

The annotated reviews - [REM comments]

**Just for your edification:**

Every novel idea in science passes through three stages.  
First, people say it isn't true (re: our PLoS Biology reviews)  
Then they say it's true but not important (these Science reviews)  
And finally they say it's true and important, but not new. (tomorrow)

**These are a couple of the sloppiest, but most artfully dismissive reviews I've seen in my 30+ years in science. I almost admire their lazy, patrician ennui.**

**But Texas boys don't go down easy.**

**I guess we forgot to add a good spoonful of eastcoast hype and a dollop of contrition. We'll those aren't in the spice kit so well use somethin' else: Fire. New bulletproof version coming up soon.**

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6 October 2009

Dr. Robert E Marc  
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University of Utah  
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Ref: 1181540

Dear Dr. Marc:

Thank you for submitting your manuscript "Exploring the retinal connectome." We have now received the [not very] detailed reviews of your paper. Unfortunately they are not positive enough to support publication of the paper in *Science* [But the negativity isn't about the science]. Although we recognize that you could likely address many of these specific criticisms in a revised manuscript, the overall nature [spin, tone, attitude] of the reviews is such that the paper would not be able to compete for our limited space. We hope that you find the comments

helpful **[not likely]** in preparing the manuscript for submission to another journal. We are grateful that you gave *Science* the opportunity to consider your work.

Sincerely,  
Peter Stern, Ph.D.  
Senior Editor

**So let's deconstruct the "not positive" reviews**

### **Review 1**

The authors generated a 16 terabyte connectome dataset from rabbit retina and explored the connectome with multi-user annotation software developed by the authors. Based on their results they suggest novel signaling modalities in the mammalian retina.

The authors took on the heroic task to assemble a retinal connectome dataset at 2 nm resolution. This highly valuable dataset makes it possible to ask in depth questions about retinal circuitry with as yet unmatched resolution and detail.

**REM: Good so far ... but this is routine reviewer-speak.**

Combined with functional data this would be truly a tool for discovery.

**REM: Oh-Oh. This is starting to go south because, you see, no one could possibly have a clue about what this reviewer has in mind. Let's see - we've got functional AGB mapping for every cell - found glutamate responses in microglia - got new circuits - oh but those are speculative because no-one's seen them before ... except for the speculative ones people have seen, and those aren't new. So it must a different kind of functional data the reviewer has in mind.**

However, the study does not live up to such expectations.

**REM: What expectations ... in particular?**

**This the "bring me a rock" game: <http://bringmearock.blogspot.com/>.**

Presenting a physical map of retinal neurons and their synapses, it is a descriptive exploration reconstructing well known retinal pathways without further analysis. As a consequence, suggested novel signaling modalities stay hypothetical and speculative.

**REM: So we *described* some new things but they remain descriptive (richly described anatomy is "descriptive" or bad, incomplete physiology is "functional" or good) and novel ideas are "speculative", or bad. It has taken us 5 years to get here and our next job is to undo all the weak physiology first before the anatomy is worth anything. It is an Atlas**

## **Shrugged moment.**

Finally, the authors describe nanoribbon synapses of ON cone bipolar cells formed in the OFF sublayer of the retina as a novel synaptic motive that significantly impacts retinal signal processing concepts, and a finding made possible by their connectome approach. However, such ON cone bipolar cell synaptic contacts, which violate the stratification rules of the retina, have already been published (Hoshi et al., J. Neuroscience, 2009).

