Vestibulo-spinal and vestibulo-ocular reflexes are modulated when standing with increased postural threat

E. N. Naranjo,1 T. W. Cleworth,1 J. H. J. Allum,2 J. T. Inglis,1,3,4 J. Lea,5 B. D. Westerberg,5 and M. G. Carpenter1,3,4

1School of Kinesiology, University of British Columbia, Vancouver, British Columbia, Canada; 2Department of Otolaryngology, University Hospital, Basel, Switzerland; 3International Collaboration on Repair Discoveries, University of British Columbia, Vancouver, British Columbia, Canada; 4Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, British Columbia, Canada; and 5BC Rotary Hearing and Balance Centre at St. Paul’s Hospital, Vancouver, British Columbia, Canada

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Naranjo EN, Cleworth TW, Allum JH, Inglis JT, Lea J, Westerberg BD, Carpenter MG. Vestibulo-spinal and vestibulo-ocular reflexes are modulated when standing with increased postural threat. J Neurophysiol 115: 833–842, 2016. First published December 2, 2015; doi:10.1152/jn.00626.2015.—We investigated how vestibulo-spinal reflexes (VSRs) and vestibulo-ocular reflexes (VORs) measured through vestibular evoked myogenic potentials (VEMPs) and video head impulse test (vHIT) outcomes, respectively, are modulated during standing under conditions of increased postural threat. Twenty-five healthy young adults stood quietly at low (0.8 m from the ground) and high (3.2 m) surface height conditions in two experiments. For the first experiment (n = 25) VEMPs were recorded with surface EMG from inferior oblique (IO), sternocleidomastoid (SCM), trapezius (TRP), and soleus (SOL) muscles in response to 256 air-conducted short tone bursts (125 dB SPL, 500 Hz, 4 ms) delivered via headphones. A subset of subjects (n = 19) also received horizontal and vertical head thrusts (~150°/s) at each height in a separate session, comparing eye and head velocities by using a vHIT system for calculating the functional VOR gains. VEMP amplitudes (IO, TRP, SCM, and horizontal and vertical vHIT gains) all increased with high surface height conditions (P < 0.05). Changes in VRT and SCM VEMP amplitudes as well as horizontal vHIT gains were correlated with changes in electrodermal activity (r = 0.44–0.59, P < 0.05). VEMP amplitude for the IO also positively correlated with fear (r = 0.43, P = 0.03). Threat-induced anxiety, fear, and arousal have significant effects on VSR and VOR gains that can be observed in both physiological and functional outcome measures. These findings provide support for a potential central modulation of the vestibular nucleus complex through excitatory inputs from neural centers involved in processing fear, anxiety, arousal, and vigilance.

Address for reprint requests and other correspondence: M. G. Carpenter, Osborne Centre Unit I, 6108 Thunderbird Blvd., Vancouver, BC, Canada V6T 1Z4 (e-mail: mark.carpenter@ubc.ca).

NEUROANATOMICAL EVIDENCE from animal research has identified strong excitatory influences on vestibular networks from neural centers that respond to threat (Balaban 2002; Balaban and Thayer 2001; Staab et al. 2013). However, evidence for similar threat-related effects on human vestibular function remains relatively limited. Recent research has shown that the threat imposed by standing at height significantly increases vestibulo-spinal reflex (VSR) gain, as suggested by a significant increase in the amplitude of vestibular evoked myogenic potentials (VEMPs) recorded from the neck and leg muscles and significant positive correlations between changes in VEMP amplitude and increased physiological arousal and fear (Naranjo et al. 2015). VEMPs involve delivery of brief, loud, auditory sounds, or bone-conducted stimuli, to activate the otoliths and thus test the entire vestibular reflex pathways from receptor to the muscle (Rosengren et al. 2010; Welgampola and Colebatch 2005) (Fig. 1). The observations of increased VEMPs with threat (Naranjo et al. 2015) corroborate observations of studies using stochastic electrical vestibular stimulation (Horslen et al. 2014; Lim 2014), thought to act primarily through irregular vestibular afferents (Fitzpatrick and Day 2004) and thus bypass the receptors. In these studies, an increase in threat was shown to increase the gain and coherence between the stochastic vestibular error signal and ground reaction forces measured through a force plate (Horslen et al. 2014) and lower leg muscle activity (Lim 2014) in standing humans. The converging results from studies using VEMPs and stochastic vestibular stimulation studies suggest that the modulation of VOR with threat most likely occurs below the level of the receptor, either at the vestibular nuclei or within the spinal cord. The latter may be due to parallel changes in other sensory afferents (Horslen et al. 2013) or other descending motor systems such as the cortico-spinal (Schutter et al. 2008) and reticulo-spinal (Paré et al. 2004) pathways that are also modulated under threatening conditions.

