Check for updates

RESEARCH ARTICLE

Functional mapping of auditory responses to complex sounds under light anesthesia in rhesus macaques

[version 1; peer review: 1 approved, 2 approved with reservations]

Régis Trapeau[1], Luc Renaud[1], Melina Cordeau[2], Yoan Esposito[1], Pascal Belin[1,3]

[1]Institut de Neurosciences de la Timone, Aix-Marseille Université, UMR 7289 CNRS, Marseille, Provence-Alpes-Côte d'Azur, 13005, France
[2]Department of Human Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA
[3]Institute of Language Communication and the Brain, Marseille, Provence-Alpes-Côte d'Azur, 13000, France

v1 **First published:** 05 Sep 2025, **5**:273
https://doi.org/10.12688/openreseurope.20953.1
Latest published: 05 Sep 2025, **5**:273
https://doi.org/10.12688/openreseurope.20953.1

Abstract

We investigated the auditory responses of anesthetized rhesus macaques to complex natural sounds using functional magnetic resonance imaging (fMRI) and a near-awake anesthesia protocol combining low-dose sevoflurane and dexmedetomidine. Eleven animals were scanned while listening to macaque vocalizations and non-vocal sounds. Robust activation was observed in primary and belt auditory cortices as well as subcortical structures, indicating preserved auditory responsiveness under anesthesia. However, unlike in awake animals, selective responses to vocalizations in anterior temporal voice areas (aTVAs) were absent. Instead, vocalization sensitivity emerged in the ventral premotor cortex, prefrontal cortex, and posterior middle temporal gyrus, regions associated with the dorsal auditory stream and multisensory integration. These findings suggest that while anesthesia preserves basic auditory processing, it modulates higher-order cortical responses involved in voice perception. Anesthetized fMRI thus offers a valuable tool for large-scale studies of primary auditory functions, while it must be approached with caution when studying higher-order auditory functions.

Plain summary

Understanding how primates process sounds, especially vocalizations, is essential to uncovering the roots of human speech and communication. Typically, this kind of brain research in monkeys is done while the animals are awake, which is challenging and time-consuming. In this study, we used a gentle anesthesia protocol in eleven macaques to see if their brains could still respond to different

Open Peer Review

Approval Status ? ? ✓

	1	2	3
version 1 05 Sep 2025	? view	? view	✓ view

1. **Mathilda Froesel**, Institut des sciences cognitives Marc Jeannerod, Bron, France
KU Leuven Department of Neurosciences (Ringgold ID: 213965), Leuven, Belgium

2. **Kirill V Nourski**, The University of Iowa, Iowa, USA
Emily Dappen, The University of Iowa Department of Neurosurgery, Iowa City, USA

3. **Alix Trouillet**, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Any reports and responses or comments on the article can be found at the end of the article.

types of sounds during functional magnetic resonance imaging. We found strong responses in early auditory regions, showing that these parts of the brain still process complex sounds under light anesthesia. However, auditory areas usually involved in processing voices in awake animals did not show the same patterns. Surprisingly, some areas linked to action and multisensory processing were still sensitive to vocalizations. This means that while light anesthesia can help with large-scale brain studies of basic hearing, it may not fully reveal how the brain processes more complex sounds like voices.

Keywords
fMRI, auditory, non-human primate, voice, anesthesia

This article is included in the European Research Council (ERC) gateway.

This article is included in the Horizon 2020 gateway.

Corresponding authors: Régis Trapeau (regis.trapeau@univ-amu.fr), Pascal Belin (pascal.belin@univ-amu.fr)

Author roles: Trapeau R: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Renaud L**: Conceptualization, Investigation, Methodology, Writing – Review & Editing; **Cordeau M**: Investigation, Methodology, Writing – Review & Editing; **Esposito Y**: Investigation, Writing – Review & Editing; **Belin P**: Conceptualization, Funding Acquisition, Methodology, Project Administration, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement no. 788240). This work was performed in the Center IRM-INT (UMR 7289, AMU-CNRS), platform member of France Life Imaging network (grant ANR-11-INBS-0006).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2025 Trapeau R *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Trapeau R, Renaud L, Cordeau M *et al.* **Functional mapping of auditory responses to complex sounds under light anesthesia in rhesus macaques [version 1; peer review: 1 approved, 2 approved with reservations]** Open Research Europe 2025, **5**:273 https://doi.org/10.12688/openreseurope.20953.1

First published: 05 Sep 2025, **5**:273 https://doi.org/10.12688/openreseurope.20953.1

Introduction

Over the past two decades, functional magnetic resonance imaging (fMRI) in rhesus macaques has become a key tool in understanding the neural mechanisms underlying auditory perception, from basic — tonotopy (Joly *et al.*, 2014; Tanji *et al.*, 2010), auditory cortical fields mapping (Petkov *et al.*, 2006; Schönwiesner *et al.*, 2015), temporal and spectral modulations (Erb *et al.*, 2019) — to higher-level processes — complex sounds (Kikuchi *et al.*, 2014), sound location (Ortiz-Rios *et al.*, 2017), multimodal integration (Froesel *et al.*, 2022; Kayser *et al.*, 2005).

Furthermore, fMRI with macaques has contributed to our understanding of the origins of speech by revealing cortical regions that are sensitive to voices and vocalizations in our close relatives (Bodin *et al.*, 2021; Joly *et al.*, 2012; Ortiz-Rios *et al.*, 2015; Petkov *et al.*, 2008), as well as to guide electrophysiological recordings to those regions (Esposito *et al.*, 2025; Giamundo *et al.*, 2024; Perrodin *et al.*, 2010; Perrodin *et al.*, 2011). Such studies have shown that conspecific vocalizations selectively activate higher-level areas of the auditory cortex, particularly in an anterior portion of the temporal gyrus, analogous to the anterior temporal voice area (aTVA) observed in humans (Aglieri *et al.*, 2018; Pernet *et al.*, 2015). Furthermore, vocalization-sensitive regions have been reported in areas beyond the temporal gyrus, specifically in the ventral premotor and prefrontal cortices (Bodin *et al.*, 2021; Ortiz-Rios *et al.*, 2015).

Auditory fMRI studies in macaques have essentially been performed in awake, behaving animals. Awake fMRI in macaques is particularly valuable because it captures neural responses under naturalistic conditions, in fully engaged, behaviorally active subjects. In addition to the findings related to voice selectivity, awake fMRI has also demonstrated its efficacy in other modalities, particularly in detecting face-selective areas (Ku *et al.*, 2011; Pinsk *et al.*, 2009; Tsao *et al.*, 2003). However, awake monkey fMRI poses substantial challenges. The animal requires extensive behavioural training to acclimate to the scanner environment, to remain motionless during long sessions of image acquisition, and to perform an auditory task (Goense *et al.*, 2010; Vanduffel & Farivar, 2014). The acquisition of awake fMRI data from as few as two naïve monkeys can easily take over a year. Consequently, the number of animals used in awake fMRI studies is limited, with only two to three animals per study due to feasibility issues. In addition, and despite the behavioral training, inevitable head and body movements occur during acquisition, even under head restraint. This results in large residual motion artifacts that degrade data quality and increase the amount of data that needs to be collected (Goense *et al.*, 2010). As a point of comparison, it has been determined that up to sixty times the amount of data acquired in humans is required to match response reliability in macaque fMRI (Norman-Haignere *et al.*, 2019).

In contrast to awake imaging, anesthetized fMRI protocols in rhesus macaques significantly simplify data acquisition. Anesthesia nearly eradicates head motion, thereby enabling the collection of higher-quality data. Most importantly, anesthesia eliminates the need for long-term behavioral training to ensure that the animal sits still for extended periods in the scanner, a hostile environment for the animal (Goense *et al.*, 2010), and the surgical procedures concomitant with head fixation. These advantages offer the potential for acquiring data on cohort sizes that are nearly equivalent to those of human fMRI studies, and at a significantly reduced time frame when compared to the whole process employed in studies involving only two awake monkeys.

