

## Novel Tools and Methods

# Ratphones: An Affordable Tool for Highly Controlled Sound Presentation in Freely Moving Rats

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## Abstract

Encoding and processing sensory information is key to understanding the environment and to guiding behavior accordingly. Characterizing the behavioral and neural correlates of these processes requires the experimenter to have a high degree of control over stimuli presentation. For auditory stimulation in animals with relatively large heads, this can be accomplished by using headphones. However, it has proven more challenging in smaller species, such as rats and mice, and has been only partially solved using closed-field speakers in anesthetized or head-restrained preparations. To overcome the limitations of such preparations and to deliver sound with high precision to freely moving animals, we have developed a set of miniature headphones for rats. The headphones consist of a small, skull-implantable base attached with magnets to a fully adjustable structure that holds the speakers and keeps them in the same position with respect to the ears.

**Key words:** behaving animals; headphones; interaural level differences; rodents; sound presentation

## Significance Statement

Presenting sensory stimulation reliably is critical in many experimental paradigms. Different methods have been used to accomplish this goal in different sensory modalities, but it has proven difficult to control sound presentation in small species, such as rats and mice. In this work, we present the Ratphones, a set of miniature headphones, and provide all necessary information for building, adjusting, and using the Ratphones to reliably present auditory stimulation to freely moving rats.

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## Introduction

Processing sensory inputs is crucial for understanding the environment and adjusting behavior accordingly. In addition to psychophysics, in which sensory evidence is critical, sensory stimuli are used in many paradigms within behavioral neuroscience. For these experiments to be valid and reliable, it is key to ensure a high degree of control over the physical properties of the stimulation that reaches the sensory organs, so that they can be accurately replicated. Moreover, precise stimulus control is critical for the experimenter to be able to interpret neural variability and its relationship with behavior; only if one can accurately repeat the same stimulus, can a distinction between internal and external neural variability be made.

In visual experiments, this requirement has been fulfilled mostly by using eye-tracking systems in head-fixed (or head-restrained) subjects (Britten et al., 1993), but also by

using head-mounted eye-trackers (Cognolato et al., 2018). Recently, a magnetic eye-tracking system that can be used in both head-fixed and freely moving mice has been developed (Payne and Raymond, 2017). For olfactory and tactile stimulation, researchers have developed high-precision devices for this purpose (Romo et al., 2002; Kepecs et al., 2006). In the auditory modality, there have been two main strategies. On the one hand, in species with relatively large heads, such as monkeys (Schroeder et al., 2001; Fishman and Steinschneider, 2009) or ferrets (Nodal et al., 2010; Keating et al., 2013), sound presentation can be controlled by using headphones. On the other hand, for smaller animals, such as rats and mice, the sound can be reliably delivered by using head-fixed (Joachimsthaler et al., 2014) or anesthetized (Yao et al., 2013) preparations. However, to our knowledge, delivering sound under strictly controlled conditions to freely moving rodents is a challenge that has been only partially solved so far in rats by chronically implanting a plastic structure into which the speakers were screwed before each behavioral session (Otazu et al., 2009).

We have designed the Ratphones, an affordable set of miniature headphones that consists of a small, skull-implantable base attached with magnets to a fully adjustable structure that holds the speakers and keeps them in the same position with respect to the ears. With the Ratphones, the experimenter only needs to implant a small base, which is less disruptive for the animals than implanting the whole structure except for the speakers, and the headphones are attached to this base with magnets, thus avoiding the need to screw (and unscrew) the speakers before (and after) every behavioral session.

These headphones allow the experimenter to control independently the sound delivered to the two ears, which is especially important for studying sound localization, where the interaural level difference (ILD) of the sound is the main cue used by the auditory system to extract azimuth in the horizontal plane (Wesolek et al., 2010). The speakers we chose are good for high frequencies (up to 40 kHz) and can deliver pure tones and narrowband noise. Thus, the Ratphones can be used in most experiments requiring highly controlled sound presentation. However, the speakers are limited in terms of sound intensity [maximum, 79 dB SPL (measured in 0.1 m distance)] and may not be the best option for experiments demanding very high sound intensities.

