# Temporal Characteristics of Neonatal Chick Retinal Ganglion Cell Responses: Effects of Luminance, Contrast, and Color

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## Abstract

We perform microelectrode array recordings of neonatal chick retina explants and report stimulus-dependent response properties of different retinal ganglion cell types to various luminance and contrast adaptation conditions. The isolated single units indicate wavelength-sensitive response properties of the retinal ganglion cell types recorded. Responses to different luminance and contrast conditions were investigated as a function of wavelength, indicating a combination of adaptation and sensitization features. We also demonstrate the presence of color opponency between blue (450 nm) and green (530 nm) light input. The analysis of their STA profiles to the full field random gaussian flicker stimulus reveals a green ON/blue OFF RGC type and a blue ON/green OFF RGC type.

## Introduction

Gallus gallus domesticus represents a diurnal vertebrate with a cone rich retina consisting of an afoveate area centralis with a high density of single and double cones and a periphery with double cones and rods. (Seifert et al., 2020) The chick-retina has been described as a model avian retina and has been recognized as a suitable cone rich retina to study eye disease and development. (Wisely et al., 2017). The importance of studying the chick retina has been highlighted in recent reviews to get a generalized understanding of the working of the vertebrate retina. (Baden et al., 2021) The structurally dense chick-retina features many neurons with complex branching patterns that divide both outer and inner plexiform layers into multiple anatomical strata, which presumably have distinct functions. The chicken follows the common vertebrate retinal organization, with three nuclear layers: the ganglion cell layer (GCL), inner nuclear layer (INL) and outer nuclear

layer (ONL), and three neuropils: the retinal nerve fiber layer (NFL), inner plexiform layer (IPL) and outer plexiform layer (OPL). Signal processing occurs along two major pathways. In the vertical pathway, bipolar cells connect photoreceptors to the retinal ganglion cells whose axons form the optic nerve. Horizontal pathways formed by horizontal cells in the OPL and amacrine cells in the IPL, modulate the vertical pathway, mediating processes such as lateral inhibition and directional selectivity. The high density of RGCs in the area centralis and the dorsal area with cone to RGC ratios averaging 1.5, resembles the primate parafovea providing high visual acuity. (Seifert et al., 2020) The understanding of chicken retina development is beneficial in the context of prosthetics that elicit specific activation of different RGC types. (Deepak et al., 2022)

Though there is now a recent wealth of anatomical information available on the chick retina with recent studies done on its connectivity in the outer plexiform layer (Gunther et al., 2021) and solving of the chick transcriptome (Yamagata et al., 2020), there is scarcity of electrophysiological studies performed on it. The utility of multi-electrode array extracellular recordings on neonatal chick retinal explants to extract the wavelength-dependent response properties of different color - opponent and non-color opponent RGCs is presented. The effect of the stimulus protocol on the elicited responses for various putative RGCs is described with responses showing dependence on ambient luminance, pulse width and the duty cycle of the periodic full field flash. Besides the direct observation of color opponency, a trend of polarity reversal in the spike triggered average with respect to contrast in non-color opponent and color opponent cells is also noticed.

## Results

Retinal explants with different full field flicker stimuli to extract different temporal characteristics of chick retinal ganglion cell responses are studied. The chick retina has been reported to describe spatial inhomogeneities in the RGC mosaic (Baden et al., 2020), to reduce variability in different preparations, retinal explants from the peripheral dorsal area were sampled across twenty explant preparations from which 470 single units were isolated though spike sorting and clustering using SpyKing circus (Yger et al., 2015). Full field flash stimuli at different luminance levels from the dark were provided to test the responsive nature of the retina to get an estimate of color dependent contrast sensitivity of the retinal explant. Responses to light intensity varying from 0.7 to 70 $mW/m^2$ are probed. A corresponding overall increase in average firing across different single units

are observed as the intensity is increased. The effect of changing luminance of the full field flash varied across different putative RGC types observed and occurred with reliable firing events at threshold intensity that depended on the light-wavelength.

**Nature of Responses**

The response of chick retinal explants to full field flashes and random flicker stimuli yielded a range of response types that differed in transiency, latency, and trial to trial variability. These recorded signals from the electrode array is a manifestation of the diverse RGCs present. In a simplified scheme, RGCs are classified functionally based on their response polarity; i.e they are ON RGCs if they fire to increasing contrasts, OFF RGCs if they fire to decreasing contrasts and ON-OFF cells if they fire to both. A large percentage of RGCs extracted in the neonatal chick retina corresponded to slow, sustained and predominantly OFF type responses. Most of the clustered RGCs were responsive to both increments and decrements in contrasts and if classified based on the R ON/ R OFF (bias index) metric can be labeled as ON-OFF cells. (Deepak et al. 2022) This bias index metric was also observed to be highly sensitive to ambient illumination (luminance) and pulse width of the full field flash indicative of integrated processes of the photons/charges generated in the RGC receptive field. A more rigorous characterization of cell types were carried out by introducing a full field random flicker stimulus where intensity values were picked from a gaussian distribution with an effective mean luminance value of 7 $mW/m^2$ and a contrast factor controlled by the variance of the distribution. The linear component of the RGC response was then estimated by performing a reverse correlation of the random flicker stimulus with the elicited spiking events of the putative RGC. The spiked triggered average (STA), average temporal contrast profile of the input that triggers a reliable RGC firing and has been used extensively to characterize RGC types across development. (Hilgen et al., 2017) Accordingly, we classify the polarity of the RGCs based on the most recent polarity of the spike triggered average (STA) estimated from a gaussian full field flicker protocol.

