Verification EVestG recordings are vestibuloacoustic signals

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Funding information
NeuralDX

Abstract

Introduction: Neural dysfunction is associated with aberrant nerve firing; thus, electrodiagnosis has the potential for objective diagnosis and quantification of neural dysfunction. Electrical stimulation alters nerve firing and may also have treatment potential. This article outlines some findings related to electrodiagnosis and electrical stimulation of the ear. The quasi-synchronous firing of many vestibuloacoustic nerve fibers can produce an extracellular potential defined as a field potential (FP). Electrovestibulography (EVestG) is a method to record vestibuloacoustic signals and detect the associated FPs. A clear picture of the muscle-, EEG-, saccade-related, or other artefactual origins, and the physiologic basis of FPs recorded with EVestG, is evolving. EVestG was applied to demonstrate the effect of electrical stimulation on spontaneous FPs in the ear canal.

Methods: Bilateral EVestG recordings were conducted on 14 guinea pigs before and after stimulation with 3–0.5 mA ipsilateral anodal electrical pulses before and after ablation via unilateral Scarpa’s ganglionectomy to elucidate the origin of the EVestG recorded spontaneous FPs.

Results: Anodal electrical stimulation suppresses the recorded activity. There was a significant reduction of the level of recorded signal observed following anodal stimulation on the ablated but not the intact side.

Conclusion: Electrical stimulation of the external auditory canal reduces spontaneous electrical activity in the ear canal, some of which is due to central nervous system activity. The EVestG recorded FPs have a major vestibuloacoustic component.

KEYWORDS
acoustic, electrical stimulation, electrovestibulography, EVestG, vestibular

1 INTRODUCTION

The diagnosis of many neurotological disorders is based on patient history and clinical observations that may be unreliable. There is a need for objective, non-invasive methods to verify and quantify clinical diagnosis, and perhaps measure treatment response, particularly for those disorders in which structural abnormalities are absent. Functional magnetic resonance imaging (fMRI) shows differences in cerebral blood flow (CBF) across various brain regions, but it is not clear that all disorders cause proportionate alterations in CBF. In addition, fMRI sampling takes considerable time and areas of activity vary over time and cannot reflect rapid changes in brain function.
This article aimed to investigate EVestG recordings on four anesthetized animals, EvestG is recorded while stationary using needle electrodes.

Initially, it was found a sensitivity and a specificity of 75% and 80%, respectively, to detect Meniere’s disease in a small, blinded study using this technique. After diagnosis, the same EvestG techniques may prove useful in assessing response to treatments such as medications or electrical stimulation.

The early evidence for the vestibuloacoustic, predominantly vestibular origin, of the EvestG recorded FPs, presented in Dastgheib et al. was based on a consideration of two human subject recordings with either a hearing or balance deficit. The second main source of evidence was based on EvestG recordings on four anesthetized guinea pigs with: (1) normal hearing and vestibular function, then (2) following cisplatin-induced auditory toxicity, and then (3) following both cisplatin auditory and gentamicin vestibular ablation. The FPs were found to be both acoustic and vestibular in origin, that is, vestibuloacoustic; albeit predominately vestibular. This finding was not surprising, considering that the recording electrode of EvestG is placed in the ear canal but understanding the nature of FPs also opens the door for possible treatments.

Aside from the small sample size of those pilot studies, the extent of ablation was not quantified. This article illustrates a larger systematic animal study with thorough, unilateral surgical ablation of auditory and vestibular function, to investigate whether EvestG detected FPs are predominantly vestibuloacoustic in origin including a central component. The aim of this study is also to examine the possibility that modulation of EvestG signals can occur through electrically-induced stimuli with a view to the treatment of inner ear disorders.

2 METHODS

The protocol was approved by the University of Manitoba, Animal Care Committee, Animal Research Ethics Board.

2.1 Sample

Fourteen guinea pigs underwent controlled EvestG testing before and 2 weeks after unilateral excision of Scapa’s ganglion. The EvestG recordings were analyzed with the NEER algorithm to detect subtle differences between ear responses pre- and postoperatively before and after electrical stimulation. Auditory brainstem response thresholds were determined before and 2 weeks after ganglionectomy confirming ablation of the inner ear by the surgical procedure.

2.2 EvestG recording

The EvestG technique is similar to electrocochleography (ECOG), except the acoustic ECOG stimulus is replaced by a passive whole-body tilt. Human EvestG recording is performed while stationary and during physical passive whole-body tilts with ECOG electrodes. In animals, EvestG is recorded while stationary using needle electrodes. We have described our methods in detail previously. Active electrodes were placed through the tympanic membrane onto the promontory. Reference electrodes traversed the ipsilateral pinna to contact the temporal bone and a ground electrode was placed subcutaneously in the abdomen under general anesthesia with intraperitoneal ketamine (60 mg/kg) and xylazine (6 mg/kg).

