MUSCLE REFLEX RESPONSES TO ACOUSTIC STIMULI

by

SARAH MARGARET LUXON

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Abstract

Loud acoustic stimuli (>115 dB) are known to evoke electromyographical (EMG) responses in human musculature that differ with body position, presentation rate, and stimulus duration. Long duration acoustic tones (40 ms) with an inter-stimulus interval of 3 – 5 s evoke small amplitude reflex responses in tonically contracted limb musculature, whereas short duration acoustic tones (0.1 – 20 ms) with an inter-stimulus interval of 0.2 – 1 s can evoke EMG responses in limb muscles that are posturally engaged. Therefore the purpose of this study was to investigate the similarities and differences of the EMG responses evoked with repeated short and long duration acoustic tones in tonically contracted axial and limb musculature of supine participants. **Methods:** Twenty subjects (aged 19 – 30) were exposed to 256 presentations of air conducted (AC) acoustic stimuli that were 7 and 40 ms in duration (500 Hz; 118 dB SPL). Two blocks of 128 AC stimuli at each stimulus duration, and one block of no stimuli were presented randomly and binaurally through calibrated headphones. Surface EMG was sampled from the right sternocleidomastoid (SCM), biceps brachii (BB), and soleus (SOL) while participants maintained low level contractions in each muscle. **Results:** Repeated 7 and 40 ms AC stimuli evoked a myogenic potential in the tonically contracted SCM, BB, and SOL in at least 80%, 75%, and 75% of participants respectively. Significant effects of stimulus duration were observed in the SCM and SOL, where significant peaks occurred 5.4 and 6.7 ms earlier in the SCM, and 9.3 ms earlier in the SOL with a shorter stimulus. No significant effects were observed in the BB. **Conclusion:** We have shown that repeated short duration acoustic stimuli presented at a short inter-stimulus interval can evoke reflex responses in tonically contracted limb muscles which has not been shown before. These observations suggest that the EMG responses observed here may differ from those that are influenced by postural engagement.

Preface

The following study was approved by the Clinical Research Ethics Board (certificate number: H11-01688) at the University of British Columbia.

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Acknowledgements

I would like to thank my supervisor Dr. Timothy Inglis for his guidance and support throughout this project. Your patience throughout this project and your continuous advice has been invaluable. I would like to thank my committee members Dr. Romeo Chua and Dr. Jean-Sébastien Blouin for their continual assistance, I am grateful for the insight and direction that you both brought to all parts of this project. A special thanks to Dr. Brian Dalton who was present, interested, and extremely helpful every single step of the way. I would not have made it this far without your assistance! Thank you to Dr. Martin Héroux who was integral to my understanding of the research process and who helped fuel my interest in research through our many conversations. A special thanks to Dr. Mark Carpenter and Dr. John Allum who patiently talked through my data with me and who were instrumental in the interpretation of the results. A big thanks goes out to Jarrod Blinch for his Matlab programming skills and extreme patience in passing on that knowledge. Thank you to all of the graduate students in the School of Kinesiology for your helpful comments and suggestions along the way. A special thanks to Scott Cowan, whose constant love and support helped keep me grounded and calm throughout the entire project. Finally, a huge thanks to my family for allowing me to have this opportunity in the first place. Words cannot describe how lucky I am to have such loving and supportive family and friends, and for that I am forever thankful.

Introduction

The vestibular system is a sensory system innate in all mammals that provides us with a sense of spatial orientation and contributes largely to our sense of balance. Information from the human vestibular system is integrated with visual and proprioceptive inputs, which combine together to allow us to subconsciously maintain a sense of balance, equilibrium, and orientation of the head and body in space (Wilson 1981). Supra-spinal motor projections that act through descending vestibulospinal and reticulospinal pathways are thought to be responsible for regulating the control of balance and posture (Groves et al. 1974; Wilson 1981; Britton et al. 1993). Interestingly short duration acoustic tones that range from 0.1 – 20 ms in duration have been shown to modulate vestibular pathways (Colebatch and Halmagyi 1992; Colebatch et al. 1994), whereas acoustic tones that are 40 ms in duration are thought to modulate activity in the caudal pontine reticular formation (Davis et al. 1982). Muscular responses to both short (0.1 – 20 ms) and long (40 ms) acoustic tones have been observed in surface EMG data of proximal (Cherchi et al. 2009; Luxon 2011) and distal human limb musculature (Watson and Colebatch 1998; Luxon 2011) which demonstrates a projection of these pathways throughout the spinal cord. Sound is thought to activate the hair cells of the otolith end organs via vibrational waves induced by the stapes footplate that travel through the saccular and utricular maculae (Ochi et al. 2001; Curthoys 2010). Once stimulated, the hair cells generate a signal that travels through the inferior division of the vestibular nerve to the lateral vestibular nucleus. The signal is then suspected to project through the spinal cord and synapse with interneurons and motor neurons of somatic muscles. There are a number of injuries and diseases that can affect the inner ear, such as Ménières disease, Benign positioning vertigo, and sensorimotor or conductive hearing loss to name a few, which may alter the muscular response observed. Therefore further research into

these systems and pathways may help elucidate the mechanisms behind inner ear dysfunction and expand our knowledge on this intricate sensory system.

An important aim of vestibular research is to develop techniques for assessing vestibular function in the clinical setting. The recent development of the vestibular evoked myogenic potential (VEMP) test has led to further investigations into short latency reflex testing and evoked responses within the vestibular system. The VEMP test is a short latency myogenic potential evoked by short but loud air conducted (AC) sound stimuli, and observed in surface electromyographic (EMG) electrodes placed over the middle third of the sternocleidomastoid (SCM) muscle belly. Animal research has demonstrated the ability of high intensity AC sounds to activate primary irregular otolith afferents in the saccular maculae of cats (McCue and Guinan 1994; McCue and Guinan 1995), guinea pigs (Murofushi et al. 1995; Murofushi et al. 1996; Murofushi and Curthoys 1997; Murofushi et al. 1999), and squirrel monkeys (Young et al. 1977). More recent research has shown that high intensity AC sound activates both the utricle and the saccule (Curthoys 2009; Curthoys 2010), but research using superior and inferior vestibular nerve sections of human vestibular patients, have determined that the EMG response evoked in the SCM arises from the saccular maculae and courses in the inferior vestibular nerve. Therefore the response evoked in the SCM with high intensity AC sounds is suspected to travel via a disynaptic pathway where the saccular hair cells project to the lateral vestibular nucleus in the brainstem via the inferior vestibular nerve. From there the pathway is suspected to project through the spinal cord via the vestibulospinal tract, and synapse with SCM motoneurons to elicit a response. Given these studies and based on an increasing amount of convergent evidence in human research, the VEMP test is now a widely accepted clinical test of saccular and inferior vestibular nerve function.

The VEMP response is usually recorded with patients lying either supine or recumbent at a 45^{o} angle to the horizontal, and holding their head up or turned to one side (Rosengren 2010; Welgampola and Colebatch 2005; Curthoys 2010). The amplitude of the VEMP response is known to increase linearly with increased background activation in the SCM so it is important to monitor this during the testing period, and normalize the amplitude of the VEMP response to background activation values. The peak to peak amplitude of the VEMP response has been shown to be maximal with a 7 ms (Welgampola and Colebatch 2001) 500 Hz tone burst (Akin et al. 2003; Rosengren et al. 2009; Todd et al. 2009), when presented bilaterally through calibrated headphones at a maximum rate of 4.3 Hz for 256 repetitions (Welgampola and Colebatch 2001). As such, these stimulus parameters are now commonly used in the clinical setting.

The VEMP response is a short latency biphasic waveform with significant positive and negative peaks at 13 and 23 ms respectively (Colebatch and Halmagyi 1992; Colebatch et al. 1994) and observed in the ipsilateral tonically contracted SCM to the stimulated ear. Studies investigating the VEMP response in patient populations with vestibular dysfunction due to Meniere's disease, vestibular shwannomas, vestibular nerve section, and other vestibular disorders have confirmed the vestibular origin of the VEMP response (Colebatch and Halmagyi 1992; Colebatch et al. 1994; Murofushi et al. 1999; Welgampola and Colebatch 2005; Curthoys et al. 2009; Rosengren et al. 2010). A second later negative-positive waveform is observed bilaterally with significant peaks occurring at approximately 34 and 44 ms respectively (Colebatch et al 1994; Welgampola and Colebatch 2005) and is suspected to be cochlear in nature. Both the VEMP response and the n34p44 response increase in amplitude with increasing acoustic intensities, up to 145 dB peak sound pressure level (SPL) (Akin et al. 2003; Huang et al. 2004; Welgampola and Colebatch 2005), but there is a high risk of inner ear damage with

acoustic stimuli beyond 120 dB in intensity. Although the original stimuli used were 0.1 ms clicks at an intensity of 145 dB, a longer stimulus duration at a lower acoustic intensity could yield the same total energy being delivered to the inner ear and may therefore be a safer form of stimulation to evoke a VEMP response.

Subsequent studies using repeated acoustic stimuli that were 12 ms or less in duration (132 – 145 dB; 500 Hz), investigated the ability to evoke a myogenic response in the tonically contracted triceps brachii (Cherchi et al. 2009) and soleus muscles (Watson and Colebatch 1998; Rudisill and Hain 2008). However, myogenic responses were only observed in those muscle groups when they were said to be posturally engaged, and not when the same acoustic stimulus was presented while they were tonically contracted.

In contrast, previous studies using repeated acoustic stimuli that were 40 ms in duration (115 – 124 dB; 1000 Hz), have observed a myogenic response in the tonically contracted biceps brachii and soleus muscles of supine participants (Nichol 2008; Luxon et al. 2011). In this case, responses were only observed in muscles that were tonically contracted (at approximately ten percent of maximal ability) (Nichol 2008; Luxon et al. 2011).

This raises the question as to whether responses evoked in tonically contracted limb musculature vary as a function of stimulus duration, posture, or a combination of both. Postural engagement is known to effect descending vestibulospinal reflex responses, and postural sway during quiet stance can influence these responses further. Given this, the supine position was selected for this study to ensure a tonic contraction while minimizing the potential effects of postural sway.

Therefore, the focus of the present study was to determine whether auditory evoked myogenic potentials: a) could be evoked in tonically contracted upper and lower limb muscles, and b) varied as a function of stimulus duration.

The overall purpose of this study was to examine the similarities and differences of the EMG responses evoked with repeated short (7 ms) and long (40 ms) duration acoustic stimuli in tonically contracted axial and limb musculature of supine participants. We investigated whether the optimal VEMP stimulus of 7 ms (500 Hz) (Welgampola and Colebatch 2001; Akin et al. 2003; Rosengren et al. 2009; Todd et al. 2009) presented at an intensity of 118 dB SPL and an inter-stimulus interval of 1 – 2 s could i) evoke a reliable and consistent myogenic response in the isometrically contracted right SCM, BB, and SOL. If a response was observed in these muscles with a repeated 7 ms acoustic stimulus, then ii) we sought to determine whether this response showed similar properties to a response evoked with repeated 40 ms acoustic stimuli of equal intensity.

All responses were compared in terms of peak latency (the time corresponding to each significant peak outside a two standard deviation bandwidth), and responses in the SCM and BB were also compared in terms of the peak to peak amplitude and peak to peak interval, to determine the effects of AC stimulus duration. Acoustic stimuli at each duration had an intensity of 118 dB SPL despite the fact that tone burst stimuli have a threshold of 114.4 dB SPL for evoking a VEMP response in the SCM (Welgampola and Colebatch 2001). This intensity level was selected based on, a) the risks associated with repeated acoustic stimuli and, b) given that 256 presentations are recommended to observe a VEMP response (Colebatch et al. 1994). According to the Canadian Center for occupational health and safety, a sound that is 118 dB SPL

has a daily exposure limit of 14 seconds. For the purpose of this study, a 118 dB SPL sound was heard for a total of 12 seconds which was just below the safety limits.

We hypothesized that:

1. Consistent with previous pilot work, repeated 40 ms AC stimuli would evoke a myogenic response in all isometrically contracted muscles, whereas repeated 7 ms stimuli would only evoke a response in the SCM and not in the tonically contracted BB or SOL while subjects were lying supine.
2. Given our previous results and those of Brown et al. (1991b), where longer onset latencies were observed with an increased distance of segmental innervation from the brainstem, that initial peak latencies observed in the BB would be shorter than those observed in the SOL.
3. That repeated 7 ms (118 dB SPL, 500 Hz) AC sound stimuli would evoke a VEMP response and n34p44 response in the isometrically contracted SCM.
4. Given that previous reports have shown a significant decrease in peak to peak amplitude of the VEMP response with acoustic stimuli that are above 10 ms and up to 20 ms in duration, we hypothesized that repeated 40 ms (118 dB SPL, 500 Hz) AC sound stimuli would evoke a VEMP response in the SCM that is smaller in amplitude compared to the VEMP response evoked with repeated 7 ms AC stimuli of equal intensity.

Materials and Methods

Subjects

Twenty subjects (7 female) aged 21 - 30 were recruited from a convenience sample of the graduate student population at the University of British Columbia. All subjects were required to give written and oral informed consent prior to participation in the study. Subjects were asked to complete a short self-report on handedness, sex, age, height, and weight. Subjects were included if they were between the ages of nineteen to thirty, and did not report any known hearing, neurological, or motor disorder. The proposed study was approved by the tri-council Ethics Committee of the University of British Columbia and all procedures were conducted in accordance with the Declaration of Helsinki.

Experimental Arrangement

Subjects were asked to lay supine on a fully reclined dental chair, with both elbows at a 45^{o} angle to the horizontal, and both feet flat against a wooden board such that both knees were extended fully and both ankles were at a 90^{o} angle (Figure 1). Subjects were asked to maintain low level isometric contractions of their right SCM, BB, and SOL simultaneously. To establish a target for the isometric low-level sustained contractions, subjects performed at least two brief maximal effort isometric contractions of each muscle group with 30 seconds of rest between contractions to limit the effects of residual fatigue. To ensure a maximal effort, subjects were given visual feedback and verbal encouragement throughout the brief effort (up to 3 s). For all isometric contractions the subjects produced a force against either a firm nylon strap or a footplate. One strap was wrapped over the forehead and secured to the chair to act as resistance for the SCM. A second strap was secured by the right hand and fixed to the ground to provide

resistance for the BB. A third strap was wrapped tightly around the dorsum of the foot to secure the right foot to a footplate and ensure an isometric contraction of the SOL.

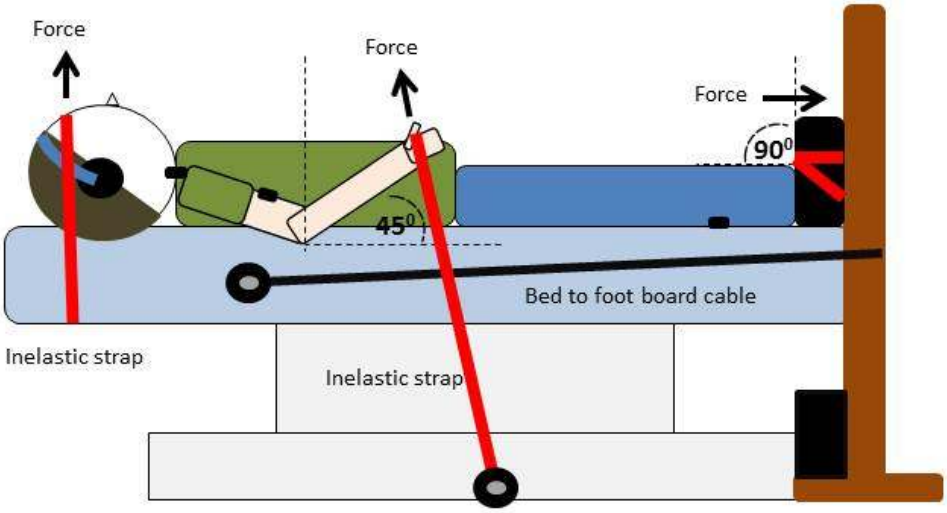


Figure 1: Experimental set up.
The red lines represent the straps, the dashed black lines show the angle the arm and ankle, the solid black arrows represent the direction of force, and the black rectangles on the neck, arm, and leg represent EMG surface electrode placement.

Electromyography

Surface EMG was recorded from the right SCM, BB and SOL using self-adhering disposable cloth electrodes (H59P Repositionable Monitoring Electrodes; Kendall, Mansfield, MA). The electrode sites were prepared by abrading and cleansing the skin with an alcohol swab. Using a 2-cm inter-electrode distance, one electrode pair was positioned on the middle third of the SCM muscle belly (Sheykholeslami et al. 2001; Rosengren et al. 2010), a second pair on the anterior surface of the BB muscle belly at the midway point, and a third pair on the lower third of the SOL muscle belly between the inferior border of the gastrocnemius muscle and above the origin of the Achilles tendon. All surface electrodes were oriented parallel to the muscle fibers with ground electrodes placed on the medial aspect of the clavicle (SCM), medial epicondyle of the humerus (BB), and medial malleolus of the tibia (SOL).

Acoustic Stimuli

A series of acoustic stimuli that were 7 or 40 ms in duration (500 Hz, 118 dB) were presented binaurally through *Telephonics* earphones via a stereo amplifier (Pioneer Stereo Amplifier; Model SA 960) while subjects were lying supine and maintaining low level muscle contractions in their SCM, BB, and SOL muscles. Subjects were presented with repeated acoustic stimuli with an inter stimulus interval of 1 – 2 s, at an impulse intensity of 118 dB SPL, and a frequency of 500 Hz (Rosengren et al. 2010; Welgampola and Colebatch 2005). Stimuli were generated by a custom written computer program (Spike 2 software), and calibrated to sound pressure level ratings on a linear scale with a Quest sound calibrator (Impulse Sound Level Meter; Model 2700).

