# ELECTROMYOGRAPHIC MUSCLE RESPONSES TO SINGLE ACOUSTIC STIMULI AND REPEATED ACOUSTIC STIMULI IN SUPINE SUBJECTS

by

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## Abstract

Electromyographical (EMG) motor responses may be elicited by loud acoustic stimuli in humans and vary based on presentation methods and body position. The purpose of this study was to investigate the EMG responses caused by different presentation methods of acoustic stimuli in a supine body position. Participants lay supine and maintained a voluntary plantar flexion contraction during trials. Auditory stimuli were presented from a speaker in front of participants' face. EMG was recorded from right orbicularis oculi (OOc) and bilaterally from sternocleidomastoid (SCM), medial gastrocnemius, deltoid and soleus muscles. Single acoustic stimuli (SAS) (40 ms, 124 dB tones), were presented to participants with ten minutes between stimuli. Repeated acoustic stimuli (RAS) (40 ms, 124 dB tones), were presented repeatedly at intervals of 3-5 sec. Ten participants in a control condition were exposed to six or more SAS and 210 RAS during testing. Pre-pulse stimuli (40 ms, 85 dB tones) were presented 100 ms before both the RAS and SAS for 8 participants in the experimental condition. These participants were exposed to 3 SAS plus pre-pulse and 3 SAS, then to a total of 200 RAS and 200 RAS plus pre-pulse presented pseudorandomly. Five participants were exposed to 210 RAS stimuli at 85 dB as a follow-up control condition. EMG signals were root mean squared and trigger-averaged to the onset of the acoustic stimulus for the different conditions. Similar responses were rendered from SAS and RAS in voluntarily contracting lower limb muscles. SAS response amplitudes were variable within single muscles across trials. RAS exposures rendered an averaged response in all participants tested which lasted for ~500 ms at a 7-8 Hz oscillation in the voluntarily contracting soleus muscles. This response appears to be similar to SAS responses but of smaller amplitude and only visible after the averaging of multiple trials. Responses to the 85 dB RAS stimuli also occurred in voluntarily contracting muscles. Pre-pulses showed inhibition in the OOc muscle in the SAS condition. The observations suggest that in humans, an EMG response may be elicited in contracting lower limb muscles by SAS and RAS and these responses may be related.

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# Introduction

A startle reflex is a motor response to an unexpected auditory, visual and/or tactile stimulus detected by the different sensory systems either independently or collectively[1] (Delwaide & Schapens, 1995; Landis & Hunt, 1939; Quednow et al., 2006; Yeomans et al., 2002). Responses to loud acoustic startle stimuli vary between participants but most people demonstrate eye closure and contraction of the neck muscles (Brown et al., 1991a). Generalized responses to sudden acoustic stimuli include trunk flexion, abduction of the arms, flexion of the elbows, pronation of the forearms (Brown et al., 1991a), extensor contractions (Rossignol, 1975), and electromyographic (EMG) responses in distal musculature[2] (Brown et al., 1991b; Nieuwenhuijzen et al., 2000). A response to a single acoustic stimulus has in the past been classified as a startle response when there is a bursting pattern of muscle EMG activity that is of greater amplitude than normal background activity in the orbicularis oculi (OOc) and sternocleidomastoid (SCM) muscles (Brown et al., 1991b; Carlsen et al., 2003). Startle responses to acoustic stimuli may be found throughout the body's musculature as the bursting pattern of muscle EMG activity following the evoking stimulus (Brown et al., 1991b; Delwaide & Schapens, 1995; Nieuwenhuijzen et al., 2000; Rossignol, 1975). Acoustic startle experiments typically use intensities above 110 dBs but no louder than 130 dB (Brown et al., 1991a; Brown et al., 1991b; Csomor et al., 2006; Delwaide & Schepens, 1995; Grosse & Brown, 2003; Nieuwenhuijzen et al., 2000; Valls-Solé et al., 1999a; Valls-Solé et al., 2005). For the purpose of the present experiment, responses that would be classified as a startle by the presence of a SCM EMG bursting activity following the stimulus were termed a *single acoustic stimulus* (SAS) response. In some literature, startle responses throughout the body are no longer defined

[1] For more information on the auditory system, see Appendix 1: The Anatomy and Neurophysiology of the Auditory System

[2] For more information on the startle reflex/SAS reflex, see Appendix 1: Single Acoustic Stimulus Reflex

as such once SCM responses no longer occur (Brown et al., 1991b; Carlsen et al., 2003). To avoid confusion of terms, the term SAS response was defined as responses to acoustic tones with long interstimulus intervals with SCM EMG responses, and as the responses to acoustic tones with short interstimulus intervals that render SCM EMG responses. Small amplitude muscle reflex responses to loud acoustic stimuli with short interstimulus intervals, with no SCM EMG response present were termed *repeated acoustic stimuli* (RAS) responses in the present study.

Both animal and human research has investigated the pathway through which the SAS response travels to evoke muscular responses[3]. The reflex is proposed to propagate through the nucleus reticularis pontis caudalis (PnC or RPC) in the reticular formation and via the reticulospinal tract. Descending nerve fibers in the reticulospinal tract then synapse at spinal levels with lower motor neurons, either directly and possibly indirectly through interneurons before reaching the neuromuscular junctions to elicit subsequent muscle responses[4] (Davis et al., 1982; Yeomans & Frankland, 1996).

The SAS response throughout the body's musculature seems to decline in amplitude, or in some cases disappears all together, after repeated exposure to the acoustic stimulus. This decline in SAS response amplitude is referred to as habituation (Brown et al., 1991b; Cadenhead et al., 1999; Geyer & Braff, 1982; Quednow et al., 2006) and is thought to be caused by a decrease in the synaptic transmission in the neural circuit involved (Carlsen et al., 2003; Kandel, 1991) or a change in receptor sensitivity (Weber et al., 2002). There is research that has demonstrated that after as few as 2 trials, the SAS response may no longer be elicited (Brown et al., 1991b). The eye blinks and neck muscle responses typically require the longest amount of exposure to habituate and do not always disappear (Davis, 1982). To elicit multiple SAS

[3] For more information on the SAS pathway, see Appendix 1: Single Acoustic Stimulus Pathway
[4] For more information on the reticulospinal tract, see Appendix 1: Reticulospinal Tract

responses in a participant, the stimuli are presented randomly with long periods of time between stimuli to ensure that one does not anticipate the SAS (Brown et al., 1991a).

Single acoustic stimulus responses are sensitive to body position in the lower limbs and EMG muscle responses in the legs are more evident in standing versus sitting conditions [5] (Brown et al., 1991a; Delwaide & Schepens, 1995; Nieuwenhuijzen et al., 2000; Rossignol, 1975). Standing also displays shorter reflex latency times with tibialis anterior (TA) latencies of 80 ms and 70 ms in the soleus (Brown et al., 1991a). In contrast, seated TA latencies are recorded at 120 ms and 130 ms in the soleus (Brown et al., 1991a). SAS while in a lying supine body position displays longer onsets than standing in the non-contracting lower limb muscles with TA responses in the range of 88-126 ms and soleus responses in the range of 98-154 ms (Bisdorff et al., 1994; Kofler et al., 2001; Stell et al., 1995). It is not known what kind of influence a supine position with lower limb muscle contraction will have on responses.

Alternative means of presenting single acoustic stimuli have been assessed in our laboratory to investigate if responses can be elicited that do not habituate. A response to sound stimuli that does not habituate could be an important clinical tool in spinal cord patients for the assessment of spinal cord intactness. In pilot studies, 124 dB acoustic stimuli were presented using short random intervals of three to five seconds between each sound for 14 minutes while participants were lying down in a supine position. This method promoted habituation of the typical SAS responses after the first 2-10 stimuli. After SAS habituation, the repeated 124 dB sounds rendered a response visible after trigger-averaging muscle EMG to the onset of the acoustic stimulus. The response was evident in participants soleus muscles which were contracting at a specific submaximal level. We have designated this presentation method as "repeated acoustic stimuli" (RAS) to differentiate it from SAS and we have named the EMG

[5] For more information on the SAS responses, see Appendix 1: SAS Responses during Static and Dynamic Tasks

response found in muscles after averaging the RAS response[6]. SAS and RAS responses are seperated by identifying and extracting SAS EMG responses from within RAS trials. The SAS response appears as a bursting pattern of SCM muscle EMG following the acoustic stimulus and are therefore not included in RAS response averaging.

Pre-pulse inhibition is a well studied effect of SAS responses (Filion et al., 1998) and may be a means of determining experimentally if a relationship exists between RAS and SAS responses. The SAS reflex may be altered by the presentation of a stimulus 30-500 ms prior to the SAS eliciting stimulus (Filion et al., 1998). The pre-stimulus decreases the amplitude or completely inhibits the SAS response and is referred to as pre-pulse inhibition[7] (Filion et al., 1998). When a pre-pulse occurs, it is thought that signals from the pedunculopontine tegmental nucleus (PPTg) are sent through cholinergic projections and inhibit the SAS centre neurons in the nucleus reticularis pontis caudalis (nRPC) in the PnC[8] (Blumenthal, 1996; Valls-Solé et al., 1999a). It is through the nRPC that the SAS stimuli are relayed to the reticulospinal tract (Davis, 1982; Yeomans & Frankland, 1996). Pre-pulse inhibition is typically measured by the change of the SAS blink reflex response of the OOc (Valls-Solé et al., 1999a). To lessen the likelihood of the pre-pulse itself eliciting a response, intensities of 95 dB or lower should be used in humans as pre-pulses of 95dB and above may evoke SAS responses (Hoffman, 1984). Pre-pulse inhibition has been investigated in OOc and SCM muscles (Valls-Solé et al., 1999a; Valls-Solé et al., 2005) but not in many of the muscles that render EMG muscle responses to SAS.

The purpose of the present experiment was to investigate the similarities and differences between single acoustic stimuli responses and the repeated acoustic stimuli responses when participants are in a supine position. Rendering reflexes in supine body positions can be difficult, as postural engagement is known to influence the occurance of other descending

[6] For more information on the RAS response, see Appendix 1: Repeated Acoustic Stimuli (RAS) Response
[7] For more information on the pre-pulse inhibition, see Appendix 1: Pre-Pulse Inhibition
[8] For more information on the pre-pulse inhibition pathway, see Appendix 1: Pre-Pulse Inhibition Processing and Pathway

reflexive responses. Without postural engagement, the vestibulspinal reflex ceases. The supine body position was chosen in this experiment to show that postural engagement is not necessary for these reflexes to occur and that they may differ from reflexes that are influenced by postural engagement. We hypothesized that SAS EMG muscle responses would be evoked by a loud acoustic stimulus (124 db) and that after repeated exposures, SAS EMG muscle responses would habituate. Once SAS responses habituate, averaged RAS EMG muscle responses within participants may be evoked in soleus muscles contracting at a specific submaximal level. We also hypothesized that SAS responses would be inhibited by the presence of pre-pulse stimuli. SAS EMG muscle response amplitude would decrease in all muscles when a 85 dB pulse preceded a 124 dB SAS pulse. Pre-pulse stimuli were not anticipated to decrease EMG response amplitude of the averaged RAS response within participants as they were believed to be separate responses from SAS responses. We anticipate that all participants will display reflexes to sounds that will not completely habituate.

# Materials and Methods

## Participants

A total of eighteen volunteers were recruited (9 males and 9 females, aged 18-30) for three testing procedures. Ten participants (5 males and 5 females, aged 18-26) were assigned to the control condition and the remaining eight participants (4 males and 4 females, aged 18-30) were assigned to the pre-pulse condition. 5 participants (2 males and 3 females, aged 18-30) were randomly selected from the entire pool of participants to complete an additional 85 dB protocol. Participants were healthy, with no known hearing deficits or disorders, past or current neurological disorders, head trauma, or sensory or motor dysfunctions of the lower extremities. Volunteers gave their informed written consent and the study was conducted in accordance with the ethical guidelines established by the University of British Columbia.

## Apparatus

Surface EMG signals were recorded from the muscle bellies of the right OOc (on the edge of the lateral orbital rim and the edge of the inferior orbital rim), the right and left SCM, the right and left medial gastrocnemius, the right and left deltoid muscles (mid-muscle belly) and the right and left soleus muscles. EMG was collected using bipolar preamplified Ag/AgCl surface electrodes using a Grass P511 AC Amplifier. Electrical impedance was decreased by the removal of excess debris at collection sites by shaving and swabbing the area with alcohol. The electrodes were placed parallel to the muscle fibers to accurately detect conduction velocity. Grounding electrodes were placed on the participant's medial and lateral malleoli, clavicles and acromion process. EMG was collected at a sampling rate of 4545.45 Hz for two participants and 3846.15 Hz for the rest of the participants with signals amplified ($2x10^4$), with a high-pass-filter at 30 Hz and a low-pass filter at 1 KHz. The signal was sent from the amplifier to the

analog/digital (AD) converter (CED Micro 1401) for sampling and was controlled by a program written with Spike2 version 5.13 software. The difference in sampling rates for the participants was due to Spike2 software assigning sampling rates and was not done intentionally.

## Stimuli

The SAS and the RAS were 40 ms duration, 1000 Hz, 124 dB pulses. The tones were delivered through a Pioneer SX-650 amplifier. The acoustic pre-pulse stimulus and the 85 dB RAS were 40 ms duration, 1000 Hz, 85 dB pulses. Both acoustic stimuli were presented via a loud speaker (Sentry/RH-30-L) placed in front of the participant's face at a distance of 30 cm from both ears. The stimulus intensities were calibrated using a Cirrus (model CR:251B) sound-level meter at a distance of 30 cm from the speaker. In pre-pulse exposures there was an interstimulus interval of 100 ms between the end of the pre-pulse stimuli and the onset of the SAS/RAS. Based on previous literature, strong inhibition typically occurs with the lead interval time (the time between the pre-pulse and SAS) within the range of 100-140ms (Blumenthal, 1996; Csomor et al., 2005; Csomor et al., 2006; Quednow et al., 2006; Schwarzkopf et al., 1993).

## Experimental Procedure

Quiet stance EMG biofeedback from each of the soleus muscles was measured with participants standing quietly upright with their feet close together but not touching and their arms at their sides for 1 minute. Background EMG of the soleus was measured for each muscle and separate horizontal cursors were set at the approximate root mean square (RMS) average EMG response seen for each muscle. The level measured in each soleus was used as a target activation level to achieve and maintain while participants were lying. This level was chosen in an attempt to achieve a constant level of activation for each participant throughout the experiment and to

replicate the level of soleus activation used during the postural task of quiet stance. Participants were asked to lie down on a clinical examination bed with their feet against a stationary platform perpendicular to the bed. To prevent movement participants' ankles were restrained to keep their feet flat against the platform and to maintain an ankle angle of 90 degrees. During the trials, participants maintained a plantar flexion contraction of both feet at the level of the horizontal cursors set from their standing position. A computer screen was placed in the participants upper visual field giving them online visual feedback of their soleus muscles level of activation as well as their target RMS activation level.

In the control condition the ten participants were exposed to two protocols. First they were exposed to the SAS protocol followed by the RAS protocol. In the experimental condition the eight participants were exposed to two protocols. First they were exposed to the SAS/pre-pulse SAS protocol, followed by the RAS/pre-pulse RAS protocol. Five participants received the 85 dB protocol following their RAS protocol (3 participants from the experimental condition and 2 from the control condition).

In the control condition, participants were exposed to the SAS protocol, which had a minimum of ten minutes in between stimuli to avoid habituation (1 hour duration minimum). The interstimulus interval of ten minutes was established through pilot work and is greater than the time used in some literature (Rossignol, 1975). A minimum of 6 SAS exposures were measured in all participants before they proceeded to the RAS protocol. The RAS protocol consisted of 210 RAS, presented with a randomly determined interstimulus interval of 3-5 seconds between the end of the last stimulus and the beginning of the following stimulus for the duration of the trial.

For the experimental condition participants were exposed to the SAS/pre-pulse SAS protocol, 3 SAS and 3 SAS preceded by pre-pulses in random order, with a minimum of ten minutes between stimuli. In pre-pulse exposures there was an interstimulus interval of 100 ms

between the end of the pre-pulse stimuli (85 dB) and the onset of the SAS/RAS (124 dB). The RAS/pre-pulse RAS protocol followed with four blocks of trials with 115 stimuli in each block. Stimuli were presented with an interstimulus interval of 3-5 seconds between the end of the last stimulus and the beginning of the following stimulus for the duration of the trials. The block consisted of 115 pseudorandomly selected RAS alone, or RAS with pre-pulse stimuli. After the four blocks of trials, over 200 RAS with pre-pulse stimuli and 200 RAS alone stimuli had been presented in total.

The 85 dB protocol was the same as the control condition RAS protocol but the RAS was an 85 dB stimulus rather than 124dB stimulus.

All trials were examined for SCM activation. SCM responses that followed acoustic stimuli onset by 20 ms or longer were deemed SAS responses and it was this criteria that was used to separate SAS responses from RAS responses. These responses were visually confirmed. The 20 ms criteria was chosen based on 20 ms being the fasted known response to a SAS. This response time of 20 ms has been found in the OOc muscles (Brown et al., 1991a).