The chick retina is a cone rich retina with a tri-stratified outer plexiform layer with rod and double cone axon segments terminating in the outermost strata, the green and red cones terminating in the

middle segment and the blue and violet sensitive cones terminating in the innermost segment of the outer plexiform layer. Behavioral studies show that the chick retina is tetrachromatic (Osario et al., 1999), hence we probed the retinal explant with narrow band blue led (430 nm), green led (530 nm) and a broad band white led to ascertain the basic wavelength dependences of the temporal response profile of different RGC types in the chick retina. The nature of responses showed a high degree of wavelength dependence in a wide variety of metrics, namely latency, transiency, and response polarity. We broadly classify responses as color opponent and non- color opponent cell types based on the time course of the STA to full field flash stimuli of different colors. Cells are labeled color opponent if their STAs show opposing polarity in specific temporal windows before a spike is elicited to different colored random flicker protocols. Figure 1A(a) shows the STA of a non-color opponent fast OFF RGC to a green gaussian full field flicker. The same cell to blue gaussian stimulus Figure 1A(b) responds with a similar temporal profile of contrast changes with minor differences in kinetics indicating similar stimulus pathways for these ganglion cells with minor differences that can be attributed to the complex photoreceptor- horizontal cell and cone type specific interconnectivity in the outer plexiform layer.(Gunther et al., 2021) The response of the same RGC to full field 100 % contrast steps of blue and green led flashes are depicted in Figure 1A(c, d). A representative example of a non-color opponent ON RGC is described in Figure 1A(e, f), where the STA computed for full field gaussian flicker for green and blue LED primaries respectively, showing identical kinetics. It was noted that in these types of RGCs, OFF RGCs showed much faster kinetics then their ON counterparts and the trends are consistent with earlier reports. (2016)

**Pulse width effects**

It was noted that the dependence of the spike firing statistics on the pulse width and duty cycles was different for different RGC types. Explants from the dorsal area of the neonatal retina with full field flashes of varying pulse widths ranging from 50 ms to 5 s were studied. Figure 1B (a) and (b) represents the response to a pulse of equal 1 s light-of light-off period and unequal 1 s light-ON, 5 s light-OFF period. It is noticed that the longer off period (5 s) cycles resulted in a clear set of spiking features Figure 1B (b), and this off-period of 5 s was maintained for all full field flash studies. In certain cells, the independence of the light-ON period width is evident from

responses shown in Figure 1B (c and d). There were also responses from other RGCs, where the light-on pulse width made a difference, as shown in Figure 1B(e and f).

**Adaptation and Sensitization**

Adaptation is a key feature of sensory systems to optimize the firing response required to reliably encode the statistics of the sensory input. Adaptation allows the retina to respond to a large range of luminance levels. Higher luminance values elicited a higher peak firing rate in all RGCs recorded and the same contrast changes at higher luminance levels also doing the same, pointing to the increased contrast sensitivity at higher ambient luminance values. (Chen et al. 2005) As seen in Figure 2A, the response of RGCs to an ambient luminance from the dark resulted in two basic features; (i) A reduction in firing rate happening over a period of a few seconds can be attributed to luminance adaptation Figure 2A (a) and was observed in a significant number of cells (75%). (ii) Many cells also describe a sensitization feature Figure 2A (b), that shows an increase in firing rate occurring after a reduction in the contrast (or after a delay after constant illumination). The luminance adaptation trend appeared to be similar to slow contrast adaptation. (Baccus and Meister 2002). The sensitization feature generally showed a peak around 15 s with a decay profile that was sharper (~2 s) than the luminance adaptation. All neurons showed one or more of these features, with slow OFF cells showing only the sensitization feature, fast ON cells showing only the luminance adaptation feature and all other RGCs showing a linear combination both features (Figure 2A (c),(d)). Adaptation and sensitization are mechanisms utilized by sensory systems to enable the dynamic modulation of the gain of different populations of RGCs by using different forms of short term plasticity. This is achieved by the differential depression and facilitation of different excitatory and inhibitory synapses of bipolar cells in the retina. (Nikolaev et al., 2013)

Firing rate changes have been attributed to the changes in the static non-linearity. Slow adaptation and sensitization both show clear changes in the firing rate with changing contrast values. (Kastner et al. 2013) The time scales of the slow contrast adaptation and the onset of sensitization seem to show significant overlap in the population of cell types that describe both features. This is indicative of both these complementary phenomena working to increase the dynamic range of operation of the retina as a whole; with sensitization compensating for the loss

of sensitivity after a high contrast switch due to contrast adaptation and adaptation compensating for the saturation of responses due to sensitization.

Luminance and contrast adaptation has been studied extensively and modeled to understand the underlying functionality. Contrast adaptation in particular has been studied for fast OFF cells in many vertebrate retinae (Baccus and Meister 2005) and seem to show a slow and fast adaptation timescale. Intracellular recordings of the rod bipolar- AII amacrine cell synapse has shown that a common vesicular transport mechanism is shared by both luminance and slow contrast adaptation. (Jarsky et al 2011) In addition the adaptation to the mean light level also involves Ca2+ channel inactivation mechanism. Thus, a shift from low to high contrast shows a similar slow decay time course as that of luminance adaptation t~10s. Figure 2B (a) and (b) describe the slow contrast-adaptation features as the contrast changes from 25% to 50%. These responses showed stronger adaptation effects (firing rate changes) than a shift from 13% to 25% contrast changes without having an effect on the background firing activity. This shift from low to high contrast results in slow increase in firing rate in the sensitizing population of cells. (Supplementary Information available).

**Precision of RGC firing events**

Subsequent to the response studies of the full field flash input as shown in Figure 3(a), the response to the random gaussian flicker stimulus was studied to gauge the repeatability and temporal jitter in the elicited firing events. Figure 3 (b) shows the STA of a fast ON ganglion cell in response to a green full field gaussian flicker. Ten trials revealed the reproducible post stimulus time histogram (PSTH) of the same fast ON cells to the same gaussian stimulus is shown in Figure 3 (c).

