

The distribution of cell and neurotransmitter markers in the auditory cortex of rats in a tinnitus model

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Introduction

- Gamma oscillations in cortex (Fig 1) are generated by the inhibitory activity of fast-spiking GABAergic parvalbumin (PV) interneurons on pyramidal cells (Pyr).
- NMDA receptor activity determines the frequency and power of oscillations¹.
- Activation of NMDA receptors stimulates synthesis of nitric oxide via neuronal nitric oxide synthase (nNOS).
- Rentisi et al. (abstract 219, this meeting) report a reduction in the peak frequency of gamma activity in auditory cortex of rats with noise-induced tinnitus.
- We hypothesised that changes in PV, NMDA receptors, and nNOS underlie tinnitus related-changes in gamma oscillations in the auditory cortex.
- We compared the distribution of these markers in the left and right auditory cortices of rats with behaviourally verified tinnitus induced by unilateral acoustic overexposure.

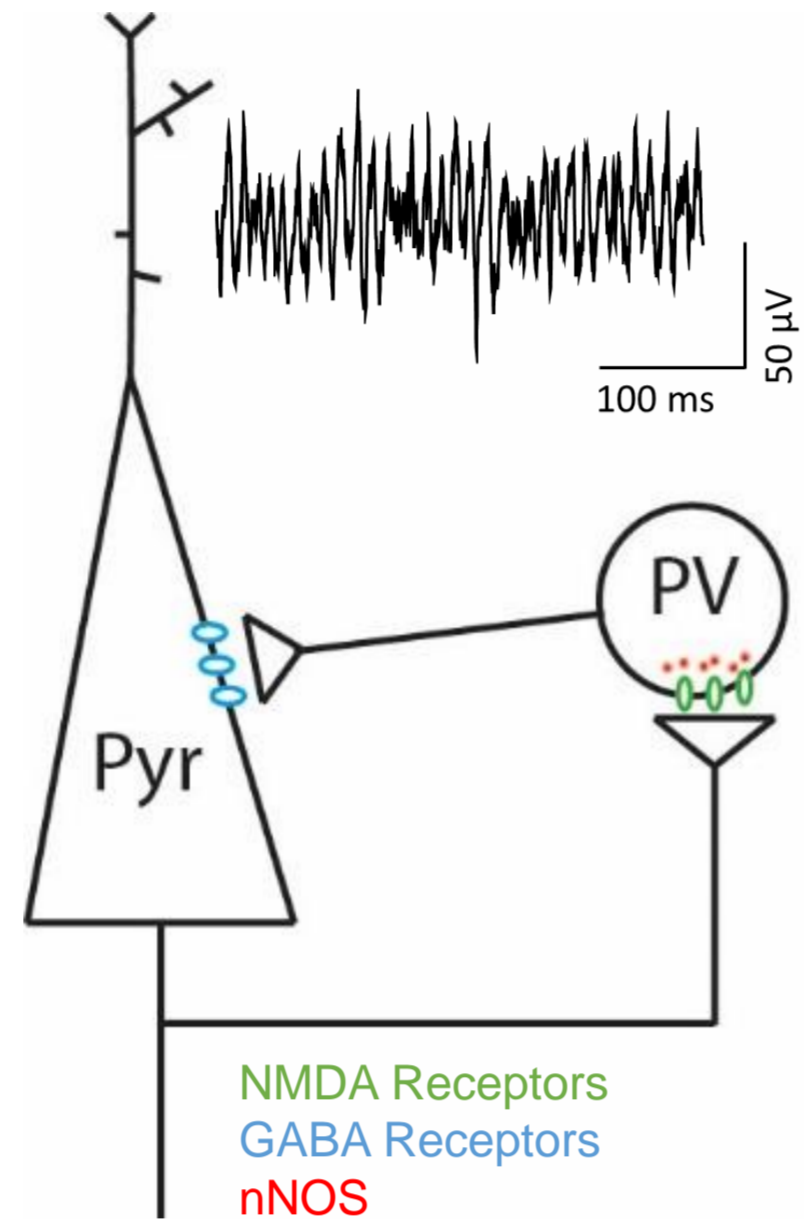


Fig 1: cortical circuit for generation of gamma oscillations. Pyr- Pyramidal cell. PV- Parvalbumin GABA-ergic interneuron.