If threat-related modulation of VSRs occurs within the central vestibular system (Balaban 2002), similar changes would also be expected within the vestibulo-ocular reflex (VOR) pathways that share a common origin at the vestibular nuclei, and VOR action is tightly coupled with VSR action during common controlled movements such as gaze stabilization (Boyle 1993). One way to test changes in VOR pathways is to record VEMPs in extraocular muscles, such as the inferior oblique (IO), contralateral to the side of the stimulus. These ocular VEMPs rely on pathways connecting the superior vestibular nuclei (Uchino and Kushiro 2011) to cranial nuclei (abducens, oculomotor, and trochlear nuclei) that control the extraocular muscles (Rosengren and Kingma 2013). To our knowledge, no study to date has examined the effects of threat on ocular VEMPs, alone or in combination with simultaneous recording of cervical and leg VEMPs during stance.

Therefore, the first aim of this study was to determine whether changes in the gain of VOR and VSR are influenced...
by threat and to determine whether these changes are associated with changes in threat-related factors such as fear, state anxiety, confidence, and physiological arousal. Threat-related changes in VSR and VOR gains were also correlated in order to determine whether these changes share common variance and thus may share a common mechanism underlying the influence of threat (Horslen et al. 2014; Naranjo et al. 2015).

While changes may be evident within an evoked potential, such as VEMPs, questions arise as to whether such changes are reflected in a more functional outcome (for a debate on this subject see Horslen et al. 2015; Reynolds et al. 2015). Therefore, the second aim of this study was to determine whether threat-induced changes in ocular VEMPs were associated with parallel changes in the functional gain of the VOR.

Evidence for increased VOR gain has been demonstrated in people with chronic anxiety (Furman et al. 2006) and healthy normal subjects experiencing increased mental stress and arousal through mental arithmetic (Yardley et al. 1995) and sleep deprivation (Quarck et al. 2006). However, these studies could not attribute the observed changes to specific changes in state anxiety or arousal because these variables were not directly measured or controlled. Furthermore, these studies relied on caloric irrigation and rotating chair tests that involve head accelerations that are relatively slow (\( \sim 30°/s^2 \)) compared with those that occur during normal gait-related balance tasks (Allum and Honegger 2013; Carriot et al. 2014) and cannot be easily performed in standing when the vestibular inputs are most relevant to balance (Luu et al. 2012).

One alternative is to use the video head impulse test (vHIT), which has the potential to assess VOR gain (Halmagyi and Curthois 1988) at higher velocities (80–250°/s) and in modified postures such as standing. The vHIT records eye and head velocities with a camera and gyroscopes, respectively, built into a set of goggles that allows the subject to fixate on a stationary target while his/her head is passively rotated by a clinician to elicit a VOR. The eyes normally remain on the target because of the compensatory VOR movement opposite to the head movement. The horizontal vHIT is recorded in the yaw plane, stimulating primarily the horizontal semicircular canals. Likewise, a vertical vHIT response is typically recorded at 45° to roll and pitch plane head movements stimulating the opposing anterior and posterior semicircular canals within these orthogonal planes (Halmagyi and Curthois 1988) as well as the otoliths. Therefore, to address the second aim of the study, the vHIT gain of the VOR in both the yaw and pitch planes was compared under conditions of increased threat. Changes in vHIT gain were correlated with changes in threat-related responses (fear, anxiety, confidence, and arousal) and changes in VEMP amplitudes.

On the basis of prior research (Furman et al. 2006; Horslen et al. 2014; Naranjo et al. 2015; Quarck et al. 2006; Yardley et al. 1995), we hypothesized that the gain of VOR and VSR would increase with threat, as evidenced by 1) a significant effect of height on the normalized VEMP amplitudes in eye, neck, and leg muscles and 2) significant correlations between VEMP amplitude and threat-related physiological and psychosocial responses. Furthermore, we hypothesized that the VOR gain measured through the vHIT would significantly increase with threat and would be significantly correlated with threat-related responses, as well as relative changes in cervical, leg, and ocular VEMP amplitudes. Because vertical (pitch) vHITs and ocular VEMPs both involve stimulation of the otoliths, we expected higher correlations between changes in vertical VOR gains and ocular VEMPs with threat.
MATERIALS AND METHODS

Twenty-five young healthy subjects (10 men, 15 women; age = 27.8 ± 5.9 yr, weight = 68.4 ± 9.2 kg, height = 168.4 ± 9.3 cm) volunteered to participate in the study. Subjects were free from any neurological or nonneurological causes of balance, hearing, or cognitive impairment, extreme fear of heights, frequent or severe headaches, pregnancy, and/or history of low blood pressure or fainting, history of chronic neck pain and/or whiplash-associated disorder, or severe neck movement restrictions, as verified by self-report. All subjects provided informed written consent prior to participation. All experimental procedures were approved by the Clinical Research Ethics committee of the University of British Columbia.

