nature portfolio

Peer Review File

Regional tuning of photoreceptor adaptation in the primate retina

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In their study, Baudin et al. compare light adaptation between foveal and peripheral cones in the primate retina. Foveal cones give rise to high-acuity vision in primates but have rarely been recorded directly. A previous study from the senior author identified striking kinetic differences between foveal (slow) and peripheral (fast) cones in macaques (Sinha, 2017). The present study shows that kinetic differences are maintained as cone responses accelerate with increasing background light levels (i.e., light adaptation). Light adaptation of the response gain is similar between foveal and peripheral cones when assessed by the response peak but is shifted to somewhat higher light levels when measured by the area under the response curve. Finally, the authors show that foveal cones adapt more slowly after a step change in ambient light levels than peripheral cones.

Overall, this study presents new observations about light adaptation in foveal cones. The quality of the data is excellent. However, the scope of the experiments and insights is narrow and seems better suited for publication in a more specialized journal. In addition, the presentation of the data on changes in adaptation kinetics is underdeveloped.

Specific comments

1.) Light adaptation of the response gain is normal when assessed by the response amplitude as usual. The authors show some changes when measuring gain adaptation by the area under the response curve (AUC). However, they provide no evidence that this measure reflects the impact of adaptation on signal processing. For example, do downstream neurons adapt differently due to this gain in the AUC?

2.) The presentation of the changes in adaptation kinetics is underdeveloped. The authors should show example traces for foveal and peripheral cones at onset and offset (and ideally for both light levels explored, i.e., 1,000 to 10,000 R* and 5,000 to 50,000 R*). They could also plot the normalized timecourse (mean +/- sd or sem) of adaptation for each foveal and peripheral population data in addition to showing the time constants.

3.) The scope of the study could be extended, and its impact increased by recording from downstream neurons, e.g., foveal and peripheral midget ganglion cells, to see if the observations made on cones propagate through the circuit. Alternatively (or in addition), the authors could probe foveal vs. peripheral adaptation by psychophysics to establish a link with their observations on cones.

4.) Another avenue for extending the scope and increasing the impact would be to perform experiments to gain insights into the mechanisms underlying the observed differences in light adaptation of foveal vs. peripheral cones.

Reviewer #2 (Remarks to the Author):

Baudin and colleagues present an excellent investigation into the differences in adaptation within foveal and peripheral cones. The authors are experts in recording from primate cones and their methodology is sound. Their work is notable for its location – the primate fovea – an essential area for visual perception. Little is known about the physiology of foveal retinal neurons and this uniquely primate area is difficult to address in other animal models, making their results very significant. The fovea is the primary fixation locus for saccades (during which light levels differ dramatically) so their account of how adaptation occurs in foveal cones will be of broad interest.

Discussion paragraph: It is a very interesting result that the slower light adaptation in foveal cones is well suited for the duration of fixation between saccades. A few additional questions on

interpretation:
- How might the kinetics of light adaptation in peripheral cones be useful for vision mediated by the peripheral retina?
- Are there any downsides to the slow adaptation of foveal cones? Are there limitations apparent in psychophysics or our visual experience?

Speaking of psychophysics, I expect this will be of considerable interest to the field as cone adaptation has been intensely studied for over a century. It might help to provide some guidance on how to convert or interpret R*/rod/sec in the context of one of their units (trolands?)

The explanatory plots for the light adaptation experiment were very helpful (Fig 3A and 3B). What were the fixed timings of the 1st, 3rd and 5th flashes? Hard to ballpark from Fig 3A. Also, it would be nice to see Fig 3B for a foveal cone as well.

Is there a 3 figure limit for this article? The data in Fig S2 are very interesting and I think worthy of inclusion in the main figures. Also, is it possible to compare time-to-peak for increments and decrements? There have been a number of studies recently on faster processing of decrements than increments in perception, V1, LGN and within the retina. I believe this is currently attributed to the differences between the ionotropic and metabotropic receptors on OFF and ON bipolar cells, but it would be interesting to know whether this arises in the cones themselves. No need for extra experiments, just a thought in case the authors already have the data necessary to weigh in on this question.

I found lines 119-121 to be very difficult to parse, particularly as the “in %” became involved. The next sentence helps but any rewording or additional clarification to the first sentence would be helpful.