Methods

Animals

- Long Evans rats; 6 control + 6 acoustically over-exposed (16kHz, 115 dB SPL, for 2 hours with left ear plugged) were used.
- Rats were tested for tinnitus with gap-pre-pulse inhibition (GPPI) of acoustic startle. A PPI of acoustic startle test was used to exclude animals with hearing loss.

Immunohistochemistry

- Rats were deeply anaesthetized and perfused with PBS and 4% paraformaldehyde.
- Coronal sections (30 μm) were cut through the left and right auditory cortices.
- Immunohistochemistry was performed using primary antibodies against PV, GluN1 (for NMDA-R), and nNOS.
- Labelling was visualized using confocal microscopy.
- The auditory cortex was identified using measurements from the rhinal sulcus, based on Paxinos and Watson (1998)².
- Image J and MatLab scripts were used to count cells and measure fluorescence intensity in regions of interest.
- Repeated measures ANOVA and post hoc t-tests were used for statistical analyses within and between groups.

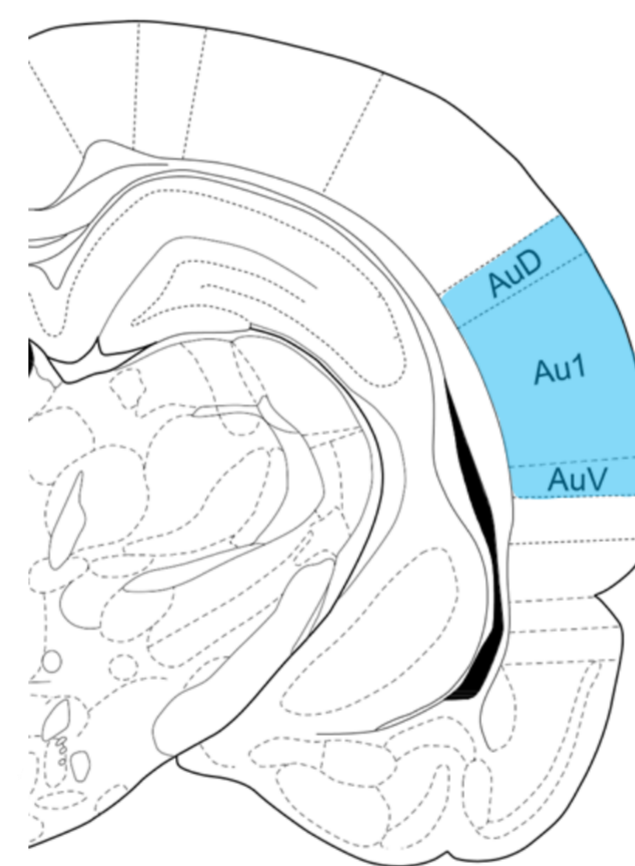


Fig 2: The auditory cortex²

Results

No difference in parvalbumin labelling in tinnitus

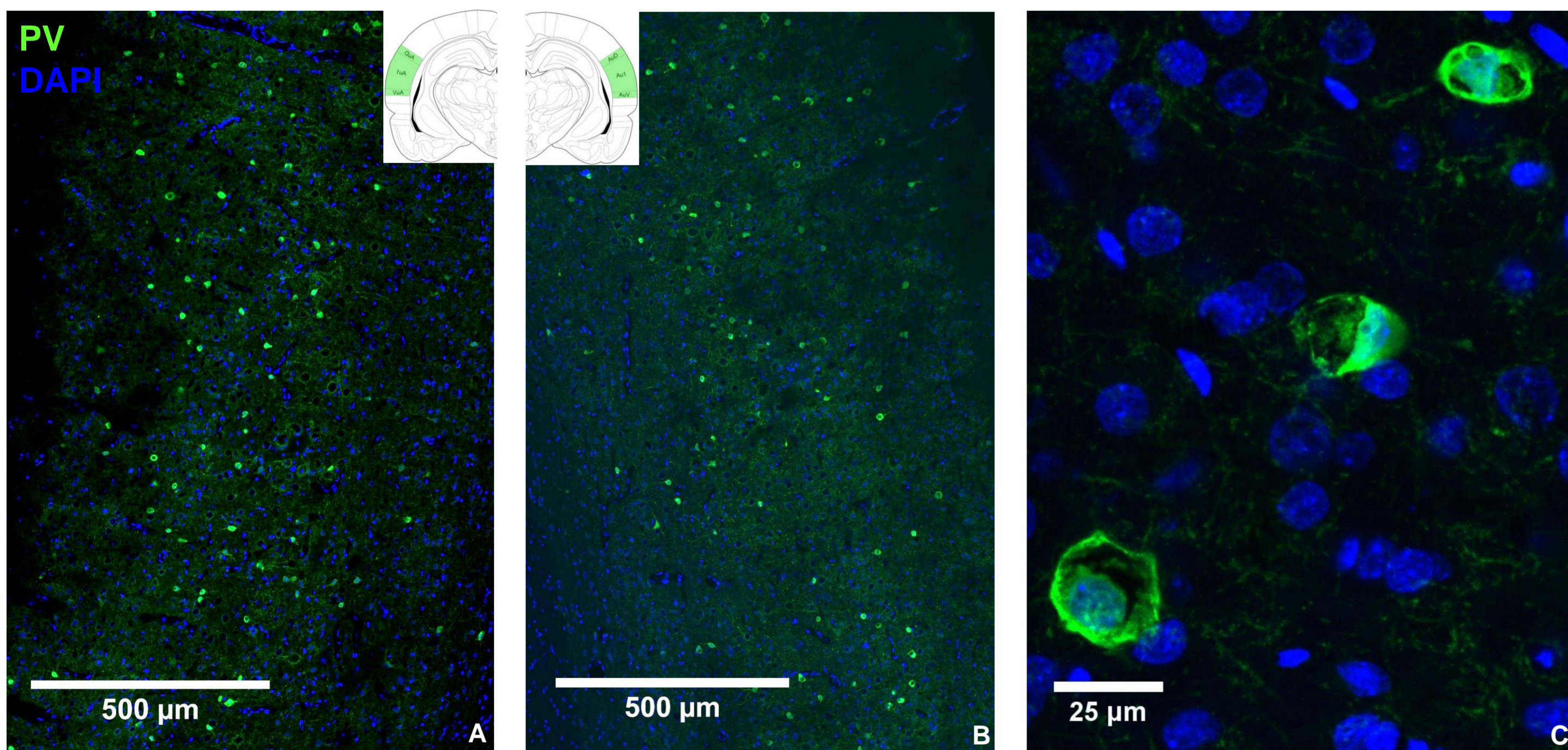
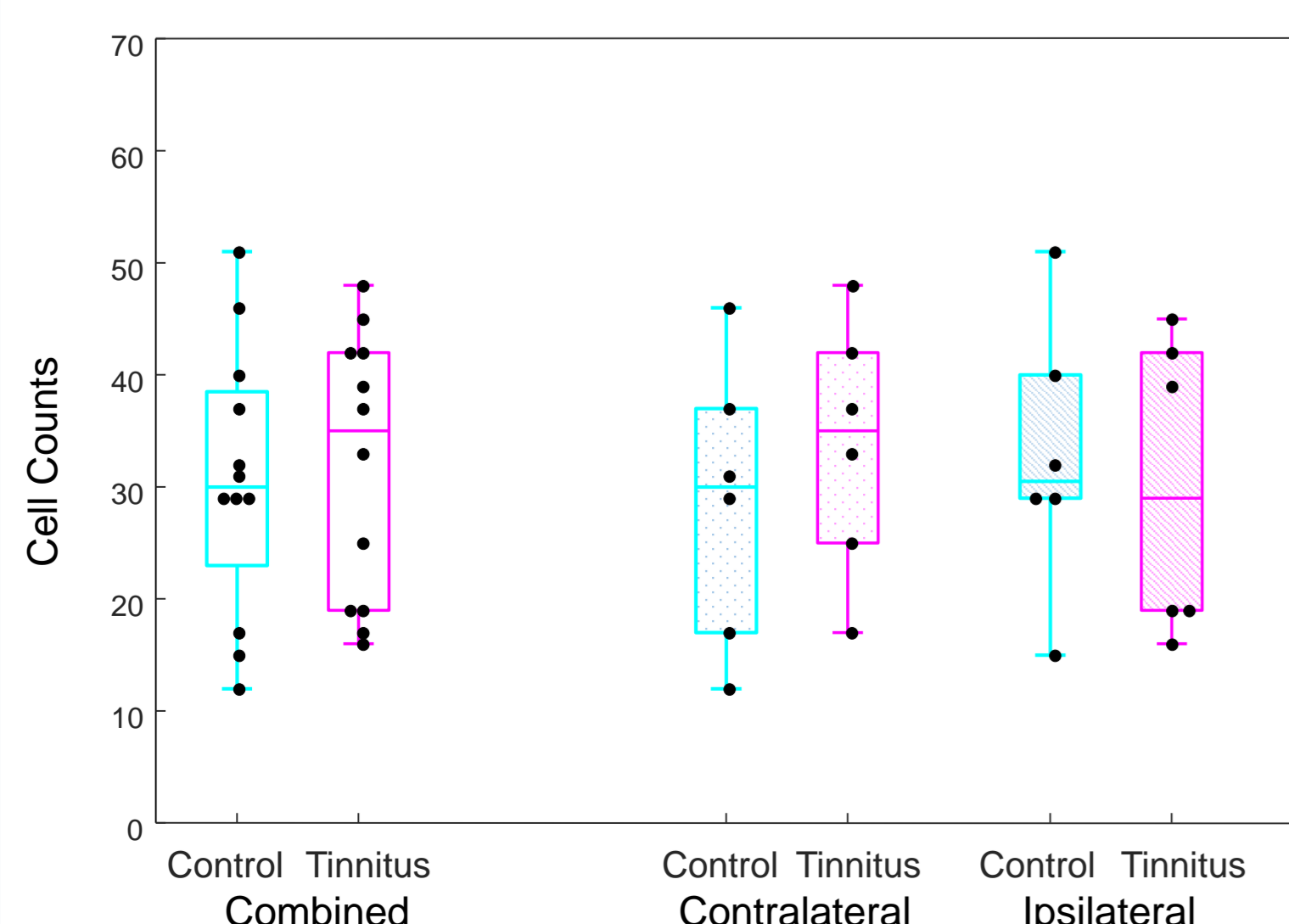