Postural threat was manipulated by having subjects stand on an hydraulic lift (Pentalift) at two different surface heights: Low (at 0.8 m above ground, away from the edge of the platform) and High (at 3.2 m high above ground, at the edge of the platform). Subjects were barefoot while standing on the platform. The order of presentation of heights was fixed in an ascending order to account for known order effects of height (Adkin et al. 2000). Subjects were accompanied by an experimenter at all times, and both the subject and experimenter were secured with a safety harness attached to the ceiling during both Low and High height conditions.

Electrodermal activity (EDA), which is a reliable measure of autonomic nervous system arousal (Mauss and Robinson 2009) and widely used in postural threat research (Cleworth et al. 2012; Davis et al. 2009; Horslen et al. 2013, 2014; Naranjo et al. 2015), was recorded by placing electrodes on the hypothenar and thenar eminences in the palm of the nondominant hand (Skin Conductance Module 2502, Cambridge Electronic Design, Cambridge, UK) and sampled at 100 Hz (Spike2 and Power 1401, Cambridge Electronic Design). EDA was recorded from the nondominant hand for the practical reason of freeing the dominant hand to write on questionnaires between trials without interfering with the EDA electrodes placed on the hand. Psycho-social measures of balance confidence, fear of falling, and perceived anxiety were collected with questionnaires that have proven validity and reliability of measures of changes between heights in young healthy adults (Hauck et al. 2008).

This study was conducted in two different experiments. All 25 subjects performed experiment 1 (VEMPs). Because of scheduling conflicts, only a subset of 19 participants that performed experiment 1 also performed experiment 2 (vHIT) (8 men, 11 women; age = 27.7 ± 6.2 yr, weight = 69 ± 9.4 kg, height = 168.5 ± 9.6 cm). Experiments were performed on separate days, at least 2 wk apart, and the order of presentation of the experiments was counterbalanced across subjects.

Experiment 1: VEMPs

Procedures. Acoustic air-conducted stimulation of the right ear was used to elicit VEMPs recorded by pairs of surface electrodes placed 2 cm apart over the muscle bellies of right sternocleidomastoid (SCM), right trapezius (TRP), right soleus (SOL), and left IO, with the ground electrode placed on the medial aspect of the right clavicle. SCM electrodes were placed over the middle third of the muscle belly. TRP electrodes were placed over the intersection between the upper and middle muscle fibers. IO electrodes were placed immediately below the left eye, following the midline of the eyeball. This electrode configuration has been shown to provide clearly observable ocular VEMP responses with low variability (Sandhu et al. 2013). SOL electrodes were placed slightly lateral to the midline of the calf immediately distal to the junction of the Achilles tendon and the belly of the lateral gastrocnemius.

Subjects performed a simple quiet standing task with their feet shoulder width apart and body oriented 90° to the edge of the platform (left shoulder toward edge). Both heels were raised with wedges (30°) to increase tonic activation of SOL. A force plate (no. K00407, Bertec, Columbus, OH) was used to monitor antero-posterior and medio-lateral moments online and provide verbal feedback, if necessary, to assist the subjects in maintaining a constant body position within and between conditions. The head was actively maintained in a position of 30° downward flexion and 60° rotation in the yaw plane to the left (toward platform edge) to increase tonic activation of SCM and TRP (McCasiun et al. 2014). A laser pointer attached to a headband provided visual feedback on the wall in front of the subject to help maintain constant head position across conditions (see Fig. 2A). During the experiment subjects were asked to look upward as much as possible along the vertical target line in order to increase tonic activation of IO muscles and were instructed to try to avoid excessive blinking.

Fig. 2. Experimental setup for experiment 1 (VEMP testing; A) and for experiment 2 [video head impulse test (vHIT) testing; B].

Experiment 1: subjects were placed side-ways at the edge of a hydraulic lift, on a force plate and on wedges to activate the soleus muscles. The lateral border of the left foot was placed at the edge of the platform, with feet centered longitudinally on the force plate. A, bottom left: the 2 height conditions, with a table placed at the low condition for removing subjects from the edge. A, top left: the subject’s head and neck position for activating the target muscles: leaving the trunk in a neutral position, the neck and head were turned 60° and flexed 30°; a laser pointer attached to the headband set and aligned with the horizontal line projected a laser beam to the wall, forcing the subject to look upward. Experiment 2: subjects were placed facing forward, with the anterior border of the first toe at the edge of the hydraulic lift, and wore the vHIT system while fixating the gaze in a central point on a wall 3 m from them. The experimenter stood on another platform behind them and moved the head randomly to different directions in each plane.
VESTIBULAR REFLEXES MODULATED WITH INCREASED POSTURAL THREAT