Experimental Protocol

Subjects were asked to perform two maximal effort contractions, for up to 3 s, in the testing position of the right SCM, BB, and SOL. A window that was approximately 3 s, was used to determine the peak root mean square value of each maximal effort contraction (MEC). This value was taken as the peak value for each trial. Subsequent MECs were recorded if the first two attempts varied in peak amplitude by >5%. The average of the peak root mean square values for each MEC attempt was used to calculate a 10% MEC value for the low level contractions. Given that the relationship between EMG and force output is not linear, there are several limitations involved with the use of EMG alone to estimate muscle force output. We attempted to maximize the fidelity of the peak RMS value as a measure of a MEC by: a) verbally encouraging subjects throughout the contraction, b) repeating the MEC twice or more if the first two values varied in peak amplitude by > 5%, c) asking subjects if they thought they were contracting maximally, and d) providing subjects with a visual display and a target for them to reach. Furthermore we did not use the peak RMS value to compare MECs between subjects, but rather used the peak RMS value to set an approximate 10% MEC level for subjects to maintain throughout each block. The VEMP response is known to increase in peak to peak amplitude with increasing background EMG, so the approximate 10% MEC level was used to ensure that subjects maintained a consistent background contraction between each block of auditory stimuli. Subjects then performed the low-level contractions with or without auditory stimuli. Verbal cues were used initially to achieve a 10% MEC level in each muscle. Once the target level was achieved the trial commenced. During the trial light taps on the contralateral muscle belly that was not being recorded from were used to inform the subject of necessary increases or decreases in contraction level. One tap indicated the need to relax the contraction, whereas two taps indicated the need to

increase the contraction level. However, similar to previous pilot work, subjects had little difficulty maintaining the required contraction levels, and only two subjects required a single reminder to increase their contraction level. Four blocks of 128 stimuli (a total of 256 exposures at 7 ms and 256 at 40 ms) and one block of no stimuli were presented while subjects maintained isometric contractions in all three muscle groups. The block of no stimuli was used to ensure that any responses observed with 7 ms and 40 ms acoustic tones were not the result of any variability involved with the EMG data recordings or signal processing methods. A computerized random number generator determined the presentation order of each block. Each block was approximately 4 minutes in duration with a 1 – 2 minute rest period between blocks to limit fatigue.

Data Acquisition and Analysis

All EMG data were pre-amplified (×10000 for the SCM; ×5000 for BB and SOL), bandpass filtered 30 Hz – 1000 Hz (Grass Instruments P511; as recommended by Rosengren et al. 2010; Welgampola and Colebatch 2005), digitally sampled at 5208.3 Hz (Spike 2 software, and Power 1401; Cambridge Electronic Design, Cambridge UK) and analyzed offline using a custom written program (Matlab 2007, Math Works Inc., Natick, MA). The first trial of each block was excluded from the analysis due to insufficient pre-stimulus data so a total of 254 trials out of 256 trials (as recommend by Rosengren et al. 2010; Welgampola and Colebatch 2005) for each stimulus were spike-trigger averaged to the acoustic stimulus using both Spike2 software and MATLAB 7. VEMP responses in the SCM are typically identified in unrectified EMG (Welgampola and Colebatch 2005; Rosengren et al. 2010) and given that a consistent response waveform was observed in the unrectified EMG data of the SCM and BB, it was possible to evaluate the peak to peak amplitude, latencies, and peak to peak intervals of the myogenic

responses. However, a consistent response waveform was not observed in unrectified EMG data of the SOL, so EMG data were rectified and analysed for peak latencies only. The mean amplitude of 250 ms pre-stimulus rectified EMG was calculated to normalize the peak to peak amplitudes of the unrectified EMG in the SCM and BB to provide a corrected reflex amplitude across subjects (Welgampola and Colebatch 2001; Lee et al. 2008; Rosengren et al. 2010). The stereo amplifier was switched off during the blocks of no stimuli, so EMG data for the sham trials were also spike-trigger averaged to acoustic tones. However no responses were found for this block of data so no further analysis was performed. The classic startle response is identified by some research groups as SCM activation onset latencies that are < 100 ms, greater than background activation, and observed in an EMG trace of a single trial (Carlsen et al. 2007). More recently, Carlsen et al. (2010) suggested that startle activity in the SCM EMG can be distinguished from other SCM activity using a time window of 30 ms to 120 ms following stimulus onset to detect SCM activity outside two standard deviations of rectified EMG data. Therefore each trial was inspected visually for each subject based on these criteria and subsequently removed from the analysis to prevent any confounding effects of the startle response (see Figure 3A for example). Significance was determined using a 2 standard deviation (SD) bandwidth based on the mean amplitude of 250 ms pre-stimulus EMG data, and only responses that exceeded this band were considered significant and included in further analysis. Peak latencies in the BB and SOL were determined by the time to the first significant peak outside the 2-SD band and labeled p1 for the first peak, and p2 for the second peak. Previous pilot work with a repeated 40 ms AC sound stimulus evoked a biphasic waveform response in the BB where the first peak occurred between 42 and 79 ms after stimulus onset. So for the

purpose of this study p1 was identified as the first peak that occurred outside the 2 –SD bandwidth between 30 and 80 ms, and the subsequent peak was labeled p2.

Since the responses observed in the soleus of previous pilot work had a large amount of variability between subjects, recordings from each subject of the present study were averaged together to create a grand mean. A window was then selected from the grand mean to determine the first significant peak latency in the soleus that ranged from 65 ms – 120 ms. Therefore the first significant peak evoked in the SOL of this study was identified by the first peak outside the 2-SD bandwidth that occurred between 65 ms and 120 ms, and labeled p1, with the subsequent peak labeled p2. In the SCM, peaks based on the p13n23 latencies and observed outside the 2-SD bandwidth were used to determine the presence or absence of a VEMP response, and were labeled p13 and n23. Given that the negative peak of the n34p44 response has a mean latency of 33.8 ms ± 2.4 ms (Colebatch et al. 1994), the first negative potential with a peak outside the 2-SD bandwidth that occurred after 30 ms in the SCM was considered the n34 component and labeled n34. Therefore, the subsequent positive peak that occurred after n34 was termed p44 and considered to be the p44 component. Therefore, the peaks of each waveform potential in the SCM retain the same terminology as the literature and are termed p13n23 and n34p44, whereas each significant peak in the BB and SOL was identified as peak 1 (p1) or peak 2 (p2).

Statistics

For each subject, peak latencies, peak amplitudes, and peak to peak intervals of each significant response were averaged. Peak amplitudes were normalized to 250 ms pre-stimulus rectified EMG and expressed as a percentage. Peak latencies of all response waveforms, and the peak amplitudes and peak to peak intervals of response waveforms in the SCM and BB were analyzed for significant effects of stimulus duration (7 ms or 40 ms) using a two tailed paired

Student t-test. Statistical significance was set to $p<0.05$. Descriptive statistics include the mean ± standard deviations (SDs) in the text and figures.

Results

Sternocleidomastoid

The p13n23 response

The effect of stimulus duration on the peak to peak amplitude of the p13n23 response in the SCM can be observed in Figures 2A and 2B which show a myogenic response in the SCM of five individual subjects in response to repeated 7 and 40 ms stimuli. Attenuation of the peak to peak amplitude can be observed in each individual EMG trace, in addition to the overlaid EMG traces of all subjects (Figure 2C -D) and the population averages (Figure 2E -F), in response to repeated 7 and 40 ms stimuli respectively.

A short latency biphasic waveform with peaks around 13 ms and 23 ms was observed in nineteen of twenty subjects (95%) with a repeated 7 ms acoustic stimulus, compared to sixteen of twenty subjects (80%) with a 40 ms stimulus (all values are presented in Table 1). However, only subjects with a biphasic waveform response to both 7 and 40 ms stimulus durations were included in the statistical comparisons. Background activation levels did not vary significantly between blocks of 7 and 40 ms stimuli, where the mean rectified 250 ms pre-stimulus values were 4.0 uV (± 2.6 uV) and 3.8 uV (± 2.5 uV) , respectively $t(19)=0.639$, $p=0.531$.

There was no significant effect of stimulus duration (n = 16) on the peak latencies of either the p13 or n23 peaks. The initial positive peak (p13) had a mean peak latency of 12.0 ms (± 1.5 ms) and 11.7 ms (± 1.8) $t(15)=0.964$, $p = 0.35$, while the subsequent negative peak (n23) had a mean peak latency of 23.0 ms (± 3.2 ms) and 23.7 ms (± 4.1 ms) $t(15)=-0.974$, $p=0.35$ for

repeated 7 and 40 ms stimuli, respectively. This finding explains why there was also no significant effect of stimulus duration on the p13n23 peak to peak interval, where the mean peak to peak interval was 11.2 ms (± 2.6 ms) for repeated 7 ms stimuli and 12.1 ms (± 3.6 ms) for repeated 40 ms stimuli $t(15)=-1.32$, $p=0.195$.

Although there was no effect of stimulus duration on peak latencies or the peak to peak interval of the p13n23 response, there was an effect of stimulus duration on the peak to peak amplitude as expected, where the normalized mean peak to peak amplitude was larger with a 7 ms stimulus at 0.9 (± 0.1), compared to a 40 ms stimulus at 0.6 (± 0.4) $t(15)=6.909$, $p=0.001$. The normalized mean peak amplitude values of the p13 and n23 peaks were larger by 7% and 26%, respectively, with repeated 7 ms stimuli compared to repeated 40 ms stimuli, and therefore both contributed to the overall larger mean peak to peak amplitude of the response with 7 ms stimuli.

Very few subjects displayed SCM activity within the 30 – 120 ms window as outlined by Carlsen et al. (2010), but Figure 3A is an example of what would be removed from the analysis process, and Figure 3B is an example of an individual trial taken from the same subject before averaging. Subjects who did display SCM activity within the 30 – 120 ms window on a single EMG trace that was outside the 2SD bandwidth, did so only in the first few trials of a given block to a maximum of five.

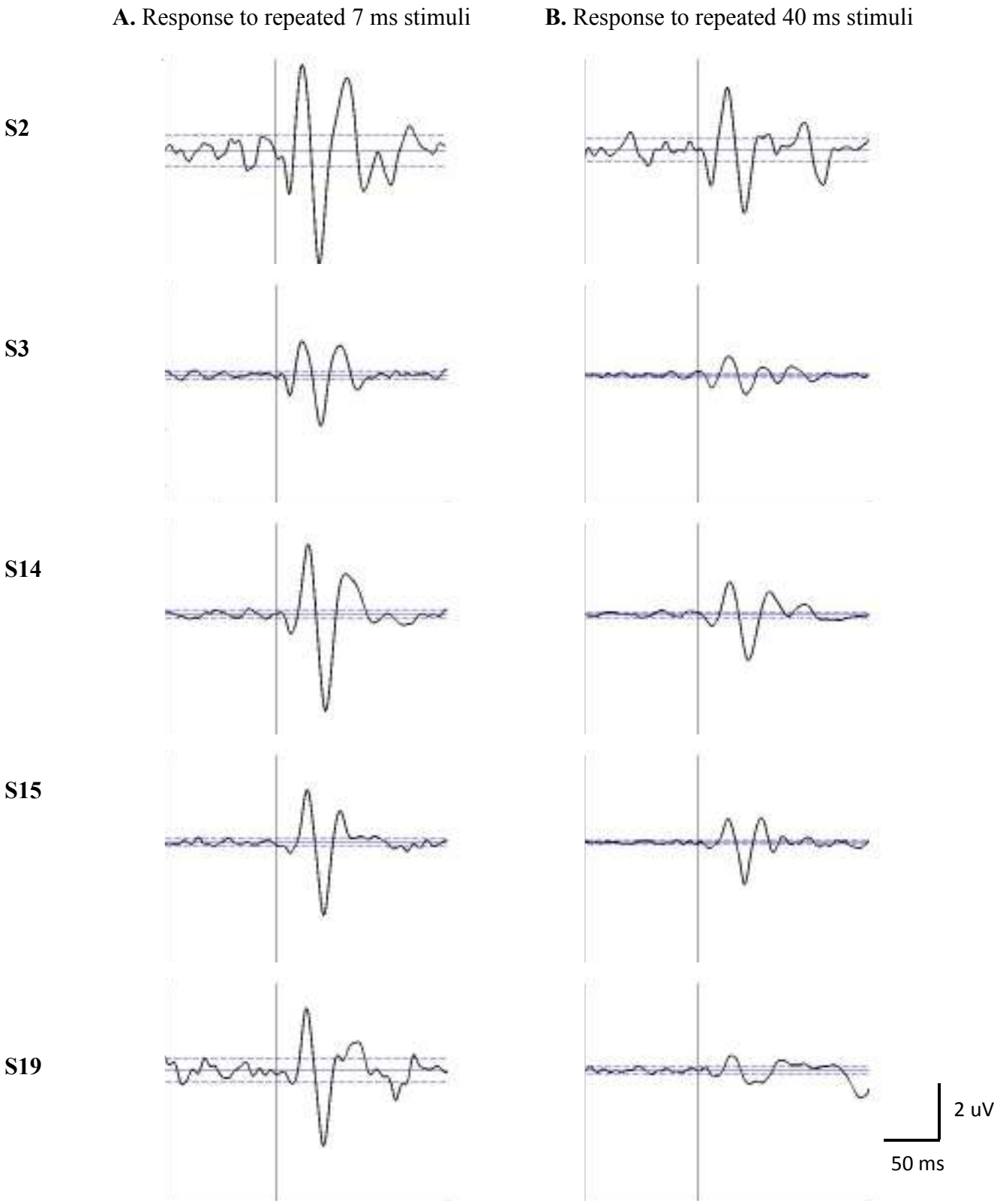


Figure 2 A-B: Individual SCM responses to 7 and 40 ms stimuli.
The average response waveforms obtained from five individual subjects in response to 254 stimuli of both 7 and 40 ms are shown in 2A and 2B respectively. The waveforms on the left hand side show the response of five individual subjects to 7 ms repeated AC stimuli, and the waveforms on the right hand side show the response of the same five subjects to 40 ms repeated AC stimuli. The vertical black bar represents stimulus onset, and the horizontal dashed lines represent the 2 SD bandwidth for each subject.

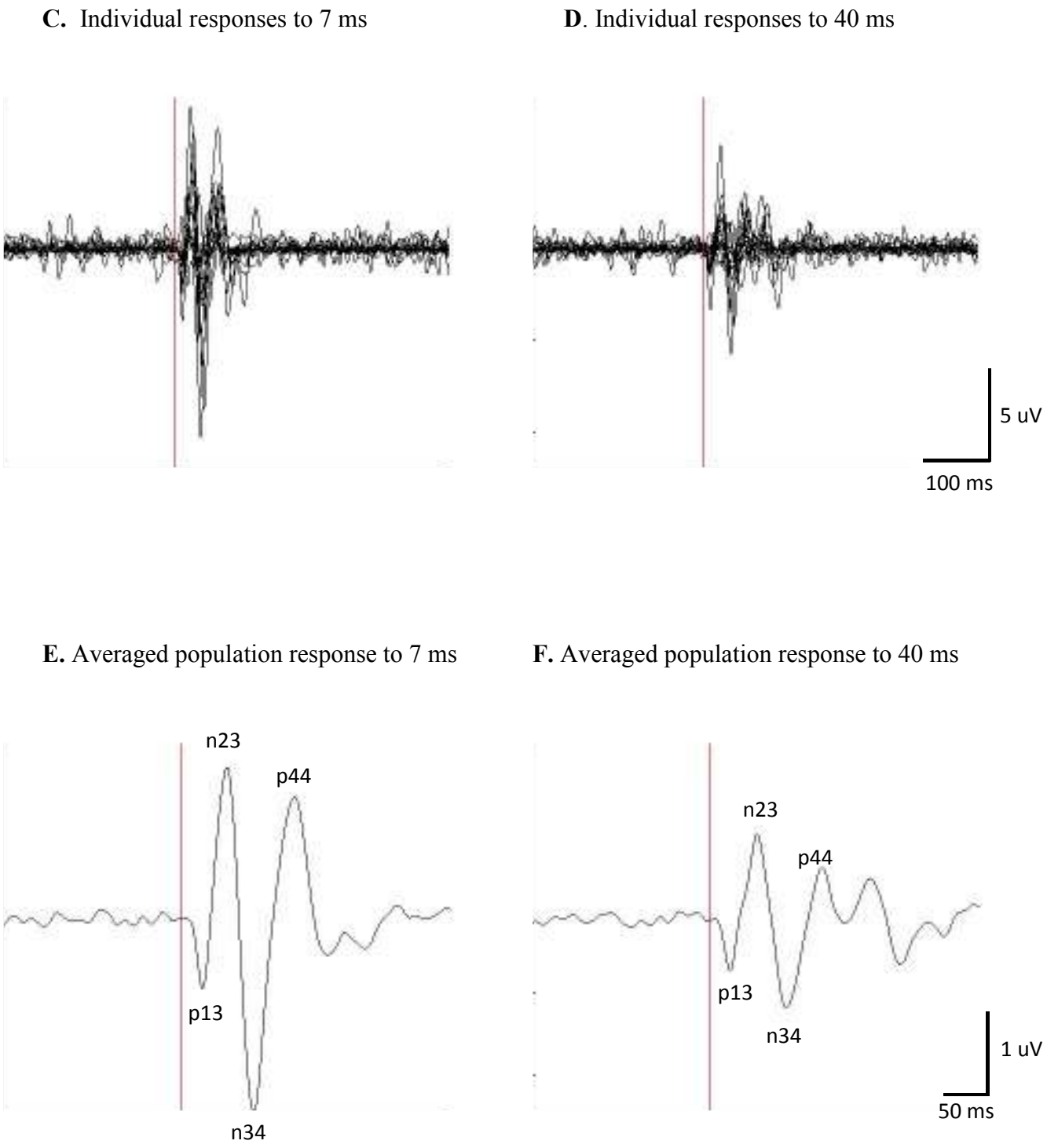
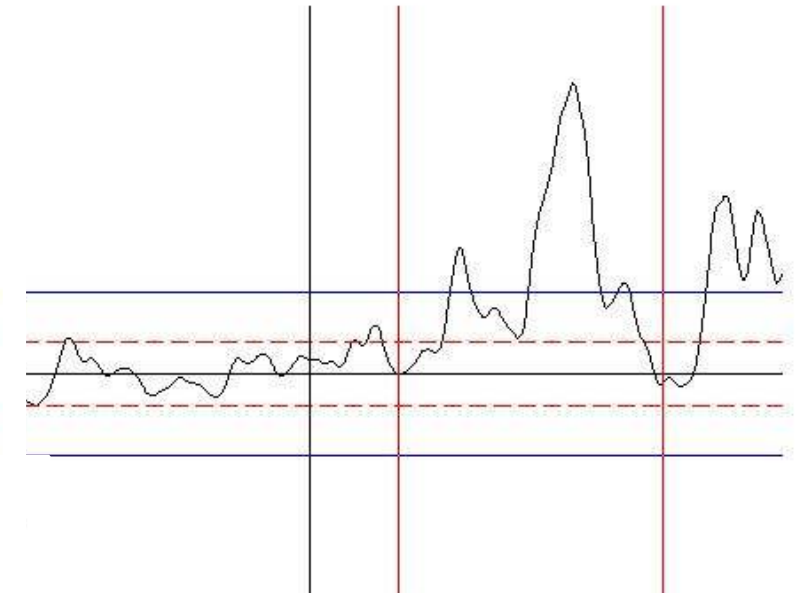


Figure 2 C-F: Population SCM responses to 7 and 40 ms stimuli.
2C and 2D display all individual averages (n=20) overlaid upon one another in response to 7 and 40 ms repeated stimuli, respectively. Each individual average represents an average of 254 stimuli. 2E and 2F are the overall average of those responses in 2C and 2D. The vertical red line represents stimulus onset.

