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OTOLOGY

Splenius capitis: sensitive target for the cVEMP in older and neurodegenerative patients

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Abstract

Background The vestibular evoked myogenic potential (VEMP) is a technique used to assess vestibular function. Cervical VEMPs (cVEMPs) are obtained conventionally from the sternocleidomastoid (SCM) muscle; however, the dorsal neck muscle splenius capitis (SPL) has also been shown to be a reliable target alongside the SCM in young subjects.

Objective This study aimed to compare cVEMPs from the SCM and SPL in two positions across young, older, and Parkinson's disease (PD) patients.

Method Experiments were carried out using surface EMG electrodes placed over the SCM and SPL. cVEMPs were measured using a 30 s, 126 dB sound stimulus with 222 individual tone bursts, while subjects were in a supine and head-turned posture (also known as the head elevation method), and in a seated head-turned posture.

Results When comparing cVEMPs across positions, the incidence of supine and seated SCM-cVEMPs diminished significantly in older and PD patients in comparison with young subjects. However, no statistically significant differences in incidences were found in seated SPL-cVEMPs when comparing young, older and PD patients. SPL-cVEMPs were present significantly more often than seated SCM-cVEMPs in PD patients.

Conclusions SPL-cVEMPs are not altered to the same extent that SCM-cVEMPs are by aging and disease and its addition to cVEMP testing may reduce false-positive tests for vestibulopathy.

Keywords Cervical vestibular evoked myogenic potential · Splenius capitis · Sternocleidomastoid

Abbreviations

VEMPs	Vestibular evoked myogenic potentials
SCM	Sternocleidomastoid
cVEMPs	Cervical vestibular evoked myogenic potentials
SPL	Splenius capitis
MG	Medial gastrocnemius
TIB	Tibialis anterior
SCM-cVEMP	Cervical vestibular evoked myogenic potential measured from the sternocleidomastoid
PD	Parkinson's disease
SPL-cVEMP	Cervical vestibular evoked myogenic potentials measured from the splenius capitis
EMG	Electromyography
MD	Meniere's disease
PSP	Progressive supranuclear palsy

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Introduction

Vestibular evoked myogenic potentials (VEMPs) are non-invasive neurophysiological tests that specifically assess the utricle and saccule and provide information about unilateral otolith organ function and dysfunction [1]. VEMPs are short-latency potentials measured from tonically active

muscles, typically the sternocleidomastoid (SCM), in response to loud sound, vibration or electrical stimulation of the vestibular system [2]. Despite the focus on the SCM, other work has measured VEMP recordings from additional muscle groups involved in maintaining balance and posture including the splenius capitis (SPL) of the neck [3, 4], as well as the medial gastrocnemius [5] and tibialis anterior muscles of the leg [6].

The typical SCM-cVEMP is most often measured in the supine head-turned posture [4, 7] where usually the subject lies supine at 30° while their head is lifted off a bed and turned sharply, contralateral to the stimulated ear. While head elevation method is the conventional position to record SCM-cVEMPs, several limitations have been identified. First, the head elevation method is often reported to be uncomfortable due to the position subjects are required to maintain [8]. Since SCM-cVEMP amplitude scales with muscle contraction [1], discomfort to the posture limits the subject's ability to maintain the appropriate muscle activation and may consequently result in diminished cVEMP amplitudes. Second, previous work has suggested that 40–50% of subjects older than 60 years of age without vestibular pathology display false-positive results [9], and thus it is possible that the method of activating the muscle target itself poses a problem for cVEMP analysis. Indeed false-positive results may relate to the discomfort experienced during cVEMP testing in the head elevation method for older patients [7, 8].

Recent work has investigated the reliability of using alternative muscle targets such as the dorsal neck rotator/extensor SPL to obtain cVEMPs in young healthy subjects [3, 4]. Camp et al. [4] showed that (1) the biphasic wave found in SCM-cVEMPs is also found in SPL-cVEMPs; and (2) SPL-cVEMPs are just as reliable as SCM-cVEMPs in simple, head-turned postures. Rosengren et al. recently measured cVEMPs in patients with hearing loss as well as patients with vestibular loss and confirmed the SPL-cVEMP to be of vestibular origin [10]. These results highlight the utility of the SPL as a cVEMP target in young healthy subjects. In addition, early work has shown that SPL-cVEMPs are altered in vestibular patients presenting with Tullio phenomenon [11]. In contrast, SCM-cVEMP parameters and response rates have been reported to be altered with increased age, particularly for those who are 60 and older [2, 9]. Similarly, a number of studies have observed changes in SCM-cVEMPs in neurodegenerative diseases including Parkinson's disease (PD) [12–14]. However, there have been no studies to date that have investigated alternative muscle targets such as the SPL in aged or PD patients. As a consequence of this gap, it is unknown whether abnormalities found in SCM-cVEMPs are indeed associated with vestibular dysfunction in these populations or associated with the limitations of the SCM as a cVEMP target. This is particularly important when considering cVEMPs measured from PD patients and interpreting absent SCM-cVEMPs from these populations.

Since the current SCM-cVEMP testing approach uses a reportedly uncomfortable head elevation method, and recent work has shown that the SPL is reliable in simpler postures in young subjects, here we asked whether SPL-cVEMPS are also reliable in older and PD patients. We show that SPL-cVEMPs are reliably elicited in both cohorts, and interestingly that previously described age-related alterations in SCM-cVEMPs are not present in SPL-cVEMPs. This finding potentially has implications for the reduction of the significant false-positive results obtained in older and patient populations.

Methods

This study was carried out in accordance with the recommendations of the National Health and Medical Research Council of Australia's statement on ethical conduct in research (2015) with approval from the Human Research Ethics Committee of the University of Sydney (2017/082). All subjects gave written consent in accordance with the Declaration of Helsinki.