The STA of an OFF RGC is described in figure 4(a), showing that it responds to sustained low contrast dips of a 400 ms duration. The response of this RGC was back calculated from a full field gaussian random flicker stimulus with a variance that was 50% of the ambient luminance value. We observed three such RGCs in the same recording that responded to the same stimulus features ;the average PSTH of the three OFF RGCs to ten trials of the same gaussian full field flicker stimulus is described in figure 4(b). A raster plot of the firing event elicited after the stimulus feature is presented is described for one of the OFF cells during the ten trials of the repeated gaussian (figure 4(c)). We observed highly precise firing events in these RGC responses with a temporal jitter of the order of 10 ms. Many the recorded responses were highly sustained in nature

and showed sluggish responses to the repeated gaussian. These cells showed clear firing rate changes to changing contrasts but showed a large temporal jitter. The neonatal nature of the retina resulted in a significant amount of spontaneous activity which affected our binning/labeling of a firing event and resulted in the STA becoming noisy. (Henning et al. 2015)

**Color opponency in the Avian retina**

The chick retina consists of 6 different types of photoreceptors. It has four single cone types expressing four different types of opsins namely, violet cones (419nm) expressing a short wavelength sensitive 1 type opsin (SWS1), a short wavelength sensitive cone expressing a SWS2 type opsin absorbing maximally at 455nm (blue light), a medium wavelength sensitive cone expressing a rhodopsin-like 2 (RH2) with peak absorption at 508nm corresponding to green light and long wavelength sensitive (LWS) opsin expressing cones with a peak absorption at 570nm corresponding to red light. A double cone and a rod photoreceptor that are touted to encode only luminance information are also expressed across the chick retina. Color discrimination behavioral experiments done on chicks have implicated at least 3 different color opponent circuits at play. Electrophysiological evidence for their existence has not been shown so far to our knowledge. The B-LEDs and G-LEDs peaked at 430 and 520 nm, with half bandwidths from 415 to 445 nm and 505 to 535 nm, respectively.

The ability to discriminate between short (400-450 nm) and longer wavelengths (500-550 nm) is observed in all organisms that live in terrestrial habitats from honeybees to primates,(Mills et al., 2014) the cone rich avian retina is no exception to that. (Osario and Vorobyev 2005) In spite of the anatomical studies that describe the densely stratified plexiform layers (Seifert et al.2020) and a wealth of behavioral color discrimination studies in birds that implicate color opponency in the avian retina, electrophysiological studies that attest to this fact are lacking. In figure 5(a, b) we describe a green ON/ blue OFF RGC and a blue ON/green OFF RGC (figure 5(c, d)) that describe color opponency to short vs long wavelengths of light. This can be seen from the time course of the STA showing complementary contrast sensitivity to the different wavelengths of the gaussian full field led flash stimulus. The color opponent responses can be attributed to the feedback inhibition from the horizontal cell to the cone. Yellow-blue(YBH) and red-green(RGH) c type horizontal cells have been implicated in the origin of color opponent responses in the turtle retina.(Twig et al. 2003).

The reptilian turtle retina expresses 4 different types of horizontal cells just like in the avian retina. (Fischer et al., 2007) This might implicate the outer plexiform layer in color opponency in the avian retina just as in the primate, reptilian, and teleost fish retinae. (Crook et al., 2011) Yet another factor that leads us to believe that this S/M color opponency is horizontal cell mediated is because the color opponent cells show a contrast level dependent switch in their polarity without compromising on their opponency. This hypothesis is further solidified from the outer plexiform layer connectivity study where the double cone which is believed to perform luminance-based coding connects to different substrata of the outer plexiform layer differently with the principal member (L cone bearing) and the accessory member (S cone bearing) describing distinct invaginating and basal contacts with other cones in the blue/violet and red/green sublayers. (Gunther et al., 2021) Color information encoding in the chick retina through correlations between adjacent cell types allows for color information transfer beyond the conventional color opponency seen as opposing contrast sensitivity to light flashes of different colors. (Zhou et al., 2005)

**Discussion**

As described in the results, RGCs predominantly exhibit significant sensitivities to both positive and negative contrasts. In the classification scheme, the polarity of the RGC cell was inferred from the most recent polarity prior to a spiking event from the STA estimate. This procedure was adopted to counteract the anti-preferred contrast firing effect that influenced the R ON/ R OFF based bias index. Three types of correlations were observed due to the concerted firing between RGCs in the neonatal chick, namely a narrow correlation between neighboring sustained firing RGCs, a medium correlation between neighboring transient firing RGCs and a broad correlation between RGCs as much as 1 mm apart. Single units were sorted clustered based on threshold crossings and clean autocorrelation functions. The polarity and kinetics of the RGC type was defined by the nature of contrast changes that elicited a firing event i.e its spike triggered average. It has been observed that bipolar cell synapses show sensitizing or adapting features depending on stimulus features such as frequency, luminance, contrast. (Niloleav et al., 2013) The present studies show that the sensitizing and or adaptive features of ganglion cells also depends on the color and irradiance of the stimulus. The results also indicates that blue light stimulus resulted in more sensitizing responses than green light responses which described more

adaptation features in the firing rate. The RGCs presumably respond to inputs from two types of bipolar cell types as they showed both the adaptation and sensitization features. The tonic and phasic bipolar cell inputs feed sustained and transient RGC types generally. (Awatarmani and Slaughter, 2000) This resulted in the STAs being offset to negative contrasts.

Most responses had low transiency, with firing events lasting between 100 ms and a few seconds. Majority of responses were slow sustained responses with preferential sensitivity to negative contrasts. The most numerous cell type recorded from was a slow OFF type RGC. The measured transiency index also described a continuum of values. The reason for most recorded RGC responses being slow and sustained is due probably to the neonatal and hence developing nature of the retina. Henning et al 2015 reported a similar trend in the neonatal mouse retina where the RGC responses became more transient the STAs more precise and smoother. Some RGCs in the neonatal chick retina showed ambipolar STAs arising likely due to fundamental nonlinearity/ multimodality in its response. This would require further spike triggered covariance (STC) to extract the temporal receptive field profile.

Most of the RGCs recorded from the peripheral Chick retina showed very low contrast sensitivity, with reliable firing elicited only for very high contrast changes (~100%). This can be attributed to the high density of retinal ganglion cells in the chick retina and thrives in a wide range of luminance conditions. (Baden et al., 2020) The high visual acuity of the chick retina in the dorsal area and the area centralis could mean a very high contrast threshold is feasible to detect wavelength dependent contrast changes a spatially dense receptive field mosaic. This could mean the chick compensates for low contrast sensitivity with high visual acuity (high spatial contrast sensitivity). Thus, chick RGCs behave as complex temporal coders that respond to wavelength dependent contrast values at specific temporal windows before an elicited firing event. Color opponency was evident in the polarity of the contrast values that elicited activity in different temporal windows before a firing event for gaussian stimuli of different colors.

