# Animal Models of Tinnitus

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## Introduction

Pharmacological, cognitive and behavioral approaches are used to treat tinnitus. These have met with limited success due to our poor understanding of tinnitus and difficulty to objectively measure it. We develop a behavioral model of noise-trauma tinnitus that includes the ability to measure physiological changes induced by tinnitus and reversed by drug treatment.

We are currently exploring a variety of drugs (Lidocaine, Steroids, Anti-Oxidants) as well as a variety of delivery methods (IV, intra-tympanic, nano-particles)

Most human imaging studies (PET, fMRI) have implicated the inferior colliculus of the auditory midbrain in the generation of tinnitus. Our preparation consists of an awake rat with a chronic multi-electrode array implanted in midbrain. In the same animal, we measure neural activity before, immediately after and several weeks after noise trauma-induced tinnitus, and after intravenous injection with Lidocaine, while simultaneously verifying the existence of tinnitus through behavior.

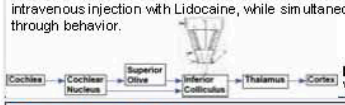



**Figure 1: The auditory pathway, simplified.** We record in inferior colliculus = auditory midbrain

## General procedure

- Implant rat with 8-electrode array and a permanent jugular catheter
- Measure single neuron activity across the span of auditory midbrain
- Induce noise-trauma
- Obtain behavioral evidence of tinnitus
- Verify hearing is otherwise normal (using ABR thresholds)
- Measure changes in single neuron activity post-trauma
- Compare neural activity with and without drugs
- Characterize molecular changes post-mortem

## Materials and methods

- Multi-electrode implant in central nucleus of inferior colliculus of 7-week old rats
- Catheter in jugular vein to deliver drugs, or intra-tympanic injection for nanoparticles
- Noise trauma induced with a 116dB, 1/3 Oct band of noise centered on 16kHz (14.2 to 17.9 kHz).

## Goals

- Characterize the changes that tinnitus induces in a behavioral task, by use of gap detection in an acoustic startle reflex procedure .
- Measure the changes in the neural responses in the collicular responses of the same awake rats.
- Determine the efficacy of pharmacological treatment with Lidocaine, Dexamethasone, N-acetyl-cistenie etc, by measuring the extent of behavioral and physiological reversal of the tinnitus-induced changes as a function of the drugs, their dosage and method of delivery.
- Characterize molecular changes correlated with tinnitus with CREB, phospho-CREB, TrkB (associated with elevated activity levels and trauma) and Parvalbumin immuno-reactivity.

## Results

### Gap detection deficits as evidence of tinnitus

The gap detection ability of rats before and after noise trauma is measured, using a method similar to Turner et al, 2006. The idea is that it is hard to hear a silent gap in a continuous pure tone if one suffers from a continuous, internally generated pure tone.
A brief burst of loud sound will elicit a "startle reflex". The reliable presence of another stimulus just before the burst will reduce the startle reflex (this is called pre-pulse inhibition). In our case, a silent gap is present 50% of the time before the startle stimulus. We compare the startle reflex with and without a gap.

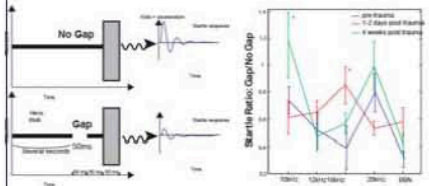



**Figure 3. Left: Startle reflex as a function of a silent gap in a tone background, measured before, just after and weeks post-trauma. Right: just after trauma, the major hearing deficit is at the center of the trauma frequencies, 16kHz. 3 weeks post-trauma, the behavioral evidence for tinnitus is at 10 kHz, with no obvious damage to the cochlea.**

Note: our rats are traumatized at 10 weeks of age and their tinnitus is either at 10-12 kHz. However, the frequency of tinnitus appears to be age dependent.

### Auditory Brainstem Response: the animal is not just deaf

The auditory brainstem response (ABR) is correlated with the health of the early parts of the auditory pathway. We measured the ABR before, just after and long after trauma. The ABR typically recovered to the pre-trauma values within a few weeks.

