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Quantitative Ultrasound Assessment of Neurotoxicity in the Young Rhesus Macaque Brain

Ivan M. Rosado-Mendez,[1,2] Laura Castañeda,[1] Chrysanthy Ikonomidou,[3]
James A. Zagzebski,[2] and Timothy J. Hall[2]

[1]*Instituto de Física, Universidad Nacional Autónoma de México, Mexico City, MEX*
[2]*Department of Medical Physics, University of Wisconsin, Madison, WI*
[3]*Department of Neurology, University of Wisconsin, Madison, WI*

Disclosures

The authors certify that they have NO financial interest in the subject matter or materials discussed in this presentation

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Background

Every year, millions of children are exposed to anesthesia worldwide[1]

Image from www.scientificamerican.com

FDA WARNING

Prolonged and repeated use of general anesthetics in children younger than 3y/o and pregnant women can have neurotoxic effects[2]

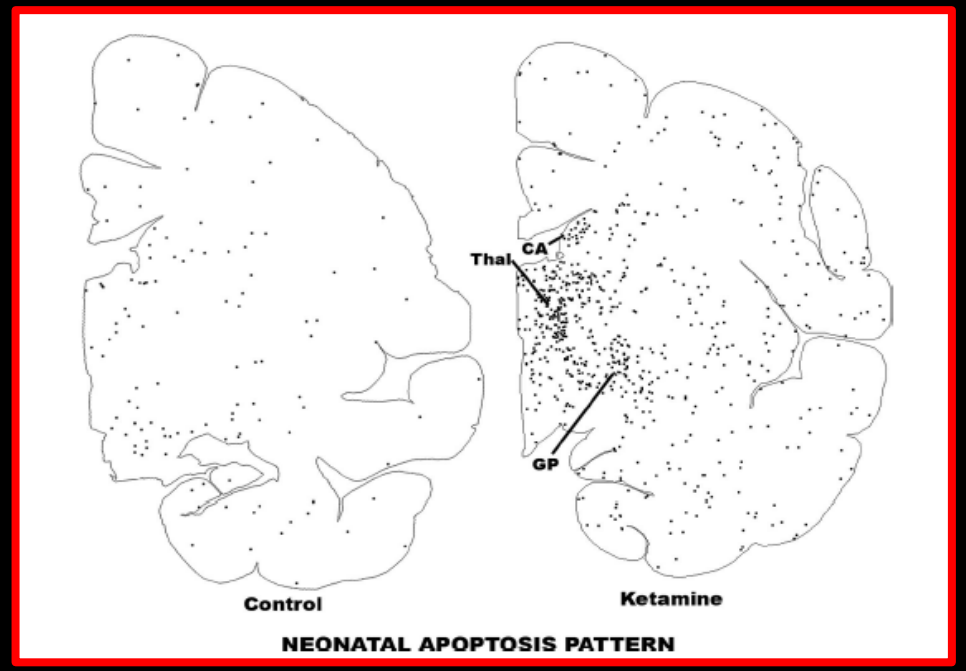
Evidence based on *ex vivo* and *in vivo* studies in animal models[3]

Neurotoxic effects in humans have not been confirmed due to lack of methods to assess these effects non-invasively in the young brain

[1]Andropolous and Greene, PMCID: 28177852
[2]https://www.fda.gov/downloads/Drugs/DrugSafety/UCM554644.pdf
[3]McGowan and Davis, PMCID: 18499584