VEMP responses were elicited by short tone bursts of 4-ms duration (alternating polarity tone burst, rise/fall time of 1 ms, and a plateau time of 2 ms) at a 500 Hz frequency, delivered at an intensity of 125 dB sound pressure level. Tone bursts were delivered at a rate of 5 Hz (i.e., 1 tone burst every 200 ms). The intensity of the short tone bursts was below safety limits determined by the Canadian Center for Occupational Health and Safety. Following clinical protocols (Rosengren et al. 2010; Welgampola and Colebatch 2005), 256 pulses were presented monaurally in the right ear through headphones (model 296D 100-1, Telephonics) via a stereo amplifier (model SX-650, Pioneer, Kawasaki, Kanagawa, Japan). Short tone bursts were generated with a custom-written computer program ( Spike2, Cambridge Electronic Design) and calibrated before every test with a sound pressure meter (model CR250, Cirrus, Hummanby, UK).

VEMP data analyses. Electromyography (EMG) data were preamplified 500x and band-pass filtered (10-1,000 Hz) (Telemayo 2400R, Noraxon) digitally sampled at 5,000 Hz (Power 1401, Cambridge Electronic Design), and analyzed off-line with a custom-written program ( Spike2, Cambridge Electronic Design). EMG data were rectified only for the SOL (Naranjo et al. 2015; Watson and Colebatch 1998). All 256 trials for each condition and muscle were spike trigger averaged based on the onset of each tone burst ( Spike2). Blink artifacts in the IO EMG were identified off-line and removed prior to trigger averaging.

Muscle background activity (BGA) of each muscle was calculated with the root mean square (RMS) amplitude of 20 ms of prestimulus unrectified EMG (i.e., for the IO, SCM, and TRP) and the mean amplitude for the rectified EMG (i.e., for the SOL), according to methods described by Welgampola and Colebatch (2005) and Rosengren and Kingma (2013). A 2-standard deviation (SD) bandwidth based on that prestimulus RMS or mean amplitude was computed in order to set the threshold of significance for different VEMP peaks. Consistent with the literature, the peaks are labeled with reference to the positive (p) and negative (n) peak latencies, such as n10-p15 for IO, p13-n23 for SCM, n1-p1 for TRP, and n1 for rectified SOL (Naranjo et al. 2015; Rosengren and Kingma 2013; Welgampola and Colebatch 2005).

Controlling for muscle background activity. Muscle BGA is known to have a strong positive linear relationship with VEMP amplitude (Rosengren et al. 2010). However, it has been shown that, with the small levels of tonic activation sustained by the subjects in this protocol, the linear correlation is not significant (Bogle et al. 2013). Therefore, methods previously used for controlling the effects of BGA on VEMPs during quiet standing were employed (Naranjo et al. 2015). This comprised two different levels of control. First, with subject instructions, the head rotation of the subjects was monitored between conditions using the laser pointer. Subjects were also instructed to look as far up as possible to have IO activation. The subject’s head was monitored constantly, using the medio-lateral torque registered with the force plate. Second, with BGA, a screening protocol was used to identify any muscle with a mean BGA that could affect the response normalization with respect to the tonic activation levels across conditions. This consisted of calculating for each subject the mean BGA obtained from the subject’s average RMS amplitude (for unrectified EMG data) or mean amplitude (for rectified EMG data) calculated from 20 ms prior to each stimulus to the subject’s average response. Normalized responses from remaining muscles were used to calculate the corrected peak-to-peak amplitude for SCM (p13-n23), IO (n1-p1), and TRP (n1-p1) and the corrected peak amplitude for SOL (n1), the latter expressed as an absolute value for comparisons with other muscles.

Experiment 2: vHIT

Procedures. A commercial vHIT system (ICS Impulse, GN Otometrics, Taastrup, Denmark) was used to measure head and eye velocities for each condition. Subjects wore goggles, tightly fitted to the head to minimize potential slippage, that incorporated a video eye recorder (on the right eye) and three gyroscopes for registering head movements in the planes of the horizontal, anterior, and posterior semicircular canals. Subjects stood facing forward, with their toes at the edge of the platform (Fig. 2B). The experimenter stood on a 40-cm-high stand directly behind the subject to perform the head impulses. Both the participants and the experimenter were secured with a harness and a safety line. Hand rails were placed at both sides of the participant at a reachable distance as an extra safety measure.

According to guidelines (Schubert et al. 2004), after calibration of the eye movements subjects were instructed to maintain the gaze at a central fixation point placed on the wall 3.10 m from the edge of the platform, at the level of their eyes, with the subject’s head flexed downward 30°. To perform the head impulses, the experimenters’ hands were placed on both sides of the subject’s head (slightly above the temporomandibular joints) for the horizontal impulses and the left hand on the mandible and the right hand on the apex for the vertical impulses. The experimenter conducted head impulses with ~30° of movement in each direction and target velocity of 100–200°/s, while randomizing the directions within a plane to avoid any anticipatory responses from the subjects.

