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Running title: Signal summation encodes response transience

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ABSTRACT

Retinal ganglion cells (RGCs) summate inputs across their receptive fields and forward a corresponding spike train code to the brain. Considering the many visual aspects carried by this code the comprehension of RGC firing kinetics and the underlying mechanisms is markedly important. RGCs can generate a maintained spiking (sustained) or a quickly decaying brief burst of spikes (transient) upon ON- and/or OFF-set of prolonged light stimuli. Our results here challenge the classical view that claims an outer retinal origin for RGC response transience and explains the observed response dichotomy with the dissimilar glutamate receptor kinetics in postsynaptic bipolar cell dendritic surfaces. We find that activation of the same glutamate receptor subtype can result in transient, sustained, and intermediate RGC responses. Moreover, even signaling via a single bipolar cell subtype can result in RGC responses with a variety of transience values. Contrary, a change in the dominance of inputs delivered by converging retinal pathways can alter RGC response transience considerably. Such response component fine-tuning occurs via inner retinal GABAergic inhibitory and gap junction mediated excitatory interactions. The above data thus indicate that RGC light response temporal characteristics are determined by inner retinal microcircuits and fine-tuned in a context dependent manner.

INTRODUCTION

Information gathered from the visual field travels through parallel intraretinal pathways and converges onto retinal ganglion cells (RGCs) that in turn summate and encrypt incoming signals into action potential trains prior to transmitting towards the brain. Light-evoked RGC responses have been characterized by their polarity (ON, OFF, and ON-OFF), sensitivity to various stimuli and kinetics. Based on response speed RGCs can be sorted into either brisk or sluggish categories, whereas the pattern can be a maintained spiking (sustained) or a brisk spike burst (transient). Both aspects of RGC response kinetics are likely important in terms of signal efficiency on postsynaptic neuronal targets in higher visual centers^{1, 2, 3}. The transient/sustained dichotomy has been documented in a variety of vertebrate species, including cold-blooded animals, primates and non-primate mammals as well^{4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14}. Since all photoreceptors generate sustained responses upon illumination¹⁵ a sustained-to-transient response transformation must occur along the retinal signal flow. Previous work in the salamander and rabbit retinas suggested that response transience is determined by the kinetics of the postsynaptic glutamate receptors (mGluR₆, AMPA, Kainate) at the site of the very first retinal contact, the photoreceptor-to-bipolar cell synapse^{16, 17}. In contrast, a recent study in the mouse retina presented a thorough analysis of the various possible sites of retinal circuits that may participate in determining response transience¹⁸. Although the conclusion of this latter study was that different RGC types use diverse mechanisms to produce sustained or transient light responses, the presented data clearly showed that outer retinal postsynaptic receptor (AMPA/kainate receptor) desensitization had only a minor effect on response transience. The discrepancy in these previous studies can be attributed to species differences (salamander vs. mammals), the difference in examined cell types (bipolar cells vs. RGCs), and the selection of examined RGC subtypes (random vs. targeted). In addition, in most of these relevant studies patch-clamp EPSC recording was the method of choice, which does not allow for the direct observation of RGC spiking, the real output signal of the retina.

To this end we utilized extracellular RGC spike recordings to examine the kinetics of the real retinal output conveyed towards visual brain centers. We show a line of evidence

supporting the view that RGC response transience is largely independent of outer retinal signal kinetics, including (i) the existence of both transient and sustained ON RGC responses contrary to a single underlying mGluR₆ receptor in cone bipolar cell dendrites; (ii) despite the difference in ON and OFF signaling glutamate receptors in bipolar cell dendrites the frequency distribution for ON and OFF RGC photopic response transience are rather similar; (iii) the existence of both transient and sustained ON and OFF cell responses under scotopic light levels, in which condition the primary rod pathway provides the sole signal conveying path. On the other hand, RGC response transience was altered considerably when stimulus intensity was altered and thereby an input dominance switch from scotopic through mesopic to photopic conditions occurred. In addition, a similar input dominance switch was also achieved pharmacologically by blocking GABAergic inhibitory and gap junction mediated excitatory inputs to RGCs. Therefore, the above data indicate that the RGC response kinetics are determined by inner retinal interactions. While direct excitatory bipolar cell inputs are summated by RGCs, inner retinal microcircuits serve as temporal filters to augment or moderate certain response components to achieve better visual performance.

RESULTS

mGluR₆ Receptor Signaling Serves both Transient and Sustained ON RGC Responses

Based on our casual RGC recordings we often observed apparent discrepancies between response decay obtained for spike trains and EPSCs of the same cell (Supplemental Figure 1). Since the retinal output to the brain is delivered in the form of spike trains it is crucial to utilize RGC spike recordings in order to evaluate if (and how) temporal features of light responses affect vision. To this end we generated peristimulus time histograms (PSTHs) and determined corresponding $PSTH_{\tau}$ values upon extracellular spike recordings according to Ganczer et al.²⁰. Once generated, $PSTH_{\tau}$ values were used to assay RGC response length (decay). Briefly, upon spike PSTHs the amplitude (A - peak frequency) and delay (D - time to peak) were determined, $PSTH_{\tau}$ values were calculated as the time required for A to drop to $1/e \cdot A$ (Supplemental Figure 1a). When both $EPSC_{\tau}$ and $PSTH_{\tau}$ values were determined for sample RGCs, they often showed significant discrepancies (Supplemental Figure 1b, c), thus further supporting our view that spike recordings are appropriate approaches to examine the topic of RGC response transience.

One goal of this work was to examine if, as it has been claimed previously^{16, 17}, transience values of RGC responses are determined in the outer retina or whether inner retinal mechanisms play a role as well. To test this, we first recorded ON-center RGC responses. In darkness, photoreceptors continuously release glutamate and upon light exposure this release decreases (or stops). Glutamate, once released into the synaptic cleft, binds to mGluR₆ receptors²¹ in the postsynaptic membrane of ON bipolar cells, initiating an intracellular cascade that involves the $G_{(o)}$ protein-mediated inactivation of adenylate cyclase and the closure of TRPM₁ bound nonspecific cation channels²². Taken together, light onset pauses glutamate release from photoreceptors, ultimately depolarizing ON center bipolar cells. Although 5-6 ON bipolar cell types and corresponding ON signaling streams are known to exist in the mammalian retina^{23, 24}, the initial signal at the photoreceptor/bipolar cell synapse is generated

by a single postsynaptic glutamate receptor type, the mGluR₆ for all ON bipolar cells. If bipolar cell glutamate receptors play a major role in shaping response transience, then the kinetics of ON-center light responses should be very similar for all ON bipolar cells and their ultimate targets, the ON RGCs. To examine this, ON RGC (n=117) spike responses were evoked by photopic (100 R*/rod/s), full-field light stimuli, and then PSTH τ values were determined for each cell in the examined population (mean: 0.125 s, SD: +/-0.121). The resultant rather high SD value reflects that the photopic ON RGC PSTH τ values varied considerably (range: 0.034-0.514 s). In addition, besides clear transient and sustained responses, a considerable fraction of cells displayed intermediary response kinetics (Figure 1), supporting our previous observations²⁰.