**REM: Hoshi et al didn't see nanoribbons - they saw some sparser, larger ectopic ribbons by confocal imaging, and missed most of the nanoribbons, and all the vetting synapses (non-canonical feedback and feedforward). Let's see, we are 90 days behind a pretty cool confocal study (July 2009) where Hoshi et al. show RIBEYE in micron-sized patches in some cone bipolar cell axons by confocal imaging. Many ribbons are transported in packs and so not all RIBEYE can be synapses, but they do show some giant ones correlated with GluR4... and miss all the nanoribbons less than 100 nm in extent and can only map a subset of bipolar cells. Our mistake was not to include the full tabulation of nanoribbons. Our direct TEM dataset with validated presynaptic and postsynaptic architecture, plus vetting synapses, and complete ground truth, in novel networks ... was deemed speculative but the confocal inferences weren't.**

**But is this all there is to the review? Wow. What happened to the DS network, the microglia, Müller cells, the first-ever multi-cell reconstructions? Damn. Talk about C- work!**

**So a slapdash review with offhand comments is rated not positive. Fair enough, but bad reviewing nonetheless**

**Let's move on**

## **Review 2**

The present study is the logical extension of a previous paper published by the same group in PLoS Biology earlier this year. The authors used a piece of rabbit retina, processed it for electron microscopy and cut serial sections in the horizontal plane of the retina. The sections were then processed for immunocytochemistry with various antibodies including the amino acids GABA, glycine or glutamate to reveal the unique signature of some of the cell types. The sections were photographed using automated transmission electron microscopy image acquisition. The images were aligned and processed for

classification using software tools as described in detail in the previous paper resulting in a database referred to as retinal connectome dataset (RC1). The senior author has a long-standing reputation in electron microscopy and in the development of elegant methods to identify cell types by their amino acid signature.

**REM: Again, so far so good. This reviewer seemed to imply knowledge of CMP ... but not really :(**

Serial reconstruction at the electron microscopic level is a tedious and very time-consuming process and only a few retinal circuits have been fully analysed to date. To my knowledge, there are currently two major attempts to speed up this process.

**REM: There are several more than that ... so this reviewer really wasn't as up-to-snuff as the first paragraph implied. Suggests that the reviewer really doesn't know that much about EM, or connectomics, but likes to act like it.**

One is the method of automatic sectioning and image capturing by the group around Denk, the other one is the approach used by the authors of the present manuscript. The major advantage of the latter approach is that synapses can easily be identified and cell types can be determined to a certain extent.

Another advantage of is that it will allow multiple users to work on the same database. **[that's not unique to us - this indicates a fundamental misunderstanding of the difference between a volume and its annotation architecture.]**

Neither of the two **[four, maybe five]** approaches has dealt with the problem of identifying ganglion cell types. **[except us!]**

**REM. Hold on kemosabe. Communication breakdown here. Where do you think the color coding in all the TEM images came from? This is what Marc RE and BW Jones 2002 Molecular phenotyping of retinal ganglion cells. J Neurosci 22:413-427, addresses in extensive detail and is exactly the technology fused with TEM in this paper. We put exactly the same signals in RC1, but this reviewer didn't notice it? Or didn't want to.**

**This is deceptive reviewing at worst, incompetent at best. It is unethical to imply you understand the work when you don't, especially since the editors can't fact-check you.**

The approach used here is excellent and innovative and will in the long run lead

to new insights into retinal circuitries. **[Throwaway sentence - maybe a twinge of conscience - but probably not - but the key is "the long run" - after we're dead]**

However, I am not convinced that the present manuscript really shows a whole lot of new data.

**REM: We'll we can't convince everyone, but the sentiment is factually wrong, and its expression in the review intentionally misleading.**

**Paraphrased, it encapsulates the essence of the entire review: "I don't want to believe it."**

**But here are a few new things that don't represent "a whole lot of new data":**

- **molecular signatures in the largest EM dataset ever built**
- **functional activity markers embedded in the dataset**
- **glutamate-sensing microglia**
- **Müller cell giant Ca buffering organelles**
- **a new candidate DS network**
- **ultra complex cone bipolar cell networks**
- **the largest reconstruction of Aii cells ever achieved**
- **nanoribbons that confocal can't see**
- **vetting synapses that confocal can't see**

**This reviewer came ready to minimize, not learn. He or she didn't read the paper.**

Furthermore, I am not convinced that the method used here will be easily reproducible in other laboratories.