Neuroelectric measurement would seem to hold promise but single units have tiny electrical potentials and there is much random activity or noise. Sampling the 80 billion or so neurons in the brain cannot be performed non-invasively. For non-random events, it seems likely that important neural activity involves populations of neurons that fire in coordinated volleys. If large enough, these volleys may be recorded extracranially, with electrodes close to the source, as field potentials (FPs). Features of FPs such as size, variability, and timing can be identified and quantified alone or in combinations, to recognize consistent changes in features that may be associated with specific disorders. Electrovestibulography (EvestG) is a technique that involves the measurement of electrical potentials from the external auditory canal and the identification of features from FPs. This article aimed to investigate the origin of the signals recorded by EvestG in guinea pigs and assess the effect of electrical stimulation on those signals.

There is hope that EvestG neuroelectric recording may identify neurological disease patterns. We have had some encouraging results using EvestG to identify Meniere’s disease and found a sensitivity and a specificity of 75% and 80%, respectively, to detect Meniere’s disease in a small, blinded study using this technique. After diagnosis, the same EvestG techniques may prove useful in assessing response to treatments such as medications or electrical stimulation.

The quasi-synchronous firing of many vestibuloacoustic nerve fibers can be defined as a “mini” FP. The EvestG methodology detects the small quasi synchronous FPs buried in noise, averages them, and displays the averaged FP and the firing time intervals between FPs using the “Neural Event Extraction Routine” (NEER). In humans, EvestG bio features have been successfully applied to the classification and or measure of the symptomology of Parkinson’s disease, depression, dementia, and vertiginous disorders. Initially, it was argued that EvestG predominantly measures the electrical activity of the vestibular hair cells, vestibular nerve, and vestibular nucleus (VN). However, contributions from muscle, EEG, saccades, or other artifacts may modulate these FPs despite some early efforts to show their vestibuloacoustic nature. Anodal electrical stimulation has been shown to suppress afferent activity in the ear; thus, it can be utilized to investigate the origin of the EvestG detected FPs with a view to therapeutic manipulation of neural activity.

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During the experiment, promontory electrical activity was recorded whilst stationary and in response to anodal (suppressive) electrical stimulation. The experimental setup consisted of a CED1902 Biological amplifier sampled at 41.67 kHz (notch filter at 60 Hz, gain 10,000) coupled to a CED1401 analog-to-digital converter connected to a PC running CED Spike2 software. The recordings were analyzed offline using the NEER V5.1 algorithm implemented in MATLAB R2017b. The recording set-up is illustrated in Figure 1. Illustrations of the extracted features are outlined in Figure 2. Figure 3 shows a typical EVestG recording.

The EVestG recordings were typically 60-s long. The first 15 s of the recording provided input to the NEER V5.1 algorithm, which outputted and stored the average FP and interval histogram for the 14 guinea pigs. Typically, the average is about 5000 FPs, depending on the signal-to-noise ratio and electrode impedance. These outputs were normalized to account for the electrode-skin impedance variations across animals and then combined to generate population-wise averages for pre- and postablation. A baseline stationary EVestG response was recorded to measure spontaneous vestibuloacoustic electrical activity for comparison with future recordings after ablation by Scarpa’s ganglionectomy. Our stationary EVestG method has been previously detailed. Briefly, an average FP was used to contrast differences in amplitudes and a 33-interval histogram was used to assess timing response differences among the various conditions.

**FIGURE 2** Features defined graphically

**FIGURE 3** Fifteen-second recordings from typical, guinea pig, preoperatively, before and after ipsilateral electrical stimulation. The scale is the same on both plots. Spike 2 software (version 7.04) was used to record real-time electrical signals. Anodal electrical stimulation reduced the signals in the ear canal.
2.3 | Extracted features

2.3.1 | Average FP

The NEER algorithm uses a complex Morlet analysis of phase and a matched filter to detect “mini” FPs buried in noise. Signals recorded for the 10 ms before and after each detected FPs are averaged over the 20-ms window to form an average FP. Using the largest FP magnitude as a central point, an average waveform like those shown in Figure 4 is the output.

2.3.2 | 33-Interval histogram (IH33)

The NEER algorithm outputs the time that each FP occurs. From these times, the interval between each 33 successive FPs is extracted. The average experimental gap between field potentials is 3.3 ms × 33 = 109 ms, that is, the system searches for approximately 10 Hz modulations of the firing rate, which has been hypothesized to be related to alpha band modulation and/or the spontaneous firing rate of vestibular efferents. These population-wise time intervals (x-axis Figure 5) provide a measure to assess temporal differences in FP firing across the various conditions.

