Electrical vestibular stimulation for human standing balance and motion perception
Effects of stimulation arrangement and signal design

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Abstract—**Vestibular information from both sides of the head converges within the vestibular nuclei to contribute to postural stabilization of the head and body, and to provide accurate perceptions of self-motion. It remains uncertain whether these responses are evoked through equivalent integration processes, because our perception of head position is not always aligned with postural responses evoked by vestibular perturbations, where postural responses are expected to align with head orientation. In this study, we examine if the contributions from bilateral vestibular organs are equal in postural responses and the perceptions of motion that are evoked during an artificial vestibular disturbance. This study also examines whether there is a linear integration of vestibular signals in the postural responses and perceived motion generated by the vestibular disturbance. Electrical vestibular stimulation (EVS) was used to evoke whole-body and compensatory ankle-muscle responses during a standing balance task or the sensation of angular velocity about a roll axis during a perceived motion task. Results obtained from stimulating one side (monaural) demonstrate that individual leg muscles receive equal inputs from both labyrinths, and that the motion perceived by activity of each labyrinth is equivalent. Principles of left-right integration were evaluated by comparing the sum of monaural responses with stimulation on both sides (binaural). The possibility of non-linear integration was demonstrated in postural responses, as the monaural sum was larger than the binaural equivalent. For perception no significant differences were found. Interestingly, comparing two binaural conditions, were one was assumed to be an independent stimulation on each side, led to equal postural and perceptual responses. Therefore it is likely that current flows in equal pathways, i.e. between the ears in both conditions. This raises uncertainty whether monaural vestibular stimulation modulates the firing rate of each labyrinth independently and about the conclusion that bilateral vestibular signals are integrated non-linearly.**

Keywords—*Vestibular disturbance, electrical stimulation, human standing balance, motion perception, bilaterality, integration*

I. INTRODUCTION

The nervous system's ability to integrate multiple sources of sensory information allows humans to interact with the surrounding environment and control body posture. The vestibular system is one of these sensory sources and encodes linear and rotational motion of the head. This sensory information is obtained from duplicate copies of two separate sensors (i.e., semicircular canals and otoliths, respectively) located on either side of the head. The signals from these separate sensors converge within the vestibular nuclei to contribute to postural stabilization of the head and body, and to provide accurate perceptions of self-motion. While the importance of vestibular signals for both posture and perception is well known, it remains uncertain whether these multiple sensory sources contribute to each task through equivalent integration processes. In this study, we examine how inputs from left and right vestibular organs are combined for the control of human balance and self-motion perception to provide a comprehensive evaluation of how the brain integrates bilateral vestibular signals.

Electrical vestibular stimulation (EVS) can be used to investigate the vestibular contribution to standing balance and self-motion perception. EVS involves delivering a mild and non-invasive electrical current using electrodes placed behind the ears to modulate the firing rate of primary vestibular afferents [1, 2]. Depending on the polarity of the stimulus (i.e. cathodal or anodal), EVS modulates afferent activity by either increasing or decreasing firing rates by an equal amount [1]. During standing balance, EVS evokes whole-body postural responses and compensatory muscle responses in equal but opposite directions in leg muscles involved in balance control [3]. The direction of these responses is dependent on the alignment of the head relative to the feet [4, 5]; in a binaural bipolar arrangement, whole-body sway evoked by EVS is along the interaural line. When applied monaurally, electrodes placed behind the ear and on the spinous process of the first thoracic vertebra (T1) deliver current that is assumed to activate a single labyrinth [3]. This approach has been used to demonstrate that leg muscles receive equal inputs from the two labyrinths, and, perhaps more interestingly, that the vector sum of left and right monaural stimulation is larger than simultaneous monaural stimulation of both sides. These observations have led to the suggestion that bilateral vestibular information is integrated non-linearly for postural control [3]. We therefore hypothesize that each labyrinth projects symmetrically to both sides of the body. We also hypothesize that bilateral vestibular information is integrated non-linearly for postural control.

Perceptually, EVS evokes a sensation of angular velocity [6] about an roll axis equivalent to the roll axis estimated from standing balance conditions [7]. However, it remains unknown whether the principles of left/right integration for posture described above also apply for perception. There is evidence for differential processing of vestibular information for posture and perception when human subjects stand with their eyes closed and their head directed over their shoulder for prolonged periods of time. Initially, a subject's perception of head direction and the postural responses evoked by EVS are aligned with the actual head orientation. Over time, both the perceptual and postural representations of head orientation drift towards a head

forward direction. However, when subjects opened their eyes, the perception of head orientation returned to normal while the postural responses remained displaced [8]. This indicates that postural responses are not always aligned with the perception of the head position and the sensory processing that occurs across these two tasks may be different. As we do not know what will happen during different stimulation arrangements, we expect the same responses as during postural control. We therefore hypothesize that bilateral vestibular information is also integrated non-linearly for perception.

An additional confound to the above postural responses is the recent observation that monaural stimulation can lead to current spread from the stimulation side of the head to the contralateral vestibular organ. In unilateral vestibular loss patients, monaural stimulation of the non-functioning side can lead to vestibular evoked reflexive eye movements [9], where an absent response was expected. This raises questions about the isolation of monaural stimulation to a unilateral vestibular organ. This puts the conclusion that the integration of postural responses is non-linear at risk since the current from one side may have spread to the opposite side. To investigate this we will compare two binaural configurations, where one includes stimulation between ears and the other one is a simultaneous but independent monaural stimulation on both sides of the head. If the two monaural stimulations are truly independent, then it is possible that different responses could be evoked when comparing to the normal binaural stimulation.

This study consists of two experiments. In our first experiment, we will re-examine the integration of vestibular signals for posture by replicating the experiments of Day et al. [3], to both replicate their results and to add to it the comparison between the two binaural configurations. In our second experiment, we will investigate the integration of vestibular signals for perception by estimating the threshold of perceived virtual rotations to monaural and binaural vestibular stimulation. Our aim is to determine if bilateral vestibular integration processes are similar for posture and perception.

II. MATERIALS AND METHODS

A. Subjects

19 healthy male subjects (age 26.1 ± 3.6 yrs (mean $\pm$ SD)) with no self-reported history of neurological disorders participated in this study, where 10 participated in the first experiment and 9 others participated in the second experiment. The experiments were conducted at Delft University of Technology, The Netherlands (see Experiment 1) and the University of British Columbia, Canada (see Experiment 2). All subjects provided written informed consent after receiving explanation of the experimental procedures. Experimental procedures were approved by the Human Research Ethics Committee of Delft University of Technology and the University of British Columbia's Clinical Research Ethics Board, and conformed to the standards of the Declaration of Helsinki.

Two separate experiments were conducted to investigate the bilateral integration of vestibular signals. Experiment 1 examined vestibular integration and the effects of signal design in vestibular evoked motor responses during standing balance. Experiment 2 examined vestibular integration in the perception of virtually evoked motion.

B. Vestibular stimuli

For all experiments, electrical vestibular stimulation was delivered using carbon rubber electrodes (Uni-Patch, Wabasha,

USA) coated with Spectral 360 electrode gel (Parker Laboratories, Fairfield, NJ, USA) and secured on the participant's head with tape. The electrical stimulus was delivered with linear isolated stimulators (STMISOLA, Biopac Systems, Goleta, USA), which received input analogue signals via a digital-to-analogue board (Experiment 1; NI USB-6211, Experiment 2; PXI-6289, both National Instruments, Austin, USA). The signals were generated using Matlab (Experiment 1; Mathworks Inc., Natick, USA) or LabVIEW (Experiment 2; National Instruments, Austin, USA), and sent out using custom LabVIEW software programs.