Horizontal and vertical vHITs were performed in separate blocks that were counterbalanced across height conditions and subjects. To be able to correlate changes in VEMPs acting in the pitch plane, vertical vHIT trials were performed in the pitch plane and not in the planes of the vertical semicircular canals (at ±45° to the pitch plane). The two directions of head thrust within each plane were randomly presented within the block until 20 correct trials were identified for each direction (left and right for the horizontal and upward and downward for the vertical) by the system’s software criteria based on head velocity and absence of artifacts due to excessive goggle slippage or blinks.

vHIT data analyses. The vHIT system used in this experiment (ICS Impulse, GN Otometrics) is designed to calculate VOR gains in the yaw plane and in the orthogonal pitch and roll planes that correspond to the right anterior semicircular canal/left posterior semicircular canal and left anterior semicircular canal/right posterior semicircular canal. To calculate vertical VOR gains in the pitch plane, the raw data from both gyroscopes that are oriented 45° to the pitch plane were extracted and analyzed off-line with a custom-made algorithm (MATLAB 2007, MathWorks, Natick, MA). First, a low-pass Butterworth filter was applied with a corner frequency set at 50 Hz to both head and eye velocity data passing once forward and backward in time. Head velocity was calculated for the vertical vHIT using the square root of the vector sum of the gyroscope signals in the two orthogonal vertical planes (±45° to the pitch plane as described above). The lateral head velocities were used directly, only rectifying the head velocity data in order to apply the same analyses as in the vertical direction. Individual HIT events were analyzed provided the peak head velocity exceeded 60°/s. Eye velocity gains were calculated with respect to head velocity over the interval starting 80 ms before peak head velocity (Weber et al. 2009) until head velocity acquired 35°/s again after its peak. Covert saccades (those occurring before the head velocity zero crossing after peak head velocity), anticipatory quick eye movements (Heuberger et al. 2014), and other artifacts (caused by excessive slippage of the goggles) were identified and...
removed by applying techniques based on previous work (Cleworth et al. 2015). The remaining plots were examined and confirmed by a blinded external evaluator to be without artifacts. After all the data were screened, head and eye velocities were compared using the VOR gains, which were calculated as the ratio obtained when dividing the sum of the eye velocities by the sum of the head velocities over the time frame described above. For statistical analyses, averages across both directions within a plane were used to calculate the horizontal VOR gain and vertical VOR gain.

**Statistical Analysis**

Paired-samples *t*-tests were used to compare differences between height conditions for all dependent measures in both experiments 1 and 2. Correlations between changes in psycho-social and autonomic measures and changes in VEMP amplitudes (experiment 1) and horizontal and vertical VOR gains (experiment 2) were calculated with Pearson’s correlation coefficients for those variables with a normal distribution and Spearman’s *r* for those with a nonnormal distribution. Normality was tested with the Shapiro-Wilk test. All levels of statistical significance were set at *P* < 0.05.

**RESULTS**

**Experiment 1: VEMPs**

Standing in the High compared with Low condition significantly increased EDA (*t*(23) = 2.95; *P* = 0.007), anxiety (*t*(24) = 4.74; *P* < 0.001], and fear (*t*(24) = 4.77; *P* < 0.001] and significantly decreased balance confidence [*r*(24) = 4.77; *P* < 0.001].

Although BGA decreased on average in all muscles from Low to High (SCM = −4.3%, TRP = −2.7%, IO = −2.5%, SOL = −2.9%), the decrease in BGA was only statistically significant for SOL (*t*(19) = 2.10, *P* = 0.049).

Peak latencies for VEMPs in the Low condition were consistent with previously reported values for each muscle (Naranjo et al. 2015; Rosengren and Kingma 2013; Rusidill and Hain 2008; Watson and Colebatch 1998; Welgampola and Colebatch 2005). However, there were no significant changes in VEMP peak latencies between conditions.