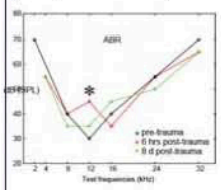



**Figure 4: Summary ABR thresholds before, just after and 8-9 days after noise trauma. While there is a significant threshold shift just following trauma, in this animal the ABR recovered after a little over a week, while behaviorally, the rat still had problems detecting the presence of gaps in a pure tone at the putative tinnitus frequency.**

## Electrophysiology

### Increased excitability

Mean spontaneous spiking rate in inferior colliculus for 3 sec before the sounds and for 3 sec after sound stimulation.
Before trauma: before stim = 77 sp/sec, post-stim 92 sp/s
Just post trauma: before stim = 45 sp/s, post-stim = 100 sp/s
3 wks post-trauma: before stim = 113 sp/s, post-stim = 139 sp/s

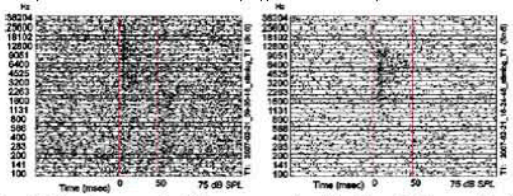
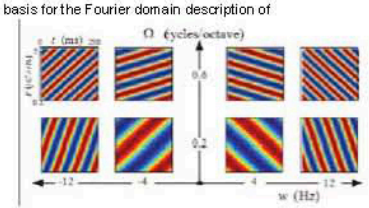



**Figure 5 . Tonal response in IC. Tone was on from 0 to 50ms. (Left) recording just prior to trauma, (Right) 2 hours post-trauma (no spike sorting). While we are probably not recording from the same cells, the electrodes have not been moved. Note change of neural response in the region corresponding to the trauma. The spectro-temporal receptive fields (STRFs) show the same change, with excitation being extinguished down to 12kHz.**

### Response to broadband, complex sounds

Auditory gratings (ripples) form a basis for the Fourier domain description of dynamic spectra.
They are characterized by spectral density and periodicity or angular frequency.




### Receptive fields

- Before trauma, we found receptive fields from 800Hz to 35kHz, about an octave in bandwidth.
- The afternoon of the trauma, it was hard to find responsive cells. The week after trauma, most cells were hyperexcitable (ratio of excitation to inhibition), see spon rate before and after sounds
- Three weeks after trauma, cells showed no responses between 9 and 11kHz. The cells that straddled the 10-12kHz region were more broadly tuned, 2-2.5 octaves, even with Lidocaine

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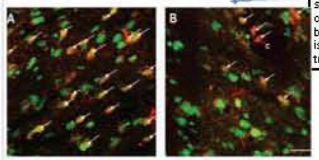
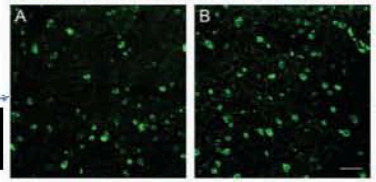
### Immunohistochemistry for CREB and phosphorylated CREB



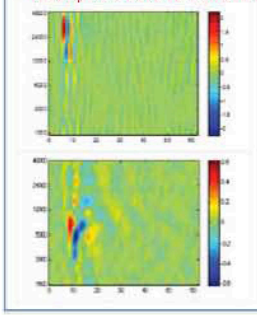

CREB (green) and p-CREB (red) post-trauma (opposite sides, same 50 µm section): Note increased cellular activation in ICC contralateral to noise trauma. Arrows point to cells that express both CREB and pCREB. A capillary in B is denoted by 'c', which is surrounded by pCREB-expressing cells, as expected in a non-traumatized situation.

### Changes in Inhibitory neural circuitry




Confocal of Immunofluorescent staining shows decreased parvalbumin-immunoreactive cells on side contralateral to noise trauma (A) compared to the ipsilateral side (B). Similar results were found in 4 of 4 rats shown to have tinnitus.

### Receptive field of collicular cells of the awake rat



### Receptive field 3 weeks post-trauma