However, anesthetized fMRI comes with its own set of drawbacks. Anesthetic agents used to induce sedation can markedly alter neural activity and neurovascular coupling (Slupe & Kirsch, 2018; Zhang, 2022), thereby potentially attenuating or even distorting the fMRI BOLD signal that reflects the underlying neuronal response to auditory stimuli (Guldenmund *et al.*, 2017; Uhrig *et al.*, 2016). To maximize the outcome of anesthetized fMRI, the anesthesia protocol must be optimized to induce a state that provides immobilization while minimally interfering with the underlying neural processes that support auditory perception. Various combinations of anesthetic agents have been used to achieve this goal. These "near-awake" protocols include: remifentanyl, an opioid analgesic, associated with mivacurium, a neuromuscular-blocking drug (Ku *et al.*, 2011; Logothetis *et al.*, 1999; Petkov *et al.*, 2006); moderate propofol sedation, with (Barttfeld *et al.*, 2015) or without (Uhrig *et al.*, 2016) muscle blocking agent (cisatracrium); sufentanil or propofol, supplemented with low-dose isoflurane (Shi *et al.*, 2021); the combination of low-dose halogenated ether (isoflurane or sevoflurane) alongside either nitrous oxide (Obliger-Debouche *et al.*, 2025; Sadagopan *et al.*, 2015) or dexmedetomidine (Autio *et al.*, 2020), a sedative that has been shown to relatively preserve functional connectivity in humans (Guldenmund *et al.*, 2017).

The objective of the present study was to map the cerebral (subcortical and cortical) response to complex natural sounds, including vocalizations, in a large group of anesthetized macaques. Eleven rhesus macaques were exposed to macaque vocalizations and non-vocal complex sounds during fMRI acquisitions, while under a near-awake anesthesia protocol involving low-dose sevoflurane combined with dexmedetomidine. Our results show robust activation of core and belt auditory cortices, suggesting that much of the fundamental neural circuitry for auditory perception remains active under this anesthesia protocol. However, in contrast with awake fMRI studies, vocalization selectivity was not observed in higher-order, anterior auditory cortices, but was found in the ventral premotor and prefrontal cortex as well as in the posterior middle temporal gyrus. This suggests different involvement of the ventral and dorsal auditory pathways during anesthesia. We conclude that near-awake anesthesia fMRI is appropriate for studying basic auditory functions in a large cohort of subjects, enabling efficient and reproducible mapping of core auditory areas without the extensive training required for

awake imaging, while it may obscure the neural mechanisms underlying complex auditory processing, such as the selective response to conspecific vocalizations.

Methods

Animals

Eleven rhesus monkeys (Macaca mulatta), 7 females and 4 males, aged between 1 and 17 years (median = 6) and weighing between 1 and 8 kg (median = 5), were scanned. The animals were sourced from the same colony and were selected based on their small head size, which was required to be compatible with the MRI head coil. All experimental procedures were in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by the Ethical board of Institut de Neurosciences de la Timone (ref 2016060618508941).

Animal preparation and anesthesia

All scans were performed under anesthesia with a protocol inspired by a previous study (Autio *et al.*, 2025). Anesthesia was induced with an intramuscular injection of glycopyrrolate (10 µg/kg), dexmedetomidine (4.5 µg/kg) and ketamine (6 mg/kg), followed by 2% sevoflurane gas administration through tracheal intubation. Two intravenous catheters were implanted into both saphenous veins to provide fluid support for dexmedetomidine and contrast agent delivery separately. Each animal was then secured in the supine position in an MRI testing chair (Rogue Research, Canada) slided feet-first in the MRI scanner. Prior to MRI acquisitions, respiratory control with mechanical ventilation was initiated, as well as continuous delivery of dexmedetomidine (2–4.5 µg/kg/hr). During anatomical scanning, the concentration of sevoflurane gas was lowered to 1–1.5% (depending on the animal), and during functional scanning, it was reduced to 0.5–1.2%. Ferrous oxide contrast agent (monocrystalline iron oxide nanoparticle, MION, 11 mg/kg) was administered at the end of anatomical scanning, some minutes before functional scanning. The animal's physiological characteristics, which includes heart and respiratory rate, capnometry, and oximetry, were monitored during scanning to ensure its health and well-being, as well as to adjust anesthesia to levels close to awakening. A warming blanket was employed to prevent hypothermia.

Stimuli & audio apparatus

Sound stimuli were a subset of the open access PrimaVoice audio stimulus set (Bodin *et al.*, 2025) and comprised two main categories: macaque vocalizations and non-vocal sounds. Each category contained 24 stimuli, for a total of 48 sound stimuli. Each main category was divided into 4 subcategories of 6 stimuli, forming 8 subcategories in total (see Figure 1). Macaque vocalizations were provided by Marc Hauser (Hauser, 1991) and included both positive (coos, n = 6, grunts, n = 6) and negative (barks, n = 6, screams, n = 6) calls. Non-vocal sounds included both natural (living, n = 6, non-living, n = 6) and artificial sounds (human actions, n = 6, or not, n = 6) from previous studies from our group (Belin *et al.*, 2000; Capilla *et al.*, 2013) or kindly provided by Christopher Petkov (Petkov *et al.*, 2008) and Elia Formisano (Moerel *et al.*, 2012). Stimuli were adjusted in duration, resampled at 48828 Hz and normalized by root mean square amplitude. Finally, a 10-ms cosine ramp was applied to the onset and offset of all stimuli. During experiments, stimuli were digitally processed by a TDT System 3 RM1 (Tucker Davis Technologies, Alachua, FL, USA) and delivered via three MRI-compatible piezoelectric speakers (Sonitron, Sint-Niklaas, Belgium). The sound was essentially delivered by bone conduction. Two smaller speakers (frequency response ranging from 1 kHz to 20 kHz) were placed on or near the animal's ears inside the antenna and gently pressed against its skull. A third, larger speaker (frequency response ranging from 300 Hz to 20 kHz), was placed against the dorsal part of the animal's head through the antenna's hole designed to house a head post.

MRI data acquisition

Images were acquired at the MRI Center of the Institut de Neurosciences de la Timone (Marseille, France) using a 3-Tesla PRISMA scanner (Siemens, Erlangen, Germany) with a 24-channels macaque head coil (Rogue Research, Canada) (Autio *et al.*, 2025). High-resolution T1 & T2-weighted anatomical volumes were first acquired for coregistration and normalization purposes (T1w: MPRAGE sequence, TR = 2.2 s, TE = 2.23 ms, TI = 900 ms flip angle: 8°, matrix size = 224 × 256 × 256, resolution 0.5 × 0.5 x 0.5 mm^3, bandwidth 270 Hz/Pixel, GRAPPA = 2, sagittal orientation; T2w: SPACE sequence, TR = 3.2 s, TE = 562 ms, matrix size = 224 × 256 × 256, resolution 0.5 × 0.5 x 0.5 mm^3, bandwidth 723 Hz/Pixel, turbo factor 314, echo train length 1234 ms, GRAPPA = 2, sagittal orientation).

Fieldmaps B0 field-maps were estimated using a pair of spin-echo EPI images with one opposite phase encoding directions (Andersson *et al.*, 2003) (LR and RL directions, 1.5 mm isotropic, TE 46.2 ms, TR 7.06 s). The spin-echo EPI scans were matched with geometry and distortion properties of fMRI acquisitions (FOV 96 × 96 mm, matrix 64 × 64, number of slices 39, phase partial Fourier 65%, bandwidth 1240 Hz/pixel, echo spacing = 0.93 ms, GRAPPA = 2, fat suppression, flip-angle = 90°, averages 3).

Functional volumes were acquired with the same coil using multiband EPI sequences obtained from the Center for Magnetic Resonance Research (CMRR, University of Minnesota), version R016a (multiband acceleration factor = 3, TA = 646 ms, TE = 16.6 ms, matrix size = 64 × 64 × 39, resolution 1.5 x 1.5 x 1.5 mm^3, flip angle = 50°, multiband LeakBlock kernel, GRAPPA = 2 with EPI calibration scan with 18 reference lines, bandwidth = 1240 Hz/Pixel, echo spacing = 0.93 ms, phase partial Fourier = 75%, fat suppression).

