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EVESTG RECORDINGS ARE VESTIBULOACOUSTIC SIGNALS

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INTRODUCTION

Electrovestibulography [1] (EVestG) has been considered as a method to record and detect vestibuloacoustic signals originating mainly from vestibular system. EVestG recordings can be made while the subject is resting (no motion) or during a whole-body tilt [1-3]. The EVestG methodology using the "Neural Event Extraction Routine" (NEER) [1] has been shown to be able to detect small field potentials (FP's) buried in noise, average them and display the averaged FP as well as the FP firing time intervals. An FP can be defined as the 'synchronous' firing of many of vestibuloacoustic nerve fibres. Classifier features are measurable properties of an observation from the averaged FP and/or their firing histogram that can be useful in providing separation of two pathologies or symptomatologies. Features extracted from EVestG recordings have, in pilot studies, been successfully applied to the classification and or measure of the symptomology of Parkinson's disease [3], Major Depressive Disorder [2] and Vertiginous Disorders [4, 5]. However, a clear picture of the likely acoustic, vestibular or vestibuloacoustic origin and the physiologic basis of these field potentials (FP's) is lacking. It is worth noting that although the cochlea is the organ that responds to acoustic signals, there is good evidence that the saccule and possibly the utricle also respond to sound, thus making the term "vestibuloacoustic" more appropriate than either cochlear or vestibular alone in this context.

Early evidence for the vestibuloacoustic, predominantly vestibular origin, of the EVestG recorded FP's presented in [1] was based on a consideration of 3 human subject recordings, 2 with profound deafness (one unilateral and one bilateral, ie. no click evoked hearing at 90dB on the profoundly deaf side(s) and normal balance) and one with a unilateral Gentamicin vestibular ablation (unilateral weakness score 90%, sum of calorics 75, slight high frequency hearing loss). A more detailed study is required to establish the FP's vestibuloacoustic and perhaps predominantly vestibular origin. This paper reports the results of the first EVestG animal study done in a controlled manner. This study's aim was to investigate whether the stationary recorded spontaneous EVestG FPs from guinea pigs are indeed vestibuloacoustic.

The NEER detection process has limitations: One, it assumes there is a Summing Potential (SP) like point on the recorded waveform and that is essentially accepted as an acoustic feature, and two, it assumes the FP waveform is similar to the acoustic FP waveform derived from click evoked Electrocochleography (ECOG) recordings. In [2, 3] it was mentioned that the SP detection module in the NEER FP extraction program, given the Signal to Noise ratio (SNR) of the recording, could be neglected without significant effect. However, analysis of the phase changes that occur across the wavelet scales (used in the detection of FPs) indicates there are detectable phase changes local to where the SP point should occur. In addition, when extracting EVestG average FPs, we have noted the waveform can be quite different from the acoustically click evoked ECOG derived waveform used as the matching template in the NEER program. Fortunately, the template used in the NEER program was designed to be flexible enough to still detect the recorded ear canal FPs despite these differences. Thus, it is necessary to determine and better define the true EVestG FP template as well as determine the origin of the EVestG FP.

Methodology

EVestG is a non-invasive diagnostic technique that measures the electrical activity of the vestibular hair cells, vestibular nerve and vestibular nucleus (VN), as well as several brain regions which broadly communicate with the VN in the pathophysiology of neurological disorders [3, 6]. The EVestG technique is similar to Electrocochleography (ECOG) [1], wherein the acoustic stimulus can be replaced by a passive whole body tilt. During the experiment, ear canal electrical activity can be recorded in response to dynamic and static phases of a computer-controlled hydraulic chair via an electrode resting proximal to the tympanic membrane. Figure 1 shows the recording system. EVestG signals were recorded at a sampling rate of 41666 Hz. Baseline ECOG recordings were made to establish hearing threshold.

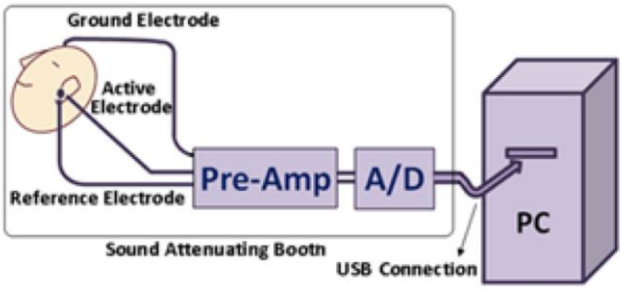

Figure 1: EVestG recording system in a human.