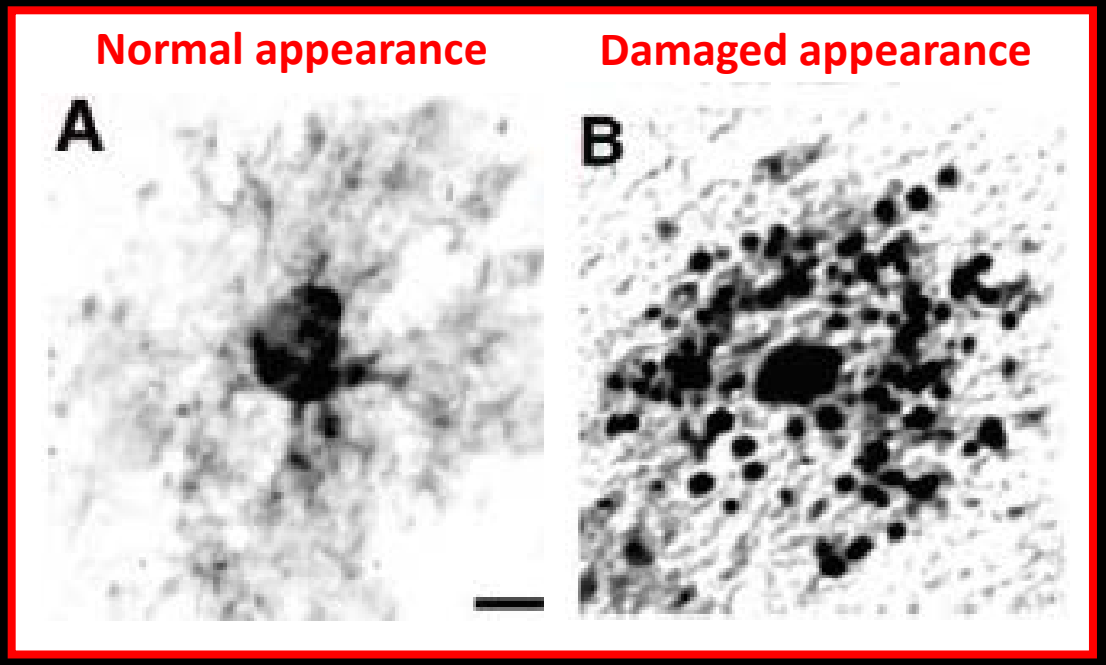
Background

N-Methyl-D Aspartate (NMDA) antagonists and γ-Aminobutyric Acid A ($GABA_A$) agonists can induce apoptosis of neurons and oligodendrocytes



Activated Caspase 3 (CASP3, an apoptotic enzime) in neonatal Rhesus macaque (*Banbrick et al., PMCID: 22222480*)

Apoptosis (programmed cell death) causes changes in tissue microstructure to which ultrasound is sensitive



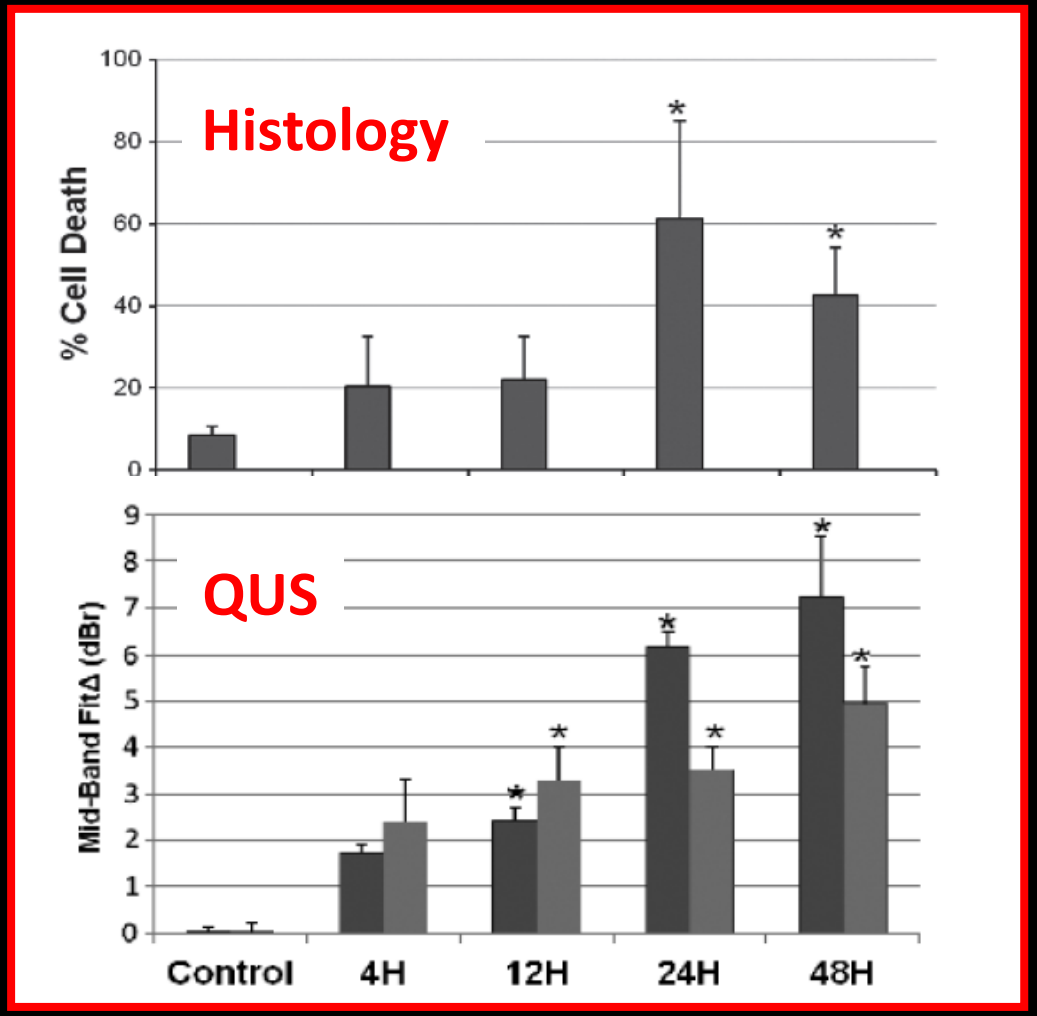
Oligodendrocytes stained for myelin basic protein after exposure of fetal Rhesus macaque to isoflurane (*Creeley et al., PMCID: 24158051*)