## Data Analysis

Surface EMG signals were root mean squared (RMS) at a time contant of 0.02 seconds and trigger-averaged to the onset of the acoustic stimulus using both Spike2 v5.13 software (Cambridge Electronic Design, Cambridge UK) and MATLAB 7 (MathWorks Inc., Natick, MA). Surface EMG signals were also examined with no RMS in their raw states and as rectified EMG using Spike2 v5.13 software. Averages were performed seperately for each participant in the control condition for their SAS and RAS responses, in the experimental condition for their SAS/pre-pulse SAS responses, RAS/pre-pulse RAS responses and for the 85 dB RAS protocol responses. The EMG averages were analyzed from 400 ms before the acoustic stimulus to 1.5 sec after the stimulus. The onset latencies, the peak latencies (time to peak) and peak response

amplitudes of the EMG responses were determined using Matlab 7.0. Onset latencies were determined based on responses at least 20 ms following stimuli that were two standard deviations above the trigger-averaged mean background EMG level prior to the acoustic stimulus. All onsets were visually confirmed. Peak latencies were determined from the time of the acoustic stimulus onset until the maximum muscle responses. Peak response amplitude was the difference in voltage between the peak response and the averaged background activation level prior to the stimulus.

## Statistical Analysis

The reliability of the size of the SAS amplitude in the control condition was examined using a intraclass correlation (ICC), a one-way random effect model based on six trials. In the control condition, a paired-samples t-test was used to examine the difference of peak response amplitude and peak response latency between SAS and RAS responses. In the pre-pulse condition, the peak amplitudes of the SAS and RAS responses, with and without pre-pulses were subjected to a two-way Tone (SAS, RAS) X Pre-pulse (present, absent) repeated measures ANOVA. A p-value of .05 was used to indicate statistical significance using SPSS 10.0 software.

# Results

## Single Acoustic Stimulus (SAS)

A representative participant's raw EMG data with no rectification may be seen in Figure 1 depicting a strong SAS response with activation of multiple muscles following the stimulus. Bursting activity is evident in the EMG of all muscles except the soleus, with the largest responses in the OOc and SCM muscles. The duration of the response ranges from 50-400 ms. A total of 94 SAS responses were elicited across participants. Table 1 summarizes the number of SAS responses from each participant from the control condition. Three SAS responses were collected from each of the 8 participants in the experimental RAS condition with and without pre-pulses.

The amplitude of the SAS responses in all muscles varied across participants over the course of the testing. Variability in amplitude was not necessarily due to habituation because some later SAS responses had a greater amplitude than earlier ones (see Figure 2). It is also evident that responses may be variable within muscle groups on different sides of the body, as responses may be larger on one side, or only present on one side (Figure 2). The amplitudes of SAS responses for the first six trials in all participants can be found in Appendix 3 for the right OOc, right and left soleus, SCM, medial gastrocnemius and deltoid muscles.

Intraclass correlation of peak response amplitude across SAS trials was 0.69 (CI 0.46 to 0.89) for right OOc responses, 0.75 (CI .54 to .91) for left SCM, 0.82 (CI .65 to .94) for right SCM, 0.61 (CI. .36 to .85) for the left medial gastrocnemius, 0.23 (CI .01 to .59) for the right medial gastrocnemius, 0.16 (CI. -.02 to .52) for the left deltoid, 0.19 (CI .01 to .55) for the right deltoid, 0.35 (CI. .12 to .70) for left soleus and 0.33 (CI .10 to .68) for right soleus. The ICCs measured the proportion of variance in response amplitudes (McGraw et al., 1996). The SAS response amplitudes were only relatively stable for the OOc and SCM muscles.

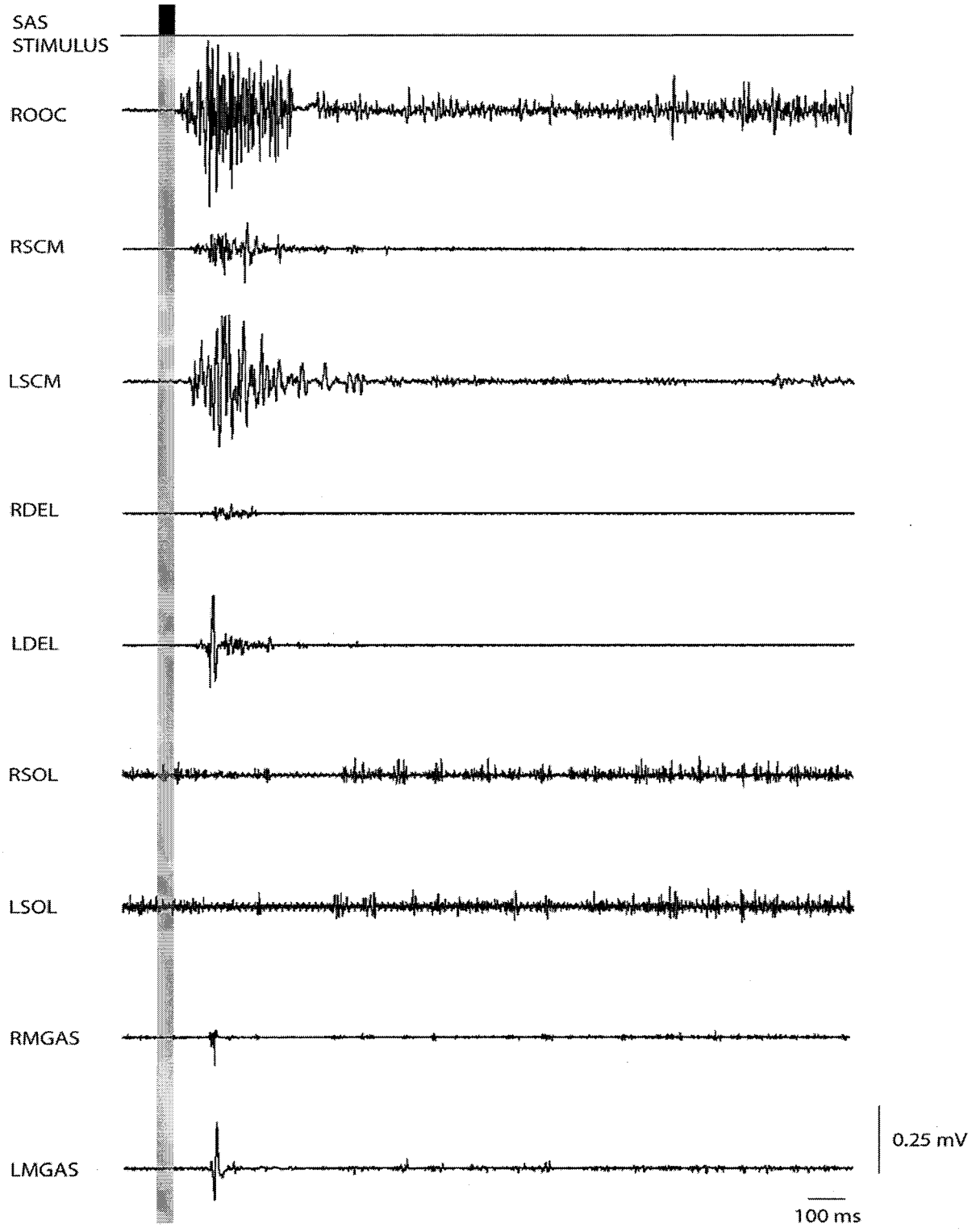


**Figure 1:** Raw EMG trace of right OOc, right and left SCM, right and left deltoid, right and left soleus and right and left medial gastroc of one participant upon exposure to one SAS stimulus. The shaded area represents the timing and duration of the SAS stimulus.

| Controls | |
|---|---|
| Participant | Number of SAS |
| 1 | 6 |
| 2 | 6 |
| 3 | 9 |
| 4 | 9 |
| 5 | 7 |
| 6 | 6 |
| 7 | 6 |
| 8 | 6 |
| 9 | 7 |
| 10 | 8 |
| total | 70 |

**Table 1.** Number of SAS exposures for each participant in the control condition

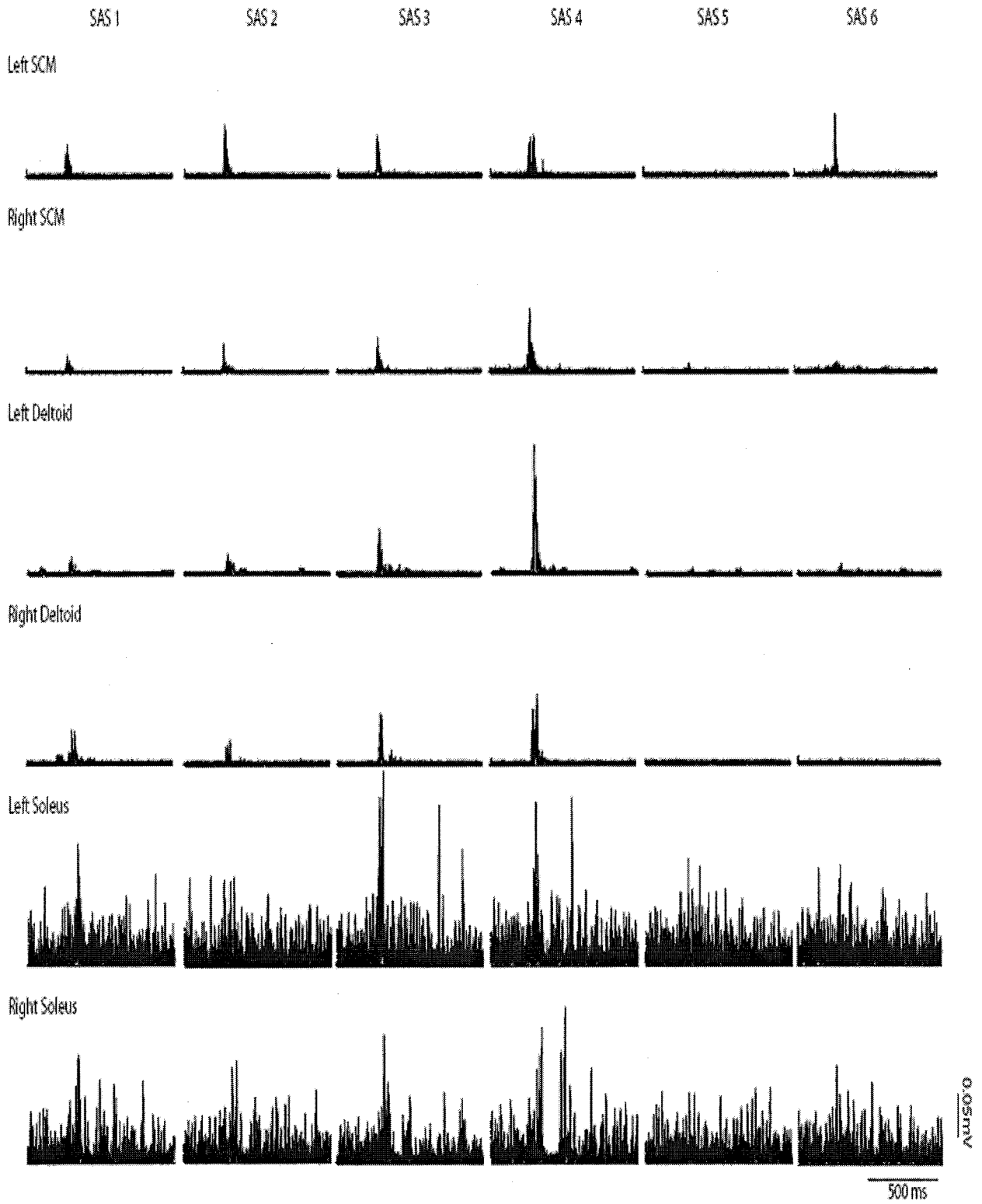


**Figure 2:** Rectified right and left SCM, right and left Deltoid and right and left Soleus responses to SAS in order of exposure of one participant. SAS1 being the first SAS exposure, SAS2 the second, through to SAS6 the sixth SAS exposure.

Responses in the tonically activated soleus muscles are not clearly visible in some of the single trials as is the case in Figure 1. However, across trials responses are present in the soleus as shown in Figure 2. The amplitude of response with respect to the level of background EMG varies between participants. Some participants display an inhibition of response followed by facilitation, while others exhibit facilitation followed by inhibition. The number of response peaks vary from one large peak to multiple and in some cases peaks are not discernable. To examine soleus responses to SAS more indepthly, responses within participants and across participants were averaged (see Figure 3). The overall average between participants seen in Figure 3 depicts a clear SAS response peak in both the right and left soleus muscles and this peak is clearly represented in many of the participants' average responses. SAS response onset latency and peak times did vary between participants as did the amplitude of the peaks (see Appendix 2). Average response onsets, peak onsets and amplitudes of the lower limb muscles may be found in Table 2.

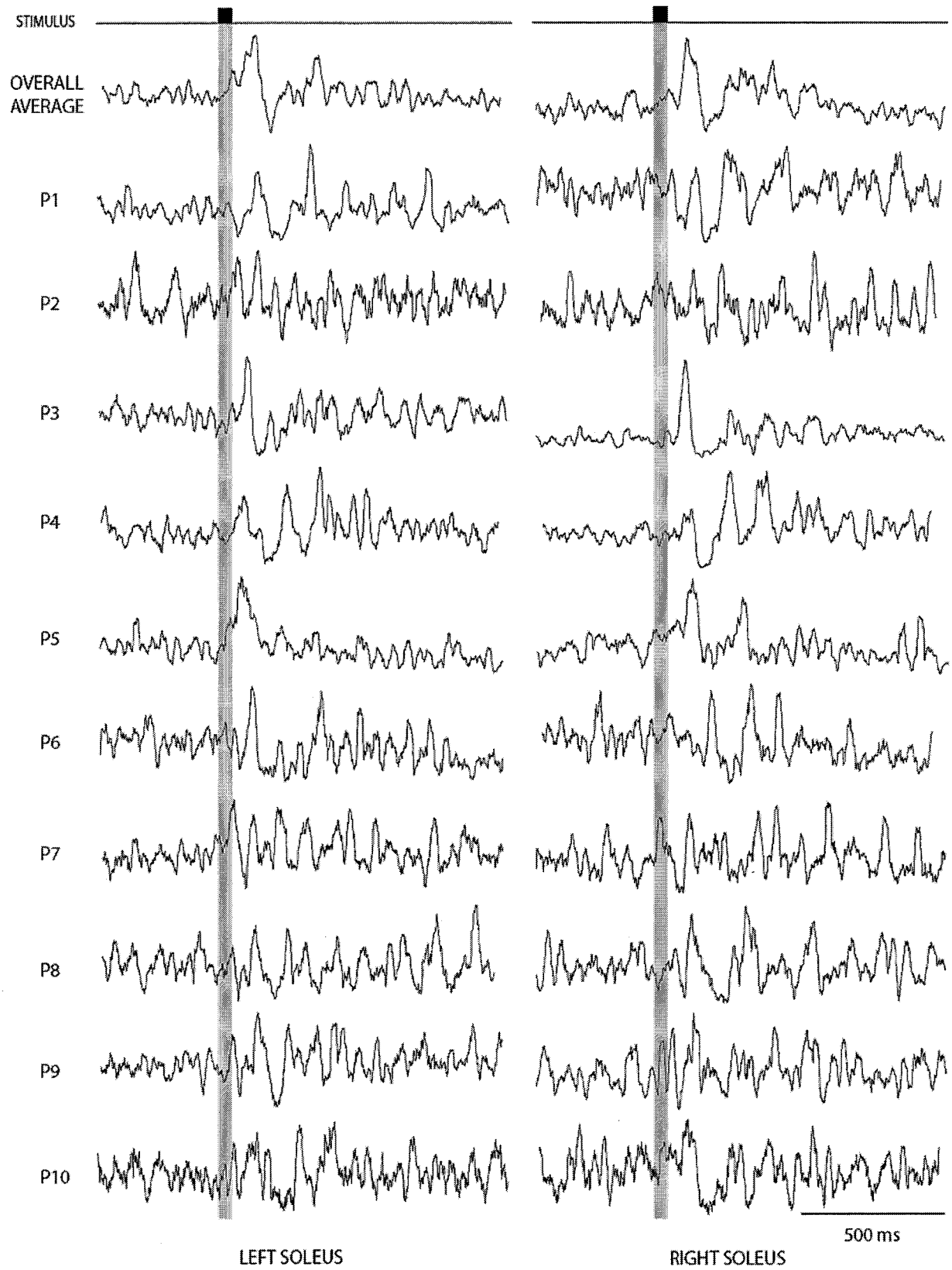


**Figure 3:** Overall group averaged EMG SAS responses of the left and right soleus muscles and individual's averaged SAS responses. All EMG responses shown are the maximum peak to peak representation. P1 through P10 represent participants one through ten.

| | SAS | | RAS | | 85 dB RAS | |
|---|---|---|---|---|---|---|
| | Average | n | Average | n | Average | n |
| Onset Latency (ms) | | | | | | |
| RSOL | 74.3 ± 13.1 | 9 | 40.9 ± 3.8 | 9 | 94 ± 6.7 | 5 |
| LSOL | 69.9 ± 11.6 | 10 | 47.3 ± 5.4 | 10 | 79.2 ± 23.1 | 5 |
| RMGAS | 67.6 ± 21.5 | 9 | 43.8 ± 5.7 | 10 | 66.6 ± 13.1 | 5 |
| LMGAS | 80.8 ± 9.8 | 8 | 42.5 ± 5.8 | 9 | 97.9 ± 23.1 | 4 |
| Peak Latency (ms) | | | | | | |
| RSOL | 111.2 ± 16.9 | 9 | 99.3 ± 5.7 | 10 | 103.0 ± 9.36 | 5 |
| LSOL | 106.4 ± 10.9 | 10 | 96.5 ± 6.5 | 10 | 118.9 ± 15.3 | 5 |
| RMGAS | 92.3 ± 14 | 10 | 101.1 ± 4.7 | 10 | 98.4 ± 7.7 | 5 |
| LMGAS | 101.1 ± 8.5 | 9 | 99.2 ± 6.2 | 10 | 137.16 ± 20 | 5 |
| Amplitude of Peak (mV) | | | | | | |
| RSOL | 0.0107 ± 0.0029 | 10 | 0.0032 ± 0.0005 | 10 | 0.0013 ± 0.00024 | 5 |
| LSOL | 0.0099 ± 0.0020 | 10 | 0.0027 ± 0.0005 | 10 | 0.0015 ± 0.0003 | 5 |
| RMGAS | 0.0052 ± 0.0015 | 10 | 0.0035 ± 0.0009 | 10 | 0.0011 ± 0.0002 | 5 |
| LMGAS | 0.0085 ± 0.0053 | 10 | 0.0025 ± 0.0005 | 10 | 0.00057 ± 0.00009 | 5 |

**Table 2**. Average onset latencies, peak onsets and amplitudes of SAS, RAS and 85dB RAS responses in the soleus and medial gastrocnemius muscles

## Repeated Acoustic Stimulus (RAS)

A RAS response was evoked in all 10 participants when they were presented with a repeated auditory stimulus of 124 dB. An average of 198 trials (range of 188-207 across subjects), were root mean squared, and trigger-averaged to the onset of the acoustic stimulus. An average EMG response from the right OOc, and bilaterally from the SCM, deltoid, soleus and medial gastrocnemius within one subject is shown in Figure 4. This average did not incorporate responses that induced a SAS response as indicated by SCM activation following the stimulus. These SAS responses were present in an average of 5.8% of trials and were removed from RAS results. However, whether or not the trial included SAS responses does not appear to change the shape of the reflex as may be seen in Figure 5 (the darker line includes all 210 trials in the average and the lighter line includes 189 of the 210 trials in the average). The RAS average including the SAS responses was not different from the RAS average excluding SAS responses. The ROOc muscle response consisted of a single peak following the stimulus. Those muscles that held tonic activation during the trials (i.e., soleus and medial gastroc) showed excitation following the stimuli. Oscillatory responses in the EMG can be seen following the initial peak and in the soleus an average of 4 oscillations occurred in participants. The oscillations finished after an average of 500 ms from stimulus onset and the frequency of these oscillations was on average 7-8 Hz. Average RAS response times and amplitudes of the lower limb muscles may be found in Table 2. There was considerable variation in the EMG onset latency of the responses in the soleus and medial gastrocnemius muscles during RAS responses (Appendix 4). The time of the first peak in the soleus and medial gastrocnemius muscles also varied between participants, but the ranges were similar for each of the four muscles. The responses in the left soleus of all control participants can be seen in Figure 6. The first facilitation/peak was not always the largest response rendered in participants and responses between participants varied. Response peak onset and amplitudes of all muscles are found in Appendix 4.