Reviewer #3 (Remarks to the Author):

Baudin and colleagues make whole-cell current clamp patch recordings in retina excised from macaque monkey eye. Small but statistically significant differences in response kinetics and light adaptation dynamics are revealed on comparing cones recorded within 0.5 mm of the cone peak density locus (center of the foveal pit) and cones recorded in peripheral retina, about 6 mm from the foveal center. Foveal cones appear to show weaker and slower kinetics (it appears by typically about 1 delta sigma) than their peripheral counterparts. The experiments appear technically sound as would be expected emanating from Dr Sinha's laboratory and the analysis is well-documented. The study would be well suited for a specialist vision-oriented journal but I am sorry to report seems unlikely to influence thinking in the field more broadly. Effects recapitulate differences almost all and in directions expected from the literature, to which Dr Sinha has made important and fundamental contributions. Most directly, the kinetics differences replicate or are easily predictable from Sinha et al, Cell, 2017, cited by the authors; increment/decrement asymmetries are thoroughly-explored in Angueyra et al, J Nsci 2022, cited by the authors. The experiments reported here do not introduce new methods or analytic approach, or address the biological cause of the small differences exhibited between foveal and peripheral retina. Therefore the authors must resort to speculation about causes and consequences of these small differences and their biological (as opposed to statistical) significance as at lines 193-196.

Minor:

17. encoding ... movements. (doesn't quite make sense, does it? The retinal image is smeared during rapid eye movements).

136 Locus of fixation during saccades (meaning?)

233 Vague, the figure 4 mins appears arbitrary. What would be convincing is a positive demonstration of response rundown that begins after 4 min. Or at least a convincing

explanation/citation to support the statement, please. Perforated patch recordings are mentioned in reference 18 but do not appear to be used here from the material supplied.

442 The flashes ... states (unclear).

Reviewer Comments:

Reviewer #1 (Remarks to the Author):

In their study, Baudin et al. compare light adaptation between foveal and peripheral cones in the primate retina. Foveal cones give rise to high-acuity vision in primates but have rarely been recorded directly. A previous study from the senior author identified striking kinetic differences between foveal (slow) and peripheral (fast) cones in macaques (Sinha, 2017). The present study shows that kinetic differences are maintained as cone responses accelerate with increasing background light levels (i.e., light adaptation). Light adaptation of the response gain is similar between foveal and peripheral cones when assessed by the response peak but is shifted to somewhat higher light levels when measured by the area under the response curve. Finally, the authors show that foveal cones adapt more slowly after a step change in ambient light levels than peripheral cones.

Overall, this study presents new observations about light adaptation in foveal cones. The quality of the data is excellent. However, the scope of the experiments and insights is narrow and seems better suited for publication in a more specialized journal. In addition, the presentation of the data on changes in adaptation kinetics is underdeveloped.

We thank the reviewer for the positive comments and helpful feedback. We have now significantly revised the manuscript and added several new elements to the story including a potential mechanism underlying the differences we see in adaptation between foveal and peripheral cones. We have also extended our comparisons of adaptation to primate blue cones which are known to exhibit slower kinetics and lack of luminance dependent adaptive filtering. We have added 2 new main figures (Fig 5,6), new data/analysis in other fgures (Fig 2F, Fig 4E,F) and 5 new supplemental figures (Supplementary Fig 1,2,4-6). We have also expanded the manuscript into a full article with distinct sections. We are quite excited about the revisions which we feel addresses several of the reviewer concerns and has sufficiently broadened the scope of our study. Unfortunately, despite our persistent efforts, getting enough robust and high-quality light-evoked recordings from ganglion cells in the macaque fovea has proven to be quite challenging due to the limited primate tissue availability. We also feel that directly relating our results to downstream circuit function and perception is beyond the scope of this current study due to technical limitations but something that we will strive to achieve in future studies. We have added a section in the discussion where we describe the potential impact of regional differences in cone adaptation on both downstream circuitry and perception.

Specific comments

1.) Light adaptation of the response gain is normal when assessed by the response amplitude as usual. The authors show some changes when measuring gain adaptation by the area under the response curve (AUC). However, they provide no evidence that this measure reflects the impact of adaptation on signal processing. For example, do downstream neurons adapt differently due to this gain in the AUC?

We thank the reviewer for this interesting suggestion to look at downstream neurons like ganglion cells but as mentioned above this has proven to be quite technically challenging to address given the limited primate tissue availability. This will be a great topic for future investigation to test if the luminance dependent changes in kinetics of cone signals persist in the ganglion cells. We can however predict that the regional differences in cone adaptation (integrated gain) will be present at the level of ganglion cells because our previous study has shown that the foveal midget ganglion cells inherit the slow response kinetics directly from the cones themselves with minimal filtering of the signals through the intermediate circuitry (Sinha et al., 2017). We have included a detailed discussion section about the impact of adaptive changes in temporal filtering and gain between foveal and peripheral cones on downstream circuitry, in particular for the midget pathway (lines 426-444).

