530 Ganglion cells
Thursday, May 07, 2015 12:00 PM–1:45 PM
4EF Mile High Brlm Paper Session
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Organizing Section: Visual Neuroscience

Program Number: 5862
Presentation Time: 12:00 PM–12:15 PM

Unexpected functional diversity among mouse retinal ganglion cell types

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Purpose: Retinal circuits compute visual features in parallel and send this information to the brain through dedicated channels represented by the retinal ganglion cell (RGC) types. Anatomical studies have suggested up to 22 morphological RGC types, indicating that as many functional channels may form the output of the retina. Here we show that this estimate may need to be at least doubled to reflect the true functional diversity.

Methods: To reliably record from every cell in the ganglion cell layer we used bulk-electroporation (Briggman & Euler, 2011) and two-photon Ca2+ imaging. A standardized stimulus set, including temporal full-field stimulation, local motion, and dense noise for receptive field mapping, was presented to the retina. Some recordings were obtained from transgenic mice (PV, Pcp2), in which distinct RGC subsets are fluorescently labelled. Also, electrical single-cell recordings were performed to relate RGC spiking to somatic Ca2+ signals and to retrieve RGC morphologies.

We implemented a probabilistic clustering framework for separating our sample of almost 10,000 cells (42 retinas) into functional clusters solely based on features extracted from their light responses using sparse PCA and mixture of Gaussians clustering. Then, the 70+ functional clusters were post-processed into “RGC groups” by a small number of cluster-split and -merge operations using meta data, such as immunolabels and morphological features.

Results: We found that RGCs can be divided into at least 30 functional types. Many of these matched known types, e.g. the “alpha” RGCs or the local-edge-detector (“W3”). In addition, we identified new RGC types, including an OFF DS RGC that does not co-stratify with starburst amacrine cells.

To test if our RGC groups indeed correspond to single RGC types, we measured how well the dendritic fields of each group covered the retinal surface. Most RGC groups had a coverage factor (CF) of ~1, suggesting that none of them has been spuriously split. Some groups had a CF >1, suggesting that they consist of multiple subtypes (e.g. the ON-OFF DS RGC had a CF ~4, presumably corresponding to the 4 cardinal directions). The summed CFs from all groups (57) indicate that there may be more than 50 types of RGCs in the mouse.

Conclusions: Taken together, our data indicates that information channels from the eye to the brain may be much more diverse than previously thought.

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Signal processing in the fovea: A specialized mode of synaptic integration by midget ganglion cells

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Purpose: Our visual perception is dominated by information conveyed from the fovea. A hallmark of the fovea is the specialized midget circuitry in which an individual midget ganglion cell (MGC), collects signal from a single cone photoreceptor resulting in the highest spatial resolution. This is very different in peripheral retina, where a single MGC pools information from 10-30 cones. Despite profound differences in the anatomy and function of the neural circuitry in the fovea and periphery, we know almost nothing about the mechanisms that underlie the signaling properties of cells in the fovea.

Methods: We used cell-attached and whole-cell patch clamp recordings to measure light-evoked spike responses and excitatory and inhibitory synaptic currents from MGCs at three retinal eccentricities i.e. foveal, central and peripheral retina. Excitatory and inhibitory postsynaptic receptors were labeled by immunohistochemistry and particle-mediated gene transfer. Dynamic clamp experiments were performed on MGCs to delineate the role of synaptic excitation and inhibition in shaping spike output.

Results: Using whole-cell voltage clamp recordings, we discovered that the responses of MGCs in macaque fovea are not shaped by pre- or postsynaptic inhibition. This is unlike most retinal ganglion cells including peripheral MGCs processing the same stimuli. This physiological finding had an anatomical correlate. Immunolabeling and particle-mediated gene expression displayed a strikingly dense expression of excitatory than inhibitory postsynaptic receptors on foveal MGC dendrites. The ratio of inhibitory to excitatory receptors expressed on foveal MGC dendrites is remarkably less compared to the ratio for peripheral MGCs and foveal wide-field ganglion cells. The light responses of MGCs also exhibited differences in their kinetics between fovea and periphery. Foveal MGCs exhibit sustained responses with slower kinetics compared to peripheral MGCs.