Functional imaging procedure

To avoid interference from the scanner noise during stimulus presentation, clustered sparse sampling of functional acquisitions was employed (Schmidt *et al.*, 2008; Trapeau *et al.*, 2024; Zaehle *et al.*, 2007). Clusters of seven fMRI volumes (4.6 s) were acquired after a 3.5 s acquisition-free period, which allowed stimuli to be presented when the scanner was silent. During each 7-minute functional run, 32 blocks of stimuli

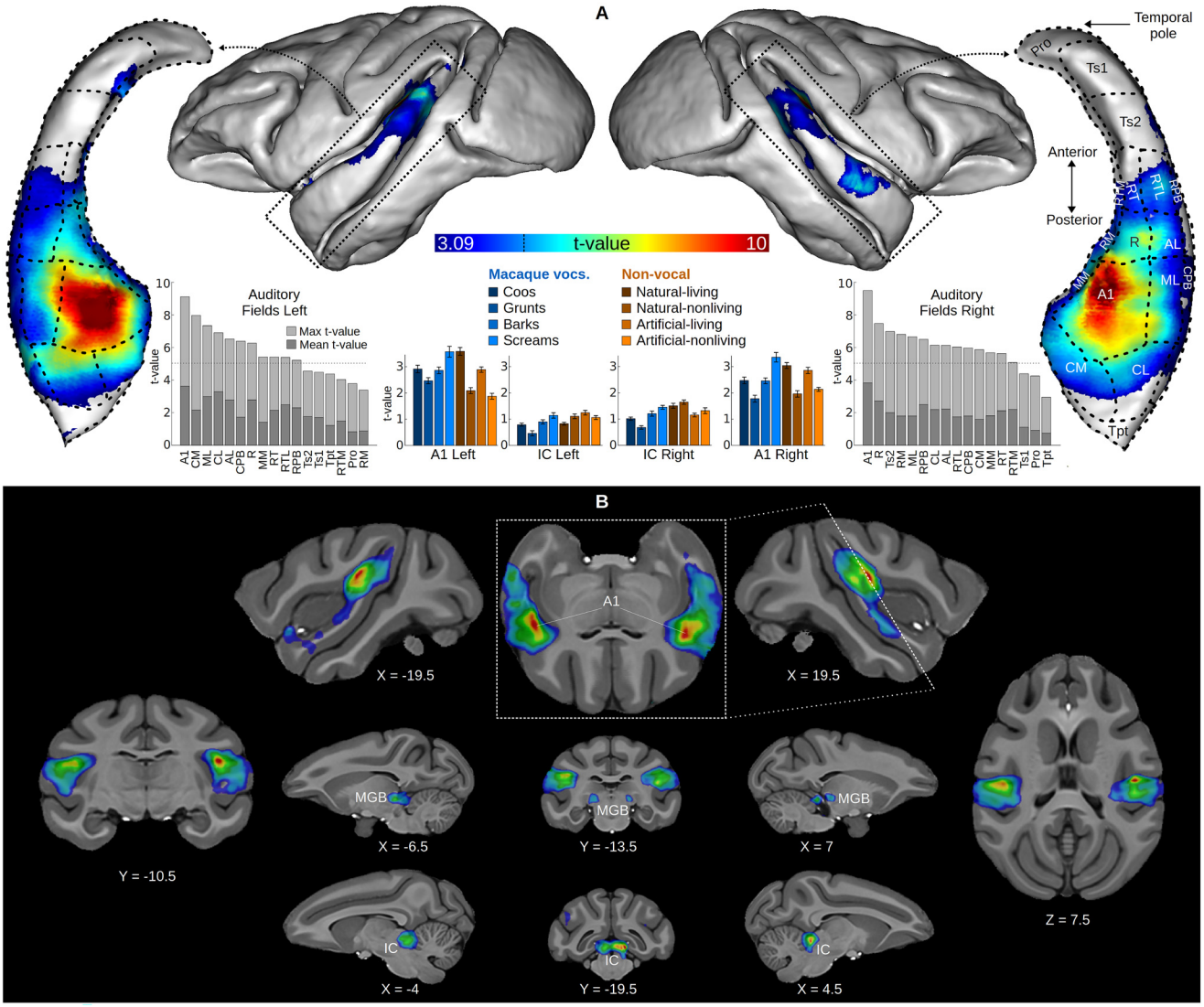

Figure 1. Group-averaged auditory activations. Surface projection and volume map of the "Sound vs. Silence" contrast in a second-level random effects group analysis. For both surface and volume, the contrast map was thresholded at $p < 0.05$ and corrected for multiple comparisons by cluster size ($p < 0.05$). The dotted line on the color bar indicates peak threshold corrected for multiple comparisons ($t = 4.93$) using Gaussian random field theory (Worsley *et al.*, 1996) at $p < 0.05$. (**A**) The contrast map is projected onto the surface of the NIMH macaque template (NMT) as well as onto isolated left and right superior temporal gyri viewed from above. Isolated superior temporal gyri include projections of the macaque auditory fields, which were provided by Christopher Petkov's group (Petkov *et al.*, 2006; Petkov *et al.*, 2008). Maximum and mean voxel t-values of each auditory field from the random effects group map are displayed next to the temporal gyri, the horizontal dotted line indicates peak threshold. The panel also shows t-values averaged across individuals (error bars indicate the standard error of the mean) of each subcategory of sound versus silence, extracted from the left and right A1 and IC ROIs (see Methods). (**B**) The contrast map is overlaid on axial, longitudinal and sagittal slices of the NMT. Top central image is an oblique slice along the superior temporal gyrus. A1: primary auditory cortex; IC: inferior colliculus; MGB: medial geniculate body.

were presented (16 blocks of each of the two main categories), interleaved with 16 silent blocks. Each block contained six stimuli (ISI = 50 ms) belonging to one of the eight subcategories. The time interval between the end of an acquisition cluster and the start of a block of stimuli was randomly jittered from 100 ms to 250 ms. A total of 188 runs were performed among the 11 subjects (one session per subject except for M1 who underwent six sessions; median of 12 runs per subject).

fMRI data preprocessing

Brain extraction and tissue segmentation was performed on the structural scans using a custom-made pipeline which included: bias field correction using the product of the T1w and T2w images (Glasser *et al.*, 2013); denoising (spatially adaptive nonlocal means, SPM); first brain extraction (FSL); registration of the NIMH Macaque Template v1.2 (Seidlitz *et al.*, 2018) to the anatomical scan, in order to provide priors to SPM's

old_segment algorithm; second brain extraction from the segmented tissues. Transformation matrices between anatomical and functional data were computed using non-linear registration (SyN, ANTS). Additionally, white matter and cerebrospinal fluid masks were eroded using a 3 mm cubic kernel in order to provide a mask that contains no region of interest to a principal component analysis of the functional data (see *fMRI data analysis*). Transformation matrices between functional, anatomical and template volumes were computed using non-linear registration (SyN, ANTS). These transformation matrices were used to register the segmented tissues to the functional data as well as to register the functional results to individual high-resolution anatomical scans and to the template.

Preprocessing of the functional data included motion correction, spatial distortion reduction using field maps, inter-runs registration and spatial smoothing. For each subject, every functional volume was realigned and unwarped (FSL) to a reference volume that was spatially the closest to the average of all runs. Spatial smoothing (SPM) was done with a full-width half-maximum 3-dimensional Gaussian kernel that was twice the size of the functional voxels (i.e., 3 mm).

fMRI data analysis

For each subject, general linear model (GLM) estimates of responses to all sounds versus silence ("Sound vs. Silence" contrast) and to macaque vocalizations against non-vocal sounds ("Macaque vs. Non-Vocal" contrast) were computed with fMRISTAT (Worsley *et al.*, 2002) using MION-based response function, manually designed to have a reversed sign, a long tail and no undershoot, similar to previous descriptions (Leite *et al.*, 2002). The GLM included covariates of no interest: the first principal components of two principal component analyses performed on eroded masks of the functional voxels identified as containing white matter and cerebrospinal fluid. The number principal components included as covariates of no interest was chosen to optimize the "Sound vs. Silence" contrast in each subject (6 for white matter, 4 for cerebrospinal fluid, on average).

Following this first level-analysis, fixed and random effects group maps were computed for both contrasts, using the template registered data from all subjects and all runs. Additionally, the group map for the "Macaque vs. Non-Vocal" contrast was computed on a selection of runs that exhibited a positive whole-brain influence on this contrast. The contribution of each run to the contrast was assessed on a per-subject basis using a jackknife procedure. This procedure systematically excluded one run from the dataset of each subject and returned the maximum t-value elicited by the remaining runs. Then, the resulting t-values of a subject were compared with the maximum t-value elicited by all runs of this subject. A lower maximum t-value after run removal meant that the run had a positive contribution to the contrast and vice versa. Note that no topographical constraints were imposed on where these maximum t-values should be, as they were computed using the whole-brain t-maps. Only the runs that showed a positive contribution to the "Macaque vs. Non-Vocal" contrast of each subject were kept, leaving 72 runs in total (median of 6 runs per subject).

