Optimization of structural imaging in auditory pathway on Chinchilla Lanigera as a pre-clinical model of blast traumatic brain injury

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Blast Traumatic Brain Injury

- Post-blast concussive symptoms reported by troops post-deployment varies based on the intensity, sequential repetitions and spacing between repetitions of the blast overpressure (BOP).
- The mechanism(s) of BOP-induced injury has been studied in different animal models such as rats, mouse (Elder et al., 2014), pig (Chen et al., 2017), Chinchilla (Hickman et al., 2018) and monkey (Lu et al., 2012).
- However, gaps remain in generalizing the pathophysiology arising from animal injury to humans.

Specific aims

1.Develop MRS methods to quantify neurotransmitters in auditory centers (CN, IC, AC) after blast injury in rats.
2.Test hypothesis that blast exposure disrupts metabolic homeostasis in auditory centers.
3.Examine susceptibility of metabolite ratios (GABA/Glutamate, Cho/Cr, NAA/Cr, Pcr/Cr) to blast injury.
4.Determine blast effects on structural connectivity using DTI in simultaneous DTI/MRS scans.

Fig.1 Chinchilla, Created with BioRender.com

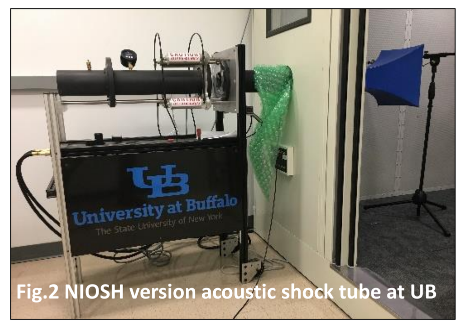


Fig.2 NIOSH version acoustic shock tube at UB

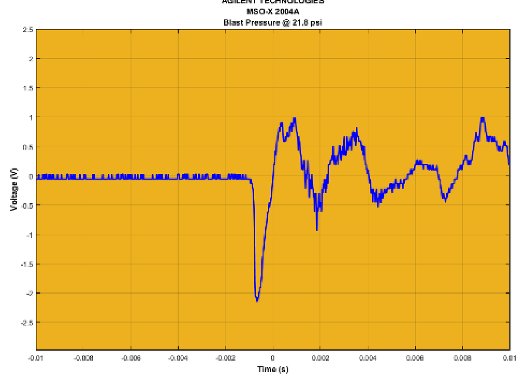


Fig.3 Impulse waveform at 21.8 psi

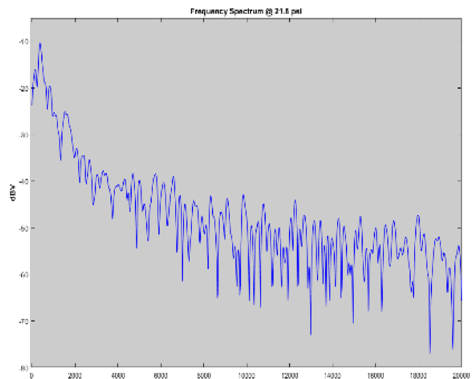
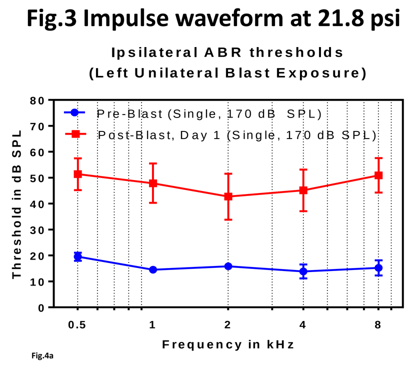
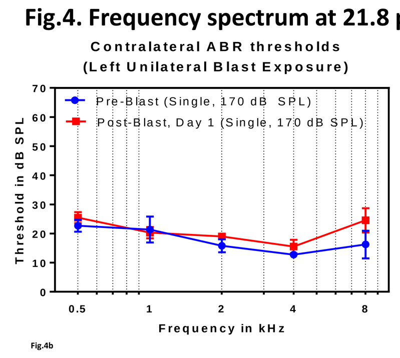