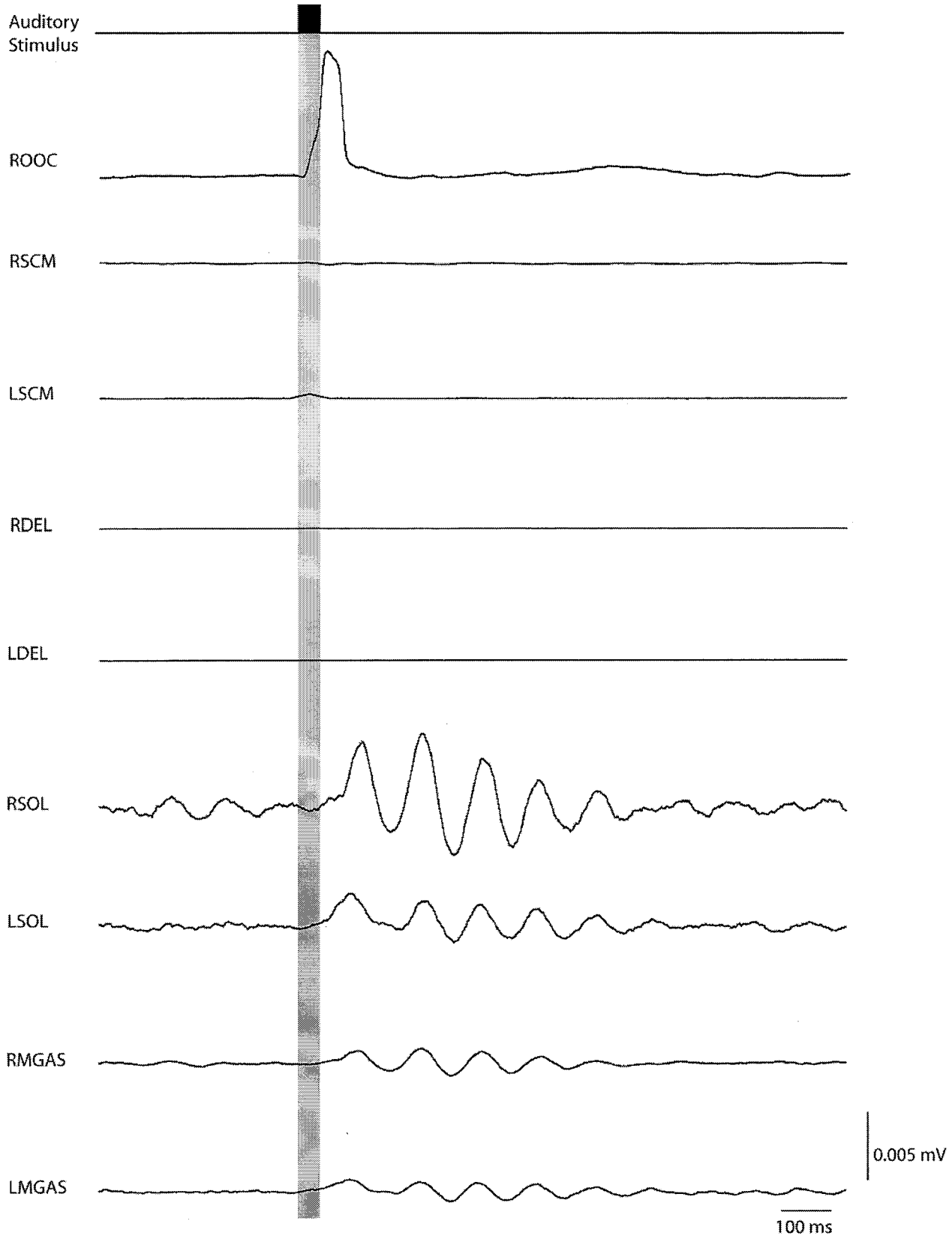


**Figure 4**: Average RAS response of the right OOc, right and left SCM, right and left deltoid, right and left soleus and right and left medial gastrocnemius of one participant.

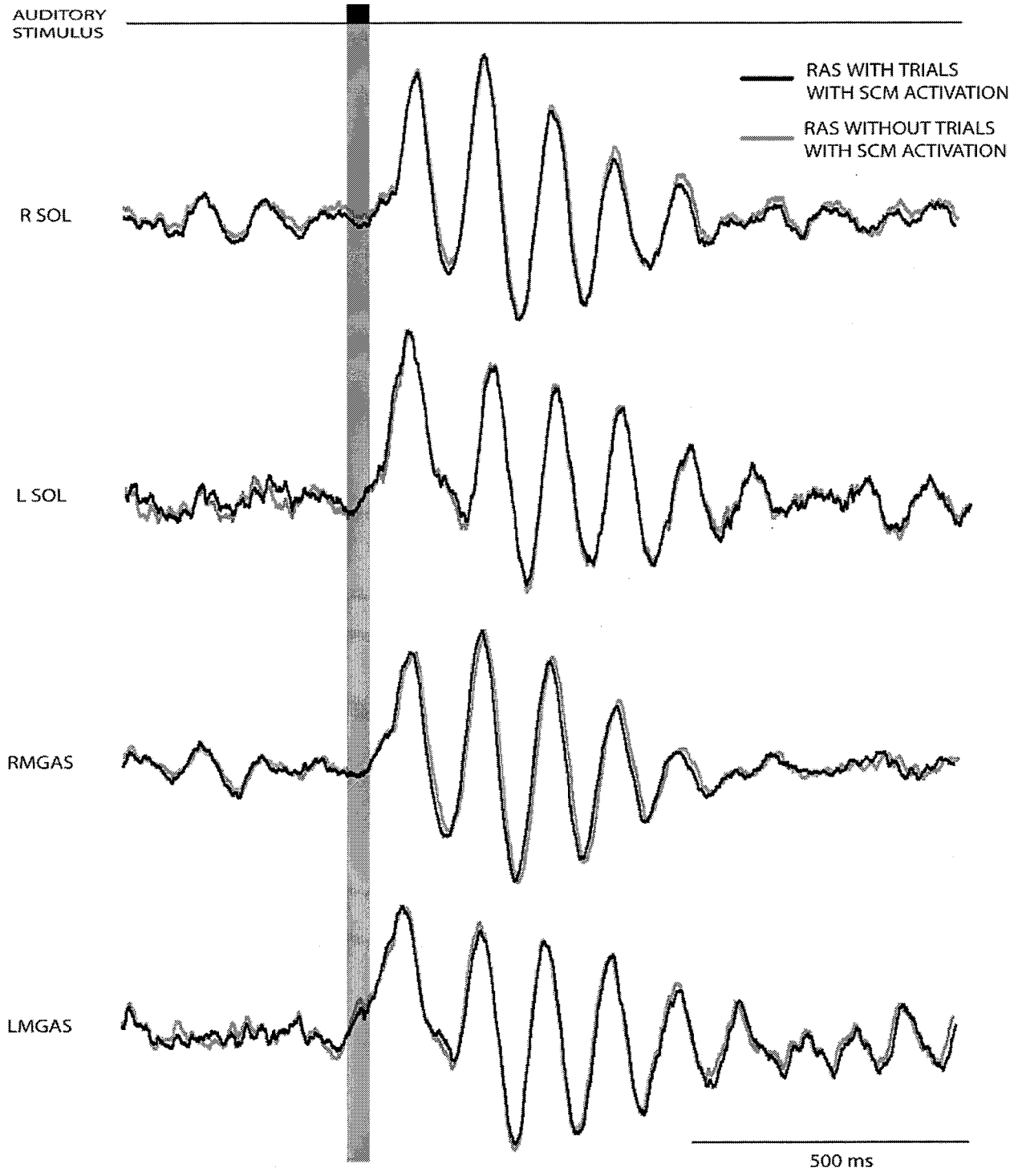


**Figure 5**: A single participants averaged RAS responses in the soleus and medial gastroc muscles. The lighter line is the trace of the average of trials where SAS responses (SCM activation) were not included in the average (21 trials were removed from the 210 total trials) and the darker line represents the averaged RAS response with all exposures included.

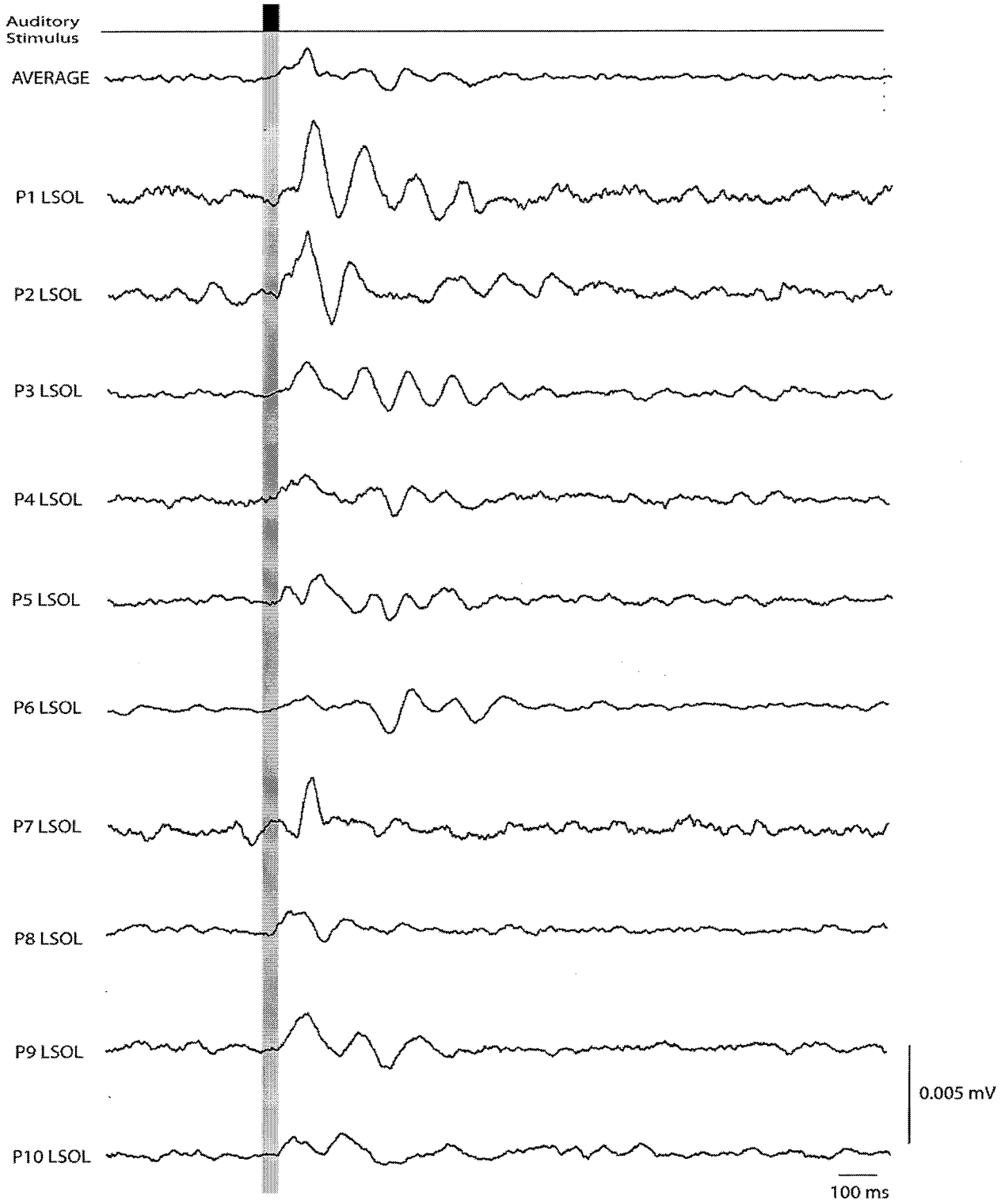


**Figure 6**: Overall group averaged EMG RAS responses of the left soleus muscles and individuals averaged RAS responses. The shaded area represents the time the auditory stimulus was presented.

No statistically significant difference in peak response time between SAS and RAS responses was found for any of the muscles tested. On the other hand, SAS evoked responses were larger than RAS responses for the right soleus [$t(1,7)=2.5$, $p= 0.042$], the left soleus [$t(1,9)=3.9$, $p= 0.003$], the right deltoid [$t(1,6)=2.7$, $p= 0.034$], the left deltoid [$t(1,9)=2.5$, $p= 0.031$], the right OOc [$t(1,9)=4.9$, $p= 0.002$], the right SCM [$t(1,8)=3.3$, $p= 0.012$] and the left SCM [$t(1,7)=3.2$, $p= 0.016$].

## Pre-pulse

Two averaged responses were determined for each pre-pulse protocol participant, a SAS response and a pre-pulse/SAS response. Decreases in the amplitude of the peak responses due to pre-pulses were only consistent in the OOc muscles in the SAS plus pre-pulse trials for all participants (see Table 3). Different participants displayed increases while others decreases in peak amplitude for remaining eight muscles in pre-pulse trials (see Appendix 5).

| ROOC | SAS | | Pre-pulse with SAS | | |
|---|---|---|---|---|---|
| Participant | Peak time (ms) | Peak Amplitude (mV) | Peak time (ms) | Peak Amplitude (mV) | Percent Amplitude of PP (PSAS/SAS*100) |
| 1 | 53.18 | 0.03913 | 49.28 | 0.02025 | 51.8 |
| 2 | 116.1 | 0.17446 | 149.12 | 0.04931 | 28.3 |
| 3 | 79.18 | 0.22775 | 139.76 | 0.012655 | 5.6 |
| 4 | 62.8 | 0.08345 | 54.74 | 0.02293 | 27.5 |
| 5 | 54.22 | 0.10491 | 180.84 | 0.07994 | 76.2 |
| 6 | 59.68 | 0.01522 | 46.42 | 0.01449 | 95.2 |
| 7 | 75.28 | 0.05576 | 74.5 | 0.02465 | 44.2 |
| 8 | 125.2 | 0.24745 | 173.82 | 0.15913 | 64.3 |

**Table 3.** Right OOc peak time and amplitudes of each participants average SAS and average pre-pulse plus SAS exposures and the percent decrease in amplitude when pre-pulse was present

An average of 219 (range of 185 to 251 pulses) RAS stimuli were root mean squared and trigger-averaged to the onset of the acoustic stimulus and an average of 224 (range of 208 to 242) pre-pulse plus RAS stimuli were root mean squared and trigger-averaged to the onset of the 124 dB RAS stimulus for each of the 8 participants. Soleus and medial gastroc muscles consistently yielded responses as were found in the RAS experiment but the influence of pre-pulses had no consistent effect among participants. No common decreases or increases were found amongst all participants for the other muscles in pre-pulse trials (refer to Appendix 6).

The peak amplitudes of the SAS and RAS responses, with and without pre-pulses were submitted to a 2 Tone type (SAS, RAS) x 2 Pre-pulse (present, absent) repeated measures ANOVA. The analysis revealed a significant Pre-pulse x Tone interaction [$F(1,7)=7.5$, $p=0.029$] in the right OOc. Tukey's post hoc analysis shows that SAS response amplitudes were larger than SAS with pre-pulse responses and RAS responses in the right OOC ($p< 0.005$). However, in all other muscles there was no effect of pre-pulse in RAS or SAS responses.