Participants

The sample included 34 participants in total (18 male, 16 female), divided into 3 experimental groups. Two healthy control groups split on the basis of age; 14 young (range 18–30 years of age; mean: 21.85; SD: 2.10) and 14 older (range 50–80 years of age; mean: 64.79; SD: 6.10) subjects. These subjects are normal control subjects. In addition, one patient group was also tested; six PD patients (range 50–80 years old; mean: 64.83; SD: 6.47). The PD patients were age matched according to the control subjects in the older group. Each subject was allocated a subject identification number in chronological order of testing. Both ears were tested for each subject, doubling the observation numbers to 28 young, 28 older and 12 PD. In most cases, each ear was tested at least twice. The age range for older subjects was based on the age range of PD patients in order to age match the control subjects to the patient group. Young subjects were either recruited from the University of Sydney or were relatives of staff and students of the University of Sydney. Older subjects and PD patients were recruited from friends and relatives of staff at the University of Sydney and from Kandos Family Medical Practice. Exclusion criteria included history of sensorineural or conductive hearing loss and/or diagnosed vestibular impairment in addition to late stage PD, as defined by the referring clinician. In addition, one young subject (11) was excluded from the analysis since it could

not be confirmed that the cVEMPs collected from the dorsal neck were indeed from the SPL as the shrug test persistently showed significant EMG activity. Table 1 below lists other information obtained from the demographics questionnaire administered to subjects such as the number of falls each subject had within the last 6 months and medication status. Note that in comparisons between older subjects and PD patients, the older group may also be referred to as age-matched controls.

Table 1 General demographics data from all subjects

Group	Subject	Falls (in 6 months)	Medication
Young	1	0	
	2	0	
	3	0	Ventolin, Seretide
	4	0	
	5	0	
	6	0	
	9	0	
	10	0	
	12	1	Seretide, Prednisone, Erythromycin
	13	0	
	14	3	
	15	0	
	18	0	
Older	7	0	
	8	0	
	16	0	
	17	0	
	20	0	
	21	0	Ibertsan, Gabapentin
	22	1 (H)	
	24	1	
	25	0	Lipitor
	26	0	
	28	0	Lipitor, Coveram, Targin
	31	0	Torvastat, Eutroxsig
	32	0	Nexium, Depresan
	33	1	Methadone, Desvenlafaxine, Valpen, Thyroxine
PD	19	0	Duodopa
	23	0	Metapar, Azilect, Paxam, Clonazapam
	27	0	Metapar, Azilect
	29	0	Levodopa, Carbidopa
	30	1	Azilect, Sinment, Florinef
	34	0	Azilect

H hospitalisation required for the fall

Sound stimulus

The sound stimulus was generated through MATLAB (Mathworks, USA) and consisted of a 500 Hz tone burst delivered at a rate of 7.4 Hz at an intensity of 126 dB sound pressure level (dBSPL). The duration of the sound stimulus was 30 s and included 222 individual tone bursts. To avoid periodicity, the stimulus was jittered by 20 ms. As described by Camp et al. [4], total sound energy is equivalent to sound intensity (peak dBSPL) + 10 log10 (stimulus length in seconds). Since the stimulus intensity used in this study was 126 dBSPL, the sound intensity totalled 120 LAeq (taking into account 3 dB attenuation due to filtering and 3 dB RMS adjustment). Each ear was exposed to four 30 s sound duration stimuli (two in each posture tested). Each tone burst was 4 ms in duration; thus, each ear was exposed to the stimulus for a total of 3.55 s during cVEMP testing. As per the equation above, 120 + 10log10(3.55 s) = 125.4 dB. Additionally, as in Camp et al. [4], the sound stimulus was shaped such that it had a 1 ms rise, 2 ms plateau, and 1 ms fall reducing the time at peak intensity and resulting in a 4 dB attenuation. Therefore, the total sound energy delivered to each subject totalled to 121.4 dB which is within the safety limits. Sound intensity calibration was achieved using a head and torso sound pressure simulator at 500 Hz (Bruel and Kjael, Denmark).

Subject preparation

Information statements outlining the objective, method, risks and benefits associated with the study were provided to participants, along with consent forms. Additionally, two questionnaires were administered. First, the Activities-Specific Balance Confidence scale (ABC16) was administered, a 16-item questionnaire measuring balance confidence in various contexts (0 = no balance confidence, 100 = complete balance confidence) (see Supplementary material 1). Second, a demographic questionnaire was administered to collect information including: age, gender, history of falls, and for patients, disease duration and medication status (see Supplementary material 2).

Prior to the testing component of this study, areas of skin overlying the target muscles were wiped with alcohol swabs in order for the sensors to adhere strongly during head positioning. Muscle activity was measured using double bar differential sensors that are 1 cm apart on the sensor (Delsys, USA). These sensors were selected due to the complex nature of the dorsal neck muscles; the orientation of the double bars ensure that signals are measured from specific muscles and reduces cross talk from muscles with different fibre orientation [4, 15]. It is important to note, however, that the polarity of the waveform may be inverted if the double bar sensors are placed outside the conventional recording zone

[16]. To ensure the sensors remained in place, skin interface stickers for the double bar sensors were attached to the sensor surface (Delsys, USA). Conductive gel was applied to the double bars of each sensor to maximise conductivity. In most participants, using an electric hair shaver was necessary to remove small hairs from target locations around the dorsal neck and beard hair obstructing the SPL and SCM, respectively. A chair fixed in position and a hospital-style bed inclined to 30° were used for testing.

Sensors were placed bilaterally on the SCM and SPL prior to testing. Identifying muscles accounted for most of the preparation time. SPL is located within the posterior triangle of the neck with components lying beneath several other muscles such as the trapezius [4, 17]. An oscilloscope was used to visually verify the sensor placement for SPL. Identifying SPL in participants required simple movements such as conducting the shrug test (asking participants to shrug their neck and hold it while monitoring the oscilloscope to ensure no muscle activity is detected from other muscles like the trapezius) as well as head rotations from side to side (looking for muscle activity in the SPL ipsilateral to the direction of the head turn). Identifying the SCM also involved head rotations in order to place sensors in the middle third of the muscle, ensuring symmetry on each side since compartmentalisation of the muscle may contribute to asymmetry [15, 18]. All sensors were placed so that the double bars were aligned perpendicular to the direction of muscle fibres of all target muscles. A ground/reference electrode was placed on the midline of the forehead.

Experimental procedure

cVEMPs were measured in two postures: (1) the head elevation method: subject lies supine while head is elevated and flexed and rotated contralateral to stimulated ear and (2) simple seated head turns at approximately 90° contralateral to stimulated ear. Testing usually began with simple seated head turns. Participants were asked to insert the earphones into their left ear and to rotate their head approximately 90° to the right. This generated cVEMPs from the ipsilateral (left) SCM to stimulated ear and contralateral (right) SPL as these muscles operate synergistically for contralateral head turns, relative to the ear being stimulated [4]. This posture was held for 30 s. cVEMPs were collected from seated head turns four times—twice per ear. To minimise muscle fatigue, the stimuli were applied to alternating ears. This posture did not maximally activate either the SCM or SPL and as such is unlikely to contribute to significant muscle fatigue. Muscle electromyography (EMG) activity was monitored visually on an oscilloscope throughout to ensure adequate muscle contraction with feedback given to the subject to adjust their posture accordingly. While a previous report in SCM suggests that a minimum background EMG measurement of 80–100 µV is required for cVEMP recording [19], work from our laboratory using a different electrode montage has shown that reliable cVEMPs can be recorded from SPL even with lower amounts of background activity (in the order of 5 uV) recorded on surface electrodes, perhaps due to the greater distance of the surface electrode from the source (see Camp et al. [4]).