Fig.4. Frequency spectrum at 21.8 psi





METHODS

Towards identifying BOP induced injury end-points, in present study, we are reporting pre & post-blast MRI characterization in chinchilla lanigera with a focus on auditory pathway, such as cochlear nucleus (CN), inferior colliculus (IC) and auditory cortex (AC). Structural integrity was also assessed using histological analysis (Cresyl fast violet – Nissl substance and Luxol fast blue – Myelin)

MRI SET-UP

MRI scanning is conducted at Center for Biomedical Imaging as part of UB core facilities which is equipped with 200mm horizontal-bore 9.4T magnet (Bruker Biospin, Biospec 94/20 USR) and a 440 mT/m imaging gradient system and a dual-element transceiver coil (Bruker Biospin) placed over the head of the anesthetized chinchilla.

Fig. 5. Bruker BioSpec 94/20USR

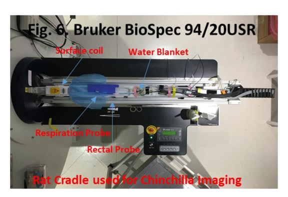


Fig. 6. Bruker BioSpec 94/20USR

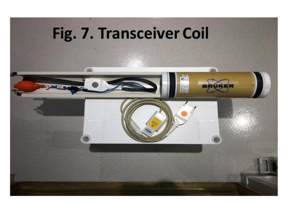


Fig. 7. Transceiver Coil

CHALLENGES WITH CHINCHILLA MRI IMAGING

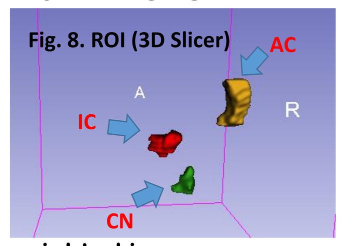
1. There is no previous literature stating Chinchilla MRI imaging. **This study is the first of its kind which provides challenges in data acquisition.**
2. We have adopted the MRI set-up specific for other rodents such as rats and mice. The rat cradle is 56mm wide in the body area and 42mm in the head area. The brain size of the chinchilla (6 months old, 600 g) matches with the active area of the coil (27x35 mm) which gave proper coverage of the brain for data acquisition.
3. The rat head surface coil is a receive-only array coil that works in combination with a transmitter coil, in our case a 1H circular polarized transceiver coil with the inside diameter of 86mm.
4. Though, surface coil matches with chinchilla's brain size, we could not able to use lateral fixation similar to other rodents which leaves more room for DTI optimization in next cohort.

DATA ACQUISITION

A series of gradient spin echo images were acquired in five chinchillas as our pilot study to acquire T1-w/T2-w and diffusion tensor images from anesthetized chinchillas. Our region of interest (ROI) is the central auditory pathway specifically on CN, IC and AC.

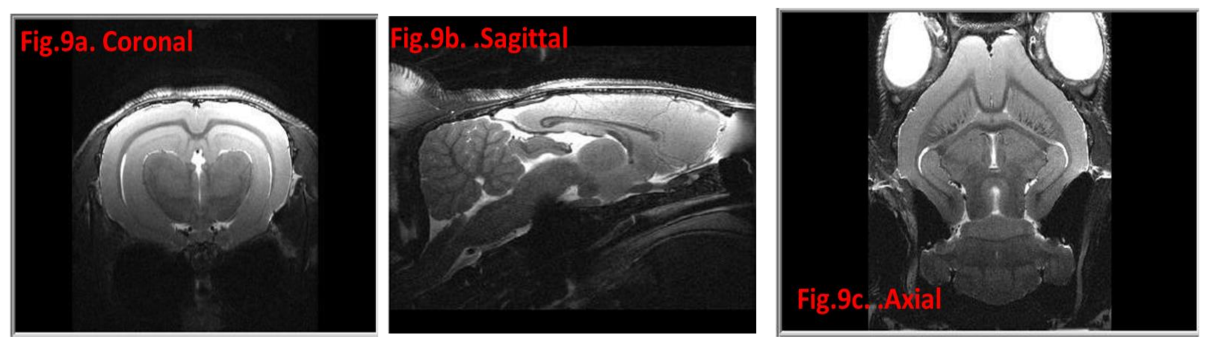
This helps to calculate the set of water diffusion tensor related parameters such as fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD) and mean diffusivity (MD) which reflects magnitude and direction of water molecule diffusion in tissues.