In a behavioral study monitoring the object fixation in chicks compared to mammals, it was shown that chicks investigate objects by looking at them with different specialized parts of the eye unlike primates that fixate objects onto the fovea. (Dawkins, 2001) The presence of centrifugal projections in the area centralis of the chick retina could enable the utilization of specialized regions in the retina to observe different aspects of the visual scene and convey

complementary sets of information about it. This is also in sync with different regions having different wavelength dependent contrast sensitivities

We recorded from two different types of blue /green opponent cells that likely mimic the S /M color opponent cells seen in the mammalian retina, (Mills et al., 2014;Sher and DeVries, 2012) corresponding to a S-OFF/M-ON RGC (Figure 5a,b) and an S-ON/M-OFF RGC respectively (Figure 5c,d).This color opponency is likely mediated by a yellow-blue horizontal (YBH ) cell as it is has been previously reported in the turtle retina.(Twig et al., 2003) These color opponent cells that show opposing contrast sensitivities observed in the time course of the STA as far as 1 second before the onset of spiking. Apart from these conventional color opponent cells, color information is also likely encoded in other features of many non-color opponent cells such as the latency and the correlations among neighboring firing ganglion cells. (Chen et al., 2005) Most RGC types also showed color dependent luminance and contrast adaptation and sensitization features. (figure 2 a,b)More cells showed sensitization features to blue light. This could be due to the blue photoreceptors terminating in the innermost layer of the outer plexiform layer. (Gunther et al., 2021) Yet another reason could be that the blue photoreceptors describe more connections with inhibitory amacrine cells which have been implicated in sensitization through adaptation of inhibition. (Nikolaev et al., 2013;Kastner and Baccus, 2011) A significant fraction of RGCs that showed sensitization features also showed an apparent contrast level dependent shift in the polarity of the STA. This is likely attributable to the differential connectivity of the double cones with different single cone types mediated by a luminosity dependent horizontal cell type. The c-type horizontal cell in the turtle retina also described response characteristics that were both color and intensity dependent. (G. Twig et al. 2002)

We record from a large subset of non-color opponent RGCs in the dorsal area of the chick retina. Non-color opponent cells show changes in latency, ISIs and transiency with varying color and luminance values. The polarity of the contrast sensitivity of these cells to different colors is the same. We estimate the temporal receptive field profile of different RGC types to green, blue, and white full field flash stimuli and present their STAs, which provide the best estimate of the linear response of the RGCs. We also observe that most RGCs exhibit stimulus dependent responses with fast ON cells showing no pulse-width dependence of the response and only a latency change with a change in stimulus color. The fast OFF cells showed significant qualitative change

in firing in the anti-preferred contrast (as a delayed-ON response) to changing pulse widths. The slow OFF cells which were the most numerous showed firing that was sensitive to the pulse duration and luminance and mainly fired an OFF response after a positive contrast step and was relatively silent to negative contrast steps from ambient illumination. We also record from color opponent RGCs in the dorsal area of the chick retina that exhibit blue/green opponency, describing contrasting temporal response profiles as observed from the time course of their STAs. These observation are indicative of the diversity of cell types and complex inter-connectivity in the outer plexiform layer of the avian retina. This architecture enables and contributes to the processing of the visual scene into behaviorally relevant features.

## Methods

### Ethics statement

All studies were performed in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and the Institutional Bioethics and Bio-safety Committee, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR). All protocols and experiments were approved by the IAEC and were performed in accordance to the guidelines followed by the Poultry Science Unit, KVAFSU (Karnataka Veterinary, Animal and Fisheries Sciences University, Hebbal, Bangalore)

### Animal Preparation and Retina isolation:

Fertilized Giriraja Gallus gallus domesticus eggs were procured from the Poultry Science Unit, KVAFSU and incubated in a Brinsea Octagon 28 incubator till they reached the appropriate stage of development (P0-P5).Neonatal chicks were obtained on the day of the experiment in a well ventilated well-lit portable brooder The neonatal chicken was sacrificed through cervical dislocation. The eyes were enucleated, the eye cup was hemisected and then the retina was isolated in ice cold aCSF under dim light conditions. The dissected retinae were preserved in freshly

prepared aCSF solution with 124 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM HEPES, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$ and 10 mM glucose at pH 7.4 that was constantly bubbled with carbogen gas (95% $CO_2$ and 5% $O_2$). A suitable healthy retina was then removed to a separate bubbled petri dish under a stereo microscope where it was cut to a desired size and placed on an annularly cut custom nylon mesh with a pore size of 100 µm with the photoreceptor side facing the nylon mesh

**Spike Sorting and Analysis**

Spyking-Circus [42] was used for spike sorting the retinal recordings. In the first step, spikes from the band-pass filtered data (300 – 3000 Hz) are detected as threshold crossing (7 S.D) and snippets of data around each crossing of appropriate duration (Nt = 5 ms) are cutout across all electrodes (Ne = 59). A maximum of Ns = 10000 snippets are gathered from each electrode for a total of Ns ∗Ne waveforms. The waveforms are in a high dimensional space due to the high sampling rate. Principal Component Analysis (PCA) was done, to reduce the dimension of the problem. This number was taken to five by setting the parameter NPCA = 5. The clusters thus obtained from spyking circus were then subjected to post processing which involved cleaning of the templates to ensure that the percentage refractory period violations per cluster was under 1%. Templates that showed a higher than 1% refractory period violations and with spike counts under 500 were discarded from the analysis.

**Firing rate** was calculated by aligning spikes to the onset of the light stimulus for 10 trials. The histogram is the average spikes in each 50 ms bin.