Experiments 1 and 2 were performed with four different stimulation arrangements using four separate electrodes. Two electrodes were attached to the skin over both mastoid processes and the other two were placed $2 \, \text{cm}$ medially from the midline at the level of the T1 spinous process. Using the constant-current stimulators, current was either applied on both sides of the head, i.e. binaural stimulation, or on only one side of the head, i.e. monaural stimulation. The four stimulation arrangements included two monaural configurations (i.e., *left* monaural - left mastoid and left T1 electrodes; *right* monaural - right mastoid and right T1 electrodes) and two binaural configurations (i.e., *left-right* binaural - mastoid and T1 electrodes on both the left and right side but always with opposing polarities; normal *binaural* - left and right mastoid electrodes). The left-right binaural configuration is thought to provide independent stimulation of each vestibular apparatus; however, it remains unknown if this configuration differs from normal binaural stimulation. Therefore, we included the normal binaural configuration for a direct comparison to the left-right binaural configuration.

The sensation of motion evoked by the electrical stimulus has been modelled based on the distribution of afferents within the vestibular organ and the assumption that afferents from both sensors are activated equally. The contribution from the otolith organs is thought to be minimal [10] due to the near symmetry of the afferent populations across the macular striola of the utricles [11]. The resultant vector summation of all otolith and canal afferents predicts a rotation vector oriented $19°$ up from Reid's plane through the centre of the skull [7, 10]. In a binaural bipolar configuration, the EVS vector sum will produce a large roll component towards the cathode and a small yaw component. When standing with the head facing forwards and the head rotated $19°$ up, corrective sway to the stimulus will be evoked towards the anodal electrode [10, 12]. The same configuration applied while seated and with the head rotated $71°$ downwards will evoke the perception of head rotation towards the cathodal electrode [6].

1) Experiment 1: Two different types of stimuli were used to modulate the firing rate of primary vestibular afferents during Experiment 1. In Experiment 1a we used square-wave galvanic vestibular stimulation (GVS) pulses, and in Experiment 1b we used stochastic vestibular stimulation (SVS). Both stimuli evoke an illusory sensation of motion and responses in muscles active in the maintenance of balance, as well as ankle torque and whole-body postural responses [13–15].

In experiment 1a, square wave GVS pulses were delivered with an amplitude of $2 \, \text{mA}$. These square wave pulses induce current flow towards the anode. In experiment 1b, the electrical stimulus was delivered as a continuous stochastic signal. The signal was designed with a 3rd order Butterworth filter, a bandwidth of $0 \, \text{Hz}$ to $25 \, \text{Hz}$ and a peak amplitude of $\pm \, 5 \, \text{mA}$

(root mean square $1.513\,\text{mA}$). The pseudorandom signal induces an oscillating current flow between the two electrodes.

2) Experiment 2: During Experiment 2, the vestibular stimuli were delivered as raised-cosine bell curves of $0.5\,\text{Hz}$ with the peak current amplitude adaptively adjusted (from $0.1\,\text{mA}$ to $5\,\text{mA}$) across trials. The skin behind the stimulation electrodes was anesthetized with AMETOP (tetracaine HCl gel $4\,\%$ (w/w), Smith & Nephew Inc., UK) $30\,\text{min}$ to $45\,\text{min}$ prior to the start of the experiment to minimize any non-vestibular cues, for example a tingling sensation behind the ears.

C. Protocol and data recording

1) Experiment 1: Experiment 1 assessed whether the contributions from bilateral vestibular organs are equal in postural responses, and whether there is a linear integration of vestibular signals into postural responses. During experiment 1, the participant needed to remain standing as described below and muscle and force responses were measured. Two different stimuli were applied; in experiment 1a, square wave GVS input was delivered as short pulse with a duration of $1.5\,\text{s}$ per pulse. Between each pulse we implemented a $1.5\,\text{s}$ to $2.5\,\text{s}$ random delay to allow subjects to return to a normal upright position prior to the next stimulus. Each trial lasted $5.5\,\text{min}$, during which a total of 40 positive and 40 negative square wave inputs was applied and the order was randomized. In experiment 1b, the electrical stimulus was delivered as a continuous stochastic signal with a duration of $1.5\,\text{min}$.

Subjects stood with their head forward and each foot on separate force plates (type 9260AA, Kistler, Winterthur, Switzerland) placed next to one another. They stood barefoot with their feet $1\,\text{cm}$ to $2\,\text{cm}$ apart in order to maximize the evoked muscular and postural responses. A blindfold was worn by subjects to diminish visual sensory information and subjects were instructed to keep their hands by their side and lean forward slightly to engage the muscles in the balance task. A laser pointer attached to the subjects' head was used to align and maintain head orientation with the chin up at an angle of $19°$. This head position aligns the EVS-evoked rotation vector with Reid's plane to maximize postural response to electrical vestibular stimulation in the mediolateral direction. Subjects were given instructions by the experimenter to maintain this head position. Force plate data were digitized using a data acquisition board (type 5695B, Kistler) at a sampling rate of $2000\,\text{Hz}$. Electromyography (EMG) was recorded bilaterally from the medial gastrocnemius (mGAS) and soleus (SOL) muscles using Ag-AgCl surface electrodes (Blue Sensor M; Ambu, Copenhagen, Denmark) and digitized at $2000\,\text{Hz}$ using a Porti amplifier (TMSi, Twente Medical Systems International, Oldenzaal, The Netherlands) on a recording computer using Polybench software (TMSi). EMG responses were high-pass filtered offline (cut-off frequency $20\,\text{Hz}$, 3rd order Butterworth), full wave rectified, and low-pass filtered (cut-off frequency $500\,\text{Hz}$, 3rd order Butterworth) prior to averaging.

2) Experiment 2: Experiment 2 assessed whether contributions from bilateral vestibular organs are equally perceived, and whether there is a linear integration of vestibular signals into the perceived signal. This experiment was performed in a dark, electrically shielded room where a participant was seated comfortably atop a memory foam-padded chair. Foam was added beneath the feet, around the chest, around the forearms, and on the legs to mitigate non-vestibular sensory

cues throughout the experiment. The participant was firmly secured to the chair by adjustable strapping to allow them to fully relax during the trials. Subjects also wore earplugs and a blindfold. The subject's head was pitched down towards the lap by $71°$ to bring the net EVS-evoked response vector in line with the chair's earth-vertical axis of rotation [7, 16], evoking the illusion of whole-body rotation around an earth-vertical axis. This head orientation was maintained by supporting the head with a padded helmet (Pro-Tec, San Clemente, USA) that was fixed to the chair.

