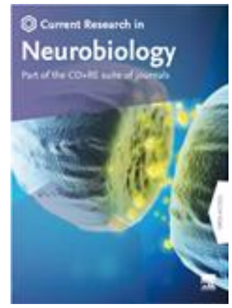
Peer Review Overview



Manuscript Title: Visual modulation of firing and spectrotemporal receptive fields in mouse auditory cortex

Received	Feb 08,2022
1st Decision	Mar 30,2022
1st Revision Submitted	Apr 26,2022
Accepted	May 06,2022

1st Decision letter

Reference: CRNEUR-D-22-00008
Title: Visual modulation of firing and spectrotemporal receptive fields in mouse auditory cortex
Journal: Current Research in Neurobiology

Dear Dr. Hasenstaub,

Thank you again for submitting your manuscript to Current Research in Neurobiology. Two peer reviewers and I have completed our evaluations of your manuscript. I am delighted to let you know that we all agree it is an interesting, well-conducted, and valuable scientific study. We suggest only minor revisions to your submitted version prior to acceptance, largely relating to the organization of your figures. Our reviews in detail can be found below.

I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Apr 29, 2022.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline in a cover letter every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

Current Research in Neurobiology values your contribution and I look forward to receiving your revised manuscript.

CRNEUR aims to be a unique, community-led journal, as highlighted in the Editorial Introduction. As part of this vision, we will be regularly seeking input from the scientific community. We encourage you and your co-authors to take the survey as part of the editorial.

Kind regards,

Kerry Walker, DPhil
Associate Editor
Current Research in Neurobiology

Comments from Editors and Reviewers:

Associate Editor:

This interesting and carefully conducted study makes important contributions to our understanding of cross-modal representations in sensory cortex. The results are insightful and well interpreted. The STRF results are particularly new and build upon previous approaches in this field.

Overall, the manuscript is well written and the data are clearly presented. However, the reviewers and I agree that the organization of figures could be improved. I agree with the reviewers' suggestion that the use of "Aa" format labelling of large numbers of figure panels is confusing. I suggest that the authors split the capital letter panals into separate figures wherever possible, for simplicity. For example, figure 1 could be easily split into 3 figures: one describing ephys collection and pre-processing (current A-D), one describing ephys analysis timepoints (Ea-b), and a third on STRF calculation (Fa-c). This would eliminate the need for many of the sub-sub-figure labels. Please consider this approach throughout all the figures. Our journal has no figure number limits. Where the authors think that sub-sub-figure labelling is unavoidable, it might be clearer to use "Ai" roman numerals rather than "Aa".

Reviewer #1:

Manuscript synopsis
The study examines the responses of auditory cortical neurons in the awake, passively listening mouse across cortical depths when presented with random double sweep (RDS) stimuli. These sounds allow characterization of spectrotemporal receptive fields (STRFs) and were presented either alone or together with a visual stimulus (contrast modulated noise) that began before and ended after the sound. The authors contrast the properties of units recorded from different depth bins (a proxy for cortical layer) and with different spike shapes (broad and narrow spiking units that may correspond to putative excitatory and inhibitory neurons respectively).

The authors use their stimulus design to first identify units that respond to visual stimuli, and then to examine whether these cells are also responsive to sound. They demonstrate that visual modulation of auditory responses differs depending on the time window in which sound-evoked activity is considered, and even without changes in overall firing rate, visual stimuli may modulate spectrotemporal receptive fields (primarily through changes in gain rather than tuning).

The STRF approach to studying multisensory integration is an interesting idea and the data provide new knowledge about the effects of multisensory integration on different cell types in the awake mouse auditory cortex. Having reviewed a previous version of the manuscript at another journal, I can see that the authors have made extensive revisions to the paper that addressed many of my original concerns. I therefore have only a few comments.

Major comments
The double-lettered system (e.g. Aa, Ab, Ba, Bb etc.) in the figures is unnecessarily confusing and caused me to look in the wrong places for data on multiple occasions. This, together with the inclusion of titles in the figures (rather than the captions) indicates that the figures are too dense. Figure 4 for example has 26 separate plots. It would be better to subdivide most figures into separate figures to help the

reader through the data.