Fig 3: PV labelling across the auditory cortex (A,B). Cortical PV cells under high power magnification (C).



- Parvalbumin cells are located across all layers of the auditory cortex (Fig 3A,B) and show strong immuno-labelling around the soma (Fig 3C).
- There was no significant difference in cell counts between tinnitus and control groups or between hemispheres within groups (Fig 4).
- There was no difference in the ratio of contralateral to ipsilateral mean intensity of labelling between tinnitus and control groups (data not shown).

Fig 4: PV cell counts

No difference in NMDA-R (GluN1) labelling in tinnitus

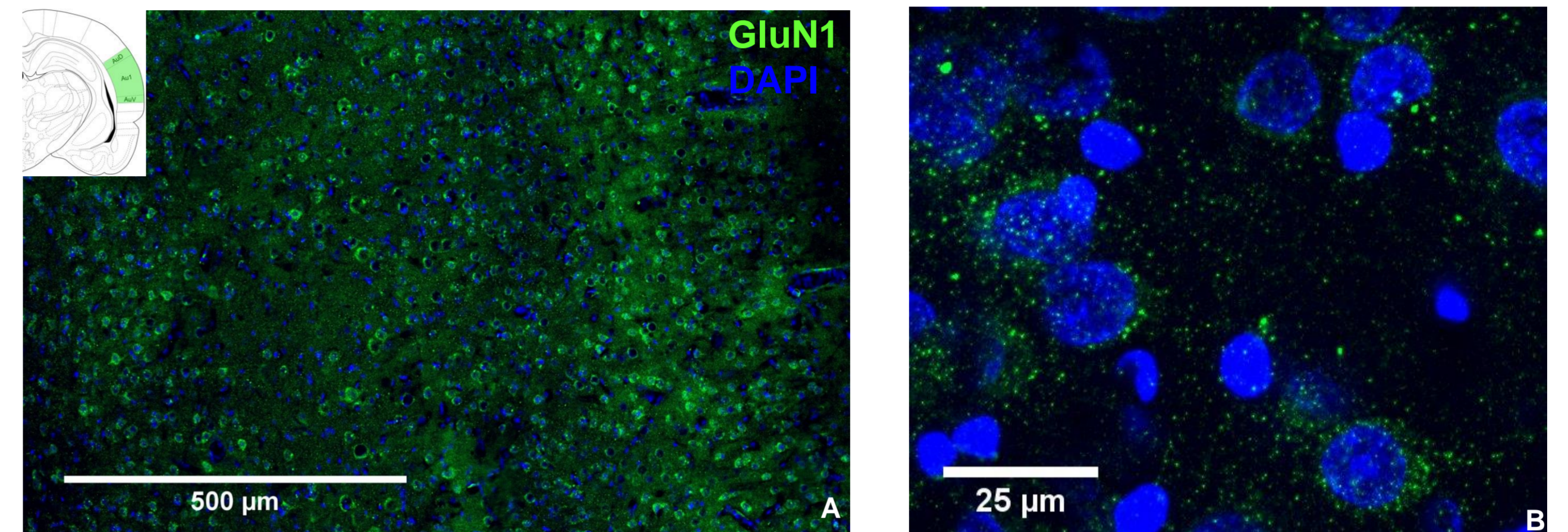


Fig 5: GluN1 labelling across the auditory cortex (A). GluN1 labelling under high power magnification (B).

- GluN1 labelled cells are distributed across the auditory cortex, but are most concentrated in the superficial layers (Fig 5A).
- Owing to the high cell density, cell counts were not performed.
- There were no differences in mean intensity between control and tinnitus groups, or between hemispheres within groups (Fig 6).