For each electrical stimulation arrangement (left monaural, right monaural, left-right binaural and normal binaural), subjects completed two trials during which they were exposed to 40 raised-cosine bell stimuli. Immediately after each stimulus, subjects had to indicate the direction of rotation by a verbal response of 'left' or 'right'. Under these conditions, subjects report vivid sensations of actually being rotated in the chair, although the chair remained stationary [6]. Electrodes were placed in such a way that a positive stimulus always evoked a leftward rotation. 40 stimuli induced a rightward rotation, while the other 40 induced a leftward rotation and the order of the direction was randomized. A Bayesian adaptive procedure [17] was used to adjust the peak electrical vestibular stimulus current. This psychophysical procedure estimates each participant's function relating stimulus amplitude (in mA) to his or her proportion of correct direction discrimination, and from this a threshold-level of direction discrimination can be extracted [18]. This threshold-level is defined as the peak stimulus level at which the participant could correctly discriminate direction with $69\,\%$ probability [6, 18]. The entire algorithm was programmed in LabVIEW, which calculated a direction discrimination threshold per condition. For more information see Peters et al. [6, 18].

D. Signal analysis and data reduction

1) Experiment 1: For data recorded during experiment 1a, we extracted the trigger averaged mediolateral (ML) force and muscle EMG responses for comparison across our stimulus conditions. Data were sectioned into $3\,\text{s}$ segments, aligned to GVS pulse onset and averaged for each experimental condition per subject. Trigger-averaged force responses are characterized by an initial short duration peak that accelerates the body in the direction of the cathode, and a larger response in the opposite direction to prevent the body from falling [19]. The force response was quantified by calculating the difference between the minimum and maximum value between stimulus onset and $300\,\text{ms}$ post-stimulus onset. Trigger-averaged EMG responses are characterized by a biphasic response consisting of a short and medium-latency peaks at $\approx 60\,\text{ms}$ and $\approx 110\,\text{ms}$ respectively [10, 20]. To quantify the EMG responses, the signal was first converted to a percentage of the background EMG, where the background EMG was defined as the mean value of the first $0.5\,\text{s}$ EMG signal per segment. This normalized EMG signal was then integrated from $100\,\text{ms}$ to $220\,\text{ms}$ post-stimulus onset in order to analyse EMG responses across conditions. We choose to use the integral rather than extract the peak responses for comparison across conditions because of the high levels of noise associated with trigger-averaged EMG responses. This approach also replicates the methods reported by Day et al. [3].

For data recorded during Experiment 1b, we estimated the autospectra for the input stimuli, muscle activity and ML force, as well as the cross-spectra of the input stimuli with the muscle activity and ML force. Data were sectioned into $2\,\text{s}$

segments prior to calculating the auto- and cross-spectra, and the resulting spectra were averaged in the frequency domain. Cumulant density functions were then calculated by taking the inverse Fourier transform of the cross-spectra [21] between input stimuli and output muscle activity or ML force. The cumulant density estimates were normalized by the product of the vector norms of the input-output signals [22]. Electrically evoked cumulant density responses are characterized by short and medium latency components, visible as biphasic peaks in both EMG and force responses, typically between $0\,\text{ms}$ to $120\,\text{ms}$ for EMG and $0\,\text{ms}$ to $250\,\text{ms}$ for forces. The difference between those two peaks (peak-to-peak value) was extracted from the cumulant density responses for each subject from $0\,\text{ms}$ to $160\,\text{ms}$ for EMG data and from $0\,\text{ms}$ to $300\,\text{ms}$ for force data.

a) Bilaterality: To assess whether each labyrinth projects symmetrically to both sides of the body, the mean EMG and force responses from the monaural stimulation conditions were combined to provide ipsilateral and contralateral estimates of the evoked muscle and force responses. With the head facing forward, the monaural stimulus evokes an excitatory response in one leg and an inhibitory response in the other, making them difficult to compare. Therefore, the responses in the same muscle to monaural stimuli of opposite polarities to each labyrinth (i.e. ipsilateral vs. contralateral) were compared. We collapsed the data across the two legs and stimulus polarities to provide estimates of ipsilateral and contralateral contributions according to:

$$\text{Ipsilateral} = \frac{X_{Lleg}(A) + X_{Rleg}(B)}{2},$$

with for experiment 1a: $A = (L_{stim}^- - L_{stim}^+)/2$, $B = (R_{stim}^- - R_{stim}^+)/2$, and for experiment 1b: $A = L_{stim}$, $B = R_{stim}$.

$$\text{Contralateral} = \frac{X_{Lleg}(A) + X_{Rleg}(B)}{2},$$

with for experiment 1a: $A = (R_{stim}^+ - R_{stim}^-)/2$, $B = (L_{stim}^+ - L_{stim}^-)/2$, and for experiment 1b: $A = R_{stim}$, $B = L_{stim}$. In both formulas X_{Lleg} is the EMG or force response of the left leg, X_{Rleg} is the EMG or force response of the right leg, L_{stim} means that the stimulation signal is delivered via the left mastoid, and R_{stim} means that the signal is delivered via the right mastoid. For the EMG responses in experiment 1a, $L_{stim}^- = $ cathode left, $L_{stim}^+ = $ anode left, $R_{stim}^- = $ cathode right and $R_{stim}^+ = $ anode right. Muscle responses to negative stimuli were inverted to account for its inhibitory effect on muscle activity.

b) Left/right integration: To assess how information from the two labyrinths is integrated, we compared the sum of responses from the left and right monaural conditions to the responses from the binaural conditions. This comparison combined responses in both limbs and stimulus polarities for both experiment 1a and 1b as follows:

$$\Sigma\text{Monaural}$$
$$= \frac{X_{Lleg}(A) + X_{Rleg}(B) - X_{Lleg}(B) - X_{Rleg}(A)}{4},$$

with for experiment 1a: $A = L_{stim}^- + R_{stim}^+$, $B = L_{stim}^+ + R_{stim}^-$, and for experiment 1b: $\Sigma\text{Monaural} = (X_{Lleg}(A) + X_{Rleg}(A))/2$ where $A = R_{stim} + L_{stim}$.

$$\text{Binaural} = \frac{X_{Lleg}(A) + X_{Rleg}(B) - X_{Lleg}(B) - X_{Rleg}(A)}{4},$$

with for experiment 1a: $A = L_{stim}^- R_{stim}^+$, $B = L_{stim}^+ R_{stim}^-$, and for experiment 1b: $\text{Binaural} = (X_{Lleg}(A) + X_{Rleg}(A))/2$ where $A = R_{stim}L_{stim}$. The same notations as previously described for bilaterality were used here.

2) Experiment 2:
a) Bilaterality: To determine if each labyrinth contributes equally to the perception of virtual motion (i.e. are bilaterally symmetric), we compared the perception thresholds obtained from left and right monaural stimulation.

b) Left/right integration: In order to assess how left and right signals are integrated to form an overall perception of virtual motion we compared the sum of responses from the left and right monaural conditions to the responses from the binaural conditions. Because monaural stimulation is thought to activate vestibular afferents on the stimulated side, we expected that monaural stimulation would result in a larger threshold compared to either binaural stimulation configuration. Subsequently, when assessing left-right integration of vestibular signals for perception, a simple summation of monaural thresholds would not reflect the expected decrease in threshold when activating both organs. Therefore, to compare monaural and binaural thresholds we estimated a summed monaural threshold from:

$$\Sigma\text{Monaural} = \left(\frac{1}{L} + \frac{1}{R}\right)^{-1},$$

where L are the thresholds obtained from stimulating on the left side, and R are the thresholds obtained from stimulating on the right side. This was compared to the thresholds obtained from the two binaural stimulations to assess left/right integration.

