hz, 0.8 nA peak-to-peak) current for 15 minutes. Because there was an inherent bias toward larger somata for which electrode impalement was more successful, we have not attempted to correlate the relative number of each ganglion cell subtype in our sample with their distribution frequency in the retina.

Histology

One hour after labeling of the last cell in an experiment, the retina was fixed overnight in a cold (4°C) solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). Retinas were then washed in phosphate buffer and soaked in a solution of 0.18% hydrogen peroxide in methyl alcohol for 1 hour. This treatment completely abolished the endogenous peroxidase activity. Retinas were then washed in phosphate buffer and reacted with the Elite ABC kit (Vector Laboratories) and 1% Triton X-100 in sodium phosphate-buffered saline (0.9% saline, pH 7.6). Retinas were subsequently processed for peroxidase histochemistry with 3,3'-diaminobenzidine (DAB), dehydrated, and flat mounted for light microscopy.

Digital images of labeled neurons were captured by a cooled CCD camera (Spot 2; Diagnostic Instruments, Sterling Heights, MI), followed by software manipulation of brightness and contrast (Photoshop; Adobe Systems, San Jose, CA). To determine the level at which dendritic processes stratified in the IPL, we examined Neurobiotin-labeled cells in flat mount under a $\times 100$ oil-immersion lens. The borders of the IPL were determined by the location at which amacrine and ganglion cell bodies were defocused with Nomarski interference contrast optics. The position of the outer margin of the IPL next to the amacrine cell bodies was defined as 0% depth, whereas the vitreal border of the IPL next to the ganglion cell bodies was defined as 100% depth. The position of cellular processes in the IPL was determined using a precision micrometer and given a depth value of 0–100. Multiple measures were made for a single cell to reveal any variations in stratification throughout the extent of its arbor.

RESULTS

Ganglion cell subtypes in the Cx36 KO mouse retina

A cohort of 136 ganglion cells in 52 Cx36 KO mouse retinas was injected with Neurobiotin and analyzed. Insofar as the main goal of this study was to compare the tracer coupling pattern of ganglion cells in WT and Cx36 KO mouse retinas, it was important to show that the different morphological subtypes of ganglion cells described in the WT (Völgyi et al., 2009) could also be distinguished in the KO mouse retina. First, we examined all our ganglion cells qualitatively to identify each of them based on our morphological characterization scheme in the WT mouse retina (Völgyi et al., 2009). During this analysis, we examined the shape of the soma, the overall morphology of the dendritic tree, the dendritic branching pattern, and looked for the existence or lack of dendritic specializations

(e.g., swellings, spines). We differentiated, based on these parameters, 15 ganglion cell subpopulations in the Cx36 KO retina with distinct soma-dendritic morphologies. Moreover, as detailed in the following sections, we were able to match these subtypes unequivocally to those (G₁, G₂, G₃, G₄, G₅, G₆, G₇, G₉, G₁₀, G₁₁, G₁₂, G₁₅, G₁₇, G₂₀, and G₂₂) described recently for the WT mouse retina (Völgyi et al., 2009).

As an adjunct to our qualitative assessment of cell morphologies and their categorization, we performed a quantitative comparison of the ganglion cell subtypes in Cx36 KO retinas with those in WT mice. Given the varied and often small samples of our cell database, we conducted a Power Analysis for the two-sample *t*-test (PASS 2008; NCSS, Kaysville, UT) to estimate the probability of rejecting a false null hypothesis for each of three morphological parameters (soma size, dendritic diameter, and level of IPL stratification) of each cell subtype. In this analysis, we used the sample size for each cell subtype and computed the difference in the means that we would have to observe in order to reject the null hypothesis (that the means of the two samples were equal) with 80% power, two-sided alpha of 0.05. Table 1 provides average values of morphometric measures and also includes comparisons to measures made of their counterparts in the WT (Völgyi et al., 2009).

In every case (a total of 39 comparisons), we found that the difference in the means that we observed was substantially smaller than the differences between the two groups that would be detectable with 80% power. For the 13 subtypes examined (two subtypes in the WT had an *n* = 1 and were not included), we calculated an averaged detectable vs. observed difference in the means of 1) 5.5 μ m vs. 1.6 μ m for soma diameter, 2) 46.9 μ m vs. 15.3 μ m for dendritic diameter, and 3) 17.7 vs. 4.8 for stratification level. As an individual example, the detectable difference in the level of dendritic stratification of G₁ cells between Cx36 KO and WT retinas with the sample sizes used would have to be greater than 10.5 to be statistically significant at the 0.05 level (two-sided) with 80% power. The observed difference in the mean level of 6.6 indicates that, given the sample size, there is no meaningful difference between the two groups, and we can conclude that the null hypothesis is not rejected. Again, this was true for each of the 39 comparisons made. Overall, the power analysis was thus consistent with our qualitative assessment indicating that the different subtypes of ganglion cells we described in WT mouse retinas can also be distinguished in KO retinas as well.

Uncoupled ganglion cells

Based on their soma-dendritic morphology (not shown), four of the 15 ganglion cell populations encountered in the Cx36 KO mouse retina corresponded to the G₅, G₉, G₁₂, and G₂₂ ganglion cells described for the WT mouse (Schubert et al., 2005b; Völgyi et al., 2009). Like their WT counterparts, these four subtypes showed no evidence of tracer coupling, so they are not included in the comparative analyses presented below. Instead, the subsequent sections focus on the remaining 11 ganglion cell subtypes in the Cx36 KO mouse retina whose WT counterparts showed extensive tracer coupling to ganglion and/or amacrine cells (Schubert et al., 2005a,b; Völgyi et al., 2009).