Conclusions: Our findings reveal a distinctive mode of synaptic integration in the foveal midget circuit such that MGC responses are largely dictated by excitatory input, unlike other retinal ganglion cells. This suggests that the retinal circuit, which mediates single cone signaling and high acuity vision, is designed to relay cone signals to higher visual centers with little downstream refinement in the retina.

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Space-time codependence of retinal ganglion cells can be explained by novel and separable components of their receptive fields

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**Purpose:** Reverse correlation methods such as spike triggered averaging are increasingly utilized to study the light sensitivity of retinal ganglion cells. While the linear receptive fields identified by these methods have a consistently identifiable spatial center, the classic antagonistic surround has proven more elusive. Studies that do identify a center-surround profile in the linear receptive field often rely on models that assume spatial and temporal filtering are independent. This assumption, referred to as space-time separability, has been questioned previously but not tested in this context.

**Methods:** Ganglion cell action potentials were recorded using a multielectrode array and single units were isolated. Binary white noise checkerboards were optically reduced to 50 micrometers per square and presented sequentially at 15Hz. Spike triggered averages were calculated by standard methods, and further data analysis was performed in Matlab (Mathworks, Natick MA). We used regional signal averaging relative to the receptive field center to circumvent common assumptions and improve signal-to-noise ratios.

**Results:** An antagonistic surround was observed in 754 of 805 mouse GCs across 16 retinas. Importantly, these RFs were frequently codependent on space and time, prescribing against the general assumption of space-time separability when modeling GC linear activity. We studied the nature of this inseparability, and discovered the overall RF can be decomposed into five spatiotemporally distinct subfilters. Each subfilter was individually space-time separable, but their relative strengths differed across space-time allowing them to account for the overall linear RF’s inseparability. This led us to propose the sum of separable subfilters (SoSS) model, which successfully accounted for the variance in our data. By leveraging this model, we were able to identify local deviations from a Gaussian profile in the receptive field surround.

**Conclusions:** The results presented here provide a new and more general model for ganglion cell linear receptive fields that links the spatial and temporal structure in a way that accounts for their observed co-dependencies. In the process we identify new functional components of GC linear receptive fields with distinct spatiotemporal organization, shedding light on the underlying synaptic circuitry and providing tools for its continued study.

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**Purpose:** In adult retina, both On and Off spiking responses were directionally selective (DS) to both cone and rod stimulation. Interestingly, we found that the Off responses for s-cone mediated DS were more poorly tuned than On responses, while the Off responses for rod mediated DS were similarly tuned to the On responses. Voltage clamp recordings showed also that inhibitory inputs to DSGCs were also directionally tuned, while excitatory inputs had equal amplitude for motion in all directions. The rod and cone mediated DS responses had different developmental time courses. Prior to eye opening (postnatal day 11), while the ON responses were tuned for both rod and cone stimulation, there was a significant difference in tuning for Off pathways. Notably only 20% of the DSGCs exhibited directional tuning in their Off response to cone stimulation while 80% of the DSGCs exhibited directional tuning in their Off response to rod stimulation. The decreased DS tuning in the cone pathway was due to an increased action potential firing to null-side stimulation.

**Conclusions:** Our data indicate that direction selectivity is computed for both rod and cone pathways, albeit via distinct circuits. The Off channel represents an important point of divergence between cone and rod-pathway both in its strength of tuning and developmental time course. These data indicate that the retina uses multiple strategies for computing DS responses across different stimulus conditions.

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**Program Number:** 5866

**Presentation Time:** 1:00 PM–1:15 PM

**The spatial and temporal contributions of melanopsin to mouse vision**

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**Purpose:** The emerging role of melanopsin and intrinsically photosensitive retinal ganglion cells (ipRGCs) in perceptual vision demands a more quantitative description of melanopsin’s input to these pathways in visually intact animals. Particularly lacking is a description of the spatial and temporal information conveyed by ipRGCs that arises exclusively from melanopsin’s photon capture, and what functional impact this may have on image forming vision.

