Single cell and population encoding in input and associative layers of mouse auditory cortex across strains

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Introduction

Sound stimuli are encoded by populations of neurons in the primary auditory cortex (A1). Sound information arrives at its input layer 4 from where activity propagates to associative layer 2/3. Given the hierarchical structure of A1, the encoding of sound information is thought to be transformed between layers, but the nature of this transformation is unclear. Since stimulus information is represented in populations of neurons, we investigated the spatiotemporal organization of neuronal population activity across layers. Mice on the C57BL/6 background are commonly used to study cortical processing, yet these mice develop high frequency hearing loss with age making them a less optimal choice for auditory research. In contrast, mice on the CBA background retain better hearing sensitivity in old age. Therefore, we performed comparative analysis of neuronal populations from both adult (~10 weeks) C57BL/6 mice and CBAxC57 mice. We used in vivo 2-photon imaging of pyramidal neurons in cortical layers L4 and L2/3 of awake mouse A1 to characterize the populations of neurons that were active both during tonal stimuli and in the absence of any stimulus. We further characterized the spatiotemporal population activity via neuronal ensembles, defined as neurons that are active within or during successive temporal windows at the temporal resolution of our imaging rate.

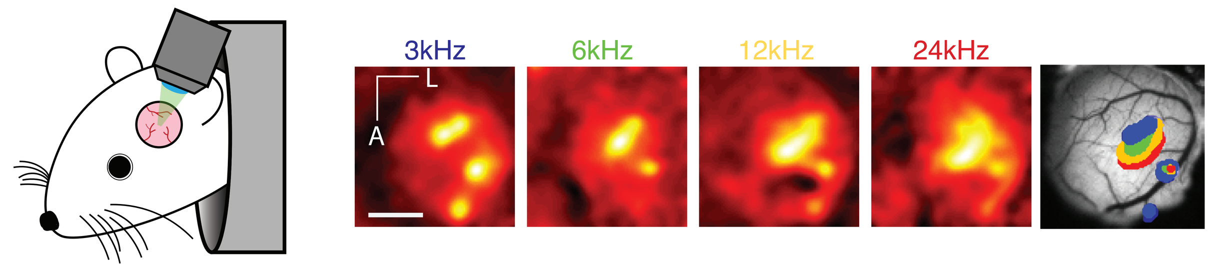