Figure 1. A Great Variety of Response Transience Across the RGC Population. a. Representative perievent raster diagrams show that individual RGCs provide light-evoked spiking responses upon full-field illumination that are rather similar across trials (four consecutive trials for each recorded cell). However, RGCs display a great variety in terms of their response length (or decay – expressed as the PSTH τ value in this work) for both the ON (cells 1 and 2) and OFF (cells 3 and 4) subpopulations. The white bar below the recordings represents the timing of the on- and offset of the stimulus in this and in all other figure panels of this paper. **b.** Frequency histogram shows the distribution of PSTH τ values for the ON (white) and OFF (black) RGC subpopulations. Clearly, the distribution of PSTH τ values appears unimodal and does not allow for the clear separation of transient and sustained RGC responses. This unimodality as well as the rather wide range are features shared by both the ON and OFF RGC subpopulations.

These results, therefore, indicate that transient, sustained and intermediary RGC responses were equally generated by various ON signaling retinal pathways all utilizing the same mGluR₆ receptor to transmit signals to ON bipolar cells.

Transience Distribution of OFF RGC Responses is Similar to ON RGC counterparts

In contrast to ON cells, photopic OFF RGC responses are generated by glutamate binding to either AMPA or kainite ionotropic glutamate receptors¹⁷. The existence of two postsynaptic receptors, in theory, suggests a greater variability in response kinetics for OFF RGCs than those mediated by the sole mGluR₆ receptor for ON cells. In fact, distinct kinetics of OFF bipolar cell responses have been shown to correspond to the two glutamate receptors expressed in rabbit OFF bipolar cell dendrites^{17, 25}. To investigate if transience of photopic OFF RGC responses show greater variability than the ON RGC counterparts a cohort of OFF responses (n=63) were recorded under photopic conditions (100 Rh/rod/sec). Akin to photopic ON cell responses, PSTH_τ values of OFF cells varied across a wide range and showed a continuum of transient, sustained, and intermediary responses (Figure 1). Moreover, the frequency histogram generated for OFF RGC responses appeared very similar to the one obtained for ON RGC counterparts (mean: 0.139 s, SD: 0.09; range: 0.034-0.554 s). Clearly, the wide range and the lack of a clear transient/sustained separation of responses were features shared by both ON and OFF cells regardless the number and type of the bipolar cell postsynaptic glutamate receptors.

The Primary Rod Pathway Carries Signals to Both Transient and Sustained RGCs

Based on the above results postsynaptic bipolar cell glutamate receptors are not the key factors in determining RGC response transience, however, dissimilar membrane properties of various bipolar cells may still play a role. In that scenario, regardless of the subtype all RGCs should maintain comparable response transience when a single bipolar cell type dominates their inputs. In contrast to photopic signals delivered by 5-6 bipolar cells to ON cell RGCs low scotopic signals reach RGCs mostly via the primary rod pathway that operates with the single rod bipolar cell type. Consequently, if bipolar cell membrane characteristics are key in determining response transience then all low scotopic RGC responses should appear similar. To investigate this, RGCs (n=19) were presented with low-scotopic stimuli (1-4.6 Rh*/rod/sec) and corresponding PSTH_τ values were determined. To test if our stimuli activated mostly the primary but not the secondary and/or tertiary rod signaling routes we blocked

mGluR₆ glutamate-mediated signaling to OFF RGCs by using the agonist L-2-amino-4-phosphonobutyric acid (APB 50 μ M). This pharmacological blockade eliminated OFF RGC responses (Supplemental Figure 2) thus proving that the dominant signaling route was the primary rod pathway under these conditions.

We found that APB sensitive low-scotopic RGC responses, though appeared somewhat more delayed and sustained (scotopic – mean: 0.155 s, SD 0.134; photopic – mean: 0.110 s, SD - 0.091; $p=0.038$), covered a PSTH τ value range similar to those of the photopic counterparts (Figure 2, 3) for both ON and OFF RGC responses.

Figure 2. Scotopic RGC Response Transience Values are Just as Diverse as Photopic Responses. **a.** Representative ON RGC light responses (raster to the left and PSTH to the right for the same RGC) were evoked first by scotopic (top) and then photopic (bottom) full-field light stimuli. This change in the stimulus strength induced a clear change in response amplitude but the overall shape (response delay and decay) of the response remained largely unchanged. **b.** Similar to the ON RGC is panel **a** this representative OFF RGC display light responses that, besides subtle changes in response amplitude, overall remain unchanged in scotopic (top) and photopic (bottom) stimulating conditions. **c.** Diagram displays PSTH τ value pairs for individual RGC light responses in scotopic (left) and photopic (right) light stimulations. Clearly, the change in stimulus strength induced the alteration of PSTH τ values for many examined RGCs, however, the range of response transience values for the entire RGC population were comparable in scotopic conditions (if not even wider) to those obtained with

photopic stimulation paradigms. **d.** Floating bar graphs show that the variety of scotopic RGC response $PSTH\tau$ values is just as great as for photopic responses for the same RGCs.