E. Statistical analysis
All statistical analyses were performed using SPSS22 (IBM, Armonk, USA) and significance was set at the $5\,\%$ level.

1) Bilaterality: To assess the bilaterality of vestibular contributions to posture we performed Student t-tests comparing ipsilateral and contralateral muscle and force responses. Similarly, to assess the bilaterality of vestibular contributions to perception we performed a Student t-test comparing the threshold after left monaural with right monaural stimulation. A Shapiro-Wilk test was used to test for homogeneity of variance. When the assumption of normally distributed data was violated a Wilcoxon signed-rank test was used instead of the t-tests.

2) Left/right integration: To assess the left/right integration of vestibular signals for posture we performed a repeated measures ANOVA to compare the sum of the left and right monaural stimulation and the two binaural arrangements for both muscle and force responses. Similarly, to assess the left/right integration of vestibular signals for perception we performed a repeated measures ANOVA to compare the sum of the left and right monaural stimulation and the two binaural arrangements. Pair-wise comparisons between conditions were evaluated using a Bonferroni correction. Mauchly's Test was used to test for sphericity, when this assumption was violated a Greenhouse-Geisser correction was used. A Shapiro-Wilk test was used to test for homogeneity of variance. When the assumption of normally distributed data was violated a Friedmann test was used instead. In between conditions were compared with a Wilcoxon signed rank test when this was the case.

III. Results

A. Experiment 1

Mean muscle and force responses from all subjects to binaural stimulation are shown in Figure 1. The biphasic shape of both the EMG and force responses matched those reported in previous studies [3, 23, 24]. Notably, muscle responses were inverted across limbs due to the mirrored symmetry of bilateral muscle pairs. EMG responses to square wave GVS demonstrate a high level of noise (Figure 1a), and the influence of noise on the responses was even more severe during monaural stimulation. In 7 out of 10 subjects, we saw no discernible biphasic response during monaural stimulation, an outcome which differs from previous studies [3]. In contrast, muscle responses evoked by SVS (i.e. cumulant density estimates, Figure 1b) in all stimulation conditions demonstrated clear biphasic peaks that are typical for this stimulus arrangement. Force responses to both square wave GVS and SVS (Figure 1c and Figure 1d) demonstrated distinguishable biphasic peaks during binaural stimulation. Unlike EMG, however, clear biphasic responses were also observed in the force responses during monaural configurations for both GVS and SVS.

1) Bilaterality: With the head facing forwards, the monaural stimulus evokes an excitatory response in one leg and an inhibitory response in the other. Therefore the responses in the same muscle to monaural stimuli of opposite polarities to each labyrinth were compared, as described in '*Materials and methods - Signal analysis and data reduction*'. Figure 2 and Figure 3 show the mean ipsilateral and contralateral responses derived from EMG and force data respectively for the assessment of bilaterality. There were no significant differences between the strengths of the ipsilateral and contralateral projections to the muscle activity for both GVS and SVS stimulation in both muscles (Figure 2, Table 1, and Table 2). There were also no significant differences between the strengths of the ipsilateral and contralateral contributions to ML force responses during both GVS and SVS (Figure 3, Table 1, and Table 2). These results match previous observations that individual leg muscles receive equal inputs from the two labyrinths [3], which results in an equal contribution of the two labyrinths to ML forces underneath the feet.

2) Left/right integration: To investigate the integration of information from the two labyrinths, the responses obtained from binaural stimulations were compared with the summed response of monaural responses. Figure 2 and Figure 3 show the mean EMG and force responses respectively, and the corresponding values and accompanying statistical outcomes can be seen in Table 1 and Table 3. EMG responses from the summed monaural estimate obtained from square wave GVS trials were on average larger than the equivalent binaural responses in both mGAS and SOL muscles (mGAS: 10%; SOL: 14%); however, this difference was not significant for either muscle. During SVS trials, in contrast, we observed significant differences between the monaural sum and both binaural equivalents for the mGAS muscle; the monaural sum was 38% larger than the left-right binaural configuration and 19% larger than the normal binaural configuration. In the soleus muscle, however, we found no significant difference across stimulation conditions, although the monaural sum was 20% and 15% larger compared to the left-right binaural and normal binaural configurations respectively.

Despite the inconsistency in muscle responses across monaural sum and binaural estimates, ML forces showed significantly larger monaural sums during both GVS and SVS conditions (see Figure 3). The monaural sum was 55% and 65% larger than the left-right binaural condition for square wave GVS and SVS respectively, and 64% and 55% larger than the normal binaural condition. The results match previous observations from Day et al. [3], where the monaural sum for both muscle and force responses was about 40% larger than the left-right binaural condition.

Interestingly, when both binaural conditions were compared no statistically significant differences in both muscle and force responses were found. During GVS the left-right binaural responses were 22%, 3% and 6% larger for respectively mGAS, SOL and ML force than the normal binaural responses. During SVS the normal binaural responses were 14%, 3% and 6% larger for respectively mGAS, SOL and ML force than the left-right binaural responses. This suggests that these stimulation arrangements generate similar responses and likely do not differ in the afferent population that they evoked during stimulation.

B. Experiment 2

A Bayesian adaptive procedure was used to map each participant's sensitivity to a direction discrimination threshold. The psychometric functions extracted from each subject are plotted together with the group average in Figure 4. Thresholds increased during monaural stimulation conditions, visible as a rightward shift in the psychometric function estimates (Figure 4a; left monaural, and Figure 4b; right monaural, compared to Figure 4c; left-right binaural, and Figure 4d; normal binaural).

1) Bilaterality: Comparison of direction detection threshold estimates across left- and right-sided stimulation revealed no significant difference (see Figure 5 and Table 4). These results match the muscle and force responses during standing balance in Experiment 1 when comparing ipsilateral and contralateral estimates, and show that information from the two labyrinths is equally perceived.

2) Left/right integration: The direction detection thresholds during left and right monaural stimulation were on average 70% larger than either binaural condition. However, the summed monaural estimate was only $\approx 15\%$ lower and was not significantly different. This result does not match with the majority of postural responses during standing balance, where larger and significant differences were found in force responses during both square wave GVS and SVS, and EMG responses from the mGAS during SVS.

