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>Reviewer: 1  
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>Comments to Author  
>The manuscript by Adams et al. presents recordings from anterior  
>cingulate slices and simulations to study cellular heterogeneity in the  
>ACC and its relationship to rhythmic activity. The authors find both  
>manual and automated classification schemes for the recorded cell types  
>to be lacking and conclude that cellular properties are distributed along  
>a continuum. Based on recordings and transection experiments, they  
>conclude that superficial layers generate primarily beta rhythms, whereas  
>deeper layers generate both beta and gamma rhythms. In simulations they  
>first show that heterogeneity broadens the range of frequencies over  
>which resonant behavior can be observed in network responses to rhythmic  
>input. Finally, they show that when two populations of interconnected  
>neurons within the same network receive sinusoidal inputs at different  
>frequencies, the ability of both populations to respond at the same time  
>is enhanced in networks with heterogeneous neurons.  
>  
>The question of how the properties of individual neurons enable different  
>rhythms to co-exist within the same network is important, and the basic  
>finding is interesting. That being said, in its current form, the  
>manuscript reads more like a collection of somewhat incomplete /  
>imperfectly related findings, as opposed to a coherent, well developed  
>story.

I hope this can be addressed with alterations to the text, a paring down the results to the main points and moving the rest to supplementary (rather than removing it).

>  
>1.     The largest section of the paper (4 out of 10 main figures) is devoted  
>to various classification schemes for the recorded neurons, all of which  
>end up being inadequate. I don¹t understand why so much time is spent on  
>what is essentially a negative result. I also don¹t understand why  
>pyramidal neurons from different layers are grouped together here.  
>Ultimately, the attempt to classify pyramidal neurons based solely on  
>electrophysiological properties, without regard to genetic markers,  
>projection targets, morphology, etc., seems ill-conceived.  It has been  
>done by countless papers in the past without much consensus.  This  
>portion of the paper should be dramatically scaled back in my opinion.  
>

>2.     The basic characterization of beta and gamma rhythms, while somewhat  
>interesting also feels a bit disconnected from the rest of the  
>manuscript.  The authors note that rhythmic IPSPs are sometimes observed  
>at frequencies that match that of the ongoing network oscillation. Based  
>on this, they model different rhythms (beta vs. gamma) simply by  
>switching the IPSC decay kinetics. This is simplistic at best, and of  
>course fails to capture the ability of both rhythms to co-exist in the  
>same network, and the ideas, mentioned by the authors, that different  
>interneuron subtypes might support the generation of distinct rhythms, or  
>that different layers may contribute differentially to beta vs. gamma  
>rhythms.  This portion of the manuscript needs to be better developed and  
>connected to the rest of the manuscript, about intrinsic heterogeneity  
>and the co-existence of rhythms within a single network.  Ideally the  
>authors would do something like explore how heterogeneity contributes to  
>the ability of a single network to display two modes of beta vs. gamma  
>oscillations, incorporate two separate beta / gamma generating modules  
>based on deep and superficial layers, etc.

The use of two different decays in the model was to simulate different external inputs to the network. The fact that a bimodal peak in decay times is not present may indeed mean that multiple populations of inhibitory neurons are not necessary for the generation of these two rhythms. However, they exist nevertheless, even in the absence of those inputs. How are we separating this concept of external dual rhythm input and internal dual rhythm generation in the text?