## Design Requirements

The main functional requirements behind this design were to have a precise, reliable, and robust relative positioning between the speaker and the pinna, while at the same time allowing flexibility to adjust this positioning to the variations between base positioning and pinna location on each individual animal. To achieve this, (1) the design contains movable pieces that can be adjusted on the anteroposterior, mediolateral, and dorsoventral axes; and (2) the procedure to configure the Ratphones consists of a first step in which the base is implanted, and a second step in which the pieces are adjusted for each individual

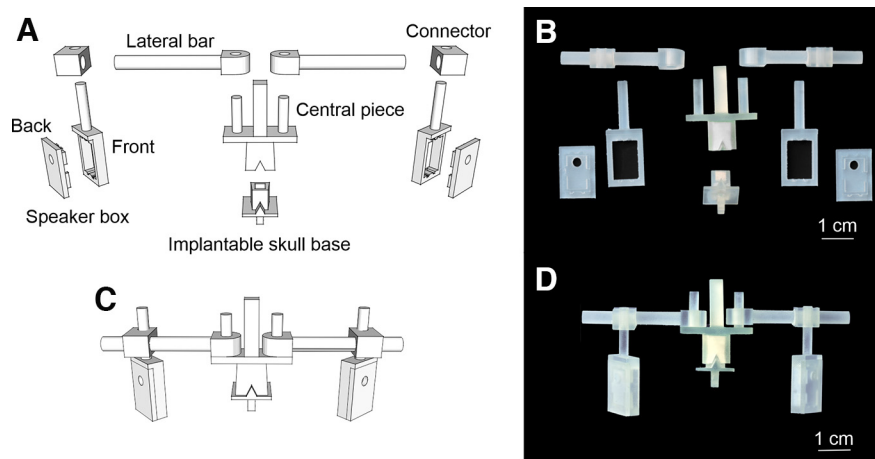
animal under anesthesia and glued in their final configuration for each rat.

Another critical design requirement for the Ratphones was to use them in behaving animals, as opposed to anesthetized or head-fixed preparations. Mostly because of this requirement, we decided to use external headphones instead of placing them in the ear. In-ear headphones in principle afford a higher degree of control, as sounds not coming from the speakers are blocked. They also allow pure monaural stimulation (i.e., one is sure that each ear only hears the sound from its corresponding speaker), which, as previously mentioned, is important for controlling ILD. They have, however, the important drawback that they are much more invasive and uncomfortable for the subject, which, especially in a behavioral context where one has to fit them on every behavioral session, is critical. If the animal starts every session stressed and uncomfortable, it will interfere with the behavioral readouts of the sensory measurements the experimenter is trying to perform. A second important drawback for internal headphones, based on human subjective experience, is the sensitivity of this configuration to slight adjustments in the positioning of the earphone (and supporting sound-isolating material) relative to the inner pinna. A bad seal can completely compromise accurate sound delivery, and the rats cannot report on a bad seal. For all these reasons, we decided to use an external design with close placement. Regarding the downsides, we tested explicitly that monaural contamination is small compared with behavioral ILD sensitivity, but we would in general recommend performing experiments in a sound-isolation box, where the possibility of interference because of a lack of the seal provided by an internal design is minimized.

In this work, we provide all necessary information for building, adjusting, and using the Ratphones to reliably present auditory stimulation to freely moving rats. Empirical data obtained with the Ratphones can be found in the study by Pardo-Vazquez et al. (2019).

## Materials and Methods

The Ratphones consist of a small, chronically implantable base and a set of movable parts (Fig. 1A,B) that can be put together to form a structure that holds the headphones in the desired position with respect to the ears (Fig. 1C,D). The structure is attached to the base using magnets. All parts, except for the magnets and speakers (Table 1), can be 3D printed using the stereolithography files (stls) we provide in <https://github.com/JosePardoVazquez/RatHeadphones>. These parts have been designed to be printed using stereolithography (Fig. 1B,D), but can be easily adapted to other 3D-printing methods. In its current form, the speaker box is designed to be used with a specific receiver model (Table 1), but it can be easily redesigned to fit other models without modifying the other parts. Below, we describe the procedure we used for implanting the base and adjusting the headphones. All procedures were reviewed and approved by the animal welfare committee of the Champalimaud Centre for the



**Figure 1.** Ratphones 3D design and 3D-printed resin pieces. **A**, Set of 3D-printable parts. **B**, Set of resin-printed parts. **C**, **D**, Front view of all the parts assembled to form the final structure that holds the speakers, in the 3D model and printed, respectively.