The perception thresholds obtained from the two binaural conditions differed less than 2%, which is comparable to the postural responses obtained in experiment 1 as no significant differences were found anywhere. The binaural direction discrimination threshold had a value of $0.8\,\mathrm{mA}$ as visible in Table 4 and Figure 5, which is comparable to previously obtained thresholds [6].
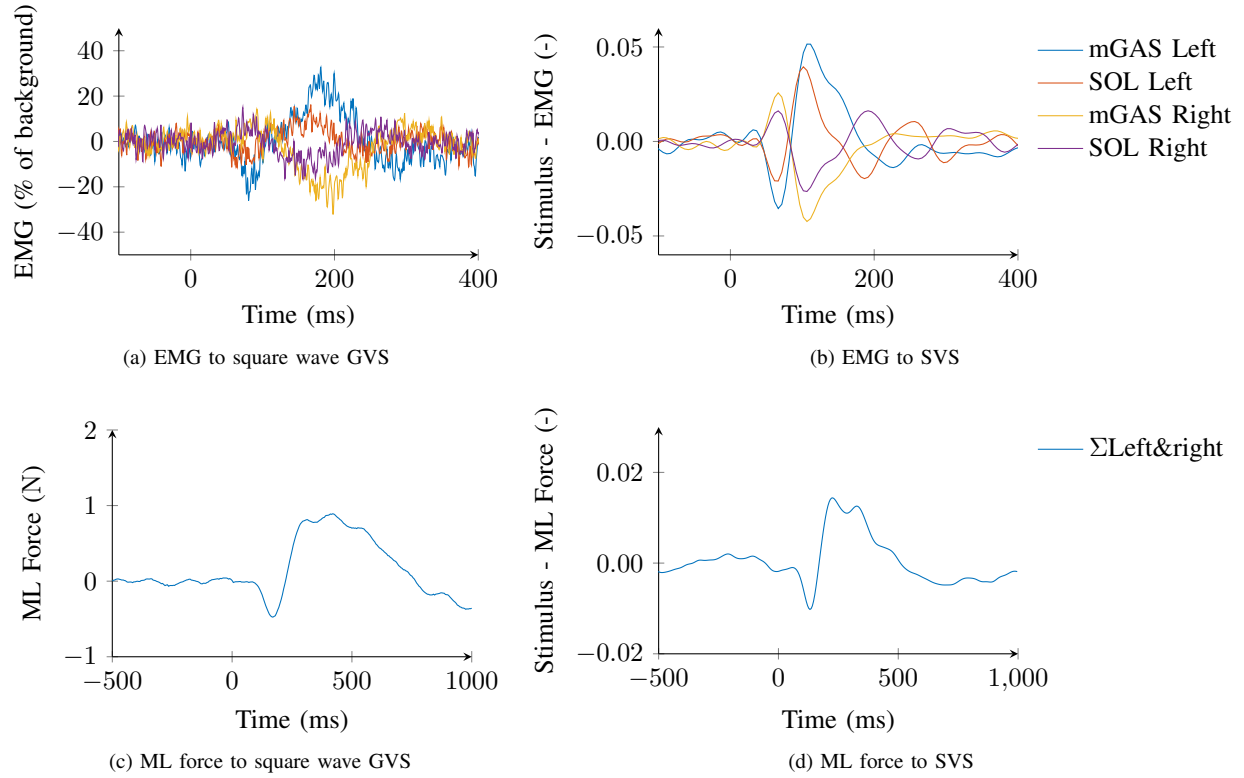
Figure 1: Mean responses of all subjects (n = 10) for a binaural arrangement. Stimulus onset at t = 0 (a) EMG responses during square wave GVS from the mGAS (left leg in blue, right leg in yellow) and SOL (left leg in red, right leg in purple) muscles using data from experiment 1a. (b) Cumulant density EMG responses during SVS from the mGAS (left leg in blue, right leg in yellow) and SOL (left leg in red, right leg in purple) muscles using data from experiment 1b. (c) ML force response during square wave GVS using data from experiment 1a, sum of left and right leg. (d) Cumulant density ML force response during SVS using data from experiment 1b, sum of left and right leg.
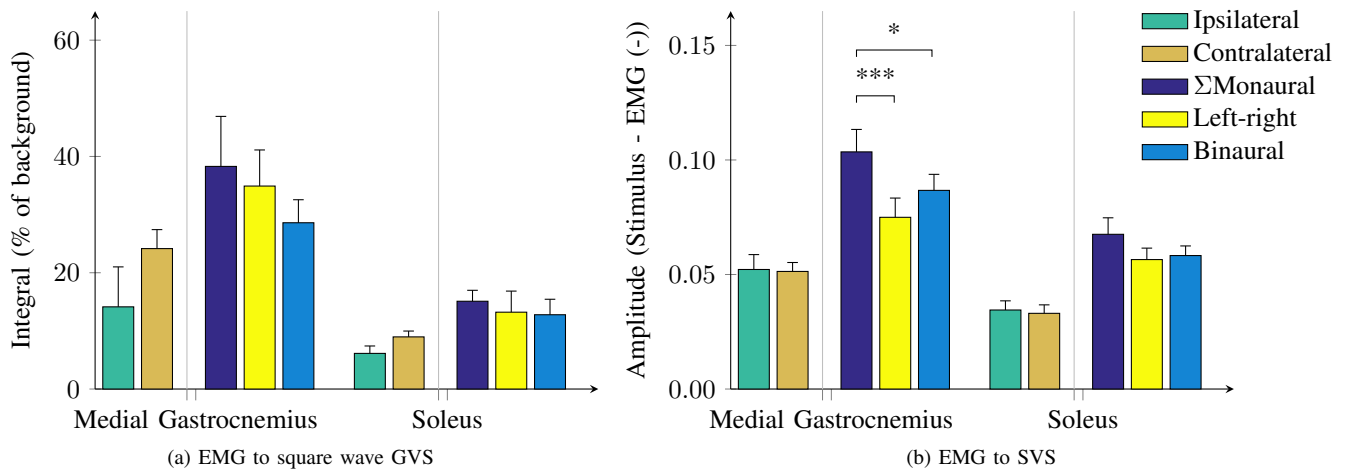
Figure 2: Group mean (+S.E.M.) mGAS and SOL EMG responses, normalised to background EMG levels across all conditions. Ipsilateral (green) and contralateral (brown) responses are plotted on the left to evaluate the bilaterality of vestibular projections. The sum of monaural responses (dark blue) is plotted on the right for comparison to left-right binaural (yellow) and normal binaural (blue) conditions to evaluate left/right integration. * $P \leq 0.05$, *** $P \leq 0.001$. (a) Responses during square wave GVS, displayed as the area under the curve, using data from experiment 1a. (b) Responses during SVS, displayed as the peak-to-peak amplitude, using data from experiment 1b.

(a) Forces to square wave GVS

(b) Forces to SVS
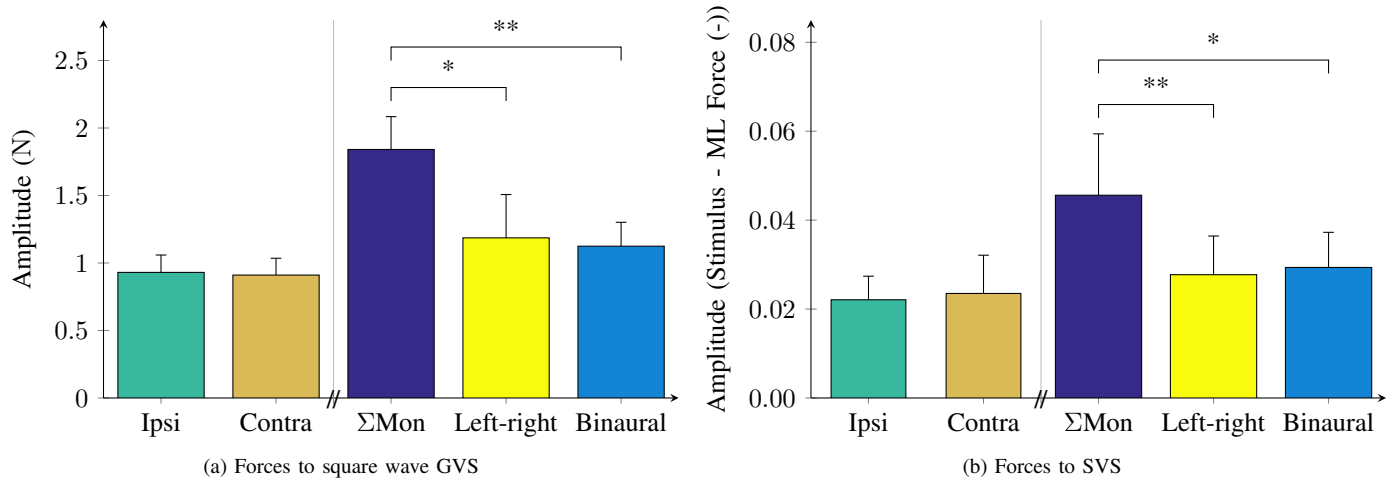
Figure 3: Group mean (+S.E.M.) ML force responses. Ipsilateral (Ipsi, green) and contralateral (Contra, brown) responses are plotted on the left to evaluate the bilaterality of vestibular projections. The sum of monaural responses (ΣMon, dark blue) is plotted on the right for comparison to left-right binaural (yellow) and normal binaural (blue) conditions to evaluate left/right integration. * P $\leq$ 0.05, ** P $\leq$ 0.01. (a) Responses during square wave GVS using data from experiment 1a. (b) Responses during SVS using data from experiment 1b.