Diffusion weighted images were corrected for eddy current distortions, and diffusion tensor parameters (Axial diffusivity (AD), Radial diffusivity (RD), Mean diffusivity (MD) and Fractional anisotropy (FA)) was calculated on voxel-by-voxel basis using DSI Studio (Yeh et al., 2013). ROI specific diffusion parameters were calculated by averaging all voxels corresponding to a specific ROI.



Segmented ROI using 3D Slicer
AC (yellow); IC (red); CN (green)

T1 and T2 weighted images were acquired in axial and coronal section (but a representative image of sagittal is shown below).



Series description/Protocol name	T1_map_Rare	T2map_MSME	T1_TurboRARE_axial	T2_TurboRARE_axial	T2_TurboRARE_cor	T1_RARE_Cor	DTI
TR (ms)	5500	4000	1500	4446.02	3711.74	1128.1	10000
TE (ms)	7.5	7.47	5.7	44	44	5.86	23.37
FA	90	90	90	90	90	90	90
Slices	48	88	42	42	35	35	2100
Spacing between thickness (mm)	1.1	1.1	0.7	0.7	0.5	0.5	0.3
Slice Thickness (mm)	0.8	0.8	0.7	0.7	0.5	0.5	0.5
No. of averages	1	1	3	3	3	3	1
Echo train Length	2	1	4	8	8	4	7

