

**Application of Electrovestibulography (EVestG) Coupled with Virtual Reality to  
Investigate Visual-Vestibular Interaction: An Exploratory Human Study**

By

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In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Biomedical Engineering Program

University of Manitoba

Winnipeg, Canada

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## **Abstract**

The integration of visual and vestibular information is an example of multisensory processing commonly applied to our daily life. The undeniable role of visual inputs in compensation, restoration, and adaptation for vestibular deficiency has been addressed in several studies. However, current literature fails to objectively measure vestibular responses following a targeted visual stimulus. In the studies of this thesis, using Electrovestibulography (EVestG), we measured participants' vestibular activity from the ear canals in response to targeted visual stimuli, developed in immersive virtual reality (VR) environments, delivered through a head-mounted display (HMD), quantitatively and non-invasively.

This dissertation consists of four sub-studies: 1. Quantifying the difference between physically (applied via a hydraulic chair)- and visually (replicas of the physical tilts designed in a VR environment)-induced vestibular responses; 2. Investigating vestibular responses to the combined and individual effects of intensity and hue of three monochromatic colored-light stimuli (blue, green, red); 3. Investigating changes in the vestibular activity when exposed to a horizontal pursuit and saccadic eye movements; and 4. Investigating vestibular responses to the visually-evoked sensation of body movement (Vection) utilizing a VR roller-coaster.

Overall from the four sub-studies mentioned above, we conclude that different visual stimuli can produce a measurably different vestibular response (measured by EVestG) depending on the VR environments' characteristics (object motion, color, etc.). As long as the applied visual stimuli do not induce a self-motion sensation, the vestibular response is generally inhibitory (sub-studies 2 and 3). Upon feeling a self-motion sensation, the vestibular response may become an excitatory response (sub-study 4).

To better understand the implications of these results, future studies should address the effect of other factors in the field of view (e.g. speed, simultaneous visual and vestibular stimuli, etc.) on the vestibular response measured by EVestG. The findings of this thesis are of significant clinical importance and can pave the way for future research in this field. This research takes us one step closer to the development of a portable EVestG technology that can become commonplace in neuro-diagnostic and clinical areas.

## Acknowledgment

Although words cannot describe my deepest feeling of appreciation and gratitude towards people who supported me throughout the Ph. D. program duration, nevertheless, I would like to express my sincere thanks to individuals and entities that made it possible for this dissertation to become a reality.

My special thanks go to my supervisors, Dr. Zahra Moussavi and Prof. Brian Lithgow for providing me this great opportunity for scholarly growth and development. Without your dedication, scientific advice, patience, and support I would not have been able to accomplish this research. I consider myself very lucky to have you as my supervisors and I am very much thankful for believing in me and trusting me with my work.

Next, I would like to extend my gratitude to my committee members and advisors, Dr. Behzad Mansouri, Dr. Gabriel Thomas, and Dr. Brian Blakley who have been available for me when I had any research questions and helped me improve in my study.

I also want to acknowledge Mitacs, NSERC (Natural Sciences and Engineering Research Council), Manitoba General Bursary, and Riverview Health Center for providing funding and research space to conduct my study. Lastly, to NeuralDX for supplying the EVestG research facility.

I would like to express my special thanks to members of the Biomedical Engineering Program (the year 2015-2020) at the University of Manitoba, who volunteered in my studies and were always there for me to support me in many aspects of my life.

Last but not the least, I would like to appreciate my amazing family for their love and unending support and my friends who brighten up my days with their presence in my life.

**You are and will always be in my heart and mind.**

### **Data Availability**

The data recorded for this study are not publicly available, and they are under the research agreement of NeuralDx Ltd Ltd. with the University of Manitoba. To access data, one may contact Mr. Charles Hider at NeuralDx to obtain permission.

## **Contribution of The Co-Authors on The Papers**

This dissertation comprises the collection of four journal papers written in grouped manuscript style (Sandwich Thesis, publication numbers 1 to 4 listed in Peer-reviewed journals section, page vii). These four journal papers are presented in Chapters 2 to 5 of this dissertation. Publication number 1 listed in the Peer-reviewed conference papers section was published in Proceedings of the 11th Augmented Human International Conference and is a part of Supplementary Material 1 presented at the end of Chapter 2 (it investigates the difference between the vestibular responses to the physical versus virtual yaw movement). This section also includes relevant complementary data on the vestibular response to other types of chair tilt (movement in the roll and vertical axis). The second listed peer-reviewed conference paper was presented as a poster at the 2017 CMBEC40 Conference but it was not published. It deals with the effect of colors on the vestibular response and provides insight complementary to chapter 3. This paper is presented in Supplementary Material 2 along with other complementary materials at the end of Chapter 3.

I was the first author of the manuscripts (Chapters 2 to 5) and was the main contributor to this body of work. The work included carrying out a literature review, submitting ethics for the study, developing different VR environments, developing electrical circuits to synchronize the onset of EVestG recordings with EOG recording, as well as VR games, recruiting participants, running questionnaires and a visual test (hue discrimination test), conducting a hearing test, performing EVestG recording, statistical analysis of the recorded data, writing papers, and responding to reviewers. Dr. Zahra Moussavi was the principal investigator of the study and helped with getting the ethics approval, reviewing the papers, foreseeing the research direction as well as providing financial support to purchase required study materials and equipment. Prof. Brian Lithgow contributed to the study design, provided scientific feedback on the results, and helped with reviewing the papers and answering the reviewers' comments. Dr. Behzad Mansouri conducted a visual examination (which was a part of the inclusion criteria for the study) for the participants. He also reviewed the papers and shared his medical perspective on the topics with us. Dr. Brian Blackley helped to review the papers. Dr. Abdelbaset Suleiman assisted me with the initial EVestG recordings and shared his thoughts about the results of the studies with me as the senior postgraduate member of the EVestG lab.

## List of Publications

The list of the published journal and conference papers, abstracts, and submitted manuscripts extracted from this dissertation is presented below.

### Peer-reviewed journals

1. M. Ashiri, B. Lithgow, A. Suleiman, B. Blakley, B. Mansouri, and Z. Moussavi (2020). Differences Between Physical vs. Virtual Evoked Vestibular Responses. *Annals of Biomedical Engineering*, 1-15, <https://doi.org/10.1007/s10439-019-02446-3>.
2. M. Ashiri, B. Lithgow, A. Suleiman, Z. Moussavi, and B. Mansouri (2018). Visio - Vestibular Interaction in Humans: Changes in the Vestibular Response Following Visual Stimuli of Different Colors. *J. Med. Biol. Eng.* 3–8, <https://doi.org/10.1007/s40846-018-0425-7>.
3. M. Ashiri, B. Lithgow, A. Suleiman, B. Mansouri, and Z. Moussavi (2020). Quantitative measures of the visually evoked sensation of body movement in space (Vection) using Electrovestibulography (EVestG). *Virtual Reality*. DOI: 10.1007/s10055-020-00488-w (in press).
4. M. Ashiri, B. Lithgow, A. Suleiman, B. Mansouri, and Z. Moussavi (2021). Electrovestibulography (EVestG) Application for Measuring Vestibular Response to Horizontal Pursuit and Saccadic Eye Movements. *Biocybernetics and Biomedical Engineering*.

### Peer-reviewed conference papers

1. M. Ashiri, B. Lithgow, B. Mansouri, and Z. Moussavi (2020). Comparison between Vestibular Responses to a Physical and Virtual Reality Rotating Chair. *In Proceedings of ACM Augmented Human Conference (AH'20)*. ACM, New York, NY, USA, 4 pages. <https://doi.org/10.1145/3396339.3396392>.
2. M. Ashiri, B. Lithgow, A. Suleiman, B. Mansouri. and Z. Moussavi (2017). Visio-Vestibular Interaction In Humans: Changes In The Vestibular Response Following Visual Stimuli Of Different Colors. *2017 CMBEC40 Conference*, Winnipeg MB, CA, 23–26.

## **Annual meetings and abstracts**

M. Ashiri, B. Lithgow, A. Suleiman, Z. Moussavi, N. Anssari, and B. Mansouri (2019). Comparative Changes In The Vestibular Response Following Horizontal Pursuit And Saccadic Eye Movements, *North American Neuro-Ophthalmology Society (NANOS)*, Omni Amelia Island, FL, USA.

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## List of Acronyms

ACS	Air conducted sound
AP	Action potential
BCV	Bone conducted vibration
CAP	Compound action potentials
CBF	Cerebral blood flow
DLPN	Dorsolateral pontine nucleus
EEG	Electroencephalography
EMG	Electromyography
ENG	Electronystagmography
EOG	Electrooculography
EVestG	Electrovestibulography
EVS	Efferent vestibular system
fMRI	Functional magnetic resonance imaging
FP	Field potentials
HMD	Head-mounted display
IH33	33- Interval histogram
LGN	Lateral geniculate nucleus
LTR	Left to right

MEMs	Middle ear muscles
MI	Magnocellular interblob
MPF	Medial prefrontal
mPFC	Medial prefrontal cortex
MST	Medial superior temporal
MT	Middle temporal/ V5 region
NEER	Neural event extraction routine
NRTP	Nucleus reticularis tegmenti pontis
PB	Parvocellular blob
PET	Positron emission tomography
PI	Parvocellular interblob
PPRF	Paramedian pontine reticular formation
RTC	Return-to-center
RTL	Right to left
SC	Superior colliculus
VALDS	Vestibular disorders activities of daily living scale
VN	Vestibular nuclei
VOR	Vestibulo-ocular reflex
VR	Virtual reality

## **Chapter 1- Introduction**

### **1.1 Background and significance**

The integration of visual and vestibular information is critical to maintain static and dynamic balance in humans. The two systems share some common brain regions to process the visual and vestibular inputs and to control many functions ranging from simple reflexes to complex hidden mechanisms that help our perception of oneself and the surrounding environment. For instance, during the 24 h cycle of a day, the illumination of the environment undergoes both intensity and spectral changes. The pupillary light reflex is one of the mechanisms that respond to the changes in the intensity of light. In addition, the dissociation between object-motion and self-motion (vection) is another example of a visual mechanism that helps us navigate and act within constantly-changing environments (both these topics are the focus of this research). The integral role of the vestibular system in controlling such visual mechanisms has been well established. Malfunction of such a complex reciprocal visual-vestibular interaction can endanger one's life.

Previous animal studies have established the interlink between the eyes to the vestibular brain regions in response to different visual stimuli including flashes of different light intensities [1], optokinetic nystagmus [2], and eye movements [3]. In addition, several human studies have described the effect of visual stimuli on vestibular activity. Some studies have applied functional magnetic resonance imaging (fMRI) to record the brain activity in response to the visual sensation of self-motion (produced by a rotation of a disk with a windmill pattern) [4], or positron emission tomography (PET) as a screening method to investigate the effect of induced self-motion sensation (produced by moving black and red dots at a constant velocity in the scene) [5]. Another study has compared the effect of exposure to black and white in comparison to colorful objects/ non-objects (having no particular shape) using fMRI to investigate human brain regional activation [6]. The aforementioned studies conclude that common visual and vestibular processing brain regions are active during exposure to those visual stimuli (e.g. insular cortex which is a region associated with visuospatial information processing).

Although the above studies provide valuable insights into the vestibular functionality, they have some shortcomings described in the next section.

## 1.2 Problem statement

Various methods have been employed by the researchers to investigate visual-vestibular interaction. However, common methods of such investigation suffer from several limitations. The main shortcoming is that the current studies in visual-vestibular have not recorded vestibular responses from their origin objectively (i.e. central or peripheral vestibular system), but rather they used indirect methods to evaluate the vestibular function. In addition, the choice of virtual reality (VR) environment to run experiments for such studies has not been justified, nor have different VR environments been compared (i.e. no explanation on why a certain VR environment is used and which part of the vestibular system is targeted by that VR stimulus). Here, we describe some of the shortcomings of vestibular-screening methods in animal and human studies.

In animal studies, invasive recordings are common. The recording procedure can range from direct electrode insertion into the optic nerve/vestibular nerve to using ototoxic drugs to promote hair cell death, or using surgically implanted head-restraining posts to apply passive head tilts. The main limitation of these animal studies is that they include invasive and irreversible procedures that cannot be applied to humans. Nor is the animal model completely compatible with human physiology [7, 8]. Several human studies have taken advantage of different imaging techniques such as PET and fMRI to screen vestibular-activity changes in cortical and subcortical brain regions [5, 9–11]. However, imaging techniques only provide an indirect evaluation of the vestibular system's response; they are expensive procedures, and also place restrictions on body motion whilst recording (they are typically done only in the supine position).

Two other techniques used for the assessment of vestibular response are questionnaires and physical condition performance tests [12–14]. Both of these techniques are inexpensive and not time-consuming, yet they impose some restrictions on the credibility of the data collection. Questionnaires can be subjective and are challenging to score the severity of the symptoms (as they are restricted to limited options such as asymptomatic, mild, moderate, and severe choices). In addition, questionnaires do not provide information over a short period of time (i.e. they are not able to measure transient and immediate changes that often disappear in a matter of seconds); thus, the results can become undifferentiated [15]. Physical condition performance test, such as the vestibular ataxia test (where participants stand on one leg for a certain amount of time and then on

the other leg while the experimenter observes any imbalance) can also be problematic due to comprehension and physical limitations introduced by natural aging or physical injury (e.g. limited sensitivity to change and responsiveness in elderly individuals) [16, 17]. As well, such evaluation methods are experienced-based, and the scores may change from one examiner to another. Other tests such as Electronystagmography (ENG) (assessing involuntary eye movements, nystagmus) can also be used to screen vestibular response change (for example when dizziness is related to brain or inner ear diseases). ENG assessment also suffers some similar complications (an indirect measure of the vestibular functionality based on oculomotor activity) and is considered more suited as a neuro-otological disease diagnostic aid rather than an assessment tool that provides features that are unique to different types of visual stimuli [18, 19].

The necessity of applying an objective and non-invasive technique that records vestibular activity from its origin (central and peripheral vestibular organs) led to the invention of Electrovestibulography (EVestG) in 2004. EVestG [20] is an objective method of measuring predominantly vestibular responses from the ear canal in response to passive whole-body tilts. The tilts are generated through a hydraulic chair and have been designed to target specific organ/organs of the vestibular periphery [21]. So far, analyzing static and dynamic vestibular responses with EVestG has been promising and successful in the detection of various types of neurodegenerative diseases such as Parkinson's disease [22] and Alzheimer's disease, psychiatric disorders such as major depressive disorder [23] and bipolar disorder [24, 25], traumatic brain injuries such as post-concussion syndrome [26–29], as well as vertiginous disorders such as Meniere's disease [30–32]. Although EVestG technology is the most direct measure of the vestibular response and EVestG signal analysis shows promising potential in the assessment of several diseases, it imposes some limitations in which restrict its extensive clinical applications. To apply vestibular stimuli a hydraulic chair is used to generate passive whole-body tilts. The recordings must be conducted in a dedicated anechoic chamber to minimize the effect of acoustic noise, environmental and hydraulic system power unit noises. Acoustic noise in particular can corrupt the 8th nerve components of the generated signal recordings. As well, the hydraulic chair itself is an expensive, and cumbersome piece of equipment that takes a large amount of space. On the other hand, we know that the contribution of visual information is important in keeping balance [33] and visual information can reach vestibular-processing brain regions and modulate their neural activities [1,

4, 34]. Thus, we questioned whether visual stimuli can evoke vestibular responses and to what extent those responses are similar to the responses evoked by physical stimuli. If visual stimuli can reasonably replace physical stimuli in evoking measurable vestibular responses, then we might be able to have the same EVestG technology but without the need for a hydraulic chair. Thus, we will be one step closer to developing a portable version of EVestG. In this research, we took advantage of visual stimuli generated/simulated in VR environments to evaluate the vestibular response. As the very first study in investigating visual-vestibular interaction by EVestG measurement, we had to design separate experiments to investigate some fundamental questions such as the effect of color and eye movement on vestibular system's response. The VR environment was presented in immersive mode using a head mounted display (HMD) to provide a stereoscopic vision and a more naturalistic experience. The EVestG recording system was modified to also record signals during the VR stimuli. The specific objectives and experimental setup for each objective are explained in the next section.

### **1.3 Objectives**

The motivation for this research was drawn from the need to develop a portable version of EVestG. As an alternative to the non-portable EVestG tilting chair used for stimulating the vestibular system, we thought of using visual stimuli delivered through immersive VR to stimulate the vestibular system. Thus, first, we investigated whether visually-evoked vestibular responses are measurable at the vestibular periphery using EVestG. Second, if those responses were measurable, we investigated how they were similar or different from the physically-induced responses. Next, we explored how some individual factors in the field of view could affect the vestibular response. The specific objectives of this research were:

1. Design a replica of the EVestG chair and its physical tilts in VR, and Compare the EVestG signals recorded during physical stimuli (current tilting chair system) with those recorded during the immersive VR chair replica to investigate the comparative vestibular response resulted from physical and visual and stimuli.
2. Investigate the effect of different colors (hue and brightness level) on the evoked vestibular responses measured with EVestG.
3. Investigate the effect of eye movement on the vestibular response.

4. Explore the influence of self-motion sensation (vection) on the vestibular response evoked by visual stimuli in an immersive VR setting.

To carry out the research addressing the above objectives different VR environments were designed for the four sub-studies specific to each objective. The first sub-study addressing the first objective was to compare EVestG signals recorded during physical stimuli (current tilting chair system) and its replica in VR. As this study is at the forefront of research by applying combined EVestG and VR to examine visual-vestibular interaction in humans, it was necessary to first characterize how the vestibular response resulted from visual inputs differs from that of an actual whole-body motion. Twenty-seven healthy participants (10 females) were tested to compare the vestibular responses to two sensory inputs: (1) physical tilts (with eyes-closed) and (2) virtual reality replica of the physical tilts (eyes-open, physically static). As the visual ability of humans is mainly the result of the interaction of light, eyes, and brain it is important to understand how ambient light (hue/intensity) can affect our vestibular system. Therefore, the second sub-study was designed to address the objective of investigating changes in the vestibular response by exposure to different colors (hue and brightness level) as stimuli. Exposure to colors can promote image-forming (making our perception of the surrounding environment) and non-image-forming responses (involved in emotion, circadian rhythm, pupillary light reflex, etc.). Vestibular-processing brain regions are engaged in the generation of these responses. In the second sub-study, the vestibular dynamic responses to three monochromatic colored light stimuli (blue, green, red) along with black and white backgrounds were measured using EVestG technology. Since the effects of intensity and hue on the visual system overlap, the combined and individual effects of the intensity and hue of the three visual stimuli (blue, green, red) on the vestibular signals were examined among a group of 16 participants ( $26.8 \pm 5.3$  yrs SD, 8 females). In addition, the reproducibility of the results was investigated by repeated measurement of the same experiment on a 30-year-old healthy female subject.

Whether we are trying to fixate our gaze, reading a text, watching TV, or tracking a moving object with our eyes in the surrounding environment, eye movements are required to stabilize the image of the object on the fovea and provide us with clear vision. The ocular and vestibular systems play a vital role in generating different types of eye movements. Thus, we investigated the effect of two types of eye movement on the vestibular activity in the third sub-study. Vestibular responses to

the horizontal pursuit and saccadic eye movements were measured in 19 healthy participants ( $27.7 \pm 5.74$  (SD) years, 11 female) to demonstrate 1) whether the vestibular response during the pursuit and saccade eye movement are different from background 2) if there is a difference between the vestibular response to the pursuit and saccade, and 3) how individual eye movements affect vestibular afferent and efferent activity. The influence of visual motion on balance perception can sometimes result in confusion between object-motion and self-motion. The multisensory integration of visual and vestibular signals plays a vital role in differentiating between object-motion and self-motion. Vection, the sensation of self-motion induced through purely visual stimulation, is due to incongruent sensory information received from visual and vestibular systems [5, 35, 36]. In the fourth sub-study, we explored vestibular-response changes following a visually-evoked self-motion sensation in 20 individuals ( $26.45 \pm 4.40$  (SD) years, 10 females). Vection was induced in the participants using an immersive VR roller-coaster.

The more detailed objectives of this thesis are listed below:

1. Developing different VR environments with the following characteristic:
  - a. A virtual replica of the current physical chair
  - b. Solid backgrounds which alternate in hue and intensity
  - c. Horizontal pursuit and saccade eye movement using a moving object in the scene
  - d. Roller coaster to induce visual Vection
2. Recruiting participants
  - a. Scheduling eye examination appointments with our research-group neuro-ophthalmologist
  - b. Hue discrimination test
  - c. Conducting a hearing test followed by recording EVestG signals of the participants
  - d. Conducting a simulator sickness, a self-designed perceived vection, and Vestibular Disorders Activities of Daily Living Scale (VALDS) questionnaires
3. Extracting vestibular responses from the recorded signals and selecting features to run statistical analysis
  - a. Average field potential
  - b. 33-Interval Histogram

4. Investigating the difference between the vestibular response to passive whole-body tilts (in pitch, yaw, roll direction as well as up-down movement) and their virtual replica
5. Inspecting the effect of closed eye condition on the vestibular response compared to open eyes condition
6. Examining the combined and individual effect of hue and intensity on the vestibular system
7. Exploring the effect of horizontal pursuit and saccade eye movements on the vestibular system
8. Investigating the effect of saccade eye movement directionality on the vestibular system
9. Examining the alpha brain-wave impact (modulation or corruption) on the vestibular response
10. Studying the effect ofvection (induced through exposure to a visual roller coaster) on the vestibular response

## 1.4 Thesis Organization

This manuscript comprises 6 chapters. Chapter 1 presents a general overview of the background, motivation, rationale, and objectives of the thesis. Chapters 2 to 5 are individual manuscripts associated with the four sub-studies that either have been published in or submitted to peer-reviewed journals. Each chapter includes a summary of the objectives behind performing the experiments and a separate abstract. The first question to be answered in this research was whether visual information can be measured using the EVestG technique and if yes, how different it is from their physical counterparts. Chapter 2 draws a comparison between visually- and physically-evoked vestibular responses (objective 1) to a pitch tilt. Three other different physical tilts and their virtual replica, including yaw, roll tilts, and up-down movements are discussed in Supplementary Material 1 at the end of Chapter 2. The difference between vestibular responses during eyes-open and eyes-closed conditions is also investigated in Chapter 2.

As light is the input for sight, the next question we faced in this research was the effect of hue and intensity on the vestibular response. In Chapter 3, the impact of color lights on the vestibular system is studied (objective 2). Particularly, the individual effect of blue color components (hue and intensity) has been examined in this chapter. A more comprehensive study considering the effect of blue, green, and red color components is presented in Supplementary Material 2 at the end of Chapter 3. A clear vision (either during stationary or gait position) is achieved through generating proper eye movements. Chapter 4 explores how eye movements affect the vestibular response (objective 3). Horizontal smooth pursuit and saccade eye movements were considered as the visual stimuli in a sample group. Object motion in the scene can sometimes produce illusory self-motion sensation during stationary position. This phenomenon is one of the hidden visual systems (hidden in plain sight) that can affect the vestibular response. The answer to how illusory self-motion sensation impact the vestibular system can be found in Chapter 5. Chapter 5 studies the vestibular activity change to a visually-induced sensation of self-motion applied to the participants by exposure to a VR roller coaster (objective 4).

The last chapter (Chapter 6) integrates and discusses the findings of the sub-studies and states the future path of the research.

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## **Chapter 2- Differences between Physical versus Virtual Evoked Vestibular Responses**

### **2.1 Summary**

The first part of this study was to design a VR replica of the tilting chair used for EVestG recordings to answer the following fundamental questions:

- 1) Can visual stimuli evoke a vestibular response measurable at the peripheral vestibular system using EVestG?
- 2) How are the visually evoked and the physically evoked vestibular responses different/alike in terms of afferent and efferent activities?
- 3) What is the effect of eyes-closed and eyes-open conditions on the generated EVestG signals in the stationary position?
- 4) How does the tilt axis (Back-forward, Rotation, Side to Side, and Up-Down) affect vestibular responses to physical versus visual stimuli?

The original EVestG recording measures vestibular responses to passive head tilts applied through a hydraulic chair (physical tilts). To address the first two questions, a VR environment similar to the existing EVestG chair and its recording chamber was designed and a virtual tilt was replicated with the pitch axis. Young, healthy, right-handed participants (both male and female) were recruited and their vestibular responses to both scenarios (physical versus virtual tilts) were recorded. Two features: 1) Average Field Potential (FP) and 2) 33-Interval Histogram (IH33 mean) were extracted to compare the physically and visually evoked vestibular responses. To provide an answer to the third question, the vestibular responses of a female participant was examined in a stationary position with eyes open and eyes closed. The results of these investigations were published in *Annals of Biomedical Engineering Journal* in January 2020, Page 1-15, DOI: 10.1007/s10439-019-02446-3. Note: The style of the manuscript is according to the submission guidelines of the paper. Authors: M. Ashiri, B. Lithgow, A. Suleiman, B. Blakley, B. Mansouri, & Z. Moussavi. Title: Differences between Physical versus Virtual Evoked Vestibular Responses.

To answer question 4, different tilts (both physical and virtual) including Rotation, Side to Side, and Up-Down movement were applied to the participants. The results of each tilt are discussed separately in Supplementary Material 1 at the end of this chapter. The results associated with the

Rotation tilt was published in Proceedings of the 11<sup>th</sup> Augmented Human International Conference (AH'20). ACM, New York, NY, USA, 4 pages. DOI: <https://doi.org/10.1145/3396339.3396392>.  
Authors: M. Ashiri, B. Lithgow, B. Mansouri, and Z. Moussavi (2020). Title: Comparison between Vestibular Responses to a Physical and Virtual Reality Rotating Chair. The outcomes of the study for the Side to Side and Up-Down movements have not been published elsewhere.

## **Differences Between Physical vs. Virtual Evoked Vestibular Responses**

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Mansouri & Zahra Moussavi

### **2.2 Abstract**

Electrovestibulography (EVestG), a technology purported to measure vestibular activity at the vestibular periphery, was used to compare the vestibular responses to two sensory inputs: (1) back-forward physical tilt (with eyes-open and eyes-closed) and (2) virtual reality replica of the back-forward tilt (eyes-open, physically static). Twenty-seven healthy participants (10 females) were tested. From each of the EVestG recordings, two feature curves: (1) average field potential (FP), and (2) distribution of time intervals between the detected FPs were extracted (IH33 mean). For the eyes-closed physical tilt, except for the background segment, the FP response curve was generally wider compared to that evoked during the virtual replica tilt ( $P < 0.05$ ). Moreover, the eyes-closed physical tilt produced longer time intervals between FP's compared to the virtual stimulus. For this measure, for the background segment, the eyes closed and open physical tilt responses were significantly different ( $P < 0.05$ ) in both ears (repeated measure experimental design). The results support: (1) both vestibular and visual inputs evoking a measurably different EVestG response, (2) the differences between physical and virtual vestibular responses are dependent on the eyes being either open or closed, and (3) for the stimuli used, the modulation of vestibular afferent activity was measurably smaller for virtual than physical stimulation.

Keywords: Virtual reality, Visual, Afferent, Electrovestibulography (EVestG).

### 2.3 Introduction

Visual and vestibular inputs are critical to maintain postural stability. These inputs are used to control motor function necessary to maintain a stationary position or dynamic gait, as well as to regulate cognitive and psychological changes [1, 2]. Neural connections originating from the vestibular system to the vision system form part of a reflex known as vestibulo-ocular reflex (VOR) [3]. Neural pathways from the visual to the central and peripheral vestibular systems also exist. The visio-vestibular neural projections (see Appendix 1 for details) make it possible for the vestibular system to be affected even in the absence of any head motion through purely visual stimuli [1]. Recordings from single units in the vestibular nuclei to light flashes of different intensities and durations [4] revealed diverse inhibitory and excitatory vestibular responses to light. In humans, utilizing the visio-vestibular link, optokinetic stimuli and/or physical rehabilitation have been applied to improve vestibular-related disorders such as dizziness [5] or other balance problems secondary to vestibular disorders [2, 6]. For example, in [6] a significant improvement was observed in postural stability, visual vertigo, and emotional state. In another study, comparing the effect of VR-based therapy with customized vestibular physical therapy [7], similar vestibular symptom (e.g. postural instability, and dizziness) improvements resulted.

The effect of visual inputs on vestibular adaptation, substitution, and habituation is undeniable, however current human literature is limited for quantitatively measuring peripheral vestibular activity to visual stimuli. Electrovestibulography (EVestG) has been proposed as an objective technique to measure responses predominantly from the vestibular periphery [8]. Using this technique may provide information towards the following research understandings: 1) how the vestibular periphery adapts to various environmental visual changes, and 2) how different is the vestibular response to a physical motion stimulus versus a virtual replica of that physical stimulus. The main focus of this paper is the second question.

In the present study, we used EVestG as a vestibular measure to compare the effect of a physical vestibular (dynamic) stimulus without any visual input (eyes-closed) with that of a visual stimulus utilizing a VR replica (vestibular-wise static) of that vestibular stimulus, as well as a physical stimulus with eyes-open (only Background segment). The objectives of this study were to 1) demonstrate EVestG can measure both static visual and dynamic vestibular evoked responses, 2) observe if these responses have any significant similarities or differences in terms of the shape of the field potential (FP) responses or time-intervals between the FPs, and 3) investigate how much

the observed similarities or differences are statistically dependent on whether the eyes are open or closed.

## **2.4 Materials and methods**

Twenty-seven (10 female) right-handed healthy volunteers (27 years  $\pm$  5.08 SD) were enrolled in the study. An eligibility questionnaire (Appendix 2) and the Vestibular Disorders Activities of Daily Living Scale (VADLS) questionnaire were used to establish the participants' physical and psychological health history. None of the participants had any history of neurological or psychological disorders. This study was approved by the Biomedical Research Ethics Board of the University of Manitoba. After the participants signed an informed consent form (Appendix 3), they were referred to a neuro-ophthalmologist (Author B.M.) to conduct a comprehensive eye examination including a check for visual acuity, color vision, extraocular motility, pupillary eye reflex, etc. They then proceeded to a hearing screening test and EVestG recording at the EVestG laboratory at Riverview Health Center. All participants underwent physical and then virtual stimuli, whilst their EVestG signals were recorded. A break of 5 min was given between the physical and virtual experiments. This break was to provide enough time for the vestibular system to return to its resting condition [9] and for the VR equipment to be set up. Besides, to compare the effect of a physical tilt with eyes-open on the vestibular response, a repeated measure experiment was conducted on a female participant.

### **2.4.1 Electrovestibulography (EVestG) recording**

EVestG is a technology used for assessing brainstem and peripheral vestibulo-acoustic predominantly vestibular activity [8]. It is a quantitative method, capable of recording spontaneous and driven (whole-body tilt) vestibular activity [8]. This method has been successfully applied to the diagnosis of (and in some cases the severity measurement of) brain disorders including Meniere's disease [10], post-concussion syndrome [11], major depression [12, 13], bipolar disorder [14] and the separation of the depressive phase of bipolar disorder and major depression [15]. In EVestG, vestibular signals are measured using extra-tympanic electrodes (Fig. 2-1a). An active electrode (soft cotton wool wick type), soaked in the solution of saline and conductive gel, is rested close to the eardrum of each ear. The same type of electrode is placed on the outer extremity of the ear canal of both ears as references (Fig. 2-1b). A common electrode is placed on

the forehead to assist in canceling out common-mode signals, such as EEG, EMG and EOG, and environmental noises (Fig. 2-1c). The recordings are detected by these electrodes, then amplified and digitized at a 41,666 Hz sampling rate. To minimize the effect of acoustically evoked cochlear potentials on the vestibular response, EVestG recordings are conducted in an anechoic chamber (< 30 dB), where participants sit in a hydraulic chair. A backward then forward tilt (pitch axis) was applied in this study. The tilt started with 20-s of recording in the stationary position (sitting upright, center position), and followed by 3 s of a smooth tilting of the chair backward through 40 degrees. The chair remained in the target position for 17 s before returning back to the center with the same velocity profile. Another 17 s recording in the center position terminated the procedure of the tilt. Participants were asked to refrain from swallowing or other movements during the recording of EVestG signals. Figure 2-1d shows the profile of the chair movement for the back-forward tilt. From 60 s of EVestG recording, six segments of interests are extracted for further analysis. These segments are: 1.5 s background measure immediately prior to the chair movement, 1.5 s of acceleration and 1.5 s of deceleration while moving to the tilted position, 1.5 s prior to the returning to the center position, 1.5 s of acceleration and 1.5 s of deceleration whilst returning back to the center position (Figs. 2-1e-g) [8]. A typical EVestG recording includes yaw, roll and pitch tilts. In this study, we focus on the back-forward tilt as, according to our participants' feedback, it produced the most sensation-wise similarity between virtual and physical stimuli compared to other tilts. Given the vestibular anatomy, for the back-forward tilt, we expect the vestibular response (predominantly the utricle plus posterior and superior semicircular canals) to produce a similarly inhibited and/or excited response in both ears.

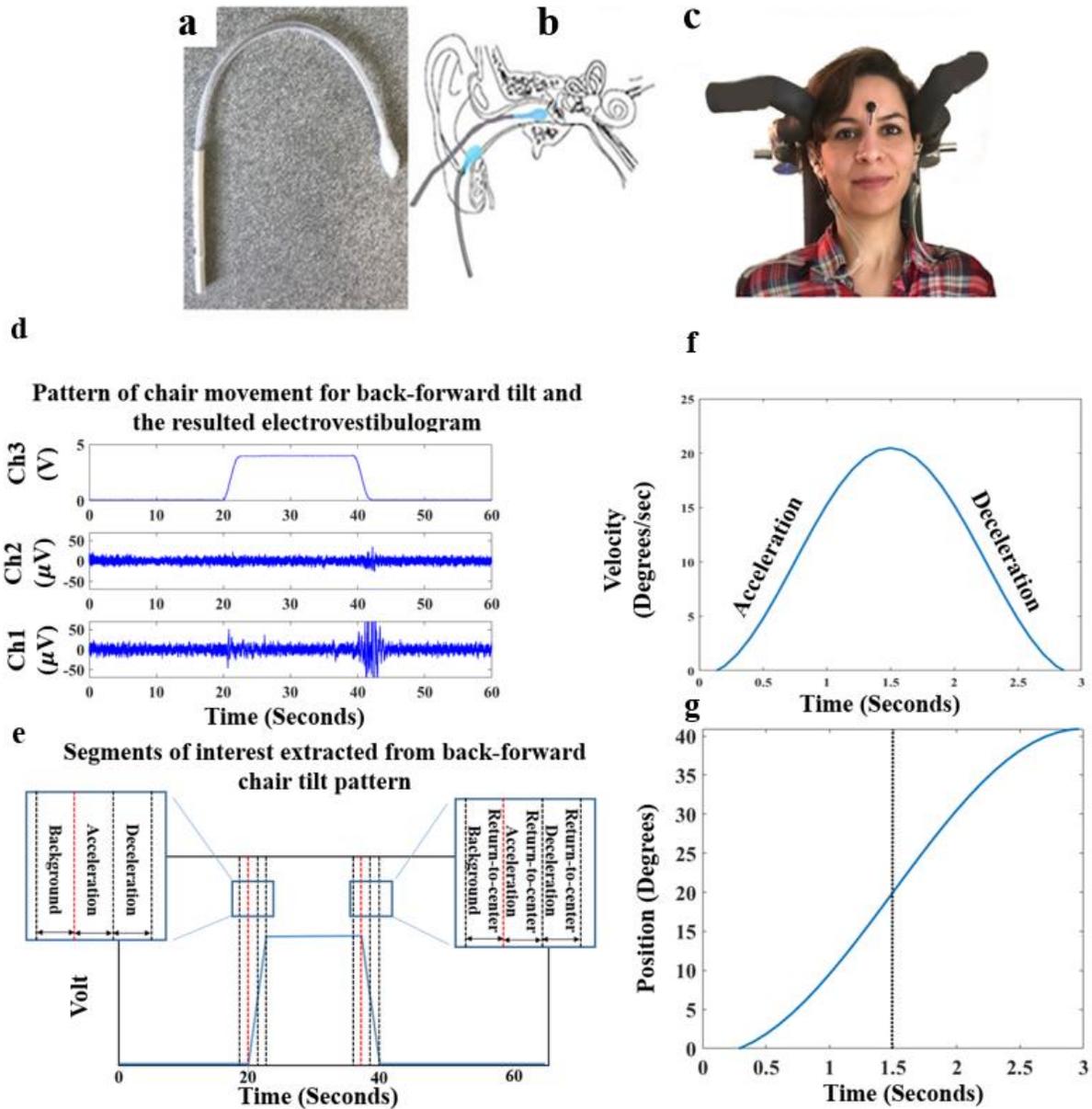


Figure 2-1: (a) Ear electrode; (b) electrode placement; (c) subject connections (This is a photo of author MA produced with her consent); (d) from top to bottom: the pattern of chair movement (Ch3), electrical signal recorded from the left ear (Ch2), electrical signal recorded from the right ear (Ch1); (e) Segments of interests with respect to the time for different tilts: background, acceleration, deceleration, return-to-the center background, return-to-the center acceleration and return-to-the center deceleration; (f) Chair velocity profile during motion; (g) Chair position [8].