Figure 3. Changes in Response Transience can be the Result of Signal Interference through Parallel Retinal Pathways. **a.** Representative ON RGC light-evoked rasters (left column) and PSTH cohorts (right column) recorded as a response to stimuli of various strength (intensity values are reflected in the right top corner of each -panel). $PSTH\tau$ values of this RGC changed non-monotonously during this experiment when the stimulus intensity was gradually increased (see also panel c). PSTHs clearly show a peak of very sensitive signal component (red arrow) evoked by weak, scotopic stimuli. This sensitive response component appears relative delayed when it is compared to the less sensitive but brisk signal component (light blue arrow). These two signal components differ in their delays but appear similar in response decay, therefore $PSTH\tau$ values are shifted towards the sustained range when the two signals are summated (mesopic conditions - 2nd, 3rd and 4th panels), whereas remain transient when only one signal is present (scotopic condition – 1st panel) or dominates over the other component (photopic conditions – 5th, 6th and 7th panels; see also panel c). **b.** Representative OFF RGC light-evoked rasters (left column) and PSTHs (right column) recorded as a response to varying intensity stimuli (intensity values are reflected in the right top corner of each -panel). $PSTH\tau$ values of this RGC clearly changed during this experiment as the stimulus intensity

was gradually increased. Similar to the cell in panel *a* this OFF RGC showed a very sensitive but rather delayed response peak (red arrow) and a faster but less sensitive (light blue arrow) peak. The two signal components differed in their delays and sensitivities and a slight alteration in $PSTH\tau$ values occurred as a result of the summation of components (mostly in mesopic conditions – middle panels). While the distinction of response components can clearly be differentiated for the ON RGC in *a*, this OFF cell (and most examined RGCs) showed a less obvious and less separable summation of incoming signals. **c and d.** Diagrams show that, similar to cells shown in panels *a* and *b* (values of these cells appear in black and red in the diagrams), most recorded RGCs displayed stimulus strength driven changes of $PSTH\tau$ values (grey curves). **e.** Diagram shows minimum/maximum $PSTH\tau$ value pairs for the recorded cells during the course of the stimulus intensity recording paradigm. The examined RGCs showed ~18-73% $PSTH\tau$ changes during the course of this experiment.

This latter observation was reflected by the (even) greater SD values in scotopic conditions and by the detected broad $PSTH\tau$ range (min/max_{scotopic}: 0.044/0.485 s; min/max_{photopic}: 0.045/0.264 s).

RGC Response Transience is Altered Due to Signal Summation

We reported previously that most examined RGCs showed some intensity-dependent alteration of $PSTH\tau$ values²⁰ and this observation was supported in our recent recordings as well (Figure 3; n=19). A considerable fraction of these cells (n=6) displayed a quasi-monotonous decrease of $PSTH\tau$ values as the illumination intensity was gradually increased (Figure 3c). This likely reflects the expected switch from a state where signals were carried dominantly by scotopic pathways (under dim stimulating conditions) to photopic channels (when stimuli were brighter). In this scheme, scotopic signals are more sustained for these cells than the low sensitive photopic counterparts. However, monotonous change in response transience could not be detected for most of our cells and many times $PSTH\tau$ values displayed local maximums or minimums under mid-range illumination. One of our examined cells (Figure 3a) clearly displayed relative transient responses to both scotopic and photopic stimuli (small $PSTH\tau$ values) but the full-field light response of the same cell appeared more sustained (large $PSTH\tau$ values) in the mesopic/low photopic range (between 10 – 100 Rh*/rod/s) when both scotopic and photopic signaling streams were active. This is clearly the result of the summation of signals carried via separate signaling pathways. Under scotopic and photopic conditions

both the PSTH peak and τ values were influenced by only one signal component (the slow and sensitive component in scotopic conditions and the fast but less sensitive component in photopic conditions), whereas mid-range illumination brought about an intermingling of the fast signal component peak and a shoulder of the slow response component, thereby increasing the PSTH τ value. The summation of these signals clearly transformed the spiking pattern of this transient cell to appear more sustained. Unfortunately, such clear separation of two (or more) signal components was only observed rarely. For most examined cells signal summation was only evident based on the faster onset of the response in photopic conditions, and intermingling signals upon mid-range illumination conditions provided less obvious combinations of a fast peak and a slow shoulder component (see Figure 3b). However, the general observation of this test was that RGCs endured a considerable stimulus intensity-dependent alteration in their response transience. The difference between the minimum and maximum PSTH τ values across the examined intensity range varied between 18.3% and 73.2% (mean 41.5 +/- 16.5 SD) for sample RGCs. These results, therefore, indicate that signal summation in fact is a significant factor to determine response transience for most cells.

RGC Response Transience is Altered by the Perturbation of Lateral Signaling

In the previous section we showed that an illumination strength-dependent dominance shift of summated scotopic and photopic signals can underly RGC response transience changes. We posit here that a similar summation of parallel signals can determine response transience even under the same stimulating conditions. The morphological substrate for this hypothesis is provided by previous studies showing that many RGC subtypes receive excitatory inputs from 2 or more bipolar cell subtypes (see Discussion). Unfortunately, parallel photopic pathways utilize the same postsynaptic receptor in the photoreceptor-to-bipolar cell synapse (mGluR₆ receptor in ON cone bipolar cell dendrites, AMPA and kainate receptors in OFF cone bipolar cells) for all vertical retinal pathways thus direct testing of their signal summation is not feasible via pharmacology. However, we can take advantage of the fact that certain vertical pathways target RGCs directly via excitatory bipolar cells, whereas others

contact them indirectly through intermediary inhibitory (mostly GABAergic) amacrine cells. In addition, many RGCs also display gap junction coupling to their RGC and/or amacrine cell neighbors^{26, 27, 28, 29, 30, 31, 32, 33}, thereby diversifying the potentially summable incoming signals. Since both GABA and gap junction mediated signals can be diminished pharmacologically while glutamate-driven bipolar cell signaling is intact, we carried out experiments in which one of the indirect signaling streams was blocked pharmacologically thereby isolating signal components carried to RGC targets via parallel streams.

To interfere with amacrine cell inhibition the nonspecific GABA_a/GABA_c receptor antagonist picrotoxin (PTX; in a concentration of 50 μ M) was utilized and changes in response characteristics were followed for both ON (n=54) and OFF (n=40) RGCs. Aside from the various physiological effects on RGC response kinetics, PTX caused a clear decrease of PSTH τ values for most RGCs (ON mean control: 0.285s $\pm$ 0.166 SD; ON mean PTX: 0.083 s $\pm$ 0.084 SD; OFF mean control: 0.159 s $\pm$ 106 SD; OFF mean PTX: 0.08 $\pm$ 0.41 SD) thus in general RGCs became more transient after the GABA inhibition blockade (Figure 4, 5).