2.) The presentation of the changes in adaptation kinetics is underdeveloped. The authors should show example traces for foveal and peripheral cones at onset and offset (and ideally for both light levels explored, i.e., 1,000 to 10,000 R and 5,000 to 50,000 R*). They could also plot the normalized timecourse (mean +/- sd or sem) of adaptation for each foveal and peripheral population data in addition to showing the time constants.*

We thank the reviewer for this suggestion and have added exemplar traces for foveal and peripheral cones for 1,000R* to 10,000 R* as well as 5,000R* to 50,000 R* (Fig 3B and Supplementary Fig 3B). As per the reviewer's recommendation we have plotted the timecourse of adaptation for foveal and peripheral cone population for the larger light step (5,000R* to 50,000 R*) which shows a slower timecourse of adaptation both at light onset and offset for foveal cones compared to peripheral cones. Therefore, we think this representation may not add any further insight and is less quantitative compared to the time constant analysis where we can extract timecourse values for individual cells. Moreover, as expected the gain values also have error bars in time-axis (not shown) due to variability in the timing of the response peak across cells and makes the statistical comparison more difficult for such a plot. However, we are happy to put this in the supplementary figure if the reviewer wants this to be included.

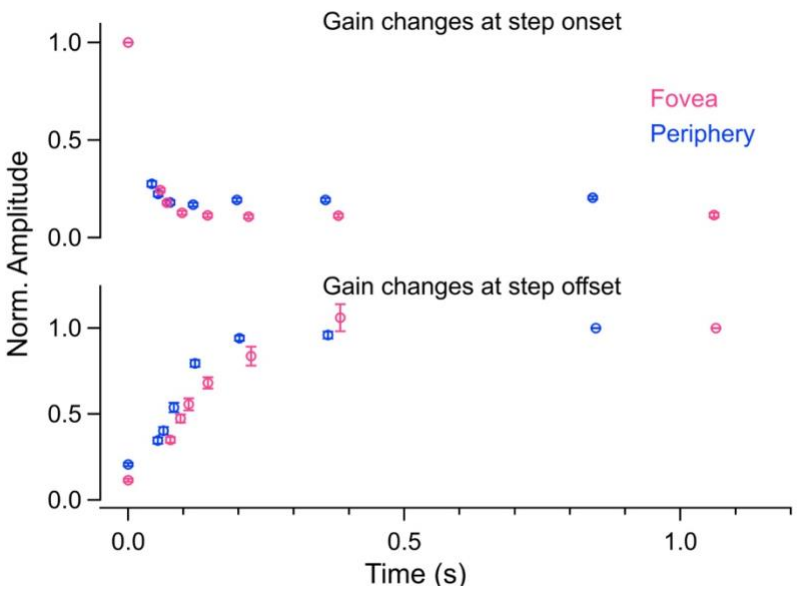

Figure: Time course of cone adaptation at light increment and decrement. Average normalized response gains for each of the light flashes presented at time = 0, 10, 20, 40, 80, 160, 320, 700 (or 1200) msec. The timepoint of the adapted flash near the end of the light step was 700 ms for a fraction of peripheral cones and 1200ms for the remaining. For foveal cones this fixed flash was always delivered at 1200ms. This does not cause any difference to the time course calculation for onset since the cones are almost fully adapted beforehand within 100 ms of the light increment. However, this makes it difficult to average the gain at that timepoint. The slower adaptation of foveal cones compared to peripheral cones is apparent and reflected in the relative magnitude and delay of the response gains of foveal cones compared to peripheral cones.

3.) The scope of the study could be extended, and its impact increased by recording from downstream neurons, e.g., foveal and peripheral midget ganglion cells, to see if the observations made on cones propagate through the circuit. Alternatively (or in addition), the authors could probe foveal vs. peripheral adaptation by psychophysics to establish a link with their observations on cones.

See response above. In relation to extending the study to psychophysics - this would be an interesting future area of study beyond the scope of the current manuscript as considerable expertise needs to be garnered for performing psychophysical experiments in humans and/or non-human primates. We have added a section in the discussion linking cone physiology to perception (lines 446-490).

4.) Another avenue for extending the scope and increasing the impact would be to perform experiments to gain insights into the mechanisms underlying the observed differences in light adaptation of foveal vs. peripheral cones.

