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Anatomy and surgical approach of rat's vestibular sensors and nerves

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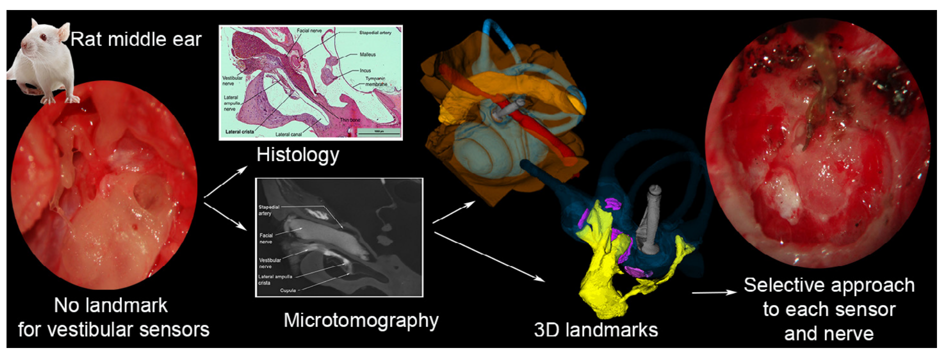
HIGHLIGHTS

- The specific role of each vestibular sensor is still unknown for complex functions such as cognition.
- Reaching each vestibular sensor and its nerve in rats requires precise landmarks.
- Histology and microtomography demonstrate precise landmarks in 2 and 3 dimensions.
- These landmarks allow a selective approach to each vestibular sensor and nerve in rat.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The rat is one of the most used species in the neurosciences, but how to selectively reach each of its 5 vestibular sensors has never been described. Besides, new functions of the vestibular system have been recently discovered in the rat involving vegetative, circadian and cognitive functions. But the central pathways sustaining these functions and the role of each of the vestibular sensors are not clear.
New methods: Here we want to describe the anatomy and look for a direct surgical approach to the 5 vestibular sensors in rats, as an indispensable technique to further study the central vestibular pathways.

To do so we studied 10 rats either by microtomography with osmium tetroxide staining, histology with hematoxilyn-eosine staining or microsurgical dissection.
Results: The microtomography allows a 3D representation of the 5 vestibular sensors and their nerves, with precise landmarks confirmed by the histological analysis. Each of the landmarks are illustrated and a selective surgical approach to each sensor and their nerves, is described step by step.
Comparison with existing method: Selective approaches to the vestibular sensors have been used in other species such as cats, monkeys and recently humans but the current study is the first allowing this technique in rats.
Conclusion: Each vestibular sensor of the rat can be reached by a selective surgical approach. This allows further techniques such as electrophysiology or neurotracing of the central vestibular pathways. This also indicates the rat as a potential model for vestibular prostheses.

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1. Introduction

The vestibular end organ contains five sensors (3 ampullae, 1 utricular macula and one saccular macula) protected by the bony labyrinth which is itself included in the temporal bone. This bone makes it difficult to reach and study each receptor and their specific roles.

Indeed, the classical techniques to stimulate the vestibular sensors fail to be selective for one sensor: stimulation of sensors by rotation always stimulates both labyrinths (the push–pull theory), otolith sensors are always stimulated by gravity, and caloric tests stimulate one lateral canal but also hearing and tactile sensors. The only known method allowing selective stimulation of the vestibular sensors is a surgical approach to each sensor in order to stimulate them electrically. This technique has been successfully used in cats (Fluur, 1959; Suzuki et al., 1969; Fluur and Mellström, 1970a,b; Goto et al., 2003, 2004), monkeys (Suzuki and Cohen, 1964) and guinea pigs (Curthoys, 1987) to study vestibulospinal and vestibulo-ocular function. Recently, the development of a vestibular prosthesis for bilateral vestibular impairment has provided new interest in these techniques, with the development of a new model in chinchilla (Tang et al., 2009; Dai et al., 2011) and the first implantations in humans (van de Berg et al., 2011; Perez Fornos et al., 2014; Guinand et al., 2015). Also other functions of the vestibular system have been recently proven, including vegetative (Yates and Bronstein, 2005), circadian rythms (Fuller and Fuller, 2006; Martin et al., 2015) and numerous cognitive functions ((Lopez, 2013; Smith and Darlington, 2013; Smith and Zheng, 2013; Jamon, 2014) see for review (Hitier et al., 2014; Besnard et al., 2015)).