>  
>3.     The most novel finding here is that heterogeneity supports the ability  
>of a network to respond simultaneously to multiple inputs at different  
>frequencies, but this also feels the least developed.  (a) Why is one of  
>the frequencies fixed at 35 Hz?  Looking at what the ³permitted²  
>combinations of frequencies are would potentially be quite informative  
>and interesting.  Showing that beta/gamma oscillations can coexist in a  
>way that other combinations of frequencies cannot would be particularly  
>interesting and help tie this portion of the manuscript together with the  
>earlier findings about beta/gamma oscillations.  (b) Why is there only  
>one simulation done for the heterogeneous case?  This makes the  
>statistical comparisons between the heterogeneous and homogenous cases  
>feel a bit artificial.  (c) Why is only one example shown and how exactly  
>is coactivity calculated? It seems like there might be other ways to  
>calculate coactivity that might better capture the ability for the  
>network to process both inputs simultaneously, i.e., coactivity as  
>defined appears to be the same if activity is split equally between both  
>populations vs. heavily weighted towards one population (the latter  
>doesn¹t seem like it counts as true ³coactivity.²).  Coactivity seems  
>like it should be 1 (or 100%) only if whenever one population is active,  
>the other population is active at the same level. (d) Does heterogeneity  
>across all intrinsic parameters matter equally or are some particularly  
>important for this effect?  Introducing heterogeneity in one parameter at  
>a time seems like it would also be very informative here.

I too don’t like that there is only one simulation for the heterogeneity example. I understand it’s origin, but would prefer a method that allows the generation of multiple comparison points.

I am not sure I agree with the statement on what constitutes coactivity, unless I am misunderstanding it. Would this point be placated with a rewording of the explanation of how co-activity is classed?

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>Reviewer: 2  
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>Comments to Author  
>The manuscript presents three different pieces of evidence to support a  
>role of cellular heterogeneity in the combinatorial computational  
>capacities of the anterior cingulate cortex. First, in vitro studies in  
>ACC slices activated with ketamine show that also in ACC beta and gamma  
>rhythms are easily generated with glutamatergic activation and the  
>rhythms are related to the decay time course of inhibitory postsynaptic  
>potentials. Second, in vitro studies in slices under pharmacological  
>blockade of glutamatergic transmission show that neurons in ACC have  
>highly variable intrinsic properties, not readily attributable to  
>distinct cellular classes. Finally, computational modeling of cellular  
>diversity in a network of excitatory and inhibitory neurons shows how the  
>network becomes able to represent rhythms with different carrier  
>frequencies within one EEG band (beta or gamma), thus making it capable  
>of combinatorial computations as expected for a hub area like ACC. There  
>is a parallel line that describes the laminar distribution of rhythms and  
>cellular properties in ACC, but this is lateral to the main line of the  
>manuscript.  
>  
>I have 3 main concerns:  
>1) The manuscript wants to make the point that cellular diversity in ACC  
>is very high and does not allow one to attribute it to separate neuronal  
>classes but rather to the broad heterogeneity of intrinsic properties  
>within a single population, and this would be particular of ACC and  
>possibly would explain its hub-like character. However, since the  
>manuscript does not compare ACC with a different area, it is not possible  
>to reach the conclusion that heterogeneity in ACC is particularly high.  
>As a matter of fact, the clustering analyses shown do find classification  
>schemes that organize neuron into different classes depending on their  
>intrinsic properties, as it has been proposed in other cortical areas.  
>The specificity of ACC that would justify asking what computational  
>implications this has thus becomes a quantitative issue, which cannot be  
>resolved from this manuscript's data. This is also not related to data in  
>the literature. For instance, the sentence "The failure of traditional  
>methods to readily segregate electrophysiological cell classes in ACC  
>based on in vitro data strongly suggests that the diversity of cell  
>intrinsic properties in ACC may be greater than in other areas" (page 22)  
>does not provide references to compare quantitatively with this  
>manuscript's data to reach the conclusion that ACC is different.

If references can’t cover this, Miles’ suggestion of delving into data already gathered will hopefully provide a solution. Though, time, it will take.