SCM	Corrected Reflex Amplitude (% of background activation)	Peak latency (ms)		Peak to peak interval
	Peak to peak	*p13*	*n23*	*p13-n23*
7 ms	*94*	*11.98*	*22.98*	*11.17*
Std Dev.		*1.47*	*3.24*	*2.55*
40 ms	*62*	*11.72*	*23.69*	*12.13*
Std Dev.		*1.76*	*4.12*	*3.65*

Table 1: Dependent measures of the p13n23 response
Mean peak amplitudes (uV), mean peak latencies (ms), and the mean peak to peak interval (ms) of the p13 and n23 peaks in response to 7 and 40 ms repeated stimuli. The mean corrected reflex amplitudes are displayed as percentages and are also included in the third column. Std Dev. (Standard Deviation).

3A. A response waveform obtained from Trial 1 of the first block in the SCM for a single subject in response to a 40 ms stimulus.



3B. Trial 16 of the first block for the same subject as in 3A.

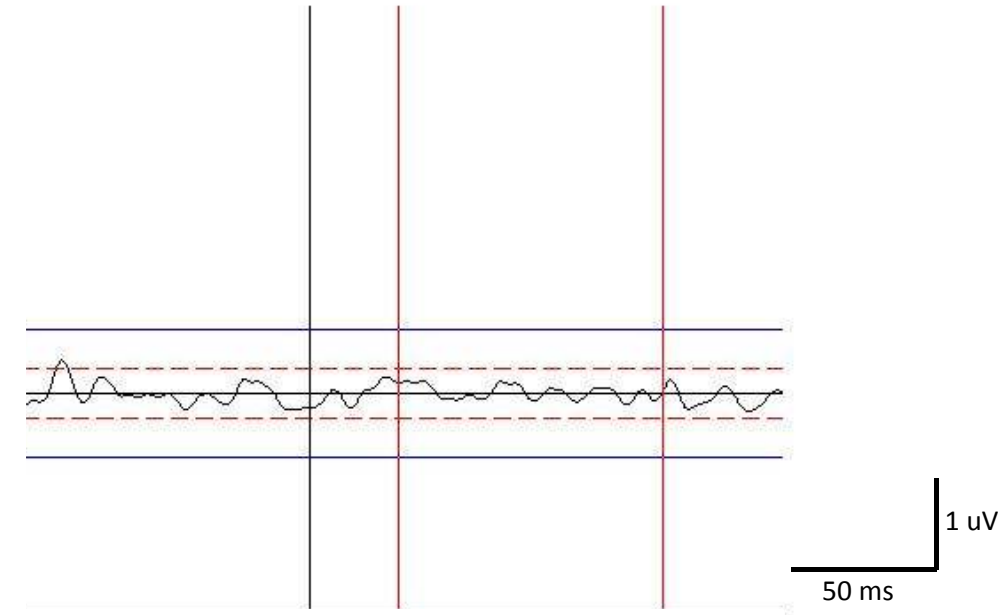


Figure 3: Two separate trials in the SCM.
EMG recordings in the SCM of a single trial in response to a 40 ms stimulus is taken from the first trial of one subject and shown in 3A. A subsequent trial (trial #16) is displayed in 3B. The vertical black line represents stimulus onset, the first red line represents 30 ms and the second red line represents 120 ms (as per the criteria outlined in Carlsen et al. 2010). The horizontal dashed red line represents 2SD and the horizontal solid blue lines represent 5SD outside the mean of 250 ms pre-stimulus rectified EMG data.

The n34p44 response

Contrary to the change in response frequency of the p13n23 waveform with a change in stimulus duration, the n34p44 waveform was observed in nineteen of twenty subjects (95%) for both stimulus durations. Since the n34p44 waveform was also recorded from the right SCM, background values were consistent with those reported for the p13n23 response and did not change between blocks of 7 or 40 ms stimuli, as reported above.

In contrast to the p13n23 response, there was a significant effect of stimulus duration on the peak latencies of both peaks pertaining to the n34p44 response, where both peaks were observed at longer latencies with a longer stimulus. In response to a 7 ms stimulus, the n34 peak had a mean peak latency of 39.4 ms (± 4.2 ms), which was significantly earlier than 44.8 ms (± 6.7 ms) observed with a 40 ms stimulus $t(18)=-3.649$, $p=0.002$. Likewise, the p44 peak was observed 6.7 ms earlier at a mean latency of 58.1 ms (± 6.8 ms) with a 7 ms stimulus, compared to 64.8 ms (± 10.5 ms) observed with a 40 ms stimulus $t(18)=-2.707$, $p= 0.014$.

However, although each peak was observed at a longer latency with a longer stimulus duration, there was no significant effect of stimulus duration on the overall n34p44 peak to peak interval, which had a mean duration of 18.6 ms (± 6.4 ms) and 20.0 ms (± 7.6 ms) for 7 and 40 ms stimuli, respectively $t(18)=-0.507$, $p=0.618$.

Similar to the p13n23 response, there was an effect of stimulus duration on the peak to peak amplitude of the n34p44 response, where the normalized mean peak to peak amplitude was almost twice as large with repeated 7 ms stimuli at 1.2 (± 0.7), compared to 40 ms at 0.7 (± 0.5) $t(18)=5.697$, $p=0.001$. The overall attenuation and shift in peak latency of the n34p44 response can also be observed in the individual traces of Figure 2A and 2B, in addition to the population averages in Figure 2E - F. All values for the n34p44 response are shown in Table 2.

SCM	Corrected Reflex Amplitude (% of background activation)	Peak latency (ms)		Peak to peak interval
	Peak to peak	*n34*	*p44*	*n34-p44*
7 ms	*119*	*39.38*	*58.07*	*18.69*
Std Dev.		*4.23*	*6.85*	*6.38*
40 ms	*74*	*44.76*	*64.79*	*20.04*
Std Dev.		*6.73*	*10.49*	*7.57*

Table 2: Dependent measures of the n34p44 response
Mean peak amplitudes (uV), mean peak latencies (ms), and the mean peak to peak interval (ms) of the n34 and p44 peaks in response to 7 and 40 ms repeated stimuli. The mean corrected reflex amplitude are displayed as percentages and are also included in the third column. Std Dev. (Standard Deviation).

Biceps

An auditory evoked myogenic response was observed in the right biceps in seventeen of twenty subjects (85%) with a 7 ms stimulus, and in fifteen of twenty subjects (75%) with a 40 ms stimulus. A significant peak was observed outside the selection window at > 90 ms in two subjects with 7 ms stimuli, and three subjects with 40 ms stimuli, however these responses were not included in the overall analysis process. Therefore statistical testing was only performed on subjects who had a response to both stimulus durations with an initial peak latency between 30 and 80 ms (n = 14).

Similar to the SCM muscle, the background activation level of the right biceps muscle did not differ significantly between blocks of 7 ms (mean of 18.5 uV ± 9.0 uV) and 40 ms stimuli (mean of 17.9 uV ± 9.0 uV) $t(19)=0.907$, $p= 0.376$. There was no significant effect of stimulus duration on the peak latencies observed with repeated 7 or 40 ms stimuli, where p1 had a mean peak latency of 47.1 ms (± 8.1 ms) and 48.9 ms (± 7.5) ms $t(13)=-0.736$, $p= 0.475$, and p2 had a mean peak latency of 62.0 ms (± 8.6 ms) and 62.6 ms (± 7.7) ms $t(13)=-0.198$, $p=0.846$, for repeated 7 and 40 ms stimuli, respectively. There was also no significant effect of stimulus duration on the normalized mean peak to peak interval which had a mean duration of 14.9 ms (± 1.9 ms) for repeated 7 ms stimuli, and 13.6 ms (± 2.6 ms) for repeated 40 ms stimuli $t(13)=1.483$, $p=0.162$.

In contrast to our hypothesis, there was no effect of stimulus duration on the normalized mean peak to peak response amplitude, which had a mean value of 0.6 (± 0.4) for both 7 and 40 ms stimuli $t(13)=-0.632$, $p<0.537$. This can be observed in individual data outlined in Figures 4A- B. In addition, Figures 4C-F demonstrate the population response data.

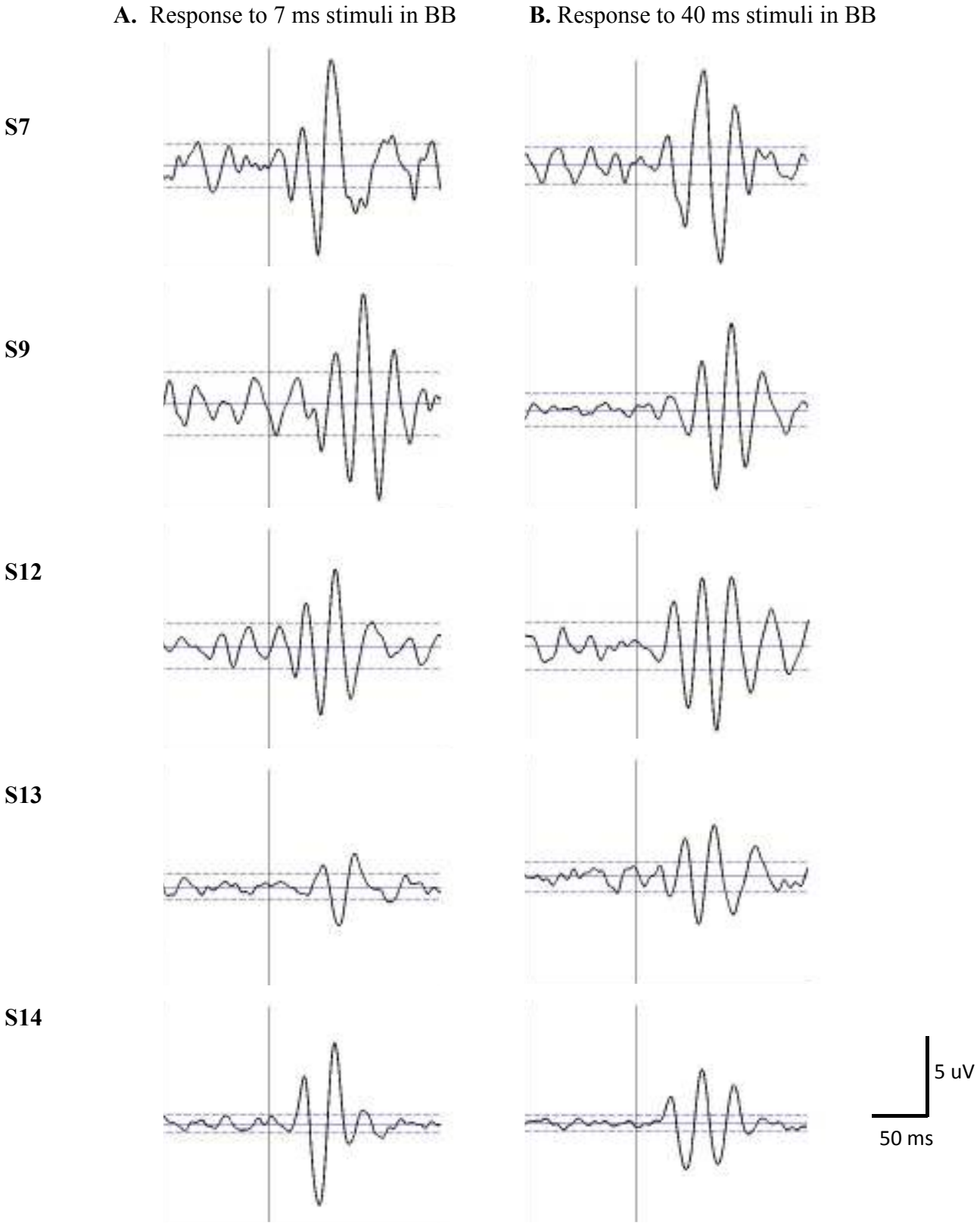
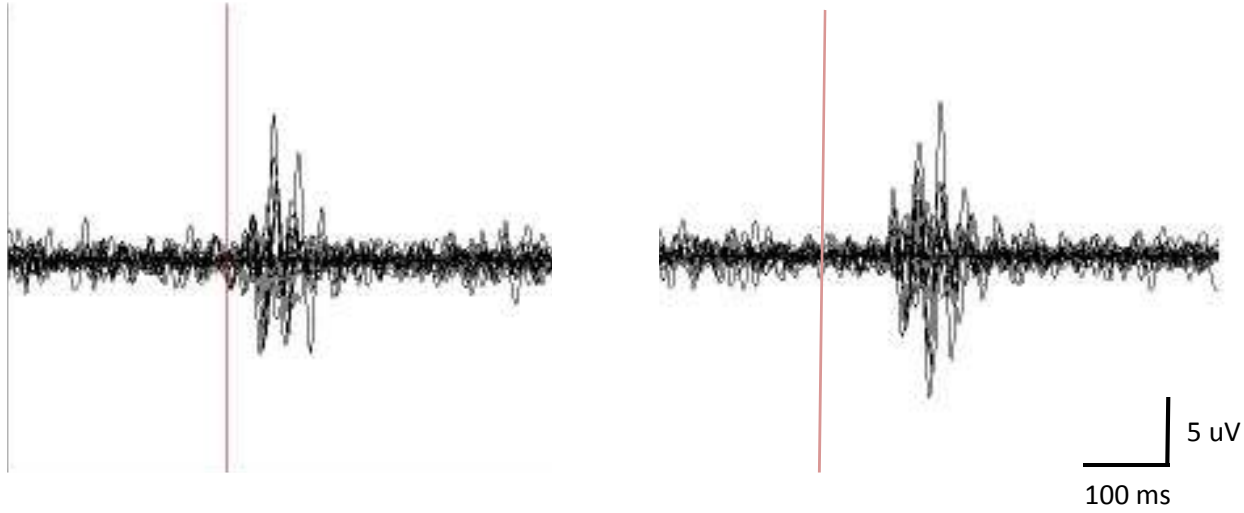


Figure 4 A-B: Individual responses in the BB to 7 and 40 ms stimuli.
The average response waveforms in the BB obtained from five individual subjects in response to 254 stimuli of both 7 and 40 ms are shown in 4A and 4B respectively. The waveforms on the left hand side show the response of five individual subjects to repeated 7 ms stimuli, and the waveforms on the right hand side show the response of the same five subjects to repeated 40 ms stimuli. The vertical black bar represents stimulus onset, and the horizontal dashed lines represent a 2 SD bandwidth for each subject.

C. Individual responses to 7 ms stimuli

D. Individual responses to 40 ms stimuli



E. Averaged population response to 7 ms

F. Averaged population response to 40 ms

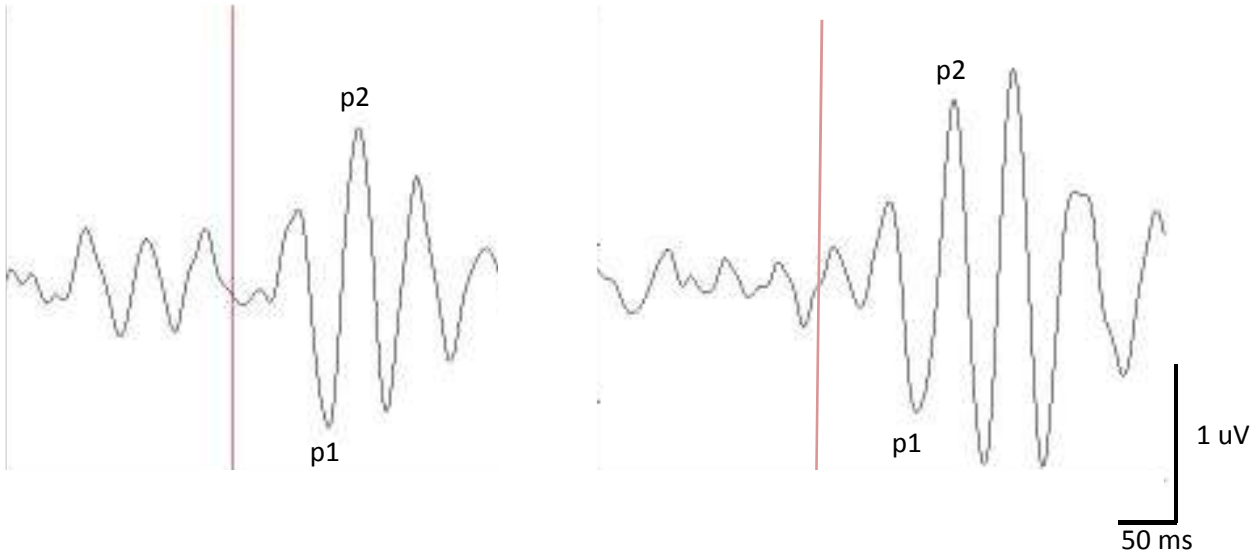


Figure 4 C-F: Population responses in the BB to 7 and 40 ms stimuli.
4C and 4D display all individual averages in the right biceps overlaid upon one another in response to 7 and 40 ms repeated stimuli, respectively. Each individual average represents an average of 254 stimuli. 4E and 4F are the averages of those in 4C and 4D.