Unknown and approved by the Portuguese Direção Geral de Veterinária (reference #0421/000/000/2019).

### Base implant surgery

Anesthesia was induced by inhalation of isoflurane at a concentration of 5% (oxygen at 2 L per min) and maintained by an injection of ketamine/xylazine (0.1 ml/100 g, i.p.). More isoflurane was occasionally administered for longer surgeries if the animal exhibited signals of pain or discomfort. The animal was shaved and fixed to the stereotaxic frame, and eye ointment was applied to the eyes. Lidocaine (0.2 ml) was injected subcutaneously at the incision site before the incision was made, for local anesthesia. The skin was cleaned using iodine, an incision (~2 cm in length) was practiced along the midline, and the skin was displaced laterally, exposing the surface of the skull. After cleaning the top region of the skull by blunt dissection, four drilling holes were made and titanium screws (length, 3 mm; thread diameter, 1 mm) were attached to the skull, allowing for most of their length to remain outside. Cement was poured on top of these screws, ensuring it reached the space between the screws and the skull for a secure attachment [using a strong dental adhesive, such as Super-Bond (Sun

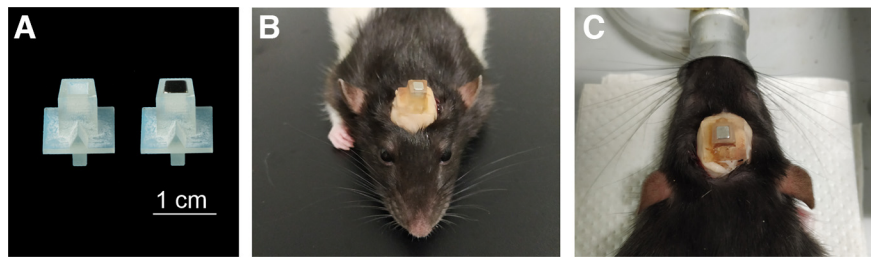
Medical), it might be possible to firmly implant the base without screws; this cement has shown high tolerance, resistance, and durability for chronic implants in different species, including mice (Lohse et al., 2021) and ferrets (Nodal et al., 2010)]. A small cube-shaped magnet (Table 1) was placed inside the resin base (Fig. 2A), which was then placed on top of the cement layer, and more cement was added around the lower part of the base until it was covered. The displaced skin was then stitched around the base, only allowing the necessary structure for the attachment of the headphones to remain visible (Fig. 2B,C). Antibiotics (8 mg/kg, s.c.; cefovecin, Convenia) and analgesics (5 mg/kg, s.c.; carprofen, Rimadyl) were administered after the surgery. The base (with the magnet) weighs ~0.9 g, but together with the cement it weighs ~2.4 g.

### Individual adjustment of the Ratphones

This procedure was performed 1 week after the base implant surgery, during which the animal was allowed to recover with free access to water and food. Under anesthesia (induced and maintained with isoflurane at 4% and 2.5%, respectively), the structure with all pieces temporarily assembled, including the speakers and

**Table 1: List of parts needed to build a set of Ratphones**

| Parts                                      | Description   |
|--|---|
| 3D-printed pieces<br>(stls files provided) | Implantable skull base<br>Central piece<br>Lateral bar (×2)<br>Speaker box, front (×2)<br>Speaker box, back (×2)<br>Lateral bar to speaker box connector (×2)   |
| Magnets                                    | B222 (K&J Magnets, Inc.) one-eighth × one-eighth × one-eighth inch thickness; grade N42, nickel-plated magnetized through thickness<br>B3301 (K&J Magnets, Inc.) three-sixteenth × three-sixteenth × one-thirty-second inch thickness; grade N42, nickel-plated magnetized thru thickness |
| Speakers                                   | Knowles active receiver (MOD 2403260 00031; “Petra”)  |
| Connector                                  | Connector header through hole 3 position 0.100 inch (2.54 mm)   |