Background

Ultrasound signals are sensitive to how tissue microstructure changes during apoptosis

This information can be obtained through the spectral (frequency-based) analysis of echo signals, i.e., **Quantitative Ultrasound (QUS)**[4]

QUS has been used *in vitro, ex vivo,* and *in vivo* to assess apoptosis and other types of cell death in various cancer types and tissues[5,6]



Cell death evaluated through histology and QUS in breast tumor xenografts in mice (*Sadeghi et al., PMID: 23761215*)

[4]Czarnota, Kolios, et al. PMCID: 10507779
[5]Vlad et al., PMCID: 21057928
[6]Tadayyon et al. PMCID:28710751

Objectives

LONG TERM GOAL

Investigate the use of Quantitative Ultrasound to detect the induction of apoptosis by anesthetics in the newborn brain

Current objectives:

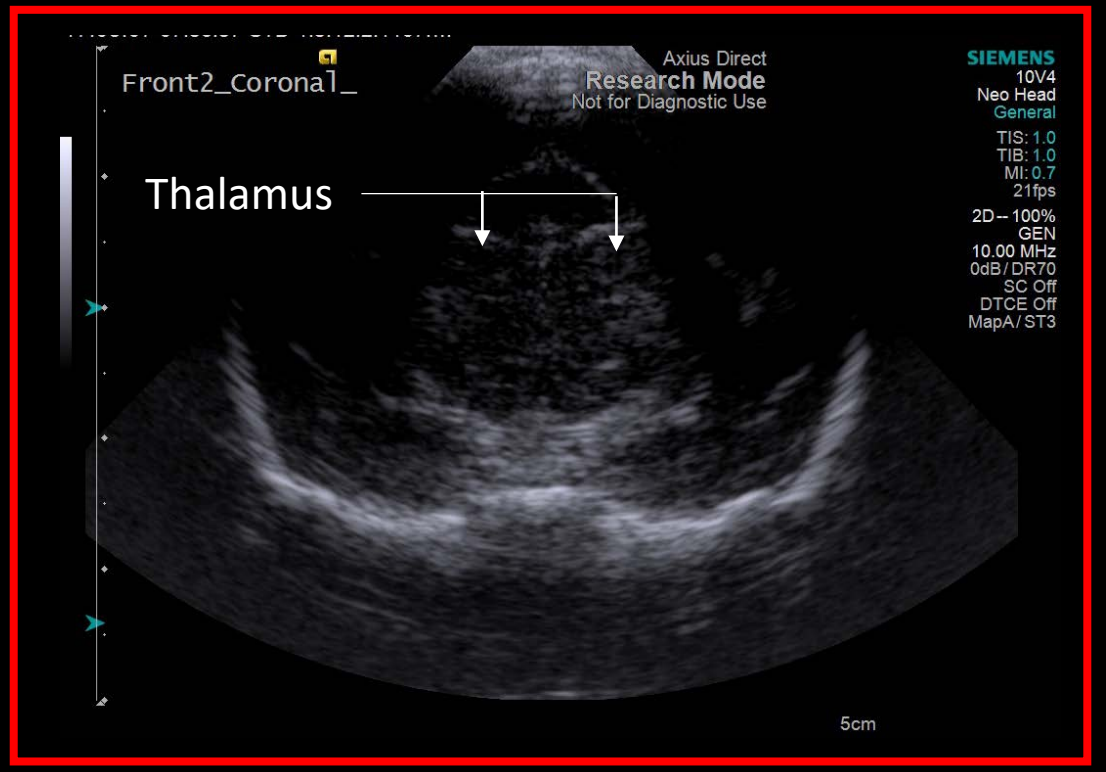
1. Perform a preliminary assessment of the sensitivity of Quantitative Ultrasound (QUS) to apoptosis induced by sevoflurane ($GABA_A$ agonist) in a non-human primate model of the newborn brain

2. Identify challenges inherent to the application of QUS techniques in the *in vivo* newborn brain

Methods

- 14 2-7 day-old Rhesus macaques from the Wisconsin National Primate Research Center
- Five-hour long exposure to sevoflurane ($GABA_A$ agonist)
- Ultrasound data acquisition at 0hrs and 6hrs after start of exposure
- Tissue harvested at 8hrs for activated caspase 3 immunohistochemistry

SIEMENS Acuson S3000
Phased array transducer @ 10MHz



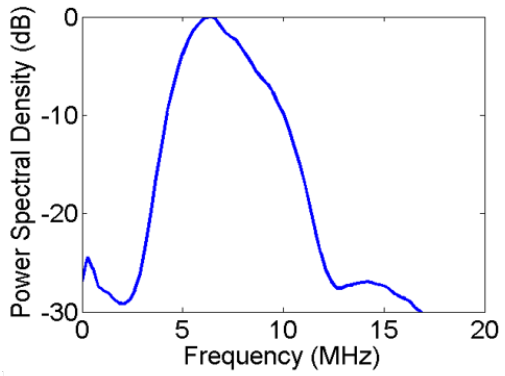
10-15 frames of “raw” radiofrequency (RF) signals from thalamus using Axius Direct User Research interfase[7]