There was a significant effect of height on VEMP amplitudes, with average increases of 17.2% for the TRP [*t*(11) = 2.38, *P* = 0.037], 30.5% for the IO [*t*(19) = 3.61, *P* = 0.002], and 30.2% for the SOL [*t*(19) = 2.10, *P* = 0.049] in the High compared with Low condition (Fig. 3). No significant changes were observed for the SCM VEMP amplitude [*t*(13) = 0.60, *P* = 0.56], with an average increase of 4% between height conditions. A significant correlation was found between changes in IO VEMP amplitude and changes in SCM VEMP amplitude (*r* = 0.57, *P* = 0.03) and changes in SOL VEMP amplitude (*p* = 0.51, *P* = 0.02). No other significant correlations between changes in VEMP amplitude were observed.
Increases in IO VEMP amplitude with height were significantly correlated with increases in EDA (ρ = 0.44, P = 0.028) (Fig. 4A) and increases in fear (ρ = 0.43, P = 0.03) (Fig. 4B). Changes in EDA with height were also significantly correlated with changes in SCM VEMP amplitude (ρ = 0.59, P = 0.014) (Fig. 4D) and approached a significant correlation with increases in SOL VEMP amplitude (ρ = 0.37, P = 0.058). No other significant correlations were observed with changes in reported anxiety or confidence in maintaining balance.

These correlations do not seem to be related to changes in BGA, as a significant negative correlation (i.e., in the opposite direction) was found between changes in IO BGA and EDA (ρ = −0.40, P = 0.046) (Fig. 4C) and no significant correlations were observed for changes in IO BGA and fear (ρ = 0.063, P = 0.39) or SCM BGA and EDA (ρ = −0.20, P = 0.23).

Experiment 2: vHIT

For both the horizontal and vertical vHIT conditions, there was a significant increase in EDA (P = 0.004; P = 0.01), fear (P = 0.004; P = 0.002), and anxiety (P = 0.003; P = 0.008) and a significant decrease in perceived confidence (P = 0.006) in the High compared with Low condition.

Mean head peak velocities were similar between conditions for horizontal (Low = 133.15 ± 38.15°/s; High = 122.49 ± 41.56°/s) and vertical (Low = 111.79 ± 3.54°/s; High = 106.18 ± 26.46°/s) vHIT. The vHIT gains in the Low condition were on average 0.99 ± 0.02 and 1.03 ± 0.03, for horizontal and vertical, respectively. Significant increases with height were observed for the horizontal VOR gains ([t(17) = 2.20, P = 0.04] and vertical VOR gains ([t(17) = 3.17, P = 0.006] measured through the vHIT tests (Fig. 5).

A significant positive correlation was found between changes in EDA and changes in horizontal VOR gain (ρ = 0.44, P = 0.03) (Fig. 4A). In contrast, no significant correlations were found between changes in EDA and changes in vertical VOR gain (ρ = 0.24, P = 0.17) or any other psychosocial outcomes (fear, anxiety, and confidence) and either horizontal VOR or vertical VOR.

A strong significant positive correlation was found between changes in the ocular VEMP amplitude and changes in vertical VOR gain with height (ρ = 0.55, P = 0.02) despite being recorded in different sessions (Fig. 4F).

**DISCUSSION**

The first aim of this study was to use VEMPs to investigate whether both VSR and VOR are modulated with increased threat. The second aim was to determine whether these threat-related changes are present in functional measures of the VOR recorded with the vHIT. To our knowledge, these studies are the first to elicit and record VEMPs simultaneously from eye, neck, and lower limb muscles in standing and also under different states of postural threat.

Manipulating postural threat using changes in surface height had a significant effect on subjects’ reported levels of fear of falling, anxiety, balance confidence, and autonomic arousal. These findings are consistent with prior studies that have used real (Adkin et al. 2000; Carpenter et al. 2001; Davis et al. 2009; Horslen et al. 2013, 2014) and virtual (Cleworth et al. 2012) heights to investigate relationships between threat-related factors and changes in balance responses and underlying sensory function.

VSRs were influenced by the manipulation of postural threat; TRP VEMP and SOL VEMP amplitudes were significantly increased with threat, and SCM VEMP amplitudes were
strongly correlated with changes in physiological arousal, with similar trends observed for SOL VEMP. These results replicate prior findings of modest, significant increases in VEMP amplitude in neck and leg muscles with threat (Naranjo et al. 2015). These results support recent evidence from other studies using electrical stimulation of vestibular afferents to examine changes in early vestibular evoked balance response gain under threatening conditions. Horslen et al. (2014) reported a higher coherence and gain between stochastic vestibular (galvanic) stimulation and ground reaction forces measured with a force plate while subjects stood at high compared with low surface heights. Similar increases in coherence and gain between galvanic inputs and EMG of posturally engaged muscles were observed when subjects were threatened with postural perturbations (Lim 2014).

One unique aspect of this study was the inclusion of simultaneous recordings of VEMPs from extraocular muscles, which involve common peripheral inputs but different central vestibular pathways. The results indicated significant increases in the IO VEMP amplitude between Low and High conditions, which parallel the changes observed in TRP and SOL VEMPs. The IO VEMPs were recorded for the first time in a standing posture; however, muscle BGA, peak amplitudes, and latencies were consistent with previous findings (Rosengren and Kingma 2013). Evidence supporting a relationship between threat-related factors and IO VEMP amplitude can be drawn from the strong significant positive correlations found between changes in IO VEMP amplitudes and changes in EDA and fear, which explain 19% and 18% of the IO VEMP amplitude variance, respectively.