### 2.4.2 Visual stimulus

An immersive VR environment, mimicking the EVestG recording chair, chamber and its associated tilts, was designed using the Unity Game Engine (version 5.3.2f1) software. Exposure to the VR environment was via a head-mounted display (Oculus Rift, Development Kit 2) connected to a battery-powered laptop (EUROCOM Sky X4, NVIDIA GTX 970 M, G-Sync Technology). Participants were immersed in the designed VR by pressing a push-button placed on the armrest of the hydraulic chair. The duration and the speed of the VR tilt movements were the same as those of the physical chair.

### 2.4.3 Feature selection

A whole-body tilt can evoke electrical activity in individual hair cells in the vestibular periphery [16]. The extracellular potentials (which include hair cells, 8<sup>th</sup> cranial nerve, and brainstem components) produced by almost simultaneous stimulation of a group of afferents together are referred to as a “field potential” (FP). FPs are recorded noninvasively via an active electrode located near the eardrum. These FPs can be averaged and used as a diagnostic feature to identify abnormalities in the observed waveforms when compared to those of healthy controls, and also to differentiate between background (lack of stimulation) and driven responses. The Neural Event Extraction Routine (NEER) [8] algorithm was used to extract FPs buried in noise from the recorded EVestG signals of each ear. The NEER algorithm uses a notch filter (to remove the power line harmonic noise), low and high pass filters [to minimize the effect of muscle artifacts (300 Hz HPF) and to remove the frequency spectrum above which neural activity is deemed relevant (4500 Hz LPF)], hydraulic jitter frequency noise (980 Hz notch filter), as well as a matched filter (to compare field potentials with a pre-recorded average template field potential). In NEER algorithm, the extracted FPs are averaged to minimize any remaining random artifacts and to form an average FP waveform or occurrence interval histogram (refer to Ref. [8] for more details on preprocessing stage of the NEER algorithm). It has been shown that the NEER algorithm can detect both spontaneous and driven field potentials of the vestibular system for different signal-to-noise ratios (down to  $< -24$  dB SNR) [8]. A typical extracted average FP of a control subject is shown in Fig. 2-2a. The distance from the zero amplitude to the minimum point of the FP curve is called action potential (AP) amplitude (Fig. 2-2a). To calculate the AP-area (red area in Fig. 2-2a), the two sample numbers corresponding to the points where the FP curve crosses the zero amplitude

immediately before and after the AP point are extracted. Next, the absolute value of the sum of each sample point amplitude on the curve between these two points was calculated as the AP area. The pre and post-potential peaks (Fig. 2-2a) are predominantly originated from changes in the vestibular peripheral activity, whilst the pre and post-potential troughs are predominantly the combined responses from the brainstem and vestibular periphery [17]. The area under different regions of the average FP, associated with the AP-area as well as the post-potential peak and post-potential trough areas, are used as characteristic features to compare different segments for the applied physical and virtual stimuli. Another useful output from the NEER algorithm is the time interval between the FPs firing, from which a '33-interval histogram (IH33) of FPs' occurrence times is extracted to produce a characteristic curve (Fig. 2-2c) [12]. Experimentally, the time interval between each two detected FPs by the NEER algorithm is approximately 3.3 ms. To generate an IH33 plot, the gap between every 33 detected FPs is calculated (~ 100 ms) (Fig. 2-2b) and the corresponding histogram is generated (Fig. 2-2c). This IH33 curve has been postulated to represent the modulation of afferent firing (by EEG activity possibly a band, 8–12 Hz or the Efferent Vestibular System activity [8, 12]). The average FP shape along with the IH33 timing curve will give us insight into the vestibular afferent and arguably the efferent activity [8, 12]. We will consider the mean of the IH33 curve as another characteristic feature for all tilts' analysis given the histograms are not skewed. In addition, we also examined the dependence of the results on eyes being open in the virtual tilt versus closed in the physical tilt. For this analysis, for each participant and stimulus (physical or virtual), the acceleration and deceleration segments were normalized relative to their own background signals by dividing by the background segment AP amplitude. Next, the difference between the FP amplitude of the acceleration and deceleration segments was calculated for each condition and changes were depicted in a form of FP response-change-curve. The reason to choose acceleration minus deceleration for plotting is that the EVestG response has been argued to be predominantly otolithic [9].

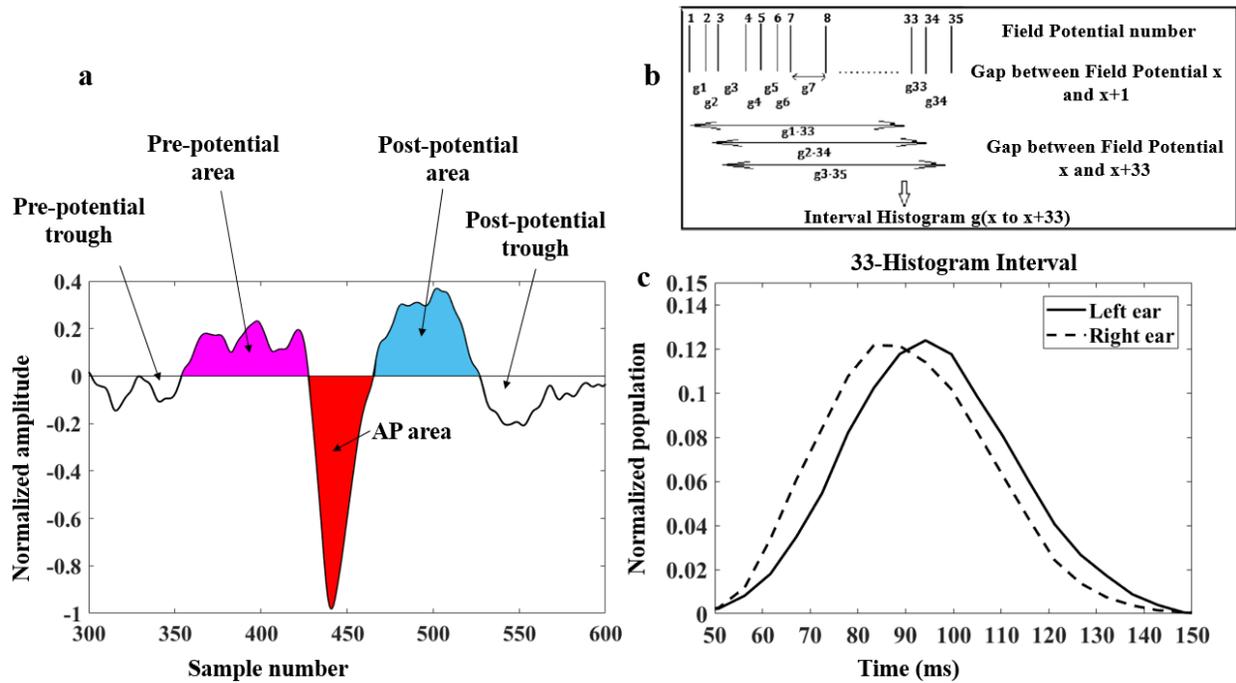


Figure 2-2: (a) A typical average field potential (FP) of a control subject extracted from NEER algorithm. (b) 33-interval histogram generation process [12], (c) Typical 33-interval histogram plots for a control subject obtained with closed-eyes.

#### 2.4.4 Statistical analysis

The Shapiro–Wilk test was used to check the normality of the features. Considering the normality of the data, two statistical tests were used: (1) paired sample  $t$ -tests were used to compare dynamic segments of interest with their Background segment as we hypothesized that the dynamic segments would generate a different vestibular response from the Background segment, and (2) Multi-Factorial ANOVA and post hoc tests were conducted to investigate the interaction between ears (left and right), segments of interest (Background, Acceleration, Deceleration, RTC-Background, RTC-Acceleration, RTC-Deceleration) and type of stimuli (physical vs. virtual), and to make comparisons between each pair of physical and virtual segments respectively; the hypothesis was that physical segments would generate a different response from the corresponding virtual segments. In all instances,  $p < 0.05$  was considered as significant. Bonferroni correction was applied to counteract the problem of multiple comparisons [18].

## 2.5 Results

To investigate the plausible differences between static and dynamic segments of each stimulus, all segments of interest were normalized to the AP amplitude of the background segment. Figure 2-3 compares the static background segment of physical tilt with the corresponding return-to-center (subject tilted as the start point) background (Fig. 2-3a), acceleration (Fig. 2-3b) and deceleration (Fig. 2-3c) segments. The same comparison was made between virtual stimulus segments (Fig. 2-3d-3f). For the physical stimulus, there is physiological evidence showing the otoliths do respond to maintained tilts [19]; this indicates we should get a difference between physically tilted (maintained tilt or during movement) and that of the upright stationary responses. When comparing the background with other segments for the physical tilt, FP changes can be observed in the AP-area as well as the pre and post-potential areas. However, for the virtual tilt, the FP of the background compared to the other segments only show small variations in these regions ( $p>0.05$ ). Table 2-1 shows all changes (%) in the areas of difference compared to that in the background. As expected, the afferent vestibular system is more strongly affected by the physical stimulus, and minimally by the visual stimulus.

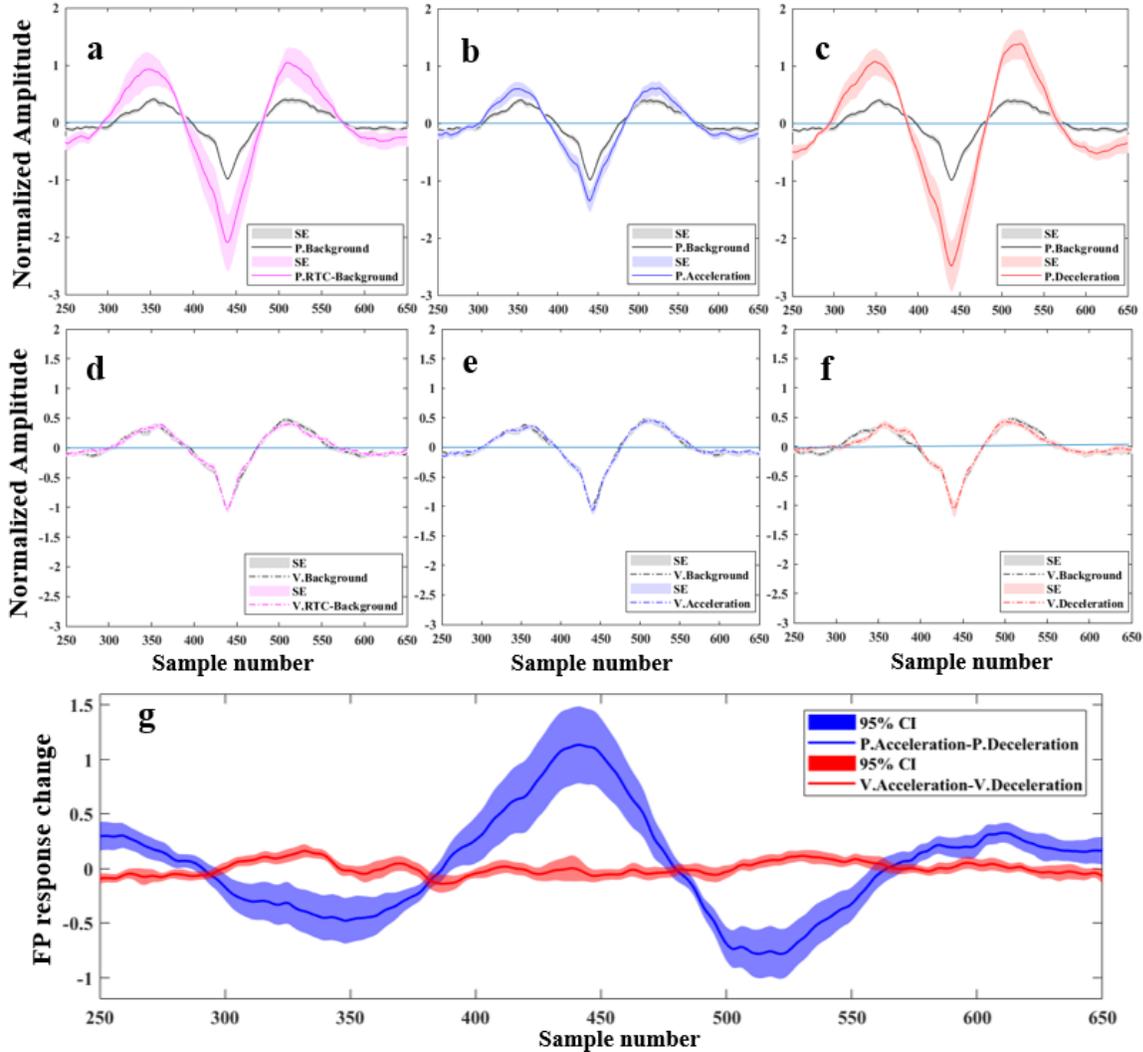


Figure 2-3: Top figures from left to right: comparison (N=27) (normalized to upright background) between physical upright background and the corresponding tilted vestibular responses; a: return-to-center background, b: acceleration, and c: deceleration. Middle figures from left to right: comparison (normalized to upright background) between virtual upright background and the corresponding virtual segments; d: return-to-center (RTC) background, e: acceleration, and f: deceleration). Afferent vestibular modulation is minimal for the virtual compared to physical stimulus. The bottom figure shows the FP response change (acceleration minus deceleration) curves for virtual and physical stimuli (g). The change was calculated by subtracting the FP amplitude of the deceleration segment from the amplitude of the acceleration segment which both had been normalized to the corresponding background segment. The areas under FP-response-change curve (acceleration-deceleration) associated with AP, post-potential peak and trough width showed significant differences ( $p < 0.015$ ) between physical and virtual stimuli in the left ear which was largest in the AP region (in the right ear, only AP-area region showed a significant difference ( $p = 0.027$ )). This can be indicative of the involvement of different pathways for processing physical versus virtual information. In the legend P stands for physical stimulus, V stands for virtual stimulus, SE is the standard error and CI is the 0.95% confidence interval.

**Table 2-1: Changes in the AP-area of different segments compared to the background segment. AP-area changes are widespread for the physical stimulus but not for the virtual stimulus.**

$$\text{Change compared to BGi} = \frac{\text{AP area of the segment of interest} - \text{AP area of BGi}}{\text{AP area of BGi}} \times 100$$

<i>Segments of Interest</i>	<i>Right ear</i>		<i>Left ear</i>	
	<i>Physical</i>	<i>Virtual</i>	<i>Physical</i>	<i>Virtual</i>
<i>Changes compared to Background (%)</i>				
<i>Acceleration</i>	94.36	-2.27	79.19	3.86
<i>Deceleration</i>	230.61*	-13.86	255.34*	-6.03
<i>RTC-Background</i>	107.72*	-16.10	185.22	-7.40
<i>RTC-Acceleration</i>	113.57*	-15.74	188.56	-5.95
<i>RTC-Deceleration</i>	159.53*	-14.40	252.24	-13.95

\* Comparison is significant at 0.01 ( $\alpha_{critical} = 1 - (1 - 0.05)^{1/5} = 0.010$ ) level.

To compare the signals between the physical (eyes-closed) and virtual stimuli (eyes-open), first, we normalized the FP curve to the magnitude of the AP amplitude (it generates an AP amplitude of -1 for all segments). The normalization was performed to eliminate the individual effect of different skin impedances and electrode placements between the individuals. Once normalized, the AP-area of each segment (except the background) during physical stimulus was found wider than the corresponding segment during the virtual tilt (Fig. 2-4a).

We conducted a 3-way repeated measure ANOVA to investigate the combined effect of side (ears) (left and right), stimuli (physical and virtual), and segments of interest (Background, Acceleration, Deceleration, RTC-Background, RTC-Acceleration, RTC-Deceleration) on the vestibular response. After removing the insignificant interactions (Ears\*Stimuli\*Segments (p-value=0.472), Ears\*Stimuli (p-value=0.379), Ears\*Segments (p-value=0.303)), as well as the main effect of ear (p-value=0.372)), we corrected the model in SPSS and reran the analysis. The interaction between Stimuli\* Segments was found to be significant with p-value<0.001, partial eta squared=0.195, observed power=0.988. Given that, we combined left and right ear data and performed a pair-sample t-test to make a comparison between the vestibular response to physical and virtual stimuli for each segment of interest. The Bonferroni correction was used to make multiple comparisons in

post hoc analysis (p-values listed in Table 2-2). Only Deceleration and RTC-Deceleration segments were significantly different between physical and virtual segments.

**Table 2-2: Mean difference between physical and virtual segments and the corresponding p-value for the Normalised AP-area.**

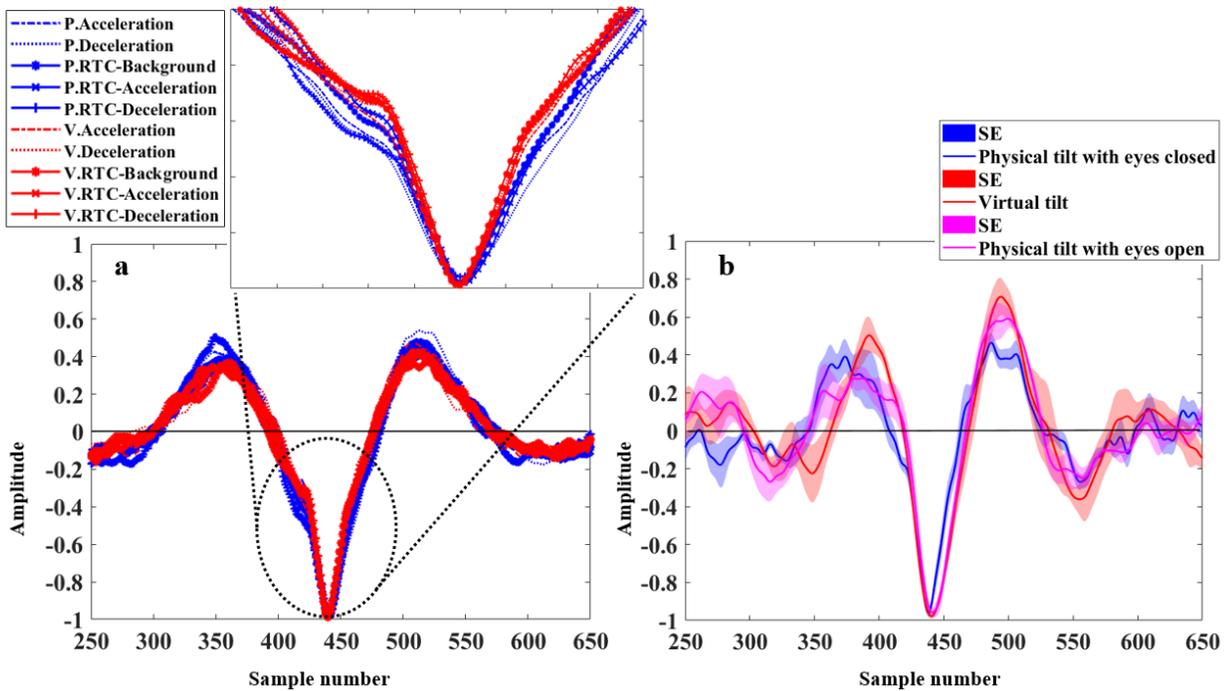
	<i>Segment of interest</i>	<i>Mean Difference ± SE</i>	<i>P-Value</i>
<b>AP Area</b>	P.Background-V.Background	-2.15 ± 1.6	0.185
	P.Acceleration-V.Acceleration	2.67 ± 1.46	0.073
	P.Deceleration-V.Deceleration	8.03 ± 1.76	0.000*
	P.RTC.Background-V.RTC.Background	2.58 ± 1.69	0.133
	P.RTC.Acceleration-V.RTC.Acceleration	2.87 ± 1.66	0.090
	P.RTC.Deceleration-V.RTC.Deceleration	5.01 ± 1.75	0.006*

\* Comparison is significant at 0.008 ( $\alpha_{critical} = 1 - (1 - 0.05)^{1/6} = 0.008$ ) level

Figure 2-4b also shows a comparison between the normalized AP-area of three different stimuli (physical tilt with eyes-open, physical tilt with eyes-closed, and virtual tilt). One female participant also underwent all different physical tilts of the EVestG (up-down, rotation, back-forward, ipsilateral and contralateral side tilts) with eyes-open. The average of five segments at the beginning of each tilt (seconds 5.5 to 7) was used for comparison of the eyes-open versus eyes-closed. This phase of the recording was chosen to restrict neuromodulation consequent to anxiety or stress feelings immediately prior to the chair movement affecting the signal [20]. As can be seen in Fig. 2-4b, the average FP of the virtual and physical stimuli with eyes-open are similar in the AP-area and post-potential area but quite different from the physical tilt with eyes-closed. Thus, it appears much of the difference seen between physical and virtual response is related to eyes being closed versus open in this particular experiment.

For the IH33 mean value feature, the eyes-closed vestibular response compared to the eyes-open virtual stimulus resulted in a larger IH33 mean value (Fig. 2-5a), i.e. IH33 was generally shifted to the right of the time axis compared to that of the virtual stimuli; the trend across all segments are seen in Fig. 2-5a. However, after conducting a 3-way ANOVA, all 3-way and 2-way interactions and the main effects of Ear, Stimuli, and Segments of Interest were found to be non-significant. The IH33 curve results, shown in Fig. 2-5b (i.e. comparison of the effects of eyes-open, eyes-closed and virtual reality stimuli) are congruent with those shown in Fig. 2-5a; the

physical tilt with eyes-closed shows a shift away from the virtual and physical tilt (with eyes-open) stimuli towards the right side.



**Figure 2-4: (a) Comparison between the normalized average FP of physical tilt with eyes-closed and virtual stimuli for all segments of interest except the background. (b) Comparison of the normalized average FP for the physical tilt with eyes-closed, physical tilt with eyes-open, and virtual stimuli for the background segment. In the legend, P stands for physical chair movement, and V for virtual reality. Physical stimulus with eyes-closed resulted in wider AP-area than the virtual reality stimulus for all segment of interest except the Background. The opposite was observed for the Background segment (AP-area for Background segment of the physical stimulus with eyes-closed was narrower than virtual reality and physical stimulus) with eyes-open.**

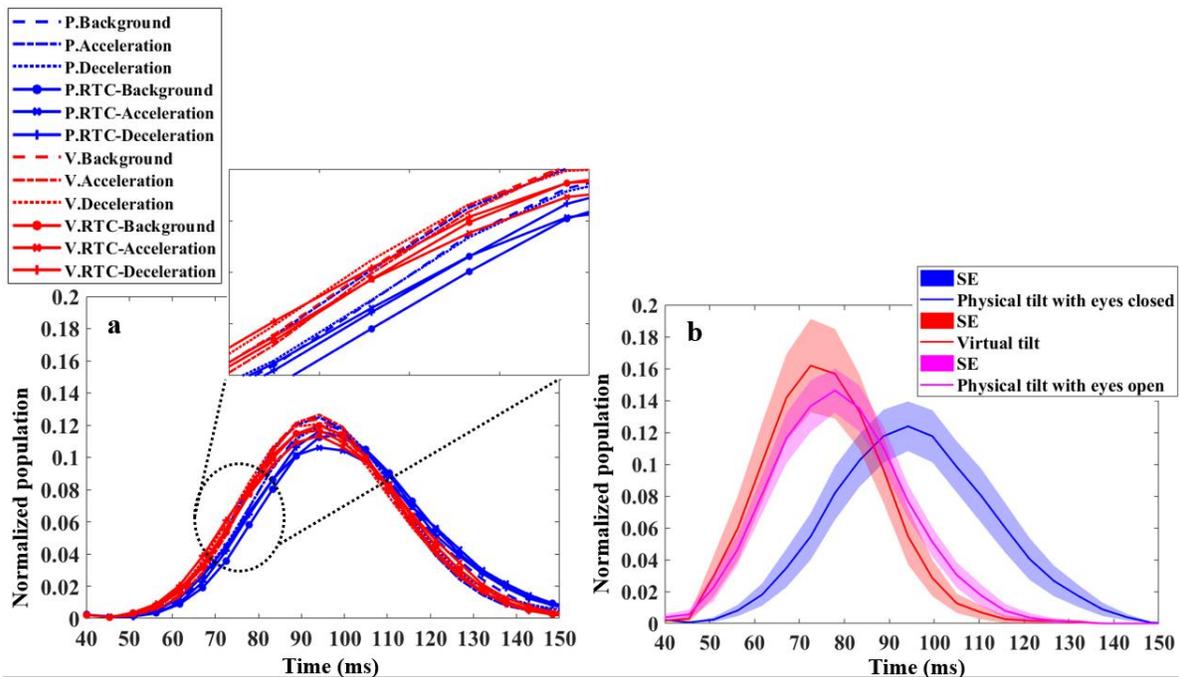


Figure 2-5: (a) Comparison between the average IH33 of the physical stimulus with eyes-closed and virtual stimulus for all segments of interests generally brought about no significant difference between the vestibular responses. (b) Comparison of the normalized average IH33 for the physical tilt with eyes-closed, physical tilt with eyes-open, and virtual stimuli for the background segment showed a significant difference between physical tilt with eyes-open and closed. As can be seen, generally, virtual reality and physical tilt with eyes-open resulted in a shorter firing pattern interval within the vestibular periphery (histogram shifts to the left).

It is worth mentioning that when comparing either the AP-area or IH33 mean as features, generally, no significant difference was found between left and right ear vestibular responses for either the physical tilt or virtual stimulus, implying there was no lateralization.

## 2.6 Discussion

The main goal of this study was to compare the EVestG-measured vestibular responses following the physical tilt and the corresponding virtual replica (visual only) of the physical tilt. Below is a summary of the findings followed by a detailed discussion for each result.

Overall, the results support the premise that the vestibular system responds to both physical and virtual stimuli, but that the pattern of response is different particularly in terms of average FP shape and size. Given the smaller pre-normalized FP size for the virtual stimulus compared to the physical stimulus, this is suggestive there are likely fewer afferents firing in a ‘quasi-synchronous manner’, resulting in a narrower spread of response.

We also investigated the effect of eyes-open or closed on background signals. A significant difference was found between the physical and virtual FP response change curve specifically in the AP-area region (Fig. 2-3g). This indicates the vestibular brain areas and pathways responsible for virtual versus physical vestibular information are not only different but the virtual input appears to have a different/limited/limiting modulatory effect on the activity of vestibular afferent nerves.

Once normalized (Fig. 2-4), the physical stimulus with eyes-closed produced a larger AP-area compared to the virtual stimulus for all segments of interest except background (Fig. 2-4a). In the upright stationary position, however, virtual stimulus and physical stimulus with eyes-open resulted in a larger AP-area compared to physical tilt with eyes-closed (Fig. 2-4b) indicating a prominent role of visual sensory inputs while stationary and vestibular sensory inputs while tilting. A central reweighting of the sensory modalities may lead to this observation. Furthermore, physical tilt stimuli with eyes-closed resulted in longer time intervals between the average afferent firings (Fig. 2-5) compared to their virtual and physical with eyes-open tilts counterparts although these differences were non-significant for the former comparison when using the IH33 population mean as the measurement feature.

### **2.6.1 Difference between spontaneous and driven EVestG signals**

In Fig. 2-3, the observed difference between background and return-to-center background as well as the acceleration and deceleration segments can be seen in the AP-area, pre- and post potential peak regions and pre- and post-potential troughs for the physical tilt. The post-potential trough part of the FP signal is mostly associated with both brainstem and peripheral vestibular activity. The pre- and post-potential peak (cf.  $N1_{RW}$  and  $P1_{RW}$  of the acoustically derived CAP [17]) can be linked to ionic transport of  $Na^+$  and or  $K^+$  across the afferent nerve membrane. The differences seen between background and return-to-center background segments for the physical stimulus indicates that even in the stationary position, peripheral vestibular and brainstem activity can change when the stimulus condition changes from stationary upright (background) to stationary tilted (return-to-center background) position [19]. However, for the virtual stimulus used in this study, these differences were small. This discrepancy might be explained as follows:

- 1) Pathway differences: for the physical stimulus, hair cells stimulate afferent vestibular fibers; however, in the virtual tilt, the efferent vestibular system modulates afferent spontaneous activity [21].
- 2) Magnitude and efficacy of the stimulus: If the efferent stimulation is not sufficiently large (or it is suppressive) an effective large afferent response is not obtained [22].
- 3) Excitatory and inhibitory inputs to VN: Visual-processing brain regions (e.g. the superior colliculus, vestibulocerebellum, and pretectum) send excitatory and inhibitory projections to the vestibular nuclei. In particular, the response of vestibular nucleus (VN) neurons to photopic stimuli (flashes of light with different intensity and duration) can be excitatory, inhibitory or a mixture of both [4]. From the left and right VN, which are connected via inhibitory commissural fibers, these responses reach vestibular efferents. Efferent vestibular responses can be excitatory or inhibitory although excitation has been reported to be the predominant response in mammals [23]. The vestibular periphery then receives bilateral efferent innervations. In our experiment, it seems the visually-driven vestibular system response is suppressive (which may be linked to a reduced efferent activity or more likely a reduction in the synchronous relationships formed around the efferent vestibular feedback loop). That is, the photopic (virtual) stimulus appears to reduce the magnitude of the FP (a measure of the almost 'synchronous' firing of small afferent groups) (Fig 2-3) but increases the number of (perhaps smaller) detected FPs (perhaps decreases the time between detected FPs) (Fig. 2-5).

Figure 2-3g demonstrates that physical and virtual stimuli are different in terms of the generated FP response when the background signal size is taken into account by normalizing the acceleration and deceleration segments to their relative background signals.

In the case of the physical chair tilt, the speed and magnitude of the chair during acceleration and deceleration results in an EVestG response, which is different to the background signal (see Table. 2-1 to see AP-area changes for different segments of interest). Correspondingly, the spatial and temporal changes in the visual field of view during visual stimulus can make the acceleration and deceleration segments look only marginally different from its background signal.

## 2.6.2 AP-area

There are standard error differences between the FP shape for physical and virtual stimuli in most regions of the average FP (see Figs. 2-2a and 2-4). Pre and post-potential peaks are associated with the depolarization and repolarization processes of the vestibular afferents. Therefore, the narrower AP-area following the virtual stimulus (for all segments of interest except the background) may be suggestive of 1) a higher rate of  $Na^+$  influx and  $K^+$  efflux within the afferent pathway system or 2) and more likely, there are fewer afferents ‘synchronously’ firing leading to a narrower spread of response.

In addition, the regions of the average FP before the pre-potential peak and after the post-potential peak referred to as pre-potential trough and post-potential trough, respectively, also show some dissimilarity for the physical and virtual stimuli. Pre-potential and post-potential troughs are assumed to correspond predominantly to the combined peripheral and brainstem (predominantly VN) activity. These two may reflect differences related to the population of the ‘synchronous’ responses recruited to form each FP. The hair cells are minimally driven for visual only stimulus. However, due to the positive feedback between efferent and afferent vestibular pathways, the efferent system can provide a synaptic interface with the calyces of type I hair cells that can potentially modulate spontaneous afferent activity [24]. These activities may modulate the spontaneously generated small narrow FPs known to occur [8, 21].

Another alternative and/or parallel explanation for the observed differences between AP-area of physical and virtual stimuli can be linked to the conflict between visual and vestibular information while the participant is exposed to the virtual stimulus. While applying a visual stimulus, a mismatch between sensory inputs can be induced to the participants as they are “in reality” in the stationary position, but do receive motion indicator signals from the visual system. This sensory conflict may modulate the action potential threshold of vestibular fibers, which then receive an altered (perhaps larger) ratio of visual to vestibular information. As a result, an increased number of smaller FPs may be produced and this may be a consequence of a loss of ‘synchronous’ firing across the afferents (smaller detected AP amplitude).

Besides, the existence of different short (such as retinotectal, retinocollicular) and long (retinocortical) visual pathways that are terminated at the VN may introduce a difference in the

arrival time of electrical potentials at VN; this too may result in a change in the synchronicity firing of populations of vestibular afferents.

The background segment response was different compared to other segments of interest having wider AP-area for the virtual compared to physical tilt with eyes-closed (not shown in Fig. 2-4a). In addition, we observed that the vestibular response to physical stimulus while eyes-open looks more like the virtual response (Fig. 2-4b). According to the literature, under constant velocity, visual inputs precede vestibular information and provide more reliable signals to the brain as the cupula in the semicircular canals and otolith organs return to their resting position [1]. However, during physical stimuli (since the stimuli consist of acceleration and deceleration parts with either eyes-closed or open) the vestibular system plays a more dominant role relative to the visual component in sending reliable sensory signals to the brain [25]. That may be why the vestibular response during stationary condition with eyes-open and virtual stimulus (backgrounds) are wider compared to that of physical tilt with eyes-closed.

### **2.6.3 IH33 mean**

The IH33 interval histogram plot indicates that eyes-open condition (either the virtual stimulus or physical tilt with eyes-open) brings about generally shorter time intervals between detected vestibular FPs compared to a physical stimulus with eyes-closed. This can happen because there is possibly more frequent but less effective efferent activity modulating the afferents (Fig. 2-5).

The presence or absence of light causes photoreceptors to decrease or increase glutamate release. These chemical changes successive to the hyperpolarization or depolarization phases of the photoreceptor are translated into temporal information, i.e. different frequencies at the ganglion cell level [26]. Due to the existence of a positive feedback loop from VN to vestibular efferent to vestibular afferent, a small change in efferent input can potentially induce a change in afferent activity resulting in shorter time intervals between detected vestibular FPs for the virtual tilt (perhaps there is more frequent irregular or simply less frequent efferent activity modulating the afferent) compared to the physical stimulus (Fig. 2-5). An increase in background afferent discharge following electrical stimulation of efferent has been reported in vertebrates [23].

Another reason for having shorter time intervals between detected FPs during open eye condition (either the virtual stimulus or physical tilt with eyes-open) compared to the physical stimulus with eyes-closed can be linked to the existence of short or long visual pathways terminating in vestibular processing brain regions. The superior colliculus and primary visual cortex have a saliency and visual response latency of 65 ms and 40 ms [27]. Other higher-order cortical visual areas can have a response latency of 100 ms or longer. Given the phase locking of irregular vestibular afferents modulated by efferents [28], the time difference between the produced responses from these structures may be linked to the shorter time intervals between efferent firings of virtual/physical tilt with eyes-open compared to the physical stimulus with eyes-closed.

Comparing the IH33 of the background segments of three stimuli (physical stimulus with eyes-open and closed as well as virtual tilt) in Fig. 2-5b, results are congruent with the study described in [29], wherein the power of brain waves (including  $\alpha$ ,  $\beta$ , and  $\delta$ ) was investigated during eyes-open and eyes-closed condition using electrocorticogram. Based on these studies, the eyes-open condition results in increased low-frequency power in both hemispheres. In addition, the inhibitory role of  $\alpha$  band brain wave has been established in several studies including [30]. Accordingly, in this study, the shift of the IH33 curve to the right side of the time axis while eyes-closed indicates longer time intervals between vestibular efferent firing.

Our data show that both virtual and physical stimuli can generate a vestibular response different from each other. The eyes-closed physical, compared to virtual evoked vestibular responses, indicate the primary involvement of peripheral versus central/cortical brain regions in modulating vestibular activity in this particular experiment. In addition, the longer time interval between afferent vestibular field potentials for the physical stimuli with eyes-closed compared to visual stimuli is suggestive of differing levels of vestibular afferent modulation i.e. potentially a modulation from either efferent vestibular or other brain wave activities. The advantage of using virtual reality stimulus over physical stimulus for EVestG recordings can be summarized as follows. The current and original EVestG recording requires sitting in a hydraulic chair in an electromagnetically shielded and soundproof chamber. By using visual stimuli and developing shielded headphones (incorporating the recording equipment within the headphones), the hydraulic chair and/or anechoic chamber may no longer be needed. Thus, EVestG recordings can be performed in any quiet room environment and the recording system can become portable. As

most clinical vestibular tests require a tilting chair that is expensive and generally only available in hospitals or specialty centers in major cities, the major advantage of such portable virtual vestibular testing is that it can be available in local and small clinical centers. Furthermore, this study investigates the functional connections and interactions between vestibular and visual systems. In the future, a variety of visual stimuli can be used to test the visio-vestibular connections in normal and patient populations with primary vestibular (e.g. benign paroxysmal positional vertigo, vestibular neuritis) and other vestibular-related diseases (e.g. Parkinson's disease, stroke). We hope that this method will be helpful in the diagnosis of the patients and help ophthalmologists, neurologists, neuro-ophthalmologists, otoneurologists, and otolaryngologists to accurately diagnose patients and prescribe the best rehabilitation strategy for each patient.