The head elevation method was often conducted last. This test measured cVEMPs in the conventional method utilised by neurological clinics worldwide. Participants were assisted in order to lie down on the inclined bed from the seated posture. Once ready, the earphone was inserted into the left ear and the participant maintained the head elevation method for 30 s; neck flexed and turned sharply away from the stimulated ear [4]. Most participants completed four trials in this posture (twice per ear) but some completed only two trials (once per ear) due to discomfort. All tests were conducted in well-lit rooms where mean luminance was approximately 750 lx.

Data collection

The sound stimulus was converted from a digital signal into an analogue signal using an audio interface (Roland, Japan) and amplified using an audio amplifier (Stewart Audio, USA). To deliver the stimulus to participants, insert earphones (Etymotic Research, USA) were used with disposable earphone attachments. Resulting muscle activity was collected using double bar differential sensors and delivered to an EMG interface (Delsys, USA) where signals were differentially amplified (data from the SCM were amplified by 1 K and SPL by 10 K) and synchronised with the sound stimulus (National Instruments, USA). Collected data were converted into a digital signal through an A/D converter (National Instruments, USA) and transmitted to the computer (Lenovo, China) for analysis with custom MATLAB (Mathworks, USA) code. Seated head turns collected data from both SCM and SPL simultaneously, since the position itself contracts both target muscles as a turning pair.

Data analysis

A cVEMP is defined as a biphasic response made up of an initial peak originally denoted as positive (p13) and a second peak originally referred to as negative (n23) [1, 20]. The latency of these peaks is usually within the window of 10–30 ms, with p13 occurring approximately at 13 ms in healthy individuals [1]. In cases where this did not happen, the following criteria were applied: (1) the first peak must fall within the latency of the mean ± 1.5 SD and (2) the latency between p13 and n23 must be within the average of the data set (approx. 6.8 ms). A cVEMP was classified as present but delayed if the peaks exceeded the mean ± 1.5 SD's but had a first peak within the expected time frame, as outlined

in the criteria above. Each peak had to exceed 1.5 SD's of the mean background EMG measured prior to the stimulus onset to be classified as a present cVEMP. For participants where more than two trials were conducted (often due to headphones falling out during a test), the best two trials were selected as cVEMPs for that participant (where best is defined as cVEMPs present in both trials or where both trials closely resembled a present cVEMP). While some subjects underwent multiple trials (in some cases due to movement of electrodes or the insert earphones mid-test), only one replication for each trial was included in the analysis. Often, this biphasic wave is followed by two more peaks between 30 and 50 ms; this has been most commonly associated with the cochlea and was disregarded in cVEMP analysis [2].

Analysis of cVEMP parameters

A number of cVEMP parameters were analysed: the positive–negative ratio represents the absolute value of p13 amplitude divided by the absolute value of n23 amplitude. Latency, as described previously, is the time between the stimulus and the first peak (p13) and between the stimulus and the second peak (n23) that make up a characteristic biphasic cVEMP wave. Peak-to-peak amplitude was determined as the addition of the absolute values of the two peaks, p13 and n23 while peak-to-peak normalised amplitude is the peak-to-peak amplitude divided by the baseline EMG (rectified mean) activity measured prior to the onset of the stimulus.

Statistical analysis

To assess the incidence of cVEMPs within each group, statistical analyses involving 2×2 contingency tables of independence were used returning a chi-squared value. Data were assessed using kurtosis and skewness measures to determine if the data were normally distributed. For data that were normally distributed, a one-way ANOVA was used. For data that were not normally distributed, the non-parametric Kruskal–Wallis tests were used instead with Dunn's correction to account for multiple comparisons. Responses from the left and right ears were compared with no differences and were subsequently pooled. The significance level for all data was set at $\alpha = 0.05$.

Results

Assessment of comfort in the clinical vs. seated posture

As part of the analyses, here a demographic questionnaire was administered prior to testing with questions about gender, age and history of falls (see Supplementary material 2). Question 14 of the demographics questionnaire asked subjects to rate the comfort of the head elevation method and the seated head turns (1 = uncomfortable, 10 = comfortable) and this part was completed following testing. Figure 1 displays the results from question 14. There was a statistically significant difference between ratings for the clinical and seated postures, where seated head turns were rated significantly more favourably than the head elevation method by all experimental groups ($p < 0.05$).

SCM- and SPL-cVEMPs

Figure 2 illustrates the characteristic p13 and n23 peaks in the two muscle targets: the SCM and SPL, in a young subject. As previously shown, [4] both muscle targets display qualitatively similar waveform structure.

Previous work has also shown that there is substantial inter-subject variability in cVEMP waveforms [4]. Figs. 3, 4 and 5 show individual cVEMPs from subjects in each of the three experimental groups (note: a subset of young subjects is shown in Fig. 3). Absence of a cVEMP as defined in Section "Data analysis" is denoted with an asterisk. cVEMPs were obtained from all subjects except for subject 19, a PD patient. Subject 19 was unable to maintain the head elevation method, and thus the response in this posture is represented in the first line of Fig. 5 by a dashed line. A number of findings can be discerned: (1) young healthy subjects are likely to have present cVEMPs in all postures and muscles and (2) SPL-cVEMPs displayed clean waveforms with distinguishable peaks despite having comparatively smaller amplitudes. From Figs. 3, 4 and 5, in almost all cases, p13 of the SCM-cVEMPs was preceded by a positive peak falling between 8 and 10 ms. This has previously been reported [4]; however, it is not clear how this peak relates to vestibular function (see "Discussion").