Tracer coupling patterns of ganglion cell subtypes in the Cx36 KO retina

By using the classification system we described recently (Völgyi et al., 2009), 11 Cx36 KO ganglion cell subtypes could be unequivocally identified as counterparts of ganglion cells with tracer coupling in the WT mouse retina. Below we 1) summarize the soma-dendritic architecture of each of these subtypes, 2) detail their tracer coupling patterns, and 3) compare these patterns with those displayed by ganglion cell counterparts in the WT retina. For further details on the morphology of these different ganglion cell subtypes, the reader is referred to Völgyi et al. (2009).

G₁ ganglion cells (n = 17)—We labeled a cohort of ganglion cells with large (~20 μm diameter), polygonal somata and large dendritic arbors that extended over 200 μm in diameter. Their dendrites showed up to sixth-order branching that occurred relatively close to the soma, resulting in long terminal dendrites. The dendrites stratified in sublamina-b of the IPL at an average depth of 71 (scale of 0–100; Fig. 1B,C). The soma-dendritic morphological features of these cells were identical to those of the G₁ subtype (Fig. 1A–C) that we described recently for the WT retina (Völgyi et al., 2009). These cells also correspond to the A1 (Sun et al., 2002), monostратified cluster 9 (Badea and Nathans, 2004), cluster 11 (Kong et al., 2005), and M10 (Coombs et al., 2006) ganglion cell subtypes described previously.

The G₁ ganglion cells in the Cx36 KO mouse were tracer coupled to amacrine cells with both small (6–9 μm in diameter) and relatively large (12–14 μm in diameter) somata displaced to the GCL (Fig. 1B,D). For the WT mouse retina, we showed previously that G₁ ganglion cells are tracer coupled to two populations of amacrine cells with both large and small somata (Völgyi et al., 2009; Fig. 1A). Here, we found a complete overlap in the diameters of coupled amacrine cell somata in WT and Cx36 KO retinas (Fig. 1D, Table 1). However, only the initial dendritic processes of coupled amacrine cells were labeled in the Cx36 KO, precluding their further morphological analysis. Taken together, our results suggest that the coupling pattern of G₁ ganglion cells was not altered following deletion of Cx36 protein.

G₂ ganglion cells (n = 7)—Another population of ganglion cells in the KO mouse had spherical somata (~18 μm in diameter) and stout, smooth dendrites that branched at acute angles with little overlap (Fig. 2B). The dendrites of these cells formed large arbors (~190 μm in diameter) and were unistratified within sublamina-b of the IPL at a depth of 67 (Fig. 2C). These morphological characteristics corresponded to those described for the G₂ subtype of ganglion cell in the WT mouse retina (Völgyi et al., 2009; Fig. 2A,C). These cells also correspond to the A2 (Sun et al., 2002), monostратified cluster 9 (Badea and Nathans, 2004), cluster 8 (Kong et al., 2005), and M8 and M9 (on) (Coombs et al., 2006) ganglion cell subtypes.

The G₂ ganglion cells in the Cx36 KO were tracer coupled to only a few faintly labeled amacrine cells with relatively small somata, 6–9 μm in diameter (Fig. 2B,D), whereas WT G₂ cells showed coupling to amacrine cells with both large (10–12 μm diameter) and small (6–9 μm diameter) cell bodies. This indicates that Cx36 deletion uncoupled G₂ ganglion cells from large, but not small, amacrine cells in the Cx36 KO.

G₃ ganglion cells (n = 23)—A third set of ganglion cells in the KO retina showed morphological characteristics corresponding to those of the G₃ ganglion cells in the WT mouse retina (Völgyi et al., 2009). These features included spherical somata, stout and smooth primary dendrites, higher order varicose dendrites that stratified in sublamina-a at depth of 36 in the IPL, and relatively dense and large dendritic arbors (~175 μm diameter; Fig. 3A–C). These cells correspond to the off alpha ganglion cells described for numerous mammalian retinas (Boycott and Wässle, 1974; Hidaka et al., 2004; Peichl et al., 1987a,b; Schubert et al., 2005a; Völgyi et al., 2005a,b). They have also been termed A2_o (Sun et al., 2002), *monostратified cluster 7* (Badea and Nathans, 2004), *cluster 10* (Kong et al., 2005), and M9 (off) (Coombs et al., 2006) ganglion cells.

The G₃ ganglion cells in the Cx36 KO displayed homologous coupling to a group of ganglion cells with large, spherical somata identical to those of the injected cells (Fig. 3B). This was consistent with G₃ ganglion cell homologous coupling in the WT mouse retina. However, WT G₃ cells also show coupling to two subpopulations of amacrine cell (Völgyi

et al., 2009), which was absent in Cx36 KO G₃ cell arrays (Fig. 3A–C). Thus, whereas the ganglion-to-ganglion cell coupling was conserved in the Cx36 KO mouse retina, the coupling between G₃ cells and amacrine cells was abolished.

G₄ ganglion cells (n = 3)—We observed ganglion cells in KO retinas with small somata (~14 μm diameter), which emitted radially running dendrites that curved and branched often to form a relatively small arbor (~145 μm diameter). Some terminal dendrites were typically two to three times longer than others, but both the short and long terminal dendrites stratified close to the middle of the IPL at an IPL depth of 41 (Fig. 3E,F). Overall, the morphology of these cells corresponded to that of the G₄ subtype of ganglion cells in the WT mouse (Völgyi et al., 2009; Fig. 3D–F), as well as the B1 (Sun et al., 2002), monostratified cluster 1 (Badea and Nathans, 2004), cluster 2 (Kong et al., 2005), and M3 (off) (Coombs et al., 2006) cells.

We found that these G₄ ganglion cells in the Cx36 KO were not coupled to any neighboring cells (Fig. 3E). In contrast, we reported that WT G₄ ganglion cells showed extensive heterologous coupling to as many as 30 amacrine cells with relatively small somata (Völgyi et al., 2009; Fig. 3D,F). Thus, deletion of Cx36 abolished all coupling between G₄ ganglion cells and neighboring amacrine cells.