2.3.3 | Electrical stimulation

After EVestG recordings of spontaneous electrical activity, the electrodes were unplugged at the biological amplifier, leaving needle placement undisturbed, and used to deliver three anodal, one-s electrical pulses of 0.5 mA, to one ear at a time using a Medtronic Xomed Varistim III surgical stimulator. After each side’s electrical stimulus, poststimulation EVestG recordings were carried out in both ears sequentially in six animals. We then developed the capability for simultaneous
recording and acquiring data from both ears in the eight remaining ani-
mals. Complete data were not available for some sessions because the
animal did not achieve adequate depth of anesthesia and/or recovered
from anesthesia before all recordings could be completed; Table 1
presents the details of the number of animals for each experiment.

2.3.4 | Scarpa's ganglionectomy

EVestG recordings, pre- and postelectrical stimulation were repeated
before and 2 weeks after trans cochlear excision of Scarpa's ganglion
of only the left ear. The ganglionectomy was performed under iso-flu-
ane general anesthesia by creating two relaxing incisions for exposure
in the lateral external auditory canal, removing the tympanic mem-
brane, then identifying and excising the cochlea in the tympanic bulla.
The dissection was then carried medial to the cochlear attachment,
removing the cochlea and all neural tissue encountered medial to the
cochlea until the empty internal auditory canal was visualized. This
procedure ablated all auditory and vestibular function on the operated
side, allowing us to determine whether the origin of the “mini” spon-
taneous FPs in the EVestG recordings is generated either centrally or
peripherally.

**FIGURE 5** Upper two panes show the IH33 gap interval histogram before and after left-side ablation on both left and right sides. The
number of detected intervals in the 15-s recordings for responses on the left-ablated side is lower after ablation. Ipsilateral anodal electrical
stimulation causes a longer gap between detected field potentials before ablation which remains unaffected on the non-ablated side. After left-
side ablation, electrical stimulation to the left has no significant effect. The x-axis is time (ms) and the Y-axis is population (%). Asterisk (*) indicates
significance for that bin.

**TABLE 1** Number of guinea pigs for each experiment

| Preoperative | Postoperative |
|--------------|---------------|
| Before and after electrical stimulation | Before and after electrical stimulation |
| Intact left ear recording | Intact right ear recording |
| 13, 12 | 8, 8 |
| Ablated left ear recording | Ablated right ear recording |
| 11, 9 | 8, 7 |
3 | RESULTS

When electrical stimulation (3 \(\times\) 0.5 mA anodal current pulses) was applied, the ipsilateral recording typically was visibly diminished, as shown in Figure 3. The suppression was consistent, but the duration of suppression was variable lasting 10s of seconds to 10–20 min.

The average FP amplitude decreases in response to the anodal electrical stimulation and the variability of the differences ±95% confidence intervals are shown in Figure, 4. Differences that exceed each other’s 95% confidence intervals were considered statistically different.

The second output extracted from an EVestG recording is the IH33 interval histogram which is normally extracted from the gap between each 33 detected FP. Figure 5 shows that left-side ablation affects the left side, but not the right side, firing pattern response significantly (shifted to the right side of the time axis meaning longer intervals between each 33 detected firings). However, the significant suppressive effect of anodal electrical stimulation on the firing pattern response is not seen on the left side following left-side ablation. On the right side (Figure 5B,C) there is a consistent electrically induced suppression (right shift) before and after opposite-side ablation.

4 | DISCUSSION

Our results indicate that EVestG signals arise largely from the inner ear (peripheral vestibular system and cochlea) and anodal electrical stimulation attenuates spontaneous electrical activity before and after ablation. In intact ears, the differences are more pronounced in the ear being stimulated, raising the possibility that attenuation with electrical stimulation might reduce tinnitus and/or hyperacusis. There is clear evidence of response change on the operative ear after Scarpa’s ganglionectomy both in the FP shape and firing pattern. This supports the detected EVestG FP’s being at least, in part, vestibulocoustic in origin. The responses observed even after ablation indicated that the central nervous system also accounts for some vestibulocoustic signal.

The NEER algorithm can detect FP’s buried in significant levels of noise by using a complex Morlet wavelet analysis of phase, that is, detecting sharp changes in phase across all scales as potential FP’s followed by using a matched filter to detect FP’s from artifacts. The trade-off for utilizing a matched filter within the detection algorithm is that the NEER algorithm will inherently detect FP’s even in pure noise. The ratio of real-detected FP’s from a noisy signal is dependent on the signal-to-noise ratio. Thus, regardless of whether there is a Scarpa’s ganglionectomy or not, the system will detect some FP’s even in noise. If that noise is modulated by alpha activity (or other brainstem components, e.g., CN or EVS or other), then the detected FP’s likely still reflect that componentry. This means the best application of NEER is to detect a change in response. The change from baseline measure used herein facilitates detecting the presence of vestibulocoustic components by measuring response to the electrical stimulus by comparisons before and after Scarpa’s ganglionectomy.

