

#### Acquiring MRI brain images in awake unrestrained sheep

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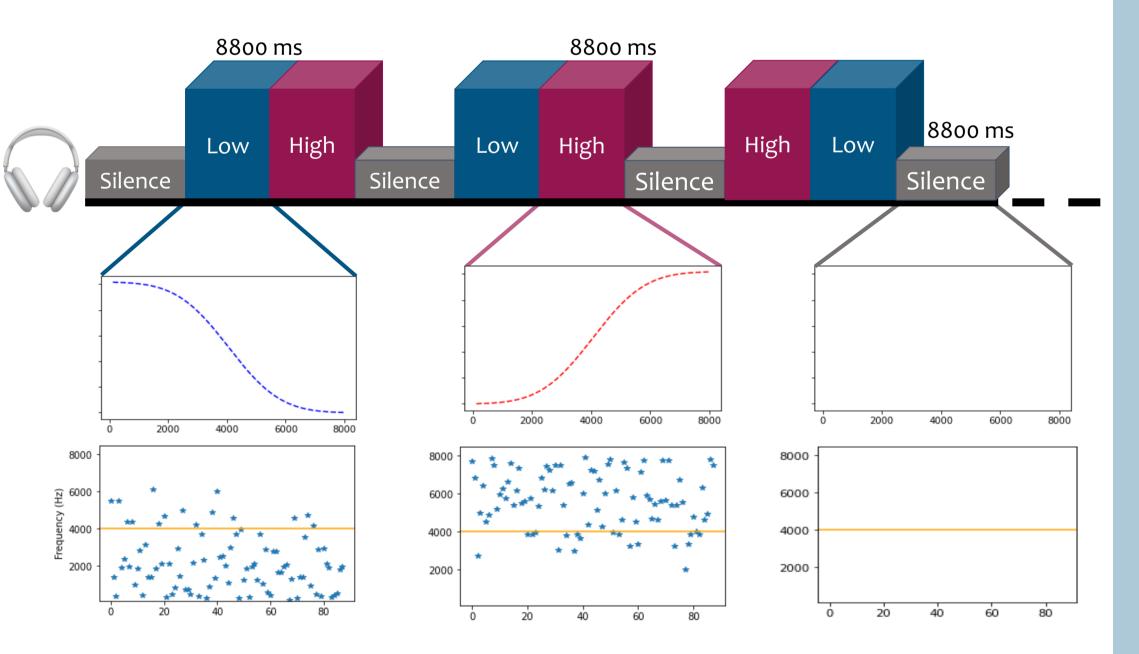
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#### Introduction

Magnetic Resonance Imaging (MRI) is a non-invasive technique that requires the participant to be completely motionless. To date, MRI in awake and unrestrained animals has only been achieved with humans and dogs (Andics et al., 2014). For other species, alternative techniques such as anesthesia (Barrière et al., 2019), restraint (Stefanacci et al., 1998) and/or sedation (Behroozi et al., 2018) have been necessary. MRI studies with sheep have only been conducted under general anesthesia. This ensures the absence of movement and allows relatively long MRI experiments. However, it can also be detrimental to health, disrupt neurovascular coupling (Aksenov et al., 2015) and does not permit the study of higher-level cognition. Here we present a proof of concept that positive reinforcement training in sheep is sufficient to be able to acquire MRI of their brain. Specifically, we are currently using functional MRI to study the mechanisms of vocalization perception in these farm animals.

#### Method

- 1. Step-by-step training protocol with 10 lambs (5  $\circlearrowleft$  5  $\circlearrowleft$ )
- → Lying motionless on the MRI table for the minimum time required for an acquisition (~10 min)
  - Sheepfold training phase (mock MRI table and coil, scanner noise desensitization)
  - MRI room training phase (table movements, scanner bore, MRI coil, headphones)
- 2. MRI acquisition
- → Localize the sheep auditory cortex and identify its functional organization
  - Auditory stimulation paradigm



## MRI sequences

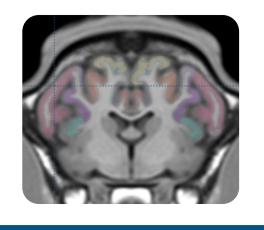
T1-weighted structural scan: TR = 2200 ms, TE = 2.64 ms, flip angle = 12°, voxel size =  $1.12 \text{ mm}^3$ , TA = 2 min 36 sec