Pre-pulse acoustic stimuli (85 dB) may have caused an early response in the soleus and medial gastrocnemius muscles that preceded the onset of the 124 dB tone (Figure 7). This response was clearly observed in 5 of the 8 participants. The effect of the 85 dB stimulus as a contaminating factor was further investigated by replacing the 124 dB tone with an 85 dB tone in the RAS protocol.

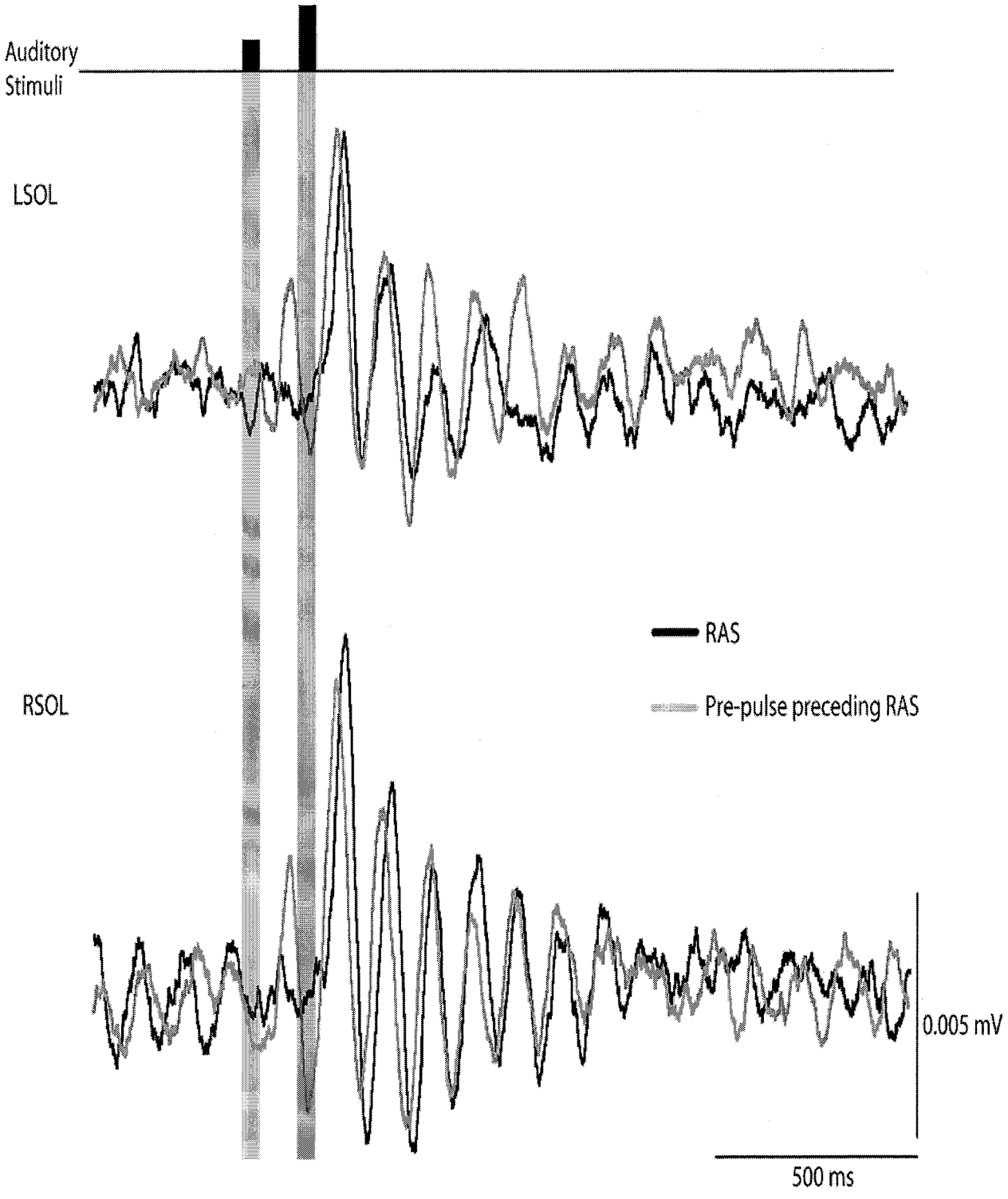


**Figure 7**: Average RAS response of the right and left soleus with and without the presence of a pre-pulse stimulus of a second participant. The shaded areas represent the duration of the auditory stimuli. The lighter line is the averaged pre-pulse with RAS trials. NOTE: A response can be seen in this subject after the 85dB pre-pulse preceding the 124 dB pulse.

An average of 197 (range of 183-203) trials were root mean squared and trigger-averaged to the onset of the acoustic stimulus for each participant and the onset and peak timings were determined (Table 2). The onset, peak time, peak amplitude and overall shape of the 85 dB control response appear to have the same characteristics as the first peak of the RAS/Pre-pulse response (bottom of Figure 8). This first peak occuring before the 124 dB stimulus was presented. In the top of Figure 8, the 85 dB control protocol response appears to have the same onset and peak time as the RAS response but at a smaller amplitude.

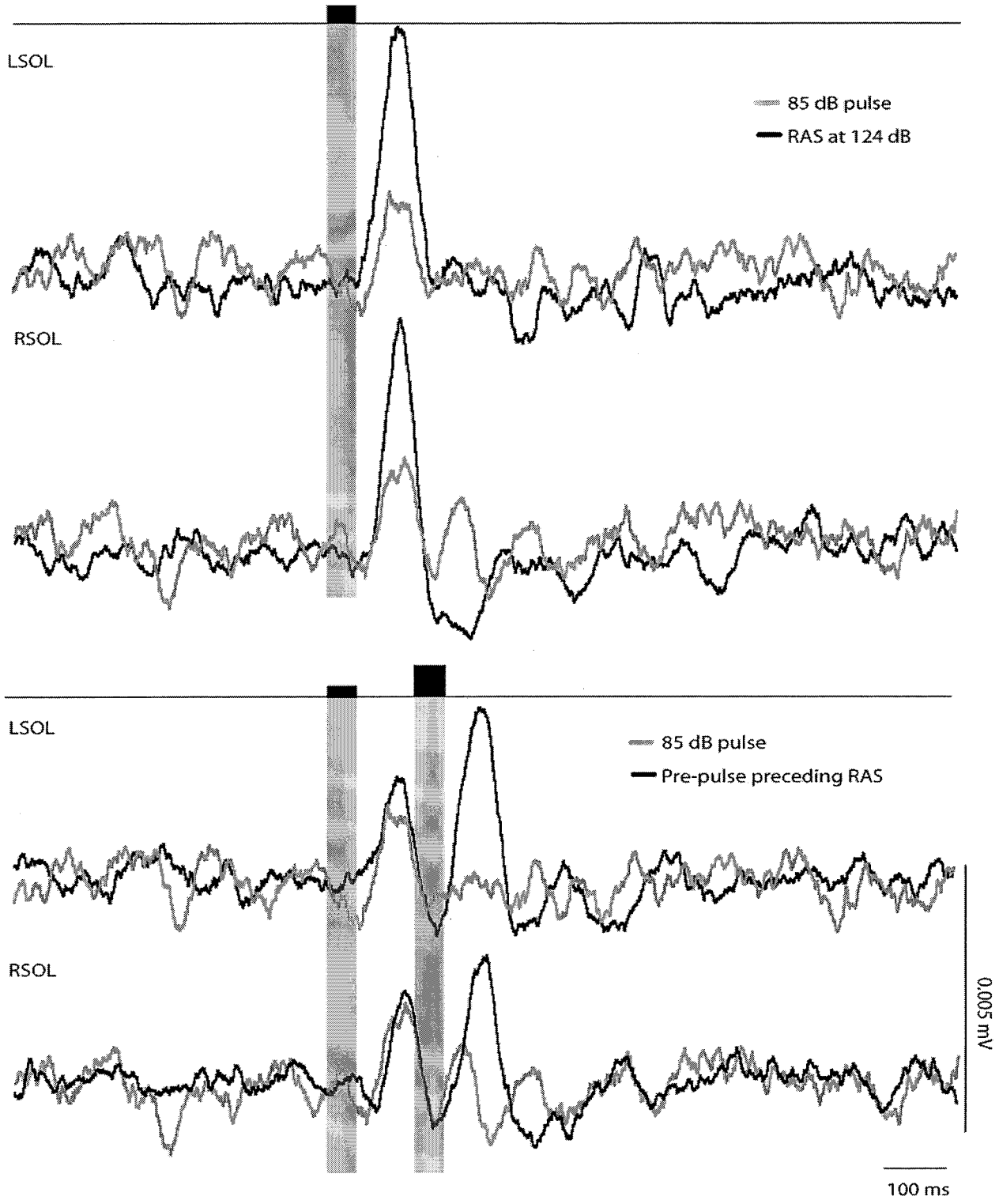


**Figure 8**: Average 85 dB responses in the soleus muscles of one participant compared to the average RAS response (Top) and average RAS plus pre-pulse response (Bottom) in the same participant

# Discussion

Responses to the single acoustic stimuli and repeated acoustic stimuli were measured in all participants. Responses to loud acoustic stimuli do not completely habituate over time as RAS responses were found in all participants after exposure to over 200 stimuli. Responses were also measured in the voluntarily contracting lower limbs in response to RAS at 85 dB. Evidence in these experiments suggests that RAS and SAS responses may be related responses and therefore may travel through the same pathway. Pre-pulse inhibition occurred in the OOc muscles in the SAS protocol but not in any other muscles tested and not in the RAS protocol.

## SAS Responses

The amplitude of the SAS EMG responses in the SCM and all muscles varied within participants over the course of the testing and did not readily habituate (Cadenhead et al., 1999; Geyer & Braff, 1982; Quednow et al., 2006). Indeed, responses sometimes were of larger amplitude later in testing than in the first trial. This variability in amplitude has been found in blink responses in a habituation study by Ornitz and Guthrie (1989), but they referred to these findings as "transient short-term sensitization," and suggested the results occurred by chance. Based on the testing procedure used in this experiment, it is impossible to predict which trials would render larger SAS responses, and even if a response would be produced at all.

The variability of the responses also had an impact on the pre-pulse trials and in turn affected what information and results could be drawn from these trials. It is impossible to know if a trial would render a small SAS response regardless of whether or not a pre-pulse was present and also whether the pre-pulse can inhibit the response.

Average onsets calculated for SAS responses were comparable to previous studies for the OOc and SCM muscles while in a supine body position (Bisdorff et al., 1994; Kofler et al., 2001; Stell et al., 1995). Onsets were faster then previously reported onsets for OOc, SCM and deltoid

muscles in the standing and seated conditions (Brown et al., 1991a; Brown et al., 1991b; Grosse & Brown, 2003). Average soleus onsets were shorter than those reported from supine participants with relaxed soleus muscles (Kofler et al., 2001). Soleus onsets were, however, similar to those recorded in standing participants at 69.9 ± 11.6 ms in the left soleus and 74.3 ± 13.1 ms in the right soleus (Brown et al., 1991a). It should be noted that in this experiment, the criteria for onset was 2 SD above the background levels of EMG RMS. In other experiments, onsets were determined by visual inspection of unrectified EMG (Brown et al., 1991a; Brown et al., 1991b) and this difference in how onsets were determined could cause this discrepancy. The fact that contracting the soleus versus not contracting the soleus while in the supine position affects onsets, poses some question. If reflexive responses are present while seated and contracting the soleus or TA, the latencies are similar to those exhibited while seated and relaxed (Delwaide & Schepens, 1995). It is not clear why there is a difference with respect to onset latencies associated with muscle contraction between the seated and supine body positions.

It is important to note that this study showed variability in response onset times both between and within participants and this is not uncommon when testing participants maintaining the same position (Brown et al., 1991b; Kofler et al., 2001). To gain a better idea of a typical response time a larger number of SAS responses should be recorded from more individuals.

## RAS Responses

RAS responses were consistently present in both the 10 RAS control participants and those 8 participants in the experimental pre-pulse condition that were exposed to RAS. RAS responses can be identified after the data has been averaged, but cannot be seen otherwise due to their small size. The oscillation in the response is not present in the average of SAS trials. SAS trials did have much fewer responses to average and it is not known what an average of 200 SAS responses would look like. The onset and duration of the RAS response oscillation coincides

with a spinal excitability reported following loud acoustic stimuli, measured through H-reflexes (Liegeois-Chauvel et al., 1989; Rossignal & Jones, 1976). This excitability begins 50 ms after acoustic stimulus onset, with a peak amplitude at 100-130 ms after stimulus and excitability lasting a mean duration of 200 ms with a range of 120-460 ms (Liegeois-Chauvel et al., 1989; Rossignal & Jones, 1976).

The RAS response maintains the same shape and amplitude with and without the inclusion of trials that contained a stereotypical SAS response (SCM activation following the stimulus) (see Figure 5). If SAS responses differed from RAS responses, one could expect a change in the outcome of the RAS results with and without the SAS response presence. If the SAS responses were unrelated to the RAS responses then they could have caused a change in amplitude, or a shift in onset, or peak time, impacting the typical RAS responses. The fact that the removal of SAS trials from within RAS trials does not greatly affect the shape of RAS responses during averaging suggests that the two responses may be related.

SAS responses may be seen on individual trials but are more clearly distinguished in the soleus muscles when averaging multiple trials as a consequence of the high variability. RAS responses are only visible after averaging. SAS and RAS responses in the soleus muscles could in fact be the same response but of differing magnitudes. When looking at Figures 3 and 6 the responses look similar and peak response times are not statistically different.

Overall peak response amplitudes were larger in muscles in the SAS condition compared to the RAS condition. RAS were loud and of similar nature to SAS but were repeated for a longer period of time. The larger response in the SAS condition may be due to the more surprising nature of the stimulus as it was unexpectedly presented and may have caused an inherent protective response. Smaller responses occurred in the RAS procedure as one became more familiar with the tones and how frequently they were presented. Some sort of inhibitory effect, or filtering of the acoustic stimuli could be occuring within the system that does not

completely attenuate the responses. Theories exist to explain the decline in SAS responses. There may be a reduction in synaptic transmission of excitatory signals as there is decrease of the neurotransmitter available (Rimpel et al., 1982), and this may be due to a change in receptor sensitivity which leads to a decrease in excitatory signaling in the PnC (Weber et al., 2002). It is possible that when the tones are more unexpected a SAS response occurs and as tones become more expected and large responses habituate, and smaller RAS responses occur. The habituation of the SAS response is well documented (Brown et al., 1991b; Cadenhead et al., 1999; Geyer & Braff, 1982; Quednow et al., 2006) but the idea that responses to acoustic stimuli have completely habituated once SCM activation ceases, is incorrect. The RAS responses clearly demonstrate that a response to the stimulus is still propagating through the spinal cord to muscles after repeated exposures. The response is of smaller magnitude, but the body is still eliciting a reaction.

It is difficult to compare responses between all the different muscles measured as it is not known what factors impact RAS responses. Voluntary muscle contraction may have had an impact as lower body muscles in the legs were contracting during trials and it is in these muscles that responses are most consistent. In previous experiments in our lab some voluntary muscle contraction work has been conducted during RAS. It is possible to elicit RAS responses in the non-contracting TA muscles while soleus muscles are contracting in some individuals, but when muscles in the legs are not contracting, no RAS responses are measured in either the soleus, or the TA. Voluntary muscle contraction also affects onset latencies, as SAS responses have different onset latencies in the soleus in the supine position when relaxed (Kofler et al., 2001) compared to when contracting, as found by this study. The SCM and deltoid muscles were not voluntarily contracting in this experiment and elicited RAS responses in only some individuals. It is not known what factors effects which muscles are responsive to RAS. It is important to note that postural engagement is not necessary for RAS responses to be evoked as participants in a

supine position during the entire experiment elicited such responses. It is unknown if responses vary at different spinal levels (e.g. upper versus lower body). The voluntary contraction of the lower limbs during the trials is driven by activation descending through the corticospinal tract. The reflex to the loud sound is thought to propagate through the reticulospinal pathway to render responses in the contracting muscles. RAS needs to be further investigated to better understand the various muscle responses of the body and what factors may alter responses.

## Pre-Pulses in SAS Trials

The right OOc muscles in all participants showed inhibition with pre-pulse exposures in the SAS condition. No other muscles had consistent inhibition across all participants. Pre-pulse inhibition is typically measured and found in the eye muscles (Blumenthal, 1996; Cadenhead et al., 1999; Csomor et al., 2005; Csomor et al., 2006; Quednow et al., 2006; Schwarzkopf et al., 1993) but is not often tested in other muscles of the body. This can be attributed to the rapid onset of habituation of SAS responses in muscles other than eye muscles, making it difficult for testing inhibition (Meincke et al., 2005). Pre-pulse inhibition has however been found in the SCM (Valls-Solé et al., 1999a; Valls-Solé et al., 2005) but under differing testing procedures from our experiment. In both of these experiments, participants were tested in a seated position, which requires more SCM activation than while in a supine position. The stimuli also varied, with a 130 dB SAS and with 70 dB pre-pulses (Valls-Solé et al., 1999a) and an electrical, tactile pre-pulse stimuli (Valls-Solé et al., 2005). A reaction time task was also a component of one experimental procedure, paired with the pre-pulses and loud stimuli (Valls-Solé et al., 2005). Loud acoustic stimuli are known to affect reaction time tasks, but the causal mechanisms relating the two are not known (Carlsen et al., 2003; Valls-Solé et al., 1999). In our experiment, some participants had larger responses in muscles with pre-pulse exposures while others had smaller responses. The lack of consistency in inhibition within participants between muscles most likely

has to do with the variability of response size to SAS. It is not known which trials would have rendered a large or a small SAS response without the presence of a pre-pulse, let alone with the presence of a pre-pulse. Therefore, a conclusion cannot be made with respect to whether any inhibition is due to a pre-pulse exposure or whether the smaller response size would have occurred regardless. It is interesting to note that the right OOc had consistent inhibition while the other muscles did not.