G₆ ganglion cells (n = 9)—A subset of ganglion cells showed round or elliptical somata (~17 μm diameter), radiate primary dendrites, and higher order dendrites with numerous swellings that occasionally overlapped (Fig. 4B). Dendrites stratified narrowly at the 64 depth of the IPL and formed a large dendritic arbor (~175 μm diameter; Fig. 4B,C). Based on these morphological features, these cells were clearly identified as the G₆ subtype described in the WT mouse retina (Völgyi et al., 2009; Fig. 4A–C), and the B3i (Sun et al., 2002), monostratified cluster 9 (Badea and Nathans, 2004), cluster 4 (Kong et al., 2005), M7 (on), and M3 (on) (Coombs et al., 2006) cells described in other studies.

The G₆ ganglion cells showed no tracer coupling in the Cx36 KO mouse (Fig. 4B,C), whereas WT G₆ cells are coupled to two populations of amacrine cells with somata displaced to the GCL (Völgyi et al., 2009; Fig. 4A–C). Thus, deletion of Cx36 abolished the heterologous amacrine cell coupling of G₆ ganglion cells.

G₇ ganglion cells (n = 11)—One subset of ganglion cells in the Cx36 KO retina showed round somata (~17 μm in diameter) and thick primary dendrites. Higher order dendrites followed a characteristic meandering course and stratified at the depth of 35 in the IPL to form a relatively large arbor (~180 μm diameter; Fig. 4E,F). These morphological features matched those described for the G₇ subtype ganglion cell in the WT mouse retina (Völgyi et al., 2009; Fig. 4D,F). They also correspond to the B3o (Sun et al., 2002), monostratified cluster 7 (Badea and Nathans, 2004), cluster 6 (Kong et al., 2005), and M6 (off) (Coombs et al., 2006) ganglion cells.

The G₇ ganglion cells in the Cx36 KO retina showed no evidence of tracer coupling (Fig. 4E). In contrast, WT G₇ cells are tracer coupled to neighboring G₇ cells as well as to two populations of amacrine cells with large or small somata (Völgyi et al., 2009; Fig. 4D,F). Thus the G₇ cells lost both homologous and heterologous coupling after deletion of Cx36.

G₁₀ ganglion cells (n = 16)—We found ganglion cells with round somata (~15 μm in diameter), which emitted five or six slender and relatively long primary dendrites that ran radially (Fig. 5B). Primary and secondary dendritic branches were relatively straight, whereas terminal dendrites were wavier and often curved back toward the soma. The large, but sparse, dendritic arbor (~195 μm diameter) lay in sublamina-b of the IPL at a 68% depth.

These morphological features corresponded to those of the G₁₀ cells in the WT retina (Völgyi et al., 2009; Fig. 5A–C), as well as the C2i (Sun et al., 2002), monostратified cluster 9 (Badea and Nathans, 2004), cluster 11 (Kong et al., 2005), and M6 (on) and unnamed bistratified cells (Coombs et al., 2006).

Neurobiotin injections of Cx36 KO G₁₀ cells revealed no evidence of coupling (Fig. 5B,C), whereas WT G₁₀ cells are tracer coupled to amacrine cells with round or elliptical somata displaced to the GCL (Völgyi et al., 2009; Fig. 5A,C). Deletion of Cx36 in the KO mouse thus abolished the heterologous coupling between G₁₀ ganglion cells and neighboring amacrine cells.

G₁₁ ganglion cells (n = 20)—A large group of ganglion cells displayed round somata (~14 μm in diameter); three to five thin, primary dendrites that branched acutely; and terminal endings that curved back toward the soma. The dendrites stratified in sublamina-a of the IPL at a depth of 38 and formed extensive dendritic arbors (~185 μm diameter; Fig. 5E,F). These cells were clearly identified as the counterparts of the G₁₁ subtype described for the WT mouse retina (Völgyi et al., 2009; Fig. 5D,F). They are also similar to the C2o (Sun et al., 2002), monostратified cluster 7 (Badea and Nathans, 2004), and M6 (off; Coombs et al., 2006) cells described in previous studies.

We found that G₁₁ ganglion cells in the Cx36 KO retinas showed no evidence of tracer coupling (Fig. 5E,F). In the WT retina, G₁₁ cells showed some variability in their tracer-coupling patterns (Völgyi et al., 2009). Most G₁₁ cells displayed homologous coupling to up to seven neighboring ganglion cells and heterologous coupling to amacrine cells with small, round somata (Fig. 5D,F). A small fraction of G₁₁ cells in WT retinas did not show homologous coupling, but only tracer coupling to amacrine cells. Irrespective of this variability in the WT, deletion of Cx36 in the KO clearly disrupted both the homologous and the heterologous coupling of G₁₁ ganglion cells.

G₁₅ ganglion cells (n = 3)—A few ganglion cells were encountered in the KO that shared morphological features with the G₁₅ cells in the WT mouse retina (Völgyi et al., 2009) as well as the C6 (Sun et al., 2002), monostратified cluster 6 (Badea and Nathans, 2004), M5a (Coombs et al., 2006), and JAM-B-positive (Kim et al., 2008) cells. These included ovoid somata (~15 μm diameter) and a single dendritic stalk that emitted undulating dendrites. Dendrites formed a medium-sized dendritic arbor (~175 μm diameter) that characteristically lay on one side of the cell body and stratified at the 27% depth of the IPL (Fig. 6B,C). G₁₅ ganglion cells in the Cx36 KO retina were uncoupled (Fig. 6B,C). In contrast, G₁₅ ganglion cells in the WT mouse retina showed heterologous coupling to a single population of amacrine cells (Fig. 6A,C). Deletion of Cx36 thus resulted in the loss of coupling between G₁₅ ganglion cells and amacrine cells.