Minor
Graphical abstract: Just a suggestion, but for the STRF panel, it might be worth specifying the gain rather than tuning changes that are reported, as it's a useful extra detail to know.

Line 467 to 476: I don't think it would change the overall results, but I think there is some room to improve the statistical testing here. Specifically, the analysis of modulation of onsets vs. sustained responses in BS and NS cells at different layers includes 10 separate Chi-squared tests, when a general linear mixed model could capture all of the factors (time window, cell type and cortical layer) in a single test. I appreciate that the authors might not feel comfortable with these approaches, and so I leave it up to them; however using such modelling approaches has the potential to more clearly isolate take-home points than an otherwise rather dense set of descriptive statistics.

Line 481, "these predictions" - please clarify that "these" refers to.

Line 484-489: The statistical test here seems slightly more complicated than necessary. Rather than binning sound evoked sustained firing rates and performing a Chi-squared test on the proportion of visually modulated units, why not just use a logistic regression instead. I doubt the results will really change, but binning tends to remove information from data in a way that isn't needed in order to answer the experimental question.

Fig. 4. Please add labels for the bar plots on the right in C-E to indicate which depth group is which. Without this information, it is hard to know whether the conclusions being drawn are valid.

Line 519: "This outcome suggests that visual modulatory influences over driven FRs and sensitivity to spectrotemporal features are dissociable in most neurons." A permutation test (in which labels for visually modulated STRF and FR were shuffled across units) would allow the authors to test this assertion.

Reviewer #2:

This study investigates the impact of visual stimulation on mouse auditory cortex neurons. This piece of work follows after a previous publication of the same team which demonstrated for the first time the presence of visual responses in deep layers of the auditory cortex in mice. The present study deepens these pionneering results by investigating into much more details the distribution and nature of visual impact on auditory cortical representations of sounds. The study combines precise receptive field measurements with identification of other responses parameters such as sustained responses.
The first important result of the study is that beyond visual responses in deep layers of the cortex there are visual modulations of auditory receptive fields that are more widespread in cortex. More over the authors bring clear evidence that the modulation of sustained responses and of linear receptive field happen in disjoint subsets of neurons indicating a fonctional specialisation of these two response types. This is interesting because it echoes with recent work indicating that cortex features different neuronal subspaces dedicated to either sensory processing or other information that arrive in cortex. These data indicate that there are subsets of neurons that encode distinct aspects of sounds, and that they are differentially impacted by vision.

The data collection in awake head fixed animals, the analysis and statistics are all very carefully

implemented. The manuscript is maybe too dense with too many figure panels (and subpanels), however in this way the decription is very exhaustive. I recommend to put secondary panels in supplementary figures, for clarity.
The pre-processed data should be made available in relevant repositories.

I have no specific comment on this study and I think it is rigourous and interesting enough to be published in this state in Current Research in Neurobiology.

1st Author Response Letter

Response to comments from Editors and Reviewers:

We thank the reviewers and editors for their reviews and helpful comments on our manuscript. We have implemented their suggestions in our revised manuscript and replied to each comment below.

In addition, while the manuscript was under review, we discovered and fixed a minor bug in one step of the STRF calculations. Whereas we reported that STRF calculations ignored the first 200 ms of the stimulus (the same as for sustained FR responses), we discovered that the calculation ignored only the first 100 ms. We corrected the error, re-ran all STRF calculations, and updated all relevant figures and results in the manuscript. As expected, the updates did not have any material impact on the results or alter any statistical outcomes.

Editor and Reviewer comments:

Associate Editor:

This interesting and carefully conducted study makes important contributions to our understanding of cross-modal representations in sensory cortex. The results are insightful and well interpreted. The STRF results are particularly new and build upon previous approaches in this field.