Table1. Scan parameters of all sequences

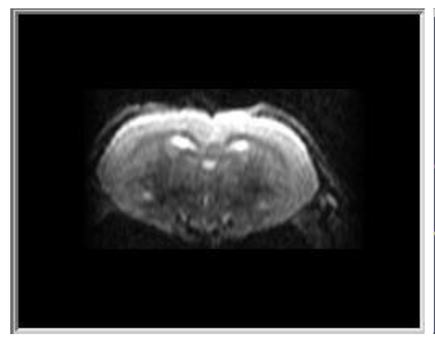


Fig.11a

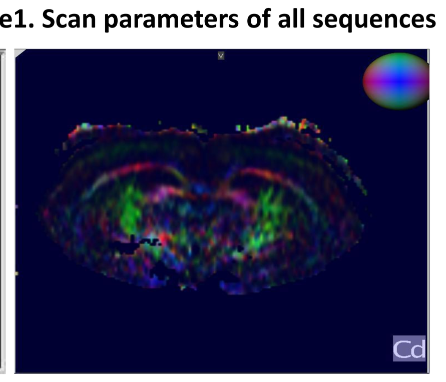


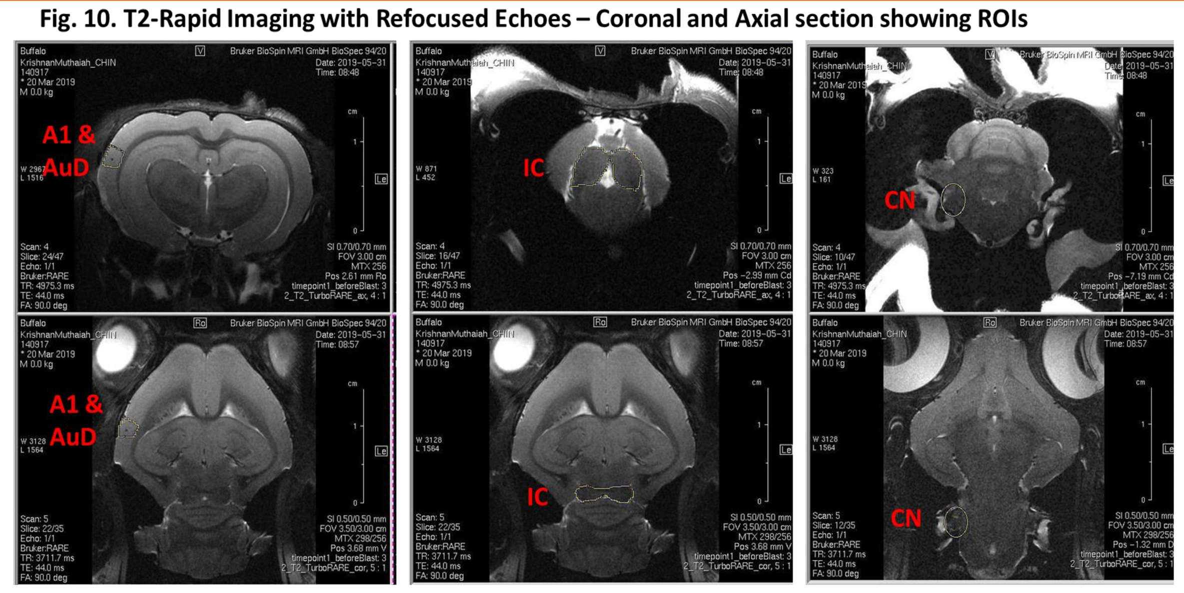
Fig.11b

Fig. 13 (a and b) shows the representative Diffusion tensor images with tractography.

Images were acquired along 30 directions (b = 1000 s/mm²)

On acquiring robust images, Fractional Anisotrophy, Radial Diffusivity and Axial diffusivity was calculated.

Fig. 10. T2-Rapid Imaging with Refocused Echoes – Coronal and Axial section showing ROIs



VOXEL BASED ANALYSIS OF TENSOR PARAMETERS

Fig. 12. Measures of Diffusion Magnitude (Axial **diffusivity**) – Principal axis of diffusion.

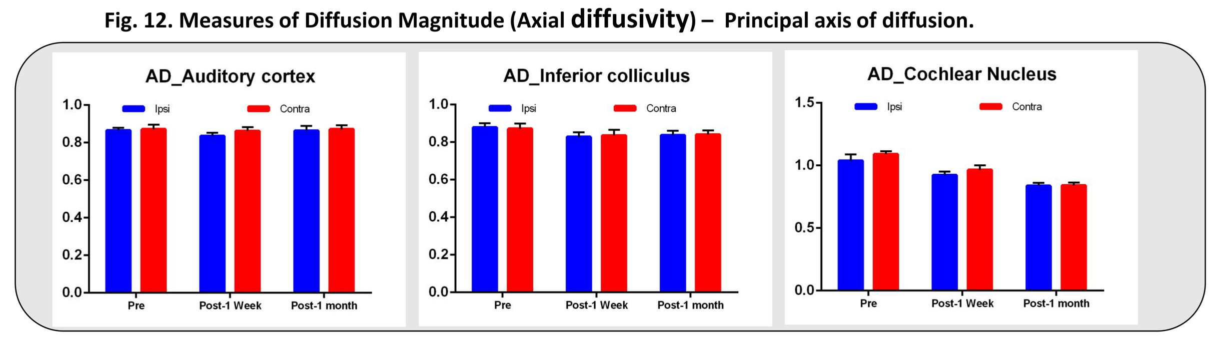


Fig. 13. Measures of Diffusion Magnitude (Radial diffusivity) – Vectors perpendicular to Principal axis of diffusion

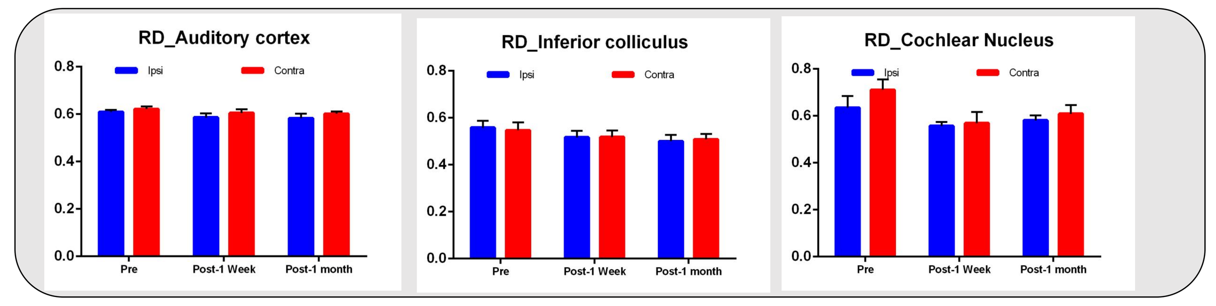


Fig. 14. Measures of Diffusion Magnitude (Mean diffusivity) – Average molecular motion – Rotationally invariant