**Gradient echo fieldmap:** TR = 500 ms, TE = 4.92/7.38 ms, flip angle= $55^{\circ}$ , voxel size = 1.5 x 1.5 x 2 mm, TA = 1 min 57 sec

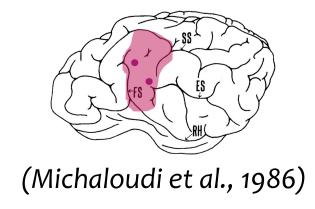
**EPI functional images:** multiband acceleration factor = 2, TR = 2000 ms, TE = 40 ms, flip angle =  $12^{\circ}$ , number of slices = 40, voxel size = 1.51 mm<sup>3</sup>, number of volumes = 112, TA = 4 min 04 sec

### Discussion

- Positive reinforcement training of sheep and positive humananimal cooperation are sufficient to enable acquiring MRI images of their brain.
- This innovative training protocol opens the door to the possibility of conducting animal welfare friendly functional MRI studies with sheep.
- The preliminary awake sheep fMRI results localize the auditory cortex similarly to the putative region proposed by Michaloudi and colleagues.



**Département PHASE** 



#### **Acknowledgements** We would like to thank UE-PAO; Platform PIXANIM and PRC workshop for their invaluable help.

# Sheep voluntarily participate in awake and unrestrained MRI sessions after positive reinforcement-based training



ACQUIRING MRI BRAIN IMAGES IN AWAKE UNRESTRAINED SHEEP 1 CNRS, IFCE, INRAE, Université de Tours, PRC, Nouzilly, France 2 INRAE, UEPAO 1297, Nouzilly, France

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LAMB'S BIRTH March 2022







- Male and female lamb's under artificial feeding
- Positive interactions with the lambs twice a day, 7 days a week
- New objects / Exercises [foam, ramp]

March 2022





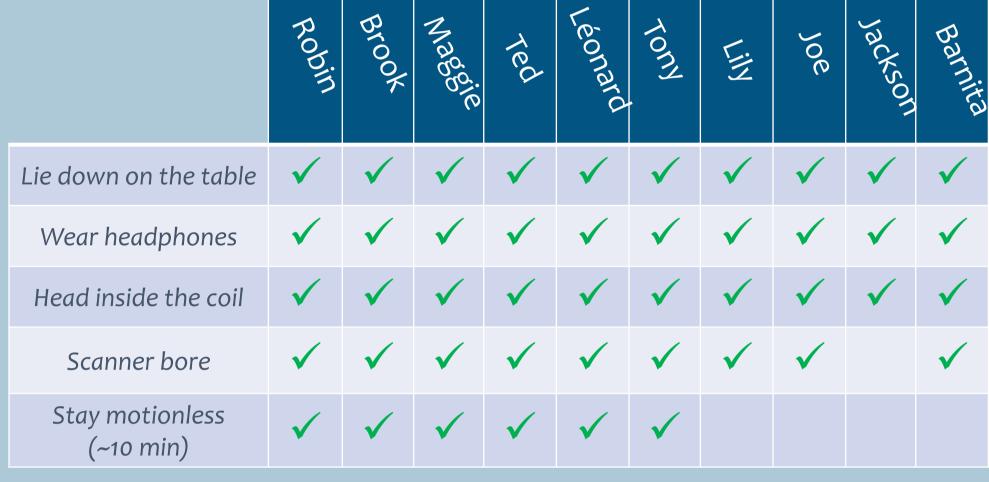
- Climb a ramp to reach the mock MRI
- ❖ Put their head inside the mock MRI coil
- Lie still on a moving table