		Mean			S.E.M.		
		mGAS (%)	SOL (%)	ML Force (N)	mGAS (%)	SOL (%)	ML Force (N)
GVS	Ipsilateral	14.1351	6.1301	0.9305	6.8723	1.2753	0.1286
	Contralateral	24.1535	8.9687	0.9106	3.2638	1.0037	0.1246
	ΣMonaural	38.2886	15.0988	1.8411	8.6024	1.8821	0.2433
	Left-right	34.9098	13.2171	1.1863	6.2086	0.2324	0.3210
	Binaural	28.5981	12.7777	1.1245	3.9593	2.6634	0.1772
		(Stim-EMG (-))	(Stim-EMG (-))	(Stim-Force (-))	(Stim-EMG (-))	(Stim-EMG (-))	(Stim-Force (-))
SVS	Ipsilateral	0.0522	0.0345	0.0221	0.0065	0.0040	0.0053
	Contralateral	0.0513	0.0330	0.0235	0.0039	0.0037	0.0086
	ΣMonaural	0.1035	0.0676	0.0456	0.0098	0.0072	0.0138
	Left-right	0.0750	0.0565	0.0277	0.0084	0.0050	0.0087
	Binaural	0.0867	0.0583	0.0294	0.0070	0.0042	0.0079

Table 1: Results for the mean and standard error of the mean (S.E.M.) during both square wave GVS and SVS for postural responses, data from experiment 1.

	t(9)			p		
	mGAS	SOL	ML Force	mGAS	SOL	ML Force
GVS	signed rank = 10	signed rank = 10	0.2826	0.0840	0.0840	0.7839
SVS	0.2038	0.5175	signed rank = 44	0.8431	0.6173	0.1055

Table 2: t and p values during both square wave GVS and SVS to test bilaterality (compare ipsilateral and contralateral contributions) for postural responses, data from experiment 1.

	F(2,18)				p		
	mGAS	SOL	ML Force		mGAS	SOL	ML Force
GVS	1.32	0.403	$\chi^2(2) = 9.6$		0.292	0.674	**0.008**
				Binaural/ΣMonaural	-	-	**0.007**
				Binaural/Left-right	-	-	0.799
				ΣMonaural/Left-right	-	-	**0.037**
SVS	14.963	5.677	$\chi^2(2) = 9.6$		**0**	**0.012**	**0.008**
				Binaural/ΣMonaural	**0.021**	0.116	**0.037**
				Binaural/Left-right	0.246	1	0.959
				ΣMonaural/Left-right	**0.001**	0.058	**0.007**

Table 3: F and p values during both square wave GVS and SVS to test left/right integration (compare sum of monaural with binaural conditions) for postural responses, data from experiment 1.

(a) Left monaural   (b) Right monaural   (c) Left-right binaural   (d) Normal binaural
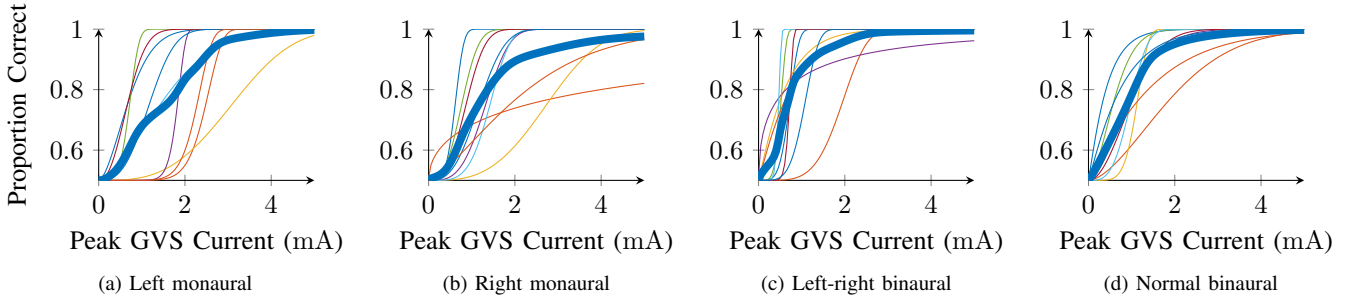
Figure 4: Individual participant (thin) (n = 9) and mean (thick) best-estimated psychometric functions for virtual direction discrimination tasks, data from experiment 2.

		Mean (mA)	S.E.M. (mA)	t(8)	p
Bilaterality				0.4851	0.6406
	Left	1.4249	0.2679	-	-
	Right	1.2630	0.1922	-	-
Left/right integration				$\chi^2(2) = 1.556$	0.459
	Binaural	0.7980	0.1160	-	-
	ΣMonaural	0.6630	0.1180	-	-
	Left-right	0.7847	0.1466	-	-

Table 4: Results for the mean, standard error of the mean (S.E.M.), t values, and p values for direction discrimination thresholds, data from experiment 2.
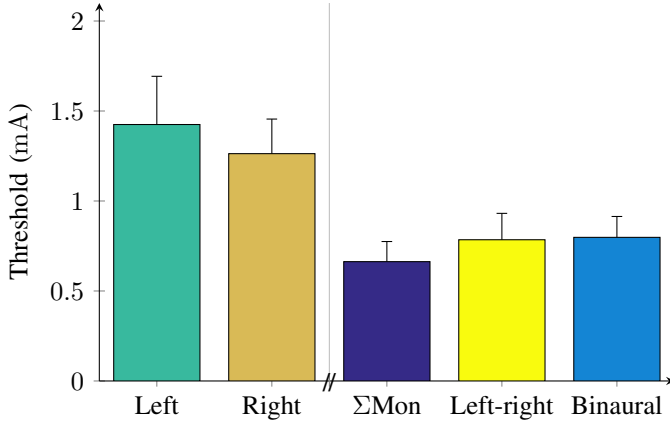
Figure 5: Group mean (+S.E.M.) (n = 9) direction discrimination thresholds. Thresholds for left monaural stimulation (green) and right monaural stimulation (brown) are plotted on the left to evaluate the bilaterality of vestibular projections. The sum of monaural responses (ΣMon, dark blue) is plotted on the right for comparison to left-right binaural stimulation (yellow) and normal binaural stimulation (blue) to evaluate left/right integration. Data from experiment 2.