## Pre-pulses in the RAS Trials

In our experiment pre-pulses inhibition was not found in RAS trials. This finding may have been confounded by the response to the 85 dB pre-pulse. The 85 dB tone has timings that are very similar to the louder 124 dB sounds as may be seen in Table 2. The amplitudes of the 85 dB responses were smaller than those found with 124 dB tones but do have similar onsets and peaks to both the SAS and RAS responses. As participants responded to the 85 dB tone and the tone itself causes RAS responses, it makes it difficult to asses fully whether pre-pulse inhibition of the main peak occurred in response to the 124 dB pulse. Based on this finding of a response to the 85 dB tone, it is possible that this confounded the SAS responses in the same way. To investigate if pre-pulses can have an inhibitory effect on RAS response amplitudes, different decibel levels would have to be investigated. Research could be done to reveal what intensity tone does not render a muscular response after repeated exposures. The pre-pulse tone would have to be at this intensity, or lower to avoid possible confounding of the SAS and RAS responses. It may not be possible however to find an intensity that does not cause a RAS response. Sounds at low intensities, like the 85 dB tone, could still excite the PnC to a certain extent and result in a small descending response.

## Pathway Associated with SAS and RAS (both to 124 dB and 85dB)

A muscular response pathway to sound seems sensitive to loud (124dB) and much quieter noises (85dB). Responses vary in amplitude depending on whether there is one exposure to the sounds or repeated exposures. Even after habituation becomes apparent in the muscles, after the data is averaged, responses may still be present in the contracting muscles of the lower limbs.

It has been proposed that the SAS response travels through the reticulospinal tract to elicit a response in the body (Brown et al., 1991a; Davis, 1982; Grosse & Brown, 2003; Yeomans & Frankland, 1996). Evidence in our experiments supports the theory that SAS and RAS responses are related, or are the same response and therefore probably propagate through the brainstem and down the spinal cord through the reticulospinal tract. If it is true that SAS and RAS responses both travel through the reticulospinal tract, then both may be used as methods to test reticulospinal tract intactness. If a spinal cord injury patient only has a partial spinal cord lesion and they maintain the ability to engage muscles below the lesion, this may be a way to test if the reticulospinal tract is intact by testing for RAS or SAS responses in those voluntarily contracting muscles.

## Limitations

In this experiment responses were measured on both sides of the body and SAS responses varied within a single trial on each side. A single trial may have activated one side of the body to a greater extent than the other side. A future study would have to be designed to investigate whether responses vary between sides of the body and look at what may cause this variability in responses.

This experiment was not designed to compare onset latencies of contracting versus non-contracting muscles while in the supine position. To test if contraction versus non-contraction of

the lower limb muscles while in the supine position affects onsets statistically, both conditions would have to be conducted in one experiment for comparison.

## Conclusions

The results of the present study have shown that similar responses may be rendered from single acoustic stimuli and repeated acoustic stimuli in voluntarily contracting lower limb muscles. SAS response amplitudes are variable within single muscles across trials. Apparent habituation to sounds in some muscles after repeated exposure does not mean that all muscles have habituated and stopped responding. RAS exposures render an averaged response in all participants tested. This response appears to be similar to SAS responses but of smaller magnitude and only visible after the averaging of multiple trials. SAS and RAS responses share multiple commonalities suggesting that they are related responses. Responses to stimuli as quiet as 85 dB also occur in voluntarily contracting muscles when repeatedly exposed that have similar timing to those much louder sounds at 124 dB. Since SAS responses are thought to propagate through the reticulospinal pathway, one may infer that RAS responses also do. Based on the results found in this present study, future testing of repeated acoustic stimuli should be carried out to better understand the relationship between RAS and SAS response, as one day this may be a means of assessing the condition of descending pathways.

# References


Bisdorff, A.R., Bronstein, A.M., & Gresty, M.A. (1994) Responses in neck and facial muscles to sudden free fall and a startling auditory stimulus. *Electroencephalography and Clinical Neurophysiology,* 93, 409-416.

Bitsios, P., & Giakoumaki, S.G. (2005). Relationship of pre-pulse inhibition of the startle reflex to attentional and executive mechanisms in man. *International Journal of Psychophysiology,* 55, 229-241.

Blumenfeld, H. (2002). *Neuroanatomy through clinical cases.* Sunderland, Massachusetts: Sinauer Associates, Inc.

Blumenthal, T.C., & Goode, C.T. (1991). The startle eyeblink response to low intensity acoustic stimuli. *Psychophysiology,* 28, 3, 296-306.

Blumenthal, T.D. (1996). Inhibition of the human startle response is affected by both pre-pulse intensity and eliciting stimulus intensity. *Biological Psychology,* 44, 85-104.

Blumental, T.D., Noto, J.V., Fox, M.A., & Franklin, J.C. (2006). Background noise decreases both pre-pulse elicitation and inhibition of acoustic startle blink responding. *Biological Psychology,* 72, 2, 173-179.

Brillinger, D.R.(1974). Cross-spectral analysis of processes with stationary increments including the stationary. *Annals of Probability,* 2, 815–827.

Brown, P., Day, B.L., Rothwell, J.C., Thompson, P.D., & Marsden, C.D. (1991a). The effect of posture on the normal and pathological auditory startle reflex. *Journal of Neurology, Neurosurgery, and Psychiatry,* 54, 892-897.

Brown, P., Rothwell, B.L., Thompson, P.D., Britton, T.C., Day, B.L., & Marsden, C.D. (1991b). New observations on the normal auditory startle reflex in man. *Brain,* 114, 4, 1891-1902.

Cadenhead., K.S., Carasso, B.S., Swerdlow, N.R., Geyer, M.A., & Braff, D.L. (1999) Prepulse inhibition and habituation of startle responses are stable neurobiological measures in a normal male population. *Biological Psychiatry,* 45, 360-364.

Carlsen, A.N., Hunt, M.A., Inglis, J.T., Sanderson, D.J., & Chua, R. (2003). Altered triggering of a prepared movement by a startling stimulus. *Journal of Neurophysiology,* 89, 1857-1863.

Carlsen, A.N., Chua, R., Inglis, J.T., Sanderson, D.J., & Franks, I.M. (2004). Prepared movements are elicited early by startle. *Journal of Motor Behaviour,* 36,3, 253-264.

Csomor, P.A., Vollenwieder, F.X., Feldon, J., & Yee, B.K. (2005). Research report: On the feasibility to detect and to quantify pre-pulse-elicited reaction in pre-pulse inhibition of the acoustic startle reflex in humans. *Behavioural Brain Research,* 162, 256-263.

Csomor, P.A., Yee, B.K., Quednow, B.B., Stadler, R.R., Feldon, J., & Vollenweider, F.X. (2006). The monotonic dependency of pre-pulse inhibition of the acoustic startle reflex on the intensity of the startle-eliciting stimulus. *Behavioural Brain Research,* 174, 143-150.

Davis, M. (1982). The mammalian startle response. In R.C. Eaton (Ed.), *Neural mechanisms of startle behavior* (pp. 287-351). New York: Plenum Press.

Delwaide, P.J., & Schepens, B. (1995). Auditory startle (audiospinal) reaction in normal man: EMG responses and H reflex changes in antagonistic lower limb muscles. *Electroencephalography and clinical Neurophysiology.* 97, 416-423.

Filion, D.L., Dawson, M.E., & Schell, A.M. (1998). Psychological significance of human startle eyeblink modifications: a review. *Biological Psychology,* 47, 1-43.

Fitzpatrick, R.C., & Day, B.L. (2004). Probing the human vestibular system with galvanic stimulation. *Journal of Applied Physiology*, 96, 2301-2316.

Germann, W.J., & Stanfield, C.L. (2002). *Principles of Human Physiology.* San Francisco: Pearson Education, Inc.

Geyer, M.A., & Braff, D.L. (1982) Habituation of the blink reflex in normals and schizophrenic patients. *Psychophysiology*, 19,1, 1-6.

Geyer, M.A., Swerdlow, N.R., Mansbach, R.S., & Braff, D.L. (1990). Startle response models of sensorimotor gating and habituation deficits in schizophrenia. *Brain Research Bulletin,* 25, 485–498.

Graham, F.K.(1975). Presidential Address, 1974. The more or less startling effects of weak pre-stimulation. *Psychophysiology*, 12, 238–248.

Grosse, P., & Brown, P. (2003). Acoustic startle evokes bilaterally synchronous oscillatory EMG activity in healthy humans. *Journal of Neurophysiology,* 90, 1654-1661.

Hoffman, H.S. (1984). Methodological factors in the behavioral analysis of startle: the use of reflex modification procedures and the assessment of threshold. In R.C. Eaton (Ed.), *Neural mechanisms of startle behavior* (pp. 287-351). New York: Plenum Press.

Hoffman, H.S., & Ison, J.R. (1992). Reflex modification and the analysis of sensory processing in developmental and comparative research. In B.A. Campbell, H. Hayne, & R. Richarson (Eds.), *Attention and information processing in infants and adults* (pp. 83-111). Hillsdale, NJ: Erbaum.

Kandel, E.R., Schwartz, J.H., & Jessell, T.M. (1991) *Principles of Neural Science: Third Edition.* Connecticut: Appleton & Lange.

Kilner, J.M., Baker, S.N., Salenius, S., Jousmaki, V., Hari, R., & Lemon, R.N. (1999). Task-dependent modulation of 15-30Hz coherence between rectified EMGs from human hand and forearm muscles. *Journal of Physiology*, 516, 2, 559-570.

Kofler, M., Müller, J., Reggiani, L., & Valls-Solé, J. (2001). Influence of age on auditory startle responses in humans. *Neuroscience Letters,* 307, 65-68.

Landis, C., & Hunt, W.A. (1939). *The startle pattern.* New York: Farrar and Rinehart.

Li, L., Steidl, S., & Yeomans, J.S. (2001). Contributions of the vestibular nucleus and vestibulospinal tract to the startle reflex. *Neuroscience,* 106,4, 811-821.

Liegeois-Chauvel, C., Morin, C., Musolino, A., Bancaud, J., & Chauvel, P. (1989). Evidence for a contribution of the auditory cortex to audiospinal facilitation in man. *Brain,* 112, 375-391.

Ludewig, K., Geyer, M.A., Etzensberger, M., & Vollenweider, F.X. (2002). Stability of the acoustic startle reflex, pre-pulse inhibition, and habituation in schizophrenia. *Schizophrenia Research,* 55, 129-137.

McGraw, K.O., & Wong, S.P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychological Methods,* 1,1, 30-46.

Meincke, U., Light, G.A., Geyer, M.A., & Braff, D.L. (2005). On the waveform of acoustic startle blink in the paradigm of prepulse inhibition- methodological and physiological aspects. *Neuropsychobiology,* 52, 24-27.

Nichol, D.D., Dakin, C.J., Elliott, B., Lee Son, G., Blouin, J.S., & Inglis, J.T. (2007). Auditory stimuli elicit lower limb electromyographic responses in habituated humans. In preparation.

Nieuwenhuijzen, P.H.J.A., Schillings, A.M., Van Galen, G.P., & Duysens, J. (200). Modulation of the startle response during human gait. *Journal of Neurophysiology,* 84, 65-74.

Nolte, J. (2002). *The Human Brain an introduction to its functional anatomy, fifth edition.* St. Louis Missouri: Mosby Inc.

Ornitz, E.M., & Guthrie, D. (1989). Long term habituation and sensitization of the acoustic startle response in the normal adult human. *Psychophysiology,* 26,2, 166-173.

Pissiota, A., Frans, Ö., Fredrikson, M., Långström, B., & Flaten, M.A. (2002). The human startle reflex and pons activation : a regional cerebral blood flow study. *European Journal of Neuroscience,* 15, 395-398.

Quednow, B.B., Kühn, K., Beckmann, K., Westheide, J., Maier, W., & Wagner, M. (2006). Attenuation of the pre-pulse inhibition of the acoustic startle response within and between sessions. *Biological Psychology,* 71, 256-263.

Riddle, C.N., Edgley, S.A., & Baker, S.N. (2007). The macaque reticulospinal tract forms monosynaptic connections with motor neurons in the cervical spinal cord controlling distal arm and hand muscles [Abstract]. *Abstract obtained* from IBRO World Congress of Neuroscience, P27.

Rimple, J., Geyer, D., & Hopf, H.C. (1982). Changes in the blink responses to combined trigeminal, acoustic, and visual repetitive stimulation, studied in the human subject. *Electroencephalography and clinical neurophysiology*, 54, 552-560.

Rosenberg, J.R., Amjad, A.M., Breeze, P., Brillinger, D.R., & Halliday, D.M.(1989). The fourier approach to the identification of functional coupling between neuronal spike trains. *Progress in Biophysics and Molecular Biology,* 53, 1–31.

Rossignol, S. (1975). Startle responses recorded in the leg of man. *Electroencephalography and Clinical Neurophysiology,* 39, 389-397.

Rossignol, S., & Melvill Jones, G. (1976). Audiospinal influence in man studied by the H-reflex and its possible role on rhythmic movements and synchronized to sound. *Electroencephalography and Clinical Neurophysiology,* 41, 83-92.

Rudell, A.P., & Eberle, L.P. (1985). Acoustic facilitation of the Hoffmann reflex. *Experimental Neurology,* 89, 592-602.

Russolo, M. (2002). Sound-evoked postural responses in normal subjects. *Acta Otolaryngol,* 122, 21-27.

Schwarzkopf, S.B., McCoy, L., Smith, D.A., & Boutros, N.N. (1993). Test-retest reliability of pre-pulse inhibition of the acoustic startle response. *Biological Psychiatry,* 34, 896-900.

Stell, T., Thickbroom, G.W., & Mastaglia, F.L. (1995). The audiogenic startle response in Tourette's syndrome. *Movement Disorders,*10, 723-730.

Tortora, G.J., & Derrickson, B. (2006). *Principles of Anatomy and Physiology.* Hoboken, NJ: John Wiley & Sons, Inc.

Valls-solé, J., Valldeoriola, F., Molinuevo, J.L., Cossu, G., & Nobbe, F. (1999a). Pre-pulse modulation of the startle reaction and the blink reflex in normal human subjects. *Experimental Brain Research*, 129, 49-56.

Valls-solé, J., Rothwell, J.C., Goulart, F., Cossu, G., & Muñoz, E. (1999b). Patterned ballistic movements triggered by a startle in healthy humans. *Journal of Physiology*, 516,3, 931-938.

Valls-solé, J., Kofler, M., & Kumru, H. (2005). Startle-induced reaction time shortening is not modified by pre-pulse inhibition. *Experimental Brain Research,* 165, 541-548.

Vidailhet, M., Rothwell, J.C., Thompson, P.D., Lees, A.J., & Marsden, C.D. (1992). The auditory startle response in the Steele-Richardson-Olszekski Syndrome and Parkinson's Disease. *Brain*, 115,4, 1181-1192.

Weber, M., Schnitzler, H.U., & Schmid, S. (2002). Synaptic plasticity in the acoustic startle pathway: the neuronal basis for short-term habituation? *European Journal of Neuroscience*, 16, 1325-1332.

Yeomans, J.S., Li, L., Scott, B.W., & Frankland, P.W. (2002). Review: Tactile, acoustic and vestibular systems sum to elicit the startle reflex. *Neuroscience and Biobehavioral Reviews,* 26, 1-11.

Yeomans, J.S., & Frankland, P.W. (1996). Review: The acoustic startle reflex: neurons and connections. *Brain Research Reviews,* 21, 301-314.

# Appendix 1: Literature Review

# Literature Review:

## The Anatomy and Neurophysiology of the Auditory System

The auditory system is one of the primary sensory systems in the human body. In circumstances where an individual is exposed to unexpected auditory stimulus, one may be startled and elicit varying responses. Understanding the auditory system is important in the understanding of the startle reflex. For the purposes of this review a startle stimulus will be referred to as single acoustic stimulus (SAS).

The external ear, or the auricle, is designed to direct sound waves into the external auditory canal. The tympanic membrane seperates the external auditory canal and the middle ear. Alternation between high and low-pressure sound waves causes the tympanic membrane to vibrate and the distance it moves is dependent on the frequency and intensity of the waves. The tympanic membrane and the round window of the inner ear are connected by small bones called the auditory ossicles. These bones are attached to the middle ear by ligaments and connected to each other by synovial joints. The malleus is the bone connected to the tympanic membrane and it transmits the vibration from the membrane to the incus, which then transmits the vibration to the stapes which is attached to the round window. The movement of the stapes causes the oval window to move in and out which give rise to fluid pressure waves within the perilymph fluid of the cochlea within the inner ear. The cochlea is a component of the bony labyrinth which is a series of cavities in the temporal bone. The cochlea is composed of three channels: the cochlear duct, the scala vestibuli and the scala tympani. The cochlear duct is seperated from the scala vestibuli by the vestibular membrane and the scala tympani by the basilar membrane. Perilymph is within the scala tympani and the scala vestibuli and endolymph is within the cochlear duct. The pressure waves move the walls of the scala vestibuli, the scala tympani and move the vestibular membrane. The movement of the vestibular membrane creates pressure waves in the

endolymph and these waves cause the basilar membrane to vibrate. The organ of Corti rests on the basilar membrane and contains hair cells, which are the receptors associated with hearing. There are inner hair cells in a single row and outer haircells composed of three rows. Each hair cell has a hair bundle of 30-100 stereocilia at the end which extend into the endolymph (Tortora & Derrickson, 2006).