Figure 4. GABA Receptor Blockade Induces Abrupt Changes in RGC Response Transience. **a** and **b**. Representative ON (a) and OFF (b) RGC light-driven responses (rasters on the top and corresponding PSTHs below) are clearly altered when the nonspecific GABA receptor blocker PTX was applied (bottom panels). The observed changes include both the disappearance (e.g. the sustained response shoulder for the OFF cell) and the unmasking (OFF pathway driven spiking for the ON cell and ON pathway driven spiking of the OFF cell; transient ON inhibition of for the ON cell) of various response components.

These changes appeared significant for both ON and OFF RGC responses ($p_{\text{ON}}=5.77 \times 10^{-8}$, $p_{\text{OFF}}=6.5 \times 10^{-5}$; Wilcoxon signed ranks test, data normality was rejected in all datasets based on both Kolmogorov-Smirnov and Shapiro-Wilk tests).

Figure 5. GABA Receptor Blockade Induces a General Decrease of RGC PSTH τ values. a and b. Apart from a handful of cells that showed no considerable change, the GABA receptor blockade induced an overall decrease of PSTH τ values for most examined ON (left) and OFF (right) RGCs. This PTX mediated decrease is also reflected in a drop of mean and median PSTH τ values (b) for both ON (left) and OFF (right) RGC subpopulations.

Besides GABAergic inhibition, many inner retinal amacrine cells provide intercellular avenues for excitatory signals to RGCs by maintaining amacrine cell/RGC gap junctions. To see if this second type of inner retinal interaction plays a role in shaping RGC response transience we utilized a pharmacological blockade of gap junctions by incubating samples in meclofenamic acid (MFA - 40 μ M; nON=123; nOFF=60). This MFA-mediated blockade of retinal gap junctions, like PTX treatment, resulted changes for most examined RGC responses (Figure 6, 7).

Figure 6. Gap Junction Blockade Induces Alterations in RGC Response Transience. a and b. Representative ON (left) and OFF (right) RGC light-evoked responses (rasters on the top and PSTHs on the bottom) are altered when the nonspecific gap junction blocker MFA was applied. These changes are less obvious when compared to the PTX induced changes, but a clear transience reduction can be observed for both the ON (left) and OFF (right) cells presented here.

Figure 7. Gap Junction Blockade Induces an Overall Decrease of RGC PSTH τ values. a and b. Although a number of RGCs showed no change or increase of PSTH τ values as a response of a pharmacological gap junction blockade via the application of MFA, most RGCs responded with a decrease of their response decays for both the examined ON (left) and OFF (right) RGCs. The observed MFA mediated decrease is also depicted by the decreased mean and median PSTH τ values (b) for both ON (left) and OFF (right) RGC subpopulations.

Although, some of the examined RGC responses became more sustained, an overall transience decrease was observed across the examined RGC population (ON mean control: 0.218s $\pm$ 0.179 SD; ON mean MFA: 0.131 s $\pm$ 0.123 SD; OFF mean control: 0.146 s $\pm$ 0.066 SD; OFF mean MFA: 0.067 $\pm$ 0.081 SD). These changes appeared significant for both ON and OFF RGC responses ($p_{ON}=6.28 \times 10^{-12}$, $p_{OFF}=6.62 \times 10^{-8}$; Wilcoxon signed ranks test, data normality was rejected in all but the control OFF dataset based on both Kolmogorov-Smirnov and Shapiro-Wilk tests). Therefore, akin to effects of GABA receptor inhibition, the closure of gap junctions also exerted an overall decrease of PSTH τ values for many examined RGCs thus making responses more transient in both the ON and OFF RGC populations.

DISCUSSION

The Distribution of PSTH τ Values Reveal a Wide Range of RGC Response Transience

The transient/sustained division of RGC light responses has been widely utilized to characterize and classify RGC subtypes and is thought to be strongly related to visual function. Transient, burst-like responses likely transmit information about 'fast-paced' and dynamic aspects of the visual field, including direction and movement whereas sustained responses provide a continuous feed of information on static aspects of the view. Therefore, transient and sustained RGC responses encode dissimilar but equally important facets of visual information. This transient/sustained dichotomy has been documented in various vertebrates including cold-blooded animals, primates and non-primate mammals^{4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 18}. However, many of the studies examined this issue based on slow-wave EPSC recordings that, as we demonstrated here, occasionally deviate from RGC spike response transience, the parameter that is inherently associated with the visual code. In addition, the most elaborate studies in this topic were restricted to only a few RGC subtypes¹⁸, most of which were the non-image-forming melanopsin expressing ipRGCs, were conducted in cold-blooded vertebrates¹⁶ and/or carried out in retinal slice preparations^{16, 17} where lateral connections were compromised. Therefore, we feel that the present reexamination of RGC response transience and related RGC spike coding is strongly justified.

We reported previously²⁰ that regardless of the analyzing method RGC light responses cannot be unequivocally divided into clear transient or sustained categories. Instead, RGC PSTH τ values were distributed over a broad continuous range. These earlier observations were reinforced here showing that the decay of RGC responses ranged from 25 ms up to 500 ms with most cells in the intermediate 0.75-225 ms subrange for both the ON and OFF subpopulations. The greater number of cells with intermediate PSTH τ values resulted in a unimodal distribution of PSTH τ values in the frequency histograms. Therefore, our results here indicate that the canonical transient/sustained categorization is a rather coarse way of RGC

response characterization. Instead, RGC response kinetics could be better depicted by using a sole number, the full-field PSTH τ .

Possible Origin of Response Transience

In addition to the postsynaptic glutamate receptor on bipolar cell dendrites^{16, 17, 34, 35, 36}, a variety of mechanisms have been proposed to affect RGC transience, including direct inhibitory amacrine cell input to bipolar cells⁷, specific membrane characteristics³⁷ and differences in the synaptic reuptake of glutamate^{38, 39}. A recent study in the mouse retina presented a thorough analysis of the various sites of the retinal hyper-circuitry that may participate in determining response transience¹⁸. Although the conclusion of this latter study was that different RGC types use diverse mechanisms to produce sustained or transient light responses, the presented data clearly showed that outer retinal postsynaptic receptor (AMPA/kainate receptor) desensitization had only a minor effect on response transience. This is in an agreement with other studies showing that exclusively the kainite receptors transmit glutamatergic signals to OFF bipolar cells in the mouse and primate retinas^{40, 41, 42, 43, 44, 45, 46} and generate transient (mouse type 2, 3a), sustained (mouse type 1, 4) and intermediary (mouse type 3b) responses. This somewhat contradicts the immunohistochemistry data^{47, 48, 49, 50} and functional work established in the ground squirrel¹⁷ that argues for an equal contribution of AMPA receptor signaling for certain OFF bipolar cells and it is unclear if this evident discrepancy is due to species differences or some other factors. Regardless of the origin of this discrepancy, however, Zhao and colleagues showed that RGCs response transience is rather determined by the balance of transient/sustained bipolar cell inputs and RGC resting membrane potential as well as the presence/lack of inhibition^{18, 50, 52}. This latter study, however, examined mostly EPSC recordings in only a subset of the RGCs, therefore it provided limited information for the entire population. Our examination here utilized RGC spikes (events that encode visual signals) and the random sampling for the analyses thus they provide a broader perspective for the topic at hand.