From the data presented herein, a clear picture of the acoustic, vestibular, muscle, EEG, saccade related, or other artefactual origin and the physiologic basis of these field potentials recorded with EVestG is still lacking. However, we have shown that the EVestG responses have vestibulocoustic input. These findings could potentially be exploited clinically.

4.1 | Comments on signal acquisition and analysis

Voluntary muscle artifact is limited by anesthesia, but respiration continue. Eye movements were not observed. Muscle signal energy is centered at about 90 Hz and in NEER V5, we high pass filter at 300 Hz, which minimizes that interference. Regarding the effect of eye movements under anesthesia condition, it has been shown that the use of ketamine/xylazine limits the eye movements significantly compared to other types of anesthetics such as isoflurane or propofol. As well, the recordings are performed in a dark chamber that helps minimize the effect of pupillary light reflex. Moreover, as the average FP’s are normalized to the AP amplitude of the baseline segment, the effect of any possible eye movement on the recorded signal can be discounted. The effect of other common mode signals such as EEG on the measured signals is also minimized through the application of the differential amplifier when using ipsilateral electrode pairs.

With respect to acoustic artifacts that may contaminate the EVestG signal, on one hand, no sound stimuli were presented during the recordings and recordings were conducted in an Eckel AB sound booth, providing 50-dB noise reduction, with added copper shielding to reduce electromagnetic field interference. On the other hand, the anatomical and physiological differences between the vestibular and acoustic systems such as the difference between the afferent resting spike rate (the spontaneous afferent acoustic activity is only 30% of that of vestibular), efferent activity (vestibular efferent effects are mostly excitatory in mammals, however, acoustic efferent effects are mostly inhibitory), and efferent innervation (the vestibular efferents have connections to the afferent and hair cells or their calyx whereas auditory efferents mostly contact the afferents through axo-dendritic processes) support arguments that vestibular responses recorded using EVestG technique are mainly of a vestibular origin.

5 | POTENTIAL CLINICAL APPLICATION

Sophisticated application of neuroelectric data has promising roles for clinical diagnosis and treatment. Clinical diagnosis may be supported by finding subtle shifts in firing patterns associated with various disorders but the specificity and sensitivity for those disorders need to be established. Return of aberrant firing patterns may not necessarily reflect return to “normal” physiology. These areas will continue to be studied by groups such as ours.

Therapeutic electrical stimulation of the ear is not a new idea either but we show here that electrical stimulation suppresses spontaneous neural activity and that suppression is not confined to the ear. Various responses after galvanic stimulation, repetitive transcranial magnetic stimulation (rTMS), transcranial direct current stimulation...
(tDCS), and other interventions have been observed but the effects are transient and require specialized equipment. Jacquemin et al. reported clinically significant improvement of the tinnitus functional index in 32% of patients treated with tDCS in a group of 39 patients but this may not be different from spontaneous improvement. A meta-analysis of tDCS and rTMS found that rTMS was more effective than tDCS or sham treatment at reducing tinnitus but it is not clear how long the effect persisted.

Treatment results for tinnitus with electrical stimulation are similarly uncertain. Our EvestG recordings illustrate that electrical stimulation of the ear may suppress spontaneous activity in the intact ear and in the brainstem without the presence of an intact ear. Perhaps electrical stimulation could be clinically applicable to disorders such as tinnitus. Although galvanic stimulation seems to have some application for vestibular disorders, albeit uncertain. Our EvestG recordings illustrate that electrical stimulation could affect both auditory and vestibular pathways. We were also unable to monitor ABR thresholds and EvestG recordings simultaneously because different hardware and set-ups are involved. Perhaps treatment efficacy can be investigated with more confidence.

Although our study demonstrates that electrical stimulation can modulate neural activity in the ear, the suppression is transient. We have not demonstrated its clinical utility in this article. The search for a more lasting effect would seem to hold promise for treatment of some otologic disorders.

5.1 Limitations

The greatest limitation of the methodology is that the applied electrical stimulus could affect both auditory and vestibular pathways. We were also unable to monitor ABR thresholds and EvestG recordings simultaneously because different hardware and set-ups are involved.

6 CONCLUSION

Spontaneous electrical activity in the ear canal is diminished by brief periods of electrical stimulation. The EvestG “mini” FPs are at least partially vestibuloacoustic and arguably predominantly vestibular in origin.

ACKNOWLEDGMENT

This study was funded by NeuralDX Pty Ltd.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Blakley B, Ashiri M, Moussavi Z, Lithgow B. Verification EvestG recordings are vestibuloacoustic signals. Laryngoscope Investigative Otolaryngology. 2022;7(4):1171-1177. doi:10.1002/lio2.862