Roy et. al 2017- Paper Summary and Discussion

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According to the Neuenschwander and Singer study, retinal ganglion cells can communicate over surprisingly long distances for contiguous large stimuli. Roy et. al aim to study the coherency of RGC spikes with disjointed stimuli. RCG coherency is dependent on many factors, later discussed in this article: time, intensity, contrast and movement. Movement as discussed in a previous study (1), can effect the subcortical and cortical oscillations - reducing neural synchrony.

Since there is limited bandwidth in the optic nerve correlation in spikes can be redundant- so only the most important, information rich, features are delivered across these gap junctions to the optic nerve. The blockade of these gap junctions reduces the ability to discern large contiguous objects from disjointed ones. In cats there can be up to 20 degrees of visual angle for a large contiguous stimuli; and this long-range coherence may point to how other animals create perceptual groupings.

However there may be groupings on a local and more global perceptive scale- as later discussed in this article - intracellular signals between RGC may be the local operator but gap junctions provide a holistic picture of all amacrine inputs and RGC. Gap Junctions are connected to amacrine cells- most of these amacrine cells are of the (polyaxonal, over 1 mm long axons)

PAC type. The electrical coupling of amacrine cells and gap junctions allows a transfer of energy from one circuit to another- this may be key to providing these long-range connections. Connexin 36 was used to block these gap junctions- to confirm the hypothesis that distant signals coincide at these junctions.

RGCs were targeted with gene therapy with KCNG4cre and Thy1-stop-YFP to mark alpha RGC subunits. Immunolabelling with the SMI32 confirmed these cells (shown in figure 1A) and Neurbiotin was used to determine celll dendritic fields and morphology(figure 1C, 2A). The sample as was first stimulated with small rectangular lights to indicate if there was overlap between the YFP-expressing cell somata of these RGCs. When the Kcng4-YFP mice were exposed to light stimuli in their receptive field they showed neural coherency (figure 2D, 2E). The alpha subunits of RGC were tested using a loose patch clamp technique- usually patch clamp techniques record a single ion channels but loose patch clamp techniques have lower electrical resistance thus lower resolution of these channels (figure 1B). For temporal data, each pair is a couple of time series- each of the cells is recorded while light stimulation was applied. To estimate the effect of these conditions on pair firing, cross- correlogram profiles were used (figure 1E, 2E). Figure 1F show a shift predictor- this “corrects” the stimulus induced relationship to show it at the same point of firing.

The Peristimulus time histogram shows the temporal firing of neurons in response to the two rectangles of light (figure 2B). Shift predictor cross- correlograms of ON RGC shows there is no communication (coherency) between cells when exposed to this non-contiguous stimuli

(figure 2C). However, an appreciable coherency was achieved for a “fused” (like a single block) stimuli (figure 2D, 2E). To show that long-range communication was important for synchrony, and as shown individual firing RGC is unrelated to long-distance coherency (figure 2G and H), MFA was binded to the RGC alpha marker to block gap junctions (figure 2F), confirmed by the blocking sites pointed out by the arrows in figure 2I.

A group of mice were treated with C57BL/6- which allowed maximal expression of mutations for Cx36- blocked connection between gap junctions. CxWT served as the lowered visual acuity control group. Both groups showed a preference for large contiguous objects, as they had for Kcng4- YFP, 18B-GA had abolished this long-range activity. In the experiment they tested pairs of distant OFF alpha RGC. OFF α-RGC filled with NB in the Kcng4-YFP shows a distinct coupling between RGC cells and PACs, showing that not all synchonry is achieved at the gap junction (figure 3A). It showed some residual coupling in OFF RGC with the inhibitor (figure 3H). Distant OFF RGC show a sustained response to the separate rectangle stimuli with stimuli offset (figure 3B, 3C) but no coherent activity (figure 3E, 3F). The contiguous rectangle also didn’t produce synchronous activity. ON α-RGCs in the produced no coherent activity with other unlabeled cell types and also showed no synchronous response to disjointed or full figures (figure 4). Pairs of different RGC subtypes, without amacrine cells or gap junctions, don’t show long range coherent activity- they are not coupled expect for homologous coupling between OFF RGCs . The “incremental fusing” of stimuli has a threshold of 20 micrometers (figure 5). Stimulus presentation had to go to at least half go the alpha RGC receptor field for coherency (figure 6K) but fired frequently in response to partial covering of receptive field centers of the

ON RGCs (figure 6E-J) but not to weak stimuli (figure 6A-D). This will stimulate PACs but it will not allow for long-range synchrony.

This could perhaps be due to dopaminergic neurons blockage to intrinsically photosensitive retinal ganglion cells, hindering sustained excitatory light responses (6). Since the blocks were only flashed for half a second there may be limited sustained responses because of this pathways- perhaps leading to lack of coherency. Intrinsically photosensitive retinal ganglion cell have long latencies, marked poststimulus persistence, and a peak spectral sensitivity of 478 nm (6), so a weak stimuli may not invoke a response because of ganglion-cell photoreceptors excitatory feedback to dopaminergic neurons is blocked.

The gap junction blockades effect global perception in specific ways, as shown by the maze study. CxWT, had acuity reduction and could only have around 1.5 or 1.0 degrees of discrimination (as relating to the visual world). MFA cut long-range correlations in most strains - this means gap junctional coupling is needed to see contiguous objects (figure 7A-F). MFA and $Cx36^{-/-}$ mice could not tell 7 degrees difference in an object (figure 7H).CxWT, MFA-injected CxWT, and $Cx36^{-/-}$ mice completed at the sinusoidal grating task, like true control C57BL/6 mice. MFA is not specific for a tier of gap junctions, it can also effect pigment epithelium cells- disabling coupling mechanist across these cells (3). It is not clear what other cells MFA and deletion of Cx36 is effecting- besides the RGC.

It is not unambiguously presented in this article, that gap junctions at the tier of amacrine connection is essential for coupling. Computation modeling showed that PAC synchronizes

RGC. Computational theories also posit that neurobiotin injected RGC will only be able to have nearest neighbor connectivity- this low conductance, limits the visual field - it is specific to certain smaller groups of cells allowing a sharper image. The dynamic range of gap junctions may be enhanced by rod-rod coupling or other factors (2). Another theory posits that RGCs are inhibited by the same PACs via gap junctions, similar to pyramidal cells in the visual cortex.

In lecture, you had mentioned that amacrine cell types have direction selectivity but that may not be the case in primate. Which suggests the question that modeling circuitry for correlate activity may be different for the animal models, perhaps different enough in rats to humans to change results. Also since there are many amacrine cell types, even in primates, not all may be related to coherency- GABA-ergic cells as mentioned in the article are related to inhibition for noisy signals but there may be "vertical integration," to contrast with lateral integration for amacrine cell types leading to different synchrony. Also the connexion blocker may also effect other interactions in the inner plexiform layer. Astrocyte to astrocyte coupling and astrocyte to Muller cell coupling may be inhibited(4), and these connection provide the propagation of intercellular Ca^{2+} waves and the regulation of intracellular ion levels which may be important to neural coherency.

This article provides a comprehensive overview of the amacrine to RGC roles for neural coherency- however, as I mentioned, many factors are left unaccounted for. It's important to consider other cellular interaction when dealing with a system as complex as the early visual system. This paper does confront the integration of amacrine lateral input by coupling is essential

for global perception- without coupling the image becomes less recognizable for holistic perception. It is undeniable that linking of this synchronous activity is compulsory for a more complete image.

Work Cited:

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