Overall, the manuscript is well written and the data are clearly presented. However, the reviewers and I agree that the organization of figures could be improved. I agree with the reviewers' suggestion that the use of "Aa" format labelling of large numbers of figure panels is confusing. I suggest that the authors split the capital letter panals into separate figures wherever possible, for simplicity. For example, figure 1 could be easily split into 3 figures: one describing ephys collection and pre-processing (current A-D), one describing ephys analysis timepoints (Ea-b), and a third on STRF calculation (Fa-c). This would eliminate the need for many of the sub-sub-figure labels. Please consider this approach throughout all the figures. Our journal has no figure number limits. Where the authors think that sub-sub-figure labelling is unavoidable, it might be clearer to use "Ai" roman numerals rather than "Aa".

We implemented the suggested figure reorganization by breaking down the original six figures into thirteen. We reduced using subpanel labels to a minority of cases in which we used capital letters for panels (“A”) and numbers for subpanels (“1.”).

Comments from Reviewer 1

Manuscript synopsis

The study examines the responses of auditory cortical neurons in the awake, passively listening mouse across cortical depths when presented with random double sweep (RDS) stimuli. These sounds allow characterization of spectrotemporal receptive fields (STRFs) and were presented either alone or together with a visual stimulus (contrast modulated noise) that began before and ended after the sound. The authors contrast the properties of units recorded from different depth bins (a proxy for cortical layer) and with different spike shapes (broad and narrow spiking units that may correspond to putative excitatory and inhibitory neurons respectively).

The authors use their stimulus design to first identify units that respond to visual stimuli, and then to examine whether these cells are also responsive to sound. They demonstrate that visual modulation of auditory responses differs depending on the time window in which sound-evoked activity is considered, and even without changes in overall firing rate, visual stimuli may modulate spectrotemporal receptive fields (primarily through changes in gain rather than tuning).

The STRF approach to studying multisensory integration is an interesting idea and the data provide new knowledge about the effects of multisensory integration on different cell types in the awake mouse auditory cortex. Having reviewed a previous version of the manuscript at another journal, I can see that the authors have made extensive revisions to the paper that addressed many of my original concerns. I therefore have only a few comments.

Major comments

The double-lettered system (e.g. Aa, Ab, Ba, Bb etc.) in the figures is unnecessarily confusing and caused me to look in the wrong places for data on multiple occasions. This, together with the inclusion of titles in the figures (rather than the captions) indicates that the figures are too dense. Figure 4 for example has 26 separate plots. It would be better to subdivide most figures into separate figures to help the reader through the data.

We implemented the suggested figure reorganization (see response to Associate editor comments).

Minor

Graphical abstract: Just a suggestion, but for the STRF panel, it might be worth specifying the gain rather than tuning changes that are reported, as it's a useful extra detail to know.

We updated the graphical abstract to specify STRF gain changes.

Line 467 to 476: I don't think it would change the overall results, but I think there is some room to improve the statistical testing here. Specifically, the analysis of modulation of onsets vs. sustained responses in BS and NS cells at different layers includes 10 separate Chi-squared tests, when a general linear mixed model could capture all of the factors (time window, cell type and cortical layer) in a single test. I appreciate that the authors might not feel comfortable with these approaches, and so I leave it up to them; however using such modelling approaches has the potential to more clearly isolate take-home points than an otherwise rather dense set of descriptive statistics.

We appreciate the suggested alternative statistical approach (GLM). We agree that it would not likely change any of the conclusions in the paper and thus retained the original approach.

Line 481, "these predictions" - please clarify that "these" refers to.

We clarified the statement as follows:

> “To determine whether our data fit the predicted inverse relationship between multisensory influences and unisensory response strength, we directly examined the relationship between auditory and visual modulation effect sizes (**Fig 7C**).”

Line 484-489: The statistical test here seems slightly more complicated than necessary. Rather than binning sound evoked sustained firing rates and performing a Chi-squared test on the proportion of visually modulated units, why not just use a logistic regression instead. I doubt the results will really change, but binning tends to remove information from data in a way that isn't needed in order to answer the experimental question.