**Figure 2.** **A**, Skull base 3D printed in resin, without and with the magnet in place. **B**, **C**, Front and rear view of a skull base chronically implanted.

their connections, was placed on the implanted base and the different angles between the pieces were adjusted so that the speakers were placed at  $\sim 5$  mm from the opening of the ear of the animal. The pieces were then fixed with cyanoacrylate, removed from the animal, and covered with flexible silicone rubber (Sugru), providing extra fixation to the resin pieces and protection to the electrical cables that connect the speakers. Initially, we covered most of the structure of the headphones with flexible silicon rubber (Pardo-Vazquez et al., 2019, their Supplementary Fig. 1), but lately we have been covering only the central piece (Fig. 3), reducing the weight of the Ratphones while keeping a strong attachment in the part that supports more tension when attaching/detaching them. A magnet (Table 1) was glued in the bottom of the structure to attach it to the implanted base during the behavioral sessions. A three-pin male connector (Table 1) was affixed to the part of the 3D-printed structure. Before each behavioral session, a standard three-wire sound cable—soldered to a matching three-pin female connector—was plugged to the male connector. A passive commutator, attached to the ceiling of the behavioral box, was used to avoid tangling. The headphones weigh 7.22 g without flexible silicone rubber and 12.8 g with a full coverage of this material.

We think that it should be possible to scale the Ratphones down to be used in freely moving mice. The 3D parts can be easily scaled; there are miniature neodymium magnets that are strong enough to keep the headphones attached to the base (e.g., a  $1.6 \times 1.6 \times 1.6$  mm neodymium cube weighs 0.06 g and can hold 90 g); and there are speakers that are light (0.6 g) and small (diameter, 10 mm) enough to be used in mice.

### Behavioral and sound attenuation tests

The Ratphones were used in freely moving rats performing a sound lateralization task (Pardo-Vazquez et al., 2019). The arena consisted in a standard Coulbourn Instruments modular box ( $30 \times 25 \times 30$  cm) equipped with three nose-pokes, one of them with a water delivery system. Before each behavioral session, the rat was placed in the box and a set of individualized Ratphones was brought near to the implanted base, until the magnets were attached, without restraining the movement of the animal. The Ratphones were plugged, through a standard sound cable, to a real time processor (RP2 by Tucker-Davis Technologies) that controlled the behavioral task, including presenting the sound and recording the responses of the animal. To avoid tangling, the cable was attached to a passive commutator.

Since we decided to minimize any physical contact between the speakers and the pinnae, it is expected that some residual sound from one speaker will reach the contralateral ear. We addressed this empirically, by playing cosine-ramped (10 ms) broadband noise (5–20 kHz) at 65, 70, and 75 dB SPL from one speaker and recording the sound with the microphone placed by the contralateral ear canal. The noise was independently generated for each presentation using a RP2 module at a sample rate of 50 kHz. The speakers were calibrated using a Brüel & Kjær Free-field one-quarter inch microphone placed in front of the speaker, 5 mm apart. We found that the head plus near-field positioning of the speaker attenuates the sound by  $\sim 22$  dB (Pardo-Vazquez et al., 2019, data published in their supplementary information). Since the just noticeable difference for lateralization of sound in this task is 2.2 dB (Pardo-Vazquez et al., 2019), this suggests that level differences played through the Ratphones are an accurate approximation of actually experienced level differences (relative to the behavioral accuracy for sound lateralization



**Figure 3.** **A**, **B**, Front and rear view of a set of Ratphones adjusted under anesthesia. **C**, Set of Ratphones assembled and glued together, including the speakers, cables, and connector.



in rats), which validates the use of the Ratphones for psychophysical testing of sound lateralization.

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