Experimental Methods

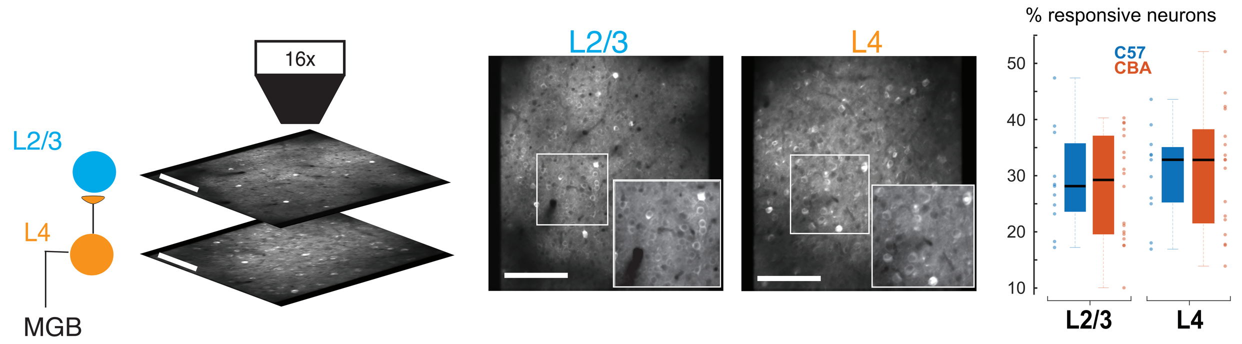
Animal preparation and Imaging

- We used transgenic mice that expressed GCaMP6s under the Thy1 promoter (Dana et al., 2014, Jax: 024274, GP4.3) bred on a C57 background which has a genetic mutation that predisposes them to early onset age related hearing loss. To address this we used the F1 generation of a hybrid mouse line that provides a non-mutated copy of the mutated gene. (CBA/CaJ x C57BL/6-Tg)
- Additionally, some L2/3 datasets were obtained from adult mice (>P40) that received injections of AAV-mRuby2-GCaMP6s in the auditory cortex and were implanted with head plate and chronic cranial window.
- 2P imaging experiments were performed on awake mice. Mean age at imaging was P75 (±21 days).
- We imaged using Ti-Saphh laser (940nm, Coherent, Vision-S) coupled to a microscope (Thorlabs, BScope2, resonant scanning, 512x512 pixels; 30 frames/s).
- Mid-frequency fields of A1 were targeted for 2P imaging based on prior widefield imaging.
- Sound evoked activity was acquired using sinusoidally amplitude modulated tones (3-48kHz, half-octave spacing, 1s duration) were presented at 3 or 4 different levels (80, 60, 40, 20dB SPL)

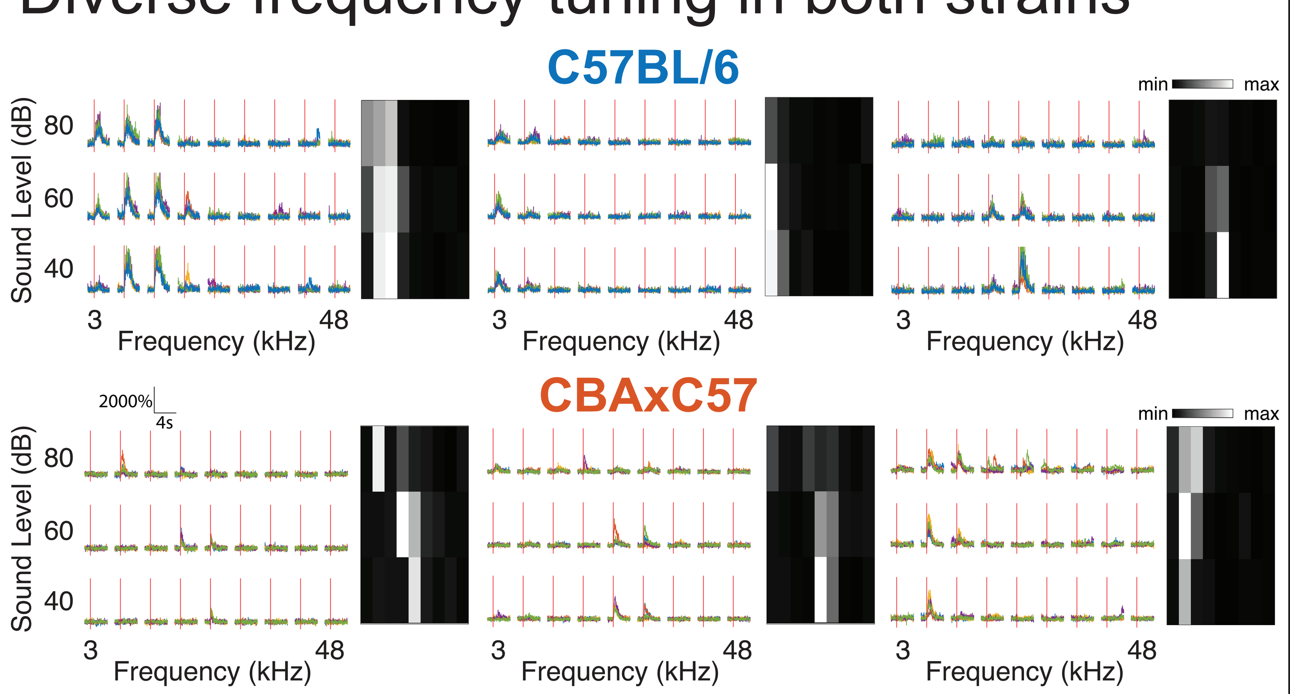
Image Processing and Data Analysis

- Individual ROIs were manually selected from the time averaged image after motion correction
- Fluorescence (F) over time was extracted from each ROI and converted to dF/F
- Significant neuronal responses were determined through comparison of baseline dF/F to stimulus dF/F
- Spike deconvolution was performed using Suite2p