G₁₇ ganglion cells (n = 4)—Some ganglion cells had medium-sized somata (~15 μm in diameter), three to five stout primary dendrites, and considerably thinner higher order dendrites that showed numerous undulations and swellings. Dendrites branched at the depths of 23 and 64 in the IPL, giving rise to a bistratified, medium-sized dendritic arbor (~170 μm in diameter; Fig. 7D,F,G). These cells were unequivocally identified as the counterparts to the G₁₇ subtype ganglion cells described for the WT mouse retina (Völgyi et al., 2009; Fig. 7A–D) and are equivalent to the on-off direction-selective ganglion cells described for mammalian retinas (Vaney, 1991, 1994; Weng et al., 2005; Xin and Bloomfield, 1997). These cells have also been termed D2 (Sun et al., 2002), *bistratified clusters* 2 and 3 (Badea and Nathans, 2004), M12, M13, M14 (Coombs et al., 2006), and *type 2* (Schubert et al., 2005b) cells.

The G₁₇ ganglion cells in the KO showed homologous ganglion-to-ganglion cell tracer coupling (Fig. 7E), a coupling pattern identical to that shown for G₁₇ ganglion cells in the WT mouse retina (Völgyi et al., 2009; Fig. 7A). The consistency of homologous coupling exhibited by all mouse G₁₇ cells (Schubert et al., 2005b; Völgyi et al., 2009) seems to contradict findings in the rabbit retina, in which only one subpopulation of on-off DS ganglion cells displays homologous coupling (Vaney, 1991, 1994). However, it must be emphasized that it remains unclear whether all four subtypes of on-off DS ganglion cells are included in our sample.

G₂₀ ganglion cells (n = 4)—The final group of ganglion cells encountered in the KO mouse retina had medium-sized cell bodies (~14 µm diameter) and relatively small dendritic arbors (~125 µm diameter). These cells typically displayed three thick primary dendrites from which a bistratified arbor (26 and 49 depths in the IPL) emerged (Fig. 8B). Both the primary and higher order dendrites were wavy and displayed many swellings. These cells were identified, based on these morphological features, as the G₂₀ subtype of ganglion cell described in the WT mouse retina (Völgyi et al., 2009; Fig. 8A), which corresponds to the monostratified cluster 4 (Badea and Nathans, 2004) and M13 ganglion cell subtypes (Coombs et al., 2006).

We found that G₂₀ ganglion cells in Cx36 KO retinas showed no evidence of tracer coupling (Fig. 8B,C). In contrast, G₂₀ ganglion cells in the WT mouse retina displayed heterologous coupling to a single cohort of amacrine cells with small somata located in the INL (Völgyi et al., 2009; Fig. 8A,C). Deletion of Cx36 in the KO thus abolished the coupling between G₂₀ cells and neighboring amacrine cells.

DISCUSSION

Ganglion cell morphology is preserved in the Cx36 KO mouse retina

In this study, we analyzed the coupling patterns of 15 ganglion cell subtypes in the Cx36 KO retina that were differentiated based on morphological descriptions of WT mouse ganglion cells (Völgyi et al., 2009). We found that the overall soma-dendritic morphologies of these different subtypes were largely conserved in the KO retinas and thus could be unequivocally identified and compared with their counterparts in the WT. In our previous study, we reported 22 ganglion cell subtypes in the WT mouse retina (Völgyi et al., 2009), so we did not encounter seven subtypes (G₈, G₁₃, G₁₄, G₁₆, G₁₈, G₁₉, G₂₁) in the KO. Many of these cells (G₁₄, G₁₆, G₁₈, G₁₉, G₂₁) were rarely encountered in the WT retina, so we believe that our failure to find them in KO mice reflects their sparse distribution and is not due to a lack of expression. For example, although we did not encounter the G₁₆ subtype in this study, Schubert et al. (2005a) described this cell in the Cx36 KO retina, showing that it maintained the same morphology and coupling pattern as seen in WT retinas. Among the remaining six ganglion cell subtypes that we did not encounter, two (G₁₄ and G₁₉) were not coupled in the WT, so only four subtypes that showed tracer coupling in the WT are missing from our sample.

It has been shown that Cx36 plays an important role in generating and promoting spontaneous ganglion cell action potentials that propagate as waves across the retina during early postnatal development (Torborg et al., 2005). These spontaneous waves subserve the segregation of ganglion cell axon terminals into eye-specific layers in the lateral geniculate nucleus. Deletion of Cx36, though causing some disruptions in the synchronous, spontaneous activity in the retina, does not affect the eye-specific layering of the LGN (Torborg et al., 2005). Thus, Cx36 gap junctions do not appear critical for the normal development of the retinogeniculate pathways. Likewise, our finding that the morphology of the ganglion cell subtypes, including the level of dendritic stratification in the IPL, changed

little if any in the KO mouse suggests that electrical synapses expressing Cx36 do not play a significant role in the maturation of retinal ganglion cells.

Cx36 disruption has a limited affect on ganglion-to-ganglion cell coupling

We previously showed that seven (G_3 , G_7 , G_{11} , G_{13} , G_{16} , G_{17} , G_{18}) of the 15 ganglion cell subtypes in the WT mouse retina display ganglion-to-ganglion cell coupling (Völgyi et al., 2009). In this study, we encountered the G_3 , G_7 , G_{11} , and G_{17} subtypes in the Cx36 KO retina, finding that the G_3 and G_{17} subtypes retained homologous coupling, whereas it was absent in G_7 and G_{11} cells. Although we did not encounter the G_{16} subtype, Schubert et al. (2005b) reported that these cells (their type 3 ganglion cell) retain homologous coupling in the Cx36 KO mouse. Taken together, these results indicate that Cx36 is not critical to most homologous coupling in the proximal mouse retina. In particular, these data indicate that the G_3 , G_{16} , and G_{17} ganglion cell subtypes express gap junctions with other connexins, such as Cx45. Indeed, it has been reported by Schubert et al. (2005b) that G_{16} and G_{17} cells (their type 2 and type 3 cells) express Cx45 protein in gap junctions made with neighboring ganglion cells.