[7]Brunke et al. PMCID 17225815

Methods

QUANTITATIVE ULTRASOUND

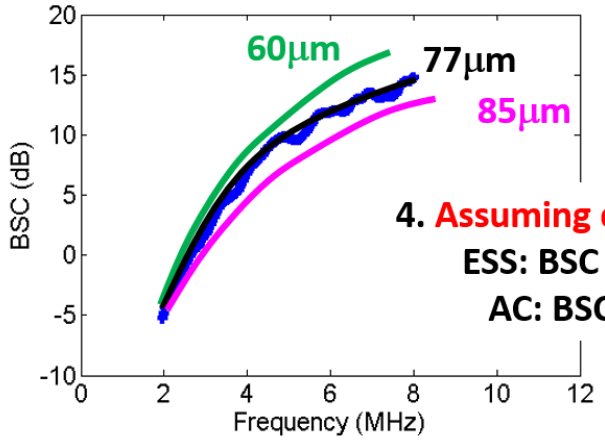
DIFFUSE SCATTERING ANALYSIS
- Biomarkers for apoptosis -



1. Echo signal power spectrum

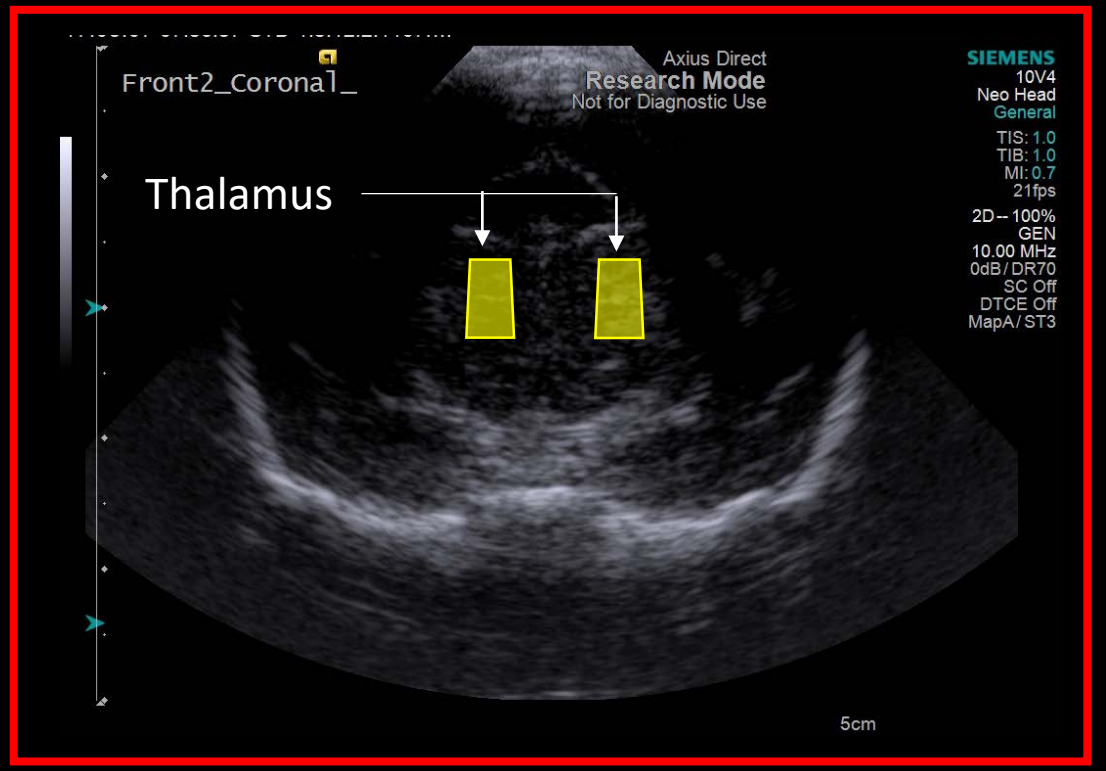
2. Attenuation compensation

3. Backscatter coefficient (BSC)



4. **Assuming diffuse scattering,**
   ESS: BSC vs. frequency
   AC: BSC magnitude

Insana & Hall, PMCID: 2299033



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Methods

QUANTITATIVE ULTRASOUND

DIFFUSE SCATTERING ANALYSIS
- Biomarkers for apoptosis -

Effective Scatterer Size (ESS)	Acoustic Concentration (AC)

(Insana & Hall, PMCID: 2299033)