Moreover, the results of this study appear to provide leads to help explain the similarity between real-world motion sickness and virtual reality sickness through the sensory mismatch theory. This study raises the following future research questions: 1) can abnormal processing of visual information cause dizziness/vertigo? 2) what environmental visual conditions can change the vestibular/visual sensory weighting? 3) how can light sensitivity intensify the symptoms of migraines?

### **Conflicts of Interest**

All authors declare no conflicts of interest and disclose no affiliations with or involvement in any organization or entity that could potentially bias the subject matter or materials discussed in this manuscript. Figure 2-1c is the author (MA) who has agreed to its publication.

### **Acknowledgment**

This study was partly supported by the Natural science and engineering research council (NSERC) of Canada.

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## Supplementary Materials 1 (S1)- Vestibular Response to Other Types of Chair Tilt

The original EVestG recording measures vestibular responses to passive head tilts produced in other axes rather than the pitch axis as well. These include tilts in yaw (rotation), roll (side tilt), and up-down movement. In this section, the results of other chair tilts are briefly discussed. Note that the procedure of the EVestG recording remains the same as presented in Chapter 2 (the same sample of twenty-seven healthy participants). The only difference is for the side tilt which takes 2 minutes to be completed (side tilt was conducted bilaterally: ipsilateral (60 s) and contralateral (60 s)). Refer to Fig. S1-1 for the pattern of the chair movement for each specific tilt.

For the purpose of analysis and to better observe the difference between each tilt, for each participant and stimulus (physical or virtual), the acceleration and deceleration segments were normalized relative to their own background signals by dividing by the background segment AP amplitude. We will consider the mean of the IH33 curve as the second characteristic feature as described in Chapter 1.

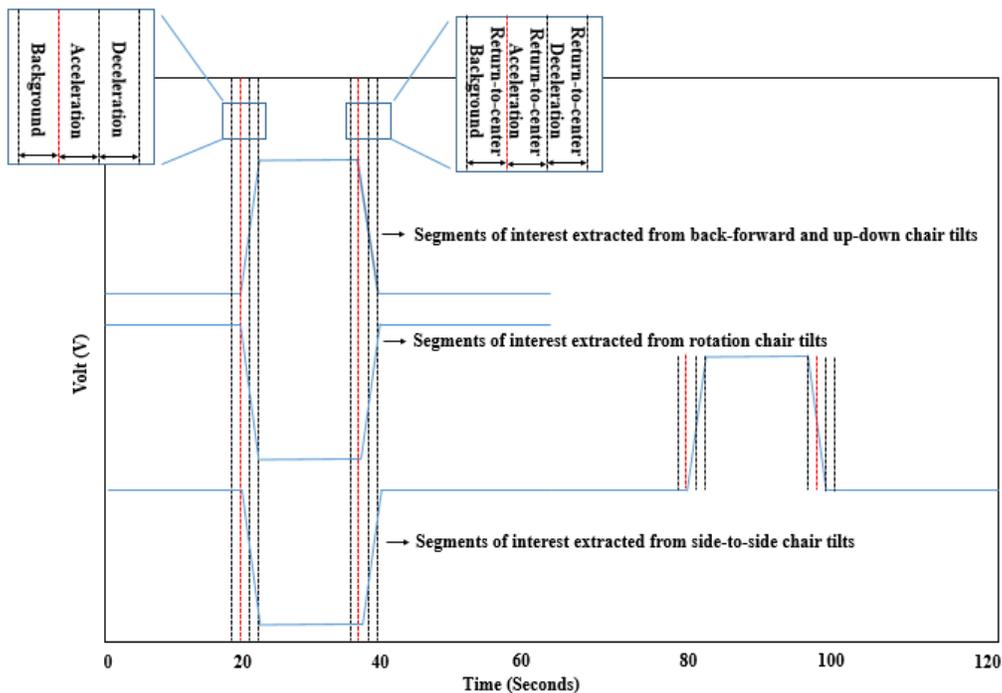


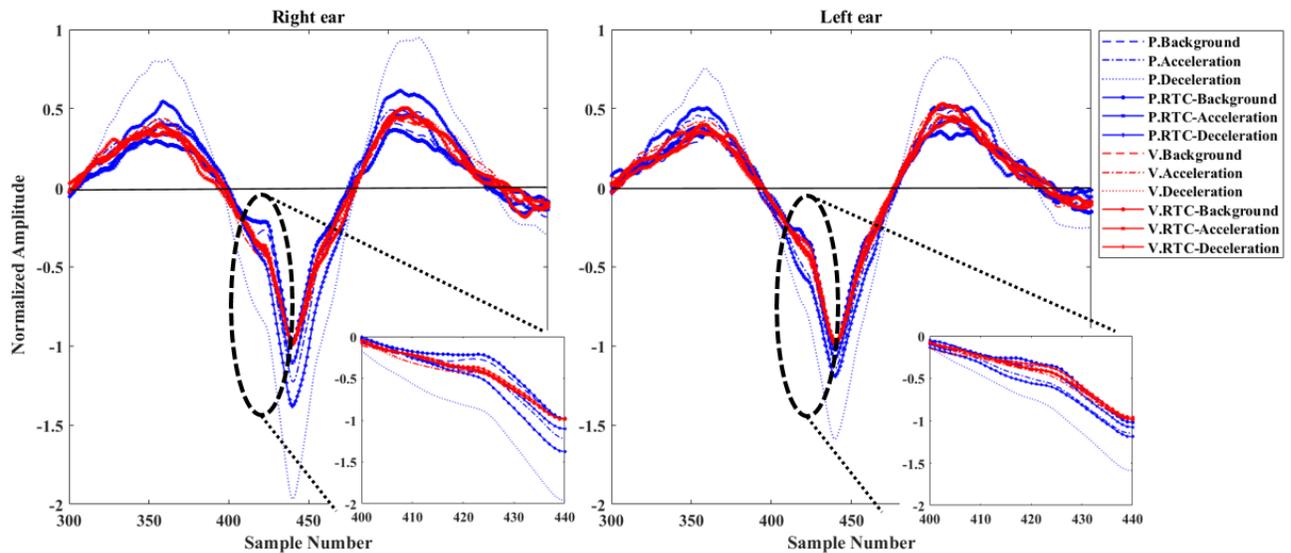
Figure S1-1: The pattern of the chair movement for different tilts generated by the hydraulic chair.

## S1.1 Results obtained from other types of chair movement

The results obtained from the three other types of chair movement (yaw, roll, up-down) are presented in the following sub-sections.

### S1.1.1 Vestibular Responses to Physical Versus Virtual Yaw Motion (Rotation)

For each AP area and IH33 mean feature, a three-way repeated measure ANOVA analysis was conducted. The main effect of three independent variables (ears, stimuli, and segments), and the two-way and three-way interactions between those variables were taken into account for analysis. Considering the AP area feature after normalizing to the Background segment (Fig. S1-2), the two- and three-way interaction of ear was not significant whilst the main effect of segments ( $P=0.028$ ) was statistically significant. Hence, we combined the left and right ear responses to make a comparison between virtual and physical stimuli. Results show no significant difference between the virtual and the corresponding physical segments.



**Figure S1-2: Average FP of all participants. In the legend, P stands for physical and V stands for virtual stimulus.**

Figure S1-3 shows the average IH33 data across all participants for physical (P) and virtual (V) segments. As can be seen in the left ear, the IH33 curve has shifted to the left side of the time axis for the virtual stimulus indicating shorter time intervals between efferent firings (modulating

afferent activity). For the IH33 mean feature, in addition to segments ( $P=0.001$ ), a statistically significant difference was observed between physical and virtual stimuli ( $P=0.014$ ).

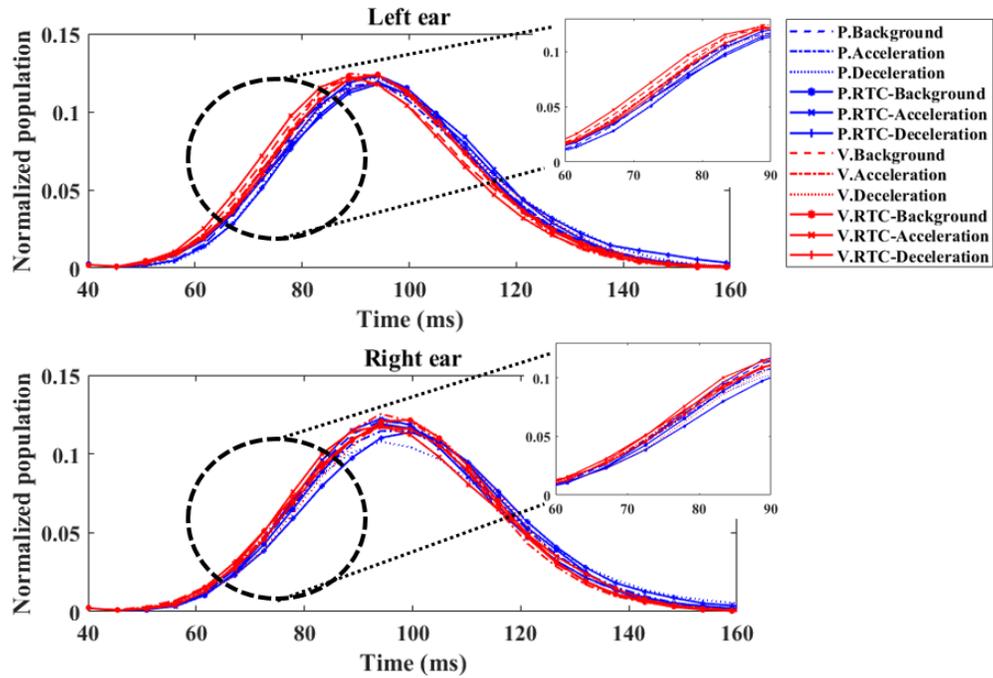


Figure S1-3: Average IH33 of all participants. In the legend, P stands for physical and V stands for virtual stimulus.

### S1.1.2 Vestibular Responses to Physical Versus Virtual Roll Motion (Side Tilt)

For each ipsilateral and contralateral side tilt, six segments were extracted to be analyzed and the AP area, as well as IH33 mean, were calculated. After accounting for differences between eyes open and eyes closed responses, once normalized to the Background segment, the data show that exposure to the physical stimulus generated larger average FPs compared to the virtual stimulus for the ipsilateral right ear and the contralateral left ear for all segments of interest (Fig. S1-4 and S1-5). Once normalized to the Background segment, physical stimuli were significantly different ( $P<0.001$ ) from the virtual stimuli for ipsilateral and contralateral side tilt. No significant difference was detected when the average FPs were normalized to their AP amplitudes. Considering the IH33 mean feature, no significant difference was observed between actual and virtual stimuli for either direction of side tilt (Fig. S1-6 and S1-7).

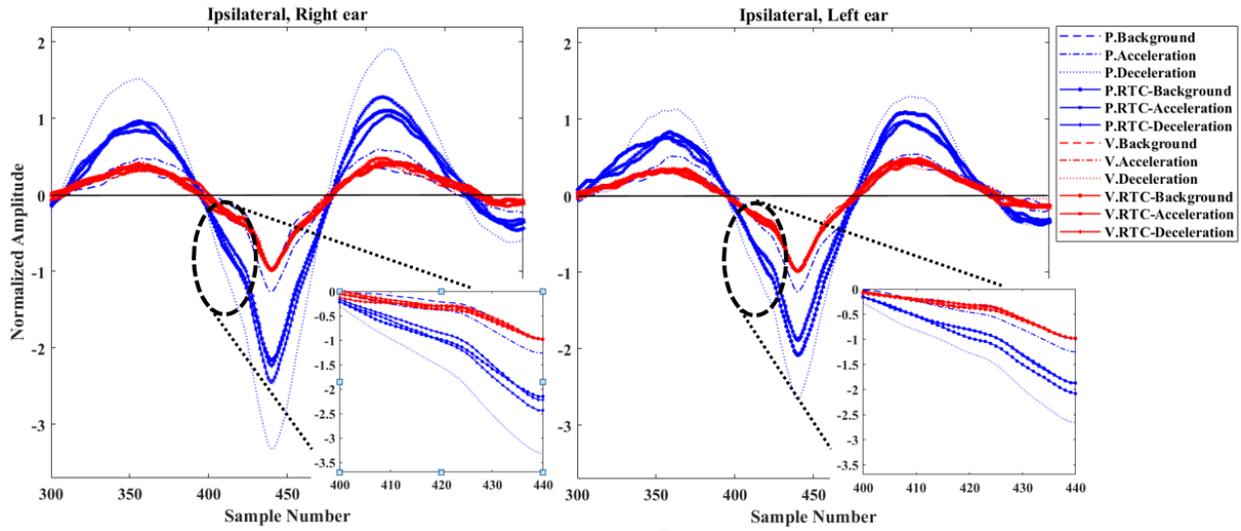


Figure S1-4: Average field potential of different segments of interest resulted from ipsilateral physical and virtual stimuli.

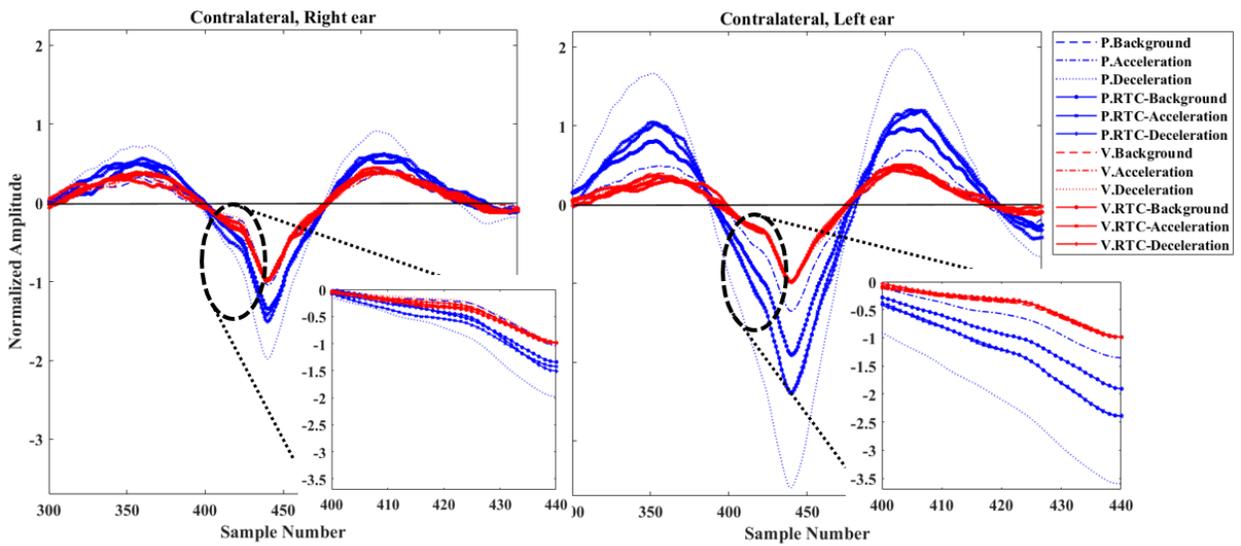


Figure S1-5: Average field potential of different segments of interest resulted from contralateral physical and virtual stimuli.

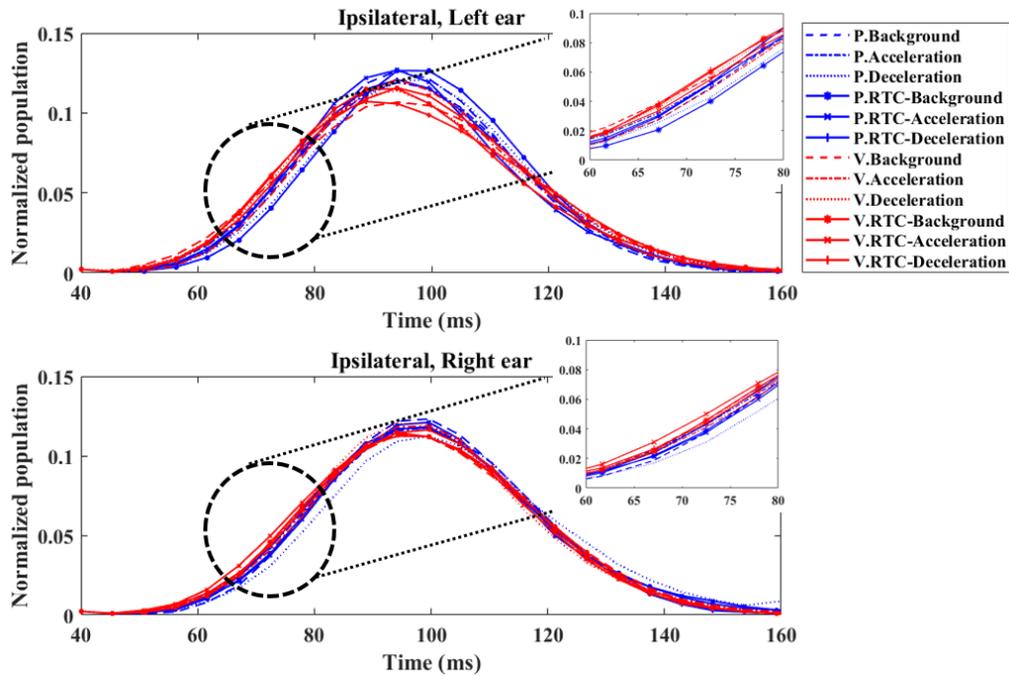


Figure S1-6: Average 33-interval histogram of different segments of interest resulted from ipsilateral physical and virtual stimuli.

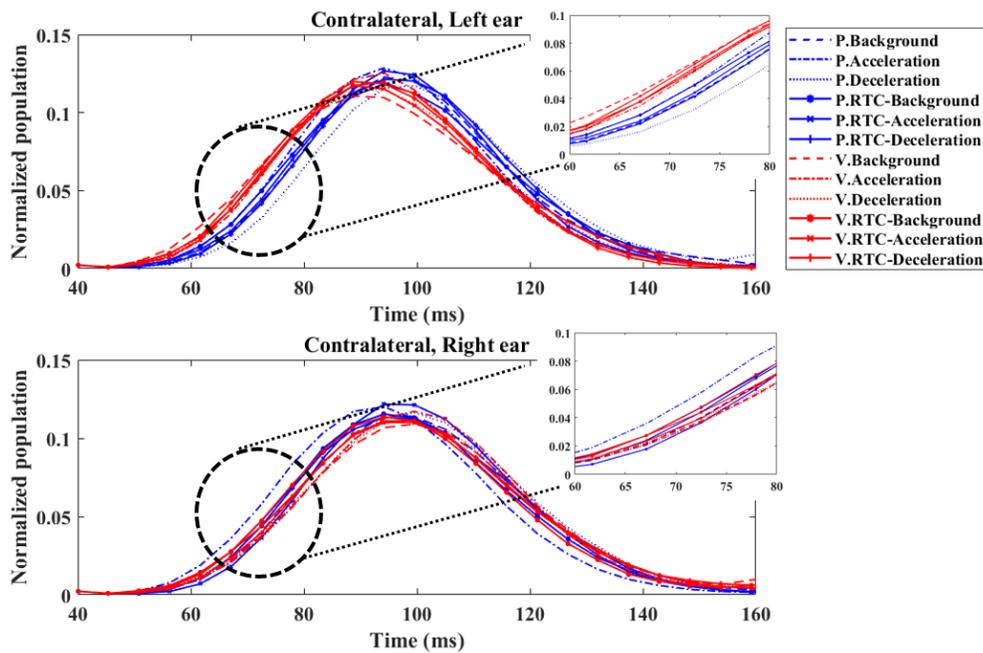


Figure S1-7 Average 33-interval histogram of different segments of interest resulted from ipsilateral physical and virtual stimuli.

### S1.1.3 Vestibular Responses to Physical Versus Virtual Up-Down Motion

In regards to average FP (Fig. S1-8), the vestibular system generally produced a larger response for the physical compared to that of the virtual stimulus (the only exceptions were Background and RTC-Background in the right ear). However, these differences were not significant when the average FPs were normalized to their AP amplitude. Once normalized to the Background segments, all two- and three-way interactions of the ear, stimuli and segments became insignificant. Therefore we combined left and right ear responses and reran the statistical test. A marginally significant difference was found between the physical and virtual stimuli ( $P=0.053$ ). Likewise, considering the second feature (IH33 mean) (Fig. S1-9), the difference between the two types of stimuli was found to be nonsignificant even after removing the nonsignificant interactions and main effects.

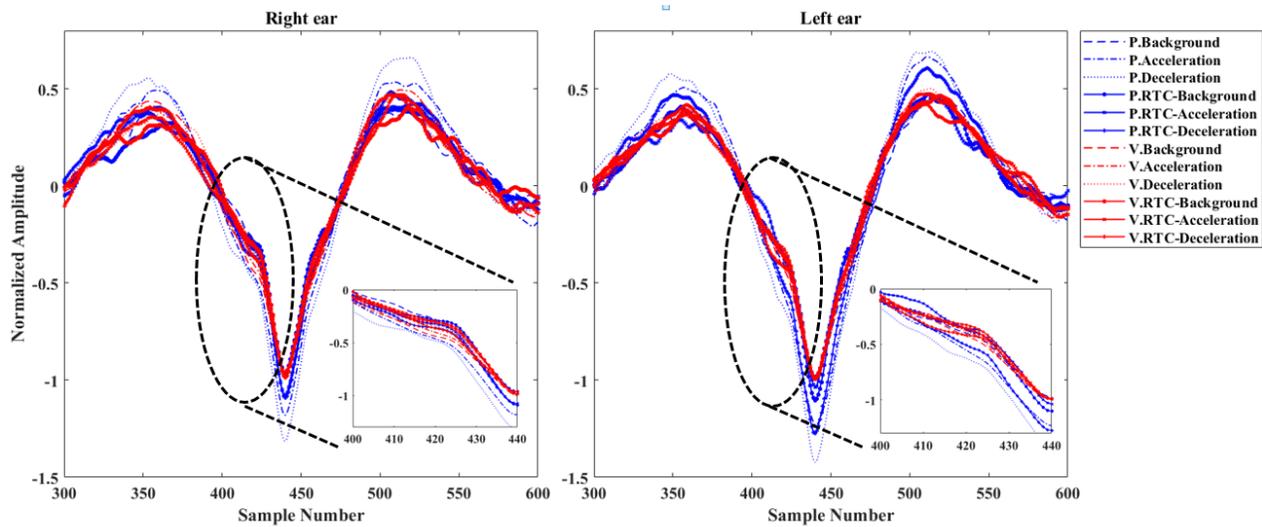


Figure S1-8: Average FP of different segments of interest for the physical versus virtual stimulus.

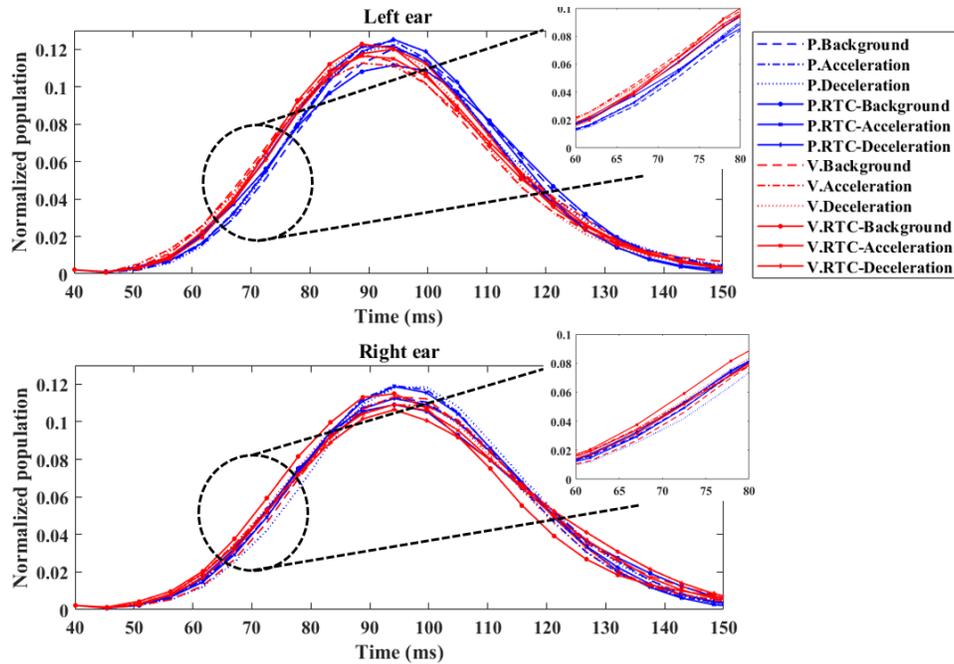


Figure S1-9: Average discharge time between efferent-mediated afferent firings for different segments of the physical versus virtual stimulus.

## S1.2 Discussion on different types of chair movement

The above results for the three different types of chair movement are discussed in detail in separate sub-sections.

### S1.2.1 Rotation tilt

Cortical (e.g. insular cortex) and subcortical (e.g. vestibular nuclei) vestibular brain regions are multisensory and respond to both visual and physical stimuli (head or body movement) [1]. This multisensory network can affect spontaneous efferent and afferent vestibular activities through reciprocal visio-vestibular pathways. As mentioned in the Discussion section of Chapter 2, the slope of the downward and upward lines forming the AP area, as shown in Fig. S1-2, represents sodium ion influx and potassium ion efflux across the afferent nerve membrane. We found no significant difference between the AP area of physical and virtual stimuli indicating that the ionic basis for neural activity within the afferent vestibular fibers in response to physical and virtual stimuli is similar for rotational movement. However, a trend can be seen in Fig. S1-2 showing the difference between visual and physical stimuli seen for the AP area is more discernable in the right ear. The reason can be explained as follows. When the chair moves to the right in the yaw axis,

the vestibulo-ocular reflex generates an eye movement towards the left. This results in excitation of contralateral (to the chair movement) extraocular motor nuclei (which in turn have connections to the contralateral vestibular nuclei), and ipsilateral semicircular canals. For this reason and also because of vestibular dominance (all participants are right-handed) the absolute value of the difference between the AP area of physical and virtual segments is larger in the right ear compared to the left ear.

Based on the explanation above, the difference observed between the efferent vestibular activity of the physical and virtual stimuli should be, and is, more observable in the left ear. The passive head rotation to the right affects the vestibular efferent in an excitatory manner (shorter time intervals between IH33 firings). The excitatory response to the virtual stimulus can be explained by the sensory mismatch theory [2, 3], in which discrepancies between sensory inputs can shift the sensory weight to a more reliable system. While being exposed to the virtual stimulus, we may ‘overuse’ the visual cues to determine our position in space. The visual cues can be misleading in this case as they signal motion when participants are stationary. This may lead to an increase in efferent vestibular activity [1]. Unreliable visual inputs may activate the sensorial reweighting system in the cerebellum and shift the sensory weight to a more reliable system (vestibular).

### **S1.2.2 Side to Side tilt**

After accounting for differences between eyes open and eyes closed responses by normalizing average FP of all segments to the AP amplitude of the corresponding Background segment, the data show that for both ipsilateral and contralateral head tilt, exposure to the physical stimulus generated larger average FPs compared to the virtual stimulus (Fig. S1-4 and S1-5). Average FP incorporates responses of vestibular hair cells, predominantly the 8th cranial nerve, and brainstem components [1, 4]. The size of FP is influenced by the number of individual hair cells as well as synchrony in the afferent firing. Therefore, the larger size of average FP for physical compared to virtual stimulus implies more ‘synchronous’ excitatory vestibular activity. The visually evoked response is produced via modulation of afferent activity by the efferents and as such would be expected to be much smaller than the direct physical evoked response produced by the movement of the peripheral hair cells.

### **S1.2.3 Up-Down movement**

The up-down movement of the chair predominantly affects the saccule of both ears in the same manner. As expected, in our data no difference was observed between the vestibular response of the left and right ear. In regards to afferent activity, the vestibular system generally produced a larger response for the physical compared to that of the virtual stimulus (the only exceptions were Background and RTC-Background in the right ear) (Fig. S1-8). This is expected since the physical stimulus directly modulates the afferent activity, however, the virtual stimulus affects the afferent activity through efferent pathways resulting in smaller average FPs. However, this difference was non-significant. Considering the second feature, no difference was found between the average efferent vestibular activity for the two types of stimuli (Fig. S1-9). This is possible since 1) the magnitude and velocity of the physical stimulus are small (compared to motion with angular acceleration) 2) vestibular nerve afferents excite the efferent which in turn excite the afferent via the positive feedback mechanism of efferent control [5]. The outcome of this study can be applied to the clinical trials wherein different optical stimuli are used for vestibular rehabilitation in patients with cognitive, psychiatric, and other vestibular-related impairments.

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## **Chapter 3- Changes in the Vestibular Response Following Visual Stimuli of Different Colors**

### **3.1 Summary**

The study presented in this chapter provides answers to the following questions:

- 1) Are there vestibular response changes following exposure to different colors (blue, green, red) as well as black and white backgrounds?
- 2) Which component of the color, hue, or intensity, has more impact on the vestibular system?
- 3) How do the obtained results vary among individuals?
- 4) Are the obtained results reproducible?

To answer question 1, ten participants were tested. We recorded the vestibular response of the participants to the combined effect of hue and intensity. The effect of Fixed Hue-Different Intensity for the blue light was also examined for one female subject over six trials. The participants were exposed to solid backgrounds through the Oculus Rift (Development Kit 2) while their vestibular responses were recorded from the left and right ear simultaneously using the EVestG technique. The results of this investigation were published as a journal paper in the Journal of Medical and Biological Engineering in May 2018. DOI: 10.1007/s40846-018-0425-7, pages: 238-243. Authors: M. Ashiri, B. Lithgow, A. Suleiman, Z. Moussavi, and B. Mansouri. Title: Visio-Vestibular Interaction in Humans: Changes in the Vestibular Response Following Visual Stimuli of Different Colors.

Later on, a more comprehensive study was conducted to investigate the combined or individual effect of hue and intensity in two samples. In the Supplementary Material 2 section at the end of this chapter, the effect of RGB colors, Fixed Hue-Different Intensity, and Fixed Intensity-Different Hues for blue, green, and red lights were investigated in 16 different individuals to answer question 3. To answer question 4, the reproducibility of the data was tested on one participant wherein 6 recordings were made in response to combined and individual effects of colors. The results of these investigations are also discussed in Supplementary Material 2. The reproducibility section of the dissertation has not been submitted/published anywhere yet.

## **Visio-Vestibular Interaction in Humans: Changes in the Vestibular Response Following Visual Stimuli of Different Colors**

Mehrangiz Ashiri, Brian Lithgow, Abdelbaset Suleiman, Zahra Moussavi, Behzad Mansouri

### **3.2 Abstract**

This paper addresses the question of whether visual stimuli of different colors can evoke a measurable vestibular response. A recently developed technique of measuring the vestibular response, called electrovestibulography (EVestG), was used in this study to assess the responsiveness of the efferent vestibular system. Visual stimuli of different colors (blue, green and red lights) along with white and black background were displayed to the participants through a virtual reality (VR) headset. Initially, the effect of each color was investigated; for each color, the corresponding red, green, or blue (RGB) value was set to 255 and the two others set to zero. Additionally, for the blue light, the impact of the intensity under well-lit (photopic) and low (close to mesopic vision range) intensity levels were separately examined. The results of this study indicate that not only does the vestibular system respond to light of different colors, but it also shows a sensitivity to the intensity of blue light. For blue light, the vestibular response changed in proportion to the intensity level, showing larger responses at higher intensities.

Keywords: Vestibular, Visual, Color, Intensity, Electrovestibulography (EVestG)

### 3.3 Introduction

Neuroscience studies indicate the presence of pathways from the visual-processing brain regions to the central and peripheral vestibular apparatus [1, 2]. These pathways can enable a visual stimulation to induce a vestibular response change [3–5]. For example, there have been extensive applications of color therapy to compensate for vestibular-related deficiencies involved in mood and emotion [6] as well as anxiety and stress [7–9]. Visual-vestibular pathways are discussed in more detail in section 4.

In a study on cats, diverse inhibitory-excitatory vestibular responses were obtained from vestibular units following exposure to light flashes of varying intensities and duration [3]. In another study investigating color perception in a macaque monkey’s brain regions, there was an activation of cortical and subcortical areas linked to the vestibular system, including the posterior inferior temporal cortex, PITd (in the superior temporal sulcus), and TEO (temporo-occipital in inferior temporal areas) [10].

Using functional MRI (fMRI), color centers in the human brain were reported to involve cortical areas V4 (the right fusiform gyrus), the superior parietal lobe, precuneus, and the right hippocampus [11]. These regions have some direct and indirect connections to the vestibular processing regions of the brain [1, 12]. Apart from the invasive methods of measuring the vestibular response in animal studies or using imaging techniques, to our knowledge, there has been no similar study measuring a direct peripheral vestibular response in humans.

In this study, the responsiveness of the human vestibular periphery to various colors is assessed using a new technology called Electrovestibulography (EVestG) [13], which is an objective method of noninvasively measuring the vestibular response.



**Figure 3-1: From left to right; Ear electrode, electrode placement, and subject connections [13].**

### **3.4 Visual-vestibular interaction**

When photons of light strike the retina they cause chemical changes in the pigments of the photoreceptors [14]. These changes produce electrical potentials, which are carried through optic nerves and reach the optic tracts through the optic chiasm. From the optic tracts, there are projections to the lateral geniculate nucleus (LGN) and cortex (i.e. retinocortical pathway), superior colliculus (i.e. retinocollicular pathway), pretectum of the midbrain, or suprachiasmatic nucleus of the hypothalamus [15]. Retinocortical pathway sends fibers to the brain's visual-processing areas including striate (V1) and extrastriate cortices (V2, V3, V4, MT) [16, 17]. From these cortices, electrical signals reach the vestibular nuclei (VN) indirectly through the inferotemporal cortex [1]. There are also some neural connections between the superior colliculus and vestibular nuclei (VN) [18]. The VN sends projections to the efferent vestibular system, which then sends efferent fibers to the vestibular periphery (afferents and type I hair cells) of the semicircular canals and otolith organs (utricle and saccule). Neural activities of the hair cells, vestibular nerve, and VN can be recorded using the EVestG technology described in the following section.

### **3.5 EVestG technology**

EVestG was first introduced in 2012 as an objective method for assessing the central and peripheral vestibular system [13]. Using this technology, static and dynamic vestibular responses are recorded noninvasively and painlessly by two electrodes inserted into the external ears. A hydraulic chair provides passive whole-body tilts as stimuli for dynamic vestibular response whereas the static vestibular response is recorded while the chair is in a stationary position. The electrodes are soft cotton wool tips soaked in a mixture of saline and conductive gel to reduce interface impedance. In each ear, one electrode is placed close to the eardrum and one electrode on the earlobe for differential recording, while the common electrode is placed on the forehead of participants to reduce the common environmental noises and other biological artifacts (e.g. EEG, EOG, EMG) (Fig. 3-1). After preparing and connecting the electrodes, participants sit in the hydraulic chair in an anechoic chamber (<30 dB), where recording is performed [13].

### 3.6 Methodology

Ten subjects, including three males, participated in this pilot study (age:  $27.5 \pm 4.9$  SD). Individuals were given an oral explanation of the procedure prior to electrode attachment. In this study, we did not use any of the dynamic stimuli (the tilts). Participants sat in the chair in the anechoic chamber without undergoing any tilts. A simple virtual reality environment consisting of a solid background was generated using the Unity Game Engine. Participants were immersed in the VR by wearing a head-mounted display (Oculus Rift, Development Kit 2) connected to a laptop (EUROCOM Sky X4, NVIDIA GTX 970M, G-Sync Technology). A sequence of colors (Black, White, Black, Blue, Black, Green, Black, Red, and Black) were shown when the participant pushed a start button located on the armrest of the chair (Fig. 3-2). For each color, in the Unity Game Engine setting, the respective red, green, or blue (RGB) value was set to 255 and the two others set to zero; RGB values were set to zero and 255, for black and white color, respectively (the intensity of RGB colors are summarized in Table 3-1).