We thank the reviewer for this excellent suggestion and have made significant progress in this direction. We have identified a mechanism responsible for the differences in cone adaptation between fovea and periphery (Fig 5, Supplemental Fig 4, 5). This mechanism relies on a hyperpolarization activated inward rectifier current (I_h) mediated by the HCN channels. These channels have previously been shown in mouse and goldfish retina to be involved in adaptive temporal filtering of cone signals but their role in primate cones remained unknown (Barrow and Wu, 2009; Howlett et al., 2017). We show that peripheral red (L) and green (M) primate cones have a prominent I_h current which is much smaller in magnitude in foveal L/M cones (Supplemental Fig 4). By using pharmacology, we further show that blocking HCN channels not only reduces the acceleration of peripheral cone response kinetics with increasing luminance but also slows the dynamics of luminance adaptation (Fig 5, Supplementary Fig 4, 5). The role of HCN channels in temporal filtering of cone signals in primate retina seems to be consistent with previous findings in goldfish and mouse retina (Barrow and Wu, 2009; Howlett et al., 2017). We have added a new results and discussion section on these new findings (lines 256-292; lines 386-424).
We were further able to show foveal red and green cones might exhibit similar properties of adaptation in comparison to blue cones and exhibit a similar smaller magnitude of the I_h current (Supplementary Fig 4B). We show that blue cones in the primate retina which are known to have a slower response kinetics as well as a lack of change in kinetics across luminance (Baudin et al., 2019) also exhibit a weaker and slower luminance adaptation compared to peripheral red and green cones (Fig 6). We have also mended the result section (lines 294-325). Together these new findings reveal novel insights into regional and type-specific differences in luminance adaptation of cones and a mechanism that is causing such differences. The inclusion of this new mechanistic data strengthens our revised manuscript.

Reviewer #2 (Remarks to the Author):

Baudin and colleagues present an excellent investigation into the differences in adaptation within foveal and peripheral cones. The authors are experts in recording from primate cones and their methodology is sound. Their work is notable for its location – the primate fovea – an essential area for visual perception. Little is known about the physiology of foveal retinal neurons and this uniquely primate area is difficult to address in other animal models, making their results very significant. The fovea is the primary fixation locus for saccades (during which light levels differ dramatically) so their account of how adaptation occurs in foveal cones will be of broad interest.

We thank the reviewer for the positive feedback and for highlighting the significance of our findings. To address the interesting points raised by the reviewer, we have expanded the discussion section.

1. Discussion paragraph: It is a very interesting result that the slower light adaptation in foveal cones is well suited for the duration of fixation between saccades. A few additional questions on interpretation:
- How might the kinetics of light adaptation in peripheral cones be useful for vision mediated by the peripheral retina?

We think a stronger adaptation and a faster time scale of adaptation in peripheral cones might be better suited to meet the demands of the higher temporal sensitivity of peripheral vision such that it is able to detect rapidly changing inputs such as those encountered during motion (Masland, 2017). Another functional reason for a stronger and quicker luminance adaptation could be because the dynamic range of signaling in peripheral cones is smaller than in foveal cones. Thus, to avoid saturation, adaptation kicks in sooner and at lower light levels in peripheral cones. We have extended the discussion section in the revised manuscript to include this (Lines 465-490).

- Are there any downsides to the slow adaptation of foveal cones? Are there limitations apparent in psychophysics or our visual experience?

We speculate that the time scale of foveal cone adaptation could be one of the determinants in setting the frequency of saccadic eye movements to 2-3 times a second. A slower adaptation allows the foveal cones to integrate more photons but limits its ability to detect rapidly changing light inputs. Thus, there is a tradeoff in the fovea of spatial over temporal resolution which is achieved at the level of cones by having a longer integration time and a smaller and slower change in response sensitivity with luminance. In fact, to test this idea at the level of perception it will be important to compare how the visual sensitivity to flickering light stimuli i.e., the critical flicker fusion frequency, changes with luminance between foveal and peripheral vision. We have extended the discussion section in the revised manuscript to include this (lines 465-481).

Speaking of psychophysics, I expect this will be of considerable interest to the field as cone adaptation has been intensely studied for over a century. It might help to provide some guidance on how to convert or interpret R/rod/sec in the context of one of their units (trolands?)*

We thank the reviewer for this suggestion. 1 photopic troland (td) is assumed to be 10–30 R*/cone/s (Crook et al., 2009; Schnapf et al., 1990). We have added this conversion in the methods section (lines 526-527).

The explanatory plots for the light adaptation experiment were very helpful (Fig 3A and 3B). What were the fixed timings of the 1st, 3rd and 5th flashes? Hard to ballpark from Fig 3A. Also, it would be nice to see Fig 3B for a foveal cone as well.

The fixed timings for the 1^{st}, 3^{rd} and 5^{th} light flashes were at 100ms, 1200ms and 2200 ms from time = 0 in Fig 3A (step duration of 1s). We also had a longer step duration of 1.5 sec instead of 1 sec for some cells. In that case the fixed timings for the 1^{st}, 3^{rd} and 5^{th} light flashes were at 100ms, 1700ms and 3200 ms from time = 0 (Fig 3B; fovea). This did not change the timescale calculation.
We have added exemplar responses for a foveal cone as well in the main figure as well as examples for both peripheral and foveal cones for the time course of adaptation for the smaller light step (1000R* -> 10000R*) (Fig 3B, Supplementary Fig 3A).