>  
>2) The computational part of the manuscript is essential in making the  
>point about how ACC heterogeneity impacts rhythmic processing (as this  
>was not experimentally addressed due to technical limitations, page 19).  
>In my view, this is the weakest part of the manuscript. The logic resides  
>in comparing a homogenous network, with clock-like oscillatory dynamics  
>(which would represent non-hub cortical areas) with a heterogeneous  
>network that represents ACC. The network that is taken as reference to  
>address the role of cellular heterogeneity is not a good model to  
>represent oscillatory cortical activity in sensory cortices, which is  
>already very irregular to start with. In other words, if instead of this  
>clock-like oscillatory network (not plausible biologically) an irregular  
>oscillatory model with homogeneous cellular properties was taken (Brunel  
>and Hakim 1999, Brunel 2000), would the role of heterogenous cellular  
>properties still be the same? Also, if irregularity in the homogeneous  
>network is increased by means of sparse connectivity, or heterogeneity in  
>coupling strengths, would there be any difference in the results? The  
>fact that random Gaussian noise (which averages in time) already improves  
>coactivity significantly in Fig. 10 is worrying. How would sources of  
>quenched noise other than pyramidal cell heterogeneity affect coactivity  
>in this clock-like network? If any quenched noise (prominent in all  
>cortical areas) leads to high coactivity, then what is specific about  
>ACC? An additional limitation of the model is the lack of interactions  
>between excitatory neurons, a prominent feature of cortical circuits.

Surely it is interesting that you do not need to add noise to get the properties that a heterogeneous network provides and this is truer physiological representation than adding noise. I believe I refrained from adding a sentence that said this in the final draft, and perhaps I shouldn’t have! Our paper addresses a specific aspect of network dynamics that can produce these results, but of course other factors may contribute – they are not the subject of this paper. I am confused as to why this is an issue. However, the extent of coactivty in the ACC network as compared to other networks may be more convincing with that other data we can try to dig out.

>  
>3) The manuscript is very scarce with statistical tests. Often important  
>conclusions are drawn without any explicit statistical test. For  
>instance, the difference in IPSP time course for beta and gamma rhythms  
>is not tested but drives all the computational modeling effort.  
>

A histogram of IPSP decays for gamma, beta and dual rhythms are shown for individual examples. If this is not sufficient, more data would be needed. However, surely the current results are illustrative of the effects we are seeing, and the results are presented as such, and no more than that (although perhaps this needs emphasizing). The fact that we use different decays in the model for input to the network, again, may need distinguishing better in the text in terms of internal vs external rhythms.

>  
>In general, the text and figures have important limitations (below). The  
>text could be significantly streamlined by reducing detail on clustering  
>analyses and laminar distributions, which are rather lateral to the main  
>story.  
>  
>Other issues:  
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>subsection "Local network excitation". This paragraph is very confusing.  
>It first refers to Fig. 2A as showing an example of the small number of  
>cells (how many? out of how many?) that did show EPSPs in control,  
>non-activated slices. Immediately after, however, it refers to Fig. 2A as  
>showing an example of how EPSPs detected in cells during KA activation.  
>Also, it states that no clear peak can be seen in the power spectra, but  
>the power spectra in Fig. 2Aii are both remarkably peaked at beta and  
>delta/theta frequencies. Also, the caption does not help. It says that  
>Fig. 2Ai shows EPSPs during evoked beta, that are not frequency or phase  
>locked to the LFP, but that cannot be deduced from panel Ai. Panel Aii is  
>not mentioned in caption. All in all, there is no clear message to learn  
>from Fig. 2A. Why is this data here?

I don’t find these peaks remarkable (perhaps they’re used to looking at in-vivo power spectra on a log scale??) and the cross-correlation inset should make it clear that the EPSPs do not follow the field.