The observed changes in VEMPs with threat could not be explained by changes in BGA. VEMPs increased in SOL, TRP, and IO; however, BGA in the same muscles decreased between the Low and High conditions. The decrease in SOL BGA with height has been observed previously (Carpenter et al. 2001), and motor neuron excitability in SOL measured with H reflexes is unchanged (Horslen et al. 2013) or decreased when standing at height (Sibley et al. 2007) or in challenging conditions (Llewellyn et al. 1990). Likewise, the positive correlations observed between changes in VEMP amplitudes and changes in fear (IO) and arousal (IO and SCM) were in contrast to correlations between these variables and BGA, which were nonsignificant (SCM) or in the opposite direction (IO).

**Effects of Postural Threat on VOR: vHIT Gains**

The results of experiment 1 provide novel evidence of simultaneous modulation of VSR and VOR pathways under conditions of postural threat. However, given the moderate effect size and predominant otolith contribution to ocular VEMPs (Rosengren and Kingma 2013; Welgampola and Colebatch 2005), it remained uncertain whether state-related electrophysiological changes would translate to functional changes in the VOR, especially those that rely primarily on stimulation of the semicircular canals. Therefore, the second aim of this study was to examine the effects of threat on VOR function in response to a natural stimulus of the vestibular receptors through fast passive head movements in two orthogonal planes.

A significant effect of threat on VOR gain was observed for both the horizontal and vertical head movements, and changes in horizontal VOR gain were significantly correlated with changes in EDA. The observed effects of threat on vertical VOR and horizontal VOR gains are consistent with prior reports of VOR gain changes associated with trait anxiety (Foruman et al. 2006), mental stress (Yardley et al. 1995), and sleep deprivation (Quarck et al. 2006). Yardley et al. (1995) observed an increase in the slow velocity phase of both the caloric and postrotary nystagmus in people with chronic anx-
Neural Modulation of Vestibular Reflexes During Height-Induced Postural Threat

The observations of parallel and highly correlated changes in VSR and VOR across threat conditions support the concept of a shared mechanism of vestibular reflex modulation with threat. As proposed previously, the vestibular nuclei are the most likely common site of modulation with threat for VOR and VSR pathways (Horslen et al. 2014; Naranjo et al. 2015; Staab et al. 2013), as these neural efferents receive excitatory inputs from neural regions involved in threat-related response (i.e., fear, anxiety, arousal, vigilance) including the parabrachial nucleus (Balaban 2002; Balaban and Thayer 2001; Staab et al. 2013), noradrenergic projections from the locus coeruleus, serotonergic projections from the dorsal raphe nucleus and the nucleus raphe obscurus (Halberstadt and Balaban 2013), and histaminergic projections from the hypothalamus (Peng et al. 2013; Serafin et al. 1993; de Waele et al. 1992; Yabe et al. 1993). Alternatively, the increased gain could be the result of other pathways that converge at the vestibular nucleus complex (cortical, somatosensory, basal ganglia, cerebellum), which are influenced themselves by threat (Staab et al. 2013).

However, there are other potential mechanisms that should be considered. For example, the peripheral vestibular receptors receive innervation from efferent cell groups located in the brain stem immediately lateral to the abducens nucleus (Rasmussen and Gacek 1958). These cell groups could at the same time have input from pathways underlying threat responses. Although it was thought that this efferent system could modulate the resting firing discharge of hair cells during active head movements, studies in monkeys have shown similar activity patterns in vestibular neurons in response to active and passive head rotations (Cullen and Minor 2002), leaving the role of these vestibular efferents still unknown. Evidence against a peripheral mechanism involving the receptors relies on observations of changes in stochastic vestibular stimulation reflexes with threat (Horslen et al. 2014), which is considered to stimulate primarily the vestibular afferents. However, it is not clear whether the galvanic current also affects the firing rate at the vestibular receptors, and thus the potential influence of the receptors during threat cannot be fully discounted.