We added the suggested logistic regression analysis, which confirmed the null results obtained by Chi-squared tests. We retained the original binned plots and Chi-square tests to aid visual representation and interpretation for readers with less developed intuitions about logistic regression. The results are as follows:

> “Extending these outcomes, auditory effect size (absolute z-score) was not significantly predictive of units with significant visual modulation effects using logistic regression (all p-values > 0.370).”

Fig. 4. Please add labels for the bar plots on the right in C-E to indicate which depth group is which. Without this information, it is hard to know whether the conclusions being drawn are valid.

We added the requested depth labels to this and all other relevant figures.

Line 519: "This outcome suggests that visual modulatory influences over driven FRs and sensitivity to spectrotemporal features are dissociable in most neurons." A permutation test (in which labels for visually modulated STRF and FR were shuffled across units) would allow the authors to test this assertion.

We added the suggested permutation test:

> “As seen in **Fig. 10**, there was surprisingly little overlap between units with visually modulated STRFs and visually modulated sustained FRs (NS: 8/24, 33.3%; BS 11/58, 19.0%). We conducted permutation tests to determine whether the observed degree of overlap (units for which visual

stimulation modulated both FRs and STRFs) fell within the limits expected by chance. By shuffling the labels of neurons with modulated FRs and modulated STRFs, we created null distributions reflecting the proportions of units for which modulation effects coincided by chance (10^4 iterations). For both unit types, the observed fractions of neurons with overlapping modulation effects were significantly smaller than expected by chance (NS: observed = 0.292, null median = 0.524, $p = 0.012$; BS: observed = 0.190, null median = 0.592, $p < 10^{-4}$). This outcome underscores the notion that units with modulated FRs and modulated STRFs occur in largely distinct neuronal populations, perhaps reflecting distinct mechanisms. In total, at least one aspect of sound encoding was modulated by visual context for roughly one in ten neurons (NS 24/212, 11.3%; BS 58/589, 9.9%)."

Comments from Reviewer 2

This study investigates the impact of visual stimulation on mouse auditory cortex neurons. This piece of work follows after a previous publication of the same team which demonstrated for the first time the presence of visual responses in deep layers of the auditory cortex in mice. The present study deepens these pionneering results by investigating into much more details the distribution and nature of visual impact on auditory cortical representations of sounds. The study combines precise receptive field measurements with identification of other responses parameters such as sustained responses.
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The data collection in awake head fixed animals, the analysis and statistics are all very carefully implemented. The manuscript is maybe too dense with too many figure panels (and subpanels), however in this way the decription is very exhaustive. I recommend to put secondary panels in supplementary figures, for clarity.

We implemented the suggested figure reorganization (see response to Associate editor comments).

The pre-processed data should be made available in relevant repositories.

As indicated in our Author Checklist and Data Availability statement, all data are available upon request to the corresponding author. At present, there is no standard format/repository for sharing the types of pre-processed data analyzed in our study, so we think direct correspondence is the best way to ensure appropriate use and analysis. We will defer to the editors' judgment if submitting the pre-processed data to a repository is deemed necessary.

I have no specific comment on this study and I think it is rigourous and interesting enough to be published in this state in Current Research in Neurobiology.

Accept Letter

Dear Dr. Hasenstaub,

Thank you for submitting your manuscript to Current Research in Neurobiology.

I am pleased to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate and value your contribution to Current Research in Neurobiology. We regularly invite authors of recently published manuscript to participate in the peer review process. If you were not already part of the journal's reviewer pool, you have now been added to it. We look forward to your continued participation in our journal, and we hope you will consider us again for future submissions.

CRNEUR aims to be a unique, community-led journal, as highlighted in the Editorial Introduction. As part of this vision, we will be regularly seeking input from the scientific community and encourage you and your co-authors to take the survey.

Kind regards,

Kerry Walker, DPhil
Associate Editor
Current Research in Neurobiology

Editor and Reviewer comments:

The authors have incorporated the most important suggestions from the reviewers, which have improved the rigor and readability of their manuscript. I congratulate them on this excellent scientific work, and am delighted to accept this manuscript for publication as a research article in Current Research in Neurobiology.

-------- End of Review Comments --------