It is important to note that certain ganglion cell subtypes may express more than one type of gap junction based on their connexin composition. For example, Cx36 may be coexpressed with other connexins to form multiple bihomotypic junctions with different neighboring cell types (Li et al., 2008). Therefore, for the ganglion cell subtypes in which deletion of Cx36 did not affect the tracer coupling seen in the WT, it cannot be concluded that they do not express Cx36. These cells may maintain gap junctions composed of other connexins through which tracer, and presumably ionic currents, can still pass after Cx36 gap junctions are ablated in the KO.

Again, the results of this study and that of Schubert et al. (2005b) indicate that the ganglion cell subtypes (G_{16} and G_{17}) that showed only homologous coupling in the WT were unaffected in the Cx36 KO retina. In contrast, both the heterologous and the homologous coupling of the G_7 and G_{11} subtypes was abolished in the KO. It has been suggested that direct ganglion-to-ganglion cell coupling does not exist in such cells that display both types of coupling (Dacey and Brace, 1992). In this scheme, the tracer movement observed for G_7 and G_{11} cells in WT retinas may have occurred only through heterologous gap junctional coupling in the sequence: ganglion cell→amacrine cell→ganglion cell. Thus, abolition of heterologous coupling in the Cx36 KO would effectively limit tracer movement into neighboring ganglion cells as well. This scenario, however, cannot be the case for G_3 cells, because deletion of Cx36 abolished ganglion-to-amacrine cell coupling, whereas ganglion-to-ganglion cell coupled was preserved. Furthermore, these results indicate that direct ganglion-to-ganglion cell gap junctions can occur in subtypes that express both homologous and heterologous tracer coupling.

Cx36 disruption has a major effect on ganglion-to-amacrine cell coupling

Previous studies have shown that 14 of the 22 ganglion cell subtypes in the WT mouse retina are coupled to distinct subsets of neighboring amacrine cells (Schubert et al., 2005a; Völgyi et al., 2005b, 2009). In this study, we injected Neurobiotin into 10 of these 14 ganglion cell subtypes (G_1 , G_2 , G_3 , G_4 , G_6 , G_7 , G_{10} , G_{11} , G_{15} , G_{20}) in Cx36 KO mouse retinas and found that only two subtypes retained all or some ganglion-to-amacrine cell coupling. This included the G_1 ganglion cells, which retained coupling to a cohort of two amacrine cell populations in the KO retina. These data indicate that the ganglion-to-amacrine cell gap junctions of the G_1 subtype are not dependent on Cx36 but rather express another connexin. Consistent with this idea, it has recently been reported that ganglion cells corresponding to our G_1 subtype express Cx30.2 protein (Pérez De Sevilla Müller L, 2008).

We found that G₂ ganglion cells retained only a portion of their heterologous coupling in Cx36 KO retinas. In the WT, G₂ cells are tracer coupled to two subtypes of amacrine cells with small or large somata displaced into the GCL; the latter display long processes, identifying them as widefield cells (Schubert et al., 2005a,b; Völgyi et al., 2009). Tracer injections of G₂ cells in the Cx36 KO mouse revealed coupling to a few amacrine cells with small somata, whereas coupling to the larger widefield amacrine cells was absent. These results indicate that, although the gap junctions between G₂ ganglion cells and widefield amacrine cells are dependent on Cx36, those made with the smaller amacrine cells are not. Thus, the G₂ ganglion cells and/or coupled amacrine cells may express multiple connexins and establish gap junctions whose connexin content is different.

Overall, our results indicate that the vast majority of heterologous ganglion-to-amacrine cell coupling in the proximal mouse retina is dependent on Cx36, insofar as it was completely eliminated in eight of 10 ganglion cell subtypes in the KO (Table 2). These data indicate that Cx36 plays a crucial role in coupling of amacrine cells to ganglion cells in these arrays. Although Cx36 is clearly the predominant subunit in these gap junctions, the complete or partial retention of heterologous coupling in some cells indicates the presence of a second type of junction with different connexin structure that also subserves ganglion-to-amacrine cell coupling in the proximal mouse retina.

Role of Cx36 in GC coupling

The extensive electrical coupling in the proximal retina would appear counterintuitive, in that it would suggest the lateral spread of signals and a corresponding loss of visual acuity just as they leave the retina for higher brain structures. However, it has been shown that the receptive fields of ganglion cells match closely the size of their dendritic arbors irrespective of the extent of coupling (Vaney, 1991, 1994; Völgyi, 2000; Xin and Bloomfield, 1997). The gap junctions in the proximal retina thus do not produce a significant lateral spread of current but likely underlie local interactions. In this regard, the extensive expression of Cx36 in ganglion cells is appropriate given the relatively low conductance of the gap junctions that they form (Hidaka et al., 2004). This contrasts with the extensive electrical syncytia formed by horizontal cells in the outer retina, whose high conductance gap junctions are comprised Cx50 and Cx57 in mammals (Ciolofoan et al., 2007; Hombach et al., 2004; Massey et al., 2003; O'Brien et al., 2006; Shelley et al., 2006).

It has been suggested that ganglion cell gap junctions underlie the synchronization of activity of neighboring cells, thereby providing additional information across the limited bandwidth of the optic nerve (Meister and Berry, 1999). Spike correlations resulting from local electrical synaptic transmission may enhance the salience of visual signals by providing temporal precision necessary for efficient summation at central targets (Alonso et al., 1996; Singer, 1999; Stevens and Zador, 1998; Usrey and Reid, 1999). Short-latency, bimodal spike synchrony is believed to reflect direct and reciprocal electrical coupling between neighboring ganglion cells, whereas the somewhat broader, unimodal function seen in cross-correlograms is thought to result from gap junctions made between amacrine cells and a common cohort of ganglion cell neighbors (Arnett and Spraker, 1981; Brivanlou et al., 1998; DeVries, 1999; Hu and Bloomfield, 2003; Mastronarde, 1983a–c). Most concerted activity takes the form of broader, unimodal correlations (Schnitzer and Meister, 2003). Thus, our finding that Cx36 is the dominant subunit at ganglion-to-amacrine cell gap junctions indicates that it likely plays a critical role in generating most synchronous activity sent from retina to brain.