Besides the above listed inconsistencies, our findings here also challenge the classical view that originates RGC response transience from the differential kinetics of bipolar cell glutamate receptors. First, the broad, unimodal peak of ON RGC PSTH τ frequency histograms indicate that the same mGluR₆ receptor in ON center bipolar cell dendrites generate signals for both transient and sustained RGCs. According to the classical hypothesis, one would expect either transient or sustained responses for all RGCs, which is obviously not the case. Interestingly, both transient and sustained mGluR₆ mediated ON polarity bipolar cell responses have also been described in the salamander retina¹⁶. These responses differed in their sensitivity for the treatment with the metabotropic glutamate antagonist, (RS)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG). However, a similar functional division of the mGluR₆ action has not been confirmed for ON polarity bipolar cells in the mammalian retina, therefore it is unclear if similar mechanisms underlie our observations for mouse ON RGCs or not. In fact, Wu and colleagues⁵³ described 12 distinct functional bipolar cell subtypes in the salamander, some of which showed ON-OFF responses, a characteristic that does not exist in the mammalian retina. This clearly indicates the existing differences in the retinal circuitry between the cold-blooded salamander and the mouse. This fact, at minimum, necessitates the reexamination of the above issue of pharmacological subdivision of mGluR₆ mediated signals for mammals. In addition, the most populous group included RGCs that displayed intermediary response transience while unequivocally transient or sustained cells were relatively rare. This, in fact, indicates that the sole postsynaptic glutamate receptor in bipolar cell dendrites is sufficient to generate a broad range of RGC response kinetics. Although intermediary responses could be explained by a mixture of two different postsynaptic receptors in the same bipolar cell dendrite, but the most parsimonious explanation is that RGC response transience is not majorly determined by the kinetics of bipolar cell glutamate receptors. This has also been suggested by the work of Zhao and colleagues¹⁸, showing that outer retinal postsynaptic receptor desensitization has only a minor effect on response transiency. In addition, one may speculate that according to the postsynaptic receptor hypothesis, the existence of the two distinct iGluR receptors in OFF polarity bipolar cell dendrites (twice as many as for ON cells)

would provide a greater variability for OFF RGC responses when they are compared to their ON counterparts. However, we found that the range and the frequency distribution profiles for OFF PSTH τ values were rather similar to those of ON RGCs. Therefore, converging evidence of this work support, the previous conclusion that the postsynaptic receptor(s) in bipolar cell dendrites are not a major factor in determining RGC response transience¹⁸. Therefore, we conclude that while bipolar cell response transience may in fact is determined by the expressed glutamate receptors located in the photoreceptor/bipolar cell synapse, RGCs do not simply inherit this feature but rather perform considerable signal transformation (summation, filtering and digitizing) before generating the RGC spiking output (see the same phenomenon mentioned below). Our results here also demonstrated that scotopic RGC responses served by a single type of bipolar cell are just as diverse in terms of transience as photopic responses (served by a variety of bipolar cell subtypes) of the same cohort of cells. This finding suggests that RGC response transience, apart from the glutamate receptor, is largely independent of other bipolar cell characteristics as well (e.g. active and passive membrane properties). On the other hand, we provided evidence showing that response transience is a subject of the interference of the signals carried by parallel intraretinal signaling streams to the same RGC targets. If such signals differ in delay, their summation may result in an RGC response that appears more sustained than the presynaptic input (Figure 8).

Figure 8. Summary Drawing of Potential Signal Summation Mechanisms that Affecting RGC Response Transience. **a.** Two bipolar cells of different subtypes provide transient inputs to the same RGC (light blue EPSC curves). These two inputs have dissimilar delays (due to differential bipolar cell signaling and/or different location of synapses over the RGC dendritic arbor) and therefore the summation of the responses results in an intermediate or sustained RGC spiking response. **b.** This RGC receives excitatory inputs from two sources, from a transient bipolar cell (light blue EPSC) and from a gap junction coupled amacrine cell (purple depolarization). If the dynamics of these two inputs differ their summation will induce intermediate and/or sustained RGC spiking. **c.** This RGC receives excitation from a bipolar cell (light blue EPSC) and delayed inhibition (red IPSC) from an amacrine cell resulting in a transient RGC response. **d.** An RGC that receives excitation from a bipolar cell (light blue EPSC) and inhibition (red IPSC) from an amacrine cell. In this scenario the two inputs have about the same delays therefore the excitation will be truncated and the RGC output is an intermediate/sustained spiking.

We showed an example where low threshold (sensitive) scotopic and high threshold photopic signals were summated. As the dominance of the two input components changed throughout the stimulus strength sequence the response delay and decay were altered as well. As a result, the spike train became more sustained upon mesopic stimulation when none of the signals dominated over the other. In this latter case response components were easily separated based on their dissimilar light sensitivities, however, a similar signal summation should occur for two or more parallel conveyed photopic signals to the same RGC targets as well. In this scheme a certain RGC subtype receives inputs from two or more signaling streams