**Table 3-1: Color intensities measured through photometer.**

<b>Color</b>	White	Green	Red	Blue	Black
<b>Intensity (lux)</b>	83.2	47.7	25.1	18.9	1.9

The duration of each color exposure was 30 seconds; thus, the recording lasted for a total of 270 seconds. The presented black background was applied between different stimuli to remove the effect of color opponency (see Hering's theory [19]). This technique has been used in previous human fMRI investigations of the emotional response to blue and green lights [20]. In addition, it takes 20-30 seconds for the vestibular response to settle after each visual stimulation [13, 21]. It is worth mentioning that to prevent any biased results, only the black data from the first exposure to a black background at the beginning of the experiment was used for analysis. We also investigated the effect of different intensities of blue light on the vestibular response. The brightness level of blue color (the value in the HSV scale) was decreased gradually from a maximum of 255 to a minimum of 115 (255 corresponds to the highest brightness in the HSV scale of the Unity Game Engine) leaving hue and saturation parameters of the light fixed. Then, the vestibular response to

the following intensities was studied: Blue1=18.6, Blue2=17.5, Blue3=15.4, Blue4=11, Blue5=7.4, Blue6=6.4 lux<sup>1</sup>. The black background was also applied between different intensities of blue color in this experiment.



**Figure 3-2: EVestG recording procedure during exposure to different colors.**

### **3.7 Signal analysis**

The recorded vestibular responses were fed into an algorithm called the neural event extraction routine (NEER) [13]. The NEER algorithm is a part of the EVestG technology which produces two outputs: 1) the average field potential (FP), and 2) the time interval between detected FPs [13, 22]. The FP refers to the extracellular potential produced as a result of the almost simultaneous firing of many vestibuloacoustic predominantly vestibular fibers. Features extracted from the FP can be used as comparison criteria for diagnostic purposes [22–24]. Practically, the average time gap between each detected FP is approximately 3.3 milliseconds [22]. Knowing that the frequency

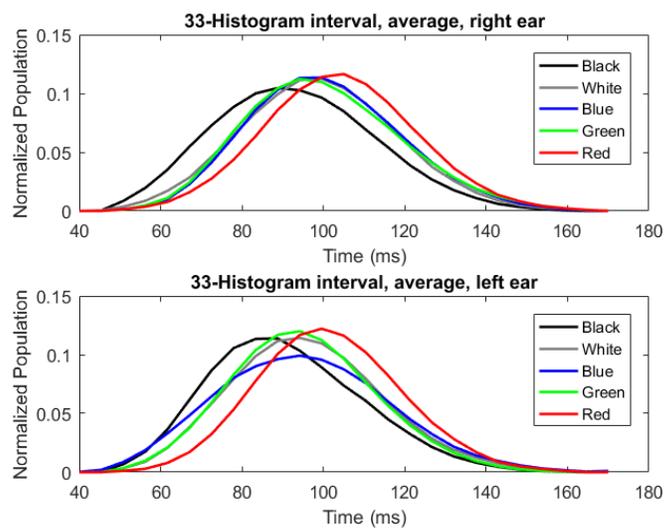
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<sup>1</sup> 1 lux =one lumen per square meter

of the spontaneous efferent vestibular activities is between 10-50 Hz, another useful feature can be extracted from the time interval signal (NEER algorithm output), called the 33-interval histogram [20]. In this case, the gap between each 33 detected field potentials is calculated (10 Hz is equivalent to 100ms; thus  $100/3.3\text{ms}=33$ ). This 33-interval histogram hypothetically corresponds to the lower range of the modulating efferent vestibular input and its effect on afferent spontaneous vestibular activity [25].

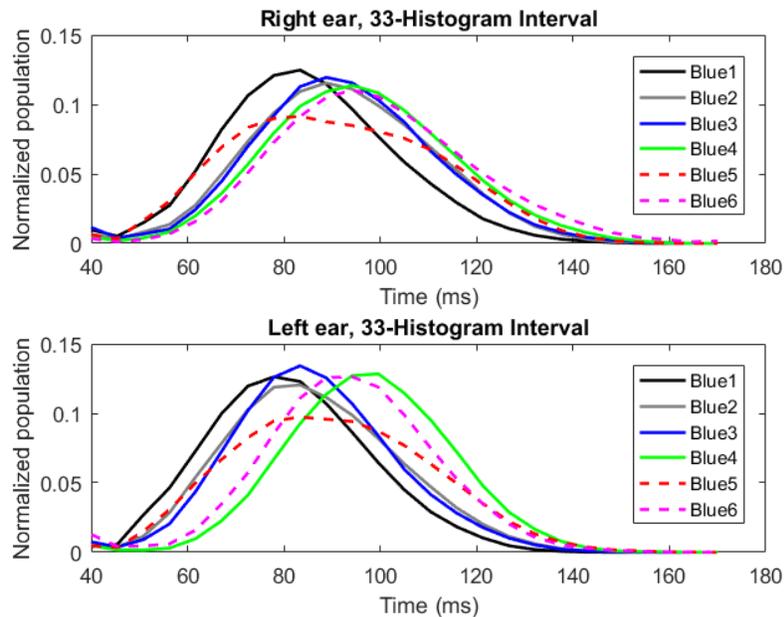
### 3.8 Results

Figure 3-3 shows the average of the 33-intervals histogram for all participants following exposure to light of different colors with the black color having the shortest average interval. First, the normality of the data was checked using the Chi-square test for normality. As all distributions were normal, a paired-samples *t*-test was conducted to compare the 33-interval histogram mean of black versus red color. The left and right ear mean separation  $\pm$  SE were  $6.77 \pm 3.05$  ms ( $p=0.054$ ) and  $10.02 \pm 3.6$  ms ( $p=0.022$ ), respectively. Comparatively, the non-parametric Wilcoxon signed-rank test, which uses sample median rather than mean, showed *p* values of 0.050 and 0.062 for the left and right ears, respectively. However, it should be noted that the intensity and wavelength were confounding variables in this experiment. Given that a single photoreceptor can respond to both variables at the same time, two scenarios need to be considered and investigated: 1) fixed intensity-different wavelengths, and 2) fixed wavelength-different intensities.



**Figure 3-3: Average 33-interval histogram for different colors.**

The fixed wavelength-different intensities scenario was examined for one female subject over six trials. Using a photometer (DT-1309 / Wide Range Professional Light Meter), a blue color screen with different intensities was applied (Blue1=18.6, Blue2=17.5, Blue3=15.4, Blue4=11, Blue5=7.4, Blue6=6.4 lx). The duration of each specific intensity was 30 seconds with the black in between of each two different intensities. The time segment of 20-25 seconds after blue onset was analyzed to remove the image aftereffect and transient responses. As it can be seen in Fig. 3-4, under well-lit condition (Blue1 to Blue4), the histogram's peak shifted to the right as the intensity of the blue color decreased, implying the vestibular response to the blue color of lower intensities had longer intervals.



**Figure 3-4: The effect of different intensities on 33-interval histogram for the blue color. Solid line; photopic region, dashed; mesopic region.**

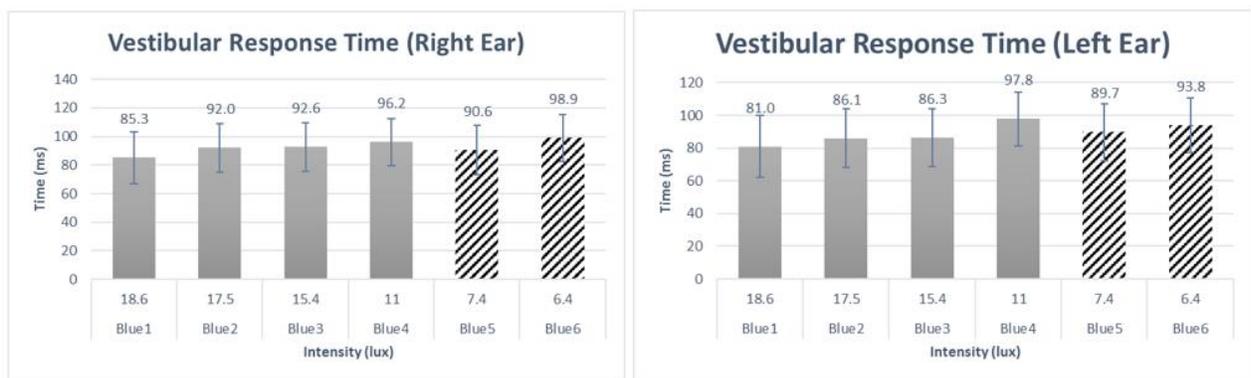
### 3.9 Discussion and conclusion

The sequence of colors' distribution from left to right being black-blue, white/green-red is difficult to interpret given the confounding wavelength and intensity variables (Fig. 3-3). Exposure to light (any color) causes photoreceptors to secrete less neurotransmitter compared to darkness [1]. Indeed, the photoreceptor circuitry is most active in darkness (the response to black color showed the shortest intervals). Comparing black and red 33-interval histograms, left and right ears show a

heterologous mean difference. This difference can originate from two main sources: 1) asymmetry between left and right vestibular responses [26], and 2) dissimilarity between the activated brain regions in the two hemispheres [10].

We also tested the fixed wavelength-different intensities scenario in which different intensities were investigated for the blue color. The results of this experiment suggest that not only can colors bring about differing vestibular responses, but so too can intensity levels. As can be seen in Fig. 3-4, vestibular activities change proportionally to intensity levels presented within the well-lit vision range (photopic vision range [27], Blue1 to Blue4). In this region, with the dominantly cone vision, by increasing the intensity, the number of detected vestibular firings increases (histogram shifted towards shorter time intervals, Fig. 3-4), and vice versa.

We also studied two other low range intensity levels (Blue5 and Blue6). These two intensities were close to the mesopic vision range [27] wherein both cones and rods are activated. Those blue color screens with intensities of 7.4 and 6.4 lx resulted in increased vestibular activity. This can be due to an increase in the eye sensitivity function at shorter wavelengths i.e. towards the mesopic region where rods gradually become the dominant photoreceptors [28]. To better understand how the vestibular response changes in relation to the intensity level, an alternative representation of Fig. 3-4 is shown in Fig. 3-5. For different intensities of blue light, the mean of the 33-interval histogram was considered as the average response time of the vestibular system.



**Figure 3-5: Average response time of the vestibular system to the blue light of different intensities. Error bars show standard errors. Solid bars represent photopic range and hatching bars represent mesopic.**

As it can be seen, within the well-lit vision range (photopic i.e. bins 1-4 of Fig. 3-5), the average response time increases when the intensity level decreases. Upon entering the low-intensity level vision range (mesopic, i.e. bins 5, 6), there is a stepped decrease in the vestibular average response time. This is equivalent to the left shift of the 33-interval histograms of Fig. 3-4 (also hypothesized to be an increase in efferent activity). In this region, following further lowering of the intensity level, the average response time of the vestibular system increases (less firing). The decline in the average response time of the vestibular system by entering the low-intensity vision range (Intensity=7.4 lux) can also be interpreted in another way. This observation might indicate that the brain uses different and shorter pathways from the eye to the vestibular system which is likely the retinocollicular pathway i.e. the superior colliculus receives input mostly from rods which are the dominant photoreceptors in the mesopic vision range [1]. This finding is very interesting per se because it shows that at higher intensities, the vestibular system uses the visual information that is provided by the visual cortex, however, in darkness, it changes to the faster and shorter retinocollicular system. The neurophysiological benefit of such a dual exclusive system is unclear and warrants further investigations in the future.

### **3.10 Limitations**

It should be noted that the fixed wavelength-different intensities scenario was investigated only for the blue color for one participant. We acknowledge the small sample size of this study that limits the reliability of the statistical analysis. However, the results of this pilot study are encouraging enough to plan a future study with a larger sample size. In addition, the effect of red and green colors with different intensities as well as the fixed intensity-different wavelengths scenario still needs to be fully examined.

Our results raise many questions such as: Does the color or intensity representation order affect the histogram response? How do changes in pupillary size and retinal light adaptation following exposure to different colors and intensities affect the vestibular response? Is the vestibular response more sensitive to wavelength or intensity? How do individual physiological/emotional effects of colors change the vestibular response? These are questions for future research in a more controlled study and larger sample size.

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## **Supplementary Materials 2 (S2)- Comprehensive Color Study**

The presence of neural pathways between the visual and vestibular systems is suggestive that visual stimuli with different qualities (e.g. wavelengths (hue) or intensities (luminance)) can influence vestibular responses. In Chapter 3, we measured the vestibular driven responses to three monochromatic colored light stimuli (blue, green, red) along with black and white backgrounds using Electrovestibulography technology (EVestG) [1] in ten participants. In addition, vestibular response to blue light of different intensities was analyzed in one participant. The following section provides more detailed information regarding visual and non-visual photoreception systems and their connection to the vestibular-processing brain regions. Also, in this section, a more comprehensive study is presented examining the individual effects of the intensity and hue of the three visual stimuli (blue, green, red) on the vestibular signals as the effect of intensity and hue on the visual system is overlapping. Two different samples are considered: 1) a 30-year-old healthy female subject (to show the reproducibility of the results), and 2) a sample of sixteen participants (8 females,  $26.8 \pm 5.3$  *SD*, to show how the results vary among individuals). The preliminary results of this investigation show that when the effective illuminance level of the three colors is constant, the evoked vestibular responses are relatively similar ( $p > 0.05$ ). However, a variation in the illuminance level (increasing or decreasing) results in a significant change ( $p < 0.05$ ) in vestibular activity relative to the intensity of the light. These findings suggest that vestibular response to visual stimuli of different colors is mostly sensitive to luminance and relatively invariant to hue.

### **S2.1 Visual and non-visual photoreception systems**

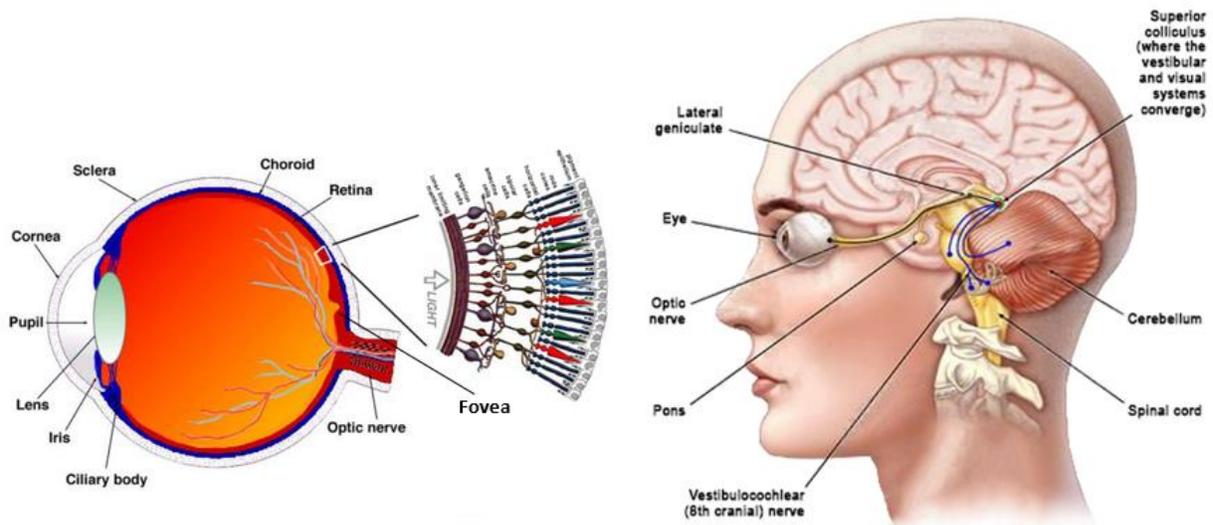
Light reaches the retina (the light-sensitive layer of the eye) after passing through the cornea, pupil and lens. These layers play different roles in adapting the refractive index between the air-eye interface [2,3], defining the amount of light reaching the retina [3,6], and focusing on the light ray to the retina [4,5]. The retina contains two general types of receptors including classical and melanopsin-expressing photoreceptors. The former is comprised of rods and three types of cones with specific spectral sensitivities. Rods are predominantly activated at low-light level (scotopic vision), and provide input for low spatial acuity and high sensitivity black and white vision [7,8]. Peak spectral sensitivity of the rod's pigments (rhodopsin) is approximately 510 nm, and they are

populated more on the periphery of the fovea rather than the foveal and macula [9]. In contrast, cones provide high spatial acuity and color vision (photopic vision).

Cones are classified into three different categories based on the sensitivity to various wavelengths including short-wavelength sensitive cones (blue), middle-wavelength-sensitive cones (green) and long-wavelength-sensitive cones (red); or, respectively: S-cones, M-cones, and L-cones. These three types of cones make it possible for us to differentiate between wavelengths and various levels of intensities. Each cone category has its unique visual pigment type. Peak absorption of the photopigments in three classes of the cones including S, M and L-cones are 430 nm, 530 nm, and 560 nm, respectively [10,11]. The population of rods is approximately twenty times higher than that of the cones (91:4.5 million) [12]. The relative proportion of L:M:S cones are approximately 12:6:1; S-cones are rarely present in the central area of the fovea [4]. Under well-lit conditions (photopic vision range) color perception is primarily mediated by the cones. Vision at low-light levels is dominated by the rods, and this is referred to as scotopic vision. Between these two vision ranges lies the mesopic vision range, in which both cones and rods are active.

Melanopsin-expressing photoreceptors were first described in [13]. It is believed that they are mostly involved in non-visual or non-image-forming responses to light such as alertness and cognition [14,15]. These photoreceptors receive some inputs from rods and cones implying that classical photoreceptors also contribute to non-visual photoreceptive tasks [16]. The wavelength sensitivity of melanopsin-expressing photoreceptors is different from the maximum sensitivity of retinal classical photoreceptors (555 nm for green light) ranging between 460-480 nm, close to the blue light [13,16,17].

Classical photoreceptors respond to the presence or absence of light in a fraction of a second (millisecond). The reduced response over time can be attributed to the fact of the rods becoming saturated. In contrast, it takes a few seconds for melanopsin-expressing photoreceptors to react and the resultant response can last up to several minutes after ceasing the light stimulus [10,17]. Fig. S2-1 depicts the general anatomy of the human eye, different layers of the retina, and some visual pathways originated from the optic nerve.



**Figure S2-1. Schematic diagram of the human eye with some key structures labeled (left), Schematic diagram of the ‘wiring’ of cells in the human retina (middle) [4,18], visual projections towards the vestibular processing brain regions (right).**

## **S2.2 Synaptic spread from the retina to visual and vestibular brain regions**

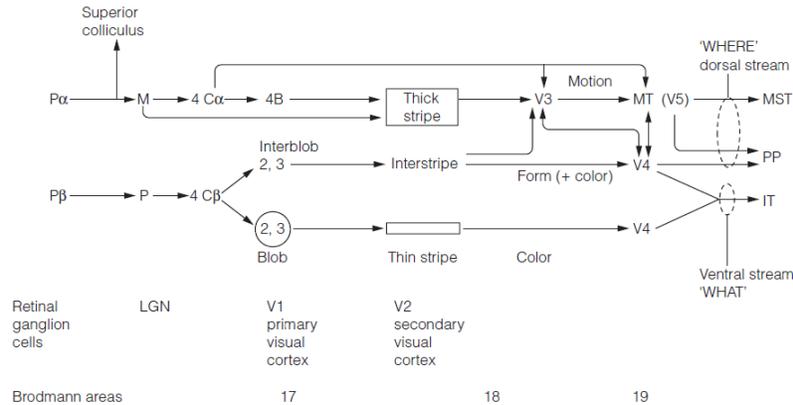
From photoreceptors, visual information reaches a special group of cells, called ganglion cells, after passing discrete arrays of cells including horizontal, bipolar and amacrine cells (closest to the ganglion cells) (Fig. S2-1) [8,18,19]. Ganglion cells lie on the surface of the retina from which they converge their axons to form the optic nerves [20]. Two general types of ganglion cells are called Parvocellular (P) ganglion cells and Magnocellular (M) ganglion cells which are arranged in the innermost layer of the retina. The population of the P ganglion cells is approximately ten times greater than the M ganglion cells in each retina. These cells are different in several aspects including size, conduction speed, input source, receptive field, and functionality [21]. All ganglion cell axons, arising from the temporal half or nasal half of the retinas, form the optic nerves. The optic nerve of the ipsilateral temporal half of the retina and contralateral nasal half of the other retina converge through the optic chiasm to form the optic tracts, each of which carries information from one-half of the visual field. From the optic tracts, there are four major projections: to the lateral geniculate nucleus (LGN), to the superior colliculus (SC) of the midbrain, to pretectum of the midbrain and to suprachiasmatic nucleus of the hypothalamus [20]. It is worth mentioning that about 20 percent of synaptic connections terminated in the primary visual cortex (V1) are

originated from LGN. The LGN also receives afferents from visual cortical areas that address visual attention (vergence) [19]. Other projections from the optic tract play different roles. The SC acts as a relay and controls eye movements (mostly involved in generating reflex saccade) [22]. The pretectal area is involved in autonomous reflexes such as pupil and accommodation reflexes. Pretectum of the midbrain and olivary pretectal nucleus mediating pupillary constriction get some input from melanopsin-expressing retinal ganglion cells and projects to the pulvinar (PULV), SC, thalamus, vestibular and vestibulocerebellar nuclei [38]. The suprachiasmatic nucleus of the hypothalamus plays role in diurnal rhythm and the reproductive cycle [16,19,20,23].

Most optic tract fibers go to the LGN which consists of six distinct layers [20]. The two ventral layers are originated from M ganglion cells and are called Magnocellular layers and connected to layer 4C $\alpha$  in the primary visual cortex (V1) (see Fig. S2-2). There are some interconnections from 4C $\alpha$  to 4B layer of V1 wherein cells, pyramidal cells, are sensitive to the direction and orientation of objects in the visual field. The secondary visual cortex (V2) gets inputs from V1 and contains thick and thin stripes. Neurons in layer 4B send output to V3 and MT (V5). From V5 there are connections to the medial superior temporal cortex (MST) and posterior parietal cortex (PP). This dorsal stream is called the “where” stream as it is engaged in motion and depth perception. The four dorsal layers of LGN are called Parvocellular layers and innervated the 4c $\beta$  layer of the V1. These layers have characteristics similar to the P ganglion cells which innervate them. Two Parvocellular pathways exist. Parvocellular-interblob (PI) pathway is colorblind and concerned with object perception (form). The Parvocellular-blob (PB) pathway is wavelength sensitive and mediates color perception. Interblob cells lie in between the blob cells in layers 2 and 3 of V1. PI and PB join each other in V4 and project to the Inferotemporal (IT) cortex. This pathway is called the “what” stream as it recognizes objects and colors.

Subcortical tracing of V4 connectivity in macaque monkeys has indicated bidirectional connections with the PULV, lateral geniculate nucleus and amygdala, efferent projections to SC and thalamic reticular nucleus, as well as afferent inputs from dorsal raphe nucleus, and locus coeruleus [24]. The role of the SC, dorsal raphe nucleus and locus coeruleus in vestibular functioning is well established [25,26]. Other structures such as PP, MST and IT have been

reported to be visual-vestibular integration centers [27,28] and have connections to vestibular-processing brain regions. Fig. S2-2 depicts M and P pathways from retinal ganglion cells.



**Figure S2-2. Flow diagram of M and P channels in the primate visual system. IT, Inferotemporal cortex; MST, medial superior temporal cortex; MT, medial temporal cortex; PP, posterior parietal cortex [21].**

### S2.3 Methods

To investigate the impact of combined hue and intensity parameters of three different colored lights on the vestibular response, two samples were tested: 1) a 30-year-old healthy female subject, and 2) a sample of sixteen participants. The participants were not in a different time zone within the last two months prior to the experiment and they had normal sleep the night before the experiments. The following criteria were checked before any recording; To ensure that the vestibular system is intact, the semi-structured questionnaire (eligibility criteria) used in the first sub-study, as well as the Vestibular Disorders Activities of Daily Living (VADL) Scale, were applied. In addition, a check for color vision deficiency, the absence of stereovision, extraocular motility, pupillary light reflex (PLR), visual field testing, standard visual acuity, and presence of any eye suppression were made by a neuro-ophthalmologist.

As the response of a single retinal photopigment is a combined response to the wavelength and intensity of color at the same time, in this study, the effects of the following confounding variables were also investigated: Fixed Intensity-Different Hues (Experiment 2) and Fixed Hue-Different Intensities (Experiment 3). In Experiment 2, the intensity value of green and red lights was reduced to the (lowest) blue level, as it has the minimum illuminance of 18.6 lux [29]. In Experiment 3, for each monochromatic light, the value of intensity level (value in the HSV scale [30]) was reduced

gradually, leaving hue and saturation parameters of the HSV scale fixed. EVestG recording was carried out for both scenarios in order to compare the individual effect of hue and intensity parameters on the vestibular system.

A head-mounted display (HMD), the Oculus Rift-DK2, was used to expose the participant to the light of different hues and intensities. The Oculus Rift was connected to a laptop (EUROCOM Sky X4, NVIDIA GTX 970M, G-Sync Technology), in which a sequence of various lights was displayed using the Unity Game Engine (version 5.3.2f1). To synchronize the onset of light exposure with the EVestG recording, a push-button was placed on the armrest of the tilt chair. In addition, to ensure that the participants pay attention and do not fall asleep during the experiment, another button was specified to be pressed when the color changes. Illuminance levels were measured from one of the Oculus lenses using a photometer (DT-1309 / Wide Range Professional Light Meter) prior to the experiments. The following criteria were considered in order to maximize the prospect that the generated vestibular response corresponds to the light stimuli:

- 1) Exposure time was set to 30 seconds to account for visual and non-visual responses to light (produced by classical and melanopsin-expressing photoreceptors) [31], as well as short and long-term vestibular responses (as a result of neural activity of the vestibular nerve & brainstem, and auditory cortex) [32,33].
- 2) A solid background was used to reduce the probability of activation of the brain areas related to object and pattern recognition [34].
- 3) A black background was presented in between the different light exposures to remove the effect of complementary color afterimage, and to provide the necessary time for the vestibular response to settle after the last light exposure [23].

In Experiment 2 (investigating the individual effect of hue), the participants were exposed to the same RGB sequence of colors (named Experiment 1 as reference) but with the difference that the illuminance of the blue, green and red lights was kept fixed at 18.6 lux. Experiment 3 was designed to take into account the individual effect of the intensity parameter on the vestibular activities regardless of any changes in hue. In this experiment, for each instance of monochromatic light, the corresponding hue and saturation factors, in the HSV scale, remained constant whilst the intensity value decreased gradually from a maximum of 255 to a minimum of 115 using a 20 stepsize (255



## **S2.4 Results of a comprehensive color experiment and its reproducibility**

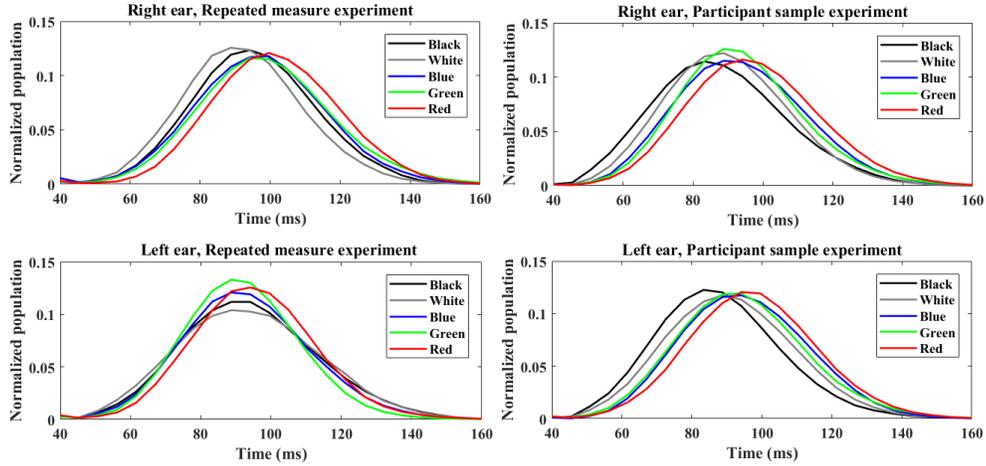
From a total of thirty seconds of each light exposure recording, a 5-second segment (20-25 sec) was selected as the segment of interest. The reason was to account for the transient response to the light stimulus and afterimage effects [6,35–37]. Performing a Shapiro-Wilk Test of Normality confirmed that all recorded data are approximately normally distributed. A nonparametric test, Friedman, was used for multiple comparisons in a sample of one participant (*P*-values of the exact test are reported as the sample size is not adequate<sup>2</sup>). A two-way repeated measure ANOVA was conducted to investigate the interaction and main effect of ears (left and right) and different colors/hues/intensities in the sample of sixteen participants (8 females,  $26.8 \pm 5.3$  *SD*). The significance level was considered 0.05 in all instances unless specified otherwise. The IH33 mean responses of Fixed Intensity-Different Hues and Fixed Hue-Different Intensities experiments showed that variation in illuminance levels influence the vestibular system significantly. However, this statement was not true for changing the hue while keeping the illuminance level fixed. The obtained result from each experiment is detailed below.

### **S2.4.1 Experiment 1: the effect of red-green-blue colors**

The average 33-interval histograms of RGB colors of different illuminances (Photopic vision range: Blue: 18.6 lux, Green: 53.6 lux and Red: 24.3) along with black and white backgrounds demonstrate that exposure to red brings about longer intervals between vestibular firings. Comparing the IH33 mean of different backgrounds for the repeated measure experiment, the result approaches a marginal level of statistical significance ( $P=0.051$ ) in the right ear (Fig. S2-4). For the participant sample experiment, only the main effect of background colors showed a significant difference ( $P<0.001$ ), but not ears. After combining the left and right ear responses, the pairwise comparisons revealed that the black background is significantly different from blue ( $P=0.002$ ), green ( $P=0.003$ ), and red ( $P=0.002$ ) backgrounds. However, it is difficult to interpret these data since a single photoreceptor response encompasses responses to the intensity and the wavelength of a color at the same time.

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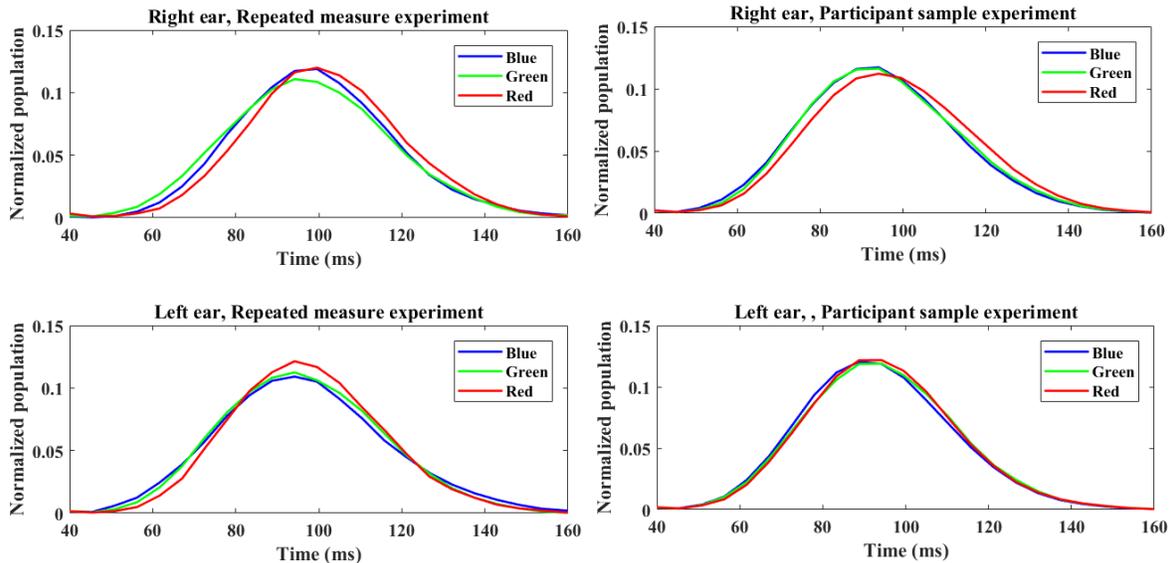
<sup>2</sup> If the calculation of the exact test faced a technical difficulty, the Monte Carlo p-value is reported [54].



**Figure S2-4.** Average 33-interval histogram resulted from exposure to light of different colors (RGB) with different intensity levels, as well as black and white backgrounds.

### S2.4.2 Experiment 2: fixed intensity-different hues

Fig. S2-5 shows the average of the IH33 mean resulted from exposure to the blue, green, and red light of the same illuminance level. No significant difference was observed between the vestibular responses in either ear for the repeated measure and participant sample experiments. This statement corresponds to measuring no substantial shifts to the left or right of the time axis for the IH33 mean feature.



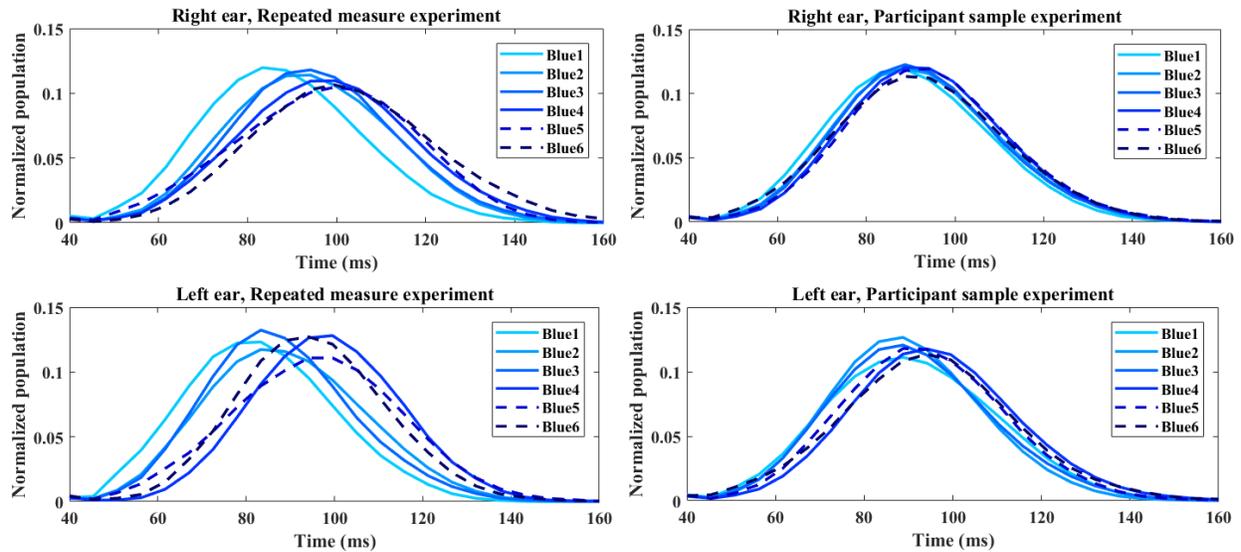
**Figure S2-5.** Average 33-interval histogram resulted from exposure to light of Fixed Intensity-Different Hues.

### **S2.4.3 Experiment 3: fixed hue-different intensities**

Generally, for the blue, green, and red light exposures, when the intensity level decreases, the number of detected FPs (corresponding to the number of vestibular efferent firings) decreases concurrently in both ears under well-lit condition. However, there are subtle differences between the vestibular responses to blue, green, and red light of different intensities. Such variance in the vestibular response to different light color exposures is not unexpected due to the spectral sensitivity of the human eyes as well as the physiological and psychiatric effect of colors; this topic is further discussed within the Discussion Section.

#### **S2.4.3.1 Blue color**

Solid lines in Fig. S2-6 show illuminances in the photopic vision range whilst the dashed lines indicate those illuminances at low light levels close to the mesopic vision range. As can be seen, in both ranges (photopic: 18.6, 17.5, 15.4, 11 lux; mesopic: 7.4, 6.4 lux), by decreasing the intensity level (or luminance level), the corresponding vestibular response shifts to the right. That means that the reduction in intensity levels leads to less firing (afferent FP detections) and or less activity of the efferent vestibular system (which can modulate the afferent response in an excitatory manner). However, a shift to the left of the time axis is notable when the illuminance of the blue light drops from the well-lit photopic region range to the values close to the mesopic region. Multiple comparisons between segments of different intensities showed a statistical significance in both ears ( $P \leq 0.010$ ). Paired sample *t*-test with Bonferroni correction (significance level=0.01 for five comparisons) revealed that vestibular responses under well-lit (Blue1 line in Fig. S2-6) and low light vision ranges (Blue6, Fig. S2-6) are significantly different in both ears ( $P \leq 0.005$ ). For the participant sample experiment, the statistical test showed a significant difference for the two-way interaction of ears\*intensities ( $P=0.018$ ) as well as the main effect of intensities (0.004). Therefore, we performed a one-way ANOVA to investigate the effect of intensity in each ear separately. Results showed a significant difference between Blue1 and Blue6 in the left ear.

