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Research Paper

In vivo recording of the vestibular microphonic in mammals

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ABSTRACT

Background: The Vestibular Microphonic (VM) has only featured in a handful of publications, mostly involving non-mammalian and *ex vivo* models. The VM is the extracellular analogue of the vestibular hair cell receptor current, and offers a tool to monitor vestibular hair cell activity *in vivo*.
Objective: To characterise features of the VM measured *in vivo* in guinea pigs, using a relatively simple experimental setup.
Methods: The VM, evoked by bone-conducted vibration (BCV), was recorded from the basal surface of either the utricular or saccular macula after surgical removal of the cochlea, in 27 guinea pigs.
Results: The VM remained after vestibular nerve blockade, but was abolished following end-organ destruction or death. The VM reversed polarity as the recording electrode tracked across the utricular or saccular macula surface, or through the utricular macula. The VM could be evoked by BCV stimuli of frequencies between 100 Hz and 5 kHz, and was largest to vibrations between 600 Hz and 800 Hz. Experimental manipulations demonstrated a reduction in the VM amplitude with maculae displacement, or rupture of the utricular membrane.
Conclusions: Results mirror those obtained in previous *ex vivo* studies, and further demonstrate that vestibular hair cells are sensitive to vibrations of several kilohertz. Changes in the VM with maculae displacement or rupture suggest utricular hydrops may alter vestibular hair cell sensitivity due to either mechanical or ionic changes.

1. Introduction

The cochlear microphonic (CM) and vestibular microphonic (VM) were first reported in 1930 and 1934, respectively (Wever and Bray., 1930; Ashcroft and Hallpike., 1934). The CM has been used extensively in auditory research to study auditory physiology and pathology, increasing our understanding of the cochlear amplifier (Legan et al., 2000; Cheatham et al., 2004), endolymphatic hydrops (Kumagami et al., 1981; Brown et al., 2009), the auditory efferent system (Guinan, 1996), hearing loss due to ototoxicity (Lodhi et al., 1980; Fitzgerald et al., 1993), acoustic trauma (Patuzzi et al., 1989a,b), genetic disorders (Steel et al., 1987) and aging (Harris and Dallos, 1984; Conlee et al., 1988). Conversely, the VM has only featured in a handful of publications mostly involving non-mammalian and *ex vivo* models (Adrian et al., 1938; Zotterman, 1943; Lowenstein and Roberts., 1951; Corey and Hudspeth, 1983). Trincker (1959) was the first to report *in vivo* mammalian recordings of the VM, detailing the effects of recording location, stimulus frequency, surgical destruction, cooling and death. Later, Wit et al. (1986) reported on the VM recorded in pigeons, and Eatock et al. (1987) performed VM recordings in bullfrogs.

It is important to note the effect of recording location and end-organ preparation on the resultant VM. *Ex vivo* preparations typically secure a vestibular end-organ within an Using chamber, allowing recordings in close proximity to the hair cells (Hudspeth, 1982; Corey and Hudspeth, 1983; Eatock et al., 1987). Alternatively, *in vivo* preparations have recorded responses from the vestibular fluids, either within perilymph as a 'global' measure where nerve and hair cell responses summate (Huizinga et al., 1951; Wit et al., 1986), or much closer to the hair cells within the endolymph where the hair cell current contribution dominates the response (Rabbitt et al., 2005).

Both otolith organs, the utricle and saccule, act as highly

Abbreviations: BCV, bone conducted vibration; CM, cochlear microphonic; DC, direct current; ECG, electrocardiography; I/O, input/output; MET, mechanoelectrical transduction; VM, vestibular microphonic

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sensitive three-dimensional linear accelerometers (Dimiccoli et al., 2013). Their non-planar and uniquely curved epithelium enables 3D-vector polarization (Curthoys et al., 1999; Jaeger et al., 2008), with both phasic and tonic vestibular pathways (Fernandez and Goldberg, 1976b). Central to these pathways are the vestibular hair cells, which are embedded in a dense gelatinous matrix, underneath the heavy otoconial layer (Kachar et al., 1990). Rotational and linear acceleration create shearing forces that displace the otoconia, resulting in apical hair bundle motion (Fernandez and Goldberg, 1976a), which alters the hair cell mechanoelectrical transduction (MET) channel conductance, and the receptor current and membrane potential (Shotwell et al., 1981). Like the CM, the VM is produced by vibration induced modulation of hair cell conductance, with the extracellular potential determined by changes in current flow through the impedance path between tissue and fluids (Corey and Hudspeth., 1983). However, whilst the CM is dominated by the hair cells local to the recording location (Patuzzi et al., 1989a,b; Cheatham et al., 2011), which are modulated in-phase for low-frequency (<1 kHz) tones, and thus can be used as a reliable estimate the MET channel gating (Patuzzi and Moleirinho, 1998), it is unclear if the same is true for *in vivo* VM recordings. That is, it is unclear whether the VM is dominated by hair cells local to the recording site, and is therefore dependent upon the orientation of the hair cells (kinocilium) at the sensory epithelium, or is rather the summated extracellular response of all vestibular hair cells (Corey and Hudspeth, 1983). Ultimately, the closer the recording electrode is to the hair cell, and the larger the extracellular impedances, the larger the hair cell response will be due to less current spread (Hudspeth, 1982).