(bipolar cells). This in fact is the case for ON alpha RGCs of the mouse retina that receive a mixture of inputs from type 6, 7 and 8 bipolar cells⁵⁴. In addition, sustained OFF alpha cells have been shown to be postsynaptic to type 2 bipolar and tGluMI cells whereas transient OFF alpha cells are targeted by type 3a and type 4 bipolar cells^{55, 56}. Therefore, mixing inputs from several parallel signaling streams seems to be a general feature for most RGCs in the mammalian retina. Furthermore, it appears that the summation of inputs with slightly different kinetics (delay, decay) and sensitivity plays a crucial role in determining the ultimate kinetics of RGC responses. Again, our presented results are not necessarily in conflict with previous reports showing that bipolar cell response transience depends on the postsynaptic glutamate receptors^{17, 24, 40}, however, we posit that this characteristic is not inherited to postsynaptic RGCs but greatly altered via signal summation. The results of such signal summation can be exemplified for some RGC subtypes that have been studied more deeply, including the sustained and the transient OFF alpha cell populations. Sustained OFF alpha RGC responses are generated by the summation of excitatory inputs from the transient type 2 bipolar cells and GluMI cells^{40, 55, 57}, whereas the synaptic partners transient OFF alpha cells are the transient type 3a and sustained type 4 bipolar cells^{55, 56}. Clearly, rather than inheriting response kinetics of presynaptic bipolar cells these two RGC subtypes performed a transformation of incoming signals. One question remains: whether the summation of excitatory signals can in fact provide an example of how transient inputs are transformed into intermediary or sustained signals by RGCs but a reverse transformation (sustained-to-transient) cannot be explained by the same mechanism. It is known that in addition to direct excitatory bipolar cell inputs to RGCs some signaling routes may provide indirect inhibitory and/or excitatory signals via intermediary amacrine cell chemical and electrical synapses, respectively. In fact, we showed that the pharmacological blockade of either GABAergic inhibition of gap junction mediated excitation greatly alters RGC response transience. Particularly, following the GABA receptor blockade examined RGC responses endured a general shift towards the more transient domain of the response range. This is in line with previous investigations showing that GABAergic amacrine cell input alters RGC response kinetics and functionality, as a fast inhibition can truncate

excitatory signals^{11, 58}, whereas delayed inhibitory inputs can shift the signal towards the more transient domain of the spectrum^{52, 59, 60}.

The closure of the electrical synapses in this study resulted in mixed changes in response transience; while a few RGC responses remained largely unaltered, most examined RGC responses changed towards either the sustained or the transient domain of the spectrum. This finding is not surprising as most RGC subtypes in the mouse retina maintain contact with neighboring RGCs and/or amacrine cells via gap junctions^{26, 27; 28, 29, 30, 31, 32, 61}. Gap junction mediated inputs are very likely combined with excitatory bipolar cell inputs and depending on the timing they contribute to either the RGC response peak or to the shoulder components thereby shifting RGC responses towards the transient or the sustained domains of the transience range, respectively. In general, the blockade of both GABAergic inhibition and gap junction mediated excitation resulted in a reduction in the RGC response diversity indicating that inputs mediated via these two latter signaling channels greatly diversify RGC responses. One may argue that changes in RGC response transience following the pharmacological blockade reflect effects on outer retinal microcircuits as well. However, the single source of GABAergic horizontal cell input to outer retinal neurons does not justify the variety of changes the blockade induces in RGC responses. Therefore, we argue that much (if not all) of the response transience changes observed following our pharmacological interventions reflect the blockade of inner retinal amacrine cell inhibitory circuits. Previous data showing that axotomized bipolar cells display a great loss of chloride currents also suggests that the GABA mediated inhibitory action occurs mostly in the inner retina⁵². Similarly, the closure of outer retinal gap junctions formed between horizontal cells and rod and cone bipolar cells that are constituents of all intraretinal signaling routes likely result in rather similar changes for all RGCs. Consequently, the variety of changes that occur after the blockade of gap junction signaling very likely reflect the pharmacological deletion of inner retinal (preferably amacrine cell to RGC) gap junctions. On the other hand, our investigation does not entirely rule out the potential involvement of outer retinal GABAergic and gap junction connections thus further

work has to be designed to address this question. In general, we conclude that RGCs collect a cohort of excitatory and inhibitory signals for summation and use them to sculpt their own output signal with kinetics that suits their function prior to sending information to the brain. This output depending on the type of summated signals and their relative timing may result in a variety of RGC response kinetics on a rather wide transient/intermediary/sustained range. According to this hypothesis, RGCs summate excitatory signals from multiple bipolar cell subtypes and gap junction coupled cell neighbors (amacrine and/or ganglion cells) and filter some of these signals out (via amacrine cell-mediated inhibition) to sway the dominance of input components to adapt to a specific visual function. This is in line with previous reports indicating that different RGC response properties fully emerge only after additional processing by currents in the bipolar cell axon terminal⁵¹, at synapses with amacrine cells⁵², and by RGCs. It is clear that the response transience of these RGCs is not simply inherited from presynaptic bipolar cells but rather transformed to suit the specific RGC visual function.

The Visual Function of RGC Transience

We reported previously that RGC light response delays are subtype-specific and they are precisely fine-tuned by inner retinal microcircuits to achieve a better RGC performance³. It has also been proposed that sustained and transient RGC spiking is another way of adaptation for various RGC subtypes to perform certain visual functions. In this scheme, sustained RGCs detect spatial contrasts and partake in form recognition, whereas transient cells perceive the movement of objects¹. This functional divergence already starts with the bipolar cells as sustained type 1 bipolar cells mediate color vision for the OFF polarity signaling stream, whereas other, more transient cells do not⁶². The 30-40 different types of mammalian RGCs⁶³,⁶⁴ cover a rather wide range of response transience therefore they can be specialized to perform a large variety of visual tasks. There has been a large collection of evidence supporting this view, including RGCs with transient responses that encode object movement^{65, 66, 67, 68}, the direction of motion^{69,70,71,72} and, also others with sustained responses that perceive luminosity contrast⁷³, color contrast⁷⁴ or object orientation⁷⁵. While the first cohort of RGCs require a quick

inactivation and corresponding decay of spiking frequency (transient response) in order to quickly recover and get ready for following changes in the visual scene, sustained RGCs allow for the summation of inputs over an extended time frame in order to get more sensitized for minuscule differences of light levels (e.g. grayscale or color) within their receptive fields. One interesting aspect of this hypothesis arises when stimulus-dependent changes in RGC response transience is taken into account. We showed one such incidence in this study when response transience values changed as a response to the modulation of stimulus intensity. Does that mean that RGCs perform better in certain stimulating conditions than in others? We think that the answer to this question is 'yes'. We experience it daily that our vision is rather limited during the night and this limitation involves the reduction of contrast sensitivity in both the spatial and temporal domains of our vision. This latter phenomenon is expressed by the Ferry-Porter Law stating that the critical fusion frequency is proportional to the logarithm of the flickering stimulus luminance⁷⁶. Therefore, the precise adjustment of RGC response temporal features including transience appears to be critical for our visual perception. Supported by our present investigation, most response transience altering mechanisms appear to be performed by the inner retinal microcircuits.