Soleus

Similar to the right SCM and right biceps, background activation levels did not differ between blocks of repeated 7 ms and 40 ms stimuli for the right soleus muscle, $t(19)=0.272$, $p=0.788$.

A significant peak that occurred outside the 2 SD bandwidth between 65 – 125 ms was observed in fifteen of twenty subjects (75%) with repeated 7 ms stimuli, and in all subjects with repeated 40 ms stimuli (100%). However it should be noted that the five subjects who did not show a significant peak within the window of 65 – 125 ms with repeated 7 ms stimuli, did show a significant peak outside the specified window where one subject had a peak latency of 58 ms, and the remaining four subjects had peak latencies that ranged from 128 ms – 178 ms. A second peak outside the 2 SD bandwidth occurred between 110 – 195 ms and was observed in only six of the fifteen subjects in response to 7 ms stimuli, and eleven of the twenty subjects in response to 40 ms stimuli. There was a significant effect of stimulus duration on the peak latency ($n=14$) where the first peak was observed 9.3 ms later with a mean peak latency of 101.4 ms (±15.7) compared to 92.1 ms (±14.7) with a 7 ms stimulus, $t(4)=-3.375$, $p=.005$. There was no effect of stimulus duration on the peak latency of the second peak ($n=5$) of the five subjects who had a second significant peak in response to both 7 and 40 ms stimuli.

Figures 5A-B display the responses observed in the soleus for three individual subjects in response to 7 and 40 ms stimuli. The three rows show subjects who had significant peaks in response to both stimulus durations. Similar to the SCM and biceps, Figures 5C-F display all individual responses, and the averaged population response. All values for the soleus and biceps can been seen in Table 4 and Table 3 respectively.

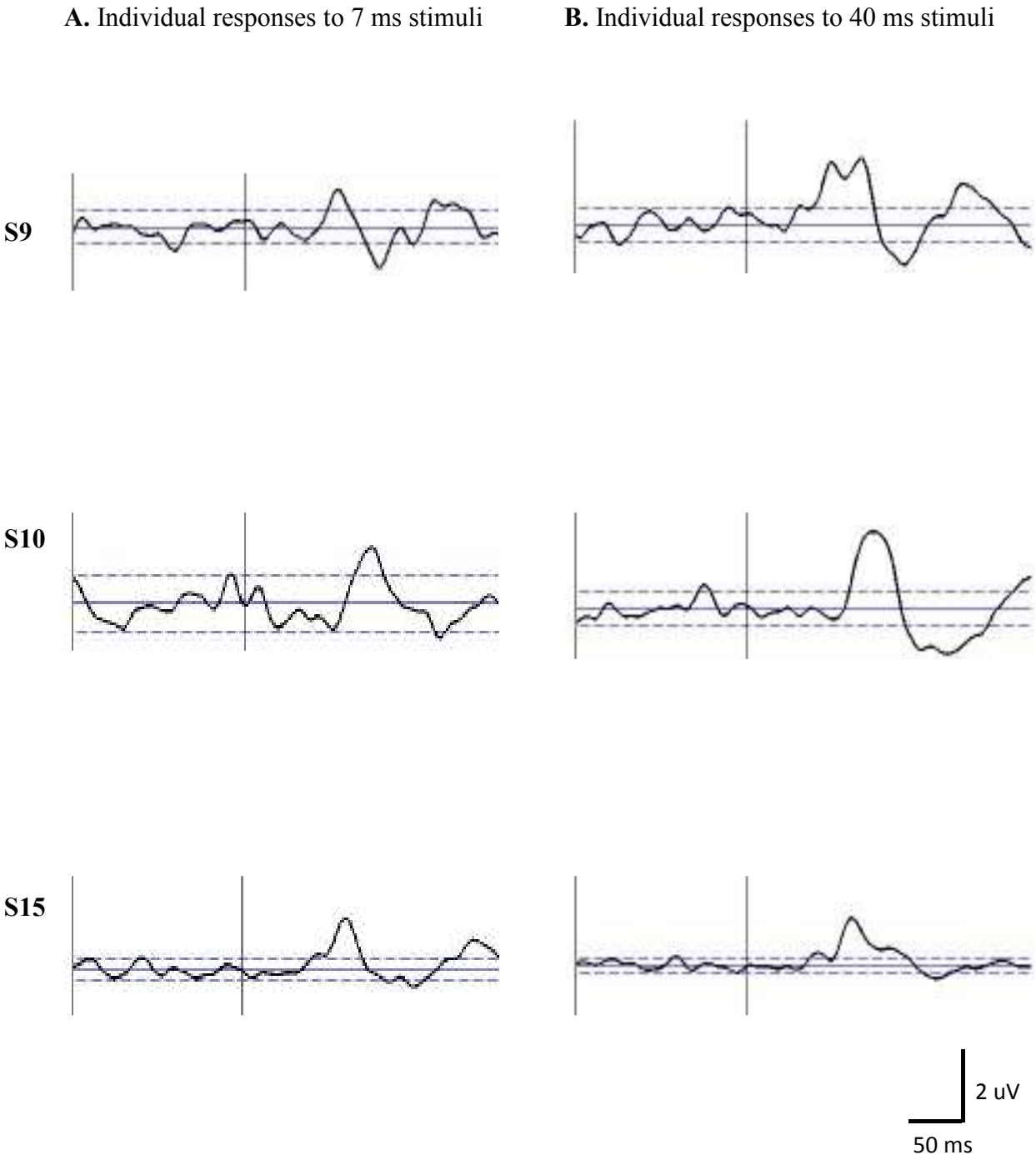
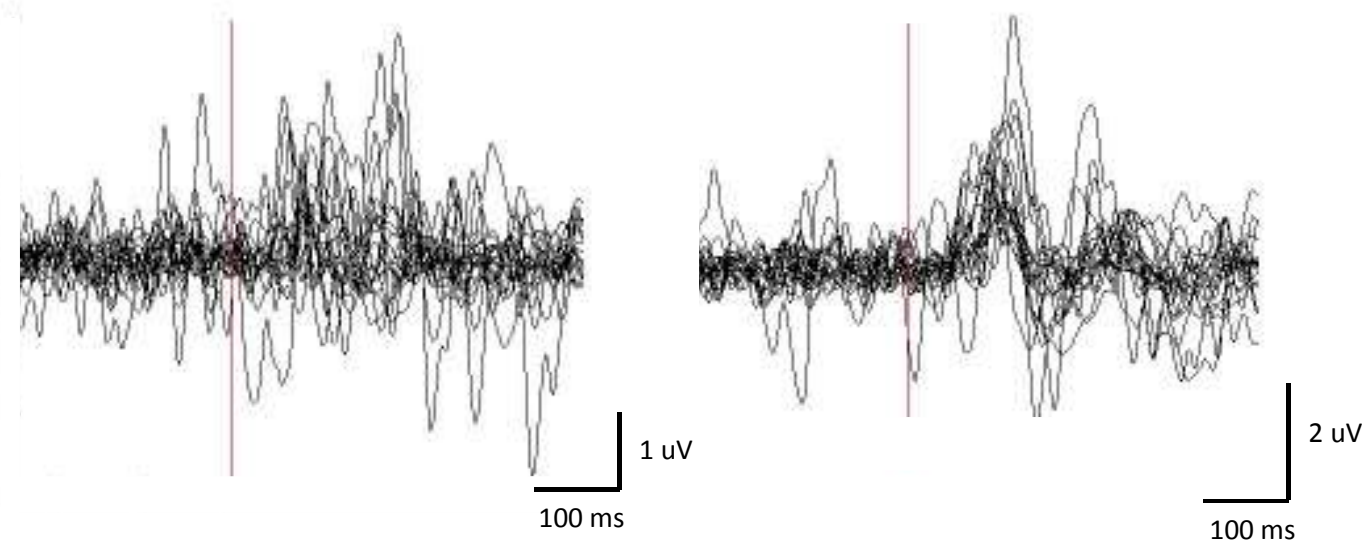


Figure 5A-B: Individual responses in the SOL to 7 and 40 ms stimuli.
The average response waveforms in the SOL obtained from three individual subjects in response to 254 stimuli of both 7 and 40 ms are shown in 5A and 5B respectively. The waveforms on the left hand side show the response of three individual subjects to repeated 7 ms stimuli, and the waveforms on the right hand side show the response of the same five subjects to repeated 40 ms stimuli. The vertical black bar represents stimulus onset, and the horizontal dashed lines represent a 2 SD bandwidth for each subject.

C. Individual responses to 7 ms stimuli

D. Individual responses to 40 ms stimuli



E. Averaged population response to 7 ms

F. Averaged population response to 40 ms

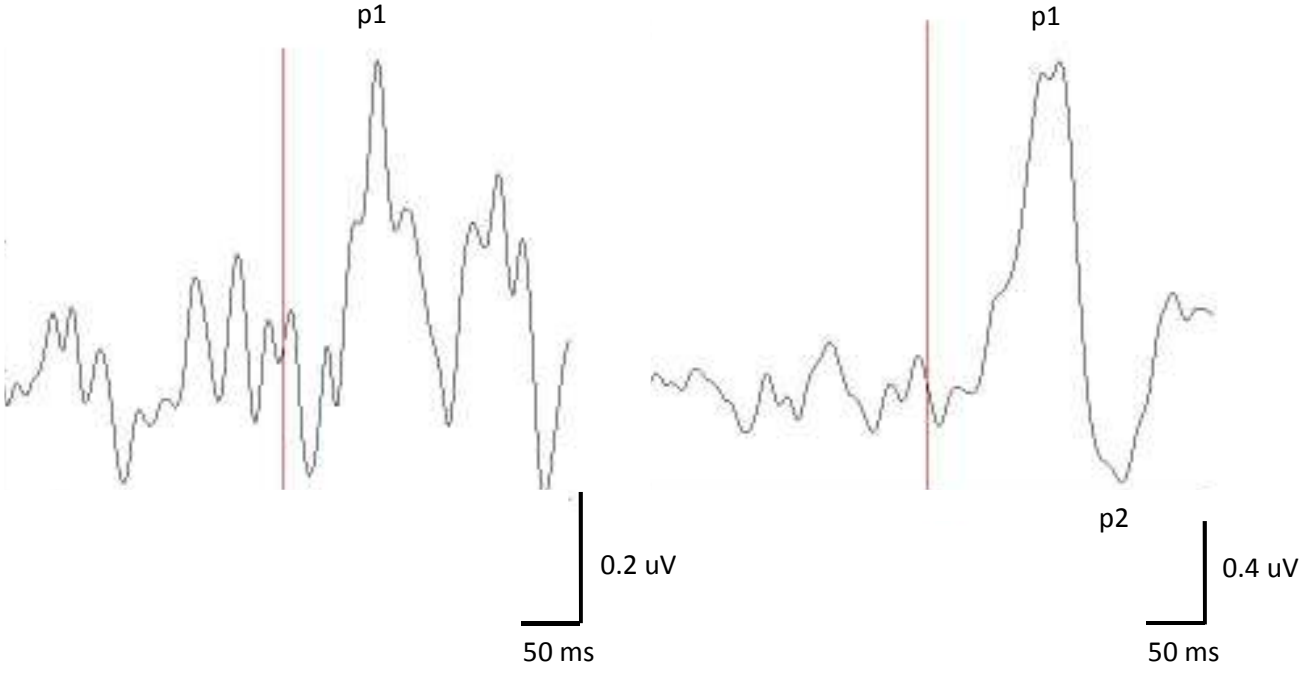


Figure 5C-F: Population responses in the SOL to 7 and 40 ms stimuli.
5C and 5D display all individual averages in the right soleus overlaid upon one another in response to 7 and 40 ms repeated stimuli, respectively. Each individual average represents an average of 254 stimuli. 5E and 5F are the averages of those in 5C and 5D.

Biceps	Corrected Reflex Amplitude (% of background activation)	Peak latency (ms)		Peak to peak interval
	Peak to peak	*p1*	*p2*	*p1-p2*
7 ms	*64*	*47.12*	*62.04*	*14.92*
Std Dev.		*8.07*	*8.64*	*1.89*
40 ms	*64*	*48.92*	*62.56*	*13.64*
Std Dev.		*7.53*	*7.75*	*2.65*

Table 3: Dependent measures of the biceps response
Mean peak amplitudes (uV), mean peak latencies (ms), and the mean peak to peak interval (ms) of the response peaks in the biceps, in response to 7 and 40 ms repeated stimuli. The mean corrected reflex amplitudes are displayed as percentages and are also included in the third column. Std Dev. (Standard Deviation).

Soleus	**40 ms stimulus**		**7 ms stimulus**	
	Peak latency (ms)	*Std Dev. (ms)*	*Peak latency (ms)*	*Std Dev. (ms)*
p1	*103.1*	*15.47*	*92.05*	*14.66*
p2	*165.85*	*16.65*	*159.53*	*31.55*

Table 4: Dependent measures of the soleus response
Mean peak latencies (ms) in response to 40 ms repeated stimuli. The mean corrected reflex amplitude is displayed as a percentage and included in the third column. Std Dev. (Standard Deviation).

Potential habituation of the responses?

The first and last twenty five trials of each block (i.e. first twenty five = trials 2-26 and 129-154, last twenty five = 102-127 and 229 – 254) for each stimulus were averaged together to assess the possibility of habituation based on the peak to peak amplitudes for both 7 and 40 ms stimuli. No significant decrease in peak to peak amplitude of the p13n23 or n34p44 response in the SCM, or the response in the biceps was observed. In fact a slight increase in peak to peak amplitude was observed in the last twenty five trials of the response in the biceps. Figures 6A-B and 7A-B show the first and last twenty five trials averaged and compared to the overall average for that block of stimuli for two separate subjects. Corrected mean peak to peak amplitude values and significance for the SCM and BB are shown in Table 5. Values for the SOL can be found in Table 6.

	p13n23			**n34p44**			**p1p2 (BB)**		
7 ms stimulus	Mean (ratio)	df	Sig.	Mean (ratio)	df	Sig.	Mean (ratio)	df	Sig.
First 25 trials	0.929	16	0.095	1.379	17	0.932	1.382	16	0.241
Last 25 trials	1.007			1.372			1.524		
40 ms stimulus									
First 25 trials	0.743	14	0.75	0.891	16	0.969	1.526	14	0.11
Last 25 trials	0.765			0.888			1.82		

Table 5: Habituation values in the SCM and BB

Mean peak to peak amplitude values have been divided by mean rectified 250 ms pre-stimulus EMG corresponding to the specified trials and displayed as arbitrary units. Data are displayed for the p13n23 and n34p44 response in the SCM, and p1p2 response in the BB, df (degrees of freedom), Sig. (significance).

6A. Lack of habituation in the SCM in response to 7 ms stimuli.

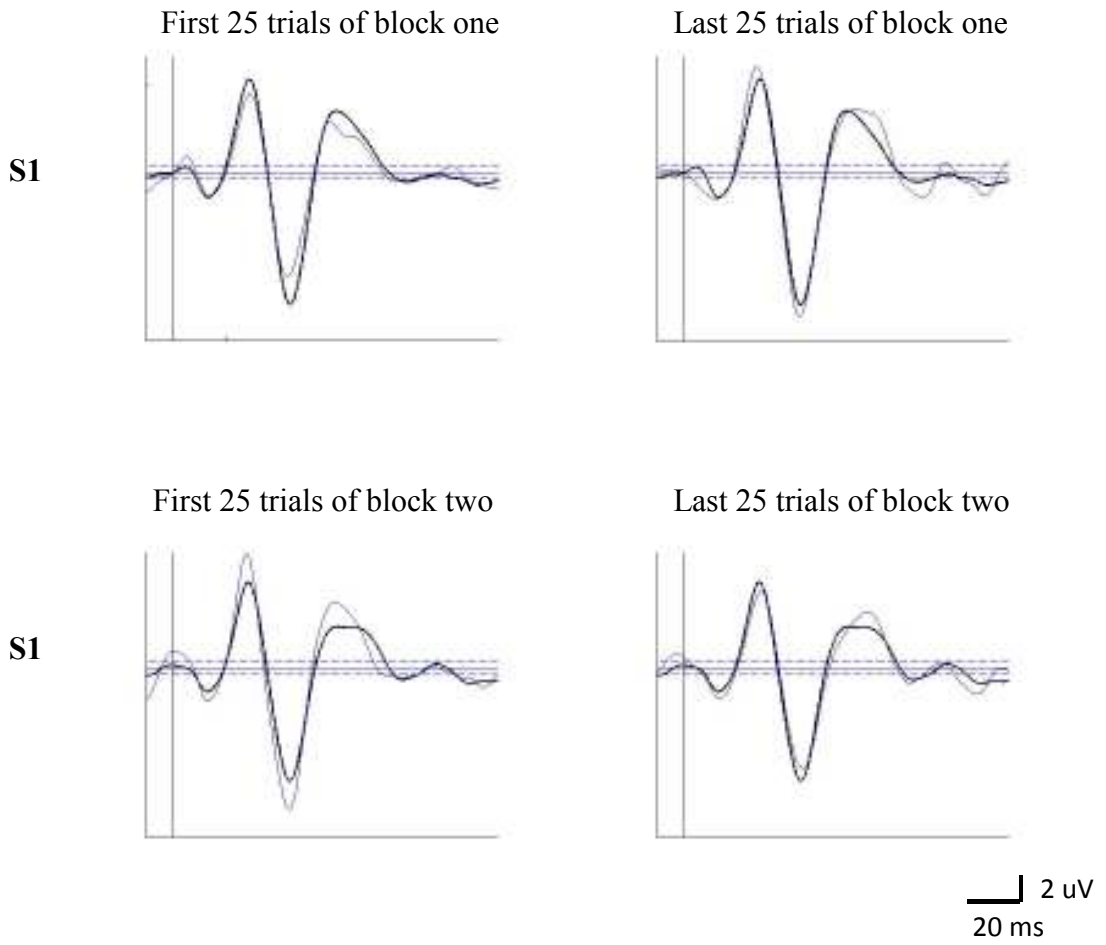


Figure 6A: Lack of habituation in the SCM to 7 ms stimuli.
SCM EMG averages of the first and last twentyfive trials in the first and second blocks of a single subject are shown in the above figures. The left hand side represents the averaged response of the first twenty trials and the right hand side represents the averaged response of the last twenty trials. The top row shows the first block of trials, while the second row shows the second block of trials. The thin blue trace represents the average of twenty trials while the thicker black line represents the overall averaged response for that block of trials. The vertical black line represents stimulus onset, and the horizontal dashed blue lines represent the 2SD bandwidth. This is shown for repeated 7 ms stimuli (6A) and 40 ms stimuli (6B).