T-values of each subcategory were extracted for each subject in several bilateral regions of interest (ROI): the primary auditory cortex (A1), which was defined, for each subject, as the intersection of the A1 auditory field identified by (Petkov *et al.*, 2006) and the "Sound vs. Silence" contrast of the subject; the inferior colliculus (IC), based on peak activations in this anatomically defined region from the random effects group map of the "Sound vs. Silence" contrast; the posterior middle temporal gyrus (pMTG) and the rostral portion of the ventral premotor cortex (F5), both based on peak activations in this region from the fixed effects group map of the "Macaque vs. Non-Vocal" contrast; and the anterior temporal voice area (aTVA) that was previously observed in three macaques during an awake fMRI study by our group (Bodin *et al.*, 2021). Each ROI size was 19 voxels in the functional space and a diameter of 4.5 mm.

Results

Auditory activations

Second-level random effects group analysis revealed strong activations in the primary auditory cortex (A1) and nuclei of the ascending auditory pathway (inferior colliculus, IC; medial geniculate body, MGB) in response to all sounds ("Sound vs. Silence" contrast, Figure 1). Cortical activation extended to the rostral field of the core auditory cortex (R), but also to belt areas (CM, CL, ML, AL, RTL, MM, RM, RTM, Figure 1A). The parabelt and higher auditory fields were marginally activated, except for the right rostral parabelt (RPB) and the temporalis superior 2 (Ts2), which showed significant activation in some voxels.

At the individual level, all subjects had significant auditory responsive clusters in IC and in the auditory cortex (Table 1). ROI t-value extraction showed that all subcategories of sounds elicited positive auditory activations in both IC and A1. (Figure 1A).

Vocalization-specific activations

We could not reproduce our previous findings of voice-selective areas in the anterior temporal lobe (aTVA) of awake macaques (Bodin *et al.*, 2021) in a second-level, random effects group analysis computed on all runs. However, a fixed effects analysis revealed activations in the rostral ventral premotor cortex (F5) and area 8a of the right hemisphere (white outline in Figure 2).

Next, we conducted the same analyses on a selection of runs that had a positive whole-brain contribution to the contrast (see fMRI data analysis). The random effects group map computed from this selection of 72 runs was very similar to the fixed effects group map computed from all 188 runs, showing activations in areas F5 and 8a in the right hemisphere. The fixed effects analysis on the selection of runs revealed much broader activations: F5 bilaterally, right anterior intraparietal area

Table 1. Individual activations in IC and AC. Peak t-value and number of significant voxels at cluster threshold (t = 3.09) in the inferior colliculi (IC) and auditory cortices (AC) of each subject.

Subject	IC peak t-value	IC extent (voxels)	AC peak t-value	AC extent (voxels)
M1	12.87	105	8.04	271
M2	5.22	27	4.2	23
M3	5.14	20	6.78	110
M4	4.07	15	6.06	257
M5	4.43	13	8.45	344
M6	7.61	40	11.26	367
M7	4.69	13	9.91	361
M8	6.79	45	3.6	11
M9	5.69	19	8.39	278
M10	6.92	32	8.34	235
M11	6.74	33	8.2	198

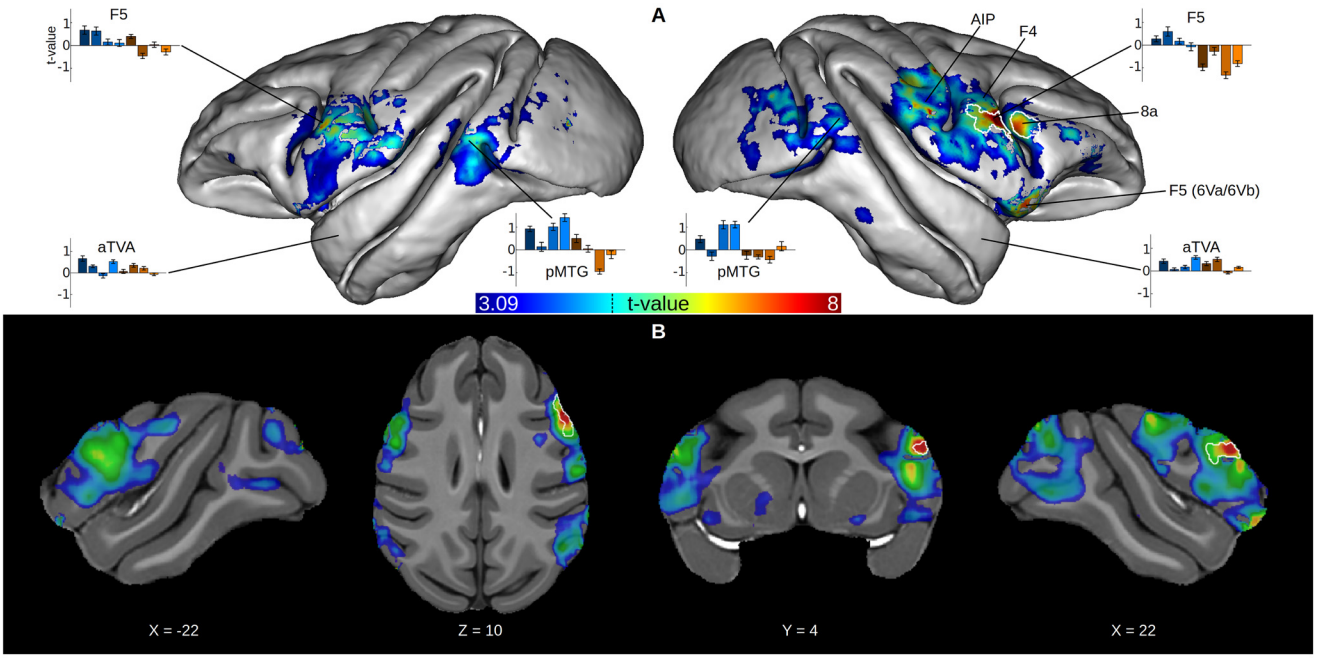

Figure 2. Group-averaged activations to macaque vocalizations. Surface projection and volume map of the "Macaque vs. Non Vocal" contrast in a second-level fixed effects group analysis, computed on a selection of runs that had a positive whole-brain influence on this contrast (see Methods). The white outlines indicate significant areas ($p < 0.05$, cluster size corrected) of the same analysis computed on all runs. For both surface and volume, the contrast map was thresholded at $p < 0.05$ and corrected for multiple comparisons by cluster size ($p < 0.05$). The dotted line on the color bar indicates peak threshold corrected for multiple comparisons using Gaussian random field theory (Worsley *et al.*, 1996) at $p < 0.05$. (**A**) The contrast map is projected on the surface of the NMT. The panel also shows t-values averaged across individuals (error bars indicate the standard error of the mean) of each subcategory of sound versus silence, extracted from the several ROIs (see Methods). (**B**) The contrast map is overlaid on axial, longitudinal and sagittal slices of the NMT. F5: rostral ventral premotor cortex; F4: caudal ventral premotor cortex; AIP: anterior intraparietal area; pMTG: posterior middle temporal gyrus; aTVA: anterior temporal voice area.

(AIP) and caudal ventral premotor cortex (F4), right 6Va/6Vb, posterior part of the middle temporal gyrus (pMTG) bilaterally.

ROI t-value extraction showed showed that auditory activations for each subcategory of sounds in F5 and pMTG were mainly positive for macaque vocalizations and negative for non-vocal sounds (Figure 2A). This difference between vocalizations and non-vocal sounds was not observed in the awake macaques aTVA, where auditory activations were mainly positive for all subcategories.

Discussion

This study aimed to map the auditory response to complex natural sounds in a large group of rhesus macaques under a near-awake anesthesia protocol involving low-dose sevoflurane and dexmedetomidine. Using a sizable sample of eleven macaques, which approaches the median of twelve subjects employed in human fMRI studies (Szucs & Ioannidis, 2020), we have obtained the first random-effects group map of auditory activation in this species. The results revealed robust responses in the core and belt areas of the auditory cortex, as well as in subcortical structures, including the midbrain (inferior colliculi) and thalamus (medial geniculate body). This suggests that essential neural circuitry supporting auditory perception remains functional under this anesthesia regimen. These findings demonstrate the efficacy of anesthetized macaque fMRI in facilitating efficient and high-quality mapping of subcortical and core auditory regions across larger cohorts, thereby circumventing the logistical challenges associated with awake imaging.

