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Sensory and cognitive neurophysiology in rats, Part 1: Controlled tactile stimulation and micro-ECoG recordings in freely moving animals

George Dimitriadis, Anne M.M. Fransen, Eric Maris*

Radboud University Nijmegen, Donders Institute for Brain, Cognition and Behaviour, The Netherlands

HIGHLIGHTS

- High-density micro-ECoG over the whole rat somatosensory system.
- A novel co-registration mof anatomical and functional brain surface data.
- A head-mounted device provides controlled tactile stimulation to the rat's snout.

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ABSTRACT

Background: We have developed a setup for rats that allows for controlled sensory input to an animal engaged in a task while recording both electrophysiological signals and behavioral output.
New method: We record electrophysiological signals using a novel high-density micro-electrocorticography (micro-ECoG) grid that covers almost the whole somatosensory system. We dealt with the well-known difficulty that the rat uses its whisker system in an active (motor-controlled) way to explore its environment by designing a head-mounted device that stimulates the rat's snout in a way unaffected by whisker movements.
Results: We replicate the spatial specificity of early evoked responses in somatosensory and auditory cortex. In a companion paper (*Cognitive Neurophysiology in Rats, Part 2: Validation and Demonstration*) we validate our setup and show for the first time that the ECoG can be used to record evoked responses in a signal that reflects neural output (spiking activity).
Comparison with existing methods: Compared with high-density wire recordings, micro-ECoG offers a much more stable signal without readjustments, and a much better scalability. Compared with head-fixed preparations, our head-mounted stimulator allows to stay closer to the rat's natural way of collecting sensory information.
Conclusions: For perceptual and cognitive research, our setup provides a unique combination of possibilities that cannot be achieved in other setups for rodents.

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1. Introduction

Neuroscience is shifting focus from spatially specific neural activity to interactions between brain areas, requiring therefore concurrent measurements from distributed brain regions (Vuilleumier and Driver, 2007). A type of recording that provides this is the electrocorticogram (ECoG), whose sensor array is a flexible substrate in which electrodes are embedded, and by means of which electrical potentials can be epi- or subdurally measured (Buzsáki et al., 2012). Coming from clinical applications in humans, this type of recording has also been used in monkeys (Bosman et al., 2012; Taylor et al., 2005) and rats (Molina-Luna et al., 2007). Especially in rats, whose brains lack gyri and sulci, the ECoG is a promising recording technology. In this paper, we extend existing rat ECoG work to brain areas that were previously inaccessible, namely the lateral surfaces of the brain. We also develop techniques for coregistration of electrodes to both anatomically and functionally defined brain regions.

The rat somatosensory system is extremely popular in sensory neurophysiology, but much less so in more cognitive research. An important reason is the difficulty in providing controlled tactile input to its main sensory apparatus, the large vibrissae, which are actively moved by the animal. In our setup, we make use of the fact that our recording elements can target the more laterally

* Corresponding author. Tel.: +31 243612651; fax: +31 243616066.
E-mail address: e.maris@donders.ru.nl (E. Maris).

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positioned primary somatosensory cortex of the small vibrissae, which are not actively moved by the animal (Mitchinson et al., 2011). In fact, we propose a stimulation paradigm that involves a head-mounted device that can stimulate the mechanoreceptors of both large and small types of vibrissae. Moreover, using a head-mounted device allows the animal to move freely while being engaged in a task and producing behavioral output.

In the following, we describe a setup that allows us to record chronically from a large part of the rat's brain surface, while at the same time providing controlled sensory input. In a companion paper (*Cognitive neurophysiology in rats, Part2: Validation and Demonstration*) we show how we can obtain signals from our ECoG recordings that relate both to neural input as well as to neural output. There, we also validate our setup by replicating some findings from sensory neurophysiology, and illustrate its usefulness by novel sensory and cognitive neurophysiology results.

2. Materials and methods

2.1. Animals

Experiments were done in 4 males, over 7 months old Long-Evans rats (Janvier). Animals were housed individually in a large cage (40 cm × 25 cm × 25 cm) and were provided with nesting material and cage enrichment. The enrichment was extra food (nuts and dried fruits) and pressed cotton squares for shredding. No large toys were used in order to avoid potential damage to the implant. The day-night schedule was reversed. Access to food and water was *ad libitum*. All animal procedures were approved by the Animal Ethical Committee of the Radboud University Nijmegen.

2.2. Surgery

Surgery was conducted under anesthesia as described in the Anesthesia protocols for surgery and recordings paragraph of Section 2. Before surgery we administer a mix of 20 μg/kg of Buprenorphine and 5 mg/kg carprofen in order to relieve post-surgery pain.

We implant the electrode grid through a 10 × 3 ± 1.0 mm trepanation hole at the top of the rat's skull. The most lateral side of the hole is adjacent to the ridge in the rat's skull to which the jaw muscles are attached (Fig. 1). The most medial side is usually about 1 mm away from the bregma-lambda growth line. To make such a large craniotomy without damaging the dura, we use a piezo-electric dentist mill (Piezosurgery, Mectron Medical Technologies, Italy) with a spherical milling bit of 3 mm in diameter. This mill can come in contact with soft tissue without damaging it in contrast to the standard dentist drills.