**Cross-Correlogram** between two putative ganglion cells was calculated by aligning the spikes of the second neuron to every spike of the first neuron and the average number of spikes of the second neuron was counted in 10ms bins. To remove stimulus correlations from the cross correlograms a **shifted cross-correlogram** was calculated by shuffling the trials and computing the cross-correlogram. This was subtracted from the initial cross-correlogram to obtain the **shift corrected cross-correlogram.**

**Spike Triggered Average (STA)** was calculated by taking the stimulus values till one(two) second(s) before each spike and averaging them during the Gaussian White Noise stimulus for

all trials. The smoothened STA trace was obtained by convolving the STA with a Gaussian kernel.

The Gaussian White Noise (GWN) stimulus was shown by changing the intensity value of the LED with a refresh rate of 10ms (25/50) picked from a Gaussian distribution. The contrast of the GWN stimulus was defined as $dI/I_0$ where dI is the variance of the Gaussian distribution and $I_0$ the mean intensity

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**References**

1. Awatramani GB, Slaughter MM. Origin of Transient and Sustained Responses in Ganglion Cells of the Retina. The Journal of Neuroscience. 2000;20(18):7087-95.
2. Awatramani GB, Slaughter MM. Intensity-Dependent, Rapid Activation of Presynaptic Metabotropic Glutamate Receptors at a Central Synapse. The Journal of Neuroscience. 2001;21(2):741-9.
3. Baccus SA, Meister M. Fast and slow contrast adaptation in retinal circuitry. Neuron. 2002;36(5):909-19.
4. Baden T, Euler T, Berens P. Understanding the retinal basis of vision across species. Nature Reviews Neuroscience. 2020;21(1):5-20.
5. Berry MJ, Warland DK, Meister M. The structure and precision of retinal spike trains. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(10):5411-6.
6. Chander D, Chichilnisky EJ. Adaptation to temporal contrast in primate and salamander retina. J Neurosci. 2001;21(24):9904-16.
7. Chen AH, Zhou Y, Gong HQ, Liang PJ. Firing rates and dynamic correlated activities of ganglion cells both contribute to retinal information processing. Brain research. 2004;1017(1-2):13-20.
8. Clarke PG, Gyger M, Catsicas S. A centrifugally controlled circuit in the avian retina and its possible role in visual attention switching. Vis Neurosci. 1996;13(6):1043-8.

9. Crook JD, Manookin MB, Packer OS, Dacey DM. Horizontal cell feedback without cone type-selective inhibition mediates "red-green" color opponency in midget ganglion cells of the primate retina. J Neurosci. 2011;31(5):1762-72.
10. Deepak CS, Krishnan A, Narayan KS. Light controlled signaling initiated by subretinal semiconducting-polymer layer in developing-blind-retina mimics the response of the neonatal retina. Journal of neural engineering. 2022;19(3).
11. Fischer AJ, Stanke JJ, Aloisio G, Hoy H, Stell WK. Heterogeneity of horizontal cells in the chicken retina. The Journal of comparative neurology. 2007;500(6):1154-71.
12. Günther A, Dedek K, Haverkamp S, Irsen S, Briggman KL, Mouritsen H. Double Cones and the Diverse Connectivity of Photoreceptors and Bipolar Cells in an Avian Retina. J Neurosci. 2021;41(23):5015-28.
13. Jarsky T, Cembrowski M, Logan SM, Kath WL, Riecke H, Demb JB, et al. A synaptic mechanism for retinal adaptation to luminance and contrast. J Neurosci. 2011;31(30):11003-15.
14. Kastner DB, Baccus SA. Coordinated dynamic encoding in the retina using opposing forms of plasticity. Nature Neuroscience. 2011;14(10):1317-22.
15. Kastner DB, Baccus SA. Spatial segregation of adaptation and predictive sensitization in retinal ganglion cells. Neuron. 2013;79(3):541-54.
16. Kastner DB, Ozuysal Y, Panagiotakos G, Baccus SA. Adaptation of Inhibition Mediates Retinal Sensitization. Current biology : CB. 2019;29(16):2640-51.e4.
17. Lall GS, Revell VL, Momiji H, Al Enezi J, Altimus CM, Güler AD, et al. Distinct contributions of rod, cone, and melanopsin photoreceptors to encoding irradiance. Neuron. 2010;66(3):417-28.
18. Mills SL, Tian LM, Hoshi H, Whitaker CM, Massey SC. Three distinct blue-green color pathways in a mammalian retina. J Neurosci. 2014;34(5):1760-8.
19. Morris VB. An afoveate area centralis in the chick retina. The Journal of comparative neurology. 1982;210(2):198-203.
20. Nikolaev A, Leung K-M, Odermatt B, Lagnado L. Synaptic mechanisms of adaptation and sensitization in the retina. Nature Neuroscience. 2013;16(7):934-41.
21. Nirenberg S, Meister M. The light response of retinal ganglion cells is truncated by a displaced amacrine circuit. Neuron. 1997;18(4):637-50.
22. Osorio D, Vorobyev M. Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. Proc Biol Sci. 2005;272(1574):1745-52.
23. Patterson SS, Kuchenbecker JA, Anderson JR, Neitz M, Neitz J. A Color Vision Circuit for Non-Image-Forming Vision in the Primate Retina. Current Biology. 2020;30(7):1269-74.e2.
24. Seifert M, Baden T, Osorio D. The retinal basis of vision in chicken. Seminars in Cell & Developmental Biology. 2020;106:106-15.
25. Shapley R. Retinal physiology: adapting to the changing scene. Current biology : CB. 1997;7(7):R421-3.
26. Shapley R, Enroth-Cugell C. Chapter 9 Visual adaptation and retinal gain controls. Progress in Retinal Research. 1984;3:263-346.
27. Sher A, DeVries SH. A non-canonical pathway for mammalian blue-green color vision. Nature Neuroscience. 2012;15(7):952-3.
28. Smirnakis SM, Berry MJ, Warland DK, Bialek W, Meister M. Adaptation of retinal processing to image contrast and spatial scale. Nature. 1997;386(6620):69-73.