Is there a 3 figure limit for this article? The data in Fig S2 are very interesting and I think worthy of inclusion in the main figures. Also, is it possible to compare time-to-peak for increments and decrements? There have

been a number of studies recently on faster processing of decrements than increments in perception, V1, LGN and within the retina. I believe this is currently attributed to the differences between the ionotropic and metabotropic receptors on OFF and ON bipolar cells, but it would be interesting to know whether this arises in the cones themselves. No need for extra experiments, just a thought in case the authors already have the data necessary to weigh in on this question.

We thank the reviewer for this comment. This article was initially submitted in the brief communication format and hence the 3-figure limit. We have now expanded the manuscript into a full-length article and made figure S2 a main figure (Fig 4) as per reviewer's recommendation.
We thank the reviewer for raising this exciting point about comparing the cone response kinetics to light increments vs decrements given previously described differences in temporal sensitivity between ON and OFF pathway in the retina, higher visual centers and at the level of perception. To compare the kinetics of cone responses to light increments vs decrements, we acquired new data where we measured responses to brief, 10ms, light increments and decrements at the brightest background luminance (50,000R*/cone/sec) where the response asymmetry is the largest. The brief flash responses allowed us to get a defined response peak which was missing in the responses to longer light decrement steps shown in Fig 4A, B. Because of a lack of a clear peak in the step response in Fig 4C-D we used the steady state current as a measure of response amplitude to compare responses to light increments and decrements. However, upon comparison of the responses to brief light flashes, we find no difference in the response kinetics (time to peak) between light increment and decrement for both foveal and peripheral cones. We have added this data to the main figure (Fig 4E-F). This is interesting and, as the reviewer mentioned, points to downstream mechanisms of speeding up the kinetics of OFF signals relative to ON signals. We have also amended the result section to incorporate this new experiment and analysis (lines 244-250).

I found lines 119-121 to be very difficult to parse, particularly as the "in %" became involved. The next sentence helps but any rewording or additional clarification to the first sentence would be helpful.

We thank the reviewer for pointing this out. Given the extended format, we have reworded the explanation for this part, and we hope this will help clarify the following result – response compression is stronger for peripheral cones with increasing luminance than for foveal cones which gives rise to a sharper decrease in integrated gain (area under the curve) for peripheral than foveal cones (lines 154-160).

Reviewer #3 (Remarks to the Author):

Baudin and colleagues make whole-cell current clamp patch recordings in retina excised from macaque monkey eye. Small but statistically significant differences in response kinetics and light adaptation dynamics are revealed on comparing cones recorded within 0.5 mm of the cone peak density locus (center of the foveal pit) and cones recorded in peripheral retina, about 6 mm from the foveal center. Foveal cones appear to show weaker and slower kinetics (it appears by typically about 1 delta sigma) than their peripheral counterparts. The experiments appear technically sound as would be expected emanating from Dr Sinha's laboratory and the analysis is well-documented. The study would be well suited for a specialist vision-oriented journal but I am sorry to report seems unlikely to influence thinking in the field more broadly.

We thank the reviewer for their comments but respectfully disagree about the significance of the findings. We think discovering how luminance adaptation in the fovea is different from that in the peripheral retina is fundamental for understanding the basis of perceptual differences between foveal and peripheral vision and especially how high-acuity foveal vision is achieved. And the first step in this direction, we think, is to

determine how cone photoreceptors adjust the gain and kinetics of their signals across varying lighting conditions. We hope that the revisions to the manuscript which shed novel insights into the mechanism underlying regional differences in cone adaptation and how such differences in adaptation are also present between cone types, address the concerns of significance and scope raised by the reviewer.

Effects recapitulate differences almost all and in directions expected from the literature, to which Dr Sinha has made important and fundamental contributions. Most directly, the kinetics differences replicate or are easily predictable from Sinha et al, Cell, 2017, cited by the authors; increment/decrement asymmetries are thoroughly-explored in Angueyra et al, J Nsci 2022, cited by the authors.