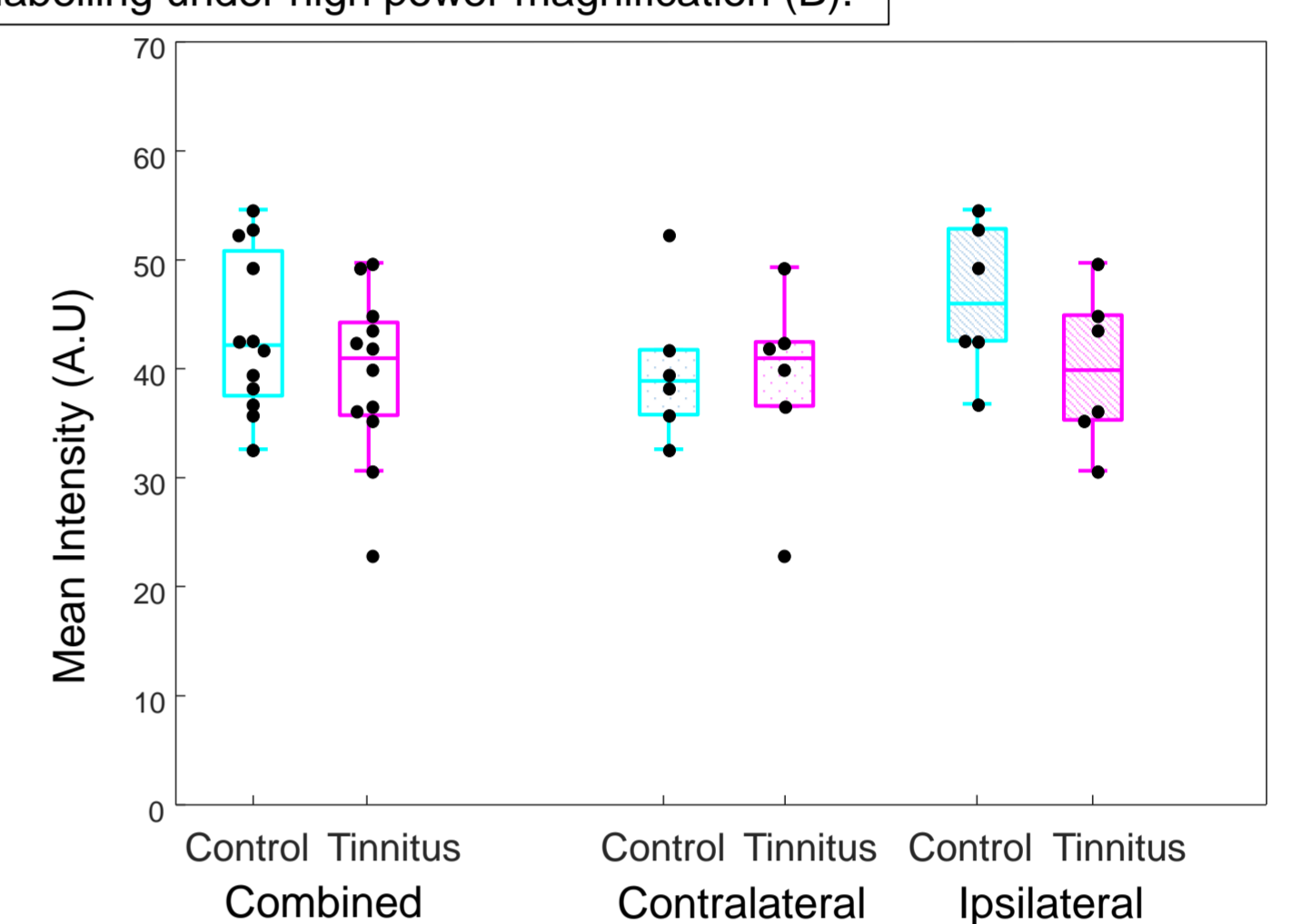
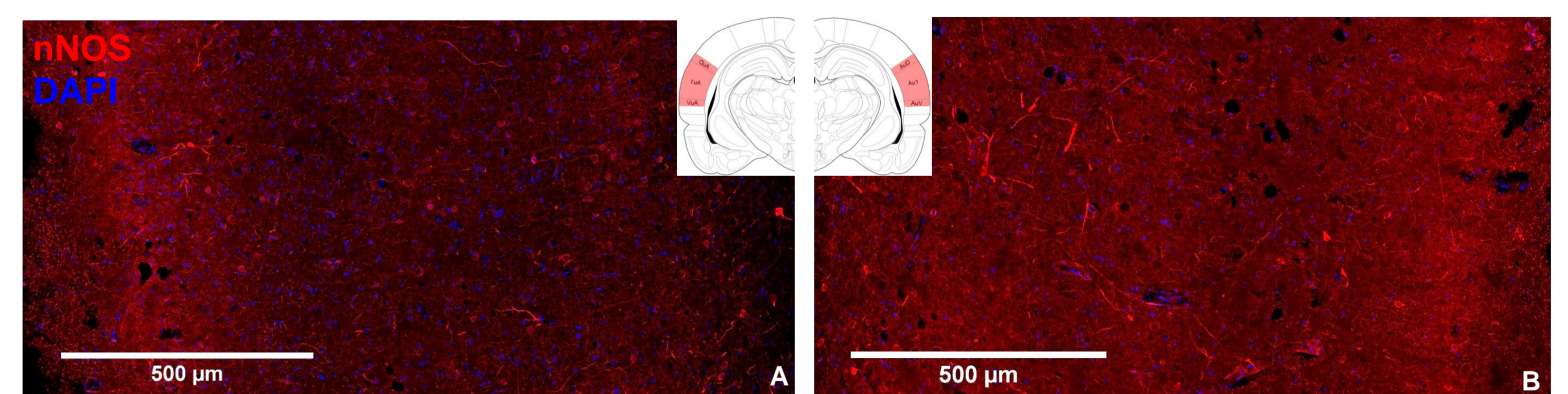


Fig 6: GluN1 mean intensity between groups.

nNOS labelling is reduced in the cortices of tinnitus rats



- nNOS labelling of cells is most dense in the superficial and deep cortical layers. There is also dense nNOS labelling of neuropil across layers (Fig 7A, B).
- There was no difference in the labelling intensity between hemispheres within animals (Fig 8).
- There was a significantly lower mean intensity of nNOS labelling in tinnitus compared to control ($F_{(1,10)} = 4.94$, $p = 0.05$, $\eta_p^2 = 0.33$), and significantly lower intensity of labelling in the contralateral auditory cortex in the tinnitus group compared to the contralateral auditory cortex in controls ($t_{10} = 3.10$, $p = 0.01$) (Fig 9).
- There was no difference in labelled cell count within or between groups (data not shown).

Fig 7: nNOS labelling in both cortices (A,B). nNOS labelling of soma and dendrite (C).