**Methods:** We have modified a commercially available projection system so that each of the R, G and B channels is instead a combination of up to five, independently controlled wavelengths ($\lambda_m$, 405, 455, 525, 561, 630nm). This allows us to present patterned stimuli that only present spatial/temporal contrast for particular photopigments. We have used multi-channel recording electrodes to record light-evoked activity in the dorsal lateral geniculate nucleus (dLGN) of urethane anaesthetised Opn1mw<sup>6</sup> mice; these mice display a long-wavelength shifted spectral sensitivity of green cones, which allows us to achieve maximal contrast for each photopigment.

**Results:** We have recorded evoked activity in the dLGN in response to spatially structured stimuli, that originate from melanopsin in isolation (49% Michelson contrast); rod opsins & cone opsins (33% Michelson contrast); or melanopsin, rod and cone opsins (49% and 33%, respectively). We have measured the spatial receptive fields in each of these conditions; when measurable, isolated melanopsin receptive fields tend to be large, extending beyond the size of rod/cone driven receptive fields recorded in the same dLGN cell. The temporal properties of these responses are also distinct from rod/cone evoked responses. Moreover, comparisons of the responses evoked by rod/cone stimuli presented with or without concurrent melanopsin
contrast reveal a defined input of melanopsin that is quite distinct from rod and cone-evoked responses. 

**Conclusions:** Visual responses arising exclusively with melanopsin are detectable at the level of the dLGN at relatively modest contrasts, and on a physiologically relevant spatial and temporal scale. Although these signals are outside the range required for high spatiotemporal acuity vision, they instead occupy a distinct sensory niche that may still be useful for pattern vision.

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**Presentation Time:** 1:15 PM–1:30 PM

**Spiking, sustained ON amacrine cells receive input from intrinsically photosensitive retinal ganglion cells (ipRGCs) through gap junctions**  
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**Purpose:** We previously reported that sustained dopaminergic amacrine cells receive input from ipRGCs via AMPA/kainate receptors (Zhang et al. *PNAS* 2008). Here, we tested the hypothesis that ipRGCs signal to additional sustained ON amacrine cells.

**Methods:** We whole-cell-recorded from >2,000 displaced amacrine cells in Ames-superfused rat eyecups and presented 10-sec light steps to find cells that depolarized for the duration of the light. Recorded cells were dye-filled and imaged using confocal microscopy. To quantify the sustainedness of a photoresponse, we measured peak amplitude and the amplitude near the end of the light step, and divided the latter by the former to calculate the final-to-peak amplitude ratio.

**Results:** 193 displaced amacrine cells had sustained ON photoresponses. 40 of these were non-spiking and their light responses were abolished when rod/cone input was blocked by the glutamate analogs L-AP4, DNQX and D-AP5. The other 153 cells spiked and showed sluggish photoresponses during rod/cone block. The photoresponses were abolished when rod/cone input was blocked by the glycine receptor antagonists, but were abolished by the gap junction blocker methocelainic acid (MFA). When MFA was added to normal Ames (which permitted rod/cone signaling), these amacrine’s light responses became less tonic, reducing the final-to-peak amplitude ratio from 0.51 to 0.37 (*p < 0.05*).

**Conclusions:** All spiking sustained ON displaced amacrine cells receive ipRGC input. Unlike the dopaminergic amacrine cells which receive TTX-sensitive glutamatergic input from ipRGCs’ axon collaterals (Atkinson & Zhang *ARVO* 2014; Yeh & Chen SJN 2014), these displaced amacrine cells are electrically coupled to ipRGCs. Such coupling could be mediated by dendrodendritic gap junctions because these cells straddle in S1 and S5 just like ipRGCs. The sustained quality of these amacrine’s light responses is due to both ipRGC input and conventional rod/cone input.