6B. Lack of habituation in the SCM in response to 40 ms stimuli (same subject as 6A).

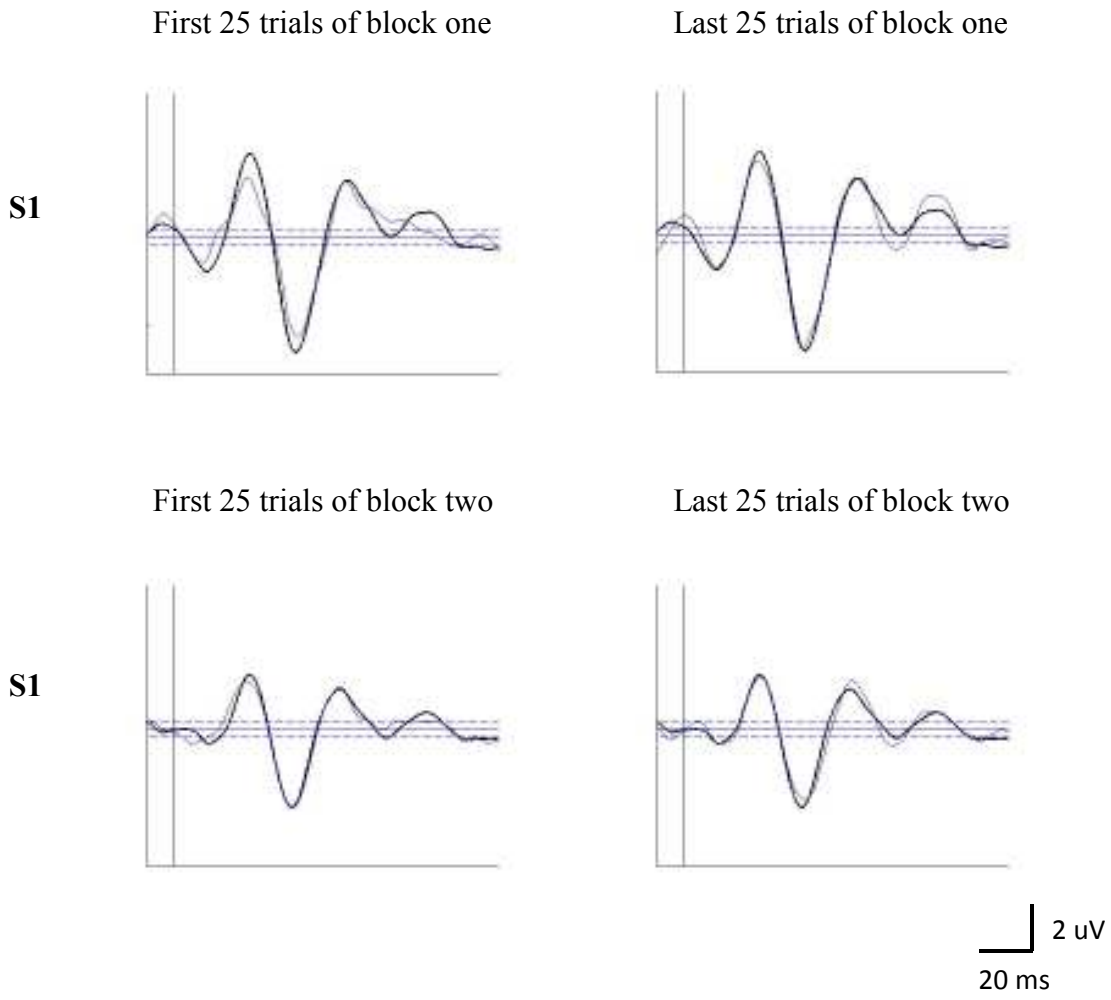


Figure 6B: Lack of habituation in the SCM to 40 ms stimuli.
SCM EMG averages of the first and last twentyfive trials in the first and second blocks of a single subject are shown in the above figures. The left hand side represents the averaged response of the first twenty trials and the right hand side represents the averaged response of the last twenty trials. The top row shows the first block of trials, while the second row shows the second block of trials. The thin blue trace represents the average of twenty trials while the thicker black line represents the overall averaged response for that block of trials. The vertical black line represents stimulus onset, and the horizontal dashed blue lines represent the 2SD bandwidth. This is shown for repeated 7 ms stimuli (6A) and 40 ms stimuli (6B).

7A. Lack of habituation in the biceps in response to 7 ms stimuli.

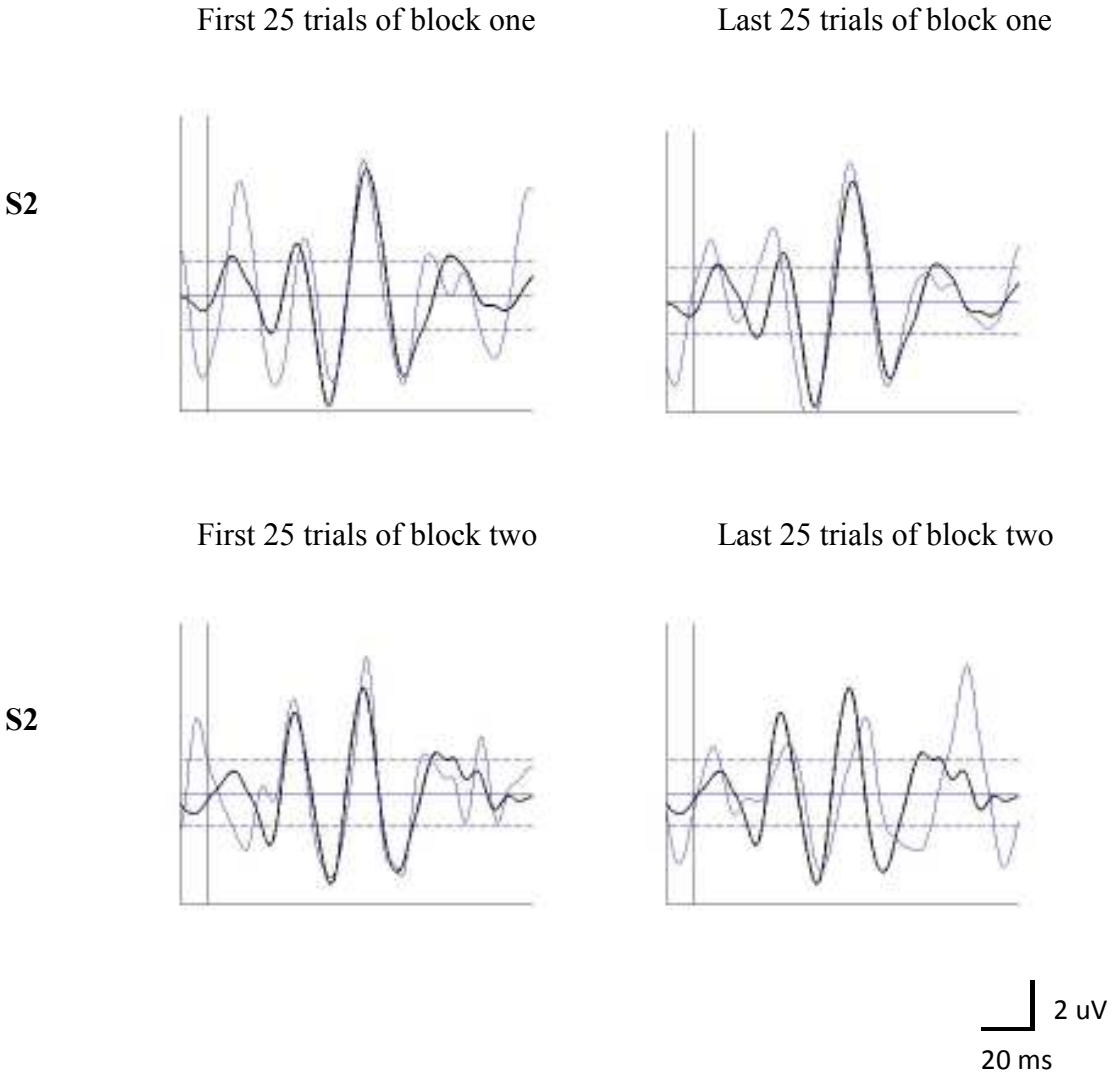


Figure 7A: Lack of habituation in the BB to 7 ms stimuli.
Averages of the first and last twenty five trials in the first and second blocks in the biceps of a single subject are shown in the above figures. The left hand side represents the averaged response of the first twenty trials and the right hand side represents the averaged response of the last twenty trials. The top row shows the first block of trials, while the second row shows the second block of trials. The thin blue trace represents the average of twenty trials while the thicker black line represents the overall averaged response for the block of trials. The vertical black line represents stimulus onset, and the horizontal dashed blue lines represent the 2SD bandwidth. This is shown for repeated 7 ms stimuli (7A) and 40 ms stimuli (7B).

7B. Lack of habituation in the biceps in response to 40 ms stimuli.

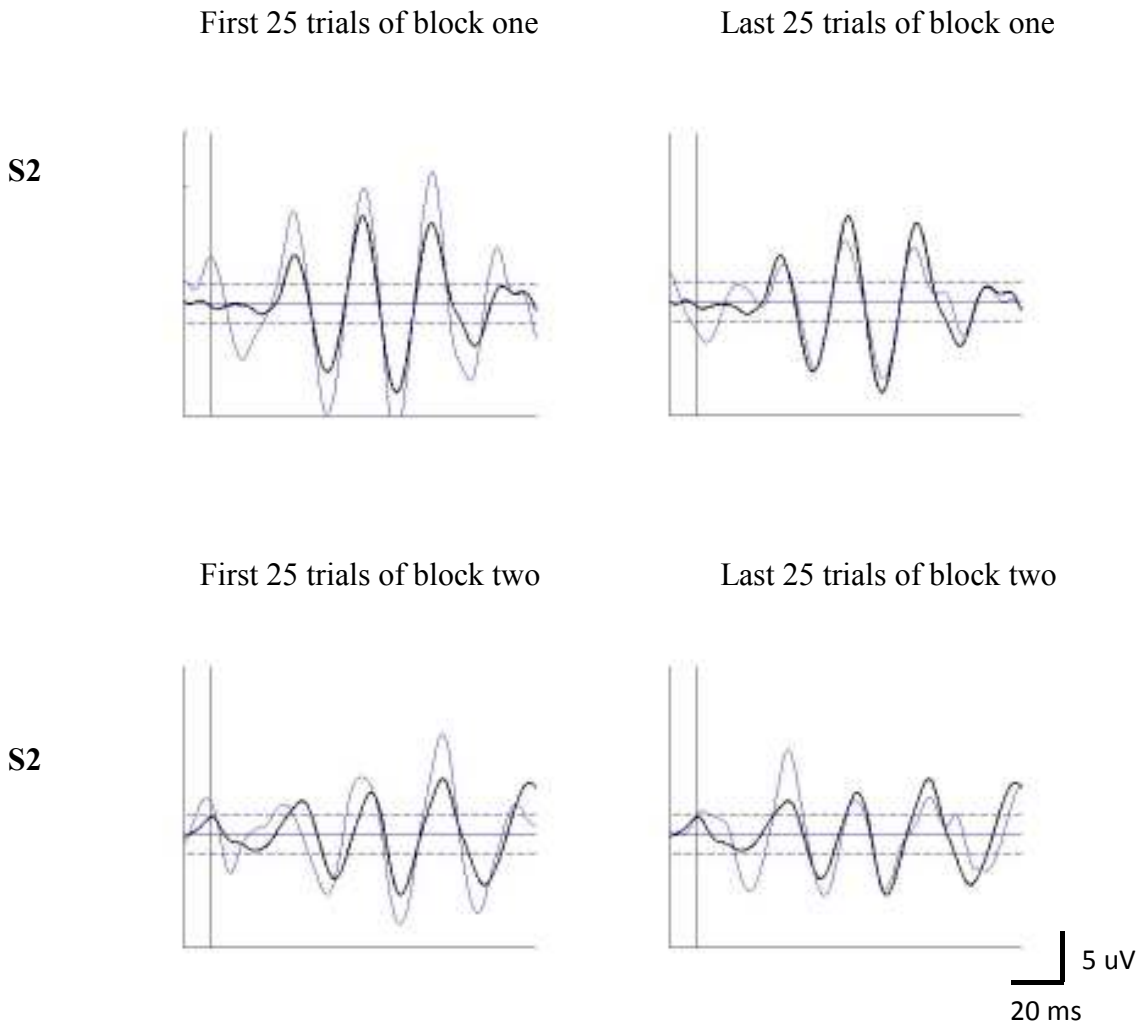


Figure 7B: Lack of habituation in the BB to 40 ms stimuli.
Averages of the first and last twenty five trials in the first and second blocks in the biceps of a single subject are shown in the above figures. The left hand side represents the averaged response of the first twenty trials and the right hand side represents the averaged response of the last twenty trials. The top row shows the first block of trials, while the second row shows the second block of trials. The thin blue trace represents the average of twenty trials while the thicker black line represents the overall averaged response for the block of trials. The vertical black line represents stimulus onset, and the horizontal dashed blue lines represent the 2SD bandwidth. This is shown for repeated 7 ms stimuli (7A) and 40 ms stimuli (7B).

Discussion

The present study sought to determine whether auditory evoked myogenic potentials: a) could be evoked in tonically contracted upper and lower limb muscles, and b) vary as a function of stimulus duration. The results indicate that indeed auditory evoked myogenic potentials can be observed in upper and lower tonically contracted limb muscles when subjects are lying supine which is contrary to previous research. The duration of the stimulus largely affected the peak to peak amplitude of the p13n23 and n34p44 response in the SCM, but had little effect on the dependent measures of the myogenic response observed in BB, which may suggest that the responses evoked in limb muscles travel via an alternate pathway.

Sternocleidomastoid

The p13n23 response

Consistent with previous reports, a short latency biphasic waveform, with significant peaks occurring around 13 and 23 ms respectively, was observed in response to repeated 7ms acoustic stimuli (500 Hz, 118 dB). The average p13 (11.8 ms) and n23 (23.1 ms) peak latencies observed with repeated 7 ms stimuli are consistent with previous studies where the p13 and n23 peaks are reported at average latencies of 13.1 and 13.3 ms and 22.8 and 22.6 ms respectively (Colebatch et al. 1994; Welgampola and Colebatch 2001). Despite the less intense stimuli (118 dB) used here, previous work has found that the p13 and n23 peak latencies do not vary as a function of click intensity (Colebatch et al. 1994; Lim et al. 1995; Akin et al. 2003), which explains why our peak latency values are consistent with previous studies.

Our study found no significant differences in latency for either the p13 or n23 peaks with a change in stimulus duration, which may be attributed to a plateau effect observed with acoustic stimuli that are greater than 7 ms. For example, Huang et al. (2005) found significantly

prolonged p13 and n23 peak latencies with no effect on the p13n23 interval, when a stimulus duration of 1 ms compared to 0.1 ms was used. This is consistent with findings reported by Welgampola and Colebatch (2001) who also found significant differences in the p13 and n23 peak latencies with six stimulus durations that ranged from 1 – 20 ms. However, successive differences were only observed with stimulus durations that ranged from 1 – 5 ms for p13, and from 1 – 7 ms for n23, meaning that there was no significant difference for the p13 and n23 peak latencies with successive stimulus durations between 7 and 20 ms. Given these observations, perhaps the rate of temporal processing from the saccular hair cell bundles to the SCM muscle fibres is greater with acoustic stimulus durations up to 7 ms, beyond which a plateau in the neural transmission rate may cause neural potentials to be processed at a similar rate. In fact, if saccular hair cells respond to acoustic stimuli similar to cochlear hair cells then a plateau effect would make sense. In the cochlea, the temporal pattern of a response to brief tone bursts is similar across auditory nerve fibres where there is an initial phasic increase in firing rate above the spontaneous level, followed by a maintained tonic discharge that persists for the duration of the tone (Kandel 1991). In this case, very brief tones, for example 1 ms, may only evoke an initial increase above spontaneous levels and result in shorter peak latencies of the response evoked in SCM motoneurons, whereas longer tones (i.e. 7 - 40 ms) would be long enough to induce a tonic discharge rate that is lower than the initial increase and result in no difference in peak latencies of the response in SCM motoneurons. Therefore, if our study had used a third stimulus duration that was less than 7 ms we may have observed a significant difference in p13 or n23 peak latencies.

No significant effect of stimulus duration on the p13n23 peak to peak interval was observed here, but as expected, there was a significant attenuation in the p13n23 peak to peak

amplitude with a 40 ms stimulus. The average corrected peak to peak amplitudes were smaller in this study compared to others (Welgampola and Colebatch 2001; Rosengren et al. 2009), which we can attribute to the lower stimulus intensity used here (118 dB verses 124 dB), but followed a similar pattern of attenuation with acoustic stimuli greater than 7 ms.

The n34p44 response

Although the n34p44 waveform has been speculated to be cochlear in nature, few studies have investigated the effects of stimulus parameters on this response.

When Colebatch et al. (1994) first described this bilateral potential in response to 0.1 ms clicks (145 dB) they observed the n34 peak at a latency of 33.8 ms, and the p44 peak at a latency of 43.7 ms. In this study, a stimulus duration of 7 and 40 ms yielded a mean n34 peak latency of 39.4 and 44.8 ms, and a mean p44 peak latency of 58.1 and 64.8 ms, respectively. Given the previously reported latencies and the results of this study it appears that the peak latencies of the n34p44 waveform vary as a function of stimulus duration. This would support a differential mechanism of activation compared to the p13n23 response, as the peak latencies of the p13n23 response did not vary as a function of stimulus duration in this study.