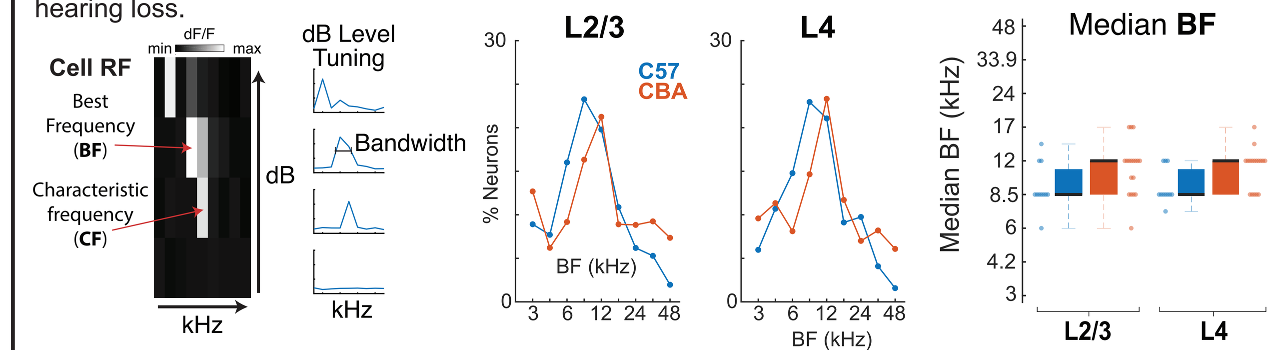


Diverse frequency tuning in both strains

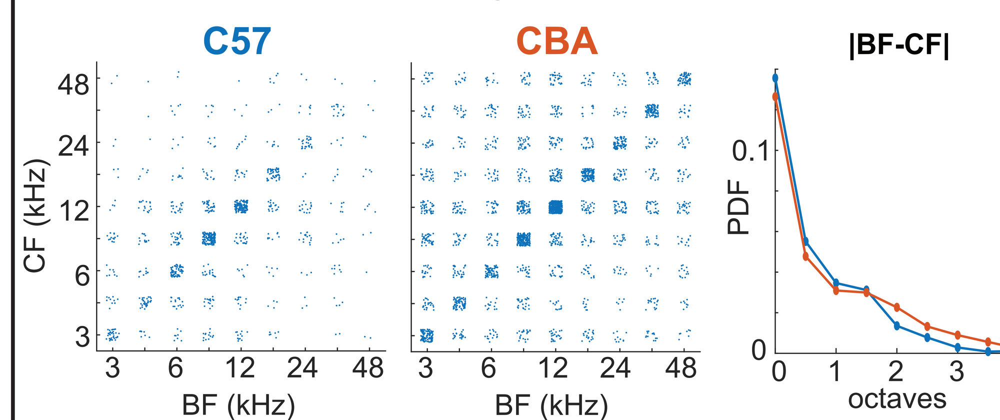


Fewer high frequency BFs in C57 mice

C57 mice had broader distribution of cells with BFs in the mid-frequency region (6-17kHz), but a reduced number of cells representing the high frequencies (33-48kHz), consistent with peripheral high frequency hearing loss.



BF and CF are less aligned in CBA mice



- BF and CF can differ as a result of nonlinear rate-level changes
- CF and BF are mostly correlated in both strains
- C57 mice have fewer neurons with a mismatched BF and CF

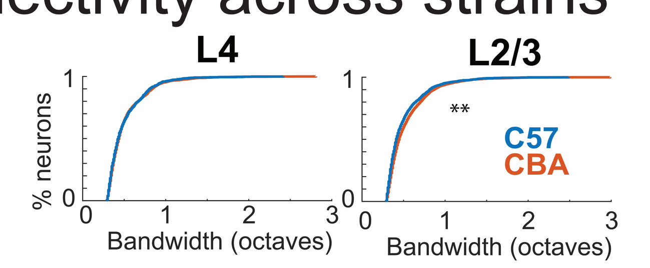
- Broader BF vs CF distribution in CBA mice could indicate that individual cells respond to a broad range of frequencies at varying SPLs, whereas cells in C57s mice are often sharply tuned.

→C57 mice have worse detection of quieter sounds.

→Each neuron may only have significant responses at one or two SPLs, causing the maximal FRA response (BF) to occur at the lowest SPL with a significant response (CF).

Similar frequency selectivity across strains

Bandwidth distributions in L4 were similar in both mouse strains. C57 mice had slightly narrower bandwidths than the CBA mice in L2/3, indicating that C57 mice had sharper tuning for tonal stimuli. However the effect size was small.