Vocalization-specific activations revealed a different picture. To our disappointment, our results revealed a conspicuous absence of vocalization selectivity in higher-order, anterior auditory cortices. This result diverges from previous findings of our group, which highlighted the crucial involvement of the aTVA in processing the same set of stimuli (Bodin *et al*., 2021), as well as from earlier awake macaque studies (Ortiz-Rios *et al*., 2015; Petkov *et al*., 2008). This outcome was somewhat unexpected, given that aTVA activation had been previously reported even in anesthetized macaques (Petkov *et al*., 2008). Nevertheless, a critical distinction exists between this study and ours with respect to the pharmaceutical agents utilized for immobilizing the animals during experimentation. In their study, Petkov and colleagues used remifentanyl and mivacurium, which is the combination of an analgesic with a neuromuscular-blocking drug. However, the absence of hypnotic agents, even at sedation levels, in this cocktail suggests that the animals remained conscious during the experiment. Conversely, we opted for a general anesthesia protocol, involving a sedative agent combined with a hypnotic agent at low dose, with meticulous physiologic monitoring and adjustment of drug levels throughout the experiment to bring the animal close to awakening. Our protocol, involving sevoflurane and dexmedetomidine, prioritized animal well-being and minimized the risk of a non pleasant mnesic experience, though possibly at the cost of reduced activation in anterior auditory regions. The inclusion of each animal in a singular session, in which some completed less than ten trials, might have also been suboptimal for detecting vocalization sensibility in higher-order auditory cortices.

Despite the absence of observed vocalization selectivity in the anterior temporal gyrus, our findings revealed the presence of voice-selective regions in the ventral premotor (F5) and prefrontal cortex (8a). The involvement of these regions in vocalization processing has been previously observed in some awake macaque fMRI studies (Bodin *et al*., 2021; Ortiz-Rios *et al*., 2015) and they have been identified as part of the dorsal auditory pathway (Arnott & Alain, 2011; Petrides & Pandya, 2009), which connects the caudal parabelt auditory regions to the ventral inferior parietal cortex (Lewis & Van Essen, 2000), and up to the frontal eye fields (Hackett *et al*., 1999; Romanski *et al*., 1999). These findings suggest that under our anesthesia regimen, the dorsal auditory stream may be preferentially engaged, speculatively as a resilient backbone for action planning, over the ventral pathway, which is more dedicated to the identification of auditory objects (Kuśmierek *et al*., 2012). More surprisingly, we found additional bilateral vocalization sensitivity in the posterior part of the middle temporal gyrus, a region previously identified as a site of audio-visual integration in humans (Beauchamp *et al*., 2004; Callan *et al*., 2004; Xu *et al*., 2009), macaques (Froesel *et al*., 2022) and marmosets (Dureux *et al*., 2024), but, to our knowledge, not reported in auditory only experiments.

In conclusion, while awake fMRI remains indispensable for probing the full complexity of the cortical processing of natural sounds such as conspecific vocalizations, our findings demonstrate that anesthetized fMRI is a powerful and efficient alternative for large-scale mapping of basic auditory function in non-human primates. Our results confirm that primary auditory pathways remain responsive under near-awake anesthesia. However, they also reveal that anesthesia, even at low levels, induces a dampening of higher-order, anterior auditory cortices, where voice-selective responses were previously identified with awake fMRI studies. Nevertheless, vocalization selectivity was observed in the ventral premotor and prefrontal cortex as well as in the posterior middle temporal gyrus, which suggests a different involvement of the ventral and dorsal auditory pathways during anesthesia. These regions have received relatively little attention in the context of vocalization processing, whether in fMRI or electrophysiological studies, where the focus has traditionally been on anterior temporal areas. In light of their pronounced involvement during anesthesia, future research endeavors should consider conducting a more thorough investigation of their specific role in the auditory processing of vocal signals.

Ethics and consent

All experimental procedures were in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by the Ethical board of Institut de Neurosciences de la Timone (ref 2016060618508941). Efforts were made to ameliorate the suffering of animals using

endpoints, environmental enrichment, and careful monitoring of health and well-being.

Data availability statement

Underlying data

Zenodo: Anesthetized Macaque fMRI data - Vocalizations and Non-Vocal Sounds, https://doi.org/10.5281/zenodo.16941258, (Trapeau & Belin, 2025)

Data are available under the terms of the Creative Commons Attribution 4.0 International

Extended data

Zenodo: Audio stimuli - PrimaVoice: A Cross-Species Stimulus Set to Study Voice Processing in Primates, https://doi.org/10.5281/zenodo.15297317, (Bodin *et al.*, 2025)

Data are available under the terms of the Creative Commons Attribution 4.0 International

Software availability statement

Source code available from: https://github.com/mdobraz/PrimaVoice-Toolbox

Archived software available from: https://doi.org/10.5281/zenodo.5075674, (Trapeau & Belin, 2021)

License: the PrimaVoice Toolbox is licensed under the MIT License.

SPM and fMRISTAT are compatible with GNU Octave which is licensed under the GNU General Public License v3.0.

Reporting guidelines

We used the ARRIVE checklist when writing our report (Percie Du Sert *et al.*, 2020).

The completed ARRIVE checklist is contained in the underlying data Zenodo record: https://doi.org/10.5281/zenodo.16941258, completed_ARRIVE_checklist.pdf, (Trapeau & Belin, 2025)

References

Aglieri V, Chaminade T, Takerkart S, *et al.*: **Functional connectivity within the voice perception network and its behavioural relevance.** *NeuroImage.* 2018; **183**: 356–365.
PubMed Abstract | Publisher Full Text | Free Full Text

Andersson JLR, Skare S, Ashburner J: **How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging.** *NeuroImage.* 2003; **20**(2): 870–888.
PubMed Abstract | Publisher Full Text

Arnott SR, Alain C: **The auditory dorsal pathway: orienting vision.** *Neurosci Biobehav Rev.* 2011; **35**(10): 2162–2173.
PubMed Abstract | Publisher Full Text

Autio JA, Glasser MF, Ose T, *et al.*: **Towards HCP-Style macaque connectomes: 24-channel 3T multi-array coil, MRI sequences and preprocessing.** *NeuroImage.* 2020; **215**: 116800.
PubMed Abstract | Publisher Full Text | Free Full Text

Autio JA, Yoshida A, Kawabata Y, *et al.*: **Development of a 24-channel 3T phased-array coil for fMRI in awake monkeys - mitigating spatiotemporal artifacts in ferumoxytol-weighted functional connectivity estimation.** *bioRxiv.* 2025–04.
Publisher Full Text

Barttfeld P, Uhrig L, Sitt JD, *et al.*: **Signature of consciousness in the dynamics of resting-state brain activity.** *Proc Natl Acad Sci U S A.* 2015; **112**(3): 887–892.
PubMed Abstract | Publisher Full Text | Free Full Text

Beauchamp MS, Lee KE, Argall BD, *et al.*: **Integration of auditory and visual information about objects in superior temporal sulcus.** *Neuron.* 2004; **41**(5): 809–823.
PubMed Abstract | Publisher Full Text

Belin P, Zatorre RJ, Lafaille P, *et al.*: **Voice-selective areas in human auditory cortex.** *Nature.* 2000; **403**(6767): 309–312.
PubMed Abstract | Publisher Full Text

Bodin C, Trapeau R, Belin P: **PrimaVoice: a cross-species stimulus set to study voice processing in primates [Audio recording].** *Zenodo.* April 28, 2025.
http://www.doi.org/10.5281/zenodo.15297317

Bodin C, Trapeau R, Nazarian B, *et al.*: **Functionally homologous representation of vocalizations in the auditory cortex of humans and macaques.** *Curr Biol.* 2021; **31**(21): 4839–4844.e4.
PubMed Abstract | Publisher Full Text | Free Full Text

Callan DE, Jones JA, Munhall K, *et al.*: **Multisensory integration sites identified by perception of spatial wavelet filtered visual speech gesture information.** *J Cogn Neurosci.* 2004; **16**(5): 805–816.
PubMed Abstract | Publisher Full Text

Capilla A, Belin P, Gross J: **The early spatio-temporal correlates and task independence of cerebral voice processing studied with MEG.** *Cereb Cortex.* 2013; **23**(6): 1388–1395.
PubMed Abstract | Publisher Full Text