The hair bundles are embedded in the tectorial membrane, a flexible gelatinous membrane that shifts with the movement of the endolymph. Stereocilia are positioned by height within a bundle, from tallest to shortest. The movement of the tectorial membrane causes the bending of the hair cell bundles. As a pressure wave travels in the endolymph the membranes oscillate causing hair cells to bend at different times yielding differing responses. When the stereocilia bend in the direction of the taller stereocilia depolarization occurs. With depolarization action potentials are sent down the hair cell to synapse with first-order sensory neurons and motor neurons from the cochlear branch of the vestibulocholear (VIII) nerve. In the case where the hairs bend away from the taller stereocilia, repolarization takes place. The inner hair cells are less abundant than the outer hair cells, but synapse with 90-95% of the first-order sensory neurons in the cochlear nerve for the relaying of auditory information to the brain. The outer hair cells mostly synapse with the motor neurons of the cochlear nerve (Tortora & Derrickson, 2006).

The first-order sensory neurons from the vestibulocochlear nerve terminate at the medulla oblongata, the inferior part of the brainstem, at the cochlear nuclei of the same side the sound was detected. Second order neurons then send auditory signals to the superior olivary nuclei on both sides of the brain. Axons from both the olivary nuclei and the cochlear nuclei relay information to the inferior colliculus of the midbrain, and from there to the medial geniculate nucleus of the thalamus. The thalamus then projects the auditory signal to the primary

auditory area located in the superior temporal gyrus of the cerebral cortex for the perception of sound (Tortora & Derrickson, 2006).

The sounds we hear are our perception of sound waves and the loudness of sound depends on the intensity of the sound wave. Sound intensity varies by the amplitude of the vibration of the wave and is measured in decibels (dB). A decibel is a logarithmic unit of measurement where an increase of one decibel represents an increase of sound intensity by tenfold. It is used to quantify sound levels and uses the reference of 0 dB as the threshold of normal hearing; where one may perceive a sound from silence. Normal conversation has been measured to be around 60 decibels (dB) while a jackhammer is approximately 90-110 dB. The level at which sounds become uncomfortable to the normal ear is roughly 120 dB. Beyond 140 dB, sound becomes painful and damaging to the ear. It is important to note that because dB are not a linear scale but a logarithmic scale, a one decibel increase represents a tenfold increase in sound. The change from 110 to 120 dB is therefore a much greater increase then the change from 80 to 90 dB (Tortora & Derrickson, 2006).

## Single Acoustic Stimulus Reflex

The Single Acoustic Stimulus reflex may be instigated by loud unexpected noises, visual stimuli and/or tactile stimuli. The SAS reflex is thought to be a mechanism common amongst most mammals that has evolved as a protective behaviour (e.g. in response to an unexpected enemy attack) (Quednow et al., 2006; Yeomans, Li, Scott & Frankland, 2002). Muscle flexion following a startling stimulus is typically seen bilaterally and is aimed to protect sensitive areas of the body for a short period of time while the startling stimuli can be assessed in order to select either a flight or fight response. Despite the loss of the motor coordination, cognitive attention, and visual input during SAS responses, the human body is still able to protect itself from physical harm (Yeomans et al., 2002).

In circumstances where the head, neck or upper body is unexpectedly hit, there are three systems that may respond independently or collectively to yield the SAS response: the somatosensory, the vestibular and/or the auditory system (Yeomans et al., 2002). The actual physical force acting upon the head will directly affect the somatosensory system (through the trigeminal system in the head). Skin and muscle can be physically displaced causing cutaneous receptors in the skin and receptors within muscles, ligaments and joints to activate in turn signaling changes to the body. Linear and angular accelerations of the head are then detected by the semicircular ducts and otolith organs of the vestibular system within the inner ear. The semicircular ducts detect angular acceleration of the head in space through hair cell receptors. Movement of the head causes fluid within circular ducts, known as endolymph, to flow in one direction and bend the hair cells, which then fire as they are directionally sensitive to acceleration. The otolith organs, the utricle and the saccule detect linear acceleration of the head through the firing of hair cell receptors within an otolithic membrane embedded with otoconia (small crystals). The movement of the head shifts the otolithic membrane and bends the hair cells, which then fire in response to the direction of acceleration associated with the path of movement (Fitzpatrick & Day, 2004). Sound waves are detected by the auditory system and relayed to the brain (Tortora & Derrickson, 2006). All three systems use rapidly conducting mechanoreceptors that elicit fast responses and relay information to the pertinent brain centers for assessment, interpretation and responses (Yeomans et al., 2002).

## Reticulospinal Tract

An unexpected blow to the head results in a combination of muscle contractions in the body that are controlled via descending input from the reticulospinal, or vestibulospinal tracts in the spinal cord (Blumenfeld, 2002; Davis, 1982). Larger amplitude responses to a SAS are elicited when a combination of signals are relayed from the acoustic, trigeminal and vestibular

systems rather than from a single system (Li, Steidl & Yeomans, 2001). A convergence of information has been proposed where the reticulospinal tract receives information from the auditory, trigeminal and vestibular systems and the vestibulospinal tract receives information from the vestibular system (Yeomans et al., 2002).

The reticulospinal tract is thought to be the main pathway through which the SAS reflex travels to the bodies musculature (Delwaide & Schepens, 1995; Li et al., 2001). The reticulospinal tract originates in the reticular formation of the brainstem. The reticular formation is an area in the central core of the brainstem with a great deal of connectivity and it is a site for the convergence and divergence of information. A single cell within this area may respond to many different stimuli and modalities and relay the information it receives to other areas within the brainstem. Specifically the reticulospinal tract is known to control movement as it connects to both the spinal cord and the cerebellum. The reticular formation itself contains neural circuitry to initiate simple and complex reflexes and complex patterns of movement (Nolte, 2002).

The reticulospinal tract descends from the medial area of the pontine reticular formation and the rostral medullary reticular formation. The reticulospinal neurons carry projections from the reticular formation that influence and control spinal motor neurons and the sensitivity of spinal reflexes. The tracts relay information from the basal ganglia, vestibular nuclei, areas of the cerebral cortex such as somatosensory and motor cortex, as well as motor commands generated from within the reticular formation itself (Nolte, 2002). It is known to send motor inputs for gait-related movements, for the maintenance of posture, and for control of fine musculature in the distal arm and hands (Blumenfeld, 2002; Davis, 1982; Riddle, Edgley & Baker, 2007).

The vestibulospinal tract sends motor inputs for head and neck positioning as well as for the maintenance of whole body posture and balance based on input it receives from the vestibular system (Blumenfeld, 2002; Fitzpatrick & Day, 2004). Second order vestibular neurons from the vestibular nucleus are thought to project to the reticular formation, specifically to the

nucleus reticularis pontis caudalis which is within the caudal pontine reticular formation (PnC) (Li et al., 2001). As the vestibular system projects to both the vestibulospinal and the reticulospinal tract, the vestibulospinal tract may in theory also influence SAS responses. The trigeminal and auditory systems have multiple synapses and also project to the PnC that then project information to the reticulospinal tract. The SAS reflex is thought to travel from the somatosensory, the vestibular and the auditory systems, through the reticular formation and then descend through the reticulospinal and/or vestibulospinal tract.

## Single Acoustic Stimulus Pathway

To elicit a response, a SAS is an unanticipated, loud and sharp stimulus (Delwaide & Schapens, 1995; Quednow et al., 2006). Much research has been done to investigate in detail the pathway through which the SAS response travels to produce the reflexive response. Animal studies have identified a SAS response pathway and it is thought that this pathway may be similar in humans. Davis (1982) has established a pathway in rats using bilateral lesioning at specific neural structures. The sound is detected in the inner ear by the hair cells of the spiral ganglion in the cochlea and synapse on to the cochlear root neurons in the cochlear nerve. The cochlear root neurons then project directly on to the posteroventral cochlear nucleus (VCN), which in turn synapses at the dorsal and ventral nuclei of the lateral lemniscus as well as the ventrolateral tegmental nucleus (VLTg). The VLTg is thought to be an area where auditory, tactile, and vestibular information are integrated. From these areas there is another synapse in the ventromedial region of the nucleus reticularis pontis caudalis (PnC or RPC). Once signals reach the PnC, axons run from their cell bodies down to the spinal cord through the reticulospinal tract. The reticulospinal tract travels down the anteromedial column of the spinal cord, specifically the medial longitudinal fasciculus on the midline, where it bifurcates and forms the ventral funiculi. Nerve fibers that travel down the reticulospinal tract then synapse in the

spinal cord with lower motor neurons directly and possibly indirectly through interneurons before reaching the neuromuscular junction to send impulses to the responding muscles (Davis, 1982; Yeomans & Frankland, 1996).

## Evidence Supporting the SAS Pathway

The SAS response is reduced or eliminated in animals with injuries to the midbrain reticular formation (Davis, 1982). This is also seen in humans with damage to their reticular formation. Steele-Richardson-Olszewski syndrome causes widespread pathological changes in the human brain stem and includes degeneration of the pontine reticular formation. People with this syndrome show a reduced SAS reflex response (Vidailhet et al., 1992). These findings help support the idea that the reticular formation acts as a SAS response relay centre, indicating the possible importance of this structure in the SAS reflex.

As described above, the PnC is located within the reticular formation and is proposed to be a main component of the SAS reflex circuit through rat models. Evidence of the SAS reflex propagating through the PnC in people has been supported by a positron emission tomography (PET) study. In a study presented by Piassiota, Frans, Fredrikson, Långström & Flaten (2002), participants were subjected to startling acoustic stimuli and had higher cerebral blood flow in an area of the pons which anatomically corresponds to the location of the PnC. The increase in activity in this area after SAS responses is thought to be evidence of the PnC having an active role in SAS response in humans.

Evidence suggests that the SAS response propagates from the caudal brainstem to the musculature in humans as it does in animals (Brown, Day, Rothwell, Thompson & Marsden, 1991a). The order of muscle recruitment may indicate circuitry through the caudal brainstem. SAS elicit SCM muscle responses more rapidly than masseter or mentalis muscle responses. The SCM is a neck muscle innervated by the eleventh cranial nerve, the masseter is innervated by the

fifth, and the mentalis by the seventh and both latter muscles are located in the face. This activation pattern would indicate that the reflex is first sent from around the eleventh cranial nerve area in the caudal brainstem, rostrally (upwards) to the superior cranial nerves involved with motor activation, as well as down the brainstem, caudally to lower levels in the brainstem and spinal cord (Brown et al., 1991a). The SAS reflex appears to follow a different route than responses elicited in muscles by transcranial magnetic stimulation of the motor cortex. Magnetic stimulation of motor neurons in the cortex elicits responses of muscles sent through the corticospinal pathway. The conduction velocities of the SAS responses in the limbs and torso appears to be moderately slow in comparison to the conduction velocities of the corticospinal responses. The reflex latencies to the SAS are greater than those elicited by magnetic stimulation of the motor cortex (Brown, Rothwell, Thompson, Britton, Day & Marsden, 1991b). This may be indicitive of the reflex following a pathway different from the corticospinal pathway, and provides further evidence that it may follow the reticulospinal pathway.

The SAS reflex results in a bilateral muscle response. These responses are seen in the firing behaviour of motor units of homologous muscles on both sides of the body with a tendency to synchronously discharge in the 10-20 Hz bandwidth (Grosse & Brown, 2003). When a participant attempts to mimick a general muscular SAS response in the absence of a SAS, bilateral coherence in the 10-20 Hz bandwidth is no longer demonstrated in homologous muscles. Intentional muscular contractions are sent from the brain via the corticospinal system, which sends separate outputs to each side of the body and drives synchronization of motor units over a 15-30 Hz bandwidth (Grosse & Brown, 2003; Kilner, Baker, Salenius, Jousmaki, Hari & Lemon, 1999). The reticulospinal tract is thought to be the pathway through which these homologous muscular responses travel at the 10-20Hz band. Responses demonstrated in the 10-20 Hz band should indicate reticulospinal drive and responses outside of this bandwith may indicate an alternate route or reflex.

## SAS Response

A SAS response may be elicited by any unexpected sound. SAS experiments typically use intensities above 110 dBs but no louder than 124 dB. The duration of these SAS tones range from 30-50 ms. The combination of sound intensity and duration will elicit an SAS response. It is also important to note that these stimuli are typically presented with a fast rise to stimulus intensity time and stimuli are typically presented binaurally (Brown et al., 1991a; Brown et al., 1991b; Csomor et al., 2006; Delwaide & Schepens, 1995; Grosse & Brown, 2003; Nieuwenhuijzen, Schillings, Van Galen & Duysens, 2000). Responses to the SAS vary between participants. Most participants generally respond with eye closure and flexion of the neck muscles. Greater responses also include trunk flexion, some abduction of the arms, flexion of the elbows, pronation of the forearms (Brown et al., 1991a), extensor contractions (Rossignol, 1975), and responses in distal musculature, like the soleus or tibialis anterior (Brown et al., 1991b, Nieuwenhuijzen et al., 2000).

The systemic SAS response seems to decline in amplitude and in some cases disappears all together after repeated exposure (Quednow et al., 2006). The decline in SAS response amplitude with repeated exposure is referred to as habituation. There is research that has demonstrated that after as few as 2 trials, the SAS response may no longer be elicited (Brown et al., 1991b). The eye blinks and neck muscle responses typically require the longest amount of exposure to habituate and do not always disappear but rather decrease in amplitude (Davis, 1982). One way to deal with the issue of habituation is to present SAS randomly over long periods of time so the stimuli may remain unexpected (Brown et al., 1991a; Nieuwenhuijzen et al., 2000; Rossignol, 1975; Russolo, 2002). For example, Brown and colleagues (1991a) randomly presented an auditory stimulus once every 20 minutes. In contrast Nieuwenheijzen et al. (2000) presented stimuli at an inter-stimulus interval of 1.5 to 2.5 minutes to avoid habituation.

The eye blink is used as a main indicator of a SAS response by some researchers, while others rely on contraction of the sternocleidomastoid (SCM) as the marker of SAS responses. Electromyography (EMG) is used to measure orbicularis oculi (OOc) during the presentation of acoustic stimulus to identify muscle activity. These blink responses vary between individuals with a range of onsets from 25 and 69 ms (Brown et al., 1991a). Blink responses are much faster than the onset of the SCM responses which range between 40 to 136 ms. Another detail to note is that eye blink and SCM responses persist in spite of the habituation in the muscle responses in the rest of the body outlined previously (Brown et al., 1991a). It appears that there is a blink reflex in response to sound that is separate from the SAS blink reflex. This blink reflex to sound is termed the auditory blink reflex and appears to have a shorter latency and shorter duration then an actual SAS blink reflex. It is suggested that the blink reflex may actually follow a different pathway than the SAS blink reflex response in the OOc and the SAS reflex responses in the muscles of the rest of the body (Davis, 1982). It has proven difficult to separate the two responses and it is thought that both pathways may travel through the nucleus reticularis pontis caudalis (nRPC) in the PnC. A weak auditory stimulus may activate a sufficient number of neurons within the nRPC to evoke an auditory blink reflex. That same auditory stimulus may be insuffient in activating a particular threshold of neurons within the nRPC for a SAS response. A subthreshold activation of neurons will not evoke an action potential to be sent down the reticulospinal tract for a SAS response. When an auditory stimulus reaches the threshold of SAS response, both auditory blink response and SAS response will be sent making it difficult to dissasociate the two (Valls-Solé, Valldeoriola, Molinuevo, Cossu, & Nobbe, 1999a). As the two reflexes are difficult to distinguish from one another, it may be incorrect to use the blink as an indicator of a SAS response and therefore a blink coupled with SCM activity may be more reliable marker of a SAS response.

## SAS Responses during Static and Dynamic Tasks

SAS responses are seen throughout the body including the lower limbs. SAS responses are seen in the soleus and TA with or without the muscles being engaged in controling posture. Standing and seated positions have been examined to identify the differences in SAS responses when body position is varied. The reflex appears as a bursting pattern of muscle EMG activity that is of greater amplitude than normal background activity present in the muscle in a stationary position (Brown et al., 1991b; Delwaide & Schepens, 1995; Nieuwenhuijzen et al., 2000; Rossignol, 1975). Standing is the most conducive position to observe SAS EMG muscle responses in the legs as they are seen about twice as frequently as seated leg responses. Standing also displays shorter reflex latency times with TA latencies of 80 ms and soleus 70 ms, while seated TA latencies of 120 ms and soleus 130 ms (Brown et al., 1991a). SAS responses may also be seen in some participants while holding a voluntary contraction of their soleus or TA when seated. Voluntarily contracting these muscles increase the excitability of the motorneuron pool to lower excitation thresholds within the muscle. If the reflexive responses were present in a subject while seated and contracting, its latency is similar to those exhibited while seated and relaxed (Delwaid & Schepens, 1995). The latency of the SAS reflex is shorter in the standing position, but still occurs in the seated position, with and without background muscle activation.

To further examine the body's response to startling stimuli in different conditions human gait has been examined. The phases of gait were examined as SAS responses may be found in both flexors and extensors with or without background activity in stationary conditions. The levels of activation due to SAS of the extensors or flexors were not solely based on the background activity of the muscle but were dependent on the phase of gait. These findings suggest that responses in distal musculature occur to maintain stability during the SAS, such as co-activation of both flexors and extensors during the stance phase (Nieuwenhuijzen et al., 2000).