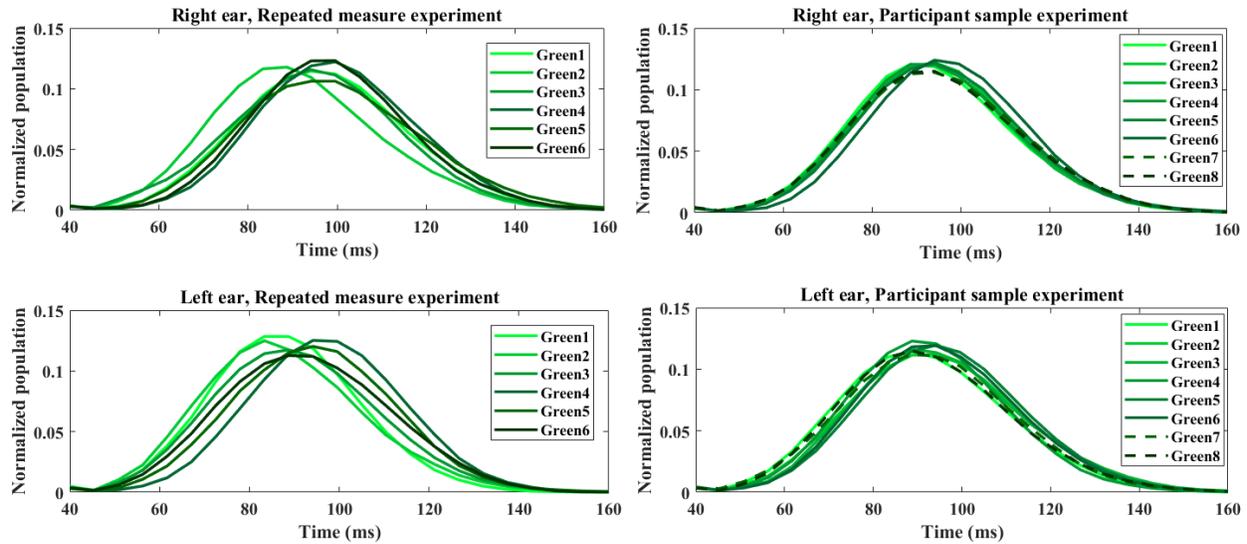


**Figure S2-6. Average of 33-interval histogram of blue light with different luminance levels (Blue1> Blue2>...>Blue6 lux).**

### S2.4.3.2 Green color

For the green light, by lowering the value parameter of the HSV scale to 115 in the Unity Game Engine, none of the intensity levels lay in the mesopic vision range for the repeated measure experiment (we designed the participant sample experiment to overcome this problem by further decreasing the intensity to mesopic vision range). In both ears, the vestibular response decreased within the photopic vision range (longer time intervals between the vestibular firing) when the illuminance of the green light decreased.

Under low light vision range (Green7 and 8, Fig. S2-7), for the participant sample experiment, when the illuminance values approach the mesopic vision range, the number of the detected FPs increases (the efferent vestibular system showed increased activity). This increase leads to a shift to the left as shown in Fig. S2-7. Vestibular changes recorded in either right or left ear to different intensities of green color were found to be non-significant for both repeated measure and participant sample experiments ( $P \geq 0.233$ ) (Fig. S2-7).

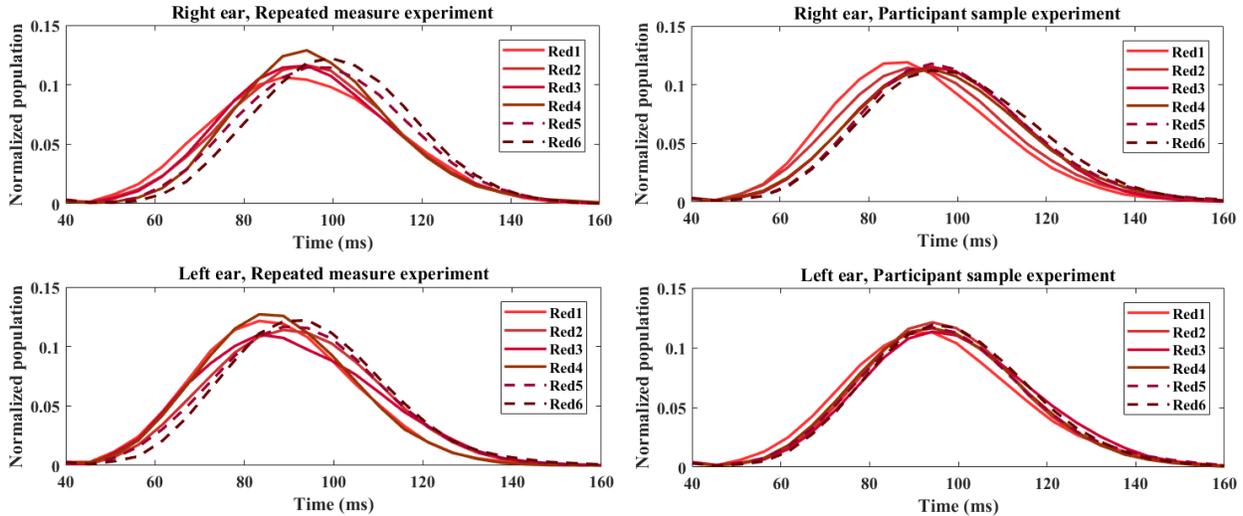


**Figure S2-7. Average of 33-interval histogram of green light with different luminance levels (Green1>Green2>...>Green6 lux).**

### S2.4.3.3 Red color

The 33-interval histogram of the red light with different intensities follows a similar pattern to that of blue light. A decrease in the vestibular response was observed by decreasing the intensity level within both the photopic and mesopic vision range (Fig. S2-8).

For the repeated measure experiment, no significant difference was found between different intensities in either ear ( $P \geq 0.426$ ). For the participant sample experiment, the main effect of intensity level showed a significant difference ( $P=0.041$ ), however, the main effect of ears and the interaction of ears\*intensities were non-significant. Hence, we combined left and right ear responses and performed a one-way repeated measure ANOVA to investigate the difference between different intensity levels. Post hoc analysis revealed a marginally significant difference ( $P=0.059$ ) between Red1 (photopic vision range) and Red6 (mesopic vision range).



**Figure S2-8. Average of 33-interval histogram of red light with different luminance levels (Red1>Red2>...>Red6 lux).**

### **S2.5 Discussion on the comprehensive color experiment and its reproducibility**

Vestibular responses to the monochromatic lights (blue, green, and red light) of short duration (30 seconds) were investigated in this study. The EVestG technique was used to measure vestibular activity, whilst participants were exposed to the light of different hues and or intensities. In addition to exposure to RGB colors (Experiment 1), two other experiments were considered and tested, separately. Initially, the illuminance level was fixed for the three different color lights (Experiment 2). Later, the illuminance level of each light was changed individually to examine the corresponding vestibular changes (Experiment 3). Collectively, the results of these experiments suggest that keeping the illuminance level fixed and only changing the hue parameter of the lights, does not impact the FP firing pattern of the peripheral vestibular system significantly; the observed IH33 for blue, green and red lights of the same intensity showed similarity in terms of time intervals between vestibular firings. In Experiments 3, a proportional relationship between the vestibular activity and the illuminance levels of the lights was observed for blue, green, and red lights, with the vestibular system showing more sensitivity to different intensities of blue compared to red and green. Our observations indicate that, in general, the vestibular activity measured via EVestG (a combination of acoustic and vestibular response but arguably predominantly vestibular) is mostly affected by the illuminance level of light rather than its hue (wavelength). In the following sub-sections, we focus on the results from both repeated measure experiment as well as

participants' experiment since although color perception is an individual experience and may vary from person to person, color exposure can have some universal effects [38,39].

### **S2.5.1 Experiment 1: the effect of red-green-blue colors**

As hue and intensity are the confounding variables of blue, green, and red in this experiment, and bearing in mind that a single photoreceptor can respond to both variables at the same time, it is difficult to interpret the results of Experiment 1. However, the trend seen for the right ear in Fig. S2-4, with black and white showing shorter time intervals between the efferent vestibular firing, can be related to the number of activated photoreceptors, differences in sensitivity and acuity of the rods and cones, as well as conduction speed and saturation of classical versus melanopsin-expressing photoreceptors [21].

### **S2.5.2 Experiment 2: fixed intensity-different hues**

Visual perception in humans is formed based on the combination of hue (wavelength) and intensity information. Although the sensitivity of human eyes in discriminating between hues is less than discriminating intensity levels [37], chromaticity (the quality of color, independent of its luminance) can provide useful information when the spatial frequency of the scene is low [40,41]. Figure S2-5 shows the vestibular responses generated by fixing the illuminance of blue, green, and red to 18.6 lux. No significant difference was observed comparing the three lights. According to the color opponent theory, which introduces three types of receptors with bipolar responses namely light-dark, red-green, and yellow-blue, the outputs of the three types of cones are summed up to produce an achromatic (lack of hue) response [37,42,43]. These opponent signals or pathways have specific temporal and spatial characteristics required for determining the color appearance, and are considered less sensitive to noise, and are more efficient. These pathways are terminated in some common/uncommon cortical and subcortical brain regions including the suprachiasmatic nucleus of the hypothalamus, the thalamus PULV, the brainstem locus coeruleus, SC, and LGN. These visual-processing brain regions send fibers to the central and peripheral vestibular apparatus resulting in modulation of vestibular activity [44]. In this study, no substantial difference was observed between the vestibular responses by exposure to light of different hues at the fixed illuminance level.

For the segment of interest, the slight shift of the 33-interval histograms to the right or left may originate from different factors: 1) photometer inaccuracy, 2) physiological aspect of the lights and the individual's response to them, and/or 3) the intrinsic difference between two general types of the photoreceptors in term of their wavelength sensitivities. Classical photoreceptors show more sensitivity to green light, while melanopsin-expressing photoreceptors are more sensitive to blue. Therefore, the small dissimilarity seen between the elicited segments is not unexpected.

### **S2.5.3 Experiment 3: fixed hue-different intensities**

Vestibular responses extracted from the left and right ears by exposure to the color lights of different illuminances show non-linear responses (Fig. S2-6). Generally, decreasing the illuminance level leads to fewer detected afferent FPs (less firing of the efferent vestibular system) and a shift to the right of the time axis of the IH33 plot under well-lit vision conditions. However, by further decreasing the intensity level to values close to the mesopic vision range, a shift to the left in the 33-interval histogram can be observed. The reason for this shift is likely because of changes in the eye's sensitivity function. Changes in the eye's sensitivity function happen when the illuminance level drops to the mesopic vision range. In this range, rods gradually become the dominant photoreceptors causing the blue light to look brighter by showing more sensitivity to shorter wavelengths [8]. Bearing in mind that rods are more populous compared to cones (91:4.5 million) [12,21], chemical changes associated with the shift in the eye sensitivity function in the pigments of the photoreceptors may produce an electrical signal large enough to affect the vestibular-processing brain regions. In our study, these changes resulted in a shift in the 33-interval histogram to the left (more firing of the vestibular system). However, by further decreasing the illuminance level within the mesopic vision range, the number of firing decreased once more (right shift on the HI33 plot) for the blue and green light, but not for the red. The reason for not observing similar results for the red color can be related to the dark-adaption mechanism which is predominantly mediated by rods and L cones. L cones (sensitive to red part of the visible light spectrum) do not become inactive when the light level decreases. In fact, for low light levels, L cones work along with rods to make our visual perception.

These results can also be interpreted physiologically. Under high luminance levels, classical photoreceptors secrete less neurotransmitter. This could lead to an increased response in the

efferent vestibular system due to the existence of a reciprocal inhibitory visual-vestibular interaction between the eyes and the vestibular apparatus [8]. Conversely, when the illuminance level of the light decreases, classical photoreceptors enter the depolarization phase and secrete more neurotransmitters. Consequently, less vestibular firing is generated (33- histogram shifts to the right on the time axis). Taking into consideration the result of RGB colors, black, and white, we can see that the longer time intervals between vestibular firings are associated with exposure to the blue, green and red compared to black and white (Fig. S2-4). Two possibilities include: 1) the difference between the number of rods and cones as mentioned above, and 2) the existence of different pathways that mediate achromatic and color visions (e. g. retino-tectal and/ or retino-cortical visual pathways) [45]. These pathways can work in parallel and affect the vestibular system through common cortical and subcortical regions. Moreover, under various luminance levels, different visual mechanisms are adapted to assist with regulating various levels of lightness/darkness entering the eyes. One of these mechanisms is the pupillary light reflex (PLR) that causes the pupil to constrict under high luminance. If the visual information is not sufficient, and because of intrinsic neural interconnection between visual, vestibular and somatosensory systems [46], the weight given to the sensory inputs can shift from less reliable sensory system to the more reliable system, the vestibular system in this case, considering the stationary position of the participant while recording.

## **S2.6 Other individual and common factors affecting the vestibular response**

Lastly, it is worth mentioning that a reaction to color might be a result of cultural, physical, or emotional conditions. Electrical signals originated from the retinal photoreceptors as a result of exposure to different hue/luminances can change the blood pressure, heart rate, body temperature, respiratory rate, etc. These signals are regulated through the hypothalamus which in turn projects fibers to the amygdala and vestibular apparatus [4,52]. The amygdala, the center of emotion in the brain, is involved in many physiological reactions such as fear [45], anxiety, stress, depression [46], memory and attention [47]. The amygdala connects to the SC, PULV, and other brainstem nuclei making it possible for the vestibular-processing brain regions to be affected by cognitive information transferred through melanopsin-expressing retinal photoreceptors. In addition, the final color appearance of an object depends on several cognitive visual mechanisms including

memory color (color association), object recognition, color constancy and discounting the illuminance [4]. Moreover, the observed differences between vestibular responses of the right and left ears can be because of human cerebral lateralization for processing colors [17,53]. For example, in [53], the largest lateralization was found in the right posterior cerebral arteries (PCA) for color stimuli and this lateralization was more noticeable for blue and red wavelengths compared to green.

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## **Chapter 4- Electrovestibulography (EVestG) Application for Measuring Vestibular Response to Horizontal Pursuit and Saccadic Eye Movements**

### **4.1 Summary**

The paper presented in this chapter seeks answers to the following questions :

- 1) How do horizontal smooth pursuit and saccadic eye movements, generated during stationary head positioning, affect the vestibular response?
- 2) Do smooth pursuit and saccade eye movements impact the efferent and afferent vestibular response in the same way?
- 3) Is the generated vestibular response during saccade eye movement sensitive to the eye movement directionality?

To answer the first two questions, two separate VR environments were designed to induce horizontal smooth pursuit and saccade eye movements. Participants were exposed to the VR stimuli while their vestibular responses were recorded simultaneously. Average AP area (representative of vestibular afferent activity), IH33 mean (representative of efferent modulated the afferent), and the number of firing in 10 ms bins (representative of changes in the firing pattern of vestibular afferent over time) were considered as features to analyze vestibular responsiveness. To find an answer for the third question, left-to-right saccade eye movements were compared with right-to-left saccade eye movements.

The results of this study were accepted for publication in Biocybernetics and Biomedical Engineering Journal in March 2021. Moreover, a part of this study was presented in the 46<sup>th</sup> annual meeting of the North American Neuro-Ophthalmology Society (NANOS) held in March 2020 in the USA. Authors: M. Ashiri, B. Lithgow, A. Suleiman, B. Mansouri, Z. Moussavi1. Title: Electrovestibulography (EVestG) Application for Measuring Vestibular Response to Horizontal Pursuit and Saccadic Eye Movements.

## **Electrovestibulography (EVestG) Application for Measuring Vestibular Response to Horizontal Pursuit and Saccadic Eye Movements**

Mehrangiz Ashiri, Brian Lithgow, Abdelbaset Suleiman, Behzad Mansouri, Zahra Moussavi

### **4.2 Abstract**

Vestibular effects linked to eye movements have been extensively investigated, however, the effect of eye movements on the vestibular is relatively unknown. In this study, vestibular responses to horizontal pursuit and saccadic eye movements were examined in healthy individuals. Visual stimuli were presented to nineteen participants ( $27.7 \pm 5.74$  (SD) years, 11 female) using a virtual reality headset whilst the vestibular responses were simultaneously recorded using Electrovestibulography (EVestG). The average field potentials (FP) of three segments 1) prior to (Pre-Background), 2) during (Movement), and 3) after ceasing the visual stimulus (Post-Background) were extracted and the action potential (AP) area used as one feature. Both pursuit and saccadic eye movements resulted in a smaller average AP area during the Movement compared to Pre-Background ( $P=0.002$ ). Pursuit and saccadic eye movements also resulted in significantly longer time intervals between the low frequency (approximately 10 Hz) modulations of FPs detected during Movement compared to the Pre-Background ( $P \leq 0.001$ ). Moreover, a comparison between rightward and leftward saccades indicated no significant difference between the two directions for the FP and time interval features ( $P > 0.37$ ). These findings suggest that pursuit and saccade eye movements inhibit the activity of both the central (postulated efferent pathways) and peripheral (afferent) vestibular system. We hypothesize that the purpose of this vestibular inhibition is to limit the vestibulo-ocular reflex and optokinetic response. Additionally, the insensitivity of the vestibular system to the saccade directions with a stationary head provides anecdotal evidence on the bilateral efferent projections to the vestibular afferent and hair cells.

Keywords: Pursuit, Saccade, Vestibular, EVestG

### 4.3 Introduction

Eye movements are a prerequisite for gaze stabilization during static and dynamic balance [1]. There is a large body of literature that establishes the multimodal integration of visual and vestibular (balance) systems to facilitate the generation of vestibular eye movements [2–4]. However, little is known about the impact of eye movements on the vestibular system. Therefore, the present study investigated the effect of two conjugate eye movements on the vestibular activity: horizontal smooth pursuits, and saccadic eye movements; Smooth pursuits are voluntary shifts of gaze that allow tracking a moving object [5]. The initiation of pursuit is evoked by seeing a moving target. Electrical potentials originating from the retina, as a result of exposure to visual inputs regarding eye movement, reach different brain regions including the lateral geniculate nucleus (LGN) and superior colliculus after passing through the optic nerves, optic chiasm, and optic tract [6]. The LGN (located in the thalamus) is the relay center for the visual pathway and makes extensive connections to the primary visual cortex. Primary visual cortex projects to the middle temporal (MT) which in turn sends fibers to the medial superior temporal (MST) region. The processing of visual information in the MT and MST is essential to determine the speed and direction of image motion. Both MT and MST make connections to the frontal eye field (FEF) which, in turn, along with MST send projections to the supplementary eye fields (SEF). The FEF as well as SEF have connections to the pontine nuclei of the brainstem [7–9]. The pontine nuclei, FEF and SEF have direct/indirect connections to the cerebellum which in turn projects to the vestibular nuclei bilaterally. The vestibular nuclei innervate the extraocular motor neurons (abducens nuclei and oculomotor in case of horizontal pursuits and saccades), and also send efferent projections to the vestibular periphery where they can affect the afferent nerves and hair cells (mainly Type I hair cells) of the otolith organs (utricle and saccule) and three semicircular canals (superior, horizontal, posterior) [9–14].

Saccades are rapid shifts in the line of sight towards the object of interest and can be executed voluntarily or involuntarily. A hierarchy of behavior can promote saccades including the sudden appearance of visual targets in the field of view, moving eyes toward a remembered point, or following a fast-moving object in a temporally or spatially predictive manner. Major visual pathways for controlling saccade eye movements are similar to the smooth pursuit, however, the cortical brain regions involved in the initiation, generation, and motor coordination of saccadic eye

movement also include the parietal eye field [15]. Information regarding saccadic inputs reaches the abducens nucleus through the paramedian pontine reticular formation (PPRF) or horizontal gaze center in the brainstem [5]. However, recent studies have shown that PPRF neurons are also active during smooth pursuit eye movement [16]. The PPRF itself receives innervations from the superior colliculus and frontal eye field. Superior colliculus contains a retinotopically organized map (a spatial map) of the visual space for generating eye movements [17]. The superior colliculus is one of the multisensory integration centers that process visual, auditory, and somatosensory information [18].

The direction of eye movements in both horizontal pursuits and saccades is determined by the activity of extraocular muscles (i.e., lateral, medial rectus) controlled by the cranial nerves arising from oculomotor and abducens nuclei.

The neuroanatomical connectivity between the visual and vestibular systems described above suggests that visual information regarding the horizontal pursuit and saccade eye movements can modulate the neural activity of the central (e.g. vestibular nuclei) and peripheral (afferent nerves and hair cells) vestibular-system via the visual-vestibular pathways. Using an objective method of measuring vestibulo-acoustic, predominantly vestibular, activity called Electrovestibulography (EVestG) [19], we recorded the vestibular activity of participants in response to eye movements from the ear canal, non-invasively. To date, there has been limited research examining the effect of visual stimuli on the peripheral vestibular response. To the best of our knowledge, except for our lab publications [20–23], no prior study has investigated vestibular response to saccade and pursuit eye movements at the vestibular periphery.

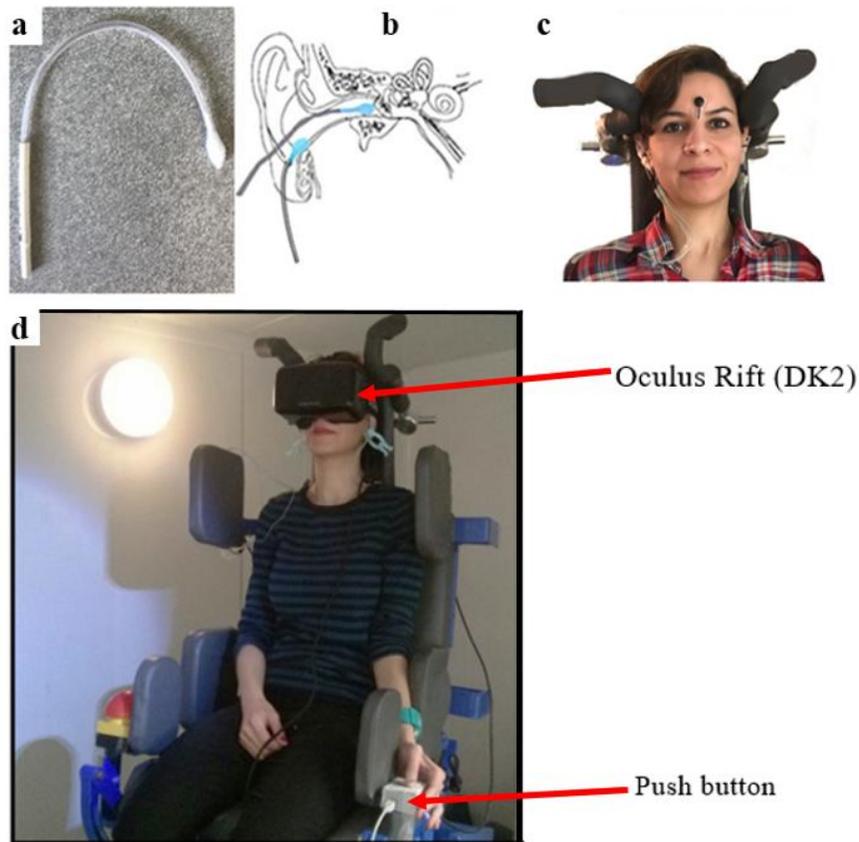
#### **4.4 Methodology**

To test our hypothesis, 19 healthy individuals ( $27.7 \pm 5.74$  (SD) years, 11 female) were recruited to participate in this study. All participants were right-handed with no history of head trauma, psychiatric or neurological disorders. This study was approved by the University of Manitoba Research Ethics Boards. After participants received and signed an informed consent form, first, a comprehensive neuro-ophthalmic examination was performed by our research group's neuro-ophthalmologist (Author BM) prior to the experiments to check visual acuity, peripheral vision,

color vision, the pupils, eyelids, retina, extraocular motility, as well as the vestibular system of the participants. These tests were done to ensure there is no eye condition affecting the vestibular response. Second, all 19 participants had visual stimuli (pursuit and saccade) applied while EVestG signals were recorded simultaneously from both ears. Furthermore, to examine vestibular-response-change to eye movements over time, we simultaneously recorded an EOG signal from one participant (right eye) and analyzed it in alignment with the average EVestG signals of all participants (N=19) in time.

#### **4.4.1 Electrovestibulography (EVestG)**

EVestG is a quantitative measure of spontaneous and evoked vestibulo-acoustic, predominantly vestibular, activity [19,24]. Using this method, the vestibular response was recorded from the ear canal noninvasively. The original EVestG technique records changes in the vestibular activity in response to passive whole-body tilts, delivered through a hydraulic chair [19,25–29]. However, for the purpose of this study, EVestG was modified and passive whole-body tilts were replaced by visual stimuli of smooth pursuit and saccade eye movements, delivered through a head-mounted display. In EVestG recording, for each ear, a non-inverting electrode was placed proximal to the eardrum, and an inverting electrode was at the opening of the ear canal. A common ground electrode was positioned on the forehead of the participants (Fig.4-1a to c). After connecting the electrodes, participants' vestibular responses were recorded in an anechoic and electromagnetically-shielded chamber while applying visual stimuli (Fig.4-1d).

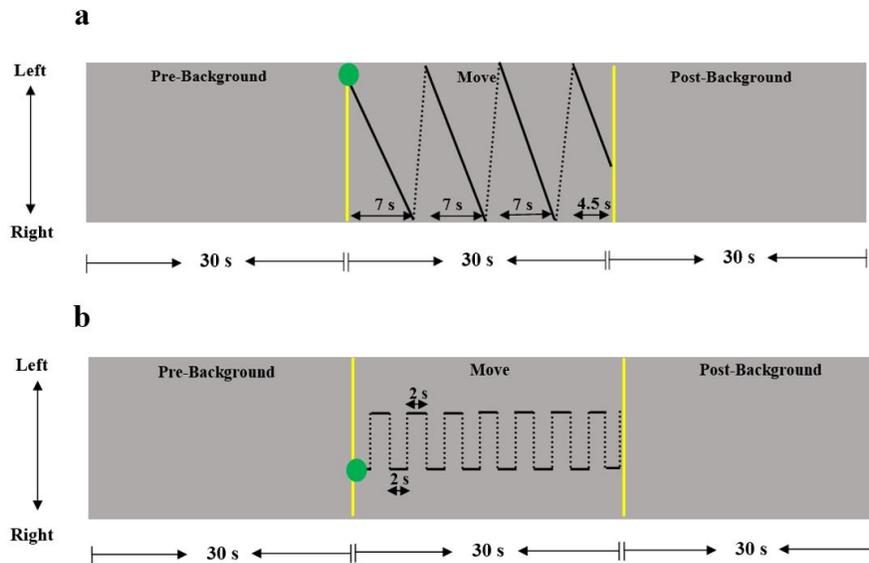


**Figure 4-1: EVestG recording procedure during exposure to virtual reality stimuli. (a) Cotton wool tip tympanic electrode. (b) Placement of active and reference electrode. (c) common electrode connection (This is a photo of author MA produced with her consent). (d) The recording settings.**

#### 4.4.2 Visual stimuli

Using a head-mounted display (Oculus Rift, Development Kit 2), participants were exposed to two basic types of conjugate eye movement: a horizontal smooth pursuit followed by a horizontal saccade. To generate the pursuit eye movement, a green circle (radius: 1 virtual unit and brightness: R=74, G=255, B=34 on a EUROCOM Sky X4, NVIDIA GTX 970M, G-Sync Technology laptop) moved with an angular velocity of  $22^\circ/\text{sec}$  from left to the right side across a grey background (Fig. 4-2a). The color of the circle was chosen to be green based on the peak spectral sensitivity of the human eye under daylight conditions (green wavelength  $\sim 555$  nm). Three and a half left-to-right pursuit eye movements were squeezed into the 30-second duration of the stimulus (Fig. 4-2a). A gap of 1.5 seconds was present between each pursuit eye movement to avoid the contribution of the quick phase of nystagmus. Dash lines in the Move section of Fig. 4-2 represent the time duration for the disappearance of the green circle. The green circle reappeared in the participants'

field of view for the next pursuit/saccade. For the saccadic eye movement, a green circle moved horizontally between two fixed points located 50 degrees apart (angular velocity of the saccade  $\cong 1450^\circ/\text{sec}$ ). For saccade testing, eight right to left (RTL) and seven left to right (LTR) horizontal gaze shifts were generated during a thirty-second recording (Fig. 4-2b). The eye movement durations were preceded and followed by displaying a 30-sec solid gray background. The experiment started when the participant pressed a pushbutton placed on the armrest of the chair (Fig.4-1d). The participants were asked to follow the circle movement while the vestibular response was recorded simultaneously in a stationary sitting position using EVestG. Head and body movements were limited by adjusting the headrest and armrests mounted on the chair.



**Figure 4-2: The Pattern of smooth pursuit (a) and saccadic (b) eye movements simulated in the virtual reality environment. Dash lines in the Move segments show the period of time that the green circle disappeared. The green circle reappeared at the end of each cycle.**

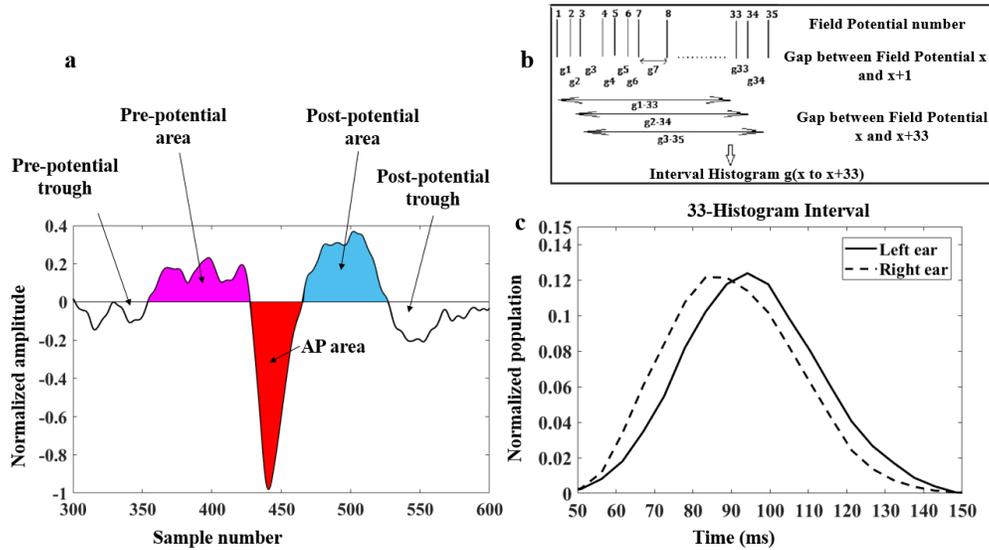
#### 4.5 Signal analysis

The total of 90 seconds of recording was divided into 2-sec segments. The 2-sec segmentation allowed for the separation of leftward and rightward saccades for investigating the effect of saccadic eye movement directionality on the vestibular system. We will refer to the first part of the signal including the grey background prior to the eye movement as ‘Pre-Background’, the saccadic or the pursuit eye movement as ‘Movement’ and the grey background after the eye movement as ‘Post-Background’ signals.

For the saccade eye movement, the Movement can be right-to-left (RTL), left-to-right (LTR), or the mix of both LTR and RTL referred to as Movement. The pursuit had one direction (left to right).

The raw recorded signals contain not only vestibular responses but also other biological artifacts (e. g. EOG, EEG, EMG) and environmental noise. An algorithm called the neural event extraction routine (NEER [19]) was used to detect vestibular responses, and to minimize or remove the effect of other biological artifacts and noise. Lithgow et. al. [19] explains the preprocessing stages of the NEER algorithm in detail. Two main outputs are generated by feeding the raw signals to the NEER algorithm: 1) average vestibular Field Potential (FP) waveform and 2) the occurrence time of the extracted FPs. The colored areas of the average FP (Pre-potential, Action Potential (AP), Post-potential areas) in Fig. 4-3a are considered the regions of interest for our visually-evoked response-change comparison purposes. To calculate the AP area, first, Movement and Post-Background segments were normalized to the AP amplitude of the Pre-Background segment (the minimum point of the average FP (AP point)). Then the two sample points where the curve crosses the vertical zero axes before and after the AP point were extracted. The absolute value of the sum of each sample point amplitude between these two points on the curve was considered as the AP area. The occurrence time of individual FPs, the second main output of the NEER algorithm, was used to generate an interval histogram plot of every 33 field potentials labeled IH33. To extract the IH33, the gap between each 33 FPs, detected by the NEER algorithm, is calculated and the associated histogram generated (Fig. 4-3b and 3c). We used the mean of IH33 histogram as our second feature for statistical analysis. This feature was selected as the distributions were not significantly skewed and did not show any kurtosis.

Furthermore, to investigate the transient vestibular responses with respect to the EOG signal of pursuit and saccade eye movements, we took into account the average number of vestibular firings within each 10ms time interval (third feature) for analysis of the different segments.



**Figure 4-3: NEER algorithm outputs. Average field potential of a control subject (a). Calculation of the time interval between each 33 detected firings (b). Generated histogram (c) based on the time intervals from (b).**

After extracting the features described above, to test the distribution normality of the AP area and IH33 mean, the Shapiro-Wilks test was used ( $\alpha=0.05$ ). As all the data were approximately normally distributed (Pre- and Post-Background as well as Movement segments), a Factorial Repeated Measure ANOVA and a post hoc analysis was conducted to investigate the main effects and the interactions (two- and three-way) of ears (left and right), types of eye movement (pursuit and saccade), and segments (Pre-Background, Movement, Post-Background) for AP area and IH33 mean. In all instances, the significance level was considered as less than 0.05 and adjusted p-values were reported. In addition, for each type of eye movement, a Pearson correlation was performed between the average firings in 10 ms bin feature of the Movement segment and the corresponding EOG signal. To compare pursuit and saccade eye movement in terms of vestibular-response-change regardless of their Pre-Background segment, paired sample t-tests were conducted (Bonferroni correction was considered for multiple pairwise comparisons based on [30],  $\alpha_{critical}=1-(1-0.05)^{(1/4)}=0.012$ ). To compare the IH33 mean of RTL and LTR saccade eye movement, the Friedman test was used instead of one-way ANOVA as the normality of distributions was violated.

## 4.6 Results

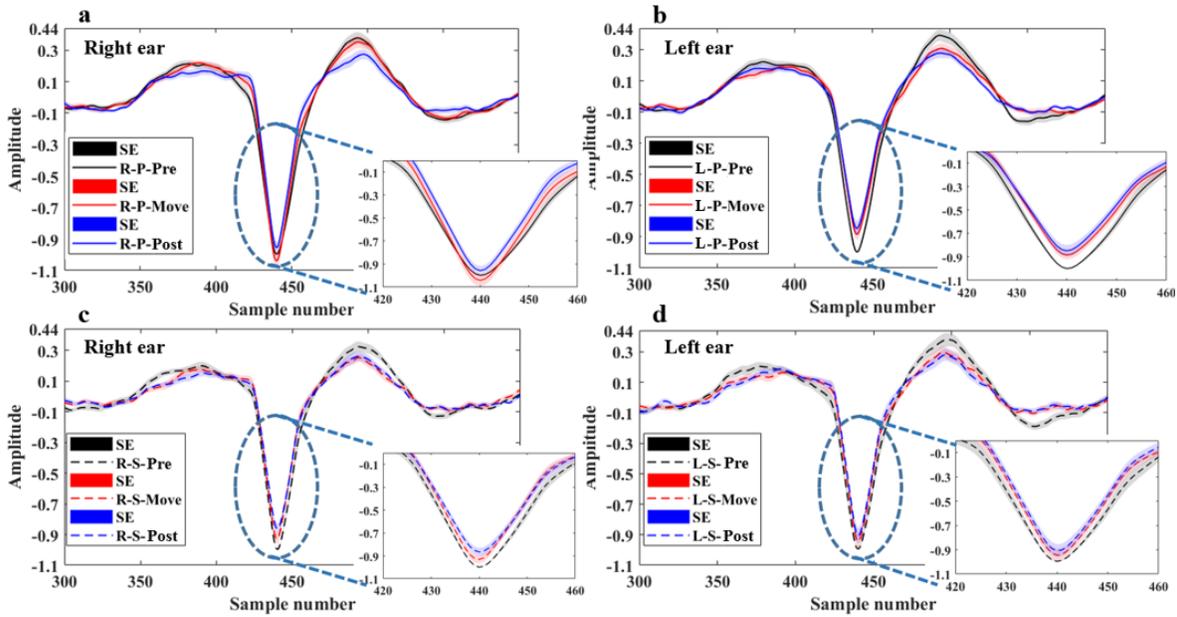
The results obtained from each feature are presented in separate subsections below.