Dureux A, Zanini A, Everling S: **Mapping of facial and vocal processing in common marmosets with ultra-high field fMRI.** *Commun Biol.* 2024; **7**(1): 317.
PubMed Abstract | Publisher Full Text | Free Full Text

Erb J, Armendariz M, De Martino F, *et al.*: **Homology and specificity of natural sound-encoding in human and monkey auditory cortex.** *Cereb Cortex.* 2019; **29**(9): 3636–3650.
PubMed Abstract | Publisher Full Text

Esposito Y, Boë LJ, Trapeau R, *et al.*: **Dynamic norm-based coding of vocal identity in the primate auditory cortex.** *bioRxiv.* 2025–01.
Publisher Full Text

Froesel M, Gacoin M, Clavagnier S, *et al.*: **Socially meaningful visual context either enhances or inhibits vocalisation processing in the macaque brain.** *Nat Commun.* 2022; **13**(1): 4886.
PubMed Abstract | Publisher Full Text | Free Full Text

Giamundo M, Trapeau R, Thoret E, *et al.*: **A population of neurons selective for human voice in the monkey brain.** *Proc Natl Acad Sci U S A.* 2024; **121**(25): e2405588121.
PubMed Abstract | Publisher Full Text | Free Full Text

Glasser MF, Sotiropoulos SN, Wilson JA, *et al.*: **The minimal preprocessing pipelines for the human connectome project.** *NeuroImage.* 2013; **80**: 105–124.
PubMed Abstract | Publisher Full Text | Free Full Text

Goense JBM, Whittingstall K, Logothetis NK: **Functional magnetic resonance imaging of awake behaving macaques.** *Methods.* 2010; **50**(3): 178–188.
PubMed Abstract | Publisher Full Text

Guldenmund P, Vanhaudenhuyse A, Sanders RD, *et al.*: **Brain functional connectivity differentiates dexmedetomidine from propofol and natural sleep.** *Br J Anaesth.* 2017; **119**(4): 674–684.
PubMed Abstract | Publisher Full Text

Hackett TA, Stepniewska I, Kaas JH: **Prefrontal connections of the parabelt auditory cortex in macaque monkeys.** *Brain Res.* 1999; **817**(1–2): 45–58.
PubMed Abstract | Publisher Full Text

Hauser MD: **Sources of acoustic variation in Rhesus Macaque (*Macaca mulatta*) vocalizations.** *Ethology.* 1991; **89**(1): 29–46.
Publisher Full Text

Joly O, Baumann S, Balezeau F, *et al.*: **Merging functional and structural properties of the monkey auditory cortex.** *Front Neurosci.* 2014; **8**: 198.
PubMed Abstract | Publisher Full Text | Free Full Text

Joly O, Pallier C, Ramus F, *et al.*: **Processing of vocalizations in humans and

monkeys: a comparative fMRI study. *NeuroImage.* 2012; **62**(3): 1376–1389.
PubMed Abstract | Publisher Full Text

Kayser C, Petkov CI, Augath M, *et al.*: **Integration of touch and sound in auditory cortex.** *Neuron.* 2005; **48**(2): 373–384.
PubMed Abstract | Publisher Full Text

Kikuchi Y, Horwitz B, Mishkin M, *et al.*: **Processing of harmonics in the Lateral Belt of macaque auditory cortex.** *Front Neurosci.* 2014; **8**: 204.
PubMed Abstract | Publisher Full Text | Free Full Text

Ku SP, Tolias AS, Logothetis NK, *et al.*: **fMRI of the face-processing network in the ventral temporal lobe of awake and anesthetized macaques.** *Neuron.* 2011; **70**(2): 352–362.
PubMed Abstract | Publisher Full Text

Kuśmierek P, Ortiz M, Rauschecker JP: **Sound-identity processing in early areas of the auditory ventral stream in the macaque.** *J Neurophysiol.* 2012; **107**(4): 1123–1141.
PubMed Abstract | Publisher Full Text | Free Full Text

Leite FP, Tsao D, Vanduffel W, *et al.*: **Repeated fMRI using iron oxide contrast agent in awake, behaving macaques at 3 Tesla.** *NeuroImage.* 2002; **16**(2): 283–294.
PubMed Abstract | Publisher Full Text

Lewis JW, Van Essen DC: **Corticocortical connections of visual, sensorimotor, and multimodal processing areas in the parietal lobe of the macaque monkey.** *J Comp Neurol.* 2000; **428**(1): 112–137.
PubMed Abstract | Publisher Full Text

Logothetis NK, Guggenberger H, Peled S, *et al.*: **Functional imaging of the monkey brain.** *Nat Neurosci.* 1999; **2**(6): 555–562.
PubMed Abstract | Publisher Full Text

Moerel M, Martino FD, Formisano E: **Processing of natural sounds in human auditory cortex: tonotopy, spectral tuning, and relation to voice sensitivity.** *J Neurosci.* 2012; **32**(41): 14205–14216.
PubMed Abstract | Publisher Full Text | Free Full Text

Norman-Haignere SV, Kanwisher N, McDermott JH, *et al.*: **Divergence in the functional organization of human and macaque auditory cortex revealed by fMRI responses to harmonic tones.** *Nat Neurosci.* 2019; **22**(7): 1057–1060.
PubMed Abstract | Publisher Full Text | Free Full Text

Obliger-Debouche M, Trapeau R, Robinet L, *et al.*: **Investigating the cortical mechanisms of conspecific voice perception in anesthetized marmosets using 3T fMRI [version 1; peer review: 2 approved with reservations].** *Open Res Eur.* 2025; **5**: 127.
Publisher Full Text

Ortiz-Rios M, Azevedo FAC, Kuśmierek P, *et al.*: **Widespread and opponent fMRI signals represent sound location in macaque auditory cortex.** *Neuron.* 2017; **93**(4): 971–983.e4.
PubMed Abstract | Publisher Full Text | Free Full Text

Ortiz-Rios M, Kuśmierek P, DeWitt I, *et al.*: **Functional MRI of the vocalization-processing network in the macaque brain.** *Front Neurosci.* 2015; **9**: 113.
PubMed Abstract | Publisher Full Text | Free Full Text

Percie Du Sert N, Hurst V, Ahluwalia A, *et al.*: **The ARRIVE guidelines 2.0: updated guidelines for reporting animal research.** *J Cereb Blood Flow Metab.* 2020; **40**(9): 1769–1777.
PubMed Abstract | Publisher Full Text | Free Full Text

Pernet CR, McAleer P, Latinus M, *et al.*: **The human voice areas: spatial organization and inter-individual variability in temporal and extra-temporal cortices.** *NeuroImage.* 2015; **119**: 164–174.
PubMed Abstract | Publisher Full Text | Free Full Text

Perrodin C, Kayser C, Logothetis NK, *et al.*: **A brain region consisting of neurons with moderate sensitivity for voices**. 2010.
Reference Source

Perrodin C, Kayser C, Logothetis NK, *et al.*: **Voice cells in the primate temporal lobe.** *Curr Biol.* 2011; **21**(16): 1408–1415.
PubMed Abstract | Publisher Full Text | Free Full Text

Petkov CI, Kayser C, Augath M, *et al.*: **Functional imaging reveals numerous fields in the monkey auditory cortex.** *PLoS Biol.* 2006; **4**(7): e215.
PubMed Abstract | Publisher Full Text | Free Full Text

Petkov CI, Kayser C, Steudel T, *et al.*: **A voice region in the monkey brain.** *Nat Neurosci.* 2008; **11**(3): 367–374.
PubMed Abstract | Publisher Full Text

Petrides M, Pandya DN: **Distinct parietal and temporal pathways to the homologues of Broca's area in the monkey.** *PLoS Biol.* 2009; **7**(8): e1000170.
PubMed Abstract | Publisher Full Text | Free Full Text

Pinsk MA, Arcaro M, Weiner KS, *et al.*: **Neural representations of faces and body parts in macaque and human cortex: a comparative fMRI study.** *J Neurophysiol.* 2009; **101**(5): 2581–2600.
PubMed Abstract | Publisher Full Text | Free Full Text

Romanski LM, Bates JF, Goldman-Rakic PS: **Auditory belt and parabelt projections to the prefrontal cortex in the Rhesus monkey.** *J Comp Neurol.* 1999; **403**(2): 141–157.
PubMed Abstract | Publisher Full Text