>  
>subsecion "Local network inhibition". The limited number of recordings  
>and diversity of the results observed limits the impact of this  
>subsection findings. It would be good to get a clear count of the  
>different behaviors: how many recordings had LFP beta, how many had both  
>beta and gamma in the LFP? how many of those in each layer? How were the  
>different behaviors in Fig. 2B distributed in layers? The comparison of  
>IPSP decay times for events rhythmic with LFP beta or with LFP gamma  
>should indicate the number of recordings in each average and a  
>statistical test to check if they are statistically different.  
>  
>Figure 2. The labeling used to refer to this multipanel figure is not  
>used consistently in the text (for instance: "see IPSPs in Fig. 2Bia:  
>modal peak at 15 ms" when the panel showing distribution of IPSP decay  
>times is in panel 2Biiia. This distribution does not have the mode near  
>15 ms, though). Panels 2Biiia and 2Biiib are identical, exactly the same,  
>which is very unlikely. Please double check. Simple visual inspection in  
>Fig. 2Bib suggests that the distribution of IPSP decays will be broader  
>for this recording, compared to Fig. 2Bia.  
>  
>subsection "ACC intrinsic cell properties". The manual grouping of cells  
>into 5 classes is not very convincing. In particular, the examples shown  
>to illustrate these classes are confusing. How can we see in Fig. 3A that  
>Group 4 has very strong spike adaptation? All this subsection is purely  
>qualitative, and the conclusions are weakly supported by the data. For  
>instance "cells in Group 3 and Group 4 were more common in layer CI than  
>any other layer (Fig. 3B,C)" is not in line with the data in Fig. 3C,  
>where the yellow sector seems as large for LV than for LVI. Statistics  
>missing here, too. Figure 3Aii shows some very faint gray traces in the  
>background that are confusing.  
>  
>subsection "Focus on Ih...". The text does not explain what criterion was  
>used to decide if one neuron had a visible Ih or not. Was this purely by  
>visual inspection or was it based on a bimodal distribution of voltage  
>deflections following a hyperpolarization step? No statistical test  
>supports the homogeneous distribution of Ih among cell classes.  
>  
>The authors spend 4 figures (3, 4, 5 and 6) to try and make sense of  
>clustering of cell properties in ACC. Each new classification has little  
>relation to the previous one and does not allow one to learn much,  
>although they all provide quite an impressive clustering. After this  
>lengthy and technically demanding exercise there is hardly a clear  
>bottomline. The reading of the authors that cells cannot be clustered is  
>hardly a straightforward conclusion. The most powerful analysis (Fig 6)  
>provides quite convincing clustering.  
>  
>Supplementary Figure 1. The order of the panels (top-middle-bottom) seems  
>to be scrambled relative to the caption description.  
>  
>Figure 7. Panel C is very confusing. Correlations shown in gray scale are  
>offset relative to the square grid that matches the axes. If I understand  
>this figure correctly, all upward pointing green triangles should be in a  
>light gray square, and all downward pointing red triangles should be  
>lying on a dark gray square, but this is not happening.  
>  
>On page 25 it is constantly emphasized that in different realizations  
>inputs are applied to different subsets of E-cells in the network, but  
>the Methods section instead says that the network only had 80 neurons. It  
>may be argued that possibly simulating different 80-neuron networks may  
>be similar to stimulating different 80-neuron subsets of a larger  
>network, but the text should be specific about what really was simulated.  
>  
>On page 25 "coactivity" is used without any proper definition of this  
>term. Although this is described in the caption, some brief intuitive  
>description is needed in the text so the logic can be followed easily by  
>the reader. Specifically, it is unclear why high coactivity should be  
>associated with better performance in ACC.  
>  
>Figure 10C. For a fair comparison n=256 heterogeneous networks should be  
>simulated and box plots of coactivity be shown and compared with  
>homogeneous network simulations. Why can homogenous noiseless reach a  
>significant value of coactivity? Where does variability in these  
>simulations come from? There is too little information regarding the  
>model in the methods.  
>  
>The biophysical neuron model used is not specified in the Methods  
>section. Please briefly mention what formalism was used (two-compartment  
>Hodgkin-Huxley model?). Is the model identical to Paptousi et al. 2014?  
>There are no equations nor parameter values provided in this manuscript.  
>What were the maximum and minimum values of the parameters that changed  
>to generate heterogeneity in cellular properties? How are inputs  
>delivered in Fig. 9 and 10? Please make sure all your simulations can be  
>reproduced using the information in the manuscript.  
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