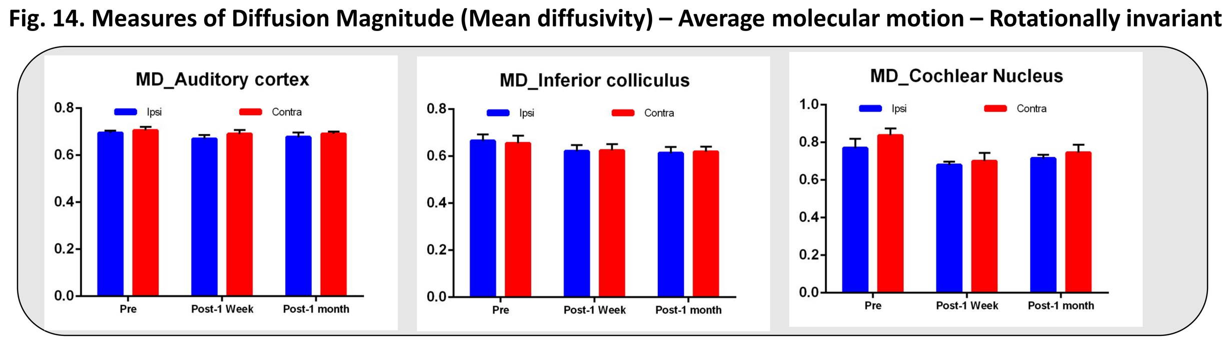
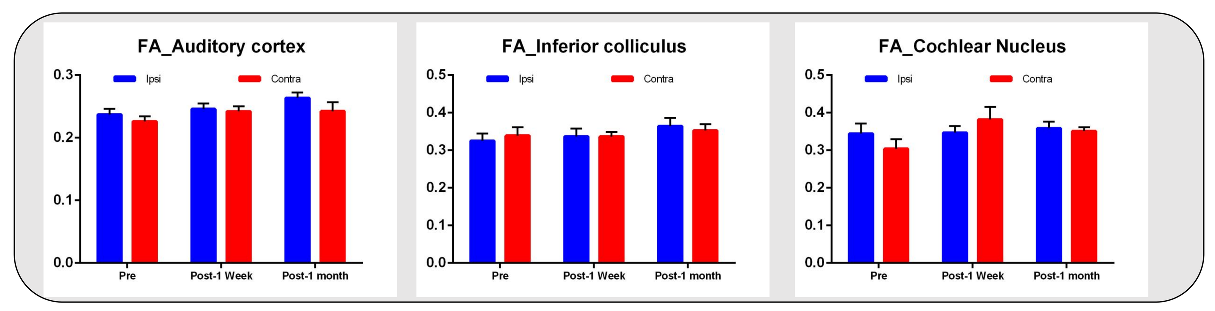


Fig. 15. Measures of Diffusion Anisotropy (Fractional Anisotropy) – Fraction of the diffusion that is anisotropic



TENSOR PARAMETERS INTERPRETATION

Differences between blast exposed groups and control group were assessed using a sample t- test. Voxels with p<0.05 was Considered significant.

1. Axial diffusivity was not significantly different in AC, IC and CN in between ipsi and contralateral ROIs. However, AD is found to be significantly decreased (both ipse and contralateral) in Cochlear Nucleus in post-blast 1 month when compared to pre-blast AD.
2. Radial diffusivity was not significantly different in AC, IC and CN in between ipsilateral and contralateral ROIs and not significantly different between time-points.
3. Mean diffusivity was not significantly different in AC, IC and CN in between ipsilateral and contralateral ROIs. However, MD of Cochlear cochlear nucleus at post-blast 1 week was significantly reduced when compared to pre-blast.
4. Fractional anisotropy was not significantly different in AC, IC and CN in between ipsilateral and contralateral ROIs and not significantly different between time-points.