Incidence of SCM- and SPL-cVEMPs

A cVEMP was determined by the presence of a biphasic waveform with peaks falling within the window of 10–30 ms as described in the "Methods"; Comparisons were made between young and older subjects, older subjects and PD patients and young subjects with PD patients. Each ear was tested more than once. Overall SCM-cVEMP incidence decreases with aging and disease while SPL is far less affected by aging or disease. Fig. 6a, b below display the incidence of cVEMPs, across experimental groups and postures. Figure 6a shows the incidence of cVEMPs organised by experimental groups. Across young subjects, there was no statistically significant difference in the incidence of cVEMPs in either posture or target muscles ($p > 0.05$). Similarly, there was no statistically significant difference in

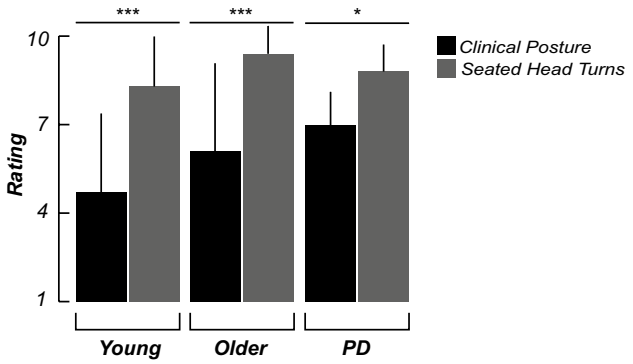

Fig. 1 Assessing comfort in testing postures across each group. All experimental groups rated seated head turns more favourably than the head elevation method. Each group rated each posture they were tested in on a scale of 1–10 (1 = uncomfortable and 10 = comfortable). There was a significant difference between how the head elevation method was rated compared with seated head turns, where subjects in the young ($n = 13$), older ($n = 14$) and PD ($n = 6$) groups rated seated head turns significantly more favourably than the head elevation method (young: $p = 0.0002$; older: $p = 0.0002$; PD: $p = 0.018$). Error bars reflect standard deviations

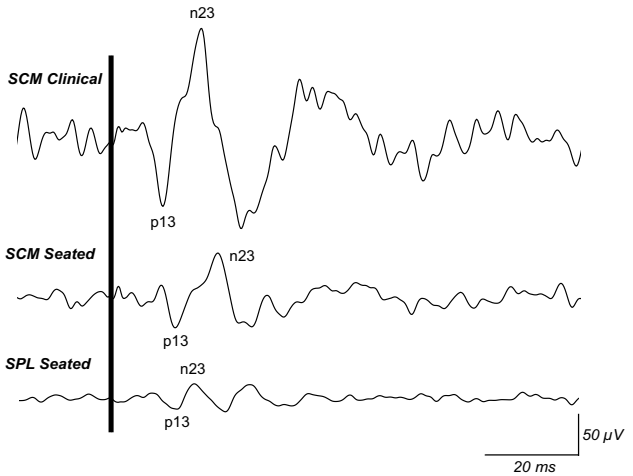

Fig. 2 Cervical vestibular evoked myogenic potentials (cVEMPs) from the SCM and SPL in seated and supine postures. cVEMPs obtained from the sternocleidomastoid (SCM) and splenius capitis (SPL) of a young subject in two positions: the head elevation method and seated head turns. cVEMPs are characterised by a first peak occurring at approximately 13 ms (p13) and a second peak occurring at approximately 23 ms (n23). While amplitudes of the SCM-cVEMPs here are larger than that of the SPL-cVEMP, the SPL-cVEMP still displays distinguishable peaks

the incidence of cVEMPs across posture or muscles in older subjects ($p > 0.05$). However, SPL-cVEMPs were detected significantly more than SCM-cVEMPs in the seated head-turned posture in PD patients (17/24 vs. 7/24, $p = 0.003$).

Figure 6b shows the same data organised by posture. SCM-cVEMPs of the head elevation method were detected significantly more in young subjects when compared with the incidence in older subjects (42/46 vs. 28/44, $p < 0.01$). Furthermore, clinical SCM-cVEMPs were also detected significantly more in young subjects compared with PD patients (42/46 vs. 7/16, $p < 0.001$). There was no statistically significant difference between older subjects and PD patients in the incidence of clinical SCM-cVEMPs. Similarly, when examining the incidence of SCM-cVEMPs in the seated posture, SCM-cVEMPs were detected significantly more in young subjects when compared with older subjects (45/52 vs. 39/55, $p < 0.05$) and when compared with PD patients (45/52 vs. 7/24, $p < 0.001$). Moreover, older subjects exhibited SCM-cVEMPs significantly more often compared with PD patients (39/55 vs. 7/24, $p < 0.001$). Of importance is the incidence of SPL-cVEMPs across all three groups such that there was no statistically significant difference in the incidence of SPL-cVEMPs across all groups tested indicating that SPL-cVEMPs are not impacted by age and disease to the extent by which SCM-cVEMPs are. This suggests that SPL is a robust target for older and clinical populations as it was shown in young healthy subjects previously [4].

cVEMP parameters

Table 2 lists the cVEMP parameters averaged across each experimental group. For the most part, cVEMP parameters were similar across groups and muscles, with a few exceptions. There were no differences between the latencies of cVEMPs in all groups.

Peak-to-peak amplitude was significantly larger for the head elevation method compared with seated SCM-cVEMPs ($p < 0.0001$), and similarly when compared with SPL-cVEMPs ($p < 0.0001$) in young subjects. Seated SCM peak-to-peak amplitude was also significantly larger than that of SPL-cVEMPs ($p = 0.0005$). For the older group, peak-to-peak amplitude was significantly larger in the head elevation method when compared with seated SCM ($p = 0.0019$); clinical SCM amplitudes were also significantly larger than SPL ($p < 0.0001$). Notably, there were no statistically significant differences in peak-to-peak normalised amplitude between all the postures within the young and PD groups, i.e. SPL-cVEMP peak-to-peak normalised amplitude was comparable to that of SCM-cVEMPs, both in seated and supine postures for these groups. However, peak-to-peak normalised amplitude was different when comparing clinical SCM with seated SCM in older subjects ($p = 0.0212$). For PD patients, as above, peak-to-peak amplitudes for clinical SCM-cVEMPs were significantly larger than those from seated SCM ($p = 0.0081$) as well as those from the SPL ($p = 0.0007$).