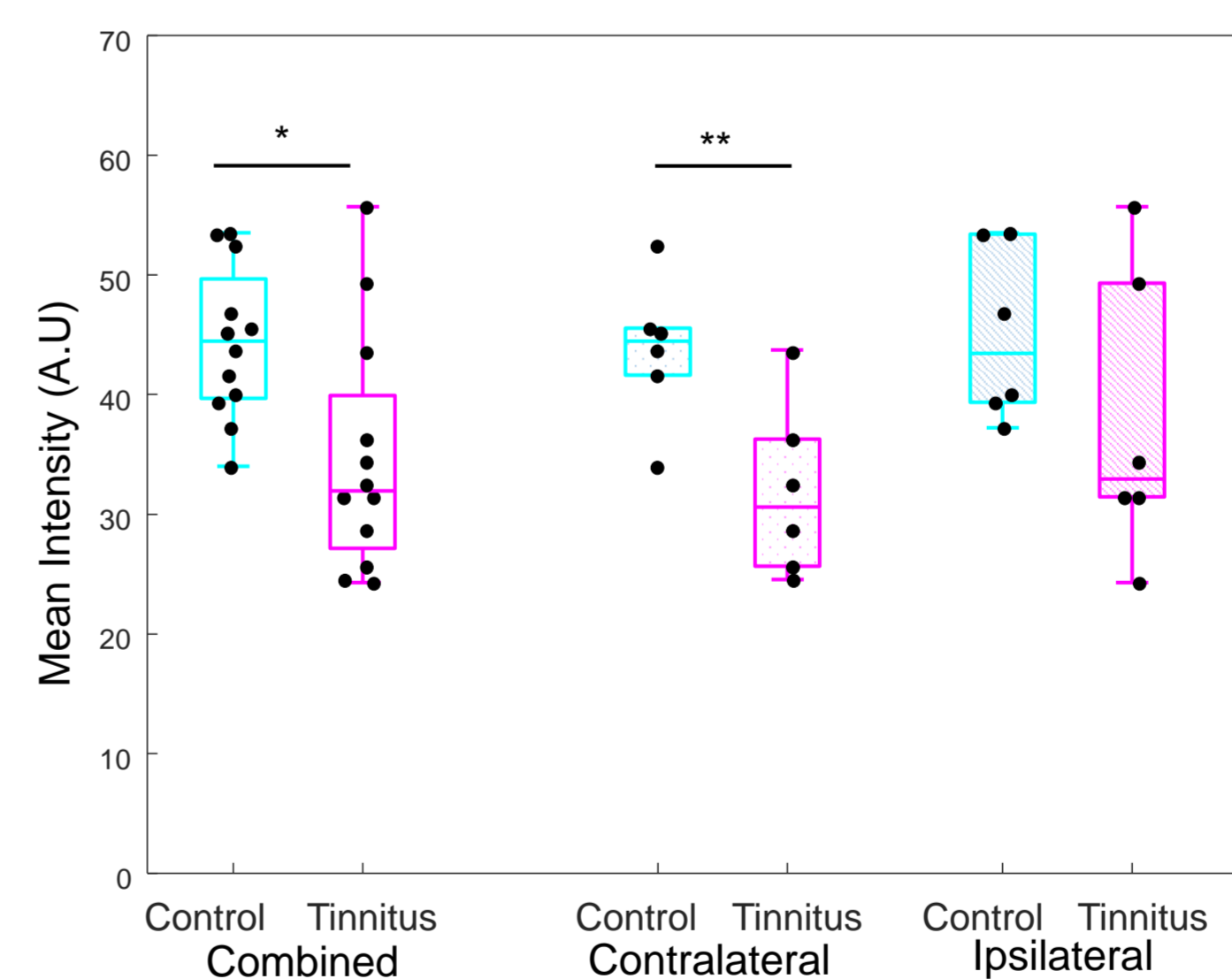


Fig 8: nNOS mean intensity between groups.