STEREOLOGICAL INTERPRETATION

Differences between blast exposed groups and control group were assessed using a sample t- test. Voxels with p<0.05 was Considered significant.

1. Cell number in CFV stain and myelin density in LFB stain was not significant between control and post-blast 1 week sample.

Fig. 16. Cresyl Fast Violet Staining of AC and CN in control and blast chinchillas (Nissl substance staining)

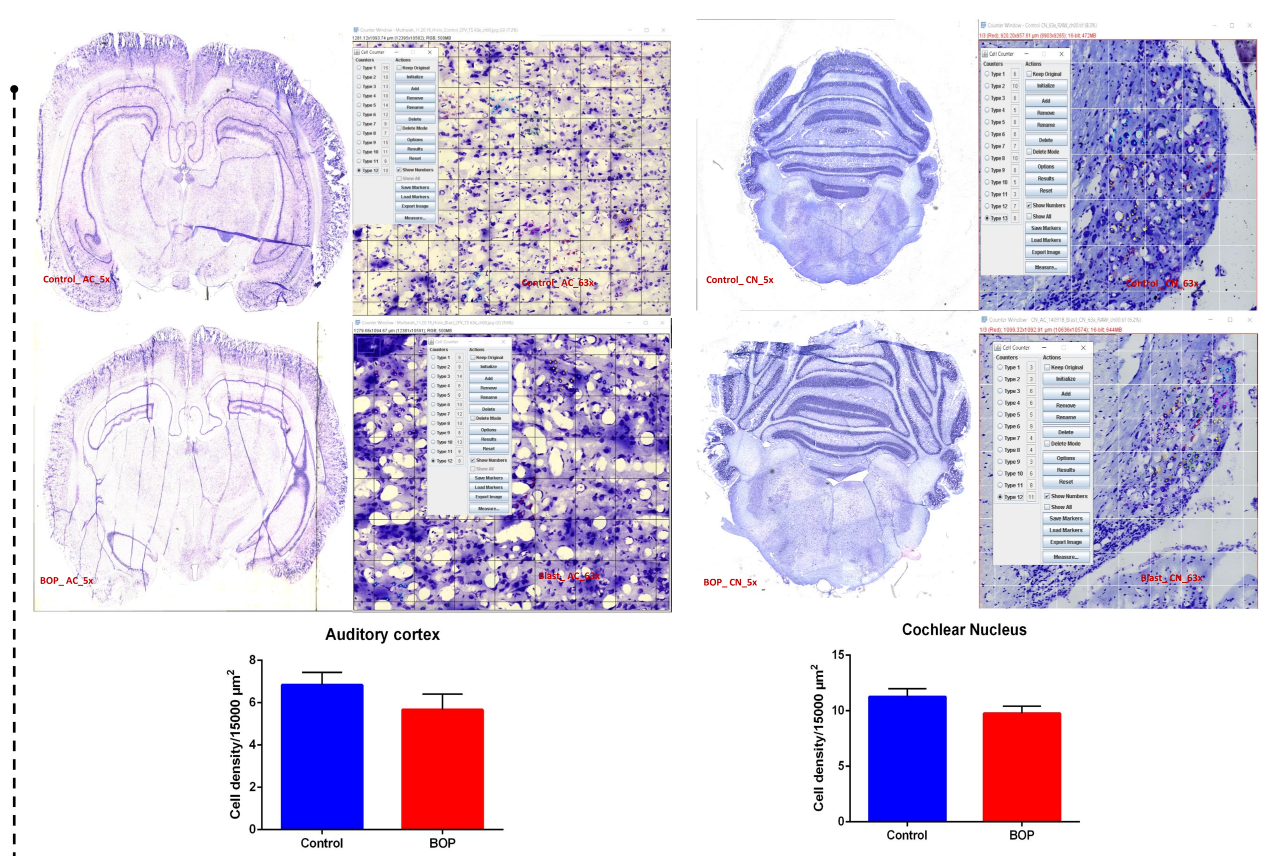
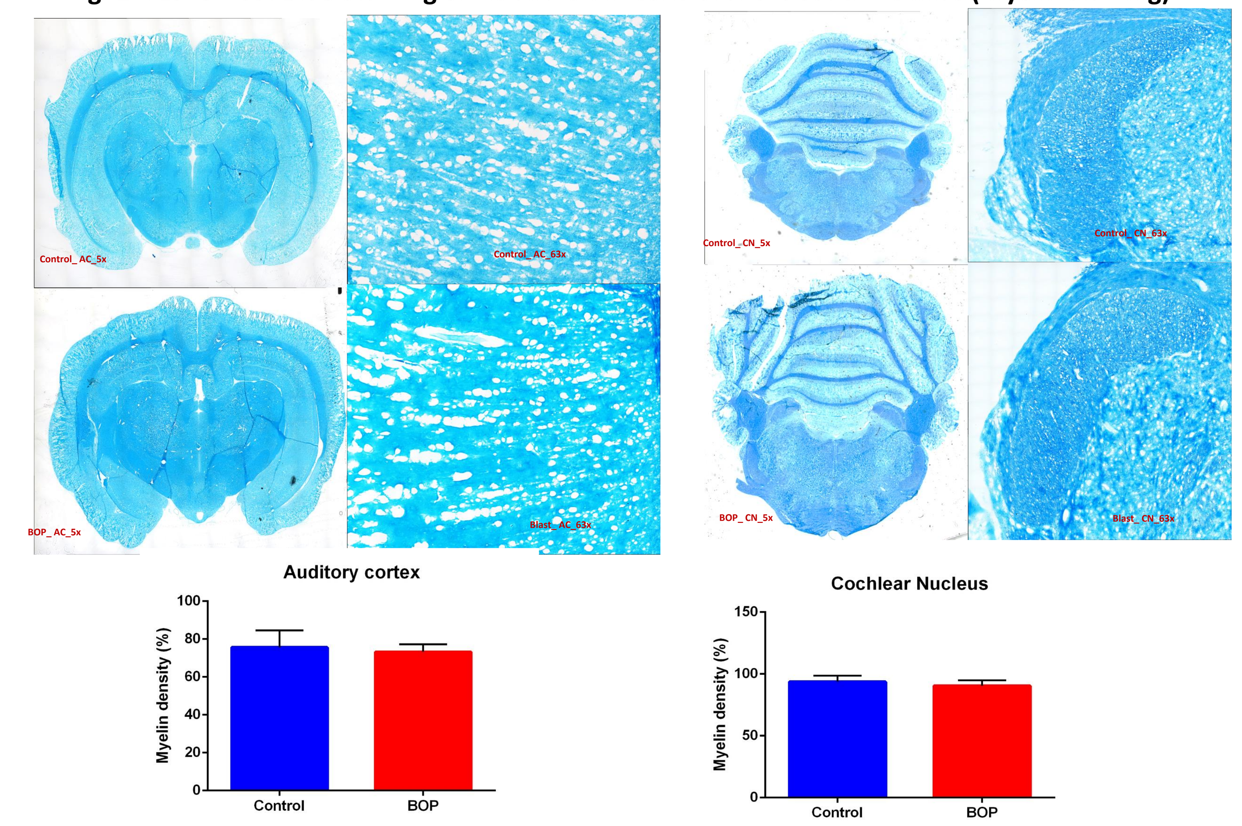


Fig. 17. Luxol Fast Blue Staining of AC and CN in control and blast chinchillas (Myelin staining)



Ongoing projects

1.Correlate MRS metabolites and DTI connectivity changes with behavioral evidence of tinnitus and hearing loss after blast exposure.
2.Evaluate efficacy of therapeutic drugs like 3AB on reversing blast-induced metabolite and connectivity changes.
3.Translate optimized MRS methods to human imaging studies of blast injury and tinnitus, “"Mapping of Metabolic profiles with the structural connectivity of the auditory pathway in blast traumatic brain injury””

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