ECOG testing was done with the active recording needle electrode placed through the tympanic membrane [7] onto the medial wall of the middle ear and held in place with foam plugs. A reference needle electrode was placed subcutaneously just below the contralateral ear and the ground electrode placed subcutaneously in the abdomen. Needle insertion and testing was performed under general anaesthesia (intraperitoneal mixture of ketamine (60 mg/kg) and xylazine 6 mg/kg). The animal was in an Eckel AB2000 sound booth with added copper electrical shielding, grounding and lining with echo-reducing carpet. Hearing thresholds (to clicks) were established using traditional ECOG's [7, 8]. Sound intensities started at 100 dB SPL and decreased in 10 dB steps to 20 dB. The auditory threshold was the highest level at which the waveform does not appear.

A baseline stationary EVestG response was then recorded to measure spontaneous "vestibuloacoustic" electrical activity for comparison with future deafened or deafened plus gentamicin vestibular ablated recordings. The stationary EVestG was recorded using the same electrode setup for ECOG but elsewise using the equipment setup as detailed in [2, 3]. At the end of these recordings the animals were injected with Cisplatin to induce hearing loss without major disruption to the vestibular apparatus [9].

Administration of cisplatin (3 doses of 4mg/kg on alternate days) to impair hearing. Cisplatin is a known ototoxic medication and causes hearing loss [10, 11]. Cisplatin affects hearing but much less so vestibular function [9-11]. We anticipate this will help us discern the origin of the EVestG responses. We aimed for a hearing loss of 50dB but ideally 70 dB (the definition of severe hearing loss).

Deafened: After waiting >30 days the GP's were anaesthetised as above and a click evoked ECOG recording made to establish the new hearing threshold. A deafened stationary EVestG response was then recorded for comparison with baseline as well as subsequent deafened plus gentamicin vestibular ablated recordings.

Vestibular Ablation. At the end of these recordings the animals were injected with Gentamicin bilaterally intratympanically with 0.1cc of Gentamicin 40 mg/cc to cause vestibular ablation [12, 13]. Gentamicin is toxic to vestibular hair cells and, to a lesser extent, to cochlear hair cells. Vestibular ablation was carried out by injecting gentamicin directly into the round window under general anesthesia. Three of the 4 GPs lost weight, did poorly and were subsequently euthanized shortly after Gentamicin treatment. Based on this sensitivity the fourth guinea pig was not subjected to the added stress of the planned caloric testing pre and post Gentamicin treatment.

Deafened and Vestibular Ablated: After >30 days the GP was anaesthetised as above and click evoked ECOG recordings made to verify the hearing loss. A hearing loss plus vestibular ablated stationary EVestG response was then recorded for comparison with baseline as well as hearing loss recordings.

Results

To ensure the signal recorded was not "artefact rich" signal wise or system wise the active electrode was retracted 5mm and EVestG recordings made. As

expected, the signal contained predominantly high frequency noise.

All 4 GP's had their hearing threshold recorded at Baseline and after Cisplatin hearing loss. Table 1 shows the hearing thresholds before and after Cisplatin.

Figure 2 shows 3 traces. To combine the GP data each animal's data on each side was scaled to 1 (baseline to AP) to allow for variable contact impedances. To obtain the NEER extracted field potentials accelerometer and other artefacts were filtered from the recordings. Trace one is the baseline EVestG response averaged across 4 right and 4 left side responses. The second trace is the EVestG response after Cisplatin "deafening" across the 4 left side and 2 right side responses that had a threshold of ≥70dB SPL. The third trace is from GP4 which was the only GP to survive both the vestibular ablation (Gentamicin) treatment and multiple anaesthesia's. This trace is made up of the left and right side traces averaged together. The recording on GP4 was repeated with a second electrode placement to ensure electrode positioning was not an issue. The second recording result was almost identical to the green trace in Fig. 2a.