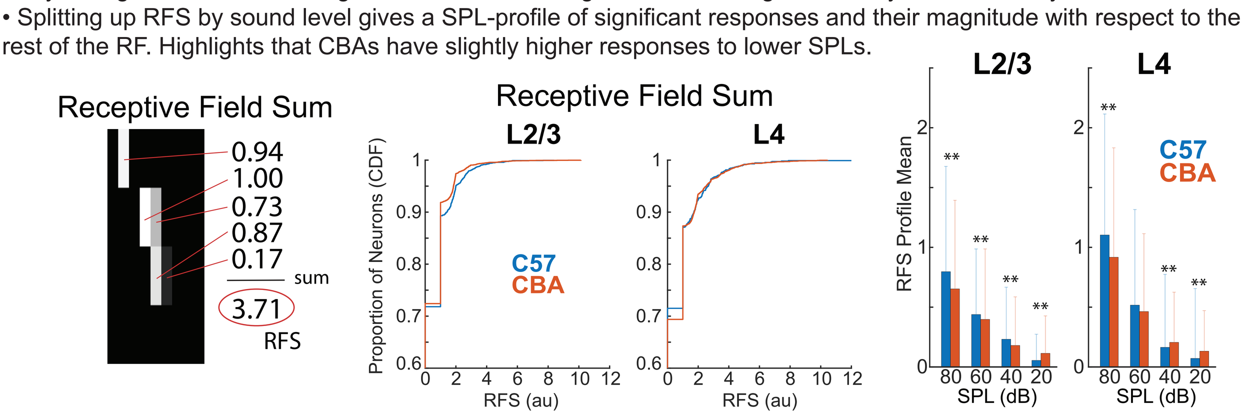


CBA have stronger responses to quiet sounds

- A1 responses can have nonlinear changes in magnitude with increasing sound level
- We created **Receptive Field Sum (RFS)** as a more inclusive measure than bandwidth to quantify and compare nonlinear receptive fields
- L2/3 neurons in CBA mice have a slightly lower distribution of RFS values than C57 mice

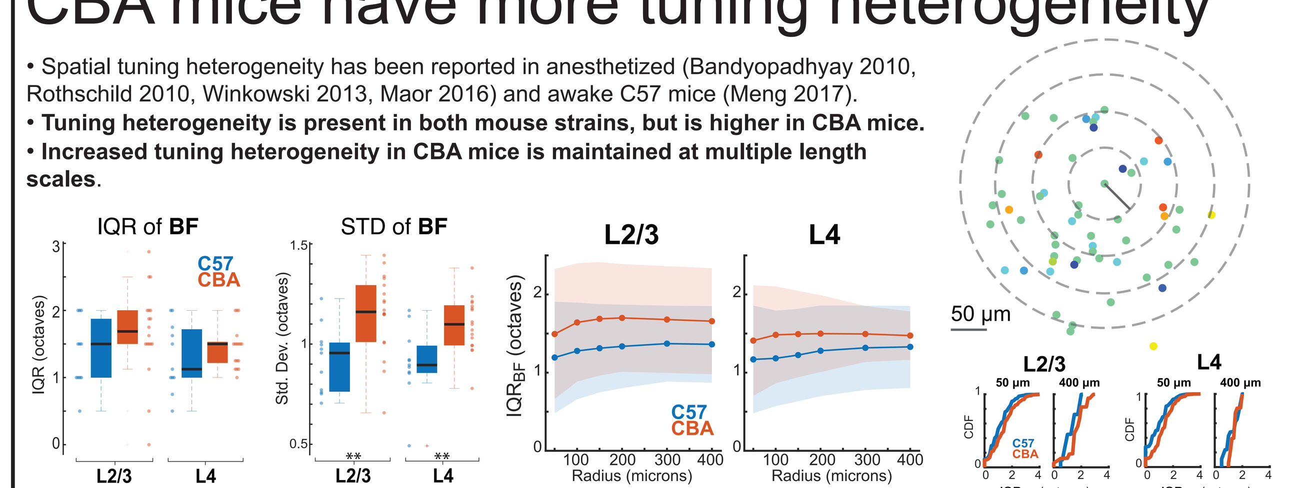
→C57 L2/3 populations have narrower bandwidth, so the higher RFS may indicate that C57 mice have a higher magnitude response to a select few stimuli in their receptive field consistent with reduced inhibition.