Our previous study (Sinha et al. Cell 2017) focused entirely on response kinetics and compared this key property at one background luminance (5000 R*/cone/sec) between foveal and peripheral cones. This does not tell us anything about adaptation of cone kinetics or gain across luminance or the timescale of luminance adaptation between these two retinal regions. To extrapolate the results at one background luminance in our previous study to how cone signals adapt across a range of luminance is at best one potential hypothesis that needs to be thoroughly tested and presented as we have done in this study. For instance, we know from another previous study of ours (Baudin et al., 2019) that blue cones in primate retina are slower than red and green cones by ~10 msec at a background luminance of 5000R*/cone/sec but what is fascinating and violates prediction from a single background luminance is that their response kinetics does not change at all, across a broad range of luminance (Baudin et al., 2019). Therefore, one cannot predict functional responses across light levels using data from a single background luminance.
Likewise, simply because foveal cones are ~two-fold slower than peripheral cones at a background light level of 5000 R*/cone/sec (Sinha et al., 2017), it is not necessarily expected that their response kinetics will change the same way across a broad range of luminance as peripheral cones. We also never expected that the peak amplitude of the foveal cone responses will be identical to that of the peripheral cones at all background luminance. In fact, an alternative prediction from our previous study could be that due to a slower time course of foveal cones, their response amplitude could have been smaller than peripheral cones at each of the background luminance so that the integrated response of foveal and peripheral cones ends up being similar. Resolving between these two scenarios (this prediction versus our presented observations) required a direct comparison of gain and kinetics across luminance between foveal and peripheral cones. Likewise, the asymmetry to light increments and decrements in foveal cone responses is also not predictive based on the previous study in peripheral primate cones (Angueyra et al, J Neurosci 2022). This needed to be tested and verified given functional differences between foveal and peripheral cones. Furthermore, revision experiments that we have additionally conducted provide new insights into mechanisms causing such differences in adaptation between foveal and peripheral cones and reveal a regional fine tuning of the cone intrinsic molecular machinery that was previously unknown.

The experiments reported here do not introduce new methods or analytic approach, or address the biological cause of the small differences exhibited between foveal and peripheral retina. Therefore the authors must resort to speculation about causes and consequences of these small differences and their biological (as opposed to statistical) significance as at lines 193-196.

We would like to address the concerns raised about methods, analytical approach, and small differences in the following section. With regard to the causes of these differences the additional experiments that we have included in the revised manuscript provide new insights into the underlying mechanisms.

Novelty of methods:
Intracellular electrical recordings from cones in the primate fovea is especially non-trivial and still novel given that primate fovea is one of the most difficult neuronal preparations to keep alive and light sensitive. The

cone inner segments in the fovea that we are patch-clamping from are ~1-2 µm in width and these cones are one of the most fragile and delicate neurons in the central nervous system to record light-evoked electrical responses from. As a result, studies using intracellular recordings from foveal cones have been far and few (~3 papers) (Baudin et al., 2019; Bryman et al., 2020; Sinha et al., 2017) despite their central role in mediating most of our everyday visual experience. Overcoming the challenges to perform patch-clamp recordings from foveal cones in an intact retinal preparation is not a small feat and should not be considered a routine method/technique. Additionally, we have now included recordings from primate blue cones in the revised manuscript which are even more difficult to target for recordings as they are sparse and comprise ~5-10% of total cone population (Grunert and Martin, 2020). We have also paired cone recordings with pharmacology to address the role of HCN channels in cone adaptation in the revised manuscript. Our employed methods thus present technological advances.

Novelty of analytical approach:
We show that using integrated response (area under the curve) as a measure of gain instead of the standard approach of using the peak amplitude (or power within a defined frequency range) as a metric for gain fully captures the differential effect of luminance on kinetics in foveal vs peripheral cone and red/green vs blue cones. In fact, this is important because this allows us to look at the combined effect of adaptive changes in response amplitude and kinetics on the gain/sensitivity of the cone photoreceptor signal. We have also added new analyses to measure the rate of kinetic acceleration (by calculating the slope; Supplementary Fig 1 and 4)), and further isolate how a smaller change (foveal cones) or lack of a change (blue cones) in kinetics has minimal impact on the gain ratio (area under the curve to peak amplitude; Fig 2F, 5D) Fig . Our analytical approach is thus not routine.

Biological cause and potential consequences of differences between foveal and peripheral retina:
We thank the reviewer for bringing up the consideration of underlying mechanisms. We have now identified a potential mechanism causing differences in adaptation between foveal and peripheral cones and between blue vs red/green cones. We have also expanded the discussion section to include more details about the potential impact of differences in cone adaptation on downstream retinal circuitry and perception – something that can be pursued in future studies.
However, we respectfully disagree that differences we see in luminance adaptation between foveal and peripheral cones or between blue and red/green cones are by any means small. The difference in the response acceleration with luminance, the strength of adaptation and the timescale of adaptation between foveal and peripheral cones are nearly two-fold. This is a massive regional difference in cone function which can cause significant perceptual differences in both absolute threshold of detection and temporal sensitivity between central and peripheral vision across background light levels (Masland, 2017).
Of note, there is strong precedence in expecting differences at the perceptual level based on differences in cone adaptation across spectral types. For instance, a rotating black and white disk, Benhams disk, produces the illusion of color because of the temporally delayed blue cone signals relative to red and green cone signals (Benham, 1894). This originates in cones and is due to the difference in response kinetics (~10 ms) between blue vs red and green cones which is enough to cause a robust perceptual effect (Baudin et al., 2019; Brindley et al., 1966; Marks and Bornstein, 1973; Smithson and Mollon, 2004). More importantly, the constancy of the blue cone response kinetics across luminance is prevalent also at the level of perception (Marks and Bornstein, 1973). Therefore, the differences in cone adaptation across spectral types although seemingly small (in absolute terms) can still be impactful at the level of human perception.