29. Tikidji-Hamburyan A, Reinhard K, Seitter H, Hovhannisyan A, Procyk CA, Allen AE, et al. Retinal output changes qualitatively with every change in ambient illuminance. Nat Neurosci. 2015;18(1):66-74.
30. Tikidji-Hamburyan A, Reinhard K, Storchi R, Dietter J, Seitter H, Davis KE, et al. Rods progressively escape saturation to drive visual responses in daylight conditions. Nature communications. 2017;8(1):1813.
31. Twig G, Levy H, Perlman I. Turtle C-type horizontal cells act as push-pull devices. Vis Neurosci. 2001;18(6):893-900.
32. Twig G, Levy H, Perlman I. Spatial-chromatic interactions in C-type horizontal cells of the turtle (Mauremys caspica) retina. Vis Neurosci. 2002;19(1):71-84.
33. Twig G, Levy H, Weiner E, Perlman I. Light adaptation and color opponency of horizontal cells in the turtle retina. Vis Neurosci. 2003;20(4):437-52.
34. Wilson M, Lindstrom SH. What the bird's brain tells the bird's eye: the function of descending input to the avian retina. Vis Neurosci. 2011;28(4):337-50.
35. Wisely CE, Sayed JA, Tamez H, Zelinka C, Abdel-Rahman MH, Fischer AJ, et al. The chick eye in vision research: An excellent model for the study of ocular disease. Progress in retinal and eye research. 2017;61:72-97.
36. Wong WT, Sanes JR, Wong RO. Developmentally regulated spontaneous activity in the embryonic chick retina. J Neurosci. 1998;18(21):8839-52.
37. Yedutenko M, Howlett MHC, Kamermans M. High Contrast Allows the Retina to Compute More Than Just Contrast. Frontiers in Cellular Neuroscience. 2021;14.
38. Zele AJ, Cao D. Vision under mesopic and scotopic illumination. Front Psychol. 2015;5:1594-.
39. Zhou Y, Chen AH, Gong HQ, Liang PJ. Color information encoded by the spatiotemporal patterns of light response in ganglion cells of chick retina. Brain research. 2005;1059(1):1-6.

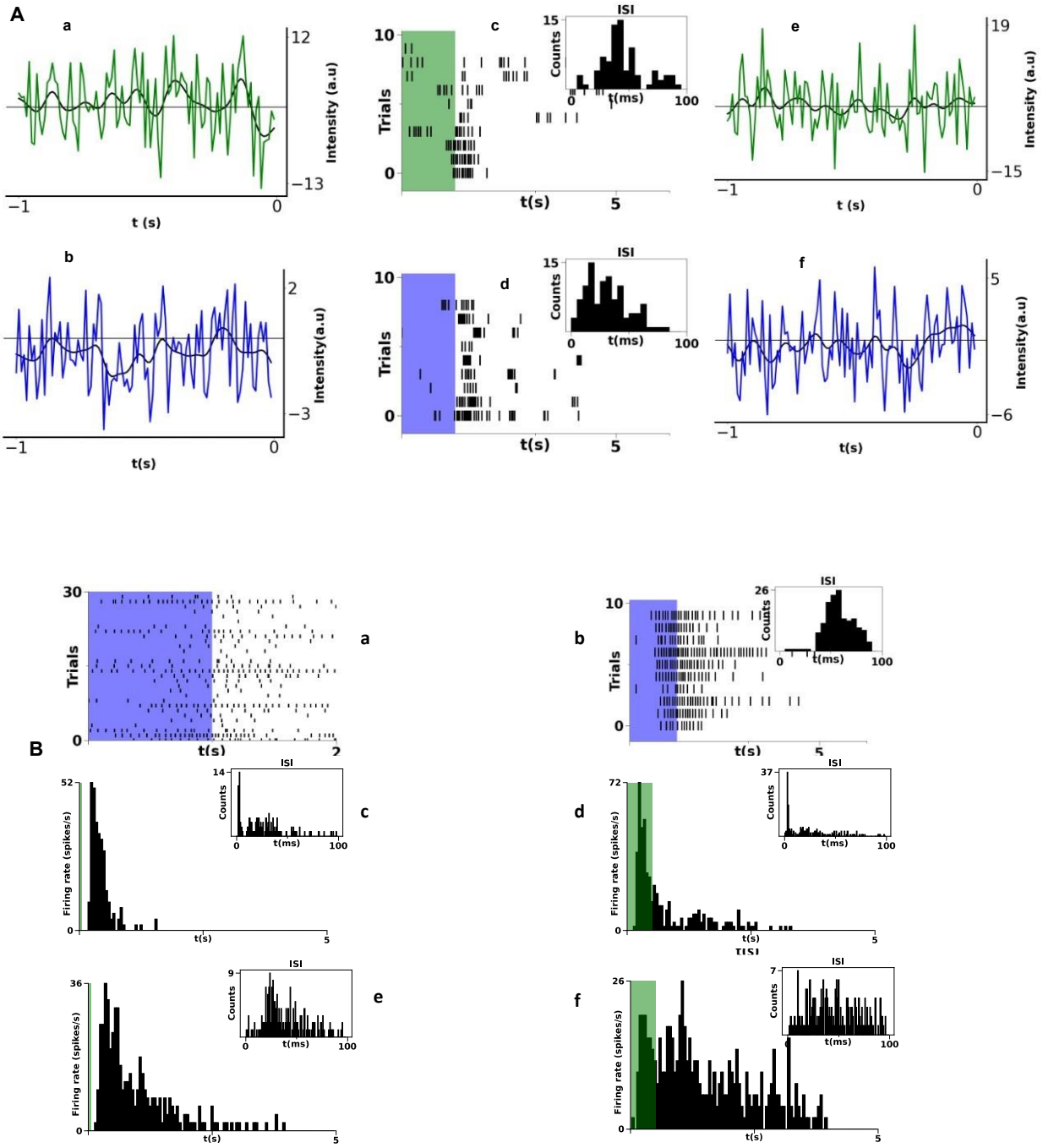


Figure 1A: (a) and (b) depicts the STA trace of a characteristic non color opponent fast OFF RGC over 1000 ms before a firing event to a full field gaussian flicker at 50% contrast and a refresh rate of 10 ms every frame averaged over 10 trials with the raw trace in green and blue and the smoothened trace in black.(c) and (d) shows the full-field flash response of the same Off cell to 100% full field contrast steps to 430 nm blue LED flash and green 530 nm flash respectively, inset represents the Interspike interval

ISI histogram 1 ms bin-size (e) and (f) The STA trace of a non- color opponent fast ON cell to green and blue full field random gaussian flicker at 50% contrast.