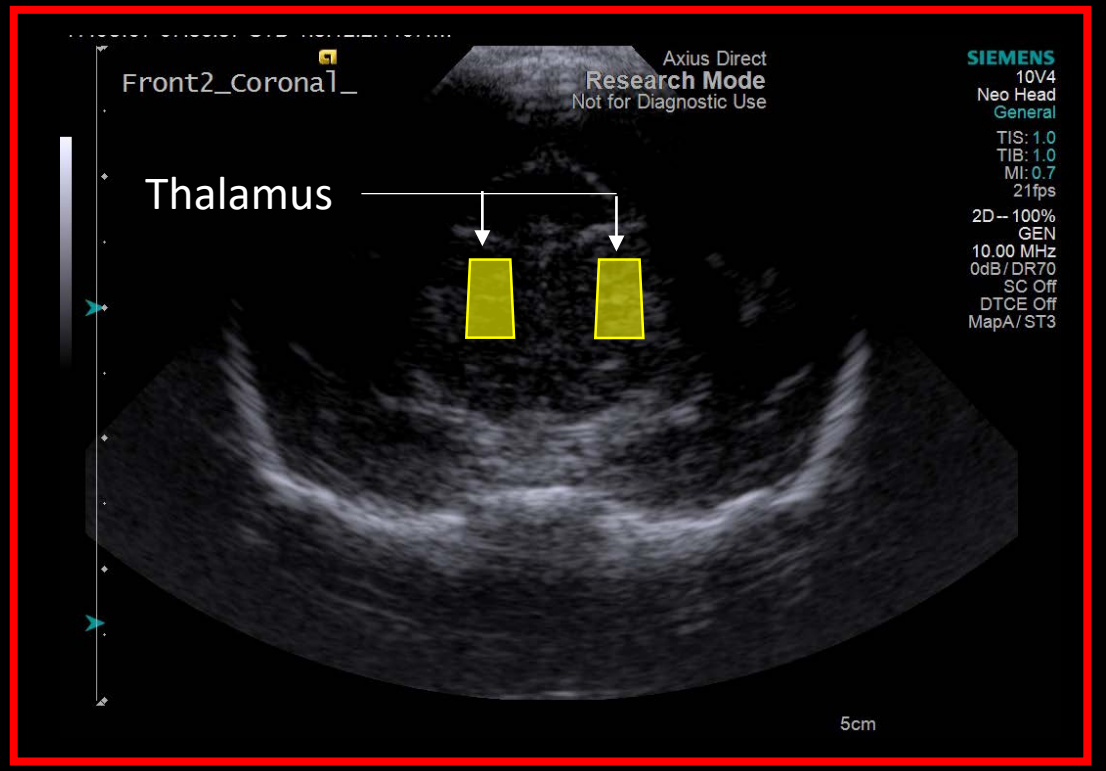
REJECTION CRITERIA

Spectral signal/noise < 15 dB,
Disagreement between results from left and right thalamus

DATA ANALYSIS

6hrs. vs. 0hrs Parameter Change (P= ESS or AC)

$$\Delta P(\%) = \frac{P(6hrs) - P(0hrs)}{P(0hrs)} \times 100\%$$



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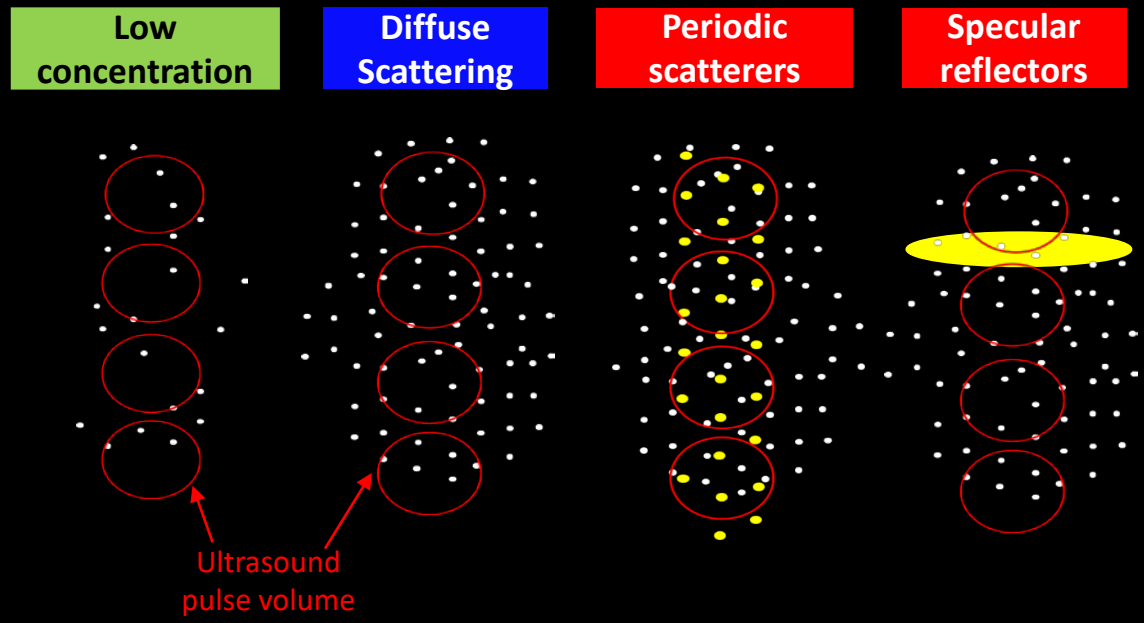
COHERENT SCATTERING ANALYSIS
- Validity of diffuse scattering assumption -