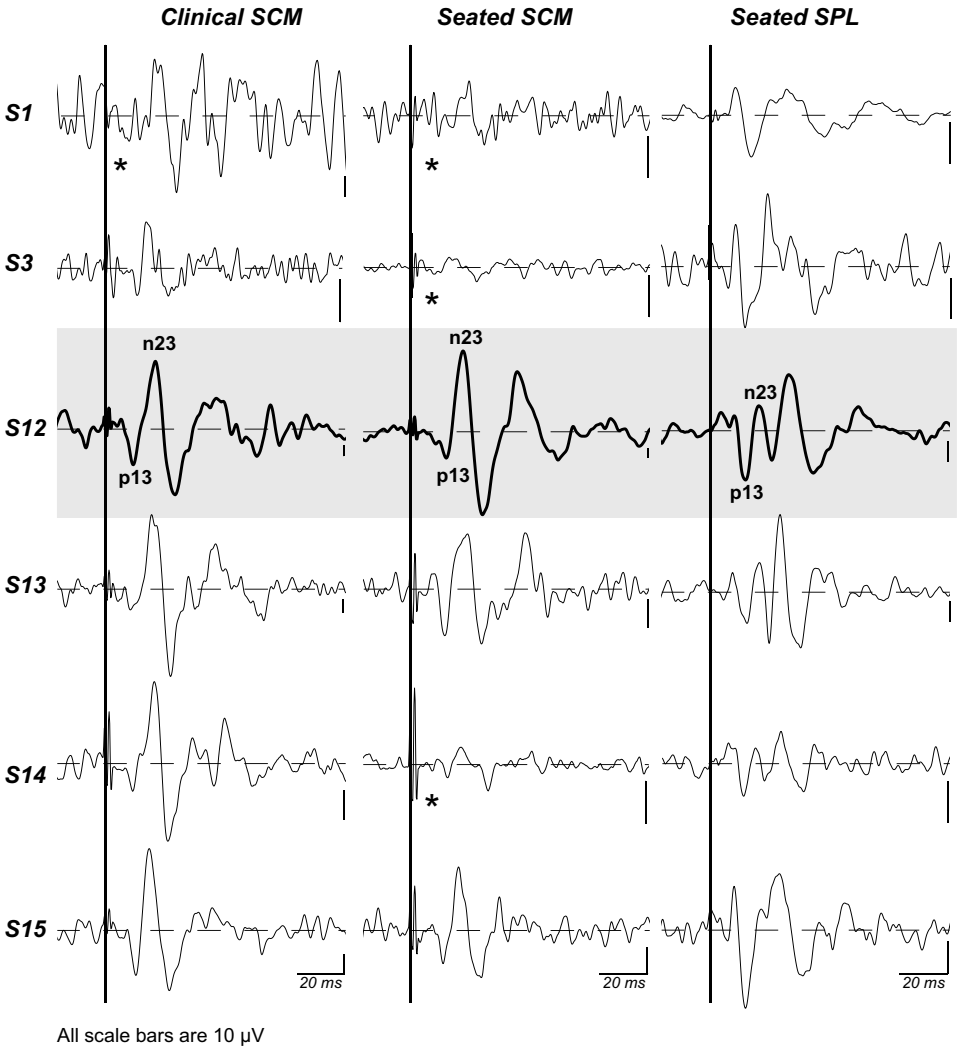

Fig. 3 Sample of cervical vestibular evoked myogenic potentials (cVEMPs) from 6 out of a total of 13 young subjects included in the analysis. cVEMPs included here are collected from both the clinical as well as seated head turn postures. Absent cVEMPs as defined in the "Methods" are denoted with an asterisk. Almost all cVEMPs were present in the young group. Subject 3, however, exhibits an absent SCM-cVEMP measured from the seated posture and seated head turn while presenting with a clear cVEMP from the SPL

Discussion

This study represents the first characterisation of SPL as a target for cVEMP measurements in clinical populations including older people and PD patients. The results show that as expected, cVEMPs from young subjects were measured reliably in both the seated head turns and the head elevation method. Further, cVEMPs were reliably measured in both the SCM and SPL of young subjects as previously reported by Camp et al. [4]. Similarly, cVEMPs were reliably measured from both muscles in older subjects, although as expected the incidence of cVEMP measurements was reduced in the SCM of older subjects. Importantly, no change in the incidence or parameters of cVEMPs was observed in the SPL of older subjects. In addition, this work compared the cVEMPs measured in the SCM and SPL from PD patients with those of age-matched controls and young subjects. As above, reliable cVEMPs can be measured from the SCM and SPL of PD patients but that the SCM-cVEMP incidence is reduced, while the SPL incidence remains unchanged. In the following, the implications of these findings relative to the literature and various points of interest are discussed.

Perceived comfort of the postures

Qualitative evidence suggests that the clinical SCM-cVEMP is limited by the discomfort associated with the position required to activate this muscle [1]. It is recognised that not everyone can tolerate the head elevation method [7, 21, 22]. Further, findings indicate that discomfort or an intolerance of the head elevation method relates to false-positive tests for vestibular pathology [7, 9]. Indeed, some suggest that false-positive tests produced by the discomfort associated with the head elevation method could account for up to 40–50% in older subjects [8, 9]. In this study, quantitative data regarding the comfort of the clinical cVEMP test in comparison