March 2022 to September 2022

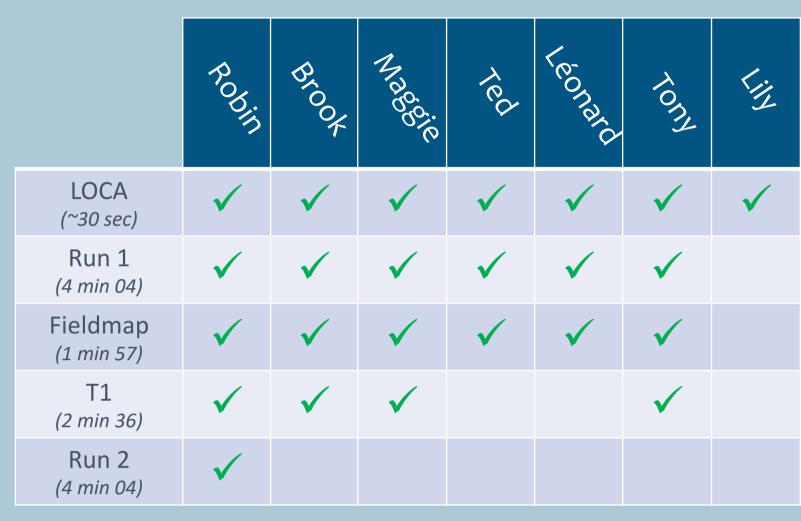


#### MRI ROOM TRAINING PHASE

- Accept headphones on their ears
- ❖ Put their head inside the real MRI coil
- Lie still on the moving table
- Stay motionless inside the scanner bore
  - September 2022 to now



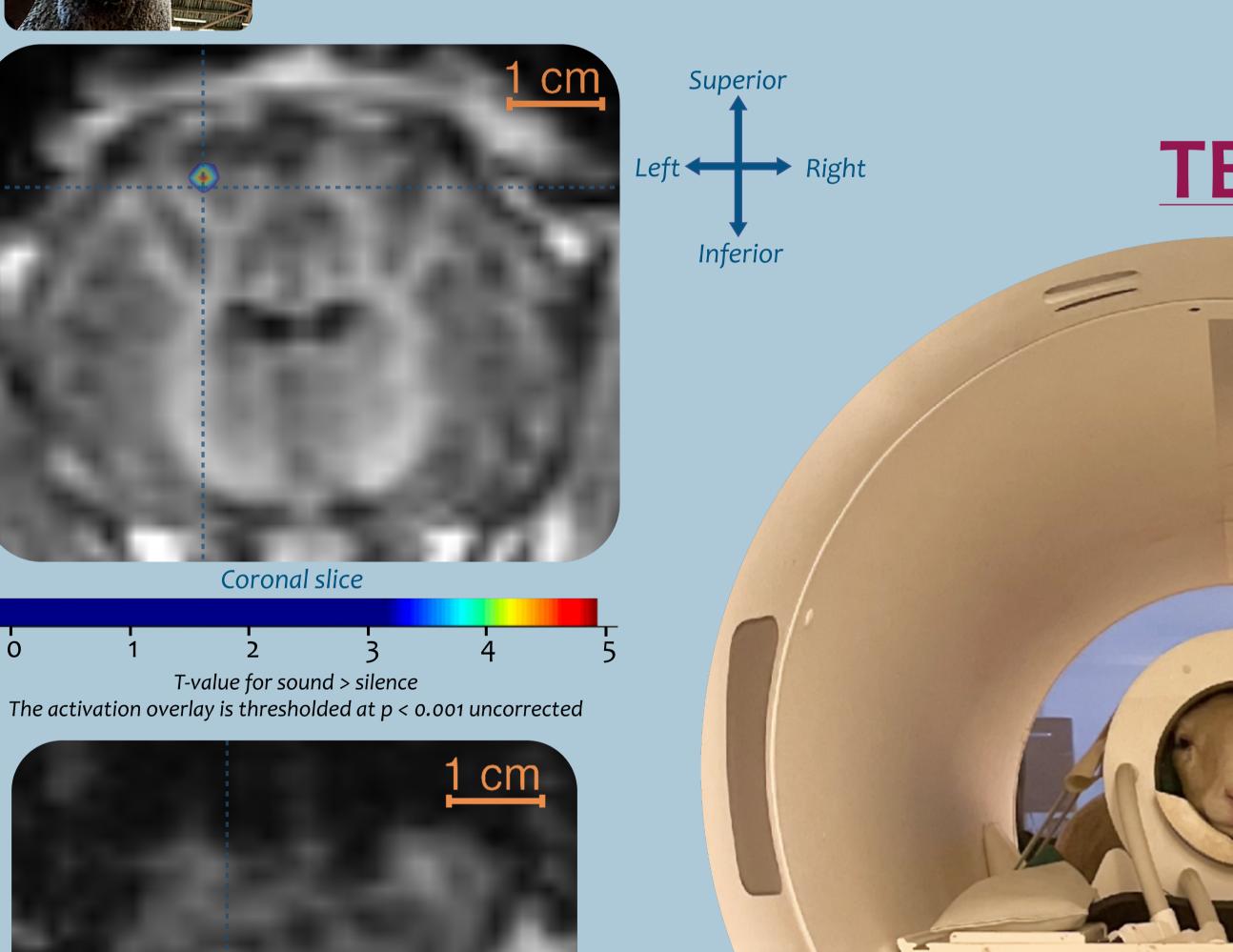
Training Progress. The different steps performed in the MRI room for each sheep  $\mathbb{Q}$ : Robin, Brook, Maggie, Lily, Barnita;  $\mathbb{O}$ : Ted, Léonard, Tony, Joe, Jackson