Sadagopan S, Temiz-Karayol NZ, Voss HU: **High-field functional magnetic resonance imaging of vocalization processing in marmosets.** *Sci Rep.* 2015; **5**(1): 10950.
PubMed Abstract | Publisher Full Text | Free Full Text

Schmidt CF, Zaehle T, Meyer M, *et al.*: **Silent and continuous fMRI scanning differentially modulate activation in an auditory language comprehension task.** *Hum Brain Mapp.* 2008; **29**(1): 46–56.
PubMed Abstract | Publisher Full Text | Free Full Text

Schönwiesner M, Dechent P, Voit D, *et al.*: **Parcellation of human and monkey core auditory cortex with fMRI pattern classification and objective detection of tonotopic gradient reversals.** *Cereb Cortex.* 2015; **25**(10): 3278–3289.
PubMed Abstract | Publisher Full Text | Free Full Text

Seidlitz J, Sponheim C, Glen D, *et al.*: **A population MRI brain template and analysis tools for the macaque.** *NeuroImage.* 2018; **170**: 121–131.
PubMed Abstract | Publisher Full Text | Free Full Text

Shi S, Xu AG, Rui YY, *et al.*: **Infrared neural stimulation with 7T fMRI: a rapid *in vivo* method for mapping cortical connections of primate amygdala.** *NeuroImage.* 2021; **231**: 117818.
PubMed Abstract | Publisher Full Text | Free Full Text

Slupe AM, Kirsch JR: **Effects of anesthesia on cerebral blood flow, metabolism, and neuroprotection.** *J Cereb Blood Flow Metab.* 2018; **38**(12): 2192–2208.
PubMed Abstract | Publisher Full Text | Free Full Text

Szucs D, Ioannidis JP: **Sample size evolution in neuroimaging research: an evaluation of highly-cited studies (1990–2012) and of latest practices (2017–2018) in high-impact journals.** *NeuroImage.* 2020; **221**: 117164.
PubMed Abstract | Publisher Full Text

Tanji K, Leopold DA, Ye FQ, *et al.*: **Effect of sound intensity on tonotopic fMRI maps in the unanesthetized monkey.** *NeuroImage.* 2010; **49**(1): 150–157.
PubMed Abstract | Publisher Full Text | Free Full Text

Trapeau R, Belin P: **Original code—functionally homologous representation of vocalizations in the auditory cortex of humans and macaques.** [Computer software]. Zenodo. 2021.
http://www.doi.org/10.5281/zenodo.5075675

Trapeau R, Belin P: **Anesthetized macaque fMRI data—vocalizations and non-vocal sounds.** [Dataset]. Zenodo. 2025.
http://www.doi.org/10.5281/zenodo.16941259

Trapeau R, Sein J, Obliger-Debouche M, *et al.*: **Comparison of auditory fMRI protocols for a voice localizer [version 1; peer review: 1 approved, 1 approved with reservations].** *Open Res Eur.* 2024; **4**: 261.
Publisher Full Text

Tsao DY, Freiwald WA, Knutsen TA, *et al.*: **Faces and objects in macaque cerebral cortex.** *Nat Neurosci.* 2003; **6**(9): 989–995.
PubMed Abstract | Publisher Full Text | Free Full Text

Uhrig L, Janssen D, Dehaene S, *et al.*: **Cerebral responses to local and global auditory novelty under general anesthesia.** *NeuroImage.* 2016; **141**: 326–340.
PubMed Abstract | Publisher Full Text | Free Full Text

Vanduffel W, Farivar R: **Functional MRI of awake behaving macaques using standard equipment.** In: *Advanced Brain Neuroimaging Topics in Health and Disease-methods and Applications.* IntechOpen, 2014.
Publisher Full Text

Worsley KJ, Liao CH, Aston J, *et al.*: **A general statistical analysis for fMRI data.** *NeuroImage.* 2002; **15**(1): 1–15.
PubMed Abstract | Publisher Full Text

Worsley KJ, Marrett S, Neelin P, *et al.*: **A unified statistical approach for determining significant signals in images of cerebral activation.** *Hum Brain Mapp.* 1996; **4**(1): 58–73.
PubMed Abstract | Publisher Full Text

Xu J, Gannon PJ, Emmorey K, *et al.*: **Symbolic gestures and spoken language are processed by a common neural system.** *Proc Natl Acad Sci U S A.* 2009; **106**(49): 20664–20669.
PubMed Abstract | Publisher Full Text | Free Full Text

Zaehle T, Schmidt CF, Meyer M, *et al.*: **Comparison of "silent" clustered and sparse temporal fMRI acquisitions in tonal and speech perception tasks.** *NeuroImage.* 2007; **37**(4): 1195–1204.
PubMed Abstract | Publisher Full Text

Zhang X: **Effects of anesthesia on cerebral blood flow and functional connectivity of Nonhuman Primates.** *Vet Sci.* 2022; **9**(10): 516.
PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Peer Review Status: ? ? ✓

Version 1

Reviewer Report 22 October 2025

https://doi.org/10.21956/openreseurope.22669.r59877

© **2025 Trouillet A.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

✓ **Alix Trouillet**
ORL, Centre Hospitalier Universitaire Vaudois, Lausanne, Vaud, Switzerland

The team looked at the auditory responses to complex natural sound (vocalization and non-vocal sounds) using fMRI in anesthetized macaques (n=11).

They show that response is mostly preserved under anesthesia (near-awake protocol) in primary and belt areas. However, they were unable to reproduce previous findings that showed activation of the aTVA in awake macaques.

Instead, they found that voice activation was found in regions associated with the dorsal auditory stream and multisensory integration.

The paper is clearly written. The study design is appropriate, the sample size is good (n=11 macaques) and the experiment seems to be replicable.

The conclusions are mainly descriptive. It consists of depicting the impact of light anesthesia on auditory brain activity.

The conclusions are not completely new in the sense that it was already known that essential neural circuitry supporting auditory perception remained functional
under anesthesia in macaques. However, they show that under this regimen unexpected regions are activated by vocal stimuli (ventral premotor and prefrontal cortex) while the anterior temporal voice area is not.

The authors conclude that fMRI under near-awake anesthesia can still be used to study basic auditory function in non-human primates but could be limiting when studying the processing of more complex sounds like voices.

While interesting, I think it is important to be very cautious when studying brain circuitry under anesthesia. Sedation protocols vary a lot depending on countries, institution and experimenters. It is known that they have a big impact on

brain function; their effects are highly variable between species and between individuals making it very difficult to draw and extrapolate clear conclusions.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I have an expertise in NHP Auditory neuroscience but more at the electrophysiological level. Extensive experience in comparing awake and anesthesized signals. However, I am unable to assess the accuracy of fMRI analysis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 27 September 2025

https://doi.org/10.21956/openreseurope.22669.r59881

© 2025 Nourski K et al. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Kirill V Nourski
The University of Iowa, Iowa, USA
Emily Dappen
The University of Iowa Department of Neurosurgery, Iowa City, Iowa, USA

This is an interesting study on the important topics of non-human primate neuroimaging in auditory research and effects of anesthesia on cortical auditory processing. The authors present results obtained in lightly anesthetized macaques and compare them with their previous work

obtained in awake animals (Bodin et al., 2021, *Curr Biol.* 2021; **31**(21): 4839–4844.e4).
Major comments:

- Introduction: there is a lot of information on why light anesthesia with fMRI is useful, and the pros and cons of both awake and anesthesia fMRI. However, the Introduction does not provide much information on why the authors are examining speech and auditory processing, why it is beneficial to study, and what it can tell us/how it is translatable to human work. Much of the discussion of prior fMRI studies would probably be better suited for the Discussion section. The authors could discuss the limitations of anesthesia fMRI but then discuss why it is still better (in this case) than awake fMRI to answer the proposed questions.
- Introduction: the hypotheses are not clearly stated.
- Methods: It is not clear how exactly was “light anesthesia” defined and confirmed during the experiments.
- Methods: As the focus of the paper is the comparison between new results obtained under light anesthesia and previously published results (Bodin et al., 2021), it should be explicitly stated that, aside from anesthesia, experimental procedures were identical between the two studies.
- Discussion: the underlying cause(s) of the differences between the present and previously published results (Bodin et al., 2021) should be presented, and alternative explanations should be considered. Is it a difference in arousal/consciousness state? Is it the effect of the drug? Are there any methodological differences between the two studies that could contribute to the observed difference in results?