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Figure 1.

Morphology and tracer coupling pattern of the G₁ ganglion cells. Note that micrographs of ganglion cells in WT animals shown in this and all subsequent figures were adapted from our previous study (Völgyi et al., 2009). **A,B:** Pair of photomicrographs showing representative G₁ ganglion cells in the WT (A) and Cx36 KO (B) mouse retinas. Asterisks indicate the somata of G₁ cells, whereas arrows and arrowheads point to amacrine cells with large and small cell bodies displaced to the GCL, respectively. G₁ ganglion cells retain both their soma/dendritic morphology and their tracer coupling pattern in the Cx36 KO mouse. **C:** Schematic drawing of the IPL showing dendritic stratification levels of injected G₁ cells and coupled amacrine cells. Black circles represent injected ganglion cell somata, whereas gray circles represent somata of tracer-coupled amacrine cells. Sizes of gray circles reflect relative size differences between coupled amacrine cell somata. Rectangles connected to somata represent the maximum vertical extent of dendritic arbors for injected ganglion cells (black), for amacrine cell dendrites (dark gray), and for amacrine cell axons (light gray with dashed frame). Note that the horizontal extent of the rectangles is arbitrary and does not reflect the lateral extent of dendritic arbors. Dendritic stratification of G₁ ganglion cells was retained in the Cx36 KO mouse retina, but dendrites of coupled amacrine cells were too faint to examine in the Cx36 KO animal. **D:** Histogram comparing soma size distribution of coupled amacrine cells in the WT (black) and the Cx36 KO (gray) mouse retinas. Clearly, two populations of amacrine cells can be distinguished in both WT and Cx36 KO retinas. Scale bar = 100 μ m.

Figure 2.

Morphology and tracer coupling pattern of G_2 ganglion cells. **A,B**: Photomicrographs showing a Neurobiotin-injected G_2 cell in WT (**A**) and in the Cx36 KO (**B**) mouse retina. Asterisks indicate the somata of injected G_2 cells, whereas arrows and arrowheads point to tracer-coupled amacrine cells with large and small cell bodies, respectively. Although G_2 ganglion cells retained their basic soma/dendritic morphology, coupling to large amacrine cells was absent in the Cx36 KO mouse. **C**: Schematic drawing shows the dendritic stratification levels of injected and coupled cells in the IPL. Conventions are the same as in Figure 1C. The G_2 ganglion cells retained their normal dendritic stratification, but the dendrites of small coupled amacrine cells were labeled too weakly to visualize their dendritic arbors. **D**: Histogram comparing soma size distributions of the amacrine cells coupled to G_2 ganglion cells in WT and Cx36 KO retinas. All the amacrine cells coupled to G_2 ganglion cell in the KO displayed somata that corresponded in size to small amacrine cells in the WT. Scale bar = 100 μ m.

Figure 3.

Morphology and tracer coupling pattern of G₃ and G₄ ganglion cells. **A,B:** Photomicrographs show tracer-injected G₃ cells in the WT (A) and the Cx36 KO (B) mouse retinas. Asterisks indicate the somata of injected cells, whereas arrows and arrowheads point to tracer-coupled ganglion and amacrine cell bodies, respectively. The G₃ ganglion cells retained homologous ganglion-to-ganglion cell coupling in the Cx36 KO mouse, whereas heterologous ganglion-to-amacrine cell coupling was eliminated. **C:** Schematic showing dendritic stratification levels of injected and tracer-coupled cells in the IPL for both WT and Cx36 KO retinas. Conventions are the same as in Figure 1C. Clearly, the stratification level of G₃ ganglion cell dendrites was the same in WT and KO retinas. **D,E:** Photomicrographs showing tracer-injected G₄ cells in the WT (D) and in the Cx36 KO (E) mouse retinas. Asterisk indicates the soma of the injected ganglion cell, whereas the arrowheads indicate the somata of tracer-coupled amacrine cells. The G₄ ganglion cells clearly lost all ganglion-to-amacrine cell coupling in the Cx36 KO mouse retina. **F:** Schematic showing dendritic stratification levels in the IPL of injected G₄ ganglion cells in both the WT and the Cx36 KO mouse strains. Conventions are the same as in Figure 1C. The stratification of dendrites was unaltered in the KO animal. Scale bars = 100 μ m in A (applies to A,B); 100 μ m in D (applies to D,E).

Figure 4.

Morphology and tracer coupling pattern of G₆ and G₇ ganglion cells. **A,B:** Pair of photomicrographs showing tracer-injected G₆ cells in the WT (A) and the Cx36 KO (B) mouse retina. Asterisks indicate the somata of injected cells, whereas arrows and arrowheads indicate tracer-coupled amacrine cells bodies with large and small cell bodies displaced in the GCL, respectively. Coupled amacrine cells with large cell bodies have been characterized as polyaxonal amacrine cells. Clearly all heterologous ganglion-to-amacrine cell coupling was lost in the Cx36 KO retina. **C:** Schematic showing dendritic/axonal stratification levels of injected and tracer-coupled cells in the IPL of WT and Cx36 KO retinas. Conventions are the same as in Figure 1C. Clearly, the level of stratification of G₆ ganglion cell dendrites was the same in WT and KO retinas. **D,E:** Photomicrographs showing tracer-injected G₇ ganglion cells in the WT (D) and in the Cx36 KO (E) mouse retina. Asterisks indicate injected ganglion cell somata, whereas arrowheads and arrows point to tracer-coupled ganglion and amacrine cell bodies, respectively. The G₇ ganglion cells clearly lost both homologous ganglion-to-ganglion cell and heterologous ganglion-to-

amacrine cell coupling in the Cx36 KO mouse retina. **F**: Schematic showing dendritic stratification levels of injected G₇ ganglion cells and tracer-coupled neurons in both WT and Cx36 KO mice. Scale bars = 100 μ m in A (applies to A,B); 100 μ m in D (applies to D,E).