MATERIALS AND METHODS

Animals and preparation

Adult (P20<) C57BL6 mice were used in this study. After overnight dark adaptation, animals were put under deep anesthesia using Forane (4%, 0.2ml/l) and terminated through cervical dislocation. Dissection and experimentation were carried out in mammalian Ringer's solution (137 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl₂, 1.0 mM MgCl₂, 10 mM Na-HEPES, 28 mM glucose, pH 7.4¹⁹) under dim red illumination. The eyes and the retina were removed and hemisected anterior to the ora-serrata. In the single-cell extracellular recordings carried out with tungsten microelectrodes, anterior optics and the vitreous humor were removed, and the resultant retina-eyecup was placed in a superfusion chamber. In MEA or patch clamp (PC) experiments, the retina was completely isolated from the eyecup and placed directly atop the array or a filter paper (Millipore) to further transfer under the PC electrophysiology setup. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Maintenance and animal housing were all carried out in accordance with the local Animal Welfare Committee guidelines and regulations. The experimental protocol was approved by the University of Pécs Animal Welfare Committee (BA02/2000-6/2006; BA/35/51-42/2016). All efforts were made to minimize pain and discomfort. We also state that this manuscript is reported in accordance with ARRIVE guidelines.

Extracellular electrophysiology

Single-cell extracellular recordings were obtained from RGCs using tungsten microelectrodes (1M Ω ; Kation Scientific LCC Minneapolis, MN, USA). An AC differential amplifier (DAM80i, World Precision Instruments) and an analog-to-digital board (Digidata 1440a; Axon Instruments, Sunnyvale, CA, USA) were also utilized in these experiments. Spiking activity was recorded digitally at a sampling rate of 20 kHz with Axoscope (Axon Instruments, Foster City, CA). In other experiments, 60 and 120 channel MEA systems (Multi Channel Systems MCS GmbH, Reutlingen, Germany) were used to detect RGC spiking activity and recordings were made using the MCRack software (Multi Channel Systems MCS GmbH, Reutlingen,

Germany). PTX (50 μ M), MFA (40 μ M) and APB (50 μ M) were applied separately and independently.

Patch clamp electrophysiology

The retinæ were maintained in oxygenated, Ringer's solution in the recording chamber, between 33–35°C. Borosilicate glass electrodes (1B150F-4; WPI, Sarasota, FL, USA), pulled with a P-87 Flaming/Brown puller (Sutter Instruments, Novato, CA, USA) to a resistance of ~5–10 MOhm were used for extracellular and intracellular patch clamp (PC) recordings. The intracellular solution contained (in mM) 120 Cs-gluconate, 10 tetraethylammonium chloride (TEA-Cl), 1.0 CaCl₂, 1.0 MgCl₂, 11 ethylene glycol-bis-(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 10 sodium N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Na-HEPES), adjusted to pH 7.2 with CsOH. The calculated ECl for this solution was -58 mV. Cell-attached and voltage-clamp recordings were carried out with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA) connected to a Digidata 1440a (Axon Instruments, Sunnyvale, CA, USA) A/D converter. Recordings were made with WinWCP (John Dempster, University of Strathclyde). Resting and full-field light-stimulated excitatory and inhibitory postsynaptic currents, EPSCs (V_h = -60 mV) and IPSCs (V_h = 0 mV) were recorded. Data were filtered at 5 kHz with a Bessel filter and were sampled at 10 kHz.

Light Stimulation

A uniform full-field stimulus was used to evoke light responses (0.5s illumination every 2s). In experiments utilizing different stimulus intensity of light stimuli values were given in terms of the rate of photoisomerization that occurs in each rod in every second ($Rh^*/rod/s$); we calculated with an average rod density of 437,000 rods/ mm² [8] and quantum efficiency of 0.67 [9]. The intensity of the light stimuli varied from 1 to 6000 $Rh^*/rod/sec$.

Data analysis

Spike sorting was carried out using Spike2 (CED, Cambridge, UK) and Offline Sorter (Plexon Instruments, Dallas, TX, USA). PSTHs measuring transiency were generated in NeuroExplorer (Plexon Instruments, Dallas, TX, USA). Gaussian smoothing (filter size: 3) was applied to all datasets. All transiency values were calculated using the $PSTH_{\tau}$ method²⁰, where $PSTH_{\tau}$ measures the time required for spiking frequency to decrease to $1/e$ of the peak firing amplitude. SPSS (v19, IBM, Armonk, NY, USA) and OriginPro (OriginLab Corp., Northampton, MA, USA) were used for statistical analysis. All responses included in this study were analyzed manually.

Data availability

All raw data of this manuscript as well as detailed protocols will be promptly available upon request.

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CONFLICT OF INTEREST

None of the authors of this manuscript have a conflict of interest.

CONTRIBUTIONS

Alma Ganczer: experiments, data analysis, manuscript writing; Gergely Szarka: data analysis, manuscript writing; Márton Balogh: data analysis, manuscript writing; Ádám Jonatán Tengölics: data analysis, manuscript writing; Tamás Kovács-Öller: experiments, data analysis, manuscript writing; Béla Völgy: funding, experiments, data analysis, manuscript writing.

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FIGURE LEGENDS

Figure 1. A Great Variety of Response Transience Across the RGC Population. **a.** Representative perievent raster diagrams show that individual RGCs provide light-evoked spiking responses upon full-field illumination that are rather similar across trials (four consecutive trials for each recorded cell). However, RGCs display a great variety in terms of their response length (or decay – expressed as the $PSTH_{\tau}$ value in this work) for both the ON (cells 1 and 2) and OFF (cells 3 and 4) subpopulations. The white bar below the recordings represents the timing of the on- and offset of the stimulus in this and in all other figure panels of this paper. **b.** Frequency histogram shows the distribution of $PSTH_{\tau}$ values for the ON (white) and OFF (black) RGC subpopulations. Clearly, the distribution of $PSTH_{\tau}$ values appears unimodal and does not allow for the clear separation of transient and sustained RGC responses. This unimodality as well as the rather wide range are features shared by both the ON and OFF RGC subpopulations.