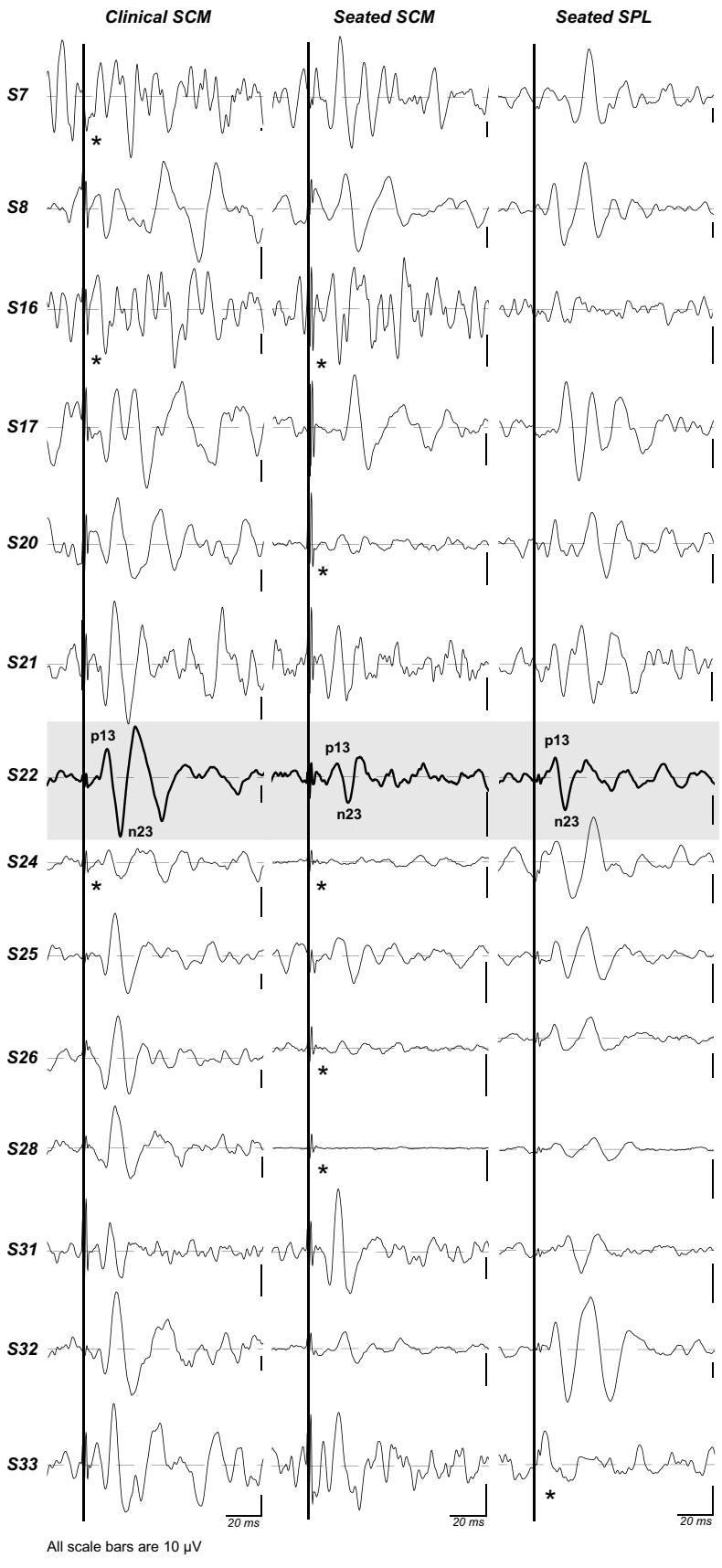

Fig. 4 Cervical vestibular evoked myogenic potentials (cVEMPs) from all the older subjects tested. All older subjects had cVEMPs measured from the sternocleidomastoid (SCM) and splenius capitis (SPL) muscles of the neck at least once on each side. Absent cVEMPs as defined in the "Methods" are denoted with an asterisk. Overall, this figure displays distinguishable SPL-cVEMPs. SPL-cVEMPs were also reliably elicited in subjects from seated and supine postures

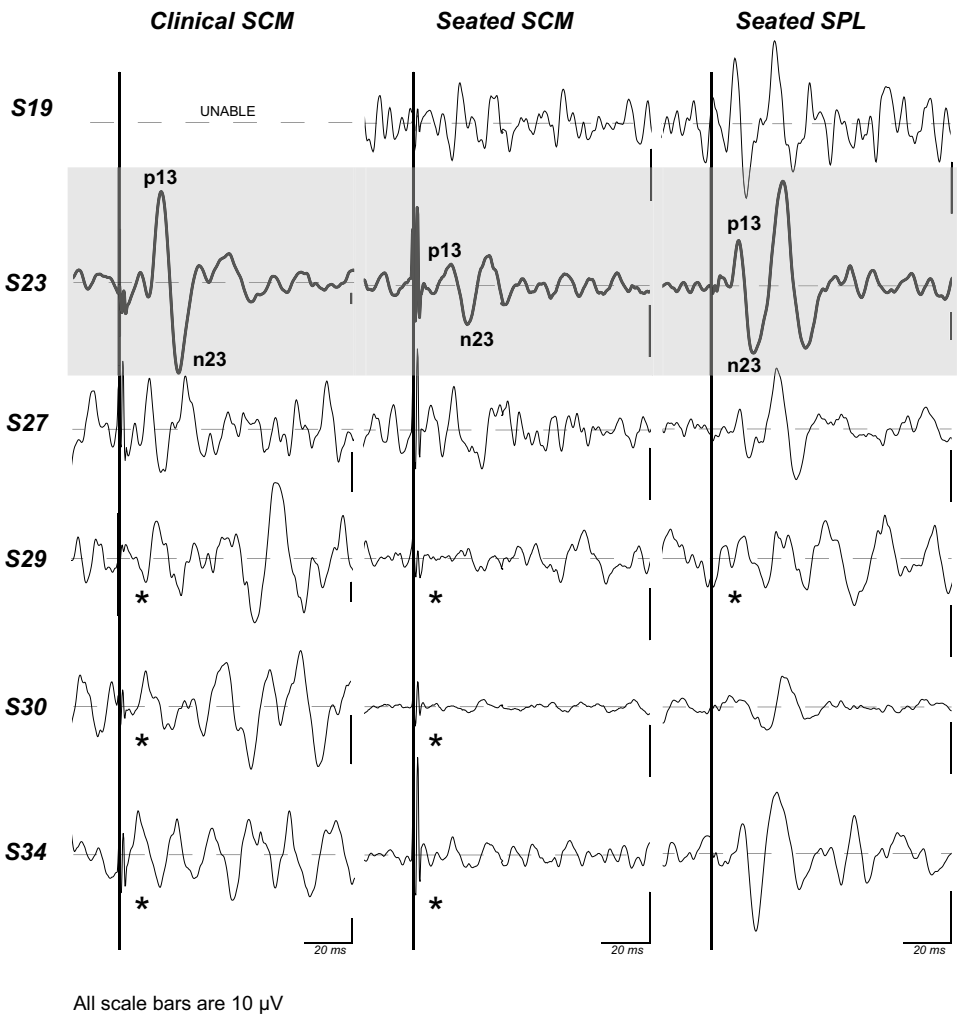

Fig. 5 Cervical vestibular evoked myogenic potentials (cVEMPs) from all the Parkinson's disease (PD) patients tested. All PD patients had cVEMPs measured from the sternocleidomastoid (SCM) and splenius capitis (SPL) muscles of the neck at least once on each side. Absent cVEMPs as defined in the "Methods" are denoted with an asterisk. Note that subject 19 was unable to lie in the clinical position for testing, further contributing to the limitations of the SCM-cVEMPs. While subject 29 was absent for cVEMPs in all postures and muscles, some subjects were present for SPL-cVEMPs, e.g. subjects 30 and 34 and not SCM-cVEMPs

with simple seated head turns are illustrated for the first time (see Fig. 1b). As predicted, each experimental group rated seated head turns as significantly more comfortable than the head elevation method. Since the SPL has been shown to be a reliable target for the cVEMP, these data suggest that the combination of simple seated head turns and SPL measurements provides a comfortable and robust technique for the assessment of vestibular impairment in all subjects.