Figure 5.

Morphology and tracer coupling pattern of G₁₀ and G₁₁ ganglion cells. **A,B:** Pair of photomicrographs showing Neurobiotin-injected G₁₀ cells and coupled amacrine cells with polyaxonal morphology in the WT (A) and the Cx36 KO (B) retina. Clearly, all heterologous ganglion-to-amacrine cell (arrows) coupling was lost in the Cx36 KO. **C:** Schematic showing dendritic/axonal stratification levels of injected and tracer-coupled cells in the IPL of both WT and Cx36 KO retinas. Conventions are the same as in Figure 1C. The stratification of G₁₀ cell dendrites was the same in WT and Cx36 KO animals. **D,E:** Photomicrographs showing tracer-injected G₁₁ cells in the WT (D) and in the Cx36 KO (E) mouse retinas. Asterisks indicate injected ganglion cell somata, whereas arrowheads and arrows point to tracer-coupled ganglion and amacrine cell bodies, respectively. The G₁₁ ganglion cells lost both homologous ganglion-to-ganglion and heterologous ganglion-to-amacrine cell coupling in the Cx36 KO mouse retina. **F:** Schematic showing dendritic stratification levels of injected G₁₁ ganglion cells and coupled amacrine cells in both WT and Cx36 KO mice. Scale bars = 100 μ m in A (applies to A,B); 100 μ m in D (applies to D,E).

Figure 6.

Morphology and tracer coupling pattern of G₁₅ ganglion cells. **A,B:** Pair of photomicrographs showing tracer-injected G₁₅ cells in both the WT (A) and the Cx36 KO (B) retina. Clearly, all heterologous ganglion-to-amacrine cell (arrows) coupling was lost in the Cx36 KO retina. **C:** Schematic showing dendritic stratification levels of injected and tracer-coupled cells in the IPL of both WT and Cx36 KO retinas. The stratification level of G₁₅ cell dendrites was the same in WT and Cx36 KO animals. Scale bar = 100 μ m.

Figure 7.

Morphology and tracer coupling pattern of G_{17} ganglion cells. **A–C:** Photomicrographs showing a tracer-injected WT G_{17} cell with plane of focus on the GCL (A), sublamina-b (B), and sublamina-a (C) of the IPL. Arrowheads point to homologously coupled ganglion cell somata, whereas the asterisk indicates the Neurobiotin-injected cell body. **D:** Schematic showing dendritic stratification of injected G_{17} cells in the IPL of WT and Cx36 KO retinas. Conventions are the same as in Figure 1C. The level of stratification of G_{17} cell dendrites was similar in WT and Cx36 KO mouse retinas. **E–G:** Photomicrographs showing a tracer-injected G_{17} ganglion cell in the Cx36 KO mouse retina plane of focus on the GCL (E), sublamina-b (F), and sublamina-a (G) of the IPL. The asterisk indicates the tracer-injected cell body, whereas arrowheads point to the coupled ganglion cell somata. Clearly, the homologous coupling of G_{17} cells was retained in the Cx36 KO mouse retina. Scale bar = 100 μm .

Figure 8.

Morphology and tracer coupling pattern of G₂₀ ganglion cells. **A,B:** Pair of photomicrographs showing tracer-injected WT G₂₀ cells in the WT (A) and in the Cx36 KO (B) retina. Arrows point to heterologously coupled amacrine cells, whereas asterisks indicate injected ganglion cell bodies. **C:** Schematic showing dendritic stratification of both G₂₀ cells in the IPL of WT and Cx36 KO retinas. The stratification of G₂₀ cell dendrites was nearly identical in the two retinas. Scale bar = 100 μ m.

TABLE 1
Quantitative Data of Morphological Parameters of Ganglion Cell Subtypes Injected in Cx36 KO and WT Mouse Retinas¹

