Title
Intra-retinal gap junction networks of ipRGCs are regulated by retinal waves in the developing mouse retina

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Study Group:

ABSTRACT

TITLE: Intra-retinal gap junction networks of ipRGCs are regulated by retinal waves in the developing mouse retina
ABSTRACT BODY:
Purpose: Previous studies demonstrate that light-activation of intrinsically photosensitive retinal ganglion cells (ipRGCs) modulates retinal waves through unknown circuits. IpRGC-dependent modulation of retinal waves is enhanced in mice lacking the ß2 subunit of nAChR, indicating that ipRGC intra-retinal circuits are enhanced in the absence of cholinergic signaling. Here we investigate the role of gap junctions in ipRGC intra-retinal signaling during development.
Methods: Experiments were performed on retinas between postnatal day 4 and 7 from transgenic mice that express GFP in ipRGCs (opn4-EGFP). For tracer coupling experiments, ipRGCs were filled with 0.5% neurobiotin using a patch pipette and subsequently stained with streptavidin and anti GFP antibodies. Light responses and gap junction-mediated spikelets of ipRGCs were characterized with voltage clamp using a cesium based internal solution containing the voltage-gated Na+ channel blocker QX 314. Light responses were also characterized with calcium imaging of retinas.
Results: We used two methods to characterize ipRGC gap junction coupling during development; tracer coupling and the presence of spikelets. Neurobiotin fills revealed that ipRGCs were tracer coupled to other ipRGCs (identified with GFP) as well as non-ipRGCs. Spikelets were observed during all light responses (9/9) and were blocked by the gap junction blocker MFA (50 µM). Interestingly, MFA also abolished light-induced currents in ipRGCs 50% of the time (6/12), indicting that some of the light responses are not intrinsic but rather generated via gap junction input from other ipRGCs. Consistent with this hypothesis, MFA reduced the number of light responsive cells as detected with calcium imaging (control, 13.33 ±4.04; MFA, 2.67 ±2.52). We next tested whether ipRGC intra-retinal signaling was modulated by retinal waves. A one-hour pharmacological blockade of cholinergic waves (8µM DHßE) increased the number of light responsive cells (control, 15.22 ±6.59; DHßE, 28.22 ±13.30). Blocking waves also increased the number cells tracer-coupled to ipRGCs (control, 13.67±6.98; DHBE 22.82±9.26), indicating that blockade of retinal waves enhances ipRGC intra-retinal signaling.
Conclusions: Our data indicate that ipRGCs directly activate nearby neurons in the developing retina via gap junction networks that are modulated by cholinergic waves.
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