IV. DISCUSSION

The aim of this study was to determine whether the integration of left and right vestibular signals is equivalent for both postural control of standing balance and the perception of self-motion. Postural and perceptual responses were evoked by electrically modulating vestibular afferent firing rates from each labyrinth. Lower leg muscle responses and horizontal ground reaction forces were measured during standing balance, and rotational detection thresholds of self-motion perception were estimated during a virtual rotation. Our results support the hypothesis that vestibular input from each labyrinth contributes equally to the postural control of standing balance and the perception of self-motion, as described in the introduction. Our results also seem to confirm the hypothesis that bilateral vestibular information is integrated non-linearly for posture, as the sum of monaural stimulation was larger than both binaural conditions, especially in mGAS responses during SVS and ML force responses. The latter hypothesis cannot be confirmed for perception. Comparing both binaural conditions did not lead to differences in the postural control of standing balance or to the perception of self-motion. Therefore it cannot be demonstrated that different stimulation pathways are established under these conditions, which raises uncertainty regarding previous conclusions that bilateral vestibular signals are integrated non-linearly.

A. Bilaterality

Ipsilateral and contralateral postural responses were compared to test for bilaterality in postural responses. From the similarity in muscle and force responses we conclude that the leg muscles receive similar input from the two labyrinths, which confirms the previous observations from Day et al. [3]. Responses from our detection discrimination threshold experiments expand the application of this principle to the perception of self-motion. Left- and right-sided stimulation resulted in similar thresholds, and both were higher than the binaural equivalents. This supports the possibility that vestibular signals are bilaterally symmetric for the higher order processing of perception as well as the contribution of these signals to motor output.

B. Left/right integration

To determine if bilateral vestibular information is integrated non-linearly for both postural control and perception, we compared the responses from the sum of monaural stimulation with responses from two binaural conditions. Although the summed response of muscle activity during monaural stimulation was larger than the response from binaural stimulation, we only

found significant differences for the mGAS muscle during SVS. The difference between GVS and SVS might be caused by the amount of noise in the signals during GVS (see *Limitations*). Differences between the two muscles might be due to the magnitudes of muscle responses, which were smaller in the SOL compared to the mGAS as visible in Figure 1 and as previously shown [20]. It was recently demonstrated that the mGAS has a larger contribution in vestibular-driven balance corrections during standing balance, as there is a larger effect on the discharge activity of motor units compared to the SOL [25]. A smaller evoked muscle response is more difficult to extract within the surrounding noise, resulting in smaller differences in the SOL muscle responses.

In contrast to the EMG responses, the forces measured were consistently significant and responses to square wave GVS and SVS were similar. Because ground reaction forces represent the motor output of all muscles involved in balance control, these responses provide a clearer overview of left-right integration of vestibular signals relative to the responses of only one muscle. It is recommended that future studies pursuing similar lines of vestibular research for balance control include at least force responses.

In contrast to postural conditions, our perception threshold responses do not demonstrate the same non-linear integration of bilateral vestibular signals. The monaural sum was not significantly different from either binaural condition. The lack of a significant difference during these perception trials may be due to the high variability of responses from the left and right monaural stimulation (see Figure 4), which was approximately double that observed from either binaural stimulation. Therefore, a significant difference might be obtained over a larger group of subjects. Another possibility is that this non-linearity mainly occurs in descending pathways that contribute to posture and to a lesser extend in ascending pathways that contribute to perception. All vestibular signals are first transmitted to the vestibular nuclei (comprised of four sub-nuclei: medial, lateral, inferior, and superior) before being sent to ascending or descending pathways. Postural responses are evoked by vestibulospinal reflexes, which arise mainly from the lateral vestibular nuclei. Perception in the vestibular cortex arises from signals from both the lateral and superior nuclei, where the superior nuclei receive input from all other sub-nuclei [26]. There is also an inhibitory effect of contralateral vestibular neurons in the superior nuclei [27, 28]. Due to the high convergence within the vestibular nuclei, spatial summation of multiple simultaneous inputs occurs [29]. Inhibitory post-synaptic potentials might in that case prevent adjacent neurons from reaching their threshold voltage, thereby not triggering action potentials [26]. The possible non-linearity in the perception signals might therefore be lost due to the integration of a larger amount of signals in the vestibular nuclei for ascending pathways.

A surprising outcome of this study was the similarity of postural and perceptual responses during left-right binaural and normal binaural stimulation. This suggests that the left-right binaural arrangement may not reflect a truly independent and simultaneous stimulation of each labyrinth. If independent stimulation would be possible than the results for left-right binaural stimulation would be closer to the monaural sum than to the normal binaural stimulation. However, due to the fact that the responses are similar, it is very likely that the current flows in equal pathways, i.e. between the two ears in both conditions. Therefore the possibly that vestibular signals are integrated non-linearly remains uncertain.

Limitations

In our analysis of force responses during the standing balance experiment, we included only the mediolateral components of the ground reaction forces, as sway was mostly expected in this direction due to the position of the head relative to the feet [5]. However, the anteroposterior forces were not exactly zero, possibly due to body rotation. This might be caused by leaning forwards, which leads to a vertical torque around the ankle joint. Even without EVS there is a frequency coupling at $7\,\text{Hz}$ to $8\,\text{Hz}$ between mediolateral shear force and vertical torque, so it is an inherent characteristic of normal stance [24]. Therefore we decided not to include anteroposterior forces as these are not a consequence of solely the applied stimulation.

Analysis of EMG responses during monaural square wave GVS stimulation revealed a substantial amount of noise, even after meaning over all repetitions and subjects. This differed from the results published by Day et al. [3] and no discernible biphasic responses were visible in monaural responses. Slight variations in our experimental design relative to Day et al. may have contributed to the differences in responses across studies. Day et al. used 50 pulses instead of our 40 and performed the integral on EMG responses over a wider time period ($120\,\text{ms}$ to $370\,\text{ms}$ post-stimulus onset instead of $100\,\text{ms}$ to $220\,\text{ms}$ post-stimulus onset). Different time periods were used to calculate the integral, but this did not lead to differences in responses as no EMG response was evoked between $220\,\text{ms}$ to $370\,\text{ms}$ post-stimulus onset. The smaller amount of pulses may have led to a reduced quality in the estimated responses. However, in an effort to compensate for this difference we used a longer stimulus time ($1\,\text{s}$ vs. $1.5\,\text{s}$) and a larger stimulus amplitude ($1.5\,\text{mA}$ vs. $2\,\text{mA}$). Therefore it is unclear why we were unable to extract the previously reported muscle responses. Despite the low quality of GVS evoked muscle responses, SVS produced consistent muscle and force responses. This is consistent with the increased signal-to-noise ratios that can be achieved using SVS compared to GVS even over shorter durations of data collection [22, 24]. In addition, GVS evoked force responses were comparable to SVS evoked force responses. Overall, consistent balance responses were evoked by both signals, but these were not visible at an individual muscle level during GVS.

V. CONCLUSION

In conclusion, during human standing balance the leg muscles receive equal input from the two labyrinths, visible in muscle responses and mediolateral ground reaction forces. In addition, the information from each vestibular system is also equally perceived. Responses to the monaural sum during standing balance were typically larger than the two binaural conditions, although not always significant, indicating the possibility of non-linear integration. For perception the results were not significant, indicating the possibility of different integration principles within the vestibular nuclei leading to perception and postural responses. Normal binaural stimulation did not lead to differences in responses compared to left-right binaural stimulation for both posture and perception, indicating that these stimulation techniques are not truly different from each other. Therefore this non-linear integration needs to be questioned, as independent activity of unilateral vestibular apparatuses may not be possible.