For the purpose of insertion, the grid is sandwiched between two 50 μm thick copper foils and we slide this sandwich between the skull and the dura, towards the target region. An important difficulty is that the rats' brain is tightly pressed against its skull and the dura is connected to the skull's inner surface at different points, especially along the growth lines. Therefore, to slide the copper-grid sandwich to the target region, we first have to severe the connections of the dura to the skull using a single copper foil.

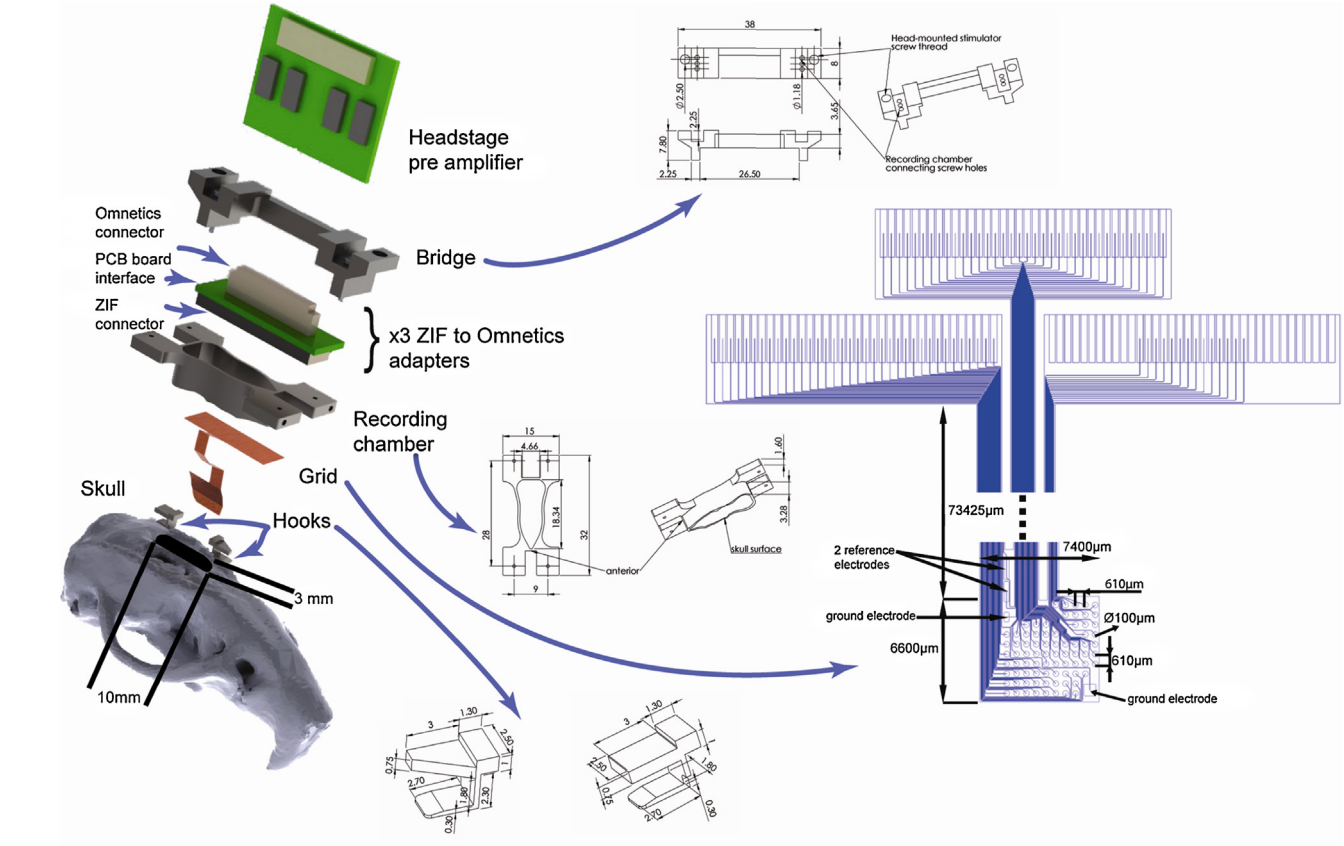

Fig. 1. Schematic representation of the surgery and the main implanted elements. The surgery involves the following steps: (1) opening of a 10 mm × 3 mm trepanation hole in the skull, (2) separating the bone from the dura over the lateral side of the brain, (3) inserting the grid and two hooks under the rostral and caudal sides of the hole, (4) positioning and gluing the recording chamber at the appropriate place, (5) inserting the soft connectors of the grid into a ZIF connector (part of an adapter that connects to an Omnetics connector), (6) filling the recording chamber cavity with glue and securing the adapter by screwing the bridge onto the recording chamber. This setup is permanently fixed onto the rat's head and allows the connection of a headstage pre-amplifier and the mounting of a head-mounted tactile stimulator.

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