The best known animal model to study spatial memory is the rat in which it has been discovered that the hippocampal neurons are specialized in spatial orientation (place cells, grid cells and border cells), rewarded by the 2014 Nobel prize in Physiology or Medicine. Studies in rats have also demonstrated the influence of vestibular input on place cells (Sharp et al., 1995; Stackman et al., 2002). But we are still ignorant of the neural networks sustaining these functions and the specific role of each vestibular sensor.

Previous studies in rat have described the surgical approach of the middle ear (Judkins and Li, 1997; Hitier et al., 2010) and the anatomy of the inner ear itself (Curthoys, 1981; Blanks and Torigoe, 1989), but without surgical landmarks to locate the vestibular sensors, or their nerves. This lack of anatomical data in rat makes it difficult to precisely reach each of the sensors without destroying one of them.

Here we propose to establish a selective approach to each vestibular sensor and nerves in rat, which is needed to better understand the central vestibular pathways and distinguish between the roles of different sensors.

2. Materials and methods

An anatomical study has been performed on 10 male Wistar rats aged 8–12 weeks old, in accordance with the Regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals and was approved by that Committee (Ethics number 55/12). The anatomy of each vestibular sensor was studied by histological sections, microtomography with 3D reconstruction and surgical dissection.

2.1. Microtomography analysis

The left temporal bone of one rat was stained with osmium tetroxide, according to the protocol described previously (Wong et al., 2013). After 3 days of staining the specimen was wrapped in parafilm with phosphate buffer solution and analyzed by microtomography (Xradia MicroXCT-400 (Xradia, CA, USA)). We set scanning parameters to 89 kV, 110 μA with an exposing time of 35 s and a rotation of 0.2° over 360° (Wong et al., 2013). Resulting images (DICOM format) were analyzed with ITK Snap software (Yushkevich et al., 2006) allowing segmentation of the anatomic structures and 3D reconstruction. The resulting Mesh Model was imported into Slicer 3D software to associate the 3D model with the microCT slices (Fedorov et al., 2012). For comparison with human anatomy, the same 3D reconstruction was used from a CT scan (120 kV, 128 × 0.6 mm, reconstruction 0.1 mm, SIEMENS, Germany).

2.2. Histological analysis

One left temporal bone was decalcified in 5% nitric acid for 3 days, followed by 5% sodium sulfate for 24 h. Specimens were embedded in paraffin wax, allowing axial sections of 5 μm thickness followed by hematoxylin eosin staining.

2.3. Surgical dissection

In five heads (10 sides) the temporal bulla was opened through a ventrolateral approach (Hitier et al., 2010) under a surgical microscope (OPMI Pico, Zeiss, Hamburg, Germany). Each vestibular sensor was located and landmarks were noted to allow the best approach.

3. Results

3.1. Bony labyrinth

Microtomography distinguished the bony labyrinth, the membranous labyrinth and the nerves in which myelin is stained by the osmium-tetroxide (Kiernan, 2007).

The 3D reconstruction of the bony labyrinth showed the 3 semicircular canals with – as in other mammals including humans – a common crus between the anterior and posterior canal (Ekdale, 2013). Each of the 3 semicircular canals is roughly perpendicular to each other (Blanks and Torigoe, 1989), as in humans (Della Santina et al., 2005; Bradshaw et al., 2010). But the vestibule, the 3 semicircular canals and the cochlea, are oriented differently in the rat skull compared with humans (Fig. 1). For instance, the lateral semicircular canal is oriented more dorsal, caudal and medial in rat compared with humans. Consequently, this canal is not visible in the rat middle ear, contrary to humans (Fig. 2). The anterior and lateral ampulla are hidden by the facial nerve in rat making it impossible to reach their sensors directly, contrary to human (Figs. 1 and 2).

3.2. Lateral ampulla sensor

According to the microtomography and histological analysis, the lateral ampulla sensors lie medially from the facial nerve with only a thin bone between them (Fig. 3). This thin bone is visible with a surgical approach lowering or removing the facial nerve (Fig. 4). With careful opening of the thin bone we can visualize the lateral ampulla sensor (Fig. 5) and follow the lumen to open the lateral semicircular canal (Fig. 5).

The nerve of the lateral sensor runs anteriorly and meets the nerve of the anterior ampulla sensor (Fig. 6).

3.3. Anterior ampulla sensor

According to the microtomography, the anterior ampulla is located just anterior to the lateral ampulla (Fig. 1). The histological analysis confirmed the position of the anterior ampulla, anterior