REFERENCES

[1] J.M. Goldberg, Charles Eugene Smith, and C. Fernandez. Relation between discharge regularity and responses to externally applied galvanic currents in vestibular nerve afferents of the squirrel monkey. *Journal of Neurophysiology*, 51(6):1236–1256, 1984.

[2] Juno Kim and Ian S. Curthoys. Responses of primary vestibular neurons to galvanic vestibular stimulation (gvs) in the anaesthetised guinea pig. *Brain research bulletin*, 64(3):265–271, 2004.

[3] Brian L. Day, Jonathan F. Marsden, Elijane Ramsay, Omar S. Mian, and Richard C. Fitzpatrick. Non-linear vector summation of left and right vestibular signals for human balance. *The Journal of Physiology*, 588(4):671–682, 2010.

[4] Lewis M. Nashner and Paul Wolfson. Influence of head position and proprioceptive cues on short latency postural reflexes evoked by galvanic stimulation of the human labyrinth. *Brain research*, 67(2):255–268, 1974.

[5] Staffan Lund and Catharina Broberg. Effects of different head positions on postural sway in man induced by a reproducible vestibular error signal. *Acta Physiologica Scandinavica*, 117(2): 307–309, 1983.

[6] Ryan M. Peters, Brandon G. Rasman, J. Timothy Inglis, and Jean-Sébastien Blouin. Gain and phase of perceived virtual rotation evoked by electrical vestibular stimuli. *Journal of Neurophysiology*, 114(1):264–273, 2015.

[7] Brian L. Day and Richard C. Fitzpatrick. Virtual head rotation reveals a process of route reconstruction from human vestibular signals. *The Journal of Physiology*, 567(2):591–597, 2005.

[8] Rasman Brandon, Brian H. Dalton, J. Timothy Inglis, and Jean-Sébastien Blouin. Spatial transformations of the vestibular control of standing balance. *9th Annual Canadian Neuroscience Meeting 2015*, Vancouver, May 24-27, 2015.

[9] Swee Tin Aw, Michael John Todd, Nadine Lehnen, Grace Elizabeth Aw, Konrad Peter Weber, Thomas Eggert, and Gabor Michael Halmagyi. Electrical vestibular stimulation after vestibular deafferentation and in vestibular schwannoma. *PloS one*, 8(12):e82078, 2013.

[10] Richard C. Fitzpatrick and Brian L. Day. Probing the human vestibular system with galvanic stimulation. *Journal of Applied Physiology*, 96(6):2301–2316, 2004.

[11] Arne Tribukait and Ulf Rosenhall. Directional sensitivity of the human macula utriculi based on morphological characteristics. *Audiology and Neurotology*, 6(2):98–107, 2001.

[12] Daniel L. Wardman, Janet L. Taylor, and Richard C. Fitzpatrick. Effects of galvanic vestibular stimulation on human posture and perception while standing. *The Journal of Physiology*, 551(3): 1033–1042, 2003.

[13] Billy L. Luu, Thomas P. Huryn, H.F. Machiel Van der Loos, Elizabeth A. Croft, and Jean-Sébastien Blouin. Validation of a robotic balance system for investigations in the control of human standing balance. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 19(4):382–390, 2011.

[14] Richard Fitzpatrick, David Burke, and Simon C. Gandevia. Task-dependent reflex responses and movement illusions evoked by galvanic vestibular stimulation in standing humans. *The Journal of Physiology*, 478(Pt 2):363, 1994.

[15] T.C. Britton, B.L. Day, P. Brown, J.C. Rothwell, P.D. Thompson, and C.D. Marsden. Postural electromyographic responses in the arm and leg following galvanic vestibular stimulation in man. *Experimental brain research*, 94(1):143–151, 1993.

[16] Richard C. Fitzpatrick, Jon Marsden, Stephen R. Lord, and Brian L. Day. Galvanic vestibular stimulation evokes sensations of body rotation. *Neuroreport*, 13(18):2379–2383, 2002.

[17] Leonid L. Kontsevich and Christopher W. Tyler. Bayesian adaptive estimation of psychometric slope and threshold. *Vision research*, 39(16):2729–2737, 1999.

[18] Ryan M. Peters, Jean-Sébastien Blouin, Brian H. Dalton, and J. Timothy Inglis. Older adults demonstrate superior vestibular perception for virtual rotations. *Experimental Gerontology*, 82: 50–57, 2016.

[19] B.L. Day, A. Severac Cauquil, L. Bartolomei, M.A. Pastor, and I.N. Lyon. Human body-segment tilts induced by galvanic stimulation: a vestibularly driven balance protection mechanism. *The Journal of Physiology*, 500(Pt 3):661, 1997.

[20] Christopher J. Dakin, Gregory M. Lee Son, J. Timothy Inglis, and Jean-Sébastien Blouin. Frequency response of human vestibular reflexes characterized by stochastic stimuli. *The Journal of Physiology*, 583(3):1117–1127, 2007.

[21] D.M. Halliday, J.R. Rosenberg, A.M. Amjad, P. Breeze, B.A. Conway, and S.F. Farmer. A framework for the analysis of mixed time series/point process data-theory and application to the study of physiological tremor, single motor unit discharges and electromyograms. *Progress in biophysics and molecular biology*, 64(2):237–278, 1995.

[22] Christopher J. Dakin, Billy L. Luu, Kees van den Doel, John Timothy Inglis, and Jean-Sébastien Blouin. Frequency-specific modulation of vestibular-evoked sway responses in humans. *Journal of Neurophysiology*, 103(2):1048–1056, 2010.

[23] Patrick A. Forbes, Christopher J. Dakin, Anoek M. Geers, Martijn P. Vlaar, Riender Happee, Gunter P. Siegmund, Alfred C. Schouten, and Jean-Sébastien Blouin. Electrical vestibular stimuli to enhance vestibulo-motor output and improve subject comfort. *PloS one*, 9(1):e84385, 2014.

[24] Raymond F. Reynolds. Vertical torque responses to vestibular stimulation in standing humans. *The Journal of Physiology*, 589 (16):3943–3953, 2011.

[25] Christopher J. Dakin, Martin E. Héroux, Billy L. Luu, John Timothy Inglis, and Jean-Sébastien Blouin. Vestibular contribution to balance control in the medial gastrocnemius and soleus. *Journal of Neurophysiology*, 115(3):1289–1297, 2016.

[26] Fitzpatrick D. Hall W.C. LaMantia A.S. White L.E. Purves D., Augustine G.J. *Neuroscience*. Sinauer Associates, fifth edition, 2012.

[27] Randall E. Merchant. Vestibular nuclei. In *Encyclopedia of Clinical Neuropsychology*, pages 2610–2611. Springer, 2011.

[28] M. Ito. The Cerebellovestibular Interaction in the Cat's Vestibular Nuclei Neurons. *Fourth Symposium on The Role of the Vestibular Organs in Space Exploration. NASA*, 187:183–198, 1970.

[29] Devon I. Rubin and Jasper R. Daube. *Basics of Neurophysiology*. Oxford University Press, third edition, 2009.