SCM- and SPL-cVEMPs

Surface and intramuscular recordings have previously been used to investigate the nature of the cVEMP response [4, 16]. All studies of the SCM have shown that the initial peak (p13) of SCM-cVEMPs reflects a reduction in muscle activity while the second peak (n23) reflects an increase in muscle activity [2]. Similarly, Camp et al. [4] showed that the SPL-cVEMP is also built on this inhibition/excitation profile. Importantly, the polarity of the cVEMP waveform depends on the sensor position relative to the motor endplate. For example, waveforms of reversed polarity will be recorded from the SPL sensor if it lies rostral or caudal to the motor endplate. Given that the exact location of the motor endplate is uncertain as SPL is a complex muscle characterised by internal tendinous inscriptions [15], the polarities shown in this paper may be reversed relative to convention. Note as well that SCM is also compartmentalised into multiple subvolumes with different attachments [15]. Further, cVEMP waveform polarity on SCM can be inverted if the active electrode in the standard belly-tendon montage is placed outside of the conventional recording zone [16, 23, 24].

Interestingly, the data often show a brief period of excitation prior to the SCM-cVEMP waveform between 8 and 10 ms after the stimulus (see Figs. 4 and 5, e.g. subjects 23, 32 and 33). This brief excitation has been previously reported [4] although no study has explicitly described this response or how it is related to vestibular function. Investigation of this component of the response may lead to additional diagnostic utility, or the potential for intra-operative cVEMP measurements where tonic muscle activation is not available.

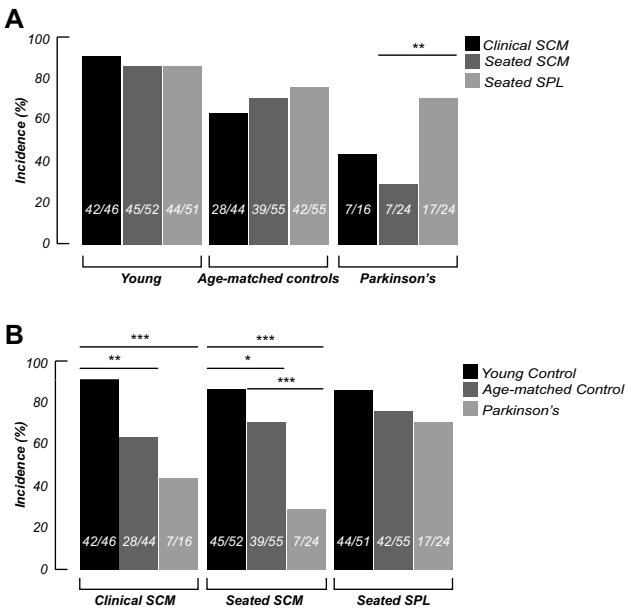

Fig. 6 Incidence of three types of cervical vestibular evoked myogenic potential (cVEMP) tests. Each subject was tested in two postures: seated head turns and the head elevation method and in two muscles: the sternocleidomastoid (SCM) and splenius capitis (SPL). **a** Incidence of cVEMPs within different experimental groups. The figure displays the robustness of SPL-cVEMPs throughout all three groups compared with SCM-cVEMPs. The incidence of cVEMPs did not significantly differ in each posture and muscle for young subjects and age-matched controls. For Parkinson's patients, SPL-cVEMPs were present significantly more than seated SCM-cVEMPs in the seated posture ($p=0.004$). **b** Incidence of cVEMP tests within different postures and muscles. The figure displays significantly greater incidence of SCM-cVEMPs in the clinical and seated postures for young groups compared with PD patients ($p<0.01$ for both). Additionally, SCM-cVEMPs were significantly more present in young subjects compared with older subjects in the head elevation method ($p=0.002$) and in the seated head turns posture ($p=0.049$). Finally, SCM-cVEMPs were also significantly more present in older people compared with PD patients in the SCM-cVEMPs measured in the seated posture ($p=0.0005$). In contrast, there was no statistically significant difference found between the groups in the presence of SPL-cVEMPs

Variability of cVEMPs

Previous work has shown that there is considerable inter-subject variability in cVEMP waveforms in young healthy subjects [4]. Figures 3, 4 and 5 similarly show variability between subjects; however, there is also considerable variability between muscles and postures. Figure 3 provides an example of this. While SPL-cVEMPs show markedly smaller peak-to-peak amplitudes in comparison to SCM-cVEMPs, perhaps due to the greater distance from the surface electrode to the muscle source, the waveforms are still distinguishable allowing for precise interpretation. In addition, akin to some of our findings, previous work has reported both high rates of bilateral SPL-cVEMPs [3] as well as low rates [4]. Interestingly, a number of subjects tested in this study presented with SPL-cVEMPs on both sides despite unilateral stimulation of the ear. This is potentially due to the bilateral projections associated with the medial vestibulospinal tract to neck muscles [25].

Incidence of cVEMPs

A limited number of previous studies have reported on the incidence of SPL-cVEMPs [3, 4, 8, 26]. Almost invariably these studies considered the SPL as a neck extensor rather than a neck turner and relied on a similar awkward position to that used in the clinical SCM-cVEMP (supine, head sharply extended) [8, 27]. Camp et al. [4] in contrast showed that SPL, acting as a head rotator synergistically with the SCM, is a reliable target for the cVEMP. Like that work, this study showed that there are no statistically significant differences in eliciting SCM-cVEMPs in simple head turns compared with the head elevation method in young healthy subjects [4]. Importantly, Camp et al. found that moderate levels of SPL muscle activation elicited by moderate head turns (45°) produced comparable cVEMP incidence to the clinical SCM-cVEMP. While this study examined SPL-cVEMPs in 90° head turns, being able to elicit cVEMPs at moderate head turns is beneficial for older and clinical populations that are not able to strongly contract their neck muscles and/or turn their heads sharply. There is concern that SCM-cVEMPs are associated with false-positive tests for vestibular pathology in those older than 60 years [9]. Indeed, this study found that the incidence of SCM-cVEMPs diminished when comparing the young group with the older group in the clinical position as well as in seated head turns, consistent with current knowledge on the alterations of SCM-cVEMPs with aging [2, 28]. While no previous studies have compared young with older subjects in terms of SPL-cVEMPs, this study found that SPL-cVEMP incidence is not affected by aging, further highlighting the reliability of the SPL as a robust target for the cVEMP especially in clinical populations including older people who are susceptible to false-positive tests.

cVEMP parameters

There are several parameters that can be used to describe cVEMPs including peak-to-peak amplitude, normalised peak-to-peak amplitude, latency and positive–negative amplitude ratio.