- Any changes in overall RF magnitude as a result of age related hearing loss is only reflected in layer 2/3.
- Splitting up RFS by sound level gives a SPL-profile of significant responses and their magnitude with respect to the rest of the RF. Highlights that CBAs have slightly higher responses to lower SPLs.



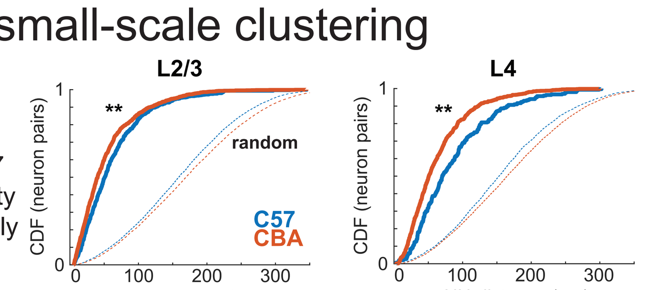
CBA mice have more tuning heterogeneity

- Spatial tuning heterogeneity has been reported in anesthetized (Bandyopadhyay 2010, Rothschild 2010, Winkowski 2013, Maor 2016) and awake C57 mice (Meng 2017).
- **Tuning heterogeneity is present in both mouse strains, but is higher in CBA mice.**
- **Increased tuning heterogeneity in CBA mice is maintained at multiple length scales.**



Similarly tuned neurons show small-scale clustering

Prior cluster (Bandyopadhyay 2010) and nearest-neighbor analysis (Zeng et al., 2019) suggest that same-tuned neurons are locally clustered. CBA mice had smaller nearest-neighbor distances than C57 mice indicating that despite the increased heterogeneity in CBA FOVs, the similarly tuned cells are more densely localized.



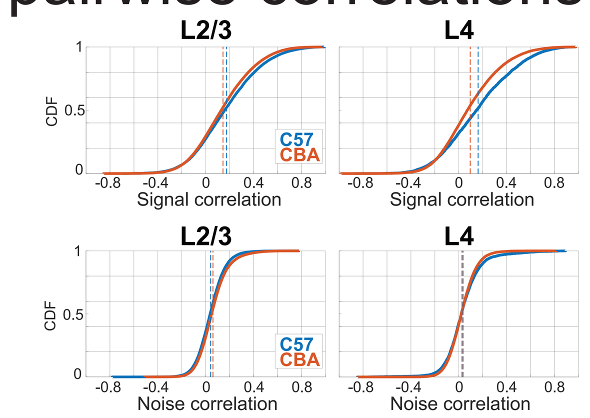
C57 mice have higher pairwise correlations

- Signal correlations infer similar feedforward input to neuronal pairs
- SC are higher in C57 mice in both L2/3 and L4

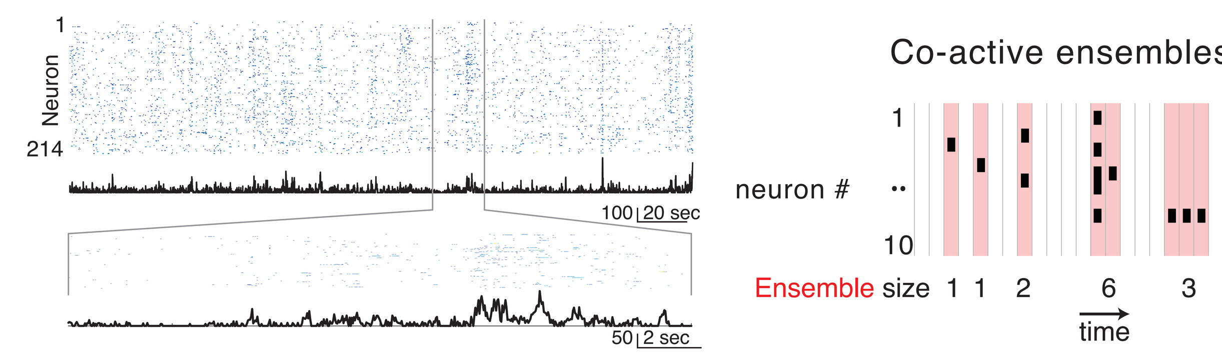
→Neuronal pairs in C57 mice have more similar inputs than in CBA mice