### 4.6.1 AP area

Figure 4-4a to d show the average FP of different segments for the pursuit (Fig. 4-4.a and b) and saccade eye movement (Fig. 4-4c and d), in the right and left ear, respectively. The mean Pre-potential peak area, AP area, and Post-potential peak area across all participants for each segment are listed in Table 4-1 for pursuit and saccade eye movements. In general, smaller AP areas were obtained during and after eye movement compared to the Pre-Background segment. Conducting a three-way Repeated Measure ANOVA on the data showed no statistically significant interaction (three-way and two-way interactions) of ears, types of eye movement, and segments ( $P \geq 0.105$ ). Simple main effect analysis showed a significant effect for segments ( $P=0.022$ , partial  $\eta^2=0.229$ , observed power=0.676,  $F(2,18)=5.344$ ), but not for ears or stimuli. Accordingly, a post hoc analysis was performed between AP areas of different segments (with a Bonferroni correction) after combining the left and right ear as well as the pursuit and saccade responses. Table 4-2 summarizes the p-values for each pairwise comparison. Note that the Pre-Background segment is significantly different from the Movement segment, but not the Post-Background segment.

**Table 4-1: Average of AP, Pre- and Post-potential areas (Mean $\pm$ SE) before, while and after pursuit and saccade eye movements. Generally, movement and Post-background segments are smaller in area than Pre-Background segment in both ears.**

<i>Type of eye movement</i>	Area	Pre-Background		Move		Post-Background	
		Right ear	Left ear	Right ear	Left ear	Right ear	Left ear
<b>Pursuit</b>	AP area	21.6 $\pm$ 0.9	22.6 $\pm$ 1.2	20.1 $\pm$ 1.3	18.7 $\pm$ 1.4	17.0 $\pm$ 1.1	17.5 $\pm$ 1.1
	Post-potential area	14.0 $\pm$ 1.1	15.6 $\pm$ 1.4	13.3 $\pm$ 1.2	12.3 $\pm$ 1.2	10.4 $\pm$ 1.1	11.3 $\pm$ 0.9
	Pre-potential area	11.2 $\pm$ 0.6	12.2 $\pm$ 0.7	11.3 $\pm$ 0.7	10.3 $\pm$ 0.8	10.1 $\pm$ 0.7	10.1 $\pm$ 0.9
<b>Saccade</b>	AP area	19.6 $\pm$ 0.9	21.8 $\pm$ 1.4	16.8 $\pm$ 0.8	18.4 $\pm$ 1.1	15.8 $\pm$ 1.0	16.9 $\pm$ 1.3
	Post-potential area	12.6 $\pm$ 1.0	15.3 $\pm$ 1.4	9.6 $\pm$ 0.8	11.2 $\pm$ 1.2	9.6 $\pm$ 1.0	10.5 $\pm$ 1.3
	Pre-potential area	10.4 $\pm$ 0.7	10.9 $\pm$ 0.8	8.8 $\pm$ 0.7	9.5 $\pm$ 0.9	8.0 $\pm$ 0.8	9.5 $\pm$ 0.7



**Figure 4-4:** The mean of the average field potentials of Pre-Background, Movement and Post-Background segments for all participants. Smaller AP areas compared to Pre-Background were observed in both ears during the pursuit and saccade eye movement, potentially representative of inhibitory activity of the vestibular system. Shaded areas show the standard error. In the legend, R stands for the right ear, L for the left ear, P for pursuit, and S for saccade eye movement.

**Table 4-2:** Pairwise comparisons between different segments of interest considering AP area feature.

(I) Segment	(J) Segment	p-value
Pre-Background	Movement	0.002*
Pre-Background	Post-Background	0.099
Movement	Post-Background	1.000

\* Comparison is significant at 0.05 level

To understand how eye movement directionality affects the generated vestibular response, a two-way repeated measure ANOVA was conducted to compare the AP area of saccade RTL and LTR segments in both ears. No significant difference was found between the vestibular response to these segments ( $P=0.38$ ).

Moreover, to take into account the difference between Pre-Background segments of pursuit versus saccade, the absolute value of the difference between the AP amplitude of the Pre-Background and Movement segments was calculated. The difference between Pre-Background and Movement was used to observe whether the amount of the vestibular-response-change is different for pursuit and saccade eye movement. Results showed no significant difference between the pursuit and saccade vestibular-response-change in either ear ( $P>0.44$ ).

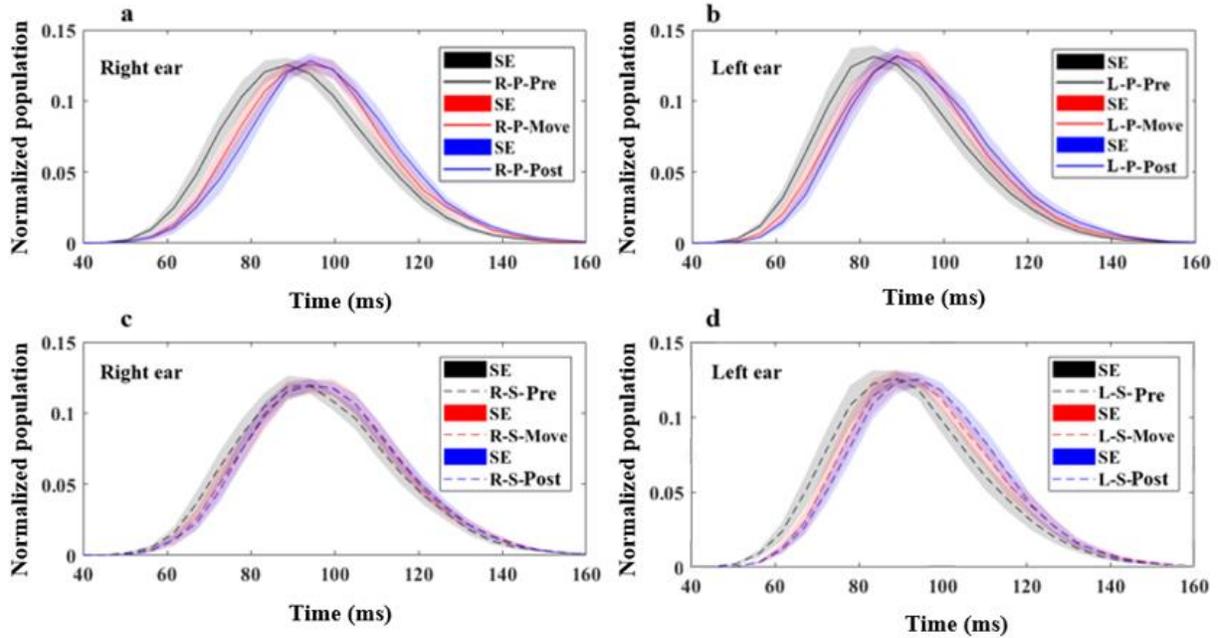
#### 4.6.2 IH33

The IH33 plots (Fig. 4-5a to d) show an overall increase in the interval (histogram shift to the right) between each 33rd detected field potential for both pursuit and saccade stimuli. Table 4-3 demonstrates the average IH33 mean of all participants for different segments of pursuit and saccade eye movements. Conducting a three-way repeated measure ANOVA to examine the interactions between ear (left and right), types of the eye movement (pursuit and saccade) and segments (Pre-Background, Movement, Post-Background) showed that all of the 3-way and 2-way interactions, as well as the main effect of ears and types of the eye movement, are non-significant ( $P\geq 0.153$ ). Indeed, no significant difference was observed between the left and right ears' signals, and also between each segment (e.g. Pre-Background of pursuit with Pre-Background of saccade) of pursuit and the corresponding saccade segment in both ears; The only significant term was associated with segments (Movement and Post-Background with Pre-Background) ( $P\leq 0.001$ , partial  $\eta^2=0.458$ , observed power=0.996,  $F(2,18)=15.229$ ). Therefore, similar to the AP result section, we combined left and right ear vestibular responses as well as saccade and pursuit data to make a comparison between different segments. Post hoc analysis was performed to make pairwise

**Table 4-3: Mean IH33±SE for different segments of saccade and pursuit eye movement. Longer time intervals between detected vestibular firings were resulted for the Movement and Post-Background segment compared to Pre-Background segment.**

<i>Type of eye movement</i>	<i>Pre-Background</i>		<i>Move</i>		<i>Post-Background</i>	
	<i>Right Ear</i>	<i>Left Ear</i>	<i>Right Ear</i>	<i>Left ear</i>	<i>Right Ear</i>	<i>Left ear</i>
<b>Pursuit</b>	99.5±1.8	97.7±2.4	104.5±2.2	100.7±2.2	106.2±1.6	100.5±2.1
<b>Saccade</b>	102.7±2.1	100.6±2.2	106.2±2.1	103.3±2.2	105.8±1.9	104.4±1.9

comparisons between each pair of segments using a Bonferroni correction. Table 4-4 lists the p-values of different pairwise comparisons between segments. As it can be seen, Pre-Background is significantly different from Movement and Post-Background, but the difference between Movement and Post-Background is non-significant.



**Figure 4-5:** IH33 plot of pre-Background, Movement and Post-Background segments for pursuit (a and b) and saccade (c and d) eye movements for all participants. Shift to the right side of the diagram for Movement and Post-Background segments is hypothetically equivalent to a decreased firing of the vestibular efferents (which can modulate afferents) compared to Pre-Background. The shaded area shows the standard error. In the legend, R stands for the right ear, L for the left ear, and P for pursuit eye movement.

**Table 4-4:** Pairwise comparison between segments of interest for IH33 mean feature.

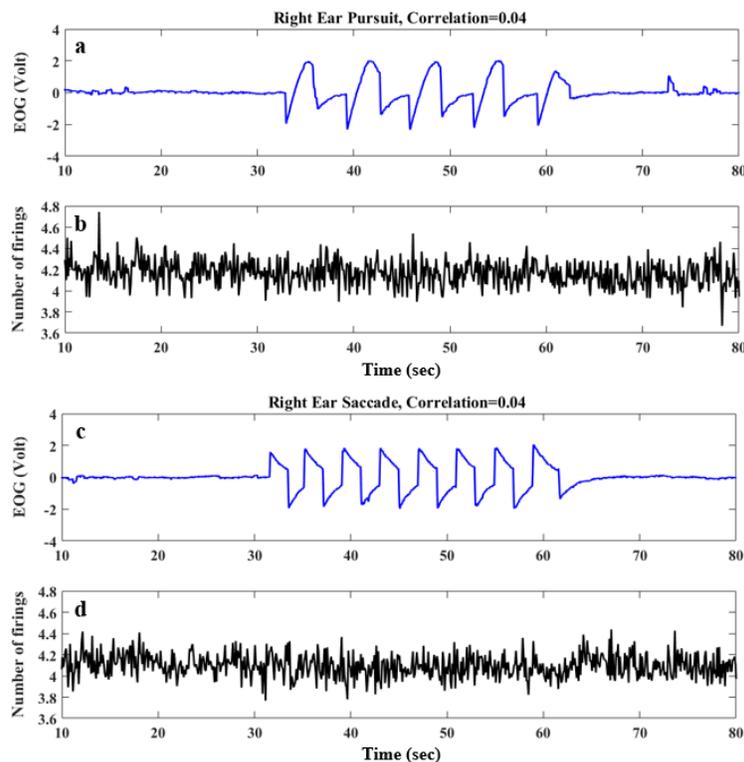
(I) Segment	(J) Segment	p-value
Pre-Background	Movement	0.000*
Pre-Background	Post-Background	0.001*
Movement	Post-Background	1.000

\* Comparison is significant at 0.05 level

To test the difference between the saccade RTL and LTR segments in both ears, the Friedman Test was performed as these data were not normally distributed. No significant difference was found between the vestibular response to saccade RTL and LTR ( $P=0.37$ ).

### 4.6.3 Number of firings in 10ms bins

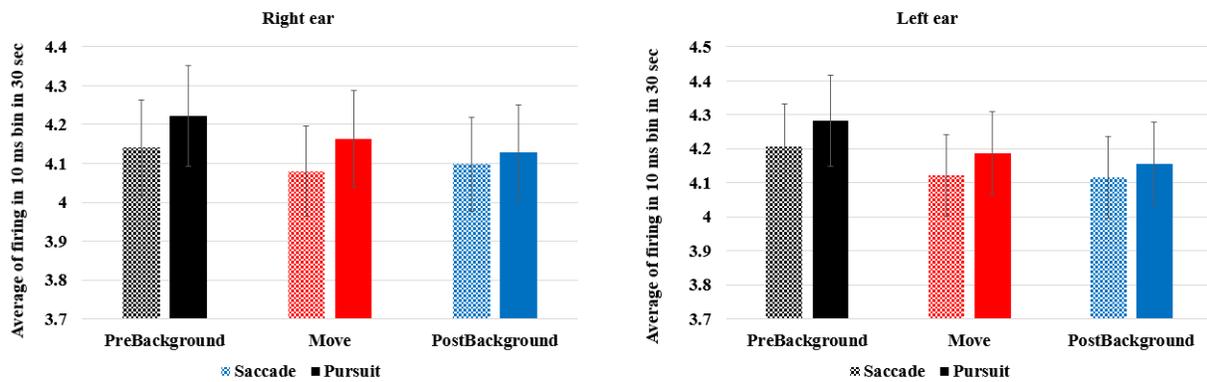
Considering the third feature, the number of firings in each 10 ms bin, the average results of the 19 participants are shown in Fig. 4-6 for pursuit (Fig. 4-6b) and saccade eye movement (Fig. 4-6d). In Fig. 4-6, the EOG output of a typical participant has been aligned with the results to show the location of the pursuit (Fig 4-6.a) and saccades (Fig 4-6.c) on the time axis. As can be seen in Fig. 4-6b and d, the two diagrams follow no obvious or specific pattern during the period of eye movement with regard to the EOG signal. An exception may be in the saccade recording (Fig. 4-6d) in which there is a marginal onset/offset response at the commencement and at the end of the overall saccade stimulus. However, no significant correlation was found between the number of firings in each of the averaged 10 ms bins of saccade or pursuit Movement with respect to their EOG signal. Additionally, all individual responses were separately examined. Out of 38 individual data, only 2 resulted in a significant correlation for the saccade Movement segment.



**Figure 4-6: Time alignment of EOG and Number of firings in 10 ms bins: pursuit EOG signal (a), Number of firings in 10 ms bins for pursuit eye movement (b), saccade EOG signal (c), Number of firings in 10 ms bins for saccade eye movement (d). The Number of firings in 10 ms bins diagram does not show any strong correlation with the pattern of the eye movement for pursuits or saccades. No significant correlation was found between the EOG signal and pursuit ( $P=0.425$ ) or saccade ( $P=0.429$ ) during the period of the eye movement.**

To obtain a better understanding, Fig. 4-7 depicts another representation of the third feature. The average of firings in each 10 ms bin was calculated over a period of 30 seconds. As can be seen, in Fig. 4-7, the number of firing decreases from the Pre-Background to Movement in both ears.

To calculate the vestibular response change independent of the Pre-Background signal, the difference from Pre-Background signal to Movement and Post-Background signal was calculated. The differences have been summarized in table 4-5. No significant different result was obtained between pursuit and saccade vestibular-response-change.



**Figure 4-7: Comparison between different segments in terms of the number of firings in 10ms bins for the pursuit and saccade eye movements. Error bars show the standard error**

**Table 4-5: Mean difference between Pre-Background and Movement/Post-Background**

<i>Changes compare to Pre-Background</i>		Move		Post-Background	
		Right	Left	Right	Left
<i>(Mean difference±SE)</i>					
Pursuit	Number of firings in 10ms bins	0.06±0.02	0.09±0.02	0.09±0.02	0.12±0.03
Saccade	Number of firings in 10ms bins	0.06±0.01	0.08±0.02	0.04±0.03	0.09±0.03

## 4.7 Discussion

The effect of eye movements (horizontal pursuits and saccades) on the vestibular system was investigated in this study. Vestibular responses were recorded while exposed to the visual stimuli using EVestG technology and corresponding features were extracted. Each of the extracted EVestG features reveals a characteristic of the vestibular function. In this section, firstly, we will explain what each outcome may imply physiologically. Secondly, we will discuss how the contribution of some visual factors (e.g. predictive eye movement) might have contributed to our results. Lastly, we will comment on the capability of the EVestG method for capturing signals from the vicinity of the eardrum and explain the criteria considered to improve signal acquisition and analysis. In general, horizontal pursuit and saccade eye movement resulted in inhibition of the vestibular efferent and afferent response compared to Pre-Background.

### 4.7.1 AP area

In section 4.1., to observe the difference between the AP area of Pre-Background and Movement/Post-Background segment, the left and right ear, as well as the pursuit and saccade responses were combined allowing for an increase in the power of the study by increasing the sample size and therefore lowering the probability of making a Type II (false negative) error. The reasons for this combination were that there was found no significant difference between the pursuit and saccade eye movements as well as between the right and left ear responses suggesting that the pursuit and saccade eye movement processing share some common brain regions (frontal eye field, supplementary eye field, cerebellum, etc.). Additionally, crosstalk between left and right ear vestibular responses is feasible through the commissural vestibular pathway.

The average FP (Fig. 4-3a) is the average of many small FPs each one being the summation of the extracellular electrical neural currents resulted from the quasi-simultaneous activity of a group of afferents. In the auditory nerve compound action potential (CAP), the pre and post-potential peaks are linked to the N1 and P1 peaks, corresponding to sodium ion influx and potassium ion efflux, respectively [31]. The potassium ion efflux is controlled through intracellular calcium ions. The vestibular nerve response resembles the auditory nerve CAP. The Pre-potential, Action Potential (AP), and Post-potential areas shown in Fig. 4-3a and 4 are associated with the depolarization and repolarization phase of the vestibular afferents as well as the vestibular nuclei and vestibular

periphery components [31]. These areas have been previously used as statistical features for the diagnosis and classification of neurodegenerative disorders in [19,25,32,33]. The smaller AP areas while performing saccade and pursuit eye movements compared to the Pre-Background segment are suggestive of a decreased rate of ion transport (efflux and influx) across the vestibular afferent, and potentially reduced vestibular response. The reduced vestibular response can appear in the AP results in two forms: 1) decreased magnitude of the AP amplitude associated with smaller FPs 2) reduced synchrony in the firing of vestibular fibers (the less quasi-simultaneous activity of a group of afferents) affecting the width of AP area. After ceasing the stimuli, no significant difference was found between Movement and Post-Background segments.

Knowing that there are specialized direction-specific V1 interblob cells that display a preference for eye movement in a particular direction, we also compared the AP area of saccade RTL and LTR segments in both ears. However, as our results indicated, the vestibular response showed insensitivity to the saccade eye movement directionality. This is possible since efferent vestibular nerves project to afferent nerves/vestibular hair cells bilaterally [14].

#### **4.7.2 IH33**

The spontaneous activity of the efferent vestibular neurons has a discharge rate of 10-50 spikes/sec (guinea pigs and macaque monkeys) [34, 35]. The experimentally measured average gap between FPs detected by the NEER algorithm is about 3.3 ms, hence, 33 correspond to 100 ms or 10 Hz. Accordingly, IH33 curves represent the distribution of time intervals between vestibular nerve firing as a result of the spontaneous efferent vestibular activity (lower range=10 spikes/sec). These efferent activities are believed to modulate the very high (300spikes/sec) spontaneous vestibular afferent activity [36]. The shift of the IH33 mean to the left or right of the time axis (hypothetically representing shorter or longer intervals between efferent modulations) may provide us useful information regarding the excitatory or inhibitory nature of central vestibular regions affecting afferent activity. Hypothetically, an increased IH33 mean (shift of the histogram to the right side of the time axis) can be related to a decrease in the firing rate of vestibular efferents and their modulatory effect/input on afferents [37]. In this study, an increased IH33 mean during and after the eye movement versus the Pre-Background segment was observed for both the pursuit and saccade experiments.

It has been investigated in [38] that the firing rate of build-up neurons in the superior colliculus (SC) show dissimilar activities for pursuit and saccade eye movement. According to that study, richer optical flow (e.g. pursuit eye movement) can trigger more firing of SC neurons; however, during saccades, the emphasis is more on the accuracy of the observations (hence less firing of SC neurons). Strictly speaking, pursuit eye movement enables speed detection of moving objects, however, saccades are mostly concerned with object position within the field of view. Furthermore, a lower action potential threshold has been identified in the SC for pursuit eye movement [39]. In another study, a pause or decreased discharge in firing occurred during saccades [40]. That can be the reason for having longer time intervals between efferent-mediated-afferent firings for saccade compared to pursuit eye movement in this study, although this difference is non-significant.

It is worth mentioning that when the pursuit stimulus stops, the eye continues in the pattern of expected object motion before stopping, indicating a memory of prior smooth tracking [41]. Yakushin et al. [42] showed that vestibular-only neurons in the medial vestibular nucleus are primarily responsible for coding velocity storage information. As the vestibular nuclei have a direct connection to the vestibular efferent, that may be the reason that the Post-Background segment, in addition to the Movement segment, is also significantly different from the Pre-background segment for the IH33 (representative of efferent-mediated afferent activities) but not AP area feature (representative of vestibular afferent activities). Moreover, the time constant of the vestibular slow component can last 20-30 seconds or more [43, 44]; therefore a vestibular response similar to that of the Movement segment should be expected within 30 seconds of ceasing the visual stimulation.

In an animal study done on monkeys [45], three major types of responsive cells were identified to be involved in generating horizontal eye movement in the reticular formation nuclei. These cell types showed different discharge rate (burst, sustained firing, or decreased firing) depending on the direction of the eye movements (responding to leftward, rightward, or both directions), type of horizontal eye movement (saccade or pursuit), and ambient light (light or complete darkness). However, in this study, no significant difference was observed between the horizontal saccade RTL-Movement and LTR-Movement in terms of IH33 (potentially efferent) interval activity

suggesting the vestibular response to the eye movement, in the head stationary position, is not affected by the eye movement direction.

### **4.7.3 Number of firings in 10ms bins**

As the visual pathways can respond to a visual stimulation within a few milliseconds [46], we calculated the number of firings in 10 ms bins; i.e. saccades have brief durations typically less than 100 msec [47]. As can be seen in Fig. 6, there is no obvious or specific pattern (or correlation) for firing within the 10 ms bins with respect to the pursuit or saccade EOG signal. Considering the whole 30-second segment of Pre-Background, Movement, and Post-Background (Fig. 7) our results are in agreement with those described in [48] (Cloherty et al, 2010), in which suppressed neural activity during saccade eye movement and enhanced neural activity after ceasing the saccade have been reported. It is believed that saccades influence hippocampal and occipital oscillations (theta-alpha) through a memory encoding, prediction or visual search tasks [49, 50]. More details on this topic have been presented in section 5.4 (Visual factors affecting the obtained results).

Shera et al. [10] have shown that the eardrum oscillates in conjunction with horizontal saccade eye movements. Two mechanisms are believed to be involved in such oscillatory eardrum displacements: 1) the activity of the middle ear muscles (MEMs) and 2) the movement of the basilar membrane of the cochlea. The vibration of ossicles as a result of MEMs and basilar membrane contraction/ expansion can be transferred to the eardrum and deflect it. For ~4 nm eardrum displacement, these eye-movement-related-eardrum movements can produce an equivalent of about 57-dB peak-equivalent sound pressure level. Knowing that the auditory efferent (arising from the medial superior olive) effect is inhibitory on the afferent and provided that vestibulocochlear nerve consists of bipolar neurons carrying both acoustic and vestibular potentials, that may be the reason for observing reduced vestibular activity during the eye movement compared to the Pre-Background segment. Given these findings and our results, two points can be highlighted regarding the number of firings in 10 ms bin feature: 1) although the saccade eye movements contribute to the acoustic activity, the effect of that input on vestibular efferent and/or afferent activity is minimal or at least not obvious in terms of the number of firings in 10 ms bins (no significant correlation) 2) although EVestG recordings are vestibulo-acoustic

signals, NEER [19], as has been previously argued, EVestG predominantly records vestibular activity rather than acoustic [25].

#### **4.7.4 Visual factors affecting the obtained results**

Prediction plays a critical role in pursuit initiation (direction change of eyes before target motion), eye movement (i.e., predicting the direction of future target motion), and termination (e.g. when the target approaches the limit of the oculus field of view). The anticipatory nature of pursuits (i.e. the same direction of target motion from left to right in this study) helps reduce the presented delays in the sensorimotor pursuit system, which can take approximately 100 ms, and recalibrate the eye velocity to follow/stop following the target in motion accurately [51, 52]. This anticipatory factor, as well as the difference between the amplitude and periodicity of pursuit versus saccade eye movements (50° versus 110°, and 7 versus 3.5 cycles considering LTR direction), can be two reasons for having a larger AP area/smaller IH33 mean (shorter time intervals between efferent-mediated afferent firings) for the pursuit compared to the saccade eye movement [39, 51, 52]. However, this difference is non-significant.

Furthermore, based on the study presented in [51], when the target disappears after a short period of pursuit eye movement, the eye velocity drops to zero within nearly 500ms. In the case of saccade eye movement, a memory-guided saccade may happen at the end of the Movement segment. According to [53], memory-guided saccades have longer latencies compared to visually-guided saccades (at least 100 ms longer) indicating the involvement of cortical processing in some brain areas such as the frontal eye field. In our experiment, the disappearance of the target at the end of each pursuit cycle or memory-guided saccades at the end of saccade eye movement may modulate the Movement or the onset of the Post-Background segments, however, since we have averaged the Movement and Post-Background segment over a 30-second time period, the effect of this modulation can be considered negligible.

#### **4.7.5 Comments on signal acquisition and analysis**

The contribution of bone-conducted vibration (BCV) stimuli in generating eye movements (with horizontal, vertical, and torsional components) and eye movements in generating eardrum oscillations are indicative of brain mechanisms that connect visual, auditory, and vestibular

information [3, 10]. The extensive neural connections arising from the visual-processing brain regions to the central and peripheral vestibular system as well as the existence of common subcortical (e.g. superior colliculus), cortical (e.g. insular cortex), and cerebellar (e.g. vestibulocerebellum) regions responsible for the processing of visual and vestibular information, makes it feasible, given the connectivity of these regions to the vestibular periphery [6–9, 12, 13, 15, 16, 35], for the electrical activity (generated as a result of chemical changes in the pigment of the photoreceptors) to travel to the peripheral organs through the efferent nerves and modulate vestibular hair cells (mainly Type 1 hair cells) and afferents [5, 9–13, 41]. These modulations can then reach the tympanic membrane (wherein the non-inverting recording electrode is placed for EVestG recording) through four possible mechanisms: 1) through the magnetically driven middle ear ossicles/tympanic membrane vibrations 2) through the activity of middle ear muscles/expansion and contraction of basilar membrane following executing eye movements 3) direct electromagnetic induction caused by the vestibular nerve far-field potentials and 4) neural connectivity. As the vestibular-evoked potentials at the tympanic membrane level are of small amplitude (in the range of microvolts [31]), in this study, averaging was utilized to compensate for low signal to noise ratios (SNRs), as well as, the low detection rate of the NEER algorithm used for extracting FPs (each 2-second segment consisted of approximately 600 FPs). Also, the interference of edge-CAPs-like fluctuations, which are summated responses from the neurons at frequencies different from tune-burst/BCV stimuli [31, 43], in the interpretation of the vestibular responses recorded by the EVestG method was essentially avoided by analyzing major peaks of the average field potential (P1 and N1 peaks) [31]. With regard to signal acquisition, to better exclude extraneous signals due to the movement of the electrodes, 3A E-A-RLINK foam ear tips, and sticky tape securing the electrode leads were used which provided a firm connection to the skin throughout the recordings. Moreover, since EVestG recordings are vestibuloacoustic signals [24], we ensured minimal acoustic contamination affecting the signal by conducting the experiments in an anechoic chamber with no auditory stimuli/artifacts. Furthermore, to minimize the effect of the conductivity/geometry variability between subjects because of skin impedance, bone structure, etc., in our analysis, we have normalized the vestibular responses to the background segment of the same recording i.e. measured subject normalized change.

It is noteworthy to mention that our proposed method, the use of a modified version of EVestG that measures vestibular activity in the stationary position in response to visual stimuli instead of physical whole-body tilts, may have some advantages over BCV and air conducted sounds (ACS) methods in terms of not introducing any jerky movement (causing electrode motion) or acoustic interference.

#### **4.8 Conclusion**

In this study, EVestG was used as an objective vestibular assessment method to measure vestibular activity in response to the horizontal pursuit and saccade eye movements, non-invasively. The findings indicate 1) a vestibular response suppression at the efferent and afferent level while executing eye movements compared with the background; 2) a vestibular insensitivity to the direction of the saccade eye movement with the head stationary. These findings may help to elucidate the nature of reciprocal inhibitory visual-vestibular interaction by avoiding vestibulo-ocular reflex when they are not necessary.

#### **4.9 Limitations of the study**

The EVestG findings in this and other EVestG studies need to be replicated in other independent studies. Currently, there are limited data evaluating the use of combined EVestG and virtual stimuli for the vestibular assessment. Aside from our lab, no other research groups have used this assessment tool, and a more in-depth investigation is required to broaden our understanding of the vestibular response to visual stimuli of eye movements and the potential clinical application of our method. Some of the topics that demand further investigation include: 1) the effect of the steady-state response of the vestibular after ceasing the visual stimulus; 2) the effect of predictive/memory-guided pursuits/saccades; 3) the effect of vertical saccades and pursuits on the produced vestibular response. It is important to note that the generated vestibular response following visual stimuli is task-dependent. Variation in color, size, speed, and place (central, peripheral) of the tracked green circle in our experiment may lead to different results. One of the shortcomings of this study was the difference between the amplitude/periodicity of saccade and pursuit eye movements ( $50^\circ$  versus  $110^\circ$  and 7 versus 3.5 cycles considering LTR direction), which may have contributed to the outcomes of our experiments. Another shortcoming can be related to the vestibuloacoustic nature of the EVestG signal that it is not possible to tease out the acoustic

response contribution from vestibular response although we minimize the acoustic response by recording the signals in an anechoic chamber. This issue might be best addressed by recording EVestG responses of a population of individuals with complete hearing or vestibular loss while performing eye movements.

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## **Chapter 5- Quantitative Measures of the Visually Evoked Sensation of Body Movement in Space (Vection) Using Electrovestibulography (EVestG)**

### **5.1 Summary**

In the paper presented in this chapter, vestibular response to perceived self-motion sensation was investigated to answer the following questions:

- 1) How does spontaneous vestibular activity change in response to a linear self-motion sensation?
- 2) How do different roller coaster trajectories influence the vestibular response?
- 3) Is there any hemispheric asymmetry during perceiving self-motion sensation?
- 4) Does comorbid anxiety or stress as a result of exposure to visual stimuli contribute to the produced vestibular response?
- 5) Do eye movements affect the perceived self-motion sensation?

To answer the first question, participants were exposed to a VR roller coaster experiment. The roller coaster experiment included four trajectories (Stationary, Up, Down, Mix), and the vestibular response recorded in each ear was compared with each other to address the second and third questions. A simulator sickness questionnaire along with another self-designed questionnaire were used to quantify participants' motion sickness symptoms as well as the degree of self-motion sensation. These data were then used to find an answer to the fourth question. The results obtained from the eye movement experiment presented in the previous chapter were used to explain the fifth question listed above.

The results of this study have been published in the Virtual Reality Journal. Authors: M. Ashiri, B. Lithgow, A. Suleiman, B. Mansouri, Z. Moussavi. Title: Quantitative measures of the visually evoked sensation of body movement in space (Vection) using Electrovestibulography (EVestG). DOI: 10.1007/s10055-020-00488-w.

## **Quantitative measures of the visually evoked sensation of body movement in space (Vection) using Electrovestibulography (EVestG)**

Mehrangiz Ashiri, Brian Lithgow, Abdelbaset Suleiman, Behzad Mansouri, Zahra Moussavi

### **5.2 Abstract**

Vection is defined as an illusory self-motion sensation induced in stationary observers that can be experienced in a real/virtual world. Vection, as a result of immersion in virtual reality (VR) environments, can subsequently lead to a sense of inability to maintain postural control and cause cybersickness symptoms. The multisensory integration of visual and vestibular (balance) information plays a vital role in vection. The etiology of vection perception, as well as, the vestibular-response-change while experiencing vection is poorly understood. This study explores vestibular-response-change following vection in 20 individuals (10 females,  $26.45 \pm 4.40$  (SD) years). Vection was induced in participants using an immersive VR roller-coaster. The vestibular response was measured simultaneously using a non-invasive method called Electrovestibulography (EVestG). The detected field potentials and the time intervals between the field potentials were extracted from the recorded EVestG signals corresponding to four segments of the VR roller-coaster trajectory namely Stationary, Up movement, Down movement, and slopes and turns (Mix). The results show that the Stationary segment is significantly different ( $P < 0.05$ ) from other dynamic segments when the average field potential of the right and left ear are subtracted. Furthermore, the Stationary segment shows longer time intervals between field potentials compared to those of the other segments in the right ear. These observations suggest that the combined effect of the visually-induced sensation of self-motion together with a concurrent/co-occurring stress/anxiety factor can affect the vestibular activity in an excitatory way. Increased excitatory vestibular activity implies increased feeling of imbalance and more likelihood of experiencing cybersickness by the participants.

**Keywords:** EVestG, Virtual Reality Vection, Vestibular, Visual, Afferent, Efferent

### 5.3 Introduction

VR technology, adapted from space research, is increasingly applied to health care, entertainment, etc. However, experiencing motion sickness symptoms such as general discomfort, headache, nausea, dizziness, vertigo, etc. whilst being exposed to VR environments have slowed its more extensive application. Vection is defined as an illusory self-motion sensation in the absence of any head or body movement, experienced in a real or virtual world [1, 2]. Vection in combination with other factors (e.g. eye movements) is usually accompanied with, and in most cases precedes, motion sickness (cybersickness in case of VR users) [1, 3, 4]. Indeed, many studies have reported that a greater likelihood of vection is positively correlated with a greater likelihood of visually-induced motion sickness.

The dominant senses involved in the vection perception are vision and balance (vestibular). The close interaction between these sensory systems controls a range of functions from reflexes to higher levels of perception [5]. It is believed that incongruent sensory inputs received from these systems trigger vection which, in turn, can give rise to cybersickness symptoms [1, 6, 7]. Investigating how vestibular response changes when participants' perception changes from object-motion to self-motion during exposure to a VR environment is beneficial to understanding the fundamentals of vection and VR motion sickness. It is hypothesized that the main anatomical link between the two phenomena is the vestibular cortex [8]. Better understanding of this link will help accelerate VR development into current and new areas of VR and VR research. Different measures have been previously used to examine the vestibular responsiveness to vection. For example, human PET and fMRI studies have proposed a reciprocal inhibitory-excitatory interaction between the visual and vestibular systems during self-motion sensations. Using these imaging techniques, cerebral blood flow (CBF), i.e. blood supply to the brain in a given period of time, was measured during visual stimuli. In one of these studies, by means of specially-designed goggles, the effect of visual stimulation through moving red and black dots in the scene (prompting vection) was compared to a solid background. Statistical subtraction analysis showed deactivation (not lack of activation) of the posterior part of the insula [8]. The authors concluded that the posterior insular could be considered as a probable region responding to the vestibular, somatosensory and optokinetic stimuli [5]. In [9], similar results were obtained when participants perception change from object motion to vection by exposure to a rotating windmill pattern.

The above-mentioned studies provide evidence for the existence of visio-vestibular neural pathways (which are explained in more detail in Appendix 1), as well as, insights into the vestibular responsiveness followingvection. However, the imaging techniques used in those studies do not measure the vestibular activity directly and objectively from the central/peripheral vestibular system and they can be expensive and time-consuming procedures. Another alternative to imaging techniques for measuring the vestibular activity is EVestG which is capable of recording spontaneous and driven vestibular responses from the ear canal non-invasively and objectively (see section 5.4.1 for details). Considering the neurological interconnection between eyes and the vestibular-processing brain regions (Fig. 5-1), we hypothesize that inducingvection in individuals can change the spontaneous activity of the vestibular-processing brain regions and these impacts can be measured using EVestG [10]. To test our hypothesis, we used an immersive roller-coaster visual stimulus to inducevection. A 3D representation (using a head-mounted display (HMD)) was used as it was found more effective in inducingvection compared to a 2D display counterpart (3D provides two images of slightly different angles similar to human's binocular vision) [11]. Compared to imaging techniques, subjective questionnaires, and invasive methods of assessing vestibular responses, EVestG technology potentially provides a quantitative measurement of vestibular activity. EVestG measures vestibule-acoustic signals predominantly emanating from the vestibular brainstem and peripheral end organs (semicircular canals and otolith organs). To the best of our knowledge, no previous study has noninvasively measured vestibular changes in humans from the vestibular periphery in response to visual stimuli.