Detection of different scattering conditions
(Rosado-Mendez et al. PMCID: 27046872)

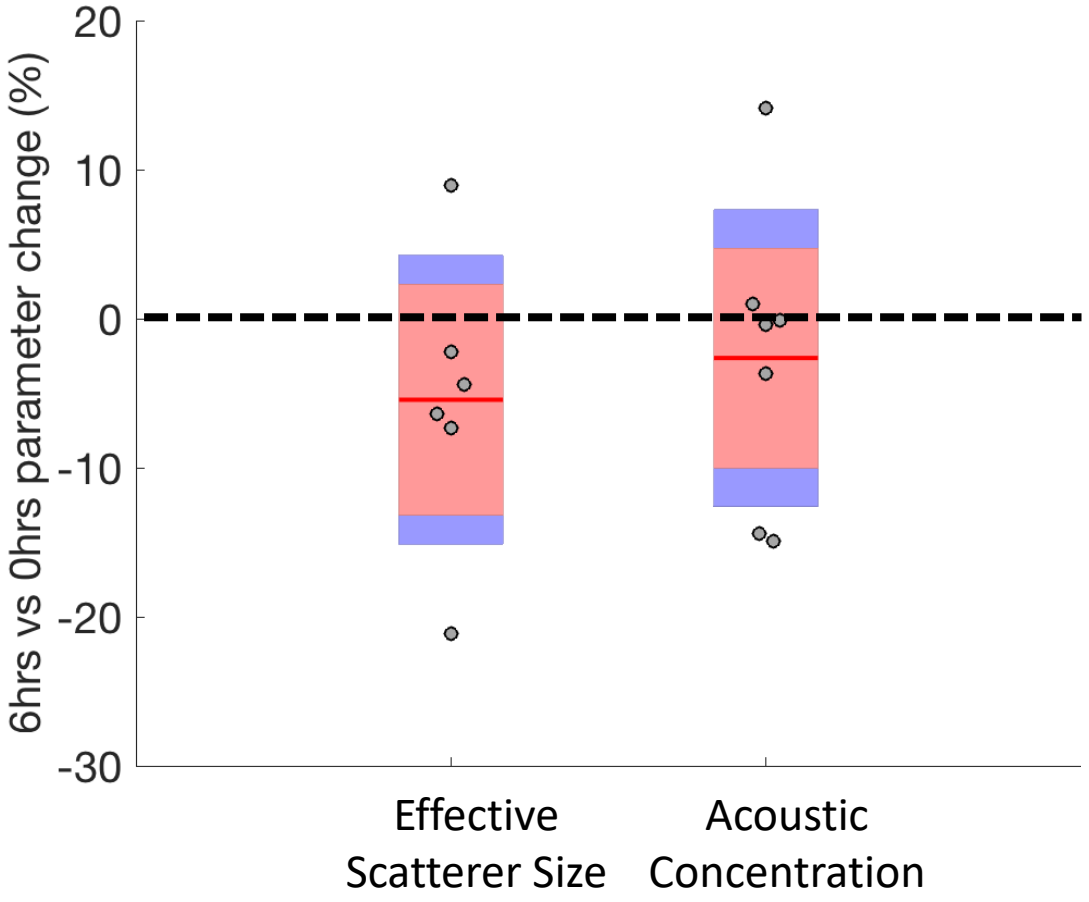


Results

Data rejection:

- Low Spectral signal/noise: 5 cases
- Disagreement left vs. right sides:
  ESS: 3 cases, AC: 2 cases
- Remaining for analysis
  ESS: 6 cases, AC: 7 cases

6hrs vs. 0hrs parameter change		
	ESS	**AC**
Mean	-5.4%	-2.6%
Range	-21.1% to 8.4%	-14.9% to 14.1%
Wilcoxon p	0.05	0.36
Significant reduction in:	5/6 subjects (83%)	4/7 subjects (57%)