→CBA mice have significant responses across more frequencies, leading to lower covariance across the neuronal population

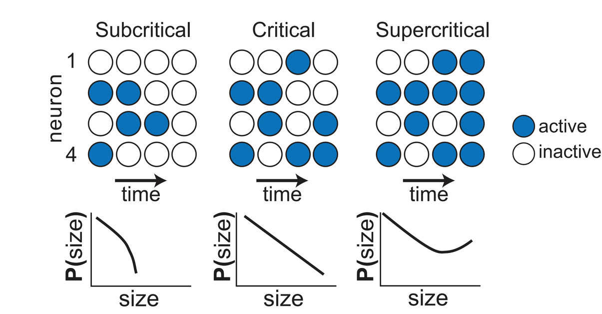
- Noise correlations infer direct connections between neurons or shared sources of trial fluctuations
- Noise correlations were largely similar in both mouse strains



A1 contains co-active neuronal ensembles



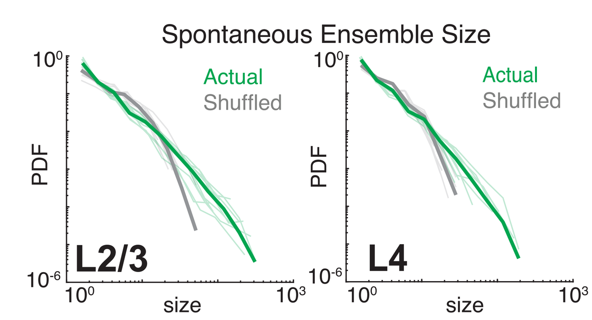
Neuronal ensembles exhibit critical dynamics



- Network activity in a critical system propagates in a balanced manner
- Characterized by power law of ensemble sizes (among several other metrics)

→Implies a healthy (E/I balance) system

→Maximizes dynamic range for input processing, fidelity of information transmission, and information capacity



- PDFs of spontaneous ensemble sizes were significantly better fit by a power law compared to an exponential function

→Temporal shuffling of each neuron's inferred spike raster abolished the power laws indicating that the temporal structure of ensemble activity is essential for critical dynamics

- **Presence of power-law distributions for ensemble size suggests that ongoing ensembles in L4 and L2/3 organize as scale-invariant neuronal avalanches.**

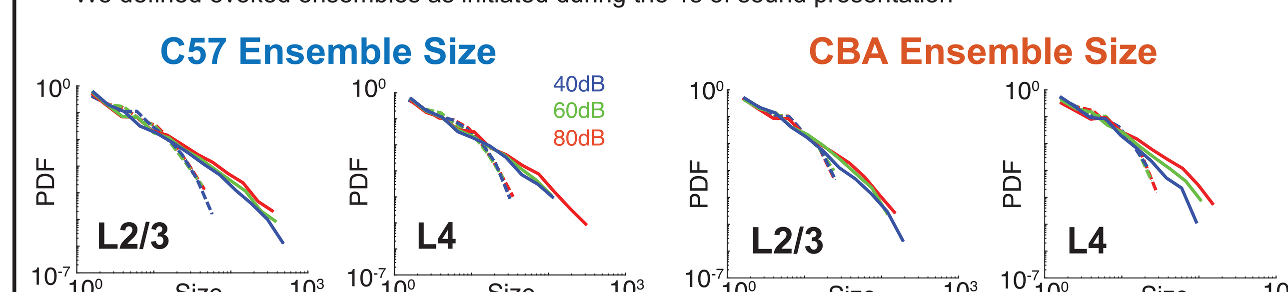
How does a sound stimulus perturb ensemble activity in A1?

- More A1 neurons fire and are recruited into ongoing ensembles

→Sound-evoked ensembles could deviate from a power law organization of avalanches by selectively increasing the incidence of large ensembles

→Alternatively, sound-evoked A1 ensemble activity could maintain scale-invariant organization by increasing the incidence of ensembles of all sizes.