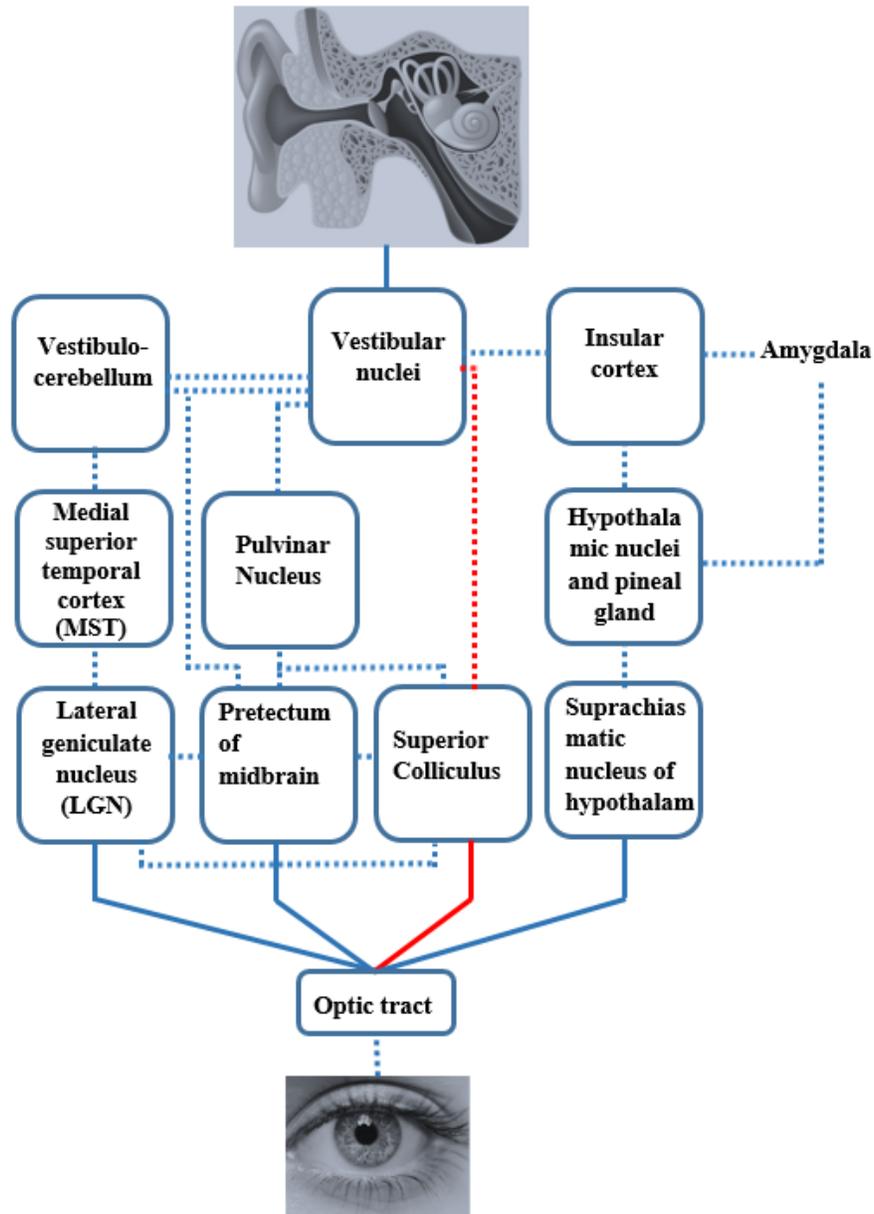


Figure 5-1: Visual-vestibular pathways. From the optic tracts, four major projections exist (via the Lateral geniculate nucleus (LGN), Pretectum of the midbrain, Superior colliculus, and the Suprachiasmatic nucleus of the hypothalamus). From these regions, optical information reaches vestibular-processing brain regions through some neural interconnection directly or indirectly. The red pathway (the retinocollicular pathway) receives inputs from magnocellular LGN predominantly involved in object-motion. This pathway (retinocolliculus) can provide a shorter processing time for visual information compared to other retinocortical pathways when changes in the visual flow are fast [12]. Modulation in the activity of the insular cortex has been reported following self-motion sensation in [9]. MST is another brain region known to be responsible for object versus self-motion detection [13].

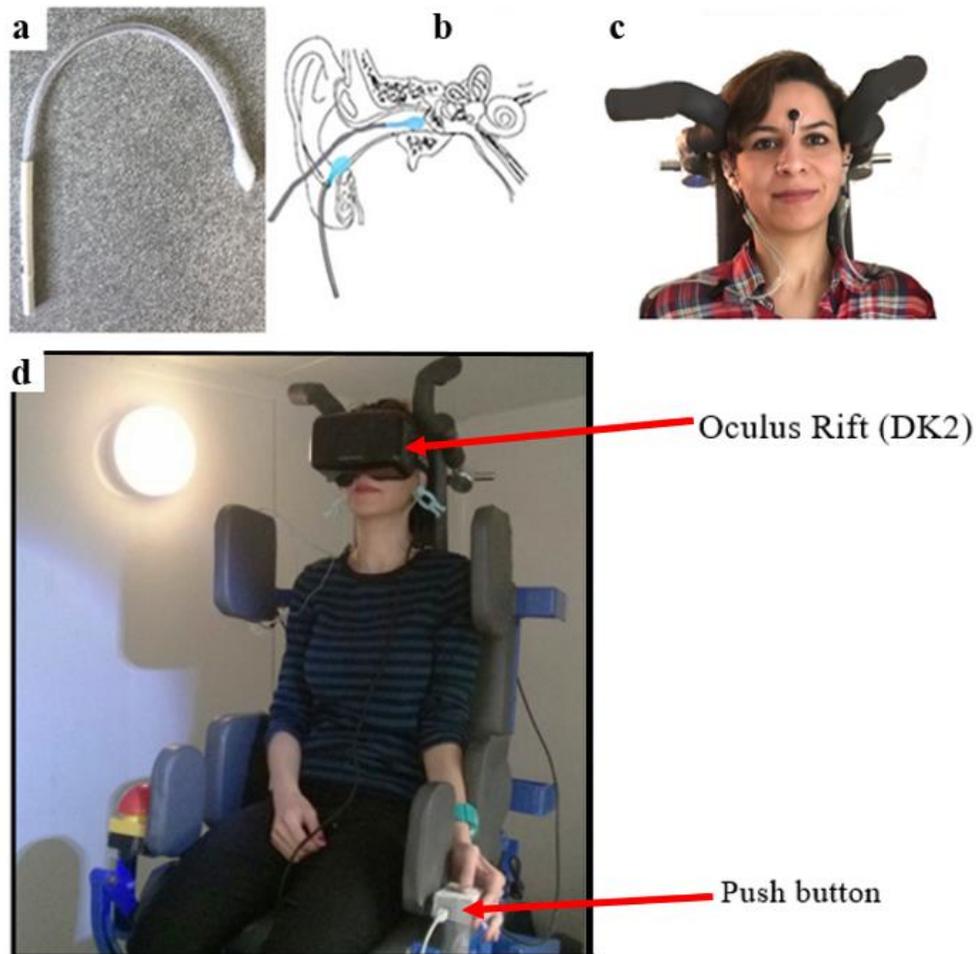
## 5.4 Methodology

We recorded EVestG signals in response tovection stimuli from 20 (10 females) healthy, right-handed, young individuals (age:  $26.45 \pm 4.40$  (SD) years). All participants were assessed by a neuro-ophthalmologist (author BM); they had a normal or corrected to normal vision and no color blindness. No history of vestibular, neurological, or psychiatric disorders was reported by the participants. The EVestG recording procedure and the visual stimuli used are detailed below.

### 5.4.1 Electrovestibulography (EVestG)

EVestG is an objective method of measuring static and dynamic vestibulo-acoustic, predominantly vestibular responses, recorded through the ear canal. EVestG has been previously applied as a diagnostic assist to measure symptomatology in Parkinson's disease [14], Meniere's disease [15], depression [16], bipolar disorder [17] and post-concussion syndrome [18]. Recording starts with the placement of one tympanic electrode (wick type with soft cotton wool saline/conductive gel-soaked tip) close to the eardrum of each ear. A reference electrode is located ipsilaterally on each earlobe. A common electrode is placed on the forehead of participants. Figure 5-2a-c demonstrates the placement of tympanic and common electrodes used for EVestG recording. The recording takes place in an anechoic chamber with a hearing attenuation factor of about 30 dB. Participants sit in a chair with adjustable headrest and armrests. The conventional EVestG measurement system records vestibular response at rest and also during whole-body tilt stimuli induced via a hydraulic chair [19]. In this study, we modified the EVestG recording system slightly so that instead of whole-body tilt stimuli, a visual stimulus was used; therefore, the signals were recorded while the chair was stationary and visual stimuli were provided through virtual reality headset (explained in the next section). A pushbutton was placed on one of the armrests to act as a trigger to synchronize the start of the visual stimuli with the computer recording the vestibular signals (Fig. 5-2d). The raw recorded EVestG signals were then analyzed using the NEER algorithm [19] in order to extract vestibular responses. The NEER algorithm uses phase analysis of the wavelet decomposed recording to detect field potentials [19]. The NEER algorithm prefilters the recorded signal [300 Hz high and 4500 Hz low-pass, 60 Hz notch, 980 Hz hydraulic jitter frequency noise removal, and after the wavelet analysis applies a matched filter (field potential template)] to detect minute field potential's and characterize them from brain artifacts (EEG, EOG, EMG, and power line artifacts).

It then averages those field potentials to produce an average field potential and to generate interval histograms of times between those detected field potentials [see [10, 19] for detail].



**Figure 5-2: EVestG recording procedure during exposure to virtual reality stimuli. (a) Cotton wool tip tympanic electrode. (b) Placement of active and reference electrode. (c) common electrode connection (This is a photo of author MA produced with her consent). (d) The recording settings.**

#### 5.4.2 Visual stimuli

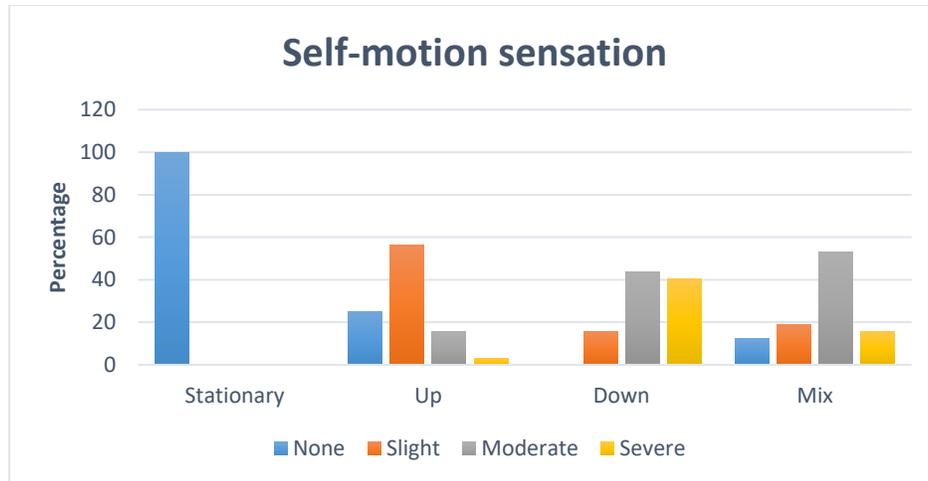
To observe vestibular changes in response to visually evoked vection, an immersive virtual reality (VR) roller-coaster (with an HMD) was used as the stimulus. The model (Animated Steel Coaster) was purchased from the Asset Store on the Unity website and was imported into the Unity Game Engine software. The roller-coaster model was modified to provide the participants with a more

realistic experience of riding in a roller-coaster by adding both sky and modified land to the scene and to have a first-person view (Fig. 5-3).



**Figure 5-3: First-person view of the visual stimulation**

Two questionnaires were used in this study: 1) a self-designed questionnaire in which degrees of self-motion sensation sensed by participants for different roller-coaster trajectory segments was evaluated (Fig. 5-4 shows the data in percentage), and 2) a simulator sickness questionnaire (SSQ) in which physical condition of the participants and motion sickness symptoms resulting from the immersion into the virtual reality environment was screened. Both questionnaires used a four-point scale including none, slight, moderate and severe [20]. The SSQ questionnaire includes some physical symptoms which are in common with the symptom of anxiety and stress including stomach discomfort, sweating, fatigue, dizziness, difficulty concentrating and nausea (Appendix 4). No prior familiarization was given to the participants about the roller-coaster experience.



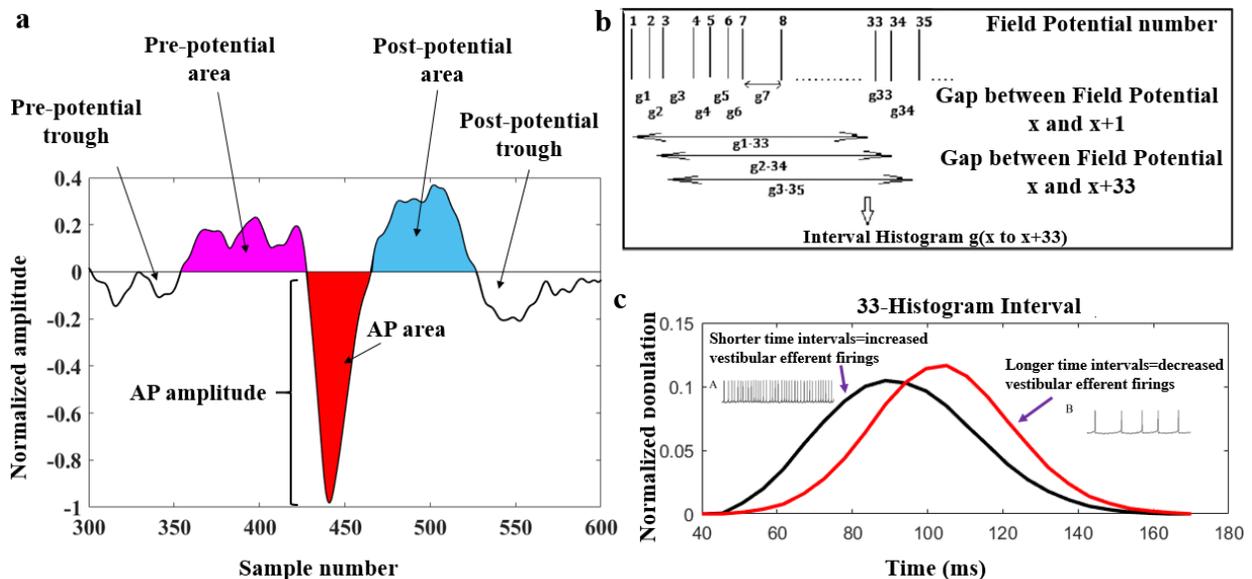
**Figure 5-4: Percentage of the self-report self-motion sensation for different roller-coaster pathways (scale response option: none, slight, moderate, severe) For the movement of the roller-coaster downhill (Down), a higher percentage of participants sensed severe (yellow) symptoms of self-motion.**

The participants of this study, observed the roller-coaster through the Oculus Rift Development Kit (DK2, refresh rate: 75 Hz, field of view: 100°, resolution: 1920 x 1080, weight: 440 g), while their EVestG signals were simultaneously recorded. Participants felt as if they were sitting in a cart that followed a trajectory with different turns and slopes. After the experiment, we extracted the EVestG signals corresponding to the Stationary segment of the cart, Up movement (when the cart goes up a ramp), Down movement (when the cart goes down a steep pathway), and Mix movement (where slopes and turns existed in the path) of the roller-coaster pathway. The duration of each extracted segment was three seconds. The roller-coaster sound was muted to avoid any acoustic stimuli.

### 5.4.3 Signal analysis

Similar to the non-invasive Electrocochleography recording (often used to measure a balance disorder called Hydrops) that records electrical potentials from the ear canal in response to sound stimuli [21], EVestG can be used to record electrical potentials using the same or modified (ipsilateral reference on outer ear canal) electrode configuration in response to virtual/ physical whole-body tilt stimuli. Using exposure to the VR roller-coaster, information regarding illusory head/body movement appears to affect the spontaneous discharge rate of the vestibular efferents (neurons that send impulses from the vestibular-processing brain regions to the vestibular end organs and have a modulatory firing rate of 10-50 Hz [16, 22]), which in turn, appear to modulate

(depolarize/hyperpolarize) the activity of vestibular afferent (neurons that receive information from the balance (vestibular end) organs (otolith and semicircular canals) fibers which then connect to the brainstem). The “quasi” simultaneous firing of a group of afferents (the detected field potentials) is then transmitted to the central nervous system. The field potential characterizes ionic transfer across the vestibular afferent nerve membranes (i.e. the sodium ion influx and potassium ion efflux). The vertical distance from the minimum point in the average field potential curve to the zero line is called the action potential (AP) amplitude. The area between the minimum point in the average field potential curve and the two points before and after this minimum point where the field potential curve crosses the zero line (area colored in red in Fig. 5-5a), is called the AP area. The areas colored in magenta and blue are called pre- and post-potential areas, respectively. Pre- and post-potential areas correspond to the activity in the vestibular periphery, while pre- and post-potential troughs are associated with peripheral and brainstem activity [23].



**Figure 5-5:** (a) Average field potential of a control subject extracted from the NEER algorithm; (b) method for calculating the time interval between each 33 detected field potential; (c) the histogram generated from the calculated time intervals. Average field potential is an extracellular signal resulted from the summed and “quasi” synchronous electrical activity of the individual hair cells. The 33-interval histogram is hypothesized to be an indicator of the low-frequency modulation of the discharge frequency of hair cells.

Normalization was performed in which the amplitude of the average field potential of each dynamic segment (Up, Down, Mix) was divided by the absolute value of the AP amplitude of the Stationary segment for each participant. This normalization was performed to observe the

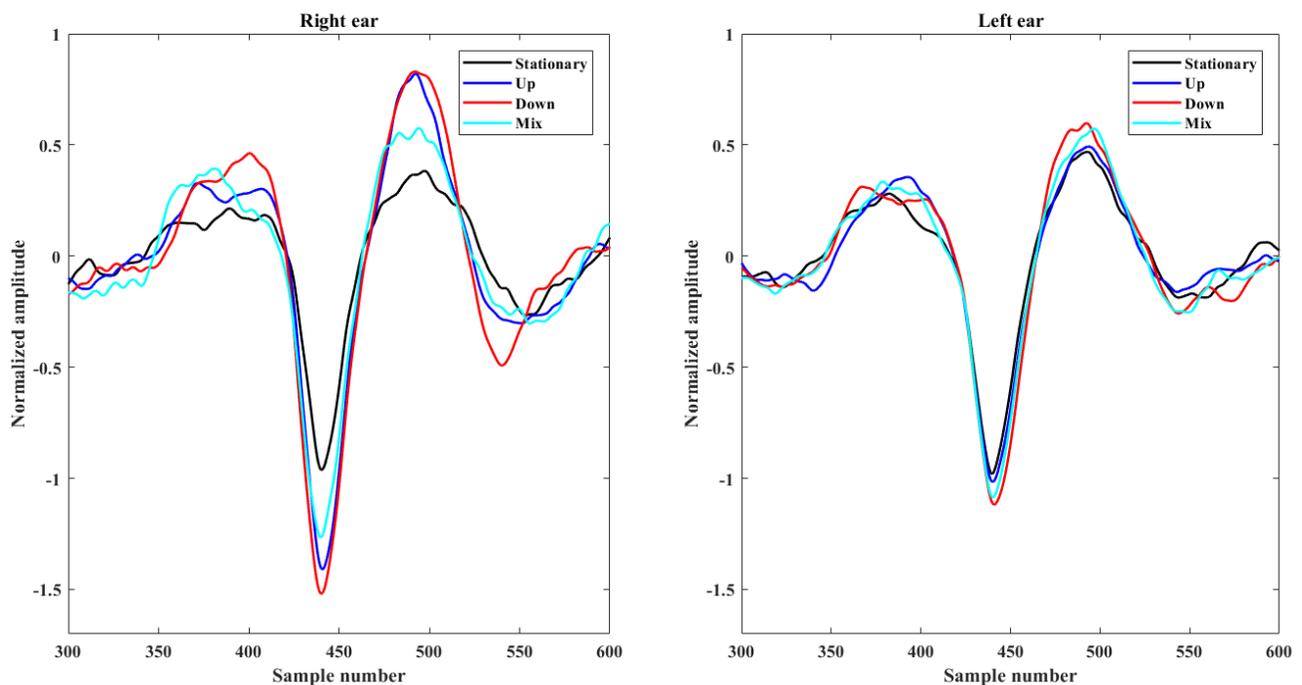
vestibular changes due tovection when compared to the static condition. Then the average (over all participants) normalized field potential of each of the four segments and the corresponding AP area were calculated. In Fig. 5-5a, larger AP areas correspond to larger AP amplitudes. Larger AP amplitudes, in turn, indicate an increased excitatory vestibular response and vice versa. Another feature can be extracted based on the time between detected field potentials. To extract this feature, the time interval between each thirty-three field potentials is calculated [16] (Fig. 5-5b) and used to create 33-interval histograms or IH33 for short (Fig. 5-5c). The spontaneous range of vestibular efferent activity ranges from 10 to 50 spikes/s [24]. The experimentally measured gap between each detected field potential is approximately 3.3ms. Therefore, 33 intervals correspond to ~100 ms (10 Hz), which is also the lower range of spontaneous vestibular efferent firing which has been postulated as a potential influencer of 33-interval histogram activity [16]. 8-13Hz is also the alpha band frequency range [25] which has also been postulated as a potential influencer of 33-interval histogram activity [16]. The 33-interval histogram provides valuable information hypothesized to reflect the spontaneous efferent vestibular activity and its ability to modulate vestibular afferent activity [16]. The 33-interval histogram mean (Fig. 5-5c) was used as our second feature in this study. The shift of the histogram-mean to the left side of the time axis is associated with shorter time intervals between efferent-mediated-afferent firings and therefore more excitatory vestibular activity.

#### **5.4.4 Statistical analysis**

First, the normality of the AP area and mean 33-interval histogram was checked using the Shapiro-Wilk test; Thirteen out of sixteen distributions ( $2_{\text{ears}} \times 4_{\text{segments}} \times 2_{\text{features}}$ ) were found to be a normal distribution. In the three non-normal distributions, we found outliers according to [26] and excluded them from the data set. Next, a Linear Mixed Model analysis was used to evaluate any plausible differences between static and dynamic segments of the roller-coaster trajectory. This analysis has been proposed as an alternative to ANOVA when dealing with missing data as it does not reduce the power of the test substantially due to listwise deletion [27]. A post hoc analysis was performed to further investigate any differences among the segment. A Bonferroni correction was applied for the purpose of multiple comparisons [28].

## 5.5 Results

Figure 5-6 shows the average field potential signals normalized to the AP amplitude of the Stationary segment of all participants. As can be seen, in both ears' signals, the Stationary segment has the smallest AP amplitude and the Down segment has the largest AP amplitude. These are associated with the smallest and largest AP area, Pre- and Post-Potential areas. Table 5-1 summarizes the average AP area, Pre- and Post-potential areas for different segments. For the AP area feature, after running the Linear Mixed Model, the test of fixed effect showed a significant effect of segments (Stationary, Up, Down, Mix) on the AP area in the right ear ( $P=0.009$ ), but not in the left ear ( $P=0.599$ ). Possible reasons for this asymmetry are discussed in the Discussion section. The post hoc analysis determined that only the AP area of the Down segment was significantly different from the Stationary segment in the right ear ( $P=0.036$ ).

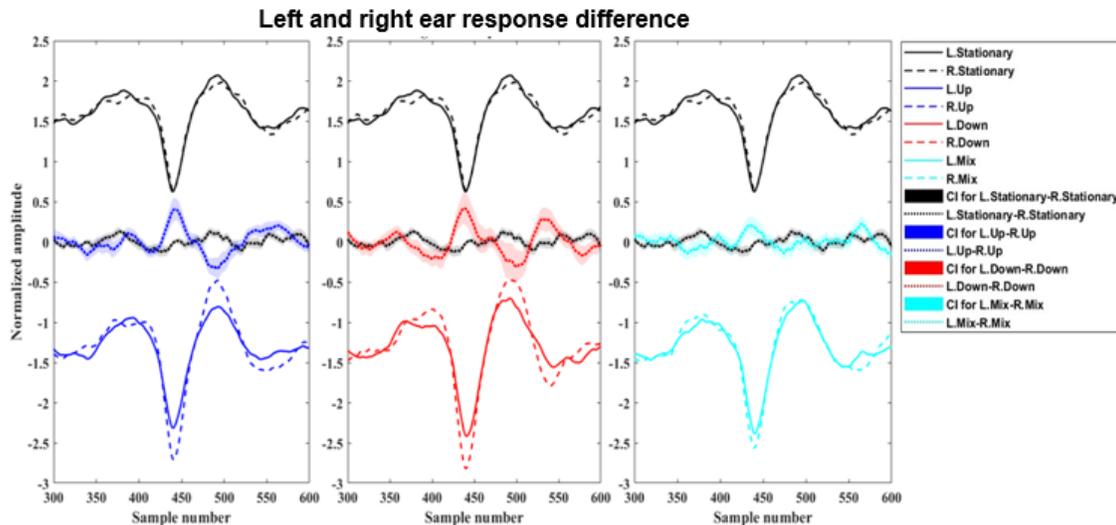


**Figure 5-6: Average field potential of various parts of the roller-coaster trajectory (normalized to the AP amplitude of the Stationary segment) for the right and left ears. The observed difference between right and left vestibular signals shows a hemispheric asymmetry in response to the sensation of self-motion. The larger AP area for the Down segment compared to the Stationary segment represents an increased excitatory vestibular activity which can be correlated with higher degrees of self-motion sensation. A significant difference was observed between the AP area of the Stationary and the Down segment in the right ear.**

**Table 5-1: Mean±SE for AP area as well as Pre- and Post-potential areas.**

	Right ear (mean±SE)				Left ear (mean±SE)			
	Stationary	Up	Down	Mix	Stationary	Up	Down	Mix
<b>Pre</b>	16.3±0.9	21.8±2.6	24.9±4.0	22.5±3.6	16.0±0.7	18.6±1.8	20.0±2.9	17.0±2.3
<b>AP area</b>	22.1±1.4	33.8±4.5	38.2±6.2	32.2±4.1	24.2±1.2	26.6±2.9	30.0±3.9	27.4±3.4
<b>Post</b>	17.9±1.6	30.9±4.5	34.8±6.4	27.6±3.7	20.7±1.8	22.1±2.6	26.7±3.4	23.4±2.8

A roller-coaster experience may induce stress and/or anxiety in participants. Given that 1) psychiatric disorders (e.g. stress, anxiety, depression) can affect vestibular-processing brain regions [29], and 2) there exist a hemispheric asymmetry related to stress and anxiety [30], we subtracted the EVestG signal (average field potential) of the right ear from the left ear (Fig. 5-7) [17] to form a new asymmetry feature. Comparing the Stationary segment with other different segments (Up, Down, Mix), a significant difference was found in the pre- and post-potential areas as well as the AP region (Table 5-2).



**Figure 5-7: Comparison of dynamic segments (Up, Down, Mix) of the roller-coaster with the Stationary segment using the difference between left and right ear vestibular responses (the dynamic segments have been normalized to the Stationary segment). Shaded areas show the 95% confidence interval. The difference between left and right ear responses is significant comparing the Stationary segment with other segments of interest.**

**Table 5-2: Pairwise comparisons between roller coaster segments considering asymmetry feature obtained from the difference between left and right ear vestibular response.**

(I) Segments	(J) Segments	Pre-potential area (sample NO. 345:420)	AP area (sample NO. 420:465)	Post-potential area (sample NO. 465:525)
		Type III Tests of Fixed Effects ( $P<0.001$ )	Type III Tests of Fixed Effects ( $P=0.004$ )	Type III Tests of Fixed Effects ( $P<0.001$ )
Stationary	Up	.004	0.020	0.021
	Down	.006	0.031	0.004
	Mix	.018	0.031	0.011
Up	Stationary	.004	0.020	0.021
	Down	.690	0.626	1.000
	Mix	1.000	1.000	1.000
Down	Stationary	.006	0.031	0.004
	Up	.690	0.626	1.000
	Mix	.346	1.000	0.246
Mix	Stationary	.018	0.031	0.011
	Up	1.000	1.000	1.000
	Down	.346	1.000	0.246

Based on estimated marginal means

The mean difference is significant at the .05 level.\*

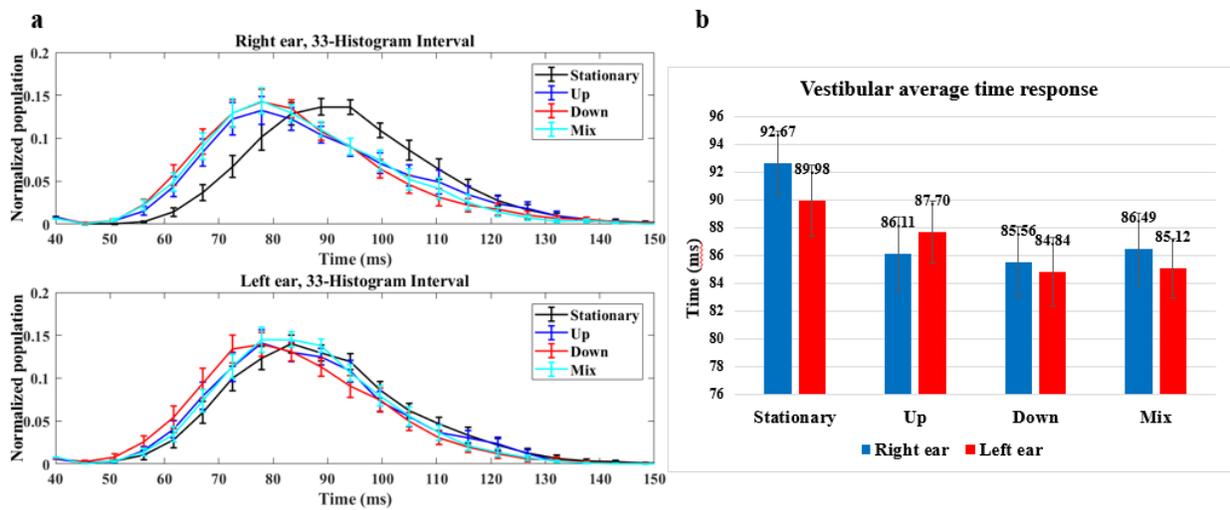
Adjustment for multiple comparisons: Bonferroni.

The average 33-interval histogram depicted in Fig. 5-8a clearly displays the Stationary segment as different from the rest of the segments. It shows longer time intervals between the vestibular firings (shift of the histogram to the right side of the time axis), especially in the right ear. On the other hand, Up, Down and Mix movements, in a similar manner, resulted in shorter time intervals between the vestibular firing (shift of the histogram to the left side of the time axis). Statistical analysis showed there was a significant effect of different roller-coaster segments on the efferent activity in the right ear ( $P=0.018$ ), but not in the left ear ( $P=0.331$ ). Multiple pairwise comparisons between the segments in the right ear revealed a statistically significant difference between the Stationary segment and the Up ( $P=0.025$ ) or Down ( $P=0.040$ ) segments.

To have a better insight into the 33-interval histogram, an alternate representation showing the mean 33-interval histogram mean is depicted in Fig. 5-8b. The bar charts in this figure show the

mean of the individual average 33-interval histogram for the four different segments for the right (blue) and left ears (red). As can be seen, comparing to the Stationary segment, the mean response time in both ears becomes shorter (but not significantly) for the Up, Down, and Mix segments.

Analogous to the AP area feature, for the 33-interval histogram mean feature, the asymmetry between left and right ear vestibular response is also observable. However, by subtracting the vestibular response of the right ear from the left ear, using both the 33-interval histogram mean and 33-interval histogram normalized population, no significant difference resulted between the segments of interest.



**Figure 5-8:** (a) Average 33-interval histogram of various parts of the roller-coaster trajectory for the right (top left) and left (bottom left) ears, and (b) vestibular mean response time (error bars show the standard error); faster vestibular firing (histogram shift to the left in Fig. 8a or shorter height of bar graph in Fig. 8b) resulted from the dynamic segments of the roller-coaster compared to the Stationary segment.

## 5.6 Discussion

Visual information can propagate through the visio-vestibular pathways and reach the vestibular periphery. These signals are hypothesized to bring about changes in the firing pattern of the vestibular afferents. Generally, when feelingvection, optic flow processing provides unreliable information required; other sensory inputs are needed to keep postural stability and/or to keep navigating through the surrounding environment [31]. Specifically, duringvection, the vestibular system assists in discriminating between self and object motions [32]. The results of this study

indicate thevection can affect the vestibular system through either individual or coinciding effect of five different mechanisms which are discussed in detail below.

### **5.6.1 Incongruent sensory information**

From a physiological perspective, the sensation of self-motion can be followed by a period of deactivation (not lack of activation) of the cortical and cerebellar vestibular-processing brain regions (e.g. parieto-insular vestibular cortex, cerebellum), which in turn have direct or indirect connections to the vestibular nuclei [8]. In this study, the feeling of self-motion was sensed to different degrees, as self-reported by all participants, especially when the movement of the cart was in the Down or Mix phases (Fig. 5-4). With regard to the EVestG response, this self-motion experience is associated with larger AP areas and shorter time gaps between efferent-mediated-afferent firings of the 33-interval histogram. One reason for having such feelings can be discrepancies between sensory information received through vision and vestibular systems. This information is sent to specific parts of the brain responsible for keeping our balance and controlling visceral autonomic symptoms (e.g. cerebellum). Under normal conditions, the brain receives congruent and harmonious signals from sensory systems about head/body position relative to other objects in space resulting in a perception that one expects to have. Conflicting sensory information disrupts the expected perception, which can lead to a sensation of self-motion [33]. If disharmony is found, the cerebellum signals a mismatch [34]. This mismatch would provide a sensory reweighing process determining the dominance of either the visual or vestibular systems according to environmental conditions, head acceleration/deceleration or constant velocity [8, 9, 35]. When a sensory input mismatch is present, based on sensorial weights, this mechanism suppresses the less reliable sensory input (vision in this study) and shifts to the dominant modality [8] (vestibular). This incongruent sensory information may affect the activity of the vestibular firing and result in an excitatory (larger AP amplitude of average field potential seen in Fig. 5-6 and Table 5-1) and an increased firing of irregular afferents (correlated with occurrence time of the field potentials detected by the NEER algorithm), which in turn may reduce the time interval between the vestibular firings especially for the Down and Mix movement compared to the Stationary condition as seen in Fig. 5-8. Prolonged mismatched visual-vestibular inputs can eventually cause simulator sickness symptoms categorized into three groups including oculomotor, disorientation, and nausea. Some of these symptoms, such as nausea, in turn, share common brain areas with motion

sickness symptoms [34]. In the current study, most participants showed either none or slight to moderate simulator sickness symptoms (only two participants felt severe symptoms of motion sickness and two other participants had no symptoms), however, all sensedvection to some extent (Appendix 4: SSQ results). This implies thatvection can be experienced without feeling motion sickness andvection in combination with other factors can lead to a sensation of motion sickness.

### **5.6.2 Anxiety and stress factors**

An alternative explanation can be associated with feeling anxiety, fear, and stress during the exposure to the roller-coaster as the visual stimulus. Several brain regions are implicated in both vestibular and cognitive/emotional processing such as the amygdala, insular cortex (a multimodal sensory processing center of the brain which also plays a role in emotional experience e.g. visceromotoric and fear), and medial prefrontal cortex (mPFC) [36]. These regions are hyperactive in individuals suffering from stress and anxiety disorders. The amygdala sends indirect projections to the vestibular nuclei through the insular cortex. The insular cortex has direct connections to the vestibular nuclei. Additionally, there are reciprocal projections to and from the amygdala to the mPFC [37]. Therefore, changes in the shape of the field potential as seen in Fig. 5-6 may be linked to experiencing emotional feelings (i.e. there is a correlation between the insular activity and the intensity of the reaction to emotional stimuli [38]). For example, whilst going Up or Down, individuals may become anxious/stressed when watching the cart moving towards the hilltop or hillside in an immersive virtual environment. These feelings can be followed by a neurotransmitter release tied to anxiety and stress [39] (classical neurotransmitters, which are mediated through synaptic transmission, have a fast excitatory postsynaptic potential in the order of ms, but neuropeptide release takes a few seconds [40]). In addition, feeling anxious/stressed can lead to glucocorticoid being released from the pituitary through other chemical mediators. The pituitary gland, in turn, has some connections with the vestibular system that may modulate vestibular afferents activity (an excitatory action of GABA on developing vestibular afferents of the chick has been shown in [41]) [42].