SAS responses are found in both engaged and unengaged muscles, but responses do seem more consistently present in cirucumstances where the maintenance of postural stability is important. Direct postural responses were examined with the presentation of SAS. Russolo (2002) found that SAS presented for longer periods of time (5 seconds) elicit sway towards the stimulus when presented unilaterally. When participants face their head's forward and the stimulus is presented to the left ear, participants swayed left. When their heads were turned over their left shoulder and the stimulus was presented in their left ear, the participant swayed forward. No sway responses were found with bilateral SAS presentation (Russolo, 2002). These findings suggest that SAS responses may be rooted around the maintenance of posture, but not completely dependent on posture as seated conditions may still yield responses similar to standing.

SAS do not only elicit reflexive responses but they also shorten the onset latency of prepared movements. The paradigm used to examine the advanced preperation of motor response is the reaction time (RT) task. In an RT task, participants are typically instructed to conduct a movement (e.g. elbow extension to flexion) in response to a visual stimuli when a 'go' signal is presented. Participants are encouraged to respond as quickly and accurately as possible. If a SAS is presented prior to the 'go' signal, motor responses are made at a shorter onset latency. The duration, timing and task accuracy in the EMG muscle responses are similar in both the non-SAS and SAS RT tasks. The speeding up of a response has been shown in ballistic reaction time tasks as well as during different components of compound movements (Carlsen, Hunt, Inglis, Sanderson & Chua, 2003; Carlsen, Chua, Inglis, Sanderson & Franks, 2004; Valls-Solé, Rothwell, Goulart, Cossu & Muñoz, 1999b). A startling sound may therefore elicit a SAS reflex response within the body, or impact upon a prepared or planned movement being made by the body.

## Pre-pulse Inhibition

The SAS reflex may be altered by the presentation of a stimulus prior to the SAS. The prestimulus decreases the amplitude or completely inhibits the SAS response when it precedes the SAS by 30-500 ms and is referred to as pre-pulse inhibition. Pre-pulse inhibition can be produced by vibrotactile, olfactory, visual or acoustic lead stimuli (Filion, Dawson & Schell, 1998). The degree of amplitude reduction is altered by the lead interval time, its intensity and the type of stimulation used (Hoffman, 1984). The level of pre-pulse inhibition varies depending on the intensity of the pre-pulse with a more intense stimuli having a greater impact, and therefore a greater inhibition effect (Bitsios & Giakoumaki, 2005; Blumenthal, 1996). Pre-pulse inhibition is typically measured by the change of the SAS blink reflex response of the OOc (Valls-Solé et al., 1999a). This response may be inhibited anywhere from 50-80% by pre-pulse inhibition in 90-100% of normal adults tested with reliable SAS blink responses (Filion et al., 1998). Both the SAS blink reflex and the auditory blink reflex are affected in the same way by pre-pulse modulation suggesting that at some point along their pathways they are influenced in the same manner (Valls-Solé et al., 1999a).

In circumstances where the pre-pulse is not an acoustic stimulus, the amount of reflex inhibition appears to be dependent on the intensity and timing of the reflex-inhibiting stimulus and independent of the intensity of the SAS reflex stimulus (Hoffman & Ison, 1992). When both pre-pulse and SAS are acoustic, the amount of SAS inhibition appears to be dependent on both sound intensities (Blumenthal, 1996). Pre-pulse inhibition studies typically present stimuli binuarally using pre-pulses with durations of 20-40 ms at dB levels lower than the SAS. Strong inhibition typically occurs with the lead interval time (the time between the pre-pulse and SAS) within the range of 100-140ms (Blumenthal, 1996; Csomor et al., 2005; Csomor et al., 2006; Quednow et al., 2006; Schwarzkopf, McCoy, Smith & Boutros, 1993).

Pre-pulses may themselves be sufficient in causing a SAS response. In rats, pre-pulse stimuli of 50-60 dB may elicit SAS responses (Blumental & Goode, 1991). In humans, pre-pulses of 95dB and above may evoke SAS responses (Hoffman, 1984). To lessen the likelihood of the pre-pulse itself eliciting a response, intentities of 95 dB or lower should be used in humans. Background noise may be used to reduce the probability of SAS responses to the pre-pulse stimuli. Csomor and colleges (2005) employed a background noise of 70 dB with pre-pulses in the range of 76-88 dB (6-18 dB above the background noise). As a result, they were still able to elicit pre-pulse inhibition of the SAS blink response using a SAS of 95, 105 and 115 dB levels. Despite the benefits of using background noise, there are nonetheless detriments. Detection of the pre-pulse stimuli may not be possible if it does not overcome the loudness of the background noise. Background noise may also reduce the level of pre-pulse inhibition of the SAS response in response to pre-pulses and may interfere with the processing of the pre-pulse stimulus (Blumenthal, Noto, Fox & Franklin, 2006). In humans using a pre-pulse of an intesity of 95 dB or lower is safe experimentally to avoid SAS response to the pre-pulse stimulus and in situations with little to no background noise. The causation of these factors is likely linked to the processing and pathway of the pre-pulse inhibition.

## Pre-Pulse Inhibition Processing and Pathway

Pre-pulse inbition is thought to occur by the processing of select information within the brain. Graham (1975) deduced that the brain attempts to protect and analyze the information of the first lower intensity stimulus it receives when another larger stimulus is presented following the first. It is a wired-in negative feedback mechanism that causes the attenuation of the louder second SAS while perceptually processing and analyzing the stimulus presented initially. This theory follows a sensory-gating mechanism wherein the brain selectively processes information and ignores other information. In general, it is thought that when a stimulus is first perceived it is

identified and analyzed while a protective process attenuates other information until the initial analysis is complete. The information that is attenuated may be external information (auditory, visual, tactile), or it may be internal stimuli such as thoughts, feelings or impulses (Geyer, Swerdlow, Mansbach & Braff, 1990). This theory is supported by clinical populations such as schizophrenic patients. These patients are characterized by their inability to regulate internal stimulation and have general inhibitory deficits. Schizophrenic patients display a lack, or lower incidence of pre-pulse inhibition (Filion et al., 1998; Ludewig, Geyer, Etzensberger &Vollenweider, 2002).

Work has been done to identify the pre-pulse pathway through which the SAS response is inhibited. Evidence suggests that the pre-pulse affects the acoustic pathway relatively early as it inhibits both the auditory blink reflex and the SAS reflex (Valls-Solé et al., 1999a). Pre-pulse signals are sent via subpallidal projections to the pedunculopontine tegmental nucleus (PPTg), which sends inhibitory signals through cholinergic projections to the nucleus reticularis pontis caudalis(nRPC) in the PnC (Valls-Solé et al., 1999a; Blumenthal, 1996). SAS are relayed through the PnC to the reticulospinal tract. When a pre-pulse occurs, signals from the PPTg are sent through cholinergic projections and inhibit the SAS centre neurons in the nRPC. An inhibitory signal is sent to the nRPC reguardless of whether a SAS follows a pre-pulse or not. The inhibitory signal may completely prevent a SAS response, or reduce the response depending on the magnitude of the SAS signal. As the intensity of the SAS is increased, a greater number of excitatory SAS centre neurons are activated in the nRPC. The pre-pulse inhibitory signal from the PPTg neurally inhibits some of SAS centre neurons within the nRPC (Blumenthal, 1996). If the number of neurons excited by input from a SAS is less than the number inhibited by the pre-pulse, then no SAS reflex should be seen. If a greater number of excitatory SAS neurons are activated than the number of those inhibited by the pre-pulse, a SAS reflex response will be seen. The magnitude of this response will be smaller than the typical SAS response in the absence of a

pre-pulse stimulus. Therefore the greater the intensity of the SAS, the larger the response that should be seen while being inhibited by the same pre-pulse intensity (Blumenthal, 1996).

## Repeated Acoustic Stimuli (RAS) Reponse

Alternative means of presenting SAS have been recently assessed. In a recent study (Nichol et al., 2007), SAS were presented with short random intervals between stimuli and this presentation method was referred to as Repeated Acoustic Stimuli (RAS). The SAS were presented at intervals of three to five seconds between each sound with 125 stimuli in a given trial. This method promoted habituation of the typical SAS response after the first few stimuli and yet still rendered an apparent reflexive response in the soleus muscles. Responses may be seen in all subjects in the experiment in Figure 9.

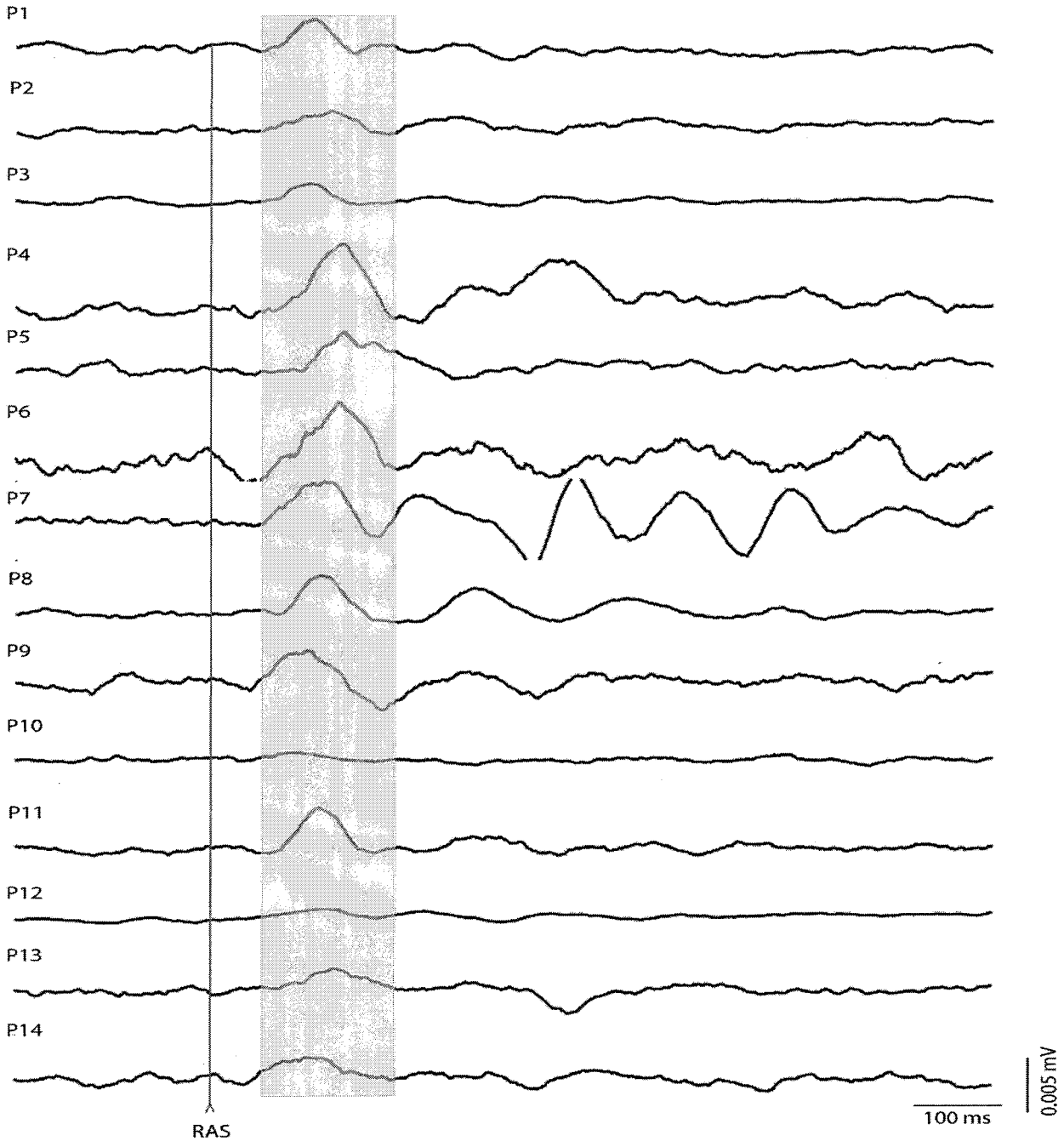


**Figure 9**: Average RAS reflexes recorded in the left soleus muscles of each participant. The shaded area highlighting the RAS reflex response peak. P1 through P14 represent the 14 different participants (Nichol et al., 2007).

Participants were positioned in the supine position with their head facing forward while maintaining a background muscle response of the soleus. The rationale for the background muscle response was to equal that of quiet stance and was determined from a standing relaxed

position. This level of background activity while laying was maintained by plantar flexion with the ankle maintained at a 90 degree angle, and the feet flat against a stationary board at 90 degrees to horizontal. RAS were presented binaurally using a horn placed directly over a participants face at a distance of 30 cm from each ear. The RAS was a 1000 Hz, 124 dB, 40 ms pulse presented randomly at 3 to 5 second time intervals between stimuli (interstimulus interval) with a fast rise to intensity. Participants surface soleus EMG responses were root mean squared and averaged without the first 10 stimuli to avoid the inclusion of typical SAS EMG responses. A reflexive response was present across all participants which may be seen in the highlighted area shown in Figure 9. All participants showed similar responses bilaterally in their soleus muscles (see Figure 10). The reflex begins as a positive deflection in the EMG followed by a return to neutral and in some participants a negative (downward) deflection that may be followed by further multi-phasic activity of lesser amplitude. The commencement of the reflex response was measured as two S.D. above background activity prior to the stimuli and was found to have an average onset latency of 65.2 ms in the right soleus (S.D.= 18.6 ms) and 69.9 ms in the left soleus (S.D.= 13.2 ms). A peak positive response is seen at 112.6 ms (S.D.= 16.2 ms) in the right soleus and 113.1 ms (S.D.=15.5 ms) in the left soleus. After the positive peak response there was a return to neutral, or a negative trough at 150-160 ms and responses varied with some participants showing multi-phasic responses of decreasing amplitude for 500 ms following the initial peak and others had no discernable peaks or troughs following the initial peak. The peak responses were still present after averaging trials with no SAS evoked SCM responses as seen in figure 11, and no SAS evoked OOc responses as seen in Figure 12.

The identifiable muscle responses to stimuli with no OOc and no SCM activity (Figures 11 & 12), typical indicators of SAS, suggests that this response may differ from a SAS response. Figures 10-12 show a discernable response at a similar onset latency to that of a standing SAS response which is 70 ms in the soleus and much shorter than the onset of the sitting SAS soleus

responses at 130ms (Brown et al., 1991a). Standing involves engagement in posture by lower limb muscles like the soleus, while seated and laying conditions do not. One might assume that the seated and laying conditions should therefore have similar onset latencies in response to SAS rather than standing and laying, although this is unknown as no laying SAS study has been conducted. As it is in fact the standing SAS and the laying condition of this experiment that have similar onset latencies, responses could be dependent on body positioning rather than engagement in posture and the responses in this experiment and SAS responses could in fact be related. To better understand what occurs with this varying form of stimulation more research must be conducted to investigate similarities and differences between SAS and these new responses.

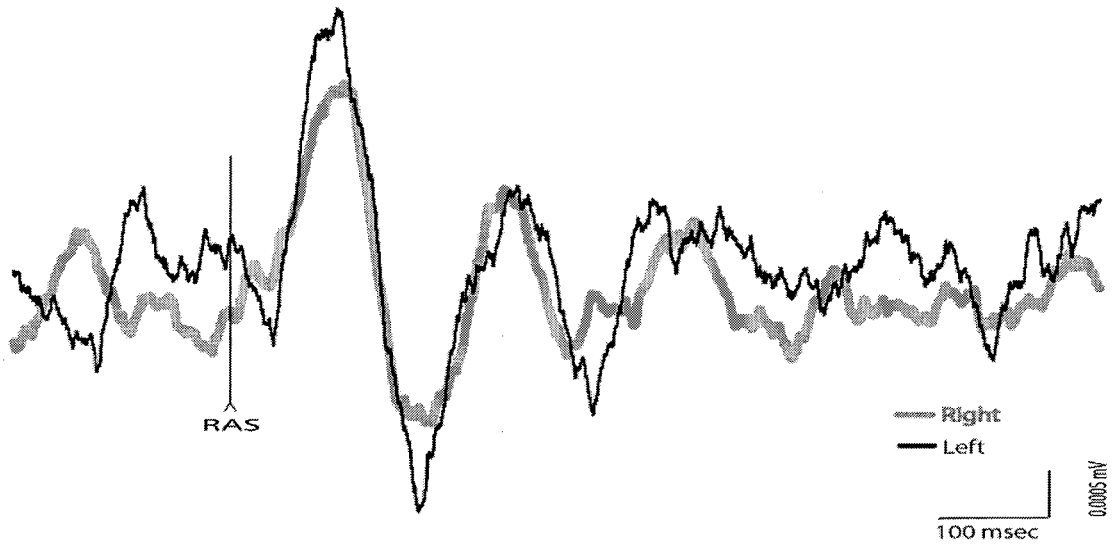


**Figure 10**: Average RAS reflexes recorded in the left and right soleus muscles of one participant (Nichol et al., 2007).