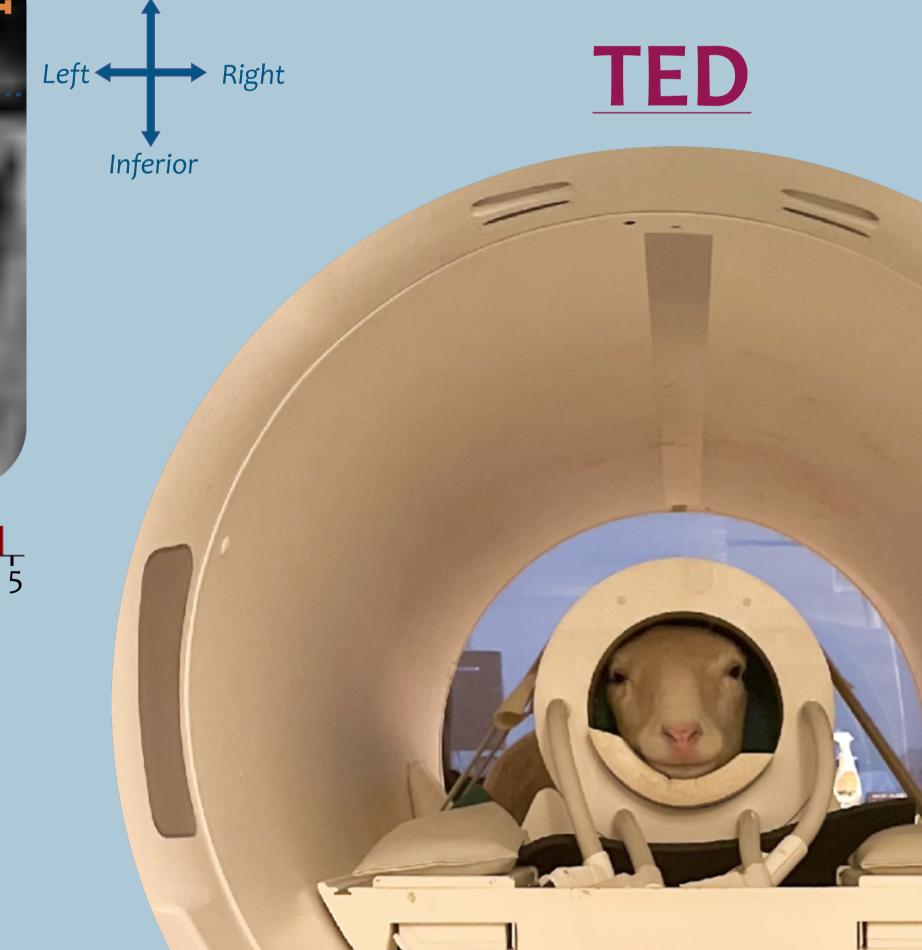


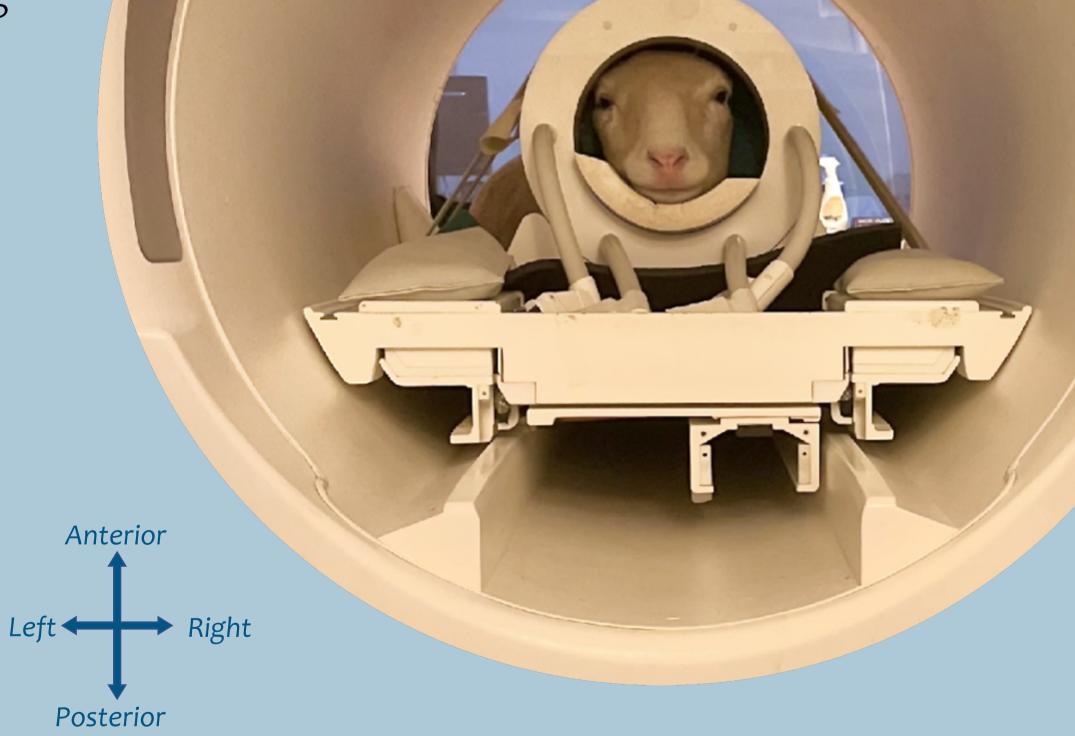
Progress in MRI data collection during a single session