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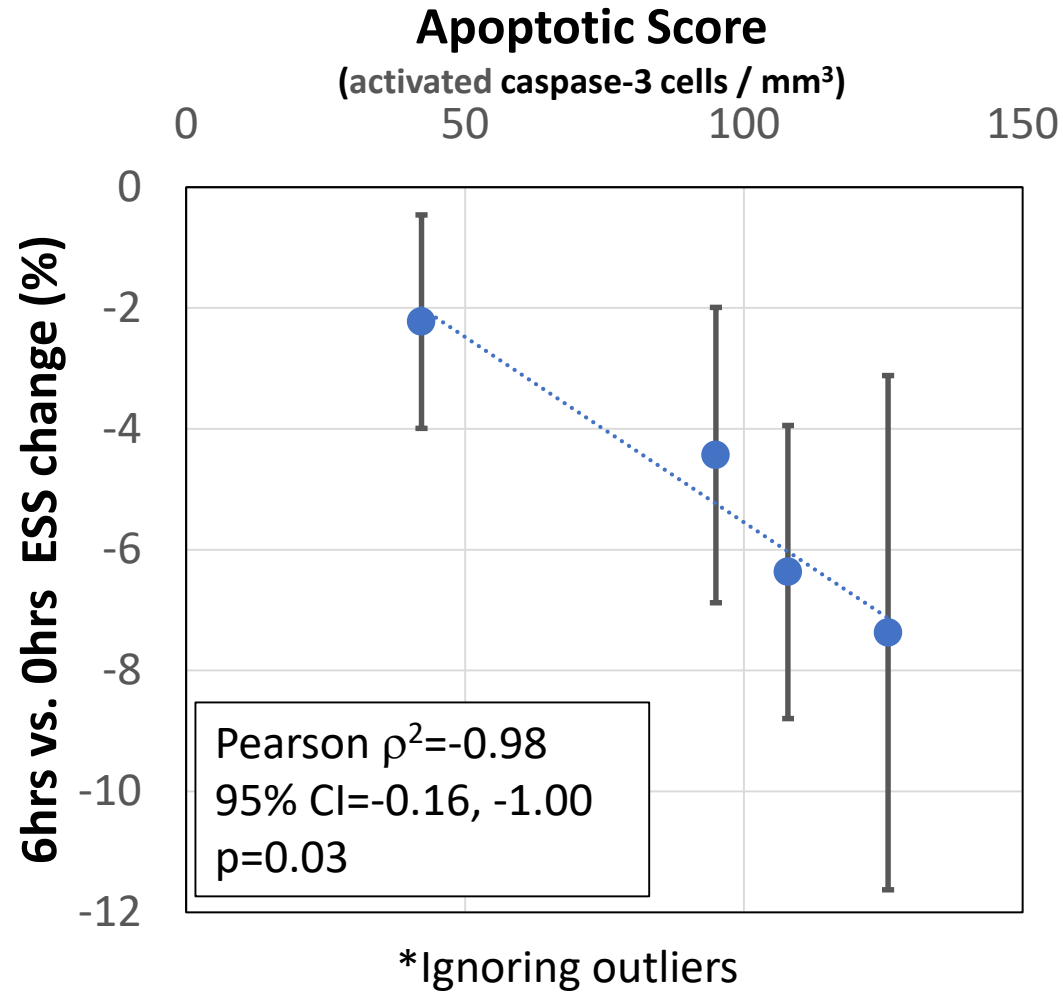
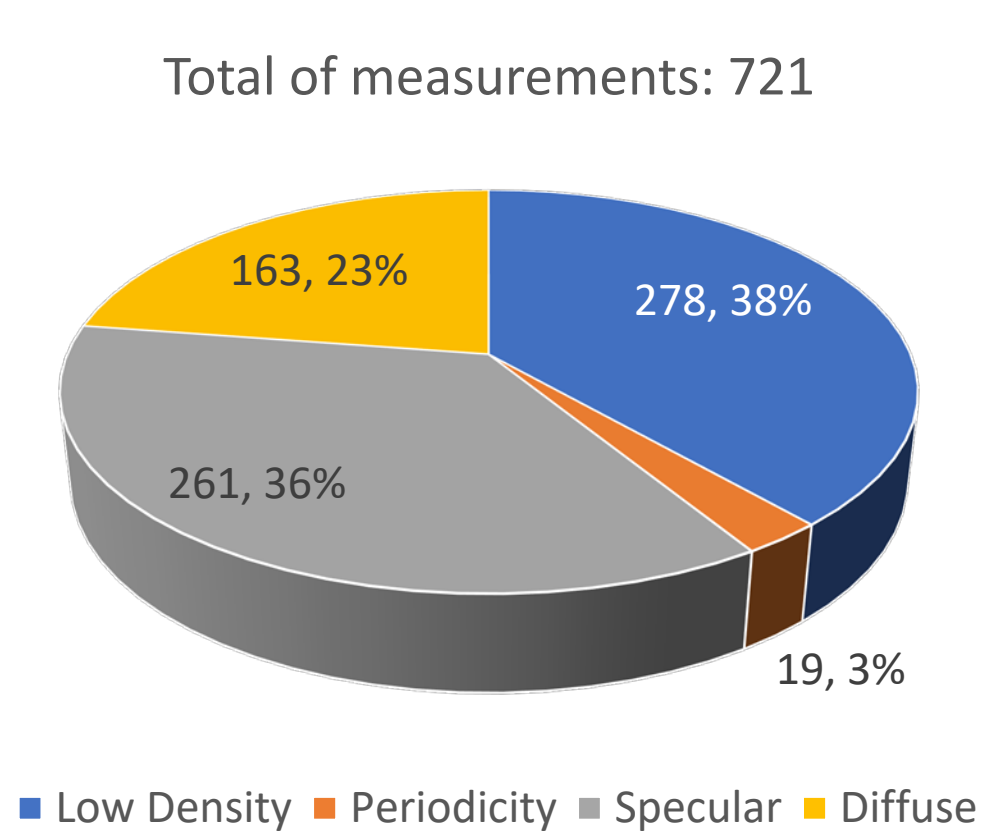
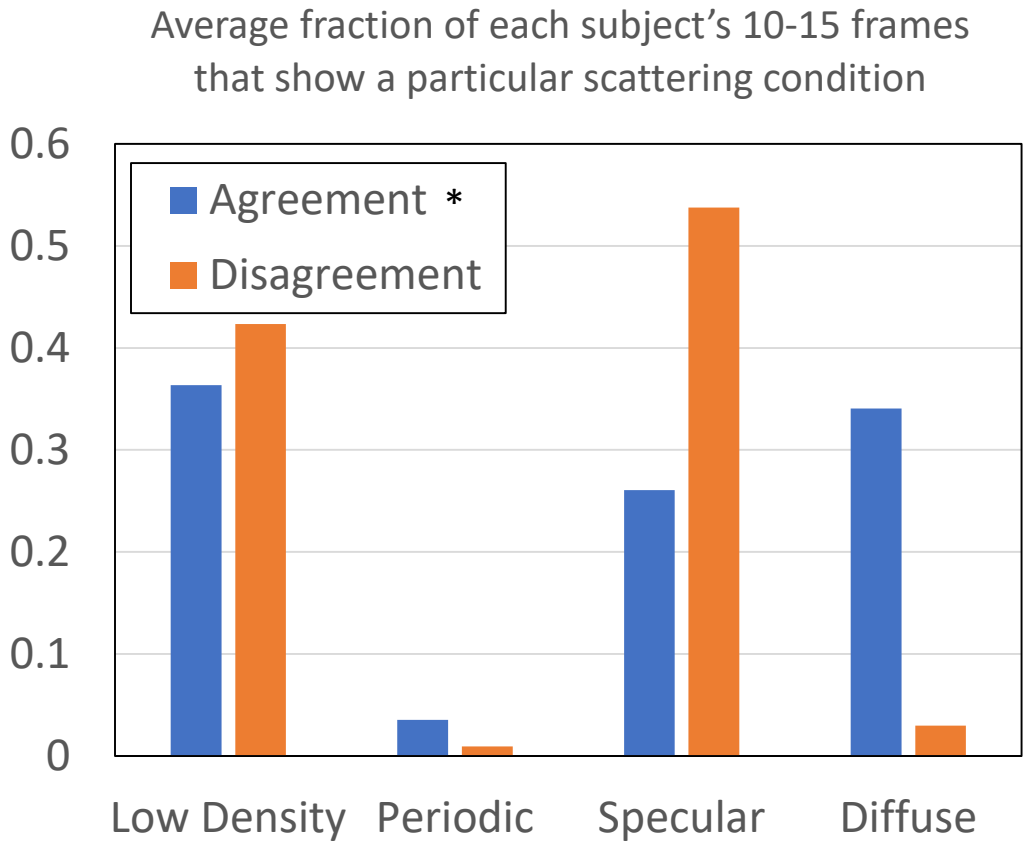