### **5.6.3 Visual flow/ eye movement**

Six types of eye movement systems (saccadic, smooth pursuit, fixation, vergence, vestibulo-ocular, and optokinetic) interact together during the visual task to meet two goals: attaining fixation

with both eyes and preventing image slip on the retina. During head tilts or object movement in the environment, these eye movements keep images on the fovea [43]. Movements of the eye is controlled by three agonist/antagonist pairs of muscles (the medial rectus, lateral rectus, superior rectus, inferior rectus, superior oblique, and inferior oblique). Each pair generates different movements, for example, the oblique muscles can produce eye rotation clockwise or counterclockwise and the medial and lateral rectus causes eye motion to left or right. The contribution of these muscles to the vestibule-ocular reflex (VOR) has been well studied. Neural projections from the eyes to the central and peripheral vestibular systems can transfer visual information including speed, direction, and color of the objects in the field of view to the vestibular-processing brain regions [12]. The question is whether the vestibular activity recorded in response to roller-coaster experience results from ocular motility. The answer to this question is the topic of another study (unpublished), in which we show that horizontal and vertical eye movements inhibit vestibular activity. However, whilst Down or during Mix movement, the cart moves faster and the visual flow is richer compared to the Stationary and Up movement. The higher temporal or spatial frequency of the field of view (increased visual flow) appears to result in more excitatory activities of the afferent (larger AP area and correspondingly larger AP amplitude) and efferent (smaller 33-interval histogram mean, i.e, shorter time intervals between detected field potentials) vestibular in our study.

#### **5.6.4 Short and long latency visual processing pathways**

Visual processing time with or without awareness can vary depending on the different visual pathways involved [44]. Subcortical pathways originating from the retina (superior colliculus, the pretectum of the midbrain, the suprachiasmatic nucleus of the hypothalamus, the nucleus of the optic tract and terminal dorsal, medial and lateral nuclei) show shorter processing time compared to retinocortical pathways [45]. These short-latency pathways are mostly responsible for primary visual functions such as position, orientation and structural characteristics of an object in the field of view, rather than visual perception (e.g. face recognition) [44]. Moreover, the neural projections from these subcortical brain regions to the amygdala, part of the brain engaged in emotional reactions, along with the pineal gland, that controls circadian rhythm, can clarify the role of visual stimuli in generating a fearful or stressful response to some extent [46]. Accordingly, as the average 33-interval histogram suggests (Fig. 5-8), Down and Mix segments of the rollercoaster

trajectory resulted in a shorter interval between efferent (modulated by afferent) vestibular firings. We speculate that the higher speed of the cart during the Down and Mix movements may trigger the shorter visual pathways in two ways: 1) faster movement of the cart may not provide enough time needed for a detailed and complete visual perception, and 2) the connections between the visual-processing subcortical regions and amygdala/ pineal gland can evoke an increased vestibular response (more firing) [42]. While moving Down or during the Mix movement, it seems that the brain assigns a larger weight to the subcortical visual-processing pathways, rather than those more cortical, in order to see the constantly changing visual field of view.

### **5.6.5 Mental imagery and perceptual anticipation**

Last but not least, our observations might, at least in part, be explained using Finks's theory [47]. Based on Finks's theory, mental imagery and visual perception share many internal neural processes. Fink asserts that forming a mental image of an object may help the initiation of information-processing mechanisms in common with visual perception. Consequently, a shorter time might be needed for visual processing when the predicted object matches the imagery [48]. Indeed, by forming a mental image, visual perception can occur at the intermediate levels of visual-processing brain regions rather than higher levels meaning subcortical pathways (short-latency pathways) may become dominant for processing of the visual information. In this study, it took a longer time for the vestibular system to respond when the cart moved Up or during the Mix movement. In this case, participants did not have any expectation/prediction of how high the cart would go away from the ground or if the cart would make a right or left turn. However, when reaching the top of the hill (in the virtual roller-coaster), they could see the cart trajectory in front of them and could make perceptual anticipation of cart movement towards downhill. As the vestibular nuclei share some connections with the subcortical visual-processing brain regions, reduction in the time of visual processing for the Down segment may correspond to a decrease in the average 33-interval histogram mean of the vestibular efferent (Fig. 5-8) i.e an increased perceived need for vestibular stability or its weighted input.

### **5.6.6 Hemispheric asymmetry**

In [9], fMRI was used to record brain activity in response to the sensation of self-motion. The sensation of self-motion was induced by exposing the participants to a rotating disk with a

windmill pattern. Perceptual switching from object motion to self-motion predominantly activated right hemispheric brain regions including the inferior and intra-parietal cortex and premotor, inferior frontal, and prefrontal cortex. In addition, right-hemispheric dominance (e.g. in the posterior parietal cortex) for visuo-spatial processing has been shown in humans [49]. For these reasons, the observed lateralization between left and right ear vestibular response should not be a surprise.

Our findings indicate that not only the vestibular system can be affected by purely visual stimuli but also the response varies depending on the visual flow, the sensation of self-motion and/or emotional feelings (stress/anxiety) [9, 29, 46, 50–53]. These results are in agreement with the current knowledge ofvection and VR motion sickness showing the implication of different factors (sensory mismatch, visual flow, emotional state, perceptual anticipation) in generatingvection and the ultimate/downstream motion sickness [1, 2, 4].

The lateralization seen in the afferent and efferent vestibular responses may be of clinical significance, helping in the diagnosis of individuals with psychiatric disorders such as acrophobia (fear of heights), stress, and anxiety. Other potential applications of this study include the development of VR games targeting to improve vestibular-related disorders and/or memory [54, 55]. Some examples include screening the vestibular response of individuals with Alzheimer's disease [56] to investigate the effectiveness of different VR cognitive training games, and screening for astronauts' imbalance due to weightlessness-induced alterations after a space mission [57].

For future studies, the VR system performance could be improved. In terms of hardware design, we aim to make use of an eye tracker to better identify and correlate signals related to eye movement that may also be intimately involved within the visually-induced motion sickness mechanism. As well, using a more advanced virtual reality headset with an interpupillary distance (IPD) adjustment option and of a lighter weight, higher frame rate, and a wider field of view could make the experience of immersion more realistic by offering more comfort, smoother motion, and a greater sense of presence in the VR environments. With regard to the software design, by knowing that the spontaneous activity of the vestibular nerve fibers can be affected by its past firing activity, a future VR design that accounts for the time constant of the vestibular slow

component (which can be 20-30 seconds long [22]) may be beneficial. This is achievable by introducing stationary pauses between changing trajectories (e.g. between Up and Down movement).

### **Conflicts of Interest**

All authors declare no conflicts of interest and disclose no affiliations with or involvement in any organization or entity that could potentially bias the subject matter or materials discussed in this manuscript. Figure 5-2c is the author (MA) who has agreed to its publication.

### **Acknowledgment**

This study was partly supported by the Natural science and engineering research council (NSERC) of Canada as well as Mitacs through the Mitacs Accelerate program.

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## Chapter 6- Conclusion

### 6.1 Summary of the Findings

The accomplishment of many daily life activities is highly dependent on the integration of visual and vestibular system information. This integrated contribution controls eye movements, stationary/dynamic balance, circadian rhythm, emotions, etc. To date, little is known about the effect of visual inputs on the vestibular system. Using EVestG coupled with VR in this study, we were able to objectively measure vestibular activity from the ear canals in response to various visual stimuli. Unlike other indirect and non-objective methods of measuring vestibular activity, EVestG recordings measure predominantly vestibular neural activity from vestibular end organs (semicircular canals and otolith organs), the vestibular nerve, and vestibular nuclei [1, 2]. The results of several sub-studies in this manuscript collectively indicate that a purely visual stimulus can evoke a vestibular response measurable at the vestibular periphery. The recorded responses are task-related and depending on the nature of the stimulus, they can evoke less or more excitatory vestibular activity. A synopsis of key findings from each study is presented below:

- The change in vestibular afferent activity was greater for the physical chair tilt compared to its virtual reality replica.
- Measured vestibular afferent (consequent to vestibular efferent activity) modulation was greater for the intensity component of colors (blue, green, red) than that for the hue component.
- Horizontal pursuit and saccade eye movement affected the vestibular activity (afferent and efferent) in an inhibitory way potentially preventing the generation of VOR/optokinetic responses.
- The visually-induced sensation of vection along with other factors (visual flow, stress/anxiety feeling, sensory mismatch) resulted in more excitatory vestibular activity (afferent and efferent) which was more prominent in the right ear compared to the left ear.

The above outcomes are elaborated in more detail and in connection to each other in the following sections.

### 6.1.1 Physical versus Virtual Stimuli

Translational and angular movements of the whole body, including the head, that occurs during a typical EVestG recording predominantly affect one of the peripheral vestibular organs (utricle, saccule, superior, posterior, and horizontal semicircular canals) housed in the inner ear. With the head stationary, we expected and observed that a visual stimulus, using a VR replica of the tilting chair, induced a change to the measured background activity/segment; we hypothesized this was due to the well-documented positive feedback loop from vestibular efferents to vestibular afferents to the vestibular nuclei [3–5]. However, the changes as a result of visual stimuli using VR were smaller at the afferent level compared to those as a result of physical stimuli (direct afferent stimuli) in which the head actually moved.

In Chapter 2, we demonstrated a significant change in the AP area of the average FP for deceleration segments of physical tilts and their virtual replica. The physically driven vestibular responses showed a large change from the background segment. Conversely, the visually stimulated segments showed a very slight change from the corresponding background segment. Additionally, vestibular responses to the visual stimulus generated smaller AP area compared to the physical stimulus (except the background segment); this result implies: 1) visual stimuli driven by VR can affect vestibular afferents through efferent pathways, and 2) efferent-mediated vestibular activity produces smaller average FPs compared to the afferent-mediated (physical movement) vestibular activity at the peripheral level.

The visual stimulus using VR generally affected the vestibular efferent-mediated-afferent in a more excitatory (shorter time intervals between efferent-mediated-afferent firings) manner in comparison to the physical stimulus for tilts including angular accelerations. However, the observed differences between the IH33 mean of the physical and visual tilts were non-significant. This is an important finding because it suggests the effect of physical and visual stimuli may be similar at the central/intermediate level of sensory information processing (vestibular nuclei and efferent), while they are different at the periphery.

### **6.1.2 Color experiment**

Following the first study, which indicated the visual stimuli can also evoke a vestibular response, we questioned how different colors could impact the evoked vestibular activity. The impact of different color light stimuli and their components (hue and intensity) on the vestibular response was presented in Chapter 3. Since ambient light mainly affects the subcortical brain regions (also associated with efferent vestibular activity) [6], to interpret the results of the experiments we focused on the IH33 mean feature, which is representative of the efferent-mediated-afferent vestibular activity. Visual information affecting these subcortical regions can, in turn, modulate the vestibular afferent activity through the efferent system. Collectively, our findings indicate that short exposure to monochromatic lights (blue, green, red), as well as black and white backgrounds, can evoke different vestibular responses. In the color experiment, exposure to the black background caused more excitatory vestibular activity, while exposure to red resulted in the least excitatory activity of vestibular efferent. The reason for such difference between black and red backgrounds can be because of the existence of short (e.g. retinotectal and retinocollicular) and long (retinocortical) visual pathways that carry different information regarding achromatic and chromatic vision and are terminated at the VN.

Investigating the effect of individual color components (hue and intensity) showed that changes in hue did not evoke substantial changes in the vestibular response if the illuminance level was fixed. On the other hand, variation in the intensity level of the lights generally resulted in the vestibular responses changing proportionally with the illuminance level. These outcomes are encouraging as this is the first time that EVestG has been used as a measure to record vestibular changes following a visual stimulus of different hues and intensities. The study shows the increased reliance/importance of intensity compared to color.

### **6.1.3 Eye movement**

The joint contributions of the vestibular and visual systems assist humans in stabilizing their gaze during head or environmental motion. The effect of two conjunctive eye movements, horizontal smooth pursuit and saccade, on the vestibular activity were investigated in Chapter 4. Both pursuit and saccade eye movements resulted in a smaller AP area as well as longer IH33 mean time interval, implying less excitatory/more inhibitory vestibular efferent and afferent activity. This

reduced vestibular activity during eye movement compared to pre-stimuli (Pre-Background) possibly helps prevent the movement of the head when it is not necessary and in doing so avoids generating VOR reflexes or optokinetic responses [7, 8].

The findings also showed more excitatory vestibular activity during the pursuit compared to the saccade eye movements although this difference was not statistically significant. This can be linked to the characteristic of each eye movement as the smooth pursuits are usually concerned with speed detection and saccades are concerned with the position of the object in the visual field [9].

#### **6.1.4 Vection**

The illusion of self-motion induced through purely visual stimulus is an interesting visual phenomenon. Utilizing a VR rollercoaster, the impact of self-motion sensation on the central and peripheral vestibular system was investigated in Chapter 5. A correlation was found between the vestibular activity and the degree of self-motion sensation as well as comorbid anxiety/stress feelings. The sensation of self-motion is explainable by mismatch theory [12, 14], in which the incongruent sensory information results in misinterpretation of the sensory inputs. The incongruent sensory signals are received by the cerebellum which is believed to have an internal conceptual model. The external feedback from the surrounding is constantly compared with the cerebellar internal model. In the presence of any sensory mismatch a sensory reweighing mechanism is activated that suppresses the less reliable sensory input and assigns more weight to the more reliable sensory modality. In our experiments, we observed that a stronger feeling of self-motion evokes a larger AP amplitude as well as shorter time intervals between the occurrence time of the FPs. These observations are associated with more excitatory afferent and efferent activities, respectively. As well, the hemispheric asymmetry seen for FP and IH33 results is in agreement with other studies [14, 15] that utilized imaging techniques to assess the vestibular response indirectly. We hypothesize that perceptual switching from object motion to self-motion as well as feeling anxious and stressed can predominantly activate the right hemispheric brain regions [16, 17] and suppress the neural activity of specific brain regions on the left hemisphere [18].

Altogether, these findings reveal the responsiveness of the vestibular system, measured by EVestG technology, to different visual stimuli. The temporal and spatial characteristics of the field of view

are two determinant factors that affect visually-induced vestibular responses. According to the results from this study, when the spatio-temporal characteristics of the VR environment change participants' perceptions from object-motion to self-motion, the activity of the vestibular system (central and peripheral) increases. Similar excitatory behavior from the vestibular system is obtained when visual inputs received through the eyes are minimum (in case of exposure to black background). Other visual stimuli (i.e. color experiment and eye movements) utilized in this body of research affect the vestibular system mainly in an inhibitory manner. This inhibitory behavior may help to keep the stationary balance while sitting by adjusting the accommodation reflex and preventing unnecessary head movements.

## **6.2 Recommendation for future work**

The presented work was conducted on healthy participants. The exploratory nature of the technique and its outcomes show potential within vestibular disorder research. The use of VR visual stimuli in this body of research will facilitate the development of a portable EVestG system (i.e. potentially without the need for a hydraulic chair or shielded/anechoic room) with the capability of being used for diagnostic and clinical application.

Further investigation on the following topics will expand our understanding of how pure visual inputs change the vestibular response.

### **6.2.1 Physical and virtual tilts in the supine position**

The differences between physical and virtual tilts were discussed in Chapter 2 of this dissertation. All physical tilts and their virtual replica were conducted while the participants were sitting in an upright position. Generating VR environments that can simulate tilts in the supine position is of scientific value knowing that each specific tilt can predominantly evoke particular (e.g. utricle) organs of the vestibular periphery. For instance, by conducting the up-down movement in the supine position the utricle will be predominantly affected and we can investigate how the generated vestibular response is different from the saccule response (predominantly affected by up-down movement in the sitting position).

### **6.2.2 Simultaneous physical and virtual tilts**

In Chapters 2 and 5, the sensorial mismatch theory is explained. According to this theory, the incongruence between sensory information can shift the sensorial reweighing mechanism controlled by the cerebellum by shifting the sensorial weight from a less reliable system to a more reliable system. It would be of significant value if physical tilts are conducted during the open-eye condition to investigate the combined effect of vestibular and visual stimuli at the same time. However, the effect of saccade artifacts (microsaccades or exogenously-driven sensorimotor saccades) on the vestibular response should be taken into account when using visual and vestibular stimuli together.

### **6.2.3 The speed of the virtual tilts**

The virtual tilts were simulated to replicate the same velocity pattern as their physical chair tilts. Investigating the impact of velocity on generated vestibular responses by exposure to virtual stimuli can broaden our insights into the temporal information processing/ velocity storage mechanism in the vestibular system.

### **6.2.4 Primary versus secondary colors and the pattern of the background**

The effect of primary RGB colors (blue, green, red) and their components (hue, and intensity) on the vestibular system were investigated in Chapter 3. Adding two primary colors together yields the secondary colors (magenta, yellow, cyan). Assessing the impact of the secondary colors on the vestibular is useful in terms of discovering how information regarding secondary lights are translated into the temporal frequency and sent to the vestibular processing brain regions (since two types of cone photoreceptors are engaged in processing of the secondary colors instead of one type for primary colors).

In this study, we considered solid backgrounds to rule out the possibility of activation of brain regions associated with pattern recognition. It has been well established that spatial frequency is an important characteristic of the visual field of view allowing us to better discriminate objects. Different black-and-white patterns (checkerboard, stripes, etc.) can be generated in the VR environment to test the effect of spatial frequency on the vestibular system.

Besides, it is noteworthy to mention that the produced vestibular response as a result of light exposure depends on many factors including age [19], ethnicity [20], genotype (being a morning or evening person), duration and intensity of light, quality of sleep, time of the day that the recording is performed, etc. [21]. In this experiment, the time of the recordings was not constant although not early morning and not late evening [22, 23]. Another shortcoming of these sets of experiments is likely the effect of color presentation order on the results [24, 25]; that can be addressed in future research.

### **6.2.5 Different types of eye movement**

Two types of eye movements (smooth pursuit and saccade) were investigated in Chapter 4. Other eye movements include vergence, optokinetic, VOR, and fixation. Each eye movement involves the activation of different extraocular muscles which are controlled through the activity of the central and peripheral vestibular regions. Hence, it is important to design VR environments that simulate other types of eye movements that have not been studied here.

### **6.2.6 Screening bio-signals and time constant of vestibular spontaneous activity**

In Chapter 5, the feeling of stress and anxiety was reported to have partial contributions to vestibular responses to the roller coaster experiment. The best practice to prove our assumption/claim is to screen participants' biomarkers such as body temperature, pulse, blood pressure, etc. The correlation between these measures and the vestibular response to each trajectory can elaborate our knowledge about vestibular function.

In addition, knowing that spontaneous activity of the vestibular nerve fibers can be affected by the past firing events, a future VR design that accounts for the time constant of the vestibular slow component (which can be 20-30 seconds long) would be beneficial.

### **6.2.7 General recommendations for future work**

In addition to the above recommendations, two major suggestions that apply to all four sub-studies and would add to the credibility of the results are: 1) recruitment of a larger sample size and 2) implementation of an eye-tracking system to better facilitate the identification of signals related to eye movements. The use of an eye-tracking system can especially help to identify saccade artifacts

when visual stimuli are present since according to the sub-study presented in Chapter 4, the effect of endogenously-driven volitional saccade tasks on the vestibular response can be significant.

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### **Thesis contribution to the current state of knowledge**

This original body of research pioneers using the EVestG technique for investigating visual-vestibular interaction in humans. The contribution and impact of this academic research can be summarized as follows:

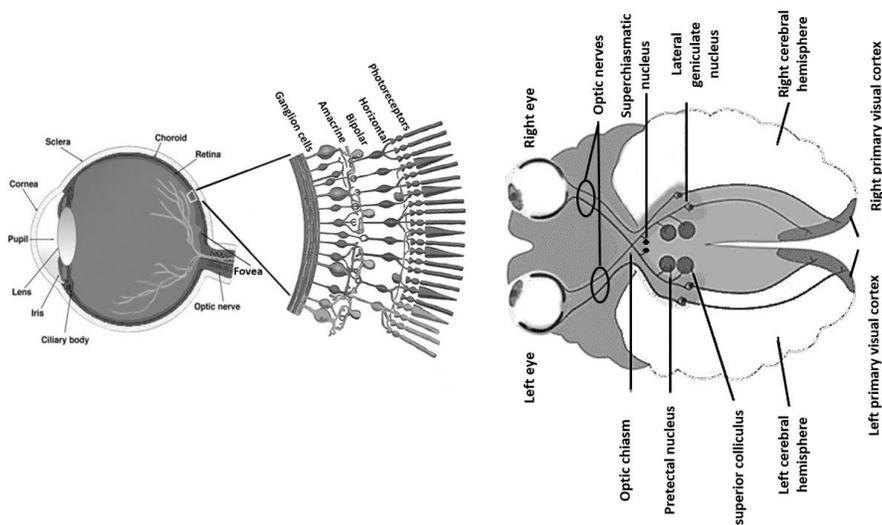
- 1) Physical and visual stimuli produce measurable vestibular responses that are different in terms of afferent activity. These differences, between physical and virtual vestibular responses, are dependent on the eyes being either open or closed.
- 2) The vestibular system responds to light of different colors (i.e. blue, green, red) showing more sensitivity to the luminance level rather than hue change.
- 3) Both pursuit and saccade eye movements inhibited the activity of both central (postulated efferent pathway) and peripheral (afferent) vestibular systems presumably altering the vestibulo-ocular reflex (VOR) and optokinetic response (eye movement in response to the movement of full visual field images).
- 4) The combined effect of the visually induced sensation of self-motion together with a concurrent/co-occurring stress/anxiety factor (perhaps because of fear of the roller-coaster ride) can affect the activity of vestibular afferents in an excitatory way.

The effectiveness of the visual stimuli in generating vestibular responses, which can be measured objectively and non-invasively utilizing the EVestG technique, offers advantages in many areas of research (e.g. space, healthcare, education) and clinical applications (diagnosis). Using the proposed test, vestibular rehabilitation, compensation, and substitution can be screened following exposure to VR training games or the test can be simply used for diagnostic purposes.

## **Appendix 1 (A1)- The Neural Pathways Connecting Visual and Vestibular Systems**

Human eyes are intricate apparatus that include a number of different structures working together to provide us the visual representation of the surrounding environment. The retina is the light-sensitive component of the eye containing layers of photoreceptors and different cells (amacrine, bipolar, horizontal). In the retina, light photons are converted to electrical signals (following chemical changes in the pigment of the photoreceptors) that are then carried to the optic nerves (axons of ganglion cells). Passing through the optic nerves, these electrical signals reach the optic tract where four major projections diverge; they are the lateral geniculate nucleus (LGN) in the thalamus, superior colliculus, pretectum of the midbrain, and suprachiasmatic nucleus of the hypothalamus (Fig. A1-1). From these regions, visual information projects to the vestibular nuclei through a number of different neural pathways (Fig. A1-1). These pathways are different in terms of visual processing time and function (e.g. responsible for elementary and complex visual function). The retino-cortical pathway, through the LGN, projects to the visual cortex wherein delicate visual processing such as the perception of emotions is performed. Based on the results of PET, fMRI and lesion studies [1], Brodmann's areas 17–21, 37, 39, and 7 of the visual cortex have been reported to contribute to the sensation of self-motion. These areas send fibers to the cerebellum and more particularly the vestibulocerebellum (the flocculus and that part of the vermis connected to it) which have a bi-directional connection to the vestibular nuclei [2]. The Vestibulocerebellar region receives inputs from the vestibular nuclei and the primary vestibular afferents and project efferents to the vestibular nuclei [2]. The processing time through retino-cortical pathway is about 100-150 ms which is longer than that of the other three subcortical visual pathways: the retino-colliculus, the retino-tectal and the retino hypothalamic pathways [3, 4]. Short-latency retino-collicular pathways involved in primary visual processing tasks, e.g. motion detection and eye movements, have connections to the vestibular nuclei, which in turn, projects fibers to extraocular muscles through the abducens, trochlear and oculomotor nuclei; from these regions, three types of voluntary eye movements including smooth pursuit (i.e. tracking a moving object), saccade (i.e. rapid gaze shift towards the object of interest) and vergence (simultaneous movement of both eyes in opposite directions to attain binocular vision) can be controlled [5]. Processing of the visual information occurs approximately within 80 ms through the retino-collicular pathway [6]. The third major projection from the optic tract that sends neurons to the vestibular nuclei is the pretectal area, which is involved in pupillary eye reflex (PLR) and

accommodation. Other projections from pretectal areas are to the thalamus, subthalamus, superior colliculus and vestibulo-cerebellum. The last major split originating from the optic tract is the suprachiasmatic nucleus, involved in the regulation of neuronal and hormonal activities, which sends fibers to the hypothalamic nuclei and pineal gland and is responsible for controlling circadian rhythms, reproduction, and human mood [7]. All these regions, directly or indirectly, have some connections with the vestibular nuclei. Vestibular nuclei send fibers via the efferent vestibular system toward the vestibular periphery, where motion-sensitive sensors (hair cells of semicircular canals and otolith organ) exist.



**Figure A1-1: On the left: Schematic diagram of the human eye with some key structures labeled and the ‘wiring’ of cells in the human retina [8]. Adapted from “How the Retina Works: Much of the construction of an image takes place in the retina itself through the use of specialized neural circuits”, by Helga Kolb, 2003, Am Sci 91 28–35. Adapted with permission. On the right: Visual projections towards the vestibular processing brain regions.**

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## Appendix 2 (A2)- Research Study Eligibility Questionnaire



UNIVERSITY  
OF MANITOBA

**Research study eligibility questionnaire    Study ID: HS21032(B2017:097)**

**Participant code:** \_\_\_\_\_

1. Is the participant age between 18 to 60 years old?

Yes  How old? \_\_\_\_\_

No

2. Does the participant has an intact vestibular system?

Yes  VESTIBULAR DISORDERS ACTIVITIES OF DAILY LIVING (VADL) SCALE? \_\_\_\_\_

No

3. Does the participant suffer from any of the following neurological/ brain/ head conditions?

A history of stroke? Yes  No

Head injury? Yes  No

Loss of consciousness? Yes  No

Epilepsy? Yes  No

Any balance disorders? Yes  No

4. Does the participant suffer from any of the following eye conditions?

Dyschromatopsia (color blindness)? Yes  No

Absence of stereovision? Yes  No



Best corrected visual acuity less than 20/40 in one or two eyes? Yes No

Eye misalignment? Yes  No

Complete suppression of one eye when both eyes are open? Yes  No

5. Does the participant suffer from any of the following disorders?

HIV? Yes  No

Hepatitis? Yes  No

Skin lesions involving the ear canal or any others? Yes  No

6. Does the patient have any other comorbidities e.g. Depression, Anxiety etc.

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## Appendix 3 (A3)- Consent Form



### Graduate Program in Biomedical Engineering

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University of Manitoba  
Winnipeg, Manitoba  
Canada R3T 5V6  
Telephone (204) 474-6469

#### RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

**Title of Study:** "Visual-Vestibular Interaction (VVI): Changes in the Vestibular Response following Different Types of Visual Stimuli"

**Protocol Version:** “\_2\_”

**Study ID:** HS21032 (B2017:097)

**Principal Investigator:** “Professor Zahra Moussavi, Electrical & Computer Engineering, University of Manitoba, 204-474-7023”

**Co-Investigators:** “Professor Brian Lithgow, Riverview Health Center, University of Manitoba, 204-474-6406 AND Dr. Behzad Mansouri, University of Manitoba”

**Sponsor:** “Natural Science and Engineering Research Council (NSERC) of Canada”

You are being asked to participate in a pilot human research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any word or information that you do not clearly understand.

This study is financially supported by the University of Manitoba and Natural Science and Engineering Research Council (NSERC) of Canada.

Disclosure – Prof. Brian Lithgow is the inventor of the EVestG technology.

## **Purpose of the Study**

The purpose of this project is to evaluate the responsiveness of the vestibular system following exposure to different visual stimuli. The vestibular system is our balance apparatus inside the inner ear which contributes to motion detection, equilibrium, and spatial orientation. A new objective method called Electrovestibulography (EVestG) is used to record vestibular responses. Various visual stimuli are applied to the participants by immersing them into different virtual reality environments. Employing visual stimulation might bring about neural activity similar to physical tilt stimulation as currently used in EVestG testing. Results of this study may offer a better insight into visual-vestibular interaction, extent and effectiveness of different visual stimuli and possibly as an alternative to the physical vestibular stimuli used in EVestG.

## **Study Procedures**

If you are interested to take part in this study, you are provided a questionnaire to check your eligibility. If you pass the first stage eligibility criteria, we will make an appointment for you to be visited by our research group-neuro-ophthalmologist for checking the second stage eligibility that involves eye and vision examinations. Since the vision system is involved in this study, it is necessary to examine visual acuity, colour blindness, astigmatism, poor eyesight or other eye conditions leading to reduced visual acuity of participants. If you meet all the eligibility criteria, you will be then enrolled in the study. The experiments will be run at Riverview Health Center (Room PE-446, Admin Building, 1 Morley Ave.). The experiments are summarized as the followings:

- having EVestG measurements taken in response to passive whole body movements (physical tilts). This involves being seated in a chair and having electrodes placed on specific spots on your forehead, in your ear and earlobes. The electrodes measure your brain's activities while you are relaxing in a chair, and during smooth tilts of the chair in different directions including up-down, forward-back as well as side to side.
- visual stimuli are applied using a headset in virtual reality environment. EVestG recording is performed in the stationary position (no chair movement) in this case. Four different environments are shown: 1) a replica of the physical chair tilt in virtual reality, 2) a sequence of colours (hue and luminance levels will be investigated individually), 3) a moving object in the scene, and 4) a roller coaster. Each visual stimulation is applied only once.
- completing two simple questionnaires to receive your feedback about the experiments.
- A hue discrimination test, in which you replace squares of different colors shown on a monitor in the hue order, will also be performed at the end of the experiment.
- These tasks can take up to 3 hours.
- In cases that your recorded EVestG signals are corrupted by noise due to events beyond our control (e.g. power failure), we may ask you to participate in a subsequent recording.

If at any time, while playing the physical or visual stimuli, you decide to stop participating in this study, you can simply leave the experiment and request the study staff to help you out of the on-going trial.

## **Benefits**

Possible benefits include comprehensive vision examination by our research group ophthalmologist and contributing to a better understanding of visual-vestibular interaction.

## **Costs**

**All clinic and professional fees, diagnostic and laboratory tests, which will be performed as part of this study, are provided at no cost to you.**

## **Risks and Discomforts**

Possible risks, side effects and discomforts are minimal. The residue of the electrode's gel in the ear after electrode removal may cause an ear infection. The residue of gel can be removed simply by washing the ears with warm water.

**You must wash your ears with warm water within a couple of hours after the experiment. Ear infection is a potential risk factor; washing the ears following the experiment minimizes the risk to a large extent.**

**Although very unlikely, the Insertion of a wick (soft cotton bud or cue tip like) electrode into your ear canal may potentially cause eardrum perforation. However, to minimize the potential discomfort only trained researchers will insert this electrode. You will be seated comfortably in a chair while the trained researcher inserts an electrode into your ear canal, to a depth of about 1cm, to rest close to but not touching the eardrum. The ear canal and earlobe electrodes are made with a soft cotton wool tip and very flexible stem to minimize any insertion risks.**

**Some of the questions in the screening questionnaire might be uncomfortable (e.g. embarrassment, feeling upset) for you to be answered. In that case, let us know and we can skip the question.**

**Simulator sickness is another potential risk that can be perceived while experiencing roller coaster trial. Some people may feel dizziness, headache or nausea. You can ask the research assistant to stop the procedure in case of having any symptom of motion sickness.**

## **Confidentiality**

Information gathered in this research study may be published or presented in public forums; however, your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law.

The University of Manitoba Biomedical Research Ethics Board may review research-related records for quality assurance purposes. All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the Riverview Health Centre. In the case of any publication of results of this study, information will be provided in such a way that you cannot be identified.

### **Voluntary Participation/ Withdrawal from the Study**

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

### **Results of the Project**

All participants have the option of receiving a lay summary of group findings. If you wish to be informed of the results of this project, please consult with research staff at the time of your participation or you may contact the principle investigator of this study, Dr. Zahra Moussavi by email (Zahra.Moussavi@umanitoba.ca).

### **Questions**

If you require further information or if you have any problems concerning this project (for example, any side effects), you can contact the researchers and/or their research assistant responsible for this project, Professor Brian Lithgow, Professor Zahra Moussavi (204-474-7023). Questions regarding your rights as a research participant, you may contact the University of Manitoba Biomedical Research Ethics Board at 204-789-3389.

### **Statement of Consent**

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Moussavi and/or her study staff. I have had my questions answered by them in a language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. Any relationship (such as employee, student or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by NSERC and The University of Manitoba Biomedical Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

I agree to be contacted in relation to this study. **Yes**  **No**

**Participant signature** \_\_\_\_\_ **Date** \_\_\_\_\_  
(day/month/year)

**Participant printed name:** \_\_\_\_\_ **Date of Birth:**  
\_\_\_\_\_

**Contact Number:** \_\_\_\_\_ **Email:**  
\_\_\_\_\_

\*\*\*\*\*  
\*\*\*\*\*

**The study coordinator Part**

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

**Printed Name:** \_\_\_\_\_ **Date** \_\_\_\_\_  
(day/month/year)

**Signature:** \_\_\_\_\_

**Role in the study:** \_\_\_\_\_ [This must be done by an authorized/qualified member of the research team i.e. investigator, study nurse, etc.]

**Relationship to study team members:** \_\_\_\_\_ [eg. supervisor, teacher/professor or family member.]

**Assigned Code:** \_\_\_\_\_

\*\*\*\*\*

**Upon completion of the experiment**

*It is confirmed that the participant feels fine after the experiment with no discomfort due to electrode insertion; and that the research assistant has checked the participant's ears after the experiment and all is well.*

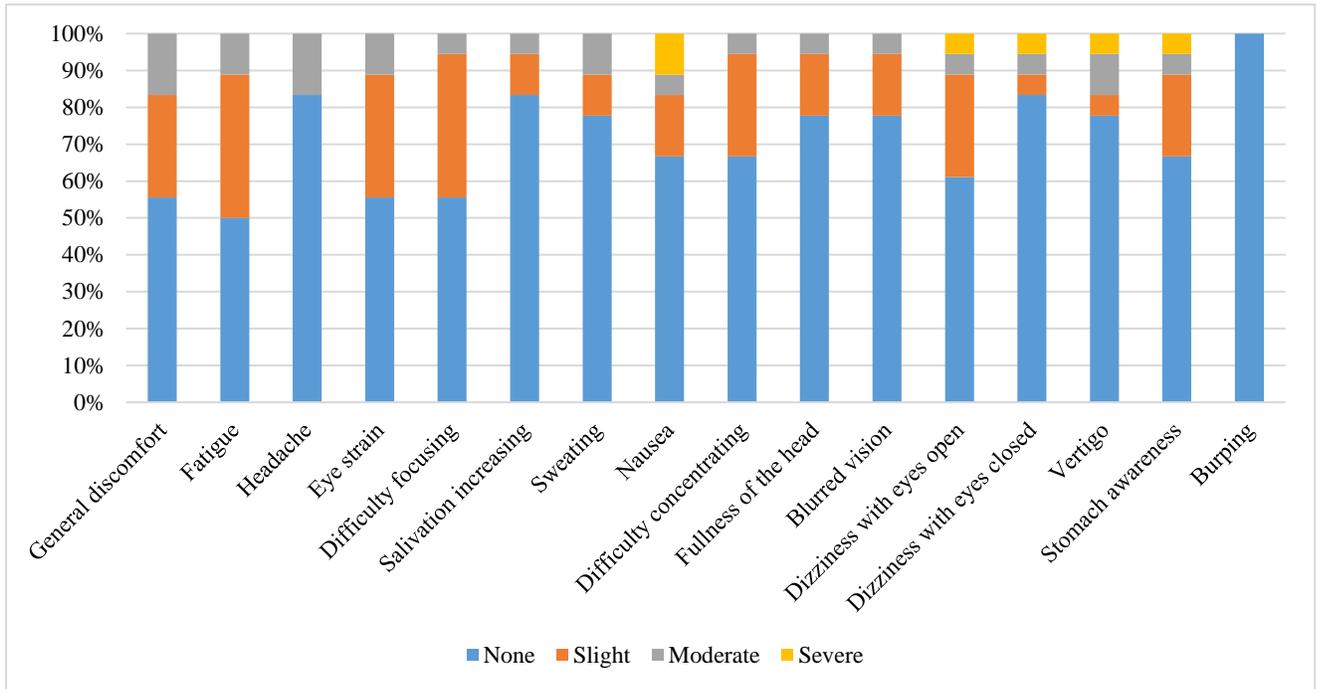
\_\_\_\_\_

Participant's Signature  
Date

\_\_\_\_\_

Research Assistant's Signature

### Appendix 4 (A4)- Simulator Sickness Questionnaire



**Figure A4-1: Percentage of physical conditions experienced by the participant using simulator sickness questionnaire**