Table 1. Click evoked ECOG Hearing Thresholds (dB SPL)

	Baseline (Right, Left)	**After Loss (Right, Left)**
GP1	(20dB, 20dB)	(50dB, 80dB)
GP2	(20dB, 40dB)	(40dB, 90dB)
GP3	(40dB, 20dB)	(80dB, 70dB)
GP4	(50dB, 20dB)	(no hearing, no hearing)

Discussion

What is apparent including from Fig. 2 is:

A. With the electrode retracted 5mm from the recording site the signal was essentially high frequency noise implying the recording with the active electrode in place is mostly vestibuloacoustic. With cisplatin plus gentamicin treatment very few FPs were detected which is also a good indicator we are detecting vestibuloacoustic FP's.

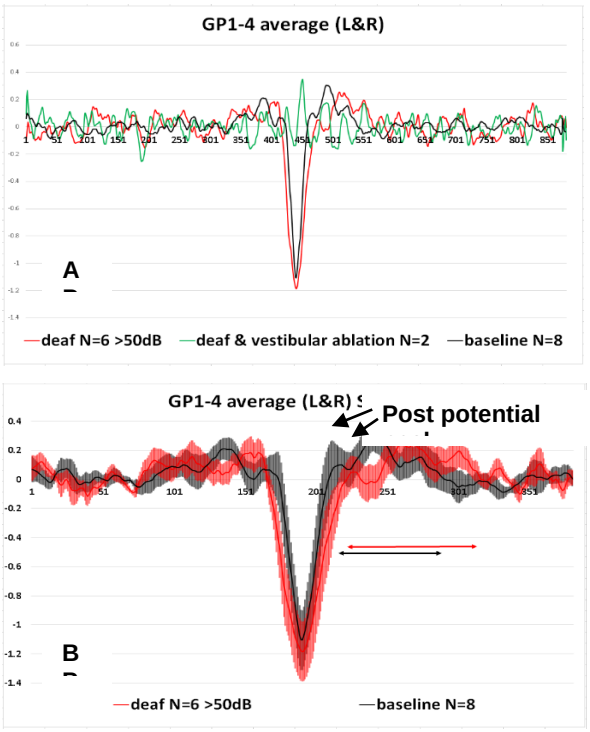

Figure 2. a. Comparison of EVestG waveform before and after deafening as well as after deafening plus vestibular ablation. **b.** Comparison of EVestG waveform before and after deafening showing Standard Error bars and regions potentially different.

After the cisplatin (hearing reduction) only treatment the curve becomes slightly wider. This increase is only significant at the Standard Error (SE) level so, if truly significant, this result is perhaps counter intuitive, given the increased vestibular (2.88um, 1.2um=SD) compared to acoustic (1.88um, 0.43um=SD) average nerve fiber diameter [14] but not the increased range (SD) of vestibular compared to acoustic fibre diameters. However, it may be that, 1) peripheral vestibular regular firing fibres with smaller diameters dominate the recordings or 2) there are K+ channel (repolarisation) differences between acoustic and vestibular fibres. K+ channels (high and low (including hyperpolarization current, I_h) voltage) have been shown to modulate the excitatory post synaptic potential (EPSP) and action potential (AP) shape [15]. The second observation on the deafened trace (again, only significant at the SE level) is that the post potential peak is slightly wider and is shifted right as would be expected from vestibular signals with longer K+ repolarization time constants [15].

C. In the baseline case the response is vestibuloacoustic. However, if the vestibular waveforms truly possess significantly wider (lower frequency) components, one could argue the vestibular signals farther away (at the recording electrode) are less attenuated than acoustic ones due, in part, to their lower frequency repolarisation (K+) components [16] and as such would be more likely/often detected. Additionally, future considerations of the resting afferent vestibular versus acoustic spontaneous rates plus the actions of their respective efferent systems might also bias the FP generation process to have a larger vestibular rather than acoustic component. This would emphasize vestibular rather than acoustic components in the baseline waveform. These issues are currently being examined in an ongoing modelling study exploring the physiological basis for the features used in EVestG pathology classification studies.

In conclusion, this study confirms that the EVestG extracted average FP is almost entirely vestibuloacoustic.

Acknowledgment

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