Rabbitt et al. (2005) recorded the microphonic (in response to 0.1–20 Hz stimulation) within the SCC ampulla of toadfish, and demonstrated the entire response was generated by the SCC hair cells. The finding that there was little-to-no contribution from neurons or otolith hair cells to the response may be due to the close proximity of the recording to the SCC hair cells, or due to some aspect specific to the stimulus used in the toadfish, or that the SCC hair cells are polarized in a single orientation. There was no suggestion that otolith hair cells do not generate microphonic responses under different recording procedures, focused on stimulating the otoliths.

Here we have performed similar experiments as the *in vivo*, mammalian VM recordings performed by Trincker (1959). However, whereas Trincker primarily used air-conducted sound to stimulate the vestibular system, and maintained fluid within the vestibule following surgical destruction of the cochlea, we have used BCV stimuli to more effectively evoke the VM, while removing the cochlea and the perilymphatic fluid within the vestibule to provide a more localized recording of the electrical response from the vestibular hair cells. This is more akin to previous VM experiments performed *ex vivo* (Furukawa et al., 1972; Corey and Hudspeth., 1983; Eatock et al., 1987).

2. Materials and methods

2.1. Animal preparation & surgery

Experiments were performed on 27 adult tri-coloured guinea pigs (*Cavia porcellus*), of either sex, weighing between 200 and 500 g. All experimental procedures were approved by The University of Sydney's Animal Ethics Committee. Animals received premedication I.P. injections of 0.1 ml Atropine Sulphate (0.6 mg/ml; Apex Laboratories, NSW, Australia), and 0.05 ml of Temgesic (Buprenorphreine Hydrochloride, 324 μg/ml; Reckitt Benckiser, Auckland, NZ). Animals were anaesthetised in an induction chamber with 4.5% isoflurane, and once sedated and lacking a foot-withdrawal reflex, were transported to the surgical table to be tracheotomised and artificially ventilated with a mixture of oxygen and isoflurane (2–3%). Blood oxygen saturation and heart rate were continuously monitored, and body temperature was maintained using a custom-made heating pad, blanket, and infrared heating lamp. Animals were secured between custom-made ear-bars, on which an electromagnetically shielded audiometric BCV stimulator (B-81, Radioear corp., PA, USA) was directly attached. Also attached to the ear-bars was a 3-axis accelerometer (Dimension engineering, OH, USA), which had a pass-band between DC – 1500 Hz. A small incision was made behind the pinna to expose the bulla from the dorsal aspect. Approximately 3 mm^2 of bone was removed from the bulla, exposing the facial nerve canal and round window. A Teflon-coated AgCl recording wire (with the very tip exposed) was then passed approximately 3 mm into the facial nerve canal to record nerve responses. A schematic illustration of the experimental setup is shown in Fig. 1.

In the ventral position, tissue and musculature overlying the bulla were carefully removed, and the bulla was opened providing a clear view of the cochlea. Several electrophysiological recordings were performed from the facial nerve electrode with the cochlea intact. Thereafter, the cochlea was completely removed, along with the stapes footplate, providing a clear view into the vestibule. From this approach, the basal surface of the utricular macula (i.e. the surface beneath the receptor hair cells) along with the utricular compartment membrane and the saccular macula (with the saccular compartment typically collapsing) were clearly visible. Fluids within the vestibule and bulla were removed using tissue wicks, taking care not to touch the otolith organs. Tissue wicks were routinely replaced as they became saturated with fluid over the course of the experiment.

2.2. Physiological measures

BCV stimuli and evoked responses were generated and recorded using custom-developed LabVIEW (National Instruments, TX, USA) programs. BCV stimuli were generated using a PCIe soundcard (Xonar Essence STX II 7.1, ASUSTek Inc. China), and amplified using an audio amplifier (AA-0488, Digitech, UT, USA). Analogue responses were amplified by 80 dB, with a 1 Hz to 10 kHz band-pass filter (IsoDAM 8, WPI, Florida USA) before being digitized at 40 kHz, 16 bit, using an analogue to digital converter (NI 9205, National Instruments, TX, USA). Averaged responses were evoked by either brief, monophasic "BCV-pulses", or 40 ms sinusoidal "BCV-bursts" with frequencies between 50 Hz and 5 kHz. The stimulus rate was 50/s for the brief stimuli, and 8.3/s for the 40 ms duration stimulus.

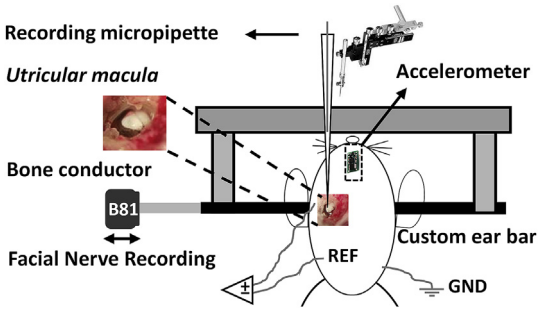

Fig. 1. Schematic diagram of our VM recording set-up using a ventral surgical approach. The animal lays supine, and was secured between custom-made ear bars, housing a B81 Bone conductor. A tri-axial accelerometer was rigidly attached to the skull vertex. The bulla was opened and the cochlea ablated, exposing the basal surface of the utricular and saccular macula. VMs were recorded from the otolithic maculae using a glass micropipette, and the facial nerve canal using an Ag/AgCl wire.