Furthermore, greater stimulus intensities evoke a greater response rate and larger peak to peak amplitudes of both the p13n23 and the n34p44 response, where a 105 dB (NHL) (approximately 145 dB SPL) acoustic stimulus evoked a significantly greater response amplitude of both the p13n23 and n34p44 potentials compared to an acoustic stimulus that was 95 dB (NHL) (approximately 135 dB) (Huang et al. 2004). This would be expected if the response originated from the cochlea, given that as the amplitude of any given frequency of sound increases, the peak amplitude of the oscillation will result in an increased displacement of the basilar membrane, and therefore a broader region of hair cells along the basilar membrane that

will be affected (Kandel 1991). Due to the similarities in hair cell activation between the otolith end organs and the cochlea, it is possible that the saccule responds in a similar fashion with increased sound intensities and may explain the differences observed in peak to peak amplitudes.

Moreover, Welgampola and Colebatch (2001) reported an n34p44 response in only one of 20 ears (ten subjects) with a 1 ms stimulus at 120 dB, whereas Colebatch et al. (1994) reported an n34 response in 15 of 20 ears (ten subjects) and p44 in 17 of 20 ears (ten subjects) with a 0.1 ms stimulus 145 dB. Therefore, given that Colebatch et al. (1994) had a greater response rate with a much shorter stimulus would lead to the assumption that the discrepancy in response rate between Welgampola and Colebatch (2001) and Colebatch et al. (1994) can be attributed to the difference in stimulus intensity. On the other hand, in the same study, Welgampola and Colebatch (2001) also reported an n34p44 response in 16 of 20 ears, in response to a 20 ms stimulus at 124 dB. Therefore it appears that the response rate of the n34p44 waveform is a function of both stimulus intensity and stimulus duration. An increase in either intensity or duration, or both, would yield a greater total energy arriving at the inner ear complex and may induce some sort of temporal summation to account for the greater response rate observed with each stimulus parameter.

In the present study, the stimulus intensity was kept constant and a change in stimulus duration significantly affected the amplitudes of both the p13n23 and n34p44 response waveforms, and the peak latencies of the n34 and p44 peaks. This suggests that the length of the acoustic stimulus affected the size of the output at a level common to both response waveforms.

If a longer stimulus yielded larger peak to peak amplitudes of the n34p44 response, then longer peak latencies would be reasonable given a longer time required to rise to peak. However, in this study, the response is significantly attenuated, while the latency of each peak is shifted

and the interval remains unchanged. The effects of stimulus duration on the n34p44 response has not been investigated before, and contrary to our hypothesis the effects of temporal summation associated with a 40 ms stimulus did not result in an increased peak to peak amplitude of the n34p44 response. Rather the n34p44 response was influenced in a similar fashion as the VEMP response where a longer stimulus duration attenuated the peak to peak amplitude, yet caused the peak latencies to increase in time which differs from the VEMP response. If both the p13n23 and n34p44 response originate from a common mechanism it is possible that the n34p44 response could be heavily influenced by the earlier waveform, which could explain the longer peak latencies. However, in order to investigate the n34p44 response much further, more detailed studies are required to determine the origin of this response.

Middle ear muscle reflex

Given that the peak to peak amplitude of both the p13n23 and n34p44 waveform potentials attenuated with a longer acoustic stimulus, it seems logical that the mechanism responsible for this observation must occur at a level similar to the origin of both waveforms (i.e. before the pressure waves reach the saccular hair cells).

Previous studies have suggested the middle ear muscle reflex involving the stapedius muscle as a potential mechanism responsible for the attenuation observed with longer stimulus durations (Welgampola and Colebatch 2001; Huang et al. 2005; Welgampola and Colebatch 2005). Contraction of the stapedius muscle dampens the oscillations of the stapes footplate and reduces the pressure waves being transmitted through the inner ear structures, including both the saccule and cochlea (Kandel 1991). The stapedius muscle inserts onto the stapes and when contracted, functions to increase inner ear impedance and prevent injury in the cochlea by controlling the amplitude of sound waves. In cats, the stapedius muscle contracts with a latency of 4.5 – 10 ms

in response to loud click stimuli (135 dB SPL) and does not habituate (Salomon 1966). A change in middle ear impedance has been shown to be directly proportional to stapedius muscle tension, and in response to a 25 ms tone, researchers showed a rise in middle ear impedance which was largest at approximately 100 ms after stimulus onset, followed by a gradual return to baseline at about 200 ms (Salomon 1966; Djupesland and Zwislocki 1971; Welgampola and Colebatch 2001). Although the inter stimulus interval of 1 - 2 s used in this study was long enough to prevent any confounding effects of middle ear impedance between successive stimuli, if the middle ear muscle reflex occurs in humans between 7 and 13 ms, then the reflex could have been evoked with each 40 ms stimulus, which may explain the attenuation of peak to peak amplitudes observed for both the p13n23 and n34p44 potentials with a 40 ms stimulus.

The relationship between the magnitude of stapedius muscle contraction as a function of tone burst duration at a constant intensity has been studied, and acoustic stimuli that were 10, 100, and 500 ms in duration were found to evoke stapedius muscle contractions that gradually increased in duration with the longer stimuli (Zwislocki 2003). If longer duration tone bursts are reflected as a temporal contraction of the stapedius muscle then this would support the suggestion of stapedial muscle activation with longer duration stimuli, and perhaps account for the attenuation observed in both the p13n23 and n34p44 potentials in this study.

Temporal processing and synaptic delays

Mechanical stimuli are known to induce pressure waves within the cochlear duct, via the stapes footplate, and cause a shearing force in the scala vestibui and scala tympani which deflects the stereocilia hair cell bundles. When a bundle is deflected towards the tallest stereocilia, a depolarization of the cell occurs resulting in increased neurotransmitter release at the synaptic cleft, and excitation of the afferent nerve fiber. Likewise, the opposite effect results in

hyperpolarization and a decrease in neurotransmitter release, resulting in inhibition. However, the hair cell bundles in the Organ of Corti are sensory transducers and do not directly fire an action potential, rather they form a receptor potential which propagates down the hair cell, when depolarized, and cause voltage gated calcium channels to open and allow an influx of calcium ions. This triggers a release of neurotransmitters at the basal end of the cell which diffuse across the synaptic cleft and bind to receptors to trigger an action potential in the nerve. Electromechanical properties of auditory hair cells allow the hair cells to phase lock with sinusoidal deflections of an acoustic stimulus and produce alternating currents in membrane potentials (Kandel 1991; McCue and Guinan 1994; Mauk and Buonomano 2004). However, although the electromechanical properties of the receptor potentials of hair cells are able to resonate at their preferred frequency, the afferent nerve fiber cannot fire at a one to one ratio of one action potential for each cycle of the sound wave due to a refractory period of approximately 1 ms (Kandel 1991). Therefore, since potentials sent through auditory neurons are brief and ion stores are readily replenished, it is likely that conduction delays will accumulate as mechanical stimuli are continuously transduced (Trussell 1999) which could account for the increased n34p44 peak latencies with a 40 ms stimulus, if the n34p44 response originates in the cochlea.

However, it is difficult to make any assumptions because there are many integrating factors at each synapse in the pathway, and we are merely observing the outcome as a global potential in SCM muscle fibers. Previous studies have tried to determine if there are any cortical neurons that discriminate between tonal durations, but this has not been found to date (Mauk and Buonomano 2004).

Biceps

The results of the present study demonstrate the ability to evoke an auditory evoked myogenic response in the tonically contracted upper and lower limb muscles, which is contrary to previous reports and has not been documented before.

The shape of the auditory evoked myogenic potential observed in the tonically contracted biceps with both stimulus durations looks similar to the waveform observed by Cherchi et al. (2009), where we observed a fluctuating waveform beginning with a significant negative deflection beyond 30 ms followed by at least four subsequent peaks in all subjects. However the peak latencies in the biceps of the present study were observed at 47.1 ms (± 8.1 ms) and 62.0 ms (± 8.6 ms) respectively, which is much later than the peak latencies observed in the triceps at 36.83 ms (± 8.42 ms) and 43.74 ms (± 8.8 ms) respectively (Cherchi et al. 2009). A likely explanation for this could be the different tasks performed in each study where subjects in the present study were supine and tonically contracting their biceps, in contrast to standing upright and posturally engaging the triceps muscle. Cherchi et al. (2009) suggest that the auditory evoked myogenic response they observe in the triceps muscle is most likely vestibular in origin. The authors base the vestibular origin of the triceps response on the fact that they could not evoke a response in the tonically contracted triceps muscle but were successful in evoking a response when the triceps muscle was involved in postural support of the body. Although their results would comply with previous reports that use galvanic stimuli to evoke a vestibular response in postural muscles of the upper and lower limbs (Britton et al. 1993; Fitzpatrick et al. 1994; Watson and Colebatch 1998; Welgampola and Colebatch 2005), it does not explain why a myogenic response in the tonically contracted biceps was observed in the present study in response to repeated 7 and 40 ms tones (500 Hz) at 118 dB SPL, when Cherchi et al. (2009) did

not observe any response in the tonically contracted triceps muscle to repeated 12 ms tones (500 Hz) at 145 dB SPL . Therefore the results of the present study would suggest that postural engagement is not necessary to elicit an auditory evoked myogenic response as subjects were lying supine and voluntarily contracting each muscle. It is unknown what pathways contribute to the response observed in the BB, but the voluntary contractions of all three muscles could suggest some descending influence via the corticospinal tract. There may also be some involvement of the reticulospinal pathway as previous studies on rats have identified some involvement of the pontine reticular nucleus in response to loud tones (Davis et al. 1982).

Indeed the VEMP response observed in the SCM is known to originate from hair cells in the saccular maculae, but acoustic stimulation is also known to activate utricular hair cells where a myogenic response can be observed in the inferior eye muscles (Curthoys 2009; Curthoys 2010). Curthoys (2010) suggests a brainstem weighting of otolith connections to each muscle to explain the alternate origins of the VEMP observed in the SCM (saccular) and the VEMP observed in the inferior eye muscles (utricular). Given this, it could be possible that the response in the upper limbs (i.e biceps) is mediated by a utricular response to acoustic stimulation. If this were the case, then the hair cell orientation within the utricular maculae would be affected with a change in head position from standing to lying supine, where the force of gravity acting on the otoconia crystals when lying supine may cause a decreased membrane potential of the utricular hair cells and allow a response to be generated in tonically contracted upper limb muscles when subjects are lying supine more easily than when standing upright. However, the utricle is known to have a lower preferential resonance frequency of 100 Hz compared to 500 Hz in the saccule (Todd et al. 2009), meaning that this may not be the only explanation to account for the myogenic response observed in the tonically contracted BB of the present study.

Another possibility is that the biceps response is a manifestation of the startle response given that the "classic" startle response involves a generalized flexion response of arms. However, this seems unlikely given that the amplitude of the startle-induced muscular response is known to attenuate with each subsequent stimulus which can occur in as few as two trials (Siegmund et al. 2003; Blouin et al. 2006). The comparison of the mean amplitude between the initial twenty five and final twenty five responses, of the total two hundred fifty four stimuli of the present study, is not significantly different which demonstrates that the biceps response does not fatigue or habituate unlike the startle response. Secondly, Brown et al. (1991b) report startle-induced myogenic responses in the right biceps muscle with the earliest latency being 67.0 ms in response to binaurally presented 1000 Hz, 50 ms tones at an intensity of 124 dB. This is in contrast to the mean peak latency observed in this study where 7 and 40 ms repeated acoustic stimuli evoked a significant peak at 47.1 ms and 49.0 ms, respectively. Therefore, the peak latencies observed in this study are much earlier in the SCM and biceps compared to those observed with a startling stimulus. Finally, previous work by Nichol (2008) and Luxon et al. (2011) demonstrates the need to average at least twenty trials in order to observe the auditory evoked response, as opposed to the ability to evoke a startle response in a single trial. As an example Figures 3A-B displays the EMG trace of a single subject to the first stimulus in the first block of 40 ms stimuli, which startled the subject, and a subsequent trial later in the same block.

Soleus

The results of the present study demonstrate that it is possible to evoke a myogenic response in the isometrically contracted soleus muscle which has not been shown before. In the present study there was a greater response of auditory evoked myogenic potentials with 40 ms stimuli compared to 7 ms stimuli of equal intensity. As mentioned above, subjects were in the

supine position and plantar flexing against a footboard where the ankle angle was fixed at 90^{o} resulting in an isometric contraction. In contrast to our results, pervious reports had greater response rates in evoking myogenic potentials in the soleus and gastrocnemius muscles with short duration acoustic stimuli (0.1 – 12 ms) at much higher intensities (132 – 145 peak SPL) (Watson and Colebatch 1998; Rudisill and Hain 2008). Fifteen subjects in the current study displayed an initial peak in rectified EMG with a mean peak latency of 92.1 ms (± 15.5 ms) in response to repeated 7 ms stimuli. This is much longer than previous reports of a mean peak latency at 49.5 ms observed in unrectified EMG of the gastrocnemius muscle in response to 12 ms tones (132 dB peak SPL) (Rudisill 2008), mean onset latencies of 49 ms and 54 ms observed in rectified EMG of the soleus muscle in response to 0.1 ms clicks (145 dB SPL), and galvanic stimulation respectively (Watson and Colebatch 1998).

The likely explanation for the discrepancy in response rate and longer peak latencies would be the effects of the lower stimulus intensity used in the present study, which has been documented in the SCM (Akin et al. 2003), but it is currently unknown what effect acoustic intensity has on the response rate characteristics of auditory evoked potentials in the lower limb. Increased stimulus intensity is known to affect the amplitude of auditory evoked myogenic responses and it is plausible that if the present study had used a higher stimulus intensity we may have observed an increased response rate with 7 ms stimuli, and earlier response latencies with both stimulus durations. Perhaps a 7 ms stimulus at 118 dB is not loud enough to evoke a consistent response in the soleus, and it was the effects of temporal summation that allowed a response to be evoked consistently with repeated 40 ms stimuli.

Previous research has shown that galvanic vestibular stimulation can evoke a response in the soleus when subjects are standing freely, but not when subjects are braced and plantar flexing

to the same level as during stance (Fitzpatrick et al. 1994). These observations suggest an ability of the human central nervous system to distinguish between active voluntary control and a passive sense of balance. During free stance, vestibular nuclei converge with ascending sensory information to influence muscle activity in order to maintain balance of the body in space. However, if the central nervous system can differentiate between passive balance and active voluntary control, then it is possible that there is a diminished influence of descending vestibular input to the appropriate postural muscles when subjects are braced and plantar flexing. Given this theory, perhaps an increased gain of vestibulospinal projections onto soleus motor neurons during free stance can account for the auditory evoked myogenic responses in the soleus of previous studies with short duration stimuli. When subjects then initiated a voluntary contraction, as in sitting and plantar flexing, it is possible that the decreased gain of vestibulospinal projections resulted in the need for a greater stimulus to evoke an auditory evoked myogenic response in the soleus muscle. Therefore an acoustic stimulus with a greater total energy may be necessary to evoke a response in the tonically contracted lower limb muscles compared to when these same muscles are being subconsciously maintained during a balance type task. Perhaps if Rudisill (2008) or Watson and Colebatch (1998) had used longer stimulus durations, they may have observed an auditory evoked myogenic response in the soleus or gastrocnemius while subjects were sitting and plantar flexing. Therefore the results of this study would suggest that the response characteristics in the lower limb may vary as a function of stimulus duration.

Interestingly both studies that were able to evoke a myogenic response in the soleus used monaural stimulation, and evoked the largest amplitude with contralateral stimulation. Perhaps some further investigation into the effects of stimulus duration in combination with the effects of

laterality of the stimulus, on auditory evoked potentials in the lower limb would offer some clarification. Finally, similar to Rudisill (2008) potentials in the lower limb were less reliable and had significantly longer latencies than the BB and SCM potentials, and may not be the best adaptation to clinical testing parameters.

Limitations

Although this study did test the effects of stimulus duration, a potential caveat to the design of this study involves the number of waveforms within each stimulus. For example, a 7 ms 500 Hz tone burst is made up of three and a half sine wave cycles, whereas a 40 ms 500 Hz tone burst is composed of 20 complete sine wave cycles. Although both tone bursts begin with the same polarity, the half cycle at the end of the 7 ms stimulus causes the tone burst to end differently than the 40 ms stimulus. This has a potential confounding effect where the final pressure wave delivered to the inner ear complex will be a rarefraction, or decrease in inner ear pressure leading to hyperpolarization of the hair cells, with a 7 ms stimulus, and conversely a compression leading to depolarization of the hair cells with a 40 ms stimulus. Therefore additional studies could be designed to evaluate the effects of opposite polarities at the beginning and end of a tone burst, on the response observed in somatic muscles.

Conclusion

In summary, repeated AC stimuli that are 7 ms or 40 ms in duration can evoke a myogenic response in the tonically contracted BB and SOL when subjects are supine which has not been observed before. This is in contrast to the click-evoked VEMP response and GVS-evoked responses which have only been observed in posturally-engaged muscles. The results of this study show that the dependent measures of the myogenic response evoked in the BB did not change with an increase in stimulus duration. These response characteristics differ from both the p13n23 and n34p44 response in the SCM where the peak to peak amplitude of both responses attenuated with a longer stimulus, but are similar in terms of the peak to peak interval which did not change for any of the observed responses in this study. These similarities and differences may help support the notion of an alternate pathway and mechanism of activation of the myogenic response observed in the BB. A myogenic response is observed in the BB simultaneously to a VEMP response in the SCM, which supports the ability of extending the clinical VEMP test and highlights the importance of investigating the pathways associated with this myogenic response further. Clinical VEMP testing is conducted with the subject either supine or lying recumbent at a 45 degree angle, and therefore the evaluation of the observed response in the BB and the subsequent pathways could become an added feature of the VEMP test.

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Appendix 1: Literature Review

Mammalian vestibular Anatomy

The vestibular system is comprised of two components: the semi-circular canal system that senses rotational movements of the head; and the otoliths which sense linear accelerations (Figure 1A). It is the combination of the signals from these two systems that allow the human brain to distinguish between acceleration and head tilt (Day and Fitzpatrick 2005).