Cell type	Soma size (μm)	DF diameter (μm)	Stratification level in IPL (%)	Coupling to GCs	Coupling to ACs in GCL	Coupling to ACs in INL
G ₁ (n = 17)	19.5 ± 2.5 (KO) 20.3 ± 3.4 (WT)	213.3 ± 24.1 (KO) 244.6 ± 29.9 (WT)	71.4 ± 8.1 (KO) 78.0 ± 8.4 (WT)	No	0–6 (m = 1.8 ± 2) large AC; ss = 12.6–14.4 μm 2–9 (m = 3.7 ± 2) small AC; ss = 6.3–9 μm	No
G ₂ (n = 7)	18.0 ± 3.6 17.6 ± 2.9	187.0 ± 31.4 200.3 ± 42.1	67.5 ± 8.9 73.3 ± 8.1	No	2–5 (m = 3.4 ± 1.3) small AC; ss = 6.3–9 μm	No
G ₃ (n = 23)	17.3 ± 2.1 17.8 ± 2.3	174.7 ± 23.1 185.4 ± 18.5	36.1 ± 10.5 32.6 ± 9.1	0–9 (m = 4.6 ± 2.2)	No	No
G ₄ (n = 3)	15.0 ± 5.5 12.7 ± 1.2	143.5 ± 35.5 142.6 ± 25.1	41.1 ± 10.7 45.7 ± 4.3	No	No	No
G ₅ (n = 4)	13.9 ± 2.7 11.4 ± 1.0	101.5 ± 14.3 105.3 ± 23.6	54.2 ± 13.3 50.1 ± 12.6	No	No	No
G ₆ (n = 9)	17.0 ± 2.8 19.0 ± 3.0	174.6 ± 29.9 189.0 ± 30.1	64.3 ± 6.6 69.9 ± 8.9	No	No	No
G ₇ (n = 11)	17.6 ± 3.1 19.0 ± 3.2	180.8 ± 26.1 196.3 ± 18.0	35.7 ± 3.6 30.0 ± 10.7	No	No	No
G ₉ (n = 5)	13.3 ± 1.0 14.5 ± 1.8	154.0 ± 33.7 165.6 ± 32.2	58.4 ± 12.7 61.6 ± 9.5	No	No	No
G ₁₀ (n = 16)	16.1 ± 2.7 18.4 ± 2.0	194.7 ± 39.8 247.5 ± 34.1	67.6 ± 14.8 71.3 ± 9.6	No	No	No
G ₁₁ (n = 20)	13.8 ± 2.1 16.9 ± 2.6	173.6 ± 30.7 183.3 ± 30.5	38.4 ± 8.1 31.4 ± 13.7	No	No	No
G ₁₂ (n = 7)	14.1 ± 3.4 14.3 ± 2.5	195.0 ± 21.3 201.2 ± 42.3	22.7 ± 8.2 (KO) 32.8 ± 11.7 (WT) 66.2 ± 7.2 (KO) 66.7 ± 8.1 (WT)	No	No	No
G ₁₅ (n = 2)	13.8 ± 3.8 18.0 ± 2.5	136.5 ± 4.9 153.0 ± 27.0	26.8 ± 8.6 24.6 ± 6.0	No	No	No
G ₁₇ (n = 4)	15.8 ± 2.3 15.3 ± 1.6	171.5 ± 12.5 159.3 ± 8.5	23.1 ± 5.5 (KO) 31.9 ± 11.2 (WT) 63.7 ± 6.7 (KO) 67.8 ± 10.3 (WT)	3–10 (m = 7.6 ± 2.7)	No	No
G ₂₀ (n = 4)	14.0 ± 5.2 14.0	124.3 ± 34.0 117.0	26.3 ± 3.8 (KO) 27.6 (WT) 48.7 ± 12.9 (KO)	No	No	No

Cell type	Soma size (μm)	DF diameter (μm)	Stratification level in IPL (%)	Coupling to GCs	Coupling to ACs in GCL	Coupling to ACs in INL
G22 (n = 4)	18.0 ± 2.5 17.0	150.5 ± 12.1 122.0	51.4 (WT)	No	No	No
			30.4 ± 3.8 (KO)			
			36.7 (WT)			
			56.7 ± 2.8 (KO) 56.7 (WT)			

¹Data for soma size, dendritic field diameter, and stratification level are average ± SD. Subtypes that show multiple data for the stratification are bistratified. DF, dendritic field; IPL, inner plexiform layer; GCs, ganglion cells; GCL, ganglion cells layer; ACs, amacrine cells; INL, inner nuclear layer; m, mean; ss, soma size (largest diameter).

TABLE 2

Comparison of Coupling Patterns of Ganglion Cell Subtypes in Cx36 KO and WT Mouse Retinas¹

	WT		Cx36 KO		Change in coupling		Cx subunit involved	
	HOM	HET	HOM	HET	HOM	HET	HOM	HET
G ₁	No	Yes	No	Yes	No		na	Cx30,2 ^e
G ₂	No	Yes	No	Yes ²	Yes		na	Cx36 ^{a,b,d,f} ; Unknown Cx ^{a,f}
G ₃	Yes	Yes	Yes	No	Yes		Unknown Cx ^{a,f}	Cx36 ^{a-c,f}
G ₄	No	Yes	No	No	Yes		na	Cx36 ^{a,f}
G ₅	No	No	No	No	na		na	na
G ₆	No	Yes	No	No	Yes		na	Cx36 ^{a,f}
G ₇	Yes	Yes	No	No	Yes		Cx36 ^{a,f}	Cx36 ^{a,f}
G ₈	No	Yes	-	-	-		na	Unknown Cx ^{a,f}
G ₉	No	No	No	No	na		na	na
G ₁₀	No	Yes	No	No	Yes		na	Cx36 ^{a,f}
G ₁₁	Yes	Yes	No	No	Yes		Cx36 ^{a,f}	Cx36 ^{a,f}
G ₁₂	No	No	No	No	na		na	na
G ₁₃	Yes	Yes	-	-	-		Unknown Cx ^f	Unknown Cx ^f
G ₁₄	No	No	-	-	-		na	na
G ₁₅	No	Yes	No	No	Yes		na	Cx36 ^{a,f}
G ₁₆	Yes	No	-	-	-		Cx45 ^c	na
G ₁₇	Yes	No	Yes	No	No		Cx45 ^c	na
G ₁₈	Yes	Yes	-	-	-		Unknown Cx ^f	Unknown Cx ^f
G ₁₉	No	No	-	-	-		na	na
G ₂₀	No	Yes	No	No	Yes		na	Cx36 ^{a,f}

	WT		Cx36 KO		Change in coupling	Cx subunit involved	
	HOM	HET	HOM	HET		HOM	HET
G ₂₁	No	Yes	-	-	-	na	Unknown Cx _f
G ₂₂	No	No	No	No	na	na	na

¹ Yes, detectable coupling; No, lack of coupling

-, lack of data in the Cx36 KO mouse; HOM, homologous ganglion-to-ganglion cell coupling; HET, heterologous ganglion cell-to-amacrine cell coupling; na, lack of coupling.

(a) Columns at right summarize connexin expression at the gap junctions of each ganglion cell subtype based on the findings of this study

(b) studies of Schubert et al., 2005a

(c) Schubert et al., 2005b

(d) Völgyi et al., 2005

(e) Pérez De Sevilla Müller et al., 2008

(f) Völgyi et al., 2009.

² Coupling is partially retained.