Minor:

17. encoding ... movements. (doesn't quite make sense, does it? The retinal image is smeared during rapid eye movements).

We thank the reviewer for pointing this out. We have revised this sentence to say 'may be well suited for maximizing collection of high-acuity information at the fovea during gaze fixation between rapid eye movements' (lines 24-26).

136 Locus of fixation during saccades (meaning?)

We have revised this sentence to "However, the timescale of adaptation remains unknown for cones in the fovea which is subject to fast and large changes in luminance as our eyes fixate from one location to next while actively sampling a visual scene" (lines 181-183).

233 Vague, the figure 4 mins appears arbitrary. What would be convincing is a positive demonstration of response rundown that begins after 4 min. Or at least a convincing explanation/citation to support the statement, please. Perforated patch recordings are mentioned in reference 18 but do not appear to be used here from the material supplied.

We thank the reviewer for this comment. We have previously tested the run-down of intrinsic responses in in primate cones after initiation of whole-cell recording (Angueyra and Rieke, 2013; Sinha et al., 2017) and use 4 mins as a cautious upper limit for collecting light-evoked responses. We have added the references in the text (Line 534).

442 The flashes ... states (unclear).

We have modified this sentence to "The light flashes following step onset and offset evoke cone responses that adapt from a lower to higher mean luminance as well as responses that adapt from a higher to lower mean luminance" (lines 774-776).

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REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have significantly expanded the scope of their study by investigating hyperpolarization-activated currents (Ih) as a mechanism of luminance adaptation in primate cones that accounts in part for the divergence in adaptation peripheral vs. foveal L/M cones and between L/M vs. S cones. The new experiments are performed to high standard and are presented clearly in the figures and text. Congratulations on an insightful study!

Minor comments
- Line 76 should be rewritten as: 'Such adaptation of gain permits cones to encode variations in luminance (i.e., contrast) independent of the mean luminance.'
- Line 91 should be rewritten as: 'Adaptive changes occur on a much slower timescale in foveal cones compared to peripheral cones.'

Reviewer #2 (Remarks to the Author):

The authors have addressed my original comments and expanded the manuscript considerably by added data from S-cones and experiments exploring the underlying mechanisms. This is a strong and significant paper, especially given the technical difficulty of recording from the fovea and the paucity of research in this critical area for human vision.

Reviewer #3 (Remarks to the Author):

The addition of data showing effects of HCN channel blocker on peripheral cones has somewhat broadened the scope of this paper, which will be a fine contribution to the literature. Foveal cones were not studied with the blocker. A specialist journal still seems the most appropriate forum for these results, which have been enhanced but not greatly enlarged by these additional data. They do indeed point to a potential causative contributor to dynamic differences between foveal and peripheral cones. Unfortunately the authors at several points make statements which could mislead readers by implying that they have measured Ih in foveal cones (#18, #22, #95, #266, #350, #404, #407).
In their response the authors argue that patch-clamp recordings from foveal cones are technically non-trivial. While having great respect for this impressive technical achievement, it is also clear that foveal cone patch clamp measurements were already reported by Sinha et al in 2017. The present paper methods appear largely identical to that study and to Baudin et al (2019). Therefore the claim to have presented here a novel method is questionable.
In their response the authors argue that their observations do not recapitulate those of their previous studies, but for example there does seem to be overlap of the conditions shown in the present submission figure 1 with figure 1 in Baudin et al (2019, cited by the authors). Many background levels overlap, and the methods section of the two papers (with exception of the HCN experiments on peripheral cones) show heavy overlap with many identical passages (e.g. #492-509 in present study is essentially a copy of Baudin et al, 2019, p16, para 1). If there are essential and extensive differences in experimental conditions (rather than analyses)

between the present study and these previous studies then it would be helpful to have them explicitly listed somewhere please.
Minor:
#22 A smaller Ih ... [this and related statements are potentially misleading, and should be clearly qualified or removed please. The authors have not measured Ih currents or effects of HCN blockers in foveal cones. Relevance of reference #21 is recognized.]
#59 .. properties .. is
#82 actively .. during eye movements [unclear]
#83 But none ... [please see above comments regarding overlap of current study with previous measures of foveal cones. The statement seems unduly sweeping]
#266 " We first measured the magnitude of HCN channel mediated currents
267 in peripheral and foveal cones ... " [Please see above, this

statement is misleading]
#91 Timescale ... timescale [unclear]
#259 ... net adaptation of gain. [unclear]
#275 Fig C-F
#371-384 [This passage is very dense and could be rewritten for clarity. For the authors' consideration]
#381 weber
#389 mechanisms both
#421 However .. will require .. [the logic here seems unduly stringent]
#449 However ... even though [unclear]

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We thank the reviewer for their positive feedback and appreciate their comments for making our study compelling.