The semi-circular canal system is comprised of three directionally sensitive semi-circular canals (anterior, posterior, and horizontal), which are positioned orthogonally to each other (Kandel 1991). The cilia of the sensory hair cells are embedded in cupula, a gel-like substance, and stored in an ampulla at the end of each canal (Kandel 1991; Fitzpatrick and Day 2004). Rotational movement of the head causes an inertial force on the endolymph fluid in each canal, which pushes on the cupula and deflects the cilia to depolarize or hyperpolarize the afferent fibers, and increase or decrease the firing rate respectively (Fitzpatrick and Day 2004). Humans are able to detect rotational movement of the head in space along three different axes due to the directional sensitivity of the canals.

The otolith organs are comprised of the utricle and saccule and are responsible for detecting linear accelerations of the head in space, and determining the position of the head with respect to gravity (Kandel 1991). Within the utricular and saccular maculae, the cilia are embedded in a gelatinous substance that sits beneath a layer of otoconia crystals, or otoliths. Gravitational forces act on the crystals that deflect the cilia to modulate the afferent firing rate. When the head is positioned upright, the macula of the utricle lies roughly in the horizontal plane with the otoliths resting directly upon it, whereas the macula of the saccule is oriented vertically. When the head is tilted or undergoes linear acceleration, the otoliths of each end organ will deform the

macula and activate receptor cells specific to the direction of force (Kandel 1991). A striola divides the utricle and saccule into two slightly unequal halves where the hair cells are aligned in opposite directions, so that the cilia are oriented towards the striola in the utricle, and away from the striola in the saccule (see Figure 1B; Fitzpatrick and Day 2004). Thus, the positional arrangement of the utricle and saccule in the head, in addition to the hair cell alignment about the striola, allows this system to detect linear accelerations in each plane of movement (Fitzpatrick and Day 2004).

Therefore, in concert with visual and proprioceptive input, information is taken from each of the above systems and relayed to the vestibular nuclei in the brainstem via the vestibular portion of the eighth cranial nerve. From here, connections are made with the spinal cord, oculomotor nuclei, and the cerebellum in an effort to maintain balance and equilibrium of the head and body in space (Wilson and Peterson 1981; Kandel 1991).

The mammalian cochlea

In close proximity to the vestibular organs is the cochlea, which contains the Organ of Corti, and makes up the auditory portion of the inner ear. In order for sounds to reach the inner ear they must first travel through the outer ear, or external auditory meatus, to reach the tympanic membrane. Sounds that reach the tympanic membrane cause it to vibrate, and the vibrations are transmitted to the inner ear via three small bones that comprise the middle ear. The three small bones are known as the inner ear ossicles and consist of the malleus which is attached to the tympanic membrane, the incus, and the stapes which is attached to the oval window of the cochlea. The cochlea is a small bony structure that is made up of three fluid-filled compartments known as the scala tympani, scala vestibuli, and the scala media (see Figure 2; Kandel 1991). When sound vibrations are transmitted through the external auditory meatus and tympanic

membrane, the vibration of the inner ear ossicles will cause the stapes footplate to push into and out of the cochlea putting pressure on the fluid in the scala vestibuli. The vibrations cause pressure waves to travel through the three fluid filled compartments and activate hair cells in the organ of Corti, which is the sensory transduction organ located on the basilar membrane in the scala media (Kandel 1991; Ramamoorthy et al. 2010). The human ear is sensitive to frequencies that range from 20 to 20,000 Hz, and due to the tapering of the cochlea from one end to the other, different frequencies of sound will affect different portions along the basilar membrane. Therefore, frequency tuning in the cochlea is based on the position of hair cells along the basilar membrane in addition to their corresponding electromechanical properties (Kandel 1991). Auditory afferent projections travel alongside vestibular afferent projections, in the eighth cranial nerve, but terminate in the cochlear nucleus of the brainstem (Kandel 1991).

Otolith sound sensitivity in animals

The mammalian cochlea developed with evolution, and in vertebrates such as the fish and frog, the utricle and saccule are responsible for both hearing and vestibular functions (Fay and Popper 2000). The development of the cochlea is suggested to have taken place early in evolutionary history and considered to be a more specialized organ for sound amplification and frequency analysis (Fay and Popper 2000). Acoustic sensitivity of the saccule has been demonstrated by Didier and Cazals (1989) who selectively eliminated the cochlear receptor in guinea pigs and observed acoustically evoked potentials in the eighth cranial nerve, in response to intense but brief AC sound stimuli. Given this finding, single neuron afferent recordings in response to short duration AC sound stimuli in the superior and inferior vestibular nerve of guinea pigs and cats were further investigated.

There are two distinct divisions of the vestibular nerve: the superior vestibular nerve which is comprised of utricular afferents, horizontal and anterior semi-circular canal afferents, and a small portion of the saccule; and the inferior vestibular nerve which carries afferents from the body of the saccule and posterior semi-circular canal (Didier and Cazals 1989; Curthoys et al. 2009; Curthoys 2010). Each neuron was tested for static and dynamic tilt responses, by tilting or rotating the head in roll, pitch, and yaw axes to determine the physiological organ from which it originated. Interestingly, in guinea pigs and cats, the majority of afferent responses to AC sound stimuli were observed in the inferior vestibular nerve, with minimal responses observed in the superior vestibular nerve, and limited responses from canal afferents (McCue and Guinan 1994; McCue and Guinan 1995; Murofushi et al. 1995; Murofushi et al. 1996; Murofushi and Curthoys 1997; Curthoys et al. 2006; Curthoys 2010). More specifically, it was the vestibular receptors that responded to static tilt (Murofushi et al. 1995; Murofushi et al. 1996) and originated in the saccular macula (Murofushi and Curthoys 1997) that responded to AC sound stimuli. Similarly, McCue and Guinan (1997) found that irregularly discharging vestibular afferents that innervated the saccule and projected to the vestibular nucleus were preferentially responsive to sound stimuli, whereas regularly discharging vestibular afferents were not. They also observed that the irregular vestibular afferents had a preferential activation frequency range of 100 – 3000 Hz, (McCue and Guinan 1997) and responded to AC sound stimuli with shorter onset latencies and higher thresholds than cochlear receptors.

It is clear how the use of indwelling recordings in the superior and inferior vestibular nerves has allowed a sufficient amount of research to investigate the acoustic sensitivity and preferential activation of the saccule in animal models such as cats (McCue and Guinan 1994; McCue and Guinan 1995), squirrel monkeys (Young et al. 1977), and guinea pigs (Murofushi et

al. 1995; Murofushi et al. 1996; Murofushi and Curthoys 1997; Murofushi et al. 1999). However, very little research into these brainstem inputs and their effects in humans has been attempted, due to the invasive nature of the technique.

Otolith sound sensitivity in humans

Although there is no direct evidence, an increasing amount of convergent research supports the concept that short-duration AC sound exposure can also activate otolith afferents in humans. A click is best defined as a short duration sound stimulus that is shorter than the time threshold required for pitch recognition (Traux 1999). The use of click stimuli evolved from auditory brainstem response testing and an initial report by von Bekesy (1935) who reported head and eye movements towards the stimulated ear in response to loud sounds. He observed these responses in subjects with profound deafness and deduced that the acoustic stimuli must be activating the vestibular system. A few decades later, responses to click stimuli (0.5 ms, 130 dB) that were presented monaurally and binaurally, were shown to be myogenic in origin as EMG activation was observed in the trapezius and unspecified cervical muscles (Bickford et al. 1964). Furthermore, the peak to peak response amplitudes increased linearly with increased muscle activation and were abolished with curare. Similar to the observations by von Bekesy (1935), the responses observed by Bickford et al. (1964) were also preserved with sensori-neural hearing loss yet normal vestibular function.

It is now well known that click stimuli activate vestibular receptors and elicit a vestibular evoked myogenic potential (VEMP) that is observed primarily in inferior extrinsic eye muscles and has been termed the ocular VEMP (oVEMP), and in various neck muscles termed the cervical VEMP (cVEMP) (Colebatch et al. 1994; Murofushi et al. 1999; Wu et al. 1999; Welgampola and Colebatch 2005; Rosengren et al. 2010). Both the oVEMP and the cVEMP can

be activated by brief bone conducted vibration or AC sound, however normal middle ear conduction and thus vibration of the stapes footplate is necessary to evoke VEMPs using AC sound (Colebatch et al. 1994; Ochi et al. 2001; Curthoys 2010). Previously, it has been shown that the oVEMP is dependent on normal utricular and superior vestibular nerve function, whereas the cVEMP is dependent on saccular and inferior vestibular nerve function (Curthoys et al. 2009; Iwasaki et al. 2009). Furthermore, research using superior and inferior vestibular nerve sections has shown that the saccule has strong projections onto SCM motoneurons; whereas the utricle projects primarily onto ocular motoneurons (Curthoys 2010). Therefore, given the sufficient amount of research over the past two decades, cVEMPs are considered a valid clinical test for assessing saccular and inferior vestibular nerve function (Colebatch et al. 1994; Welgampola and Colebatch 2001; Welgampola and Colebatch 2005).

The VEMP response

The cVEMP response was described originally by Colebatch and Halmagyi (1992) and is characterized by a short latency inhibitory waveform (Colebatch and Rothwell 2004) observed in the tonically contracted sternocleidomastoid (SCM) muscle ipsilateral to the stimulated ear. The VEMP response is characterized by an initial positive-negative myogenic potential with peaks at 13 ms (p13) and 23 ms (n23), respectively, after stimulus onset and observed in surface EMG recordings (see Figure 3; Colebatch and Halmagyi 1992; Colebatch et al. 1994; Colebatch and Rothwell 2004). A second, later component is observed bilaterally in the tonically contracted SCM muscles, and referred to as the n34p44 waveform. This later response is characterized by a second negativity (n34) followed by a second positivity (p44) occurring at approximately 34 ms and 44 ms, respectively (Colebatch et al. 1994; Welgampola and Colebatch 2005). Consistent with the early reports by von Bekesy (1935) and Bickford et al. (1964), click evoked cVEMPs

are attenuated or abolished completely in patients with absent or severely decreased vestibular function (due to vestibular neuritis, late Meniere's disease, and vestibular schwannomas) whereas the later n34p44 component is retained. On the other hand cVEMPs are preserved in those with sensori-neural hearing loss whereas the later component is absent (Colebatch and Halmagyi 1992; Colebatch et al. 1994; Murofushi et al. 1999; Welgampola and Colebatch 2005; Curthoys et al. 2009; Rosengren et al. 2010). Therefore it is clear that the cVEMP response is dependent on the integrity of the vestibular apparatus whereas the later component is not well understood and speculated to be cochlear in nature (Colebatch and Halmagyi 1992; Colebatch et al. 1994; Welgampola and Colebatch 2005; Rosengren et al. 2010).

Evoking a VEMP response

The optimal stimulus parameters that are required to evoke a VEMP response have been studied in detail by a number of research groups. In the literature, intense clicks that are 140 – 145 decibels (dB) sound pressure level (SPL) and 0.1 milliseconds (ms) in duration are presented at a rate of 1 – 5 Hz monaurally or binaurally via calibrated headphones, and used in clinical testing for vestibular function (Welgampola and Colebatch 2005). At least two blocks of 100 stimuli are required, with a typical number of trials being 256 for each ear (Colebatch et al. 1994; Murofushi et al. 1996; Murofushi et al. 1999). Huang et al. (2005) observed a more prominent VEMP waveform with a 0.5 ms click compared to a 0.1 ms click, with an increased response rate of 100% compared to 94% respectively. Short tone bursts can also evoke a VEMP response similar to clicks (Murofushi et al. 1999) and require a lower acoustic intensity. Short tone bursts that are 2 -10 ms in duration, with a preferred frequency of 500 Hz (Akin et al. 2003; Todd et al. 2009; Rosengren et al. 2010), have been shown to evoke the largest VEMP amplitude response and require stimulus intensities of 120 dB SPL, compared with 140 dB SPL (required for clicks)

(Murofushi et al. 1999; Welgampola and Colebatch 2001; Welgampola and Colebatch 2005). Furthermore, the peak to peak amplitudes of cVEMP responses have been shown to increase with AC stimuli up to 7 to 10 ms and decrease thereafter with stimuli up to 20 ms (Welgampola and Colebatch 2001; Welgampola and Colebatch 2005), but it remains unclear if the VEMP response is abolished with AC sound stimuli beyond 20 ms (Welgampola and Colebatch 2001). The vestibular tuning properties of the VEMP response have also been investigated, and a preferential tuning frequency of 500 Hz has been shown to evoke a larger VEMP response than AC stimuli that are 1000 Hz or 2000 Hz (Murofushi et al. 1999; Akin et al. 2003; Welgampola et al. 2003; Rosengren et al. 2009; Curthoys 2010; Rosengren et al. 2010). Finally, in regards to AC sound presentation, Wang and Young (2003) determined that there is no difference in onset latencies or amplitudes with binaural or monaural acoustic stimulation, and recommended the use of binaural acoustic stimulation to decrease collection time and the subsequent muscular effort required by patients (Wang and Young 2003).

Posture and the click evoked response

While click evoked VEMP responses in the SCM have been studied in detail, sound evoked myogenic potentials have also been observed in unrectified surface EMG of the triceps brachii (Cherchi et al. 2009) and rectified surface EMG of the soleus (Watson and Colebatch 1998) but only when these muscles are engaged in a postural task. For example, Cherchi et al. (2009) presented repeated tone burst stimuli at a rate of 4.3 Hz that were 12 ms in duration (500 Hz, 132 dB SPL) and observed a myogenic response in the ipsilateral triceps muscle of all subjects when the triceps muscle was contributing to postural support. The myogenic response waveform consisted of an initial positive-negative potential in all subjects with peak latencies of 35.69 ms ± 7.40 and 44.29 ± 9.51 respectively. When subjects then used their triceps muscle to

push against a weight that was not relevant to balance, the sound evoked myogenic response potentials were abolished. These results are similar to those observed by Watson and Colebatch (1998) where repeated 0.1 ms clicks (approximately 145 dB SPL) presented at 3 Hz evoked short latency myogenic potentials with a mean onset latency of 51 ± 6.6 ms in the soleus muscle contralateral to the stimulated ear of six subjects (n=8) when they were standing. To compare the click evoked responses to a validated test of vestibular function a subsequent group of twelve subjects then underwent click and transmastoid galvanic stimulation (4-mA, 20ms). Here, eleven of twelve subjects showed a short latency response to click stimulation with an onset latency of 54 ± 6.3 ms and all subjects showed a short latency response to galvanic stimulation with an onset latency of 49 ± 9.1 ms. Furthermore, four of the twelve subjects were then studied under a range of postures and interestingly none of these subjects showed responses to either type of stimulation when they were sitting and maintaining comparable levels of tonic activation to standing in the soleus muscles. These reports are similar to responses observed with galvanic vestibular stimulation (GVS) where short and medium latency responses are only observed in muscles that are contracting and actively involved in a balancing task (Fitzpatrick et al. 1994).

It is clear that sound, click, and GVS evoked myogenic potentials were not observed in the tonically contracted triceps brachii (Cherchi et al. 2009) or soleus muscle (Watson and Colebatch 1998) of either study. However these results are in contrast to previous pilot work at the University of British Columbia involving repeated acoustic stimuli that are longer in duration and lower in intensity than the acoustic stimulus parameters mentioned above.

Repeated acoustic stimuli

Longer duration AC sound stimuli (90 ms, 110 dB) have been used to investigate the startle response and contributing pathways (Groves et al. 1974; Davis et al. 1982). Interestingly,

the startle response associated with loud auditory stimuli (40 ms, 124 dB, 1000 Hz) is known to evoke EMG activity in human axial and lower limb musculature (Brown et al. 1991a) and has been used to investigate reticulospinal inputs onto motorneurons (Davis et al. 1982). The induced muscular response is known to attenuate with repeated exposure, termed habituation, and is characterized by a decreased muscle response with each subsequent stimulus. Habituation of the muscle response is rapid and robust when the stimulus is unexpected (Siegmund et al. 2003; Blouin et al. 2006). More recent research has examined the muscular response characteristics of habituation and compared the effects of six single unexpected acoustic stimuli (10 minute interstimulus interval) to repeated acoustic stimuli (3 – 5 second interstimulus interval) of identical intensity (40ms, 124 dB, 1000Hz) (Nichol 2008). Indeed the single unexpected acoustic stimuli evoked a startle response with the associated charactersitics of habituation after each stimulus. In contrast, the repeated acoustic stimuli evoked a response that was similar to the startle response but smaller in amplitude and only visible after averaging multiple trials (Nichol 2008). Moreover, the startle response was observed in all muscles that were recorded in contrast to the response observed with repeated acoustic stimuli, which was only observed in those muscles that were tonically active (right orbicularis occuli; bilateral soleus; bilateral medial gastrocnemius) (Nichol 2008).

Based on these previous observations, pilot work at the University of British Columbia in the Neurophysiology Lab has further examined the physiological mechanisms involved in the response to repeated acoustic stimuli that do not induce a typical startle response. In the literature, the startle response is identified by a bursting pattern of muscle EMG activity in the SCM that is greater than background activation and observed in the EMG trace of a single trial (Brown et al. 1991b; Carlson 2003; Carlson 2007). Typically, acoustic stimuli that are 124 dB

(40 ms, 1000 Hz) are used to induce a startle response (Carlson 2003; Carlson 2007). Therefore, to observe the response characteristics in the absense of a startle response, repeated acoustic stimuli (3 – 5 second interstimulus interval) that were 95 and 115 dB (40 ms, 1000Hz) were used. Interestingly, this pilot work (Luxon 2011) found similar observations to Nichol (2008) where small amplitude waveforms, only visible with the averaging of multiple trials, were observed in tonically contracted limb muscles and absent in quiescent musculature. Based on these findings and those observed by Nichol (2008), it appears that the response to reapeated acoustic stimuli differs from the startle response. More specifically, we observed a persistent reflexive response at stimulus intensities of 95- and 115 dB with initial peak latencies that increased with the distance of each segmental innervation in the spinal cord. For example, the average peak latency observed in the left biceps and soleus (n = 8) to a 40 ms tone (1000 Hz, 115 dB) was 45.7 ms ± 10.5 ms and 68.9 ± 18.3 ms, respectively.