Alternatively, VSR and VOR could be modulated through nonvestibular pathways that project onto spinal and cranial motor nuclei that control neck, leg, and eye muscles, for example, the reticular formation, which is active during states of increased threat (Paré et al. 2004) and also increases readiness to react to postural adjustments (Prentice and Drew 2001). The reticular formation sends excitatory projections to the motor neuron pools of the spinal cord, via the reticulospinal pathways (Peterson et al. 1979), and the oculomotor nuclei (Büttner-Ennever and Büttner 1988). Likewise, serotonergic projections from the raphe nuclei could also connect with the motor neuron pools, via excitatory interneurons in the spinal cord (Todd 2002), and the oculomotor nuclei (Peyron et al. 1998). Other descending motor pathways, including tectospinal and cortico-spinal pathways, may be active under threatening conditions (Paré et al. 2004; Schutter et al. 2008) and could influence the spinal and oculomotor nuclei resting states as well. Evidence against the potential contribution of increased activation of spinal/cranial motor nuclei can be drawn from a lack of observable increases (and in some case decreases) in BGA and alpha-motor neuron excitability using H reflexes (Horslen et al. 2013; Sibley et al. 2007). Furthermore, the changes in gain and coherence observed using stochastic vestibular stimulation are known to be independent of changes in BGA (Horslen et al. 2014). However, these results are limited, and thus further studies are required to understand the extent to which changes at the spinal/cranial motor nuclei may mediate VSR and VOR changes with increased threat, through either common or independent mechanisms.

Limitations

Compared with the increases observed in VEMP amplitudes in the present and previous (Naranjo et al. 2015) studies (range = 17.2–30.5% and 9–12.7% respectively), the magnitude of changes in vHIT gains with threat were relatively small (2.3–4.3%). While these changes in vHIT gains were similar to the VOR increase (2–4%) observed in subjects with trait anxiety compared with healthy control subjects (Durman et al. 2006), they were also lower than increases reported in the postrotatory nystagmus mean slow phase velocity for subjects under mental stress compared with minimal alerting (≈12%; Yardley et al. 1995) and healthy persons subjected to sleep deprivation (16%; Quarck et al. 2006). Smaller changes may be expected with vHIT given the potential ceiling effect of the head-eye velocity gain in healthy control subjects. The relatively high vHIT gain observed at low height (0.99 and 1.02 for horizontal and vertical, respectively) allows little room for increases compared with rotating chair measures that have lower gains recorded in the dark (0.41–0.5; Durman et al. 2006) and VEMPs. Alternatively, the differences may be explained by different mechanisms associated with trait anxiety (Durman et al. 2006; Yardley et al. 1995) and state anxiety (Spiegelberger 1972). The observed changes in vHIT gains may still have functional significance, as small (10%) changes in the VOR gain are required with multifocal lenses. Such small changes could create nonstable visual feedback, possibly delaying vestibular recovery (Michaeldes and Schutt 2014). Most importantly, our results confirm that threat effects are not limited to changes in electrophysiological measures but are observed in functional VOR measures as well. Although it was a limitation that a similar functional outcome measure was not collected for the VSRs, prior studies have shown that threat-related changes

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in vestibular evoked muscle responses are also evident in ground reaction forces recorded with a force plate (Horslen et al. 2014).

Given that VEMPs and vHITs were performed in stance, we acknowledge the limitation of the potential for threat-related changes in postural sway to influence the outcome measures. For example, the increased frequency of sway observed previously at height (Adkin et al. 2000; Carpenter et al. 2001) could arguably have an effect on the vestibular system. While this could explain to some extent the increases observed in VEMPs at height, it is unlikely to explain changes observed in vHITs since the head was held by the experimenter, reducing body sway oscillations. Furthermore, previous results with stochastic vestibular stimulation would probably not have been affected by changes in underlying head movements (Horslen et al. 2014).

Another potential limitation is that the vHIT methods used in the present study had to be modified from standard clinical guidelines (Schubert et al. 2004) in order to allow for vHITs to be measured during stance and using head impulses along the pure pitch plane instead of in the plane of the vertical semicircular canals. These modifications included 1) having the subject standing instead of sitting and 2) keeping gaze position fixed centrally for vertical vHITs (McGarvie et al. 2015). Despite these deviations, all procedures were kept constant across height conditions and thus should not have any influence on the observed effects of threat. Furthermore, the head velocities produced in this study were within recommended ranges, met criteria for acceptance within the standard clinical software, and were found to be consistent across height conditions.

Conclusions

The observed increases in VEMP potentials and vHIT gains are likely associated with stronger VOR and VSR. This study confirms the effects of height-induced fear, anxiety, and arousal on VSR gain, as reflected in a significant increase in the amplitude of ipsilateral TRP VEMPs and SOL VEMPs and provides evidence of the same effect on VOR gains, recorded with physiological and functional methods. These effects were supported by significant correlations between changes in threat-related responses and changes in the vestibular reflex gains. The observed increases in the vestibular reflex gains with increased threat are most likely mediated at the vestibular nuclei complex, which receives direct excitatory projections from multiple fear processing regions. The new findings provide further insight into the potential mechanisms for threat modulation of vestibular reflexes and unique neurophysiological evidence to support the growing clinical observations of vestibular-emotional links underlying many pathological conditions (Furman et al. 2006; Staab et al. 2013).

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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