Other specific comments:

Abstract:

“Instead, vocalization sensitivity emerged in… posterior middle temporal gyrus, regions associated with the dorsal auditory stream and multisensory integration”. Posterior MTG is typically considered part of the ventral rather than dorsal stream (e.g., Hickok & Poeppel, 2015, Handb Clin Neurol 129:149-160).

Keywords:

“auditory” and “anesthesia” are used verbatim in the title and thus are redundant as keywords.

Methods

Pg4, P3: The description of auditory stimuli used is insufficient given the scope of the study. It’s unclear what the “living” and “non-living” stimuli were. It is states that the stimuli were “adjusted for duration”, but the actual duration is not specified. Please consider providing comprehensive details of the stimuli used as a summary table and/or examples of stimulus waveforms or spectrograms in Supplementary materials.

Figure 1: Referencing figure 1 in the methods was a bit confusing. It might be worth separating out figure 1 into two different figures, one as a methods figure describing the different stimuli and the second with the results.

Pg5, P1: Please spell out ISI. Also please explain why an inter-stimulus-interval of 50 ms was used. Is this typical for auditory paradigms in rhesus monkeys?

Results:
Pg6, P8: "We could not reproduce our previous findings of voice-selective areas in the anterior temporal lobe (aTVA) of awake macaques (Bodin *et al*., 2021) in a second-level, random effects group analysis computed on all runs." As is, adding this sentence adds confusion – perhaps rephrasing to just highlight the result then discussing how this diverges from previous findings in the discussion (as the authors have done) would improve clarity.

Pg8, P2: "This difference between vocalizations and non-vocal sounds was not observed in the awake macaques aTVA, where auditory activations were mainly positive for all subcategories." Please cite Bodin et al (2021) here if that's the awake study that is referred to in this sentence.

Figure 2: Please make the bar plots larger – it is hard to see the error bars and what color each bar is. Please also include a key for what the bar colors mean.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Auditory neurophysiology, consciousness neuroscience.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 26 September 2025

https://doi.org/10.21956/openreseurope.22669.r59878

© 2025 Froesel M. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Mathilda Froesel
[1] Institut des sciences cognitives Marc Jeannerod, Bron, Auvergne-Rhône-Alpes, France
[2] Neurosciences, KU Leuven Department of Neurosciences (Ringgold ID: 213965), Leuven, Flanders, Belgium

This study addresses the important question of the neural basis of auditory perception in macaques, utilizing a "near-awake" anesthesia protocol. The authors should be commended for collecting a valuable dataset from a large cohort of eleven subjects, which is a significant effort and a precious resource for the community. The goal of mapping auditory responses and comparing them to findings from awake-animal studies is highly relevant and of great interest. The paper has the potential to make a significant contribution to our understanding of the effects of anesthesia on auditory processing. However, several major methodological concerns must be addressed to ensure the validity and robustness of the conclusions, particularly those regarding vocalization selectivity.

Major concerns:
1) Circular Analysis in Run Selection My most significant concern relates to the procedure for selecting runs for the vocalizations-specific activation analysis. Selecting runs (72 out of 188, meaning over 60% of the data was discarded) based on their "positive contribution" to the contrast of interest is a form of circular analysis ("double-dipping"). This method invalidates the statistical inference, artificially inflates the results, and prevents reliable conclusions from being drawn from the data.
Recommendation: The authors must present the analysis performed on all valid runs (after standard quality control based on objective measures like motion, level of anesthesia or heart rate during this run, or SNR) as their primary result. The analysis based on the selected runs can, at best, be considered supplementary.
2) Circular Analysis in Region of Interest (ROI) Definition Similarly, the definition of the functional ROIs (pMTG, F5) from the group maps, which are then used to analyze the same data, is another form of circularity. This procedure virtually guarantees an effect but one that is biased and whose magnitude is inflated.
Recommendation: The standard method to avoid this bias is to use an independent localizer approach. For example, the authors could split the data for each subject in half: one half to define the ROIs, and the other, independent half to extract values and perform statistical analyses.
3) Main contrasts
Recommendation: To significantly increase the impact of the current study and facilitate a direct comparison with the rich existing literature, it would be highly valuable for the authors to present the whole-brain activation maps for the 'Vocalizations > Silence' and 'Non-Vocalizations > Silence' contrasts, computed on all valid runs. Showing these fundamental activation patterns would allow the reader to visually compare the results with those from awake-fMRI studies, such as Bodin et al. (2021) and Froesel et al. (2025). This would also permit a more nuanced interpretation of the findings regarding the anterior temporal voice area (aTVA). The reported absence of aTVA selectivity is not necessarily a critical flaw; indeed, other studies on awake macaques have also reported variability in identifying this specific anterior patch. Presenting the basic activation maps

would situate the current findings within this ongoing scientific discussion and would constitute a valuable contribution, independent of the outcome of the direct contrast between vocalizations and non-vocalizations.
4) Ambiguity in the Interpretation of ROI Results The discussion of the ROI results (in F5, pMTG) is ambiguous. The authors report a difference between "vocalizations" and "non-vocalizations" but do not show whether each condition is significantly activated or deactivated relative to the baseline (silence). This makes it impossible to know if the effect is driven by activation to vocalizations, suppression by other sounds, or both.
Recommendation: For a correct interpretation, statistical tests to indentify whether these responses for each condition are significantly different from silence have to be provided.

Suggestions for improvements:
1) Transparency of Statistical Methods: The cluster correction method is incompletely described. For reproducibility, the initial voxel-level threshold used to form the clusters should be specified (also maybe the authors were too stringent with that threshold).
2) Data Visualization: To improve the clarity of the figures, the authors could consider using inflated surfaces to better visualize activity within the sulci. The addition of anatomical atlas outlines on the activation maps is also highly recommended to help with the identification of areas.
3) Details on Subjects and Consistency of Results: A table with the age and sex of each subject would be welcome. Showing individual activation maps (in supplementary materials) would allow an assessment of the consistency of the results across subjects.
4) Suggestion for Exploratory Analysis: Given the presence of both males and females, the authors have a valuable opportunity to conduct an exploratory analysis of sex-based differences, a fascinating and understudied topic that would add great value to their paper. 5) Acoustic Properties of Stimuli: While the stimuli were normalized for RMS amplitude, this only accounts for overall loudness. To properly rule out low-level acoustic features as a potential confound for the "Vocalization vs. Non-Vocal" contrast, the authors should provide a more detailed acoustic analysis comparing the two categories on other key parameters (e.g., spectral content, temporal modulation, harmonic-to-noise ratio). At a minimum, a discussion of these potential uncontrolled differences is warranted.
6) Sound Presentation Level: For the sake of reproducibility, the intensity level at which the stimuli were delivered should be explicitly reported. Please specify the sound pressure level (e.g., in dB SPL) and briefly describe how it was calibrated at the animal's head.
7) Task Design Figure: The Methods section would be greatly clarified by the addition of a figure illustrating the task structure. A schematic depicting the sequence and timing of events within a single trial (e.g., stimulus presentation, inter-trial interval) would help readers quickly and clearly understand the experimental paradigm.
8) Spatial Normalization Template: The Methods section should explicitly state which standard template was used for spatial normalization. The figure legend suggests it is the NMT, but this essential information needs to be clearly stated in the text for reproducibility.
9) Spatial Smoothing and Subcortical Structures: The authors use a 3 mm smoothing kernel. This choice is particularly critical for the analysis of small subcortical structures, as the kernel may average out and obscure genuine activations in nuclei within the thalamus, midbrain, etc. It would be valuable to know if the subcortical findings are robust to this parameter, or to discuss this potential limitation.
In conclusion, this paper presents a potentially rich dataset, but the main conclusions regarding vocalization selectivity in areas F5 and 8a are not currently supported by a rigorous and unbiased

methodology. The issues of circular analysis are critical and must be addressed. Nevertheless, I am confident that the authors can resolve these points by re-analyzing their data using standard, unbiased methods. The study's potential to clarify the limitations of imaging under anesthesia is very real. Indeed, demonstrating that even with a large cohort, widespread activity outside the lateral sulcus is not necessarily present under light anesthesia would be a fundamental methodological finding for the primate imaging community. The authors are encouraged to develop the paper further along this axis, as this provides crucial guidance for researchers designing future studies with anesthetized animals. For these reasons, I recommend Approved with Reservations

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: fMRI, NHP, sensory perception

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.