Appendix 2: p13n23 response data

SCM data for repeated 7 ms stimuli: p13n23 response

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	p13 (uV)	n23 (uV)	Corr. P13	Corr. N23	Difference (n23-p13)	Normalized	p13	n23	p13n23
S1	4.04									
S2	10.09	-4.03	7.50	0.40	0.74	11.54	1.14	12.48	23.81	11.33
S3	4.29	-1.95	2.91	0.45	0.68	4.86	1.13	11.90	22.66	10.76
S4	1.73	-0.57	0.62	0.33	0.36	1.19	0.69	10.56	19.01	8.45
S5	7.56	-1.61	2.15	0.21	0.28	3.77	0.50	11.71	19.39	7.68
S6	3.64	-0.42	0.57	0.12	0.16	0.99	0.27	10.94	26.69	15.75
S7	4.79	-1.64	3.55	0.34	0.74	5.20	1.08	16.13	26.30	10.17
S8	7.62	-1.06	2.78	0.14	0.37	3.84	0.50	10.37	18.24	7.87
S9	2.78	-1.12	1.15	0.40	0.41	2.27	0.82	10.94	20.35	9.41
S10	5.05	-2.57	2.25	0.51	0.45	4.82	0.95	12.10	20.54	8.44
S11	1.22	-0.46	0.56	0.37	0.46	1.02	0.83	11.14	23.42	12.28
S12	2.20	-0.58	0.63	0.26	0.29	1.21	0.55	10.94	19.20	8.26
S13	1.82	-0.47	0.21	0.26	0.12	0.68	0.37	10.18	17.66	7.48
S14	3.76	-1.90	6.12	0.50	1.63	8.02	2.13	12.86	28.22	15.36
S15	4.30	-0.79	4.89	0.18	1.14	5.68	1.32	12.29	27.26	14.97
S16	3.07	-1.11	1.84	0.36	0.60	2.95	0.96	11.71	24.96	13.25
S17	2.34	-1.06	2.14	0.45	0.92	3.20	1.37	10.94	25.15	14.21
S18	1.01	0.08	0.30	0.08	0.30	0.22	0.22	10.94	26.69	15.75
S19	8.02	-1.02	5.74	0.13	0.72	6.76	0.84	14.21	26.50	12.29
S20	0.96	0.11	0.34	0.11	0.35	0.23	0.24	11.33	22.66	11.33
Means	4.02	-1.17	2.43	0.30	0.56	3.60	0.84	11.77	23.09	11.32
Std. Dev.		0.98	2.20	0.14	0.37	2.99	0.48	1.43	3.44	2.92

SCM data for repeated 40 ms stimuli: p13n23 response

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	p13 (uV)	n23 (uV)	Corr. P13	Corr. N23	Difference (n23-p13)	Normalized	p13	n23	p13n23
S1	4.41									
S2	10.49	-3.39	5.44	0.32	0.52	8.83	0.84	11.71	25.92	14.21
S3	2.84	-1.29	1.53	0.45	0.54	2.82	0.99	12.29	26.88	14.59
S4	1.90	-0.57	0.62	0.30	0.33	1.19	0.63	10.56	19.01	
S5	5.58	-0.69	1.02	0.12	0.18	1.71	0.31	10.56	19.97	8.45
S6	4.93									
S7	5.42	-0.85	1.75	0.16	0.32	2.60	0.48	15.17	23.81	8.64
S8	9.10	-1.40	0.96	0.15	0.11	2.35	0.26	9.79	25.92	16.13
S9	2.73	-0.79	0.54	0.29	0.20	1.32	0.48	10.94	17.09	6.15
S10	4.63	-1.59	0.45	0.34	0.10	2.05	0.44	11.52	18.43	6.91
S11	1.05	-0.27	0.14	0.25	0.14	0.41	0.39	11.14	24.77	13.63
S12	3.39	-0.48	0.61	0.14	0.18	1.09	0.32	9.22	16.32	7.10
S13	2.06									
S14	2.75	-1.22	2.75	0.44	1.00	3.97	1.44	12.29	28.03	15.74
S15	3.05	-0.36	2.27	0.12	0.74	2.63	0.86	10.18	26.69	16.51
S16	3.40	-1.04	1.14	0.31	0.33	2.18	0.64	11.71	24.19	12.48
S17	2.12	-1.04	1.50	0.49	0.71	2.54	1.20	11.71	26.88	15.17
S18	1.39									
S19	4.61	-0.38	1.51	0.08	0.33	1.88	0.41	15.74	28.99	13.25
S20	1.08	0.08	0.29	0.08	0.27	0.21	0.19	13.06	26.11	13.05
Mean	3.85	-0.95	1.41	0.25	0.37	2.36	0.62	11.72	23.69	12.13
Std. Dev.		0.79	1.30	0.14	0.26	1.97	0.36	1.76	4.12	3.65

Appendix 3: n34p44 response data

SCM data for repeated 7 ms stimuli: n34p44 response

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	n34 (uV)	p44 (uV)	Corr. N34	Corr. P44	Difference (p44-n34)	Normalized	n34	p44	n34p44
S1	4.04									
S2	10.09	-10.30	6.26	1.02	0.62	16.56	1.64	38.78	63.36	24.58
S3	4.29	-4.70	2.52	1.10	0.59	7.22	1.68	39.17	56.06	16.89
S4	1.73	-0.90	0.11	0.52	0.06	1.01	0.58	35.71	44.35	8.64
S5	7.56	-2.80	2.09	0.37	0.28	4.89	0.65	36.29	52.22	15.93
S6	3.64	-1.45	1.16	0.40	0.32	2.61	0.72	44.74	64.7	19.96
S7	4.79	-3.26	3.38	0.68	0.70	6.64	1.38	42.05	67.97	25.92
S8	7.62	-5.11	2.46	0.67	0.32	7.57	0.99	33.79	61.06	27.27
S9	2.78	-1.20	0.54	0.43	0.20	1.74	0.63	35.33	62.4	27.07
S10	5.15	-3.24	3.10	0.63	0.60	6.34	1.23	51.07	64.51	13.44
S11	1.22	-1.09	0.34	0.89	0.28	1.43	1.17	38.4	52.8	14.4
S12	2.20	-1.33	0.62	0.60	0.28	1.94	0.88	33.6	45.12	11.52
S13	1.82	-0.84	0.49	0.46	0.27	1.32	0.73	37.06	58.18	21.12
S14	3.76	-8.79	3.45	2.34	0.92	12.24	3.25	43.39	60.29	16.9
S15	4.30	-6.37	2.99	1.48	0.69	9.36	2.17	41.66	56.06	14.4
S16	3.07	-2.88	2.12	0.94	0.69	5.00	1.63	41.47	60.1	18.63
S17	2.34	-2.60	1.21	1.11	0.52	3.81	1.63	39.17	52.22	13.05
S18	1.01	0.04	0.40	0.04	0.39	0.36	0.35	36.48	68.93	32.45
S19	8.02	-6.60	1.53	0.82	0.19	8.12	1.01	41.47	53.95	12.48
S20	0.96	-0.02	0.27	0.02	0.28	0.30	0.31	38.59	59.14	20.55
Mean	4.02	-3.34	1.84			5.18	1.19	39.38	58.07	18.69
Std Dev.		2.94	1.57			4.37	0.71	4.23	6.85	6.38

SCM data for repeated 40 ms stimuli: n34p44 response

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	n34 (uV)	p44 (uV)	Corr. N34	Corr. P44	Difference (p44-n34)	Normalized	n34	p44	n34p44
S1	4.41									
S2	10.49	-5.81	1.04	0.55	0.10	6.85	0.65	40.90	53.57	12.67
S3	2.84	-1.84	0.55	0.65	0.19	2.39	0.84	42.24	57.22	14.98
S4	1.90	-0.43	0.29	0.23	0.15	0.73	0.38	49.54	62.78	13.24
S5	5.58	1.30	-1.77	0.23	0.32	3.07	0.55	35.52	50.88	15.36
S6	4.93	-1.39	1.15	0.28	0.23	2.55	0.52	51.07	85.82	34.75
S7	5.42	-1.22	2.01	0.23	0.37	3.23	0.60	48.38	67.01	18.63
S8	9.10	-1.72	2.91	0.19	0.32	4.62	0.51	43.58	61.25	17.67
S9	2.73	-0.89	0.41	0.32	0.15	1.30	0.48	54.91	71.81	16.90
S10	4.63	-2.27	2.71	0.49	0.59	4.98	1.07	55.68	86.02	30.34
S11	1.05	-0.78	0.17	0.74	0.16	0.95	0.91	39.55	56.06	16.51
S12	3.39	-1.16	0.72	0.34	0.21	1.88	0.55	33.60	66.05	32.45
S13	2.06	-0.86	0.35	0.42	0.17	1.21	0.59	42.05	58.37	16.32
S14	2.75	-4.28	1.85	1.55	0.67	6.12	2.22	44.54	62.98	18.44
S15	3.05	-3.57	2.36	1.17	0.77	5.93	1.94	40.90	55.68	14.78
S16	3.40	-1.31	0.90	0.38	0.27	2.21	0.65	47.23	61.82	14.59
S17	2.12	-1.21	0.57	0.57	0.27	1.78	0.84	43.39	76.99	33.60
S18	1.39	0.05	0.38	0.04	0.28	0.33	0.24	56.45	70.46	14.01
S19	4.61	-1.06	1.08	0.23	0.23	2.13	0.46	45.50	74.11	28.61
S20	1.08	0.06	0.22	0.06	0.20	0.16	0.14	35.33	52.22	16.89
Mean	3.85	-1.49	0.94			2.76	0.74	44.76	64.79	20.04
Std. Dev.		1.62	1.08			2.03	0.52	6.73	10.49	7.57

Appendix 4: Biceps response data

Biceps data for repeated 7 ms stimuli

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	p1 (uV)	p2 (uV)	Corr. P1	Corr. P2	Difference (p1-p2)	Normalized	p1	p2	p1p2
S1	20.28	-4.42	-0.06	0.22	0.00	4.36	0.22	34.75	49.92	15.17
S2	22.66	-9.90	3.23	0.44	0.14	13.13	0.58	58.18	75.84	17.66
S3	16.41	-5.76	0.47	0.35	0.03	6.23	0.38	52.42	68.74	16.32
S4	23.63	-7.10	5.09	0.30	0.22	12.19	0.52	57.79	74.69	16.90
S5	6.22									
S6	10.89	-4.95	-0.06	0.45	0.01	4.89		92.35 *	107.5 *	
S7	29.84	-13.61	10.48	0.46	0.35	24.09	0.81	42.82	54.53	11.71
S8	22.68	-5.88		0.26		5.88		99.65 *		
S9	33.09	-8.24	3.87	0.25	0.12	12.11	0.37	45.89	58.56	12.67
S10	3.61	-2.73	-2.37	0.76	0.66	0.36	0.10	56.06	73.73	17.67
S11	23.62	-14.38	16.03	0.61	0.68	30.42	1.29	38.78	54.53	15.75
S12	19.71	-10.99	6.99	0.56	0.35	17.98	0.91	45.12	57.98	12.86
S13	14.34	-7.24	1.60	0.50	0.11	8.84	0.62	61.63	74.88	13.25
S14	12.72	-12.53	7.46	0.99	0.59	19.99	1.57	44.16	57.60	13.44
S15	27.68	4.52	-6.95	0.16	0.25	11.48	0.41	47.23	65.28	18.05
S16	4.67	-2.94	-2.12	0.63	0.45	0.82	0.18	50.88	67.58	16.70
S17	12.16	-4.78	-0.28	0.39	0.02	4.50	0.37	34.37	47.62	13.25
S18	8.72	0.01	6.17	0.00	0.71	6.16	0.71	43.01	57.41	14.40
S19	30.91	-4.56	10.10	0.15	0.33	14.66	0.47	57.79	74.11	16.32
S20	25.50	-2.75	9.67	0.11	0.38	12.42	0.49	37.44	52.80	15.36
Mean	18.47	-6.22	3.85	0.40	0.30	11.08	0.59	47.55	62.69	15.15
Std. Dev.		4.73	5.69	0.24	0.24	7.88	0.39	8.69	9.68	1.99

Biceps data for repeated 40 ms stimuli

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	p1 (uV)	p2 (uV)	Corr. P1	Corr. P2	Difference (p1-p2)	Normalized	p1	p2	p1p2
S1	20.19	-5.78	0.83	0.29	0.04	6.61	0.33	67.20	81.41	14.21
S2	19.10	-6.44	0.42	0.34	0.02	6.87		92.93 *	108.3 *	
S3	18.07	-8.78	4.02	0.49	0.22	12.80	0.71	50.69	64.32	13.63
S4	24.88	-8.10	1.89	0.33	0.08	9.98	0.40	57.02	73.92	16.90
S5	5.91	-3.06	-1.16	0.52	0.20	1.90	0.32	54.72	70.08	15.36
S6	13.31	-5.51	-0.20	0.41	0.01	5.31		94.46 *	109.2 *	
S7	27.79	-13.43	14.23	0.48	0.51	27.66	1.00	43.39	59.71	16.32
S8	20.31	-12.35	3.71	0.61	0.18	16.06		104.6 *	119.6 *	
S9	33.13	-9.28	10.36	0.28	0.31	19.65	0.59	45.89	57.79	11.90
S10	4.02									
S11	22.49	-14.43	6.00	0.64	0.27	20.44	0.91	39.55	55.10	15.55
S12	23.60	-12.20	8.29	0.52	0.35	20.49	0.87	44.74	57.02	12.28
S13	18.75	-10.05	5.61	0.54	0.30	15.67	0.84	54.91	68.54	13.63
S14	9.66	-9.79	5.88	1.01	0.61	15.67	1.62	44.54	58.37	13.83
S15	27.33	3.79	-7.53	0.14	0.28	11.32	0.41	47.42	64.70	17.28
S16	4.80	-2.81	-2.26	0.59	0.47	0.55	0.11	51.26	58.37	7.11
S17	6.33									
S18	10.30	-0.32	5.08	0.03	0.49	5.40	0.52	42.62	56.64	14.02
S19	28.25	-2.41	10.72	0.09	0.38	13.13	0.46	54.34	65.09	10.75
S20	19.85	-1.59	7.55	0.08	0.38	9.14	0.46	41.28	54.91	13.63
Mean	17.90	-6.81	4.08	0.41	0.28	12.15	0.64	49.30	63.06	13.76
Std. Dev.		5.01	5.29	0.24	0.18	7.20	0.37	7.41	7.71	2.59

Appendix 5: Soleus response data

Soleus data for repeated 7 ms stimuli

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	p1 (uV)	p2 (uV)	Corr. P1	Corr. P2	Difference (p1-p2)	Normalized	p1	p2	p1p2
S1	7.08	7.82		1.11				177.8 *		
S2	11.39	12.60		1.11				128.4 *		
S3	18.75	20.44		1.09				87.55		
S4	12.68	13.81		1.09				103.50		
S5	31.27	33.50		1.07				107.90		
S6	17.60	19.63		1.12				58.37 *		
S7	9.12	10.02		1.10				130.9 *		
S8	10.54	11.68		1.11				114.40		
S9	12.49	13.54	11.40	1.08	0.91	2.15	0.17	80.06	116.20	36.14
S10	11.14	12.67	10.19	1.14	0.91	2.48	0.22	109.20	169.20	60.00
S11	10.68	11.53		1.08				82.18		
S12	3.99	4.39		1.10				90.82		
S13	6.49	7.06		1.09				83.33		
S14	9.70	11.49	9.29	1.18	0.96	2.20	0.23	61.25	136.50	75.25
S15	6.29	7.69	5.82	1.22	0.92	1.88	0.30	90.43	150.70	60.27
S16	11.52	12.30	10.71	1.07	0.93	1.59	0.14	110.00	181.10	71.10
S17	12.16	6.40		0.53				77.38		
S18	6.16	7.09	5.86	1.15	0.95	1.22	0.20	89.66	203.50	113.84
S19	7.83	8.19		1.05				93.12		
S20	15.25	16.83	14.25	1.10	0.93	2.58	0.17	163 *	181.6 *	18.6 *
Mean	11.61	12.43	9.64	1.08	0.93	2.01	0.20	92.05	159.53	69.43
Std. Dev.		6.53	3.02	0.14	0.02	0.48	0.05	14.66	31.55	25.65

Soleus data for repeated 40 ms stimuli

		P-P AMPLITUDE						ONSETS (ms)		INTERVAL (ms)
Subject	Background (uV)	p1 (uV)	p2 (uV)	Corr. P1	Corr. P2	Difference (p1-p2)	Normalized	p1	p2	p1p2
S1	7.42	8.44	0.00	1.14				110.60		
S2	11.42	14.01	10.65	1.23	0.93	3.36	0.29	122.70	180.50	57.80
S3	19.25	20.92	0.00	1.09				88.32		
S4	16.51	18.42	15.29	1.12	0.93	3.13	0.19	104.30	160.10	55.80
S5	32.23	36.50	29.86	1.13	0.93	6.64	0.21	120.00	166.70	46.70
S6	19.67	21.62	17.00	1.10	0.86	4.62	0.23	86.40	147.10	60.70
S7	10.23	11.72	0.00	1.15				100.40		
S8	10.80	12.61	10.30	1.17	0.95	2.31	0.21	117.90	193.70	75.80
S9	16.45	18.71	15.07	1.14	0.92	3.63	0.22	101.80	139.20	37.40
S10	8.56	11.02	7.12	1.29	0.83	3.90	0.46	111.60	175.10	63.50
S11	9.80	10.46	0.00	1.07				105.60		
S12	4.64	5.67	0.00	1.22				109.60		
S13	7.13	8.22	0.00	1.15				80.83		
S14	8.09	9.73	7.06	1.20	0.87	2.67	0.33	65.47	157.20	91.73
S15	4.90	6.41	4.48	1.31	0.91	1.93	0.39	92.74	167.00	74.26
S16	12.92	13.89	12.02	1.08	0.93	1.87	0.15	122.50	145.00	22.50
S17	5.84	7.00	0.00	1.20				111.70		
S18	6.83	8.70	0.00	1.27		8.70		92.93		
S19	7.24	8.60	7.00	1.19	0.97	1.60	0.22	95.66	182.00	86.34
S20	9.03	11.86	0.00	1.31				100.40		
Mean	11.45	13.23	6.79	1.18	0.91	3.70	0.26	102.07	164.87	61.14
Std. Dev.		7.22	8.12	0.08	0.04	2.11	0.09	14.85	17.12	20.66