Figure 1B (a) The response of a putative ganglion cell to 1s ON-1s OFF blue full field flash and (b)1s ON-5s OFF blue LED flash. The 5 s duty cycle results in more reliable firing event, with each response being equivalent to the first response. The response of a transient ON RGC to (c) 50 ms-ON 5s-OFF green LED flash and (d) the response of the same ganglion cell to 500ms periodic green LED flash with a duty cycle of 5 seconds. The response of the ON transient is invariant of the pulse width of the full field flash. The response of an OFF ganglion cell to a 50 ms flash with a duty cycle of 5 s(e) and the response of the same ganglion cell to a 500 ms light pulse delivered periodically with a duty cycle of 5 s. The OFF RGC shows qualitative changes in firing in the anti-preferred contrast with varying pulse widths. The inset in (b-f) is the ISI histograms.

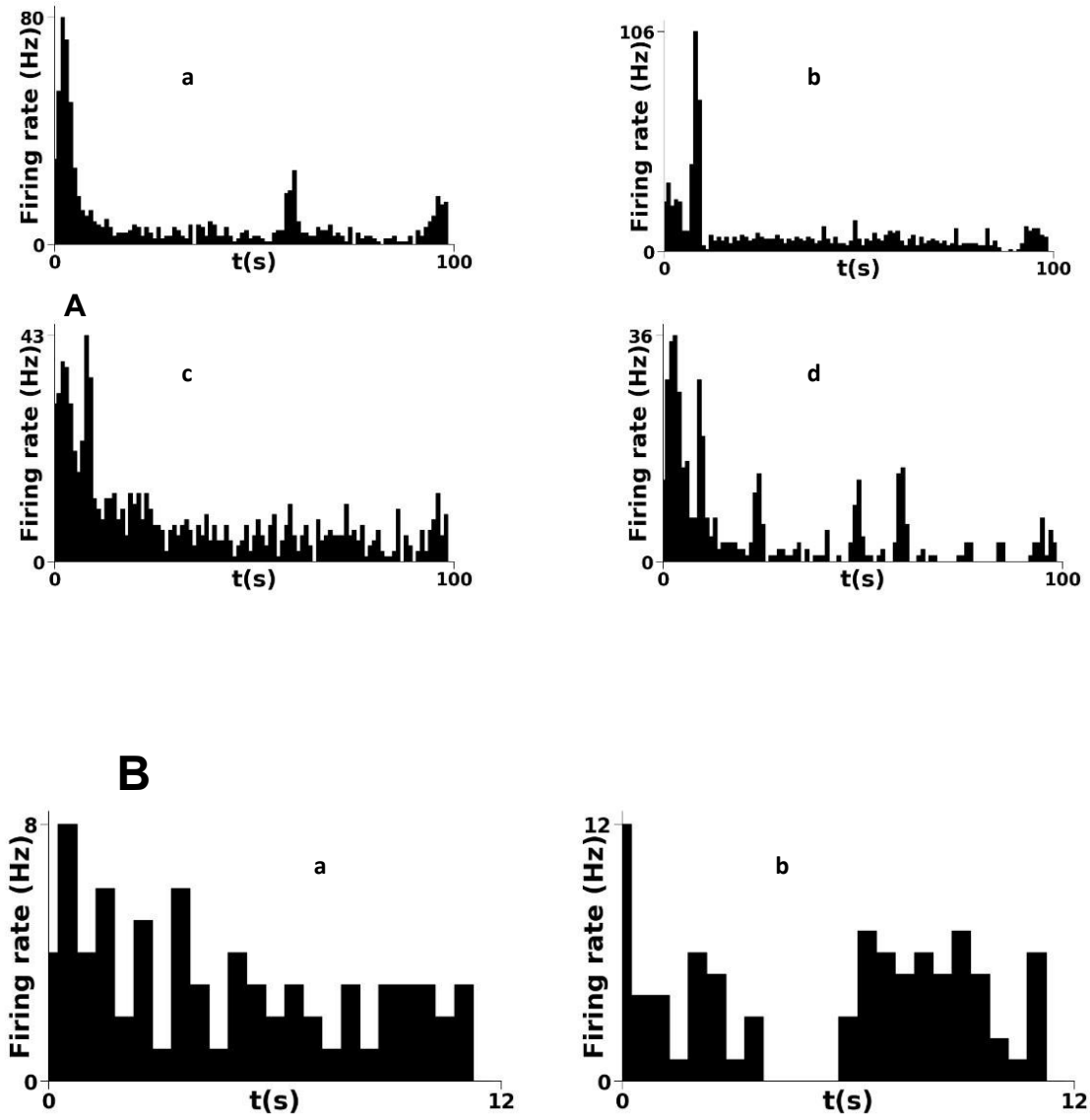


Figure 2A: Luminance adaptation effects in different cells (clockwise from top left). (a)A characteristic fast OFF cell showing only slow adaptation effects. (b)An OFF cell showing only sensitization c) OFF cell showing minor adaptation and more significant sensitization effects. (d) an OFF cell showing minor sensitization and greater adaptation effects. Figure 2B: The response of a representative fast OFF RGC type from low (50%) to high contrast (100% contrast) Gaussian flicker stimuli for blue (a-left) and green (b-right) full field random gaussian flicker stimuli. The higher contrast stimulus results in sharper firing events without a change in the baseline firing of the RGC.