Figure 2. Scotopic RGC Response Transience Values are Just as Diverse as Photopic Responses. **a.** Representative ON RGC light responses (raster to the left and PSTH to the right for the same RGC) were evoked first by scotopic (top) and then photopic (bottom) full-field light stimuli. This change in the stimulus strength induced a clear change in response amplitude but the overall shape (response delay and decay) of the response remained largely unchanged. **b.** Similar to the ON RGC is panel **a** this representative OFF RGC display light responses that, besides subtle changes in response amplitude, overall remain unchanged in scotopic (top) and photopic (bottom) stimulating conditions. **c.** Diagram displays $PSTH_{\tau}$ value pairs for individual RGC light responses in scotopic (left) and photopic (right) light stimulations. Clearly, the change in stimulus strength induced the alteration of $PSTH_{\tau}$ values for many examined RGCs, however, the range of response transience values for the entire RGC population were comparable in scotopic conditions (if not even wider) to those obtained with photopic stimulation paradigms. **d.** Floating bar graphs show that the variety of scotopic RGC response $PSTH_{\tau}$ values is just as great as for photopic responses for the same RGCs.

Figure 3. Changes in Response Transience can be the Result of Signal Interference through Parallel Retinal Pathways. **a.** Representative ON RGC light-evoked rasters (left column) and PSTH cohorts (right column) recorded as a response to stimuli of various strength (intensity values are reflected in the right top corner of each -panel). $PSTH_{\tau}$ values of this RGC changed non-monotonously during this experiment when the stimulus intensity was gradually increased (see also panel **c**). PSTHs clearly show a peak of very sensitive signal component (red arrow) evoked by weak, scotopic stimuli. This sensitive response component appears relative delayed when it is compared to the less sensitive but brisk signal component (light blue arrow). These two signal components differ in their delays but appear similar in response decay, therefore $PSTH_{\tau}$ values are shifted towards the sustained range when the two signals are summated (mesopic conditions - 2nd, 3rd and 4th panels), whereas remain transient when only one signal is present (scotopic condition – 1st panel) or dominates over the other component (photopic conditions – 5th, 6th and 7th panels; see also panel **c**). **b.** Representative OFF RGC light-evoked rasters (left column) and PSTHs (right column) recorded as a response to varying intensity stimuli (intensity values are reflected in the right top corner of each -panel). $PSTH_{\tau}$ values of this RGC clearly changed during this experiment as the stimulus intensity was gradually increased. Similar to the cell in panel **a** this OFF RGC showed a very sensitive but rather delayed response peak (red arrow) and a faster but less sensitive (light blue arrow) peak. The two signal components differed in their delays and sensitivities and a slight alteration in $PSTH_{\tau}$ values occurred as a result of the summation of components (mostly in mesopic

conditions – middle panels). While the distinction of response components can clearly be differentiated for the ON RGC in *a*, this OFF cell (and most examined RGCs) showed a less obvious and less separable summation of incoming signals. **c and d.** Diagrams show that, similar to cells shown in panels *a* and *b* (values of these cells appear in black and red in the diagrams), most recorded RGCs displayed stimulus strength driven changes of PSTH τ values (grey curves). **e.** Diagram shows minimum/maximum PSTH τ value pairs for the recorded cells during the course of the stimulus intensity recording paradigm. The examined RGCs showed ~18-73% PSTH τ changes during the course of this experiment.

Figure 4. GABA Receptor Blockade Induces Abrupt Changes in RGC Response Transience. a and b. Representative ON (*a*) and OFF (*b*) RGC light-driven responses (rasters on the top and corresponding PSTHs below) are clearly altered when the nonspecific GABA receptor blocker PTX was applied (bottom panels). The observed changes include both the disappearance (e.g. the sustained response shoulder for the OFF cell) and the unmasking (OFF pathway driven spiking for the ON cell and ON pathway driven spiking of the OFF cell; transient ON inhibition of for the ON cell) of various response components.

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Figure 8. Summary Drawing of Potential Signal Summation Mechanisms that Affecting RGC Response Transience. a. Two bipolar cells of different subtypes provide transient inputs to the same RGC (light blue EPSC curves). These two inputs have dissimilar delays (due to differential bipolar cell signaling and/or different location of synapses over the RGC dendritic arbor) and therefore the summation of the responses results in an intermediate or sustained RGC spiking response. **b.** This RGC receives excitatory inputs from two sources, from a transient bipolar cell (light blue EPSC) and from a gap junction coupled amacrine cell (purple depolarization). If the dynamics of these two inputs differ their summation will induce intermediate and/or sustained RGC spiking. **c.** This RGC receives excitation from a bipolar cell (light blue EPSC) and delayed inhibition (red IPSC) from an amacrine cell resulting in a transient RGC response. **d.** An RGC that receives excitation from a bipolar cell (light blue EPSC) and inhibition (red IPSC) from an amacrine cell. In this scenario the two inputs have about the same delays therefore the excitation will be truncated and the RGC output is an intermediate/sustained spiking.

Supplemental Figure 1. Calculation of RGC τ Values Obtained by Various Recording Methods. a. Schematic drawings depict the steps of how τ values can be calculated for RGC

signals recorded with extracellular (left) and whole-cell patch clamp (right) recordings. After determining the delay (D) and amplitude (A) of RGC responses, A/e can be calculated and τ values can be obtained for both signal types. **b.** Pairs of light evoked responses were recorded in cell attached and whole-cell modes in order to detect spike responses (top) and EPSCs (bottom) for the same cells. Representative ON RGC shows a very transient spike output and corresponding small τ , however, the same cell displayed rather intermediate EPSCs and greater τ values in whole-cell recording mode. The diagram to the right shows the τ value obtained for this cell based on spike responses and PSTHs (black column), and τ values gathered for each EPSC responses (grey; individual response τ values are also shown as red circles). **c.** Pair of spike and EPSC recordings of a representative OFF RGC (same recording paradigm as in b) where response transience appear similar and corresponding τ values are very similar as well.

Supplemental Figure 2. Scotopic OFF RGC Responses are Completely Blocked via the Blockade of mGluR₆ receptors. a and b. Both raster diagrams and PSTHs show that APB in an equal concentration of 50 μ M blocked light evoked scotopic OFF RGC responses attesting that we utilized the adequate light conditions in our scotopic experiments.

Figures

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