**REM: Wrong. And negative wishful thinking. If any method can be reproduced easily, it is ours. This suggests again that the reviewer came with a negative attitude and was ready to overlook all the novel things we did accomplish.**

Major points

1) The authors show the scotopic pathway, which is probably the best-studied pathway in the retina. It is reassuring to see that the results obtained here confirm previous manual reconstructions, but it is not clear to me how the present results extend previous knowledge.

**REM: We did make a mistake here. Thank you for noticing. We didn't show the full dataset documenting the 12 different kinds of contacts these cells**

make including a vast number of new partners for these cells. We only showed five fully reconstructed cells ... in the same tissue, all connected to each other ... for the first time in neuroscience. This is in a set of over 100 cells in RC1 associated with rod vision.

2) The authors' claim that they found novel network motifs needs to be explained more clearly. They introduce the terms 'nanoribbons' and 'vetting synapses' but it is not clear to me why these structures were interpreted as novel synaptic motifs.

**REM: Where else have you seen 100nm-scale ribbons making synapses on crossing GABA amacrine cell axons? Where have you seen a single complete dendrite traced from a bona fide GABAergic amacrine cell pick up two ribbons (nanoribbons) and then target another BC in the ON layer. Where else have you seen large inhibitory terminals on bipolar cell axons? Where is the prior physiological data that predicts these structures? That's how weak the physiology is ... and I guess how speculative ground truth is.**

If the authors think these structures are important new features, they should provide some quantitative data.

**REM: Our mistake for not folding yet another paper into this one. I think the reviewer knew we had it, though.**

The implication is that these structures were not detected previously, but it is not clear why this should be the case.

**REM: Say that again, slowly ten times. Maybe it will make sense. Oh ... maybe not.**

Based on these presumed novel synaptic motifs, the authors arrive at physiological conclusions, which are highly speculative.

**REM: We use ground truth and map connections that really there, comment on the implications and that is speculation.**

Physiology uses incomplete models, doesn't include nanoribbons, vetting synapses, serial inhibitory chains, ultra-complex motifs, laminar crossing GABA AC axons, glutamate-sensing microglia, etc. but is OK because it is functional?

**Any bets on who is right kids?**

Minor points:

Page 6, para 1, lines 8, 9. The references given for ON responses in melanopsin ganglion cells and dopaminergic amacrine cells are incorrect. Please check.

**REM: My mistake.**

The authors should also consider citing the recent paper in JCN by Dumitrescu et al. (2009) showing ribbon synapses formed by ON cone bipolar cells in the OFF sublamina.

**REM: Another data subset using optical imaging - not quite as good as Hoshi, but again misses all the real nanoribbons. And so EM validation is second class to confocal papers? I'm exhausted.**

The reconstruction shown in Figure 3A is confusing. What are the red dots? Are these synapses? And if they are how can they be distinguished from the processes belonging to the OFF cone bipolar cell C478?

**REM: Wow. The first multi-cell 3D rendering in history of the retina (based on ground truth) and the reviewer is on about red dots which were never mentioned. We debated removing the markers (we had no room to discuss them), because someone might use them to complain. How prophetic. I guess we should have added more supplemental data imagery, but since they didn't really look at what we had carefully it to begin with, who knew what they would choose? Did anybody miss those amazing gap junctions?**

I strongly suggest providing scale bars instead of giving the width of the images in nm. Scale bars would allow the reader to see immediately which images are shown at the same scale.

**REM: irrelevant?**

The scale is missing completely for Figs. 3D and E.

**REM: True**

Supporting material:

Please indicate the size of the piece of retina sectioned, and the number of sections used for the analysis.

**REM: That's in the main manuscript and PLoS, which the reviewer claims to know something about.**

I assume this method uses one section per EM grid, this information should be given somewhere.

**REM: We reference the method in PLoS Biology.**

Page 18, line 5. A reference is given to a manuscript (Anderson et al. in review). How is this manuscript different from the current manuscript and the previously published one?

**REM: It describes how to build Viking? Interesting?**