Minor comments
- Line 76 should be rewritten as: 'Such adaptation of gain permits cones to encode variations in luminance (i.e., contrast) independent of the mean luminance.'
- Line 91 should be rewritten as: 'Adaptive changes occur on a much slower timescale in foveal cones compared to peripheral cones.'

We have made the above changes.

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We thank the reviewer for recognizing our efforts to revise the manuscript which have broadened the scope of the study. We have indeed measured I_h in foveal cones and plotted the data in Supplementary figure 4B. To make this clearer, we have now included an example foveal cone and a peripheral S cone data in Supplementary Fig 4A. As shown in Supplementary Fig 4B the magnitude of I_h is significantly smaller in foveal M/L cones and peripheral S cones compared to peripheral M/L cones.

2. In their response the authors argue that patch-clamp recordings from foveal cones are technically non-trivial. While having great respect for this impressive technical achievement, it is also clear that foveal cone patch clamp measurements were already reported by Sinha et al in 2017. The present paper methods appear largely identical to that study and to Baudin et al (2019). Therefore the claim to have presented here a novel method is questionable. In their response the authors argue that their observations do not recapitulate those of their previous studies, but for example there does seem to be overlap of the conditions shown in the present submission figure 1 with figure 1 in Baudin et al (2019, cited by the authors). Many background levels overlap, and the methods section of the two papers (with exception of the HCN experiments on peripheral cones) show heavy overlap with many identical passages (e.g. #492-509 in present study is essentially a copy of Baudin et al, 2019, p16, para 1). If there are essential and extensive differences in experimental conditions (rather than analyses) between the present study and these previous studies then it would be helpful to have them explicitly listed somewhere please.

We have addressed this point raised by the reviewer in detail in the previous round of revision (point 2). In brief, the novelty is in measuring properties of luminance adaptation in foveal cones across a range of light levels and making a systematic comparison with peripheral cones. These measurements of light adaptation in foveal cones and regional comparison of cone adaptation were not present in Baudin et al 2019 or Sinha et al 2017 or any other previous studies. Thus, we feel this is a significant advance in retinal neurobiology. Primate retinal tissue preparation, patch-clamp recordings and light levels were intentionally kept identical to previous studies for the purpose of reproducibility and robust comparisons across studies – a practice heavily encouraged by all institutions for enhancing rigor and reproducibility.

Minor:
#22 A smaller Ih ... [this and related statements are potentially misleading, and should be clearly qualified or removed please. The authors have not measured Ih currents or effects of HCN blockers in foveal cones. Relevance of reference #21 is recognized.]

We have indeed measured I_h in foveal cones (Supplementary Fig 4A-B). However, we have removed this sentence and modified it to reflect the results.

#59 .. properties .. is

Done

#82 actively .. during eye movements [unclear]

Removed ‘actively’.

#83 But none ... [please see above comments regarding overlap of current study with previous measures of foveal cones. The statement seems unduly sweeping]

This is an accurate sentence and claim. In Baudin et al 2019, we looked at luminance adaptation in *peripheral* M/L cones vs *peripheral* S cones but not in the same detail as in this study. In Sinha et al 2017, we didn’t

study luminance adaptation at all. So, none of the features of light adaptation were known for *foveal* cones – the focus in the current study.

#266 " We first measured the magnitude of HCN channel mediated currents 267 in peripheral and foveal cones ... " [Please see above, this statement is misleading]

We measured I_h currents in foveal cones (see response above).

#91 Timescale ... timescale [unclear]

Removed ‘Timescale’.

#259 ... net adaptation of gain. [unclear]

Revised it to ‘gain adaptation’. (line 264)

#275 Fig C-F

Changed to Fig 4C-F (line 280)

#371-384 [This passage is very dense and could be rewritten for clarity. For the authors' consideration]

We have rewritten this for clarity. (lines 378-393)

#381 weber

Changed

#389 mechanisms both

Revised this sentence (line 397)

#421 However .. will require .. [the logic here seems unduly stringent]

Revised this sentence

#449 However ... even though [unclear]

Revised this sentence. (line 430)