Results

Coherent scattering leads to data paucity and increased variance[8,9]





[8]Rubert et al., PMCID 24726800
[9]Luchies et al. PMCID: 22622974

*Agreement between QUS results from left and right thalamus

Conclusion

- The Effective Scatterer Size, a Quantitative Ultrasound parameter based on assuming diffuse scattering, shows promise as a biomarker for apoptosis induced by exposure to sevoflurane in the *in vivo* young Rhesus macaque brain

- The presence of coherent scattering conditions severely constrained our data. Current work is focusing on overcoming these limitations through data compounding and other strategies (Liu et al. PMCID: 7521044, Herd et al. PMCID: 22033132, Luchies et al. PMCID: 22622974)

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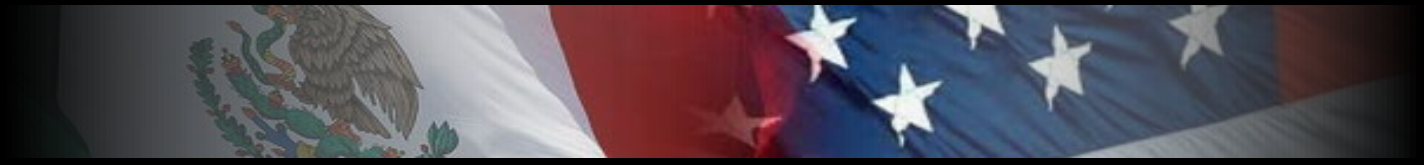

Contact information

Ivan M. Rosado-Mendez, PhD
Research associate, Instituto de Física
Universidad Nacional Autónoma de México

irosado@fisica.unam.mx