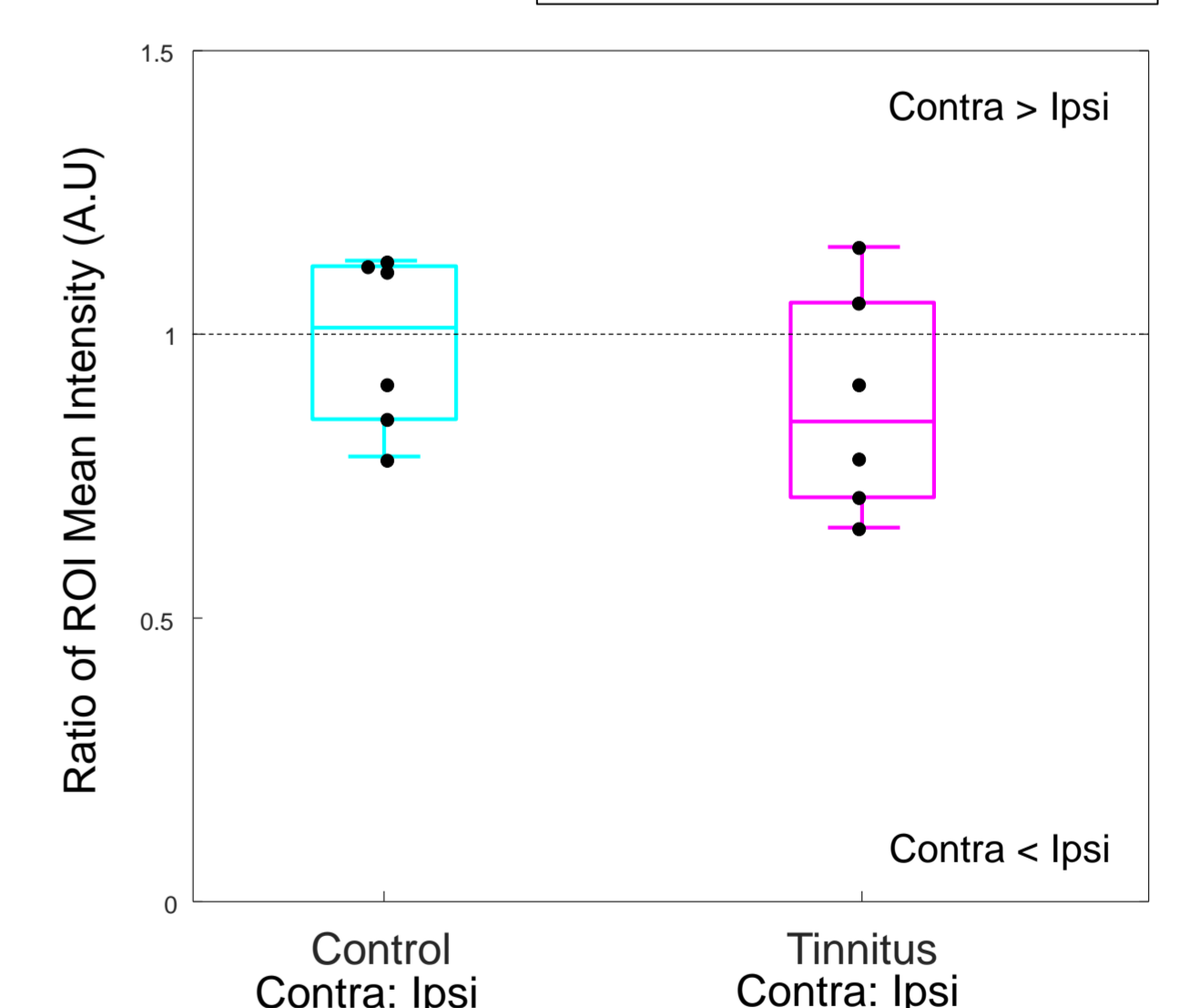


Fig 9: Ratios of ROI mean intensity within animals.

Discussion

- The distribution and expression of labelling for PV and GluN1 are not affected by induction of tinnitus by acoustic overexposure.
- There was a significant reduction in the mean intensity of nNOS labelling in the auditory cortex in tinnitus animals, suggesting that expression of nNOS is affected by acoustic overexposure. This contrasts with the increase in nNOS labelling found in the ventral cochlear nucleus in rats with tinnitus³.
- The difference in nNOS labelling between tinnitus and control groups appears to be primarily driven by reduced labelling in the cortex contralateral to the sound-exposed ear.
- Could the reduced expression of nNOS, and thus reduced production of nitric oxide, in interneurons underlie observed differences in gamma oscillations in tinnitus animals?

References

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