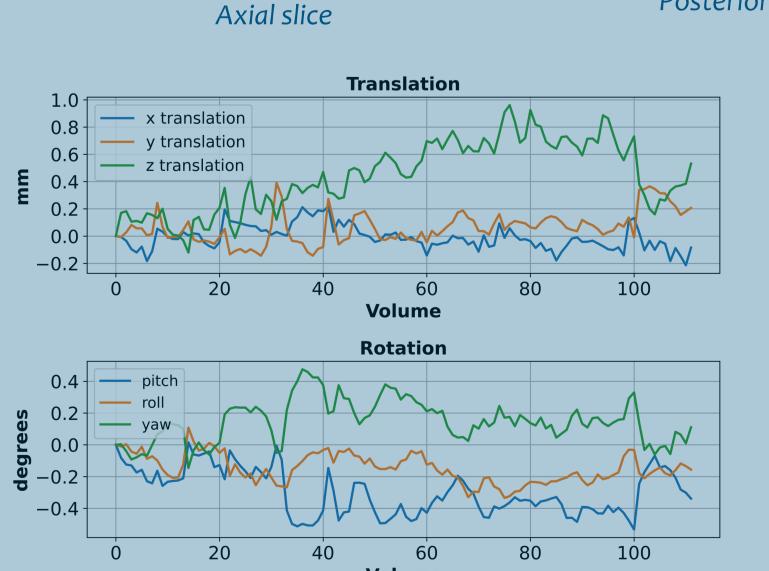


## LÉONARD





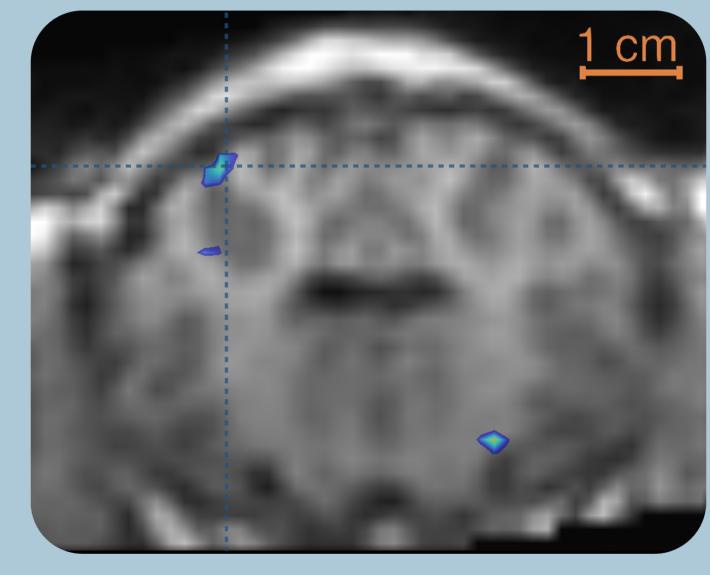




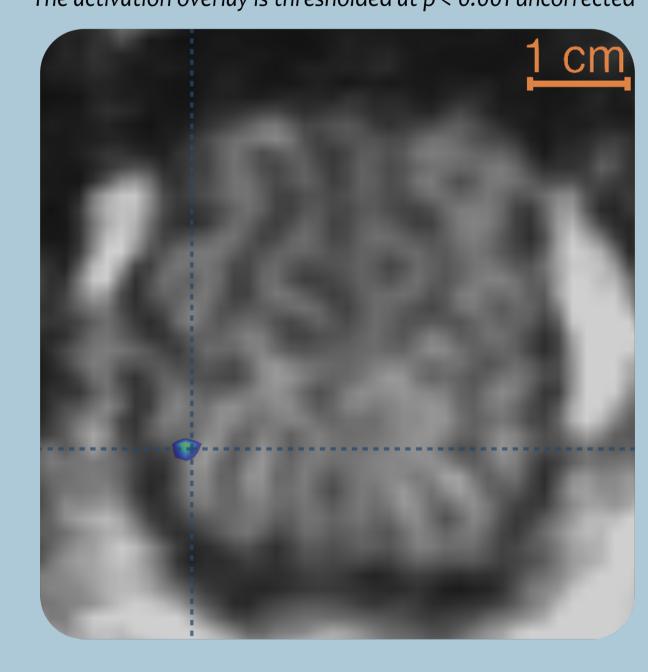
Motion parameters representing the amount of motion in each of the six directions for each volume acquired.

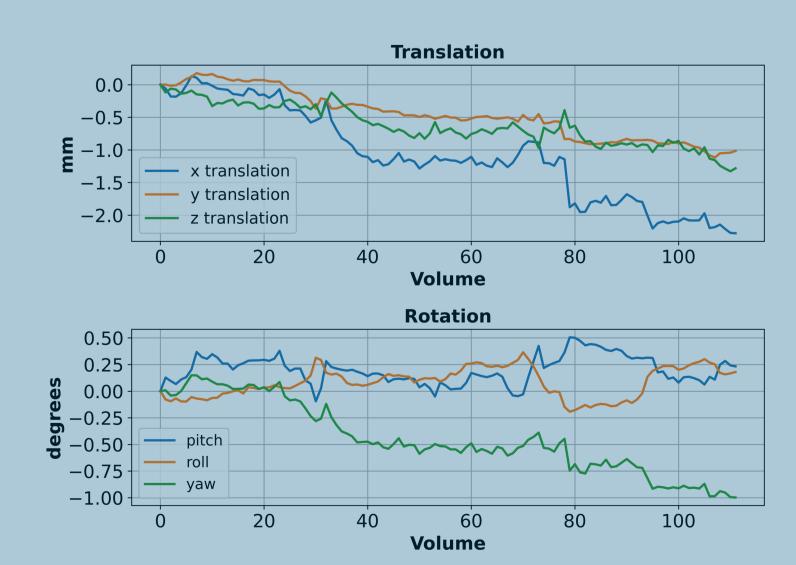
# **BROOK**





T-value for sound > silence The activation overlay is thresholded at p < 0.001 uncorrected





Motion parameters representing the amount of motion in each of the six directions for each volume acquired.



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