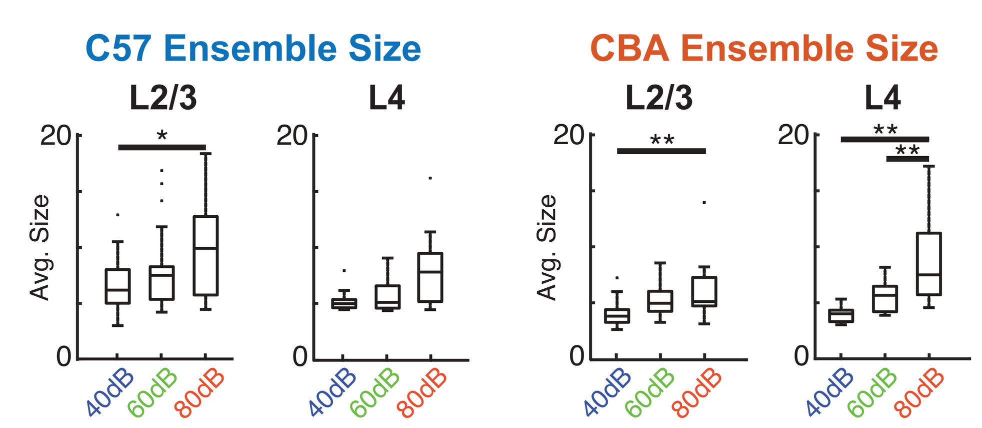
- We defined evoked ensembles as initiated during the 1s of sound presentation



- PDFs of sound-evoked ensemble sizes retained a power law distribution at all sound levels

→Suggests that evoked activity in L4 and L2/3 organizes as scale-invariant neuronal avalanches across different sound amplitudes

- Population activity patterns obey the same statistical rules despite their diversity in size and duration.
- **Scale-invariant avalanche dynamics in both layers L4 and L2/3 are preserved even in the presence of distinct sound input to A1 and across different sound amplitudes.**



- Given the increase in activity of single neurons by sound stimulation it is expected that there are specific changes in the activity patterns due to the sensory stimulus
- Average size and duration of evoked ensembles both increased systematically with sound level (i.e., input strength).