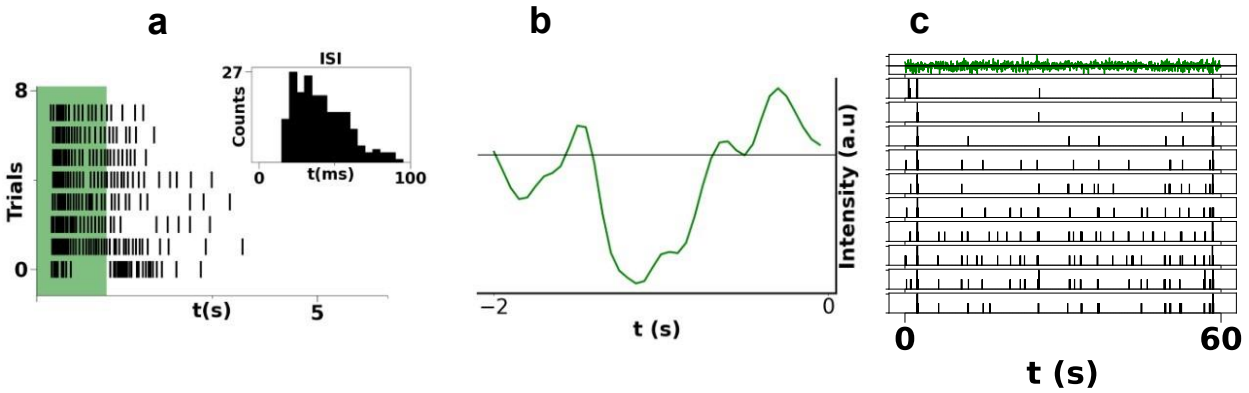


Figure 3. Here the temporal response characteristics of an ON RGC is described.3(a) A trial by trial raster plot of said cluster to 10 trials of a 1 s ON- 5 s OFF full field flash stimulus is shown.(b) The STA estimated by averaging the contrast changes preceding a firing event for the ON cell. The RGC is labelled as an ON RGC because the most recent contrast change prior to firing is a positive contrast change (c) The PSTH of the green ON ganglion cell to 10 trials of the same repeated gaussian stimulus is shown, describing its consistent firing to distinct contrast changes.

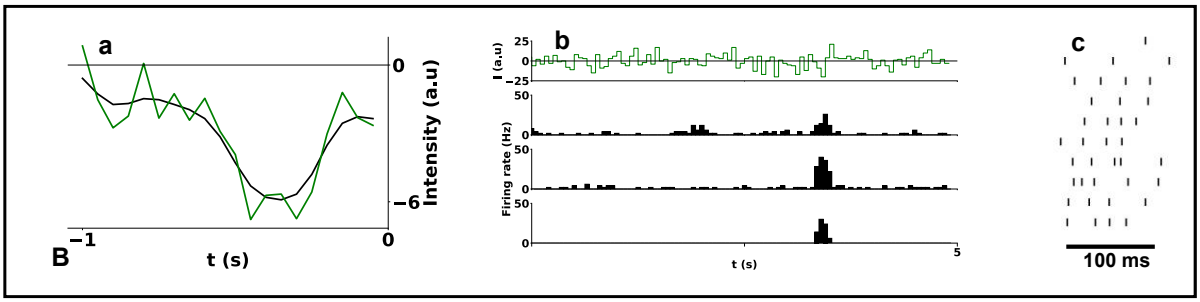


Figure 4: The STA for a representative OFF RGC described in (a). The PSTH of 3 OFF RGCs to 10 trials of a 30 s gaussian full field flicker (50% contrast) stimuli is shown in (b). (c) Represents the enlarged view of the firing events of OFF RGC is shown in a 100 ms scale. The neuron shows sparse and precise firing with a temporal jitter of ~ 10 ms.

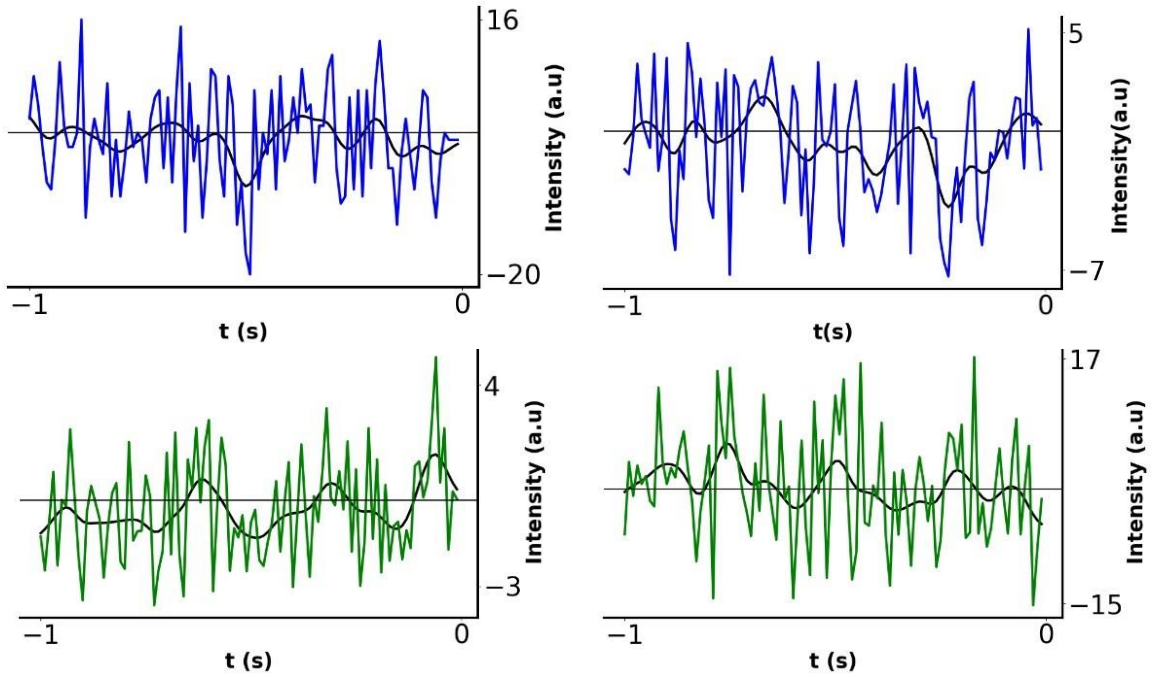


Figure 5 (a,b) The STA trace of blue OFF/ green ON RGC indicating color opponency. Green ON- blue OFF RGC STA averaged over 10 trials of a random green gaussian full field flicker. (c and d) represents the time course of the STA of a blue ON green OFF RGC