Amplitudes of cVEMPs

Previous work has examined the utility of absolute amplitude values in comparison to alternative measures such as left-to-right asymmetry [2] as well as peak-to-peak normalised amplitude which corrects for baseline EMG activity

Table 2 Characteristics and cVEMP parameters relating to groups tested (mean ± SD)

	Young	Older	PD
No. of subjects	13	14	6
Gender (M/F)	7/6	6/8	5/1
Mean Age	21.85 ± 2.1	64.79 ± 6.1	68.67 ± 6.5
p13 latency average (ms)			
Clinical SCM	14.23 ± 3.4	14.15 ± 3.0	14.79 ± 3.0
Seated SCM	16.67 ± 3.5	15.81 ± 3.7	15.61 ± 2.7
Seated SPL	12.80 ± 3.1	14.00 ± 3.7	14.40 ± 4.8
n23 latency average (ms)			
Clinical SCM	20.87 ± 4.0	20.78 ± 4.1	20.55 ± 3.5
Seated SCM	23.52 ± 4.1	21.89 ± 4.4	20.87 ± 3.2
Seated SPL	18.23 ± 4.0	20.83 ± 4.7	20.36 ± 5.6
Peak-to-peak amplitude (μv)			
Clinical SCM	113.62 ± 76.4*†	52.79 ± 33.3**‡‡	91.91 ± 45.6§***
Seated SCM	48.92 ± 36.2‡	29.28 ± 24.3	14.13 ± 5.0
Seated SPL	21.37 ± 11.3	18.44 ± 15.1	16.99 ± 12.9
Peak-to-peak normalised (ratio)			
Clinical SCM	41.69 ± 67.8	64.30 ± 119.0††	52.09 ± 83.1
Seated SCM	31.93 ± 53.2	29.59 ± 75.2	14.51 ± 6.7
Seated SPL	35.71 ± 46.2	29.71 ± 38.8	24.97 ± 24.1
Positive:Negative			
Clinical SCM	0.72 ± 0.3	0.79 ± 0.4	0.74 ± 0.3
Seated SCM	0.72 ± 0.2	0.83 ± 0.3	0.69 ± 0.2
Seated SPL	0.91 ± 0.4	0.79 ± 0.4	0.75 ± 0.2

Significant findings, down the columns

Young group:

*Peak-to-peak amplitude, clinical SCM v. seated SCM ($p < 0.0001$)

†Peak-to-peak amplitude, clinical SCM v. SPL ($p < 0.0001$)

‡Peak-to-peak amplitude, seated SCM v. SPL ($p = 0.0005$)

Older group:

**Peak-to-peak amplitude, clinical SCM v. seated SCM ($p = 0.0019$)

‡‡Peak-to-peak amplitude, clinical SCM v. SPL ($p < 0.0001$)

††Peak-to-peak normalised amplitude, clinical SCM v. seated SCM ($p = .0212$)

PD group:

§Peak-to-peak amplitude, clinical SCM v. seated SCM ($p = 0.0081$)

***Peak-to-peak amplitude, clinical SCM v. SPL ($p = 0.0007$)

[4, 29]. SCM-cVEMPs appear to have large amplitudes while SPL-cVEMPs tend to have smaller amplitudes when comparing absolute peak-to-peak amplitude. Table 2 lists the associated parameters from the collected cVEMPs and while there are differences in peak-to-peak amplitudes, when amplitude is normalised for baseline EMG activity, there are no differences between the SCM and SPL in all three experimental groups. This highlights the reliability of the SPL as a muscle target for cVEMPs.

Latency of cVEMPs

Demyelination in the brainstem has generally been hypothesised to reduce the speed of conduction in vestibular-related tracts [30]. This externalises as longer latencies to VEMP peaks, whereas complete absence of VEMPs may reflect more severe axonal damage and neurodegeneration [30]. No significant differences were found in clinical and seated SCM-cVEMPs when comparing with the SPL both within and between experimental groups. Murofushi [27] reports relationships between patients with multiple sclerosis and cVEMP results with prolonged latency, previously described as being related to demyelination within the brainstem. On the other hand, the same paper describes PD patients presenting with cVEMP abnormalities such as latency delays and diminished amplitudes [27]. The data presented here, however, found no significant differences in latency between age-matched controls and PD patients in any of the postures/

muscles tested. This finding may reflect no pathology related to demyelination within central vestibular structures of this sample of PD patients [29].

Positive–negative ratios of cVEMPs

Positive–negative ratios are calculated by dividing the amplitude of the initial peak, p13, by the amplitude of the subsequent peak, n23, of the cVEMP and can be thought of as a measure of the waveform shape. This study represents the first time that the positive-negative ratio has been used in cVEMP analysis. The data collected from each group returned comparable ratios, both across all three groups as well as between each posture. These data reflect (1) the utility of SPL as a reliable and robust target for cVEMPs and (2) positive–negative ratios are not affected by aging or PD. The utility of positive–negative ratios in the assessment of the cVEMP to differentiate between clinical diseases is not clear from this work.

Limitations

The difficulty associated with accessing clinical population means that a small sample of PD patients was assessed. However, other EMG studies have reported on similar numbers of subjects [17, 18]. Further, while the sample size may appear small, it actually reflects measurements from 12 individual ears/sides as described in the Methods section.

Conclusion

This study investigated cVEMPs in two muscle targets (SCM and SPL) and in two postures (the head elevation method and seated head turns). While conventional methods have consistently utilised the SCM as a target for the cVEMP, this study as well as previous work indicate the utility of introducing an additional muscle target into the clinical testing battery; specifically, the SPL. SPL-cVEMPs were not altered in terms of incidence by aging or disease to the same extent that SCM-cVEMPs were, indicating that age- and disease-related changes in SCM-cVEMPs reported previously may reflect changes in the SCM muscle itself rather than vestibular pathology. This work shows that the addition of the SPL to cVEMP testing improves the sensitivity of the test, it addresses problems associated with discomfort of the head elevation method, and it may reduce the chances of obtaining false-positive diagnoses for vestibular pathology since a positive test would require absence of cVEMPs in both muscles.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving humans and animal rights This study was carried out in accordance with the recommendations of the National Health and Medical Research Council of Australia's statement on ethical conduct in research (2015) with approval from the Human Research Ethics Committee of the University of Sydney (2017/082).

Informed consent All subjects gave written informed consent in accordance with the Declaration of Helsinki.

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