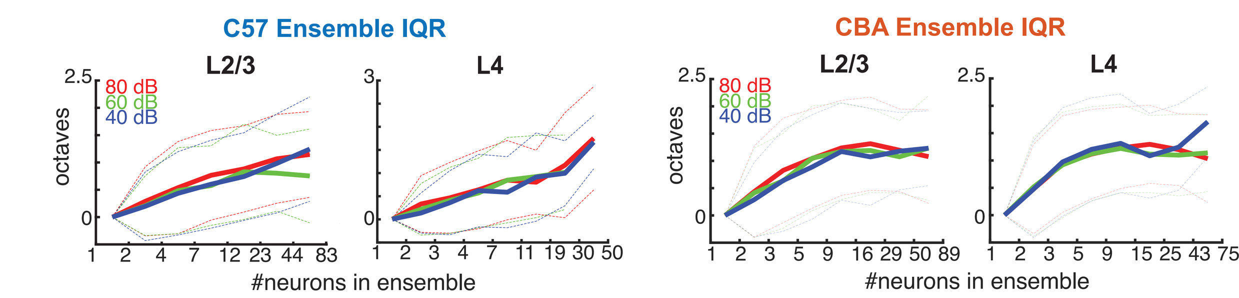
→Relatively larger change in CBA mice

Tuning of ensembles is similar in both strains

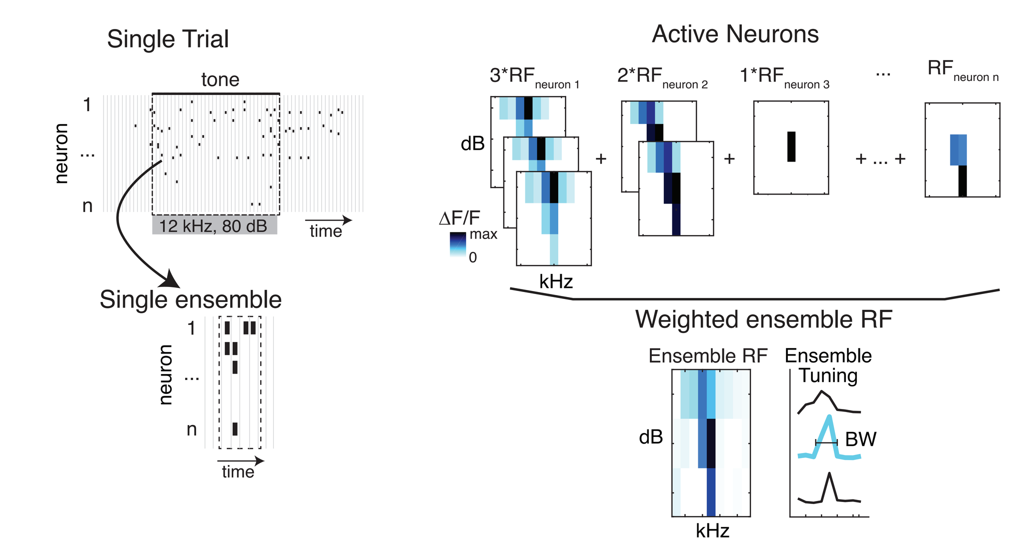
- Evoked avalanches in A1 recruit neurons with widely varying tuning preference
- Most behaviorally relevant sound stimuli are suprathreshold and thus recruit neurons with varying tuning preference
- We observe a large range of ensemble sizes and duration

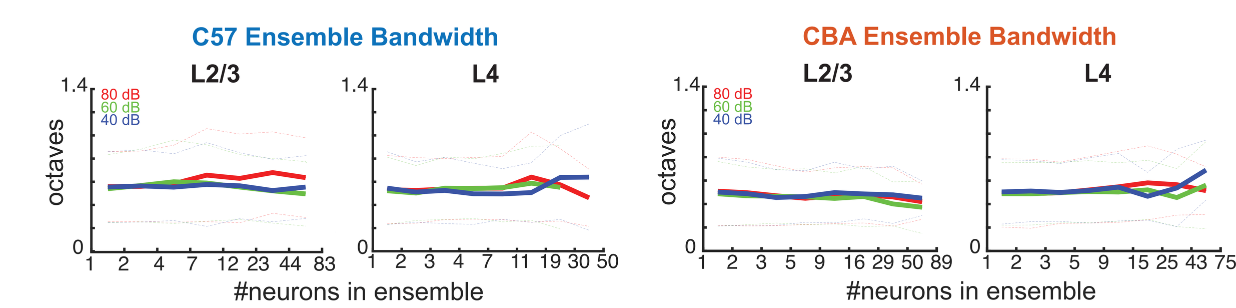
→Does the range of frequency preference vary with ensemble size and duration?

- **IQR_{BF} systematically increases with ensemble size (and duration) in C57 mice**
- **CBA mice reach max IQR_{BF} at smaller ensemble sizes, likely as a result of increased overall tuning diversity**



- A neuron's BF gives a limited description of a neuron's overall receptive field
- **Ensemble Receptive Field**: weighted average of the receptive fields of neurons active an ensemble
- **Bandwidth of ensemble RFs were not altered with respect to the size or duration of ensembles, indicating that stimulus selectivity is scale-invariant in the population activity.**
- Ensemble RF may serve as a useful population encoding measure in future studies





Summary & Conclusions

- Pure tones recruited neurons of widely ranging frequency preference in both layers and strains
- Mid-frequency regions of A1 in C57 mice contain neurons with a frequency preference that is shifted towards lower frequencies than that of CBA mice
- Frequency selectivity and responsiveness of individual neurons is slightly higher in C57 than CBA mice, particularly in L2/3.
- Signal correlations were higher in C57 mice, likely as a result of reduced tuning diversity
- Neuronal ensemble sizes distributed according to power laws, the hallmark of neuronal avalanches, and were similar across sound levels.
- Ensembles were composed of neurons with diverse tuning preference, yet with selectivity independent of avalanche size.

→Single cell and ensemble activity is largely similar in A1 of adult C57BL/6 and CBAxC57 mice with CBA mice showing more tuning diversity and sound-level sensitivity in responses.

→Spatial heterogeneity in frequency preference does not depend on mouse strain and thus is a feature of rodent auditory cortex.

→Neuronal ensembles in both strains exhibit network dynamics characteristic of criticality which is linked to maximal dynamic range and optimal information capacity and transmission.

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