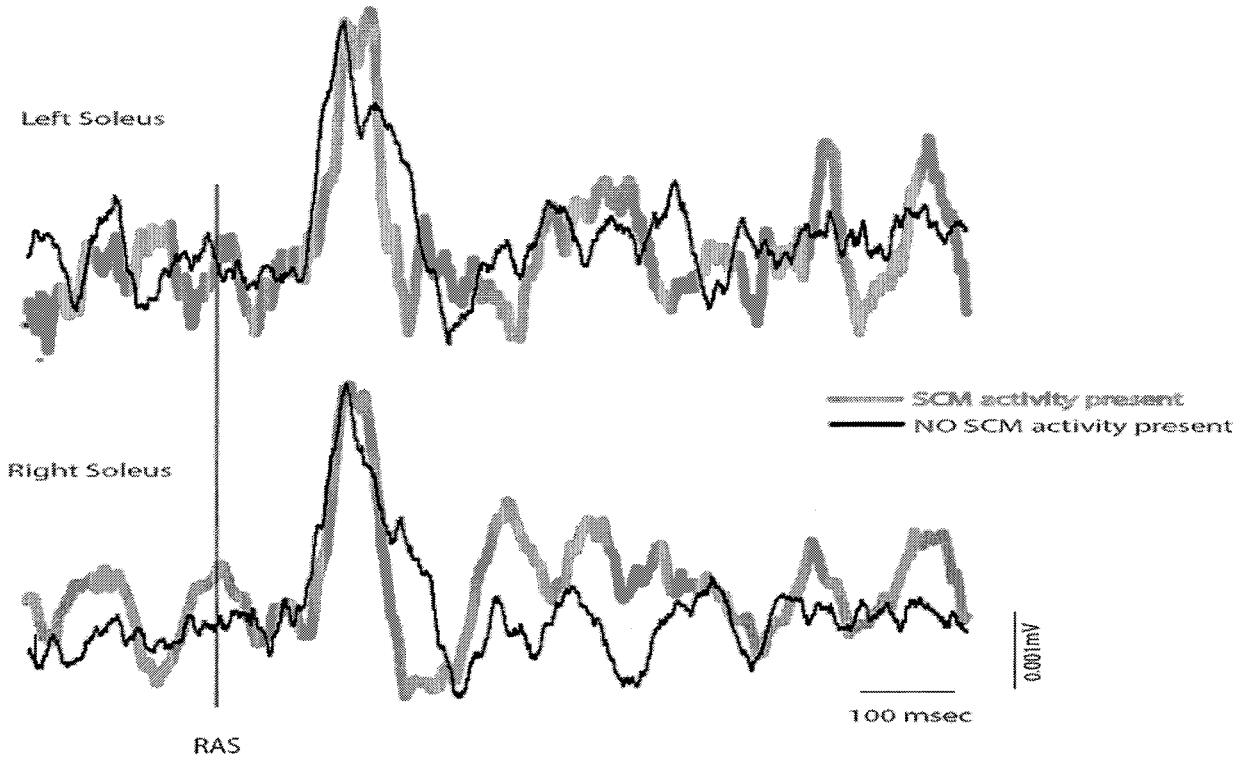


**Figure 11:** Average RAS reflexes recorded in the left and right soleus muscles of one participant with and without the presence of SCM activity (Nichol et al., 2007).

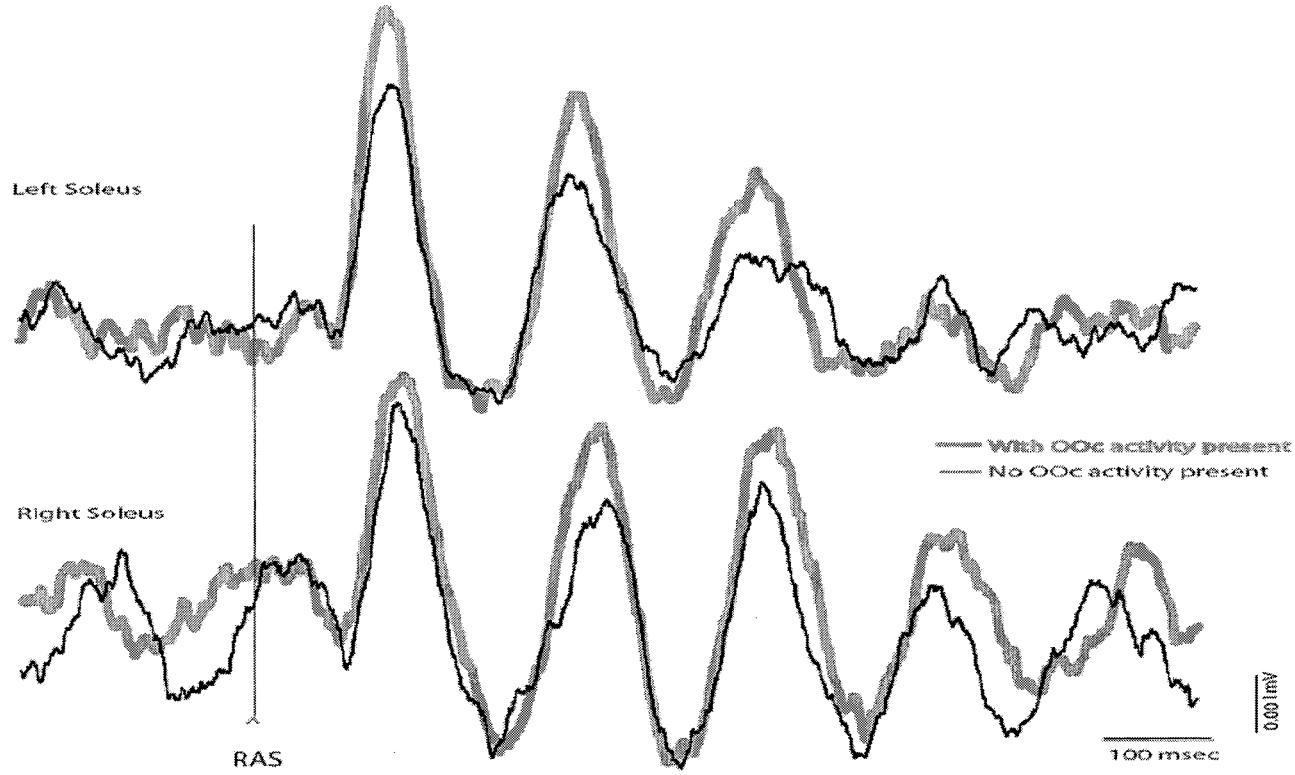


**Figure 12**: Average RAS reflexes recorded in the left and right soleus muscles of one participant with and without the presence of OOc activity (Nichol et al., 2007).

The results found in this experiment show similar timing to sound-induced variations in H-reflex amplitude. A spinal excitability has been reported following loud acoustic stimuli. This excitability begins 50 ms after acoustic stimulus onset, with a peak amplitude at 100-130 ms after stimulus and excitability lasting a mean duration of 200 ms with a range of 120-460 ms (Liegeois-Chauvel, Morin, Musolino, Bancaud & Chauvel, 1989; Rossignal & Jones, 1976). This audiospinal facilitation is considered a possible physiological substrate of SAS (Liegeois-Chauvel et al., 1989). The potentiation of the H-reflex by the acoustic stimuli also appears more resistant to habituation than a SAS response and remains present after repeated stimuli (Rossignal & Jones, 1976). The auditory facilitation is noted with loud stimuli of 110 dB (Rossignal & Jones, 1976), as well as more ordinary environmental sounds of 80 dB (Rudell & Eberle, 1985). The facilitation is bilateral and symmetrically distributed with the same timing of the soleus H-reflex in both limbs with monoaural stimulation. There is suggestion that the facilitation may be due to an increase in motoneuron excitability in the soleus and/or due to a decrease in the presynaptic inhibition of soleus Ia fibers (Liegeois-Chauvel et al., 1989). Due to the similarities between RAS responses and the timing of the sound-induced H reflex response, it is possible that the two are related. The RAS reflex is a new area of investigation. It is not known if it shares commonalities with the SAS reflex, or if it is a completely separate response by the body.

# Appendix 2: Single Acoustic Stimulus Responses

| RSOL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 138.12 | 140.98 | 0.003745 |
| 2 | 50.56 | | 0.002525 |
| 3 | 79.7 | 102.32 | 0.029065 |
| 4 | 95.82 | 127.28 | 0.005995 |
| 5 | 40.44 | 124.68 | 0.023255 |
| 14 | 44.6 | 195.66 | 0.01397 |
| 15 | | 14.18 | 0.006255 |
| 16 | 110.9 | 124.42 | 0.0139 |
| 17 | | 60.46 | 0.00597 |
| 18 | 93.48 | 111.16 | 0.0026 |
| Mean | 74.34444 | 111.2378 | 0.010728 |
| stdev | 39.25023 | 50.86951 | 0.009195 |
| sterror | 13.08341 | 16.9565 | 0.002908 |

| LSOL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 121.18 | 139 | 0.016525 |
| 2 | 51.44 | 55.18 | 0.00341 |
| 3 | 51.36 | 101.02 | 0.011145 |
| 4 | 76.32 | 96.08 | 0.00671 |
| 5 | 32.38 | 84.38 | 0.023555 |
| 14 | 111.68 | 119.48 | 0.012495 |
| 15 | 32.38 | 51.62 | 0.00605 |
| 16 | 127.02 | 141.58 | 0.0068 |
| 17 | 43.82 | 139.76 | 0.009035 |
| 18 | 51.62 | 135.6 | 0.00336 |
| Mean | 69.92 | 106.37 | 0.009909 |
| stdev | 36.80797 | 34.45071 | 0.006315 |
| sterror | 11.6397 | 10.89427 | 0.001997 |

| RDEL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 22.84 | 108.2 | 0.09122 |
| 2 | 82.9 | 95.88 | 0.00034 |
| 3 | 63.06 | 115.06 | 0.01818 |
| 4 | 32.12 | 119.22 | 0.08852 |
| 5 | 60 | 101.8 | 0.01244 |
| 14 | 69.08 | 151.98 | 0.0131 |
| 15 | 100.5 | 114.54 | 0.00086 |
| 16 | 70.4 | 90.62 | 0.00424 |
| 17 | 36.8 | 83.08 | 0.0259 |
| 18 | | 197.48 | 0.06637 |
| Mean | 59.74444 | 117.786 | 0.032117 |
| stdev | 25.15065 | 33.84639 | 0.035891 |
| sterror | 8.383551 | 10.70317 | 0.01135 |

| LDEL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 30.76 | 106 | 0.0393 |
| 2 | | | 0.00006 |
| 3 | 20.16 | 117.92 | 0.09632 |
| 4 | 57.34 | 130.4 | 0.01067 |
| 5 | 48.5 | 80.48 | 0.02265 |
| 14 | 67.22 | 163.16 | 0.11518 |
| 15 | 27.18 | 170.96 | 0.00175 |
| 16 | 93.22 | 97.9 | 0.00178 |
| 17 | 42.52 | 124.94 | 0.03878 |
| 18 | 60.98 | 200.08 | 0.01037 |
| Mean | 49.76444 | 132.4267 | 0.033686 |
| stdev | 22.85276 | 38.51273 | 0.040805 |
| sterror | 7.617587 | 12.83758 | 0.012904 |

| RGAS | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 67.72 | 79.82 | 0.006305 |
| 2 | 37.8 | 47.26 | 0.001045 |
| 3 | 47.46 | 108.3 | 0.017335 |
| 4 | 51.36 | 105.18 | 0.00059 |
| 5 | 25.62 | 105.18 | 0.003335 |
| 14 | 47.46 | 101.8 | 0.002645 |
| 15 | 33.94 | 199.04 | 0.00527 |
| 16 | | 57.6 | 0.00872 |
| 17 | 232.84 | 53.96 | 0.00431 |
| 18 | 61.24 | 64.88 | 0.003155 |
| Mean | 67.27111 | 92.302 | 0.005271 |
| stdev | 63.44494 | 44.32421 | 0.00488 |
| sterror | 21.14831 | 14.01655 | 0.001543 |

| LGAS | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | | | 0.003775 |
| 2 | 113.04 | 116.34 | 0.000635 |
| 3 | 46.68 | 117.92 | 0.056145 |
| 4 | 78.92 | 95.56 | 0.00389 |
| 5 | 48.5 | 60.98 | 0.005275 |
| 14 | 108.82 | 115.32 | 0.002525 |
| 15 | 108.82 | 110.64 | 0.00347 |
| 16 | | 68 | 0.00297 |
| 17 | 57.6 | 139.24 | 0.002395 |
| 18 | 84.12 | 86.2 | 0.003585 |
| Mean | 80.8125 | 101.1333 | 0.008467 |
| stdev | 27.69713 | 25.54644 | 0.016796 |
| sterror | 9.792415 | 8.515481 | 0.005311 |

| RSCM | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | | 58.26 | 0.074305 |
| 2 | | 64.2 | 0.0029 |
| 3 | 42.26 | 113.76 | 0.017415 |
| 4 | | 69.82 | 0.147975 |
| 5 | . | 69.04 | 0.01195 |
| 14 | 58.38 | 135.6 | 0.03582 |
| 15 | 54.74 | 71.38 | 0.00382 |
| 16 | 41.22 | 69.56 | 0.02852 |
| 17 | 25.62 | 74.76 | 0.127795 |
| 18 | 21.98 | 200.08 | 0.119225 |
| Mean | 40.7 | 92.646 | 0.056973 |
| stdev | 14.76654 | 44.97884 | 0.055848 |
| sterror | 6.028413 | 14.22356 | 0.017661 |

| LSCM | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | | 63.1 | 0.092325 |
| 2 | 23.28 | 200.16 | 0.00085 |
| 3 | 37.58 | 93.48 | 0.07324 |
| 4 | | 66.44 | 0.034445 |
| 5 | | 65.92 | 0.01588 |
| 14 | 56.04 | 127.54 | 0.04497 |
| 15 | | 69.3 | 0.01042 |
| 16 | | 73.98 | 0.021385 |
| 17 | 25.1 | 70.34 | 0.16618 |
| 18 | 38.62 | 199.3 | 0.07818 |
| Mean | 36.124 | 102.956 | 0.053788 |
| stdev | 13.14814 | 54.52285 | 0.050236 |
| sterror | 5.880025 | 17.24164 | 0.015886 |

| ROOC | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | | 50.78 | 0.10045 |
| 2 | 28.12 | 57.38 | 0.03041 |
| 3 | | 156.66 | 0.1111 |
| 4 | | 60.72 | 0.07881 |
| 5 | | 77.62 | 0.07357 |
| 14 | | 56.3 | 0.02732 |
| 15 | 30.04 | 53.7 | 0.01858 |
| 16 | 20.2 | 54.48 | 0.02742 |
| 17 | | 52.14 | 0.02516 |
| 18 | 21.98 | 78.66 | 0.06311 |
| Mean | 24.88 | 69.844 | 0.055593 |
| stdev | 5.026039 | 32.09499 | 0.034214 |
| sterror | 2.513019 | 10.14933 | 0.010819 |

# Appendix 3: Single Acoustic Stimuli Across Six Trials

| ROOc | SAS 1 AMPLITUDE (mV) | SAS 2 AMPLITUDE (mV) | SAS 3 AMPLITUDE (mV) | SAS 4 AMPLITUDE (mV) | SAS 5 AMPLITUDE (mV) | SAS 6 AMPLITUDE (mV) |
|---|---|---|---|---|---|---|
| 1 | 0.17813 | 0.12181 | 0.17218 | 0.0726 | 0.09858 | 0.19502 |
| 2 | 0.02864 | 0.00882 | 0.01056 | 0.04775 | 0.03527 | 0.06182 |
| 3 | 0.1486 | 0.30862 | 0.16732 | 0.22527 | 0.1223 | 0.15882 |
| 4 | 0.09337 | 0.11507 | 0.08988 | 0.11633 | 0.24352 | 0.08975 |
| 5 | 0.06962 | 0.08147 | 0.10761 | 0.16134 | 0.09084 | 0.04919 |
| 14 | 0.02153 | 0.04237 | 0.04787 | 0.01934 | 0.03185 | 0.01323 |
| 15 | 0.01765 | 0.00724 | 0.00553 | 0.01838 | 0.03195 | 0.04582 |
| 16 | 0.00495 | 0.04104 | 0.04113 | 0.03767 | 0.02778 | 0.02375 |
| 17 | 0.02558 | 0.02538 | 0.03944 | 0.02616 | 0.03911 | 0.04205 |
| 18 | 0.09769 | 0.14652 | 0.14611 | 0.1368 | 0.0463 | 0.12594 |

| LSCM | SAS 1 AMPLITUDE (mV) | SAS 2 AMPLITUDE (mV) | SAS 3 AMPLITUDE (mV) | SAS 4 AMPLITUDE (mV) | SAS 5 AMPLITUDE (mV) | SAS 6 AMPLITUDE (mV) |
|---|---|---|---|---|---|---|
| 1 | 0.05951 | 0.018895 | 0.149355 | 0.12301 | 0.087775 | 0.166245 |
| 2 | 0.00404 | 0.00174 | 0.001355 | 0.00195 | 0.001335 | 0.00253 |
| 3 | 0.18494 | 0.07139 | 0.18096 | 0.13166 | 0.06131 | 0.083235 |
| 4 | 0.034815 | 0.042825 | 0.05449 | 0.037245 | 0.02006 | 0.034555 |
| 5 | 0.00061 | 0.015465 | 0.026635 | 0.02548 | 0.02519 | 0.000535 |
| 14 | 0.03516 | 0.06947 | 0.17716 | 0.058755 | 0.1735 | 0.00961 |
| 15 | 0.00501 | 0.002855 | 0.010365 | 0.004815 | 0.036775 | 0.017415 |
| 16 | 0.001735 | 0.04553 | 0.019245 | 0.03127 | 0.02936 | 0.00448 |
| 17 | 0.22122 | 0.21339 | 0.197935 | 0.210215 | 0.175445 | 0.20558 |
| 18 | 0.15624 | 0.14269 | 0.127685 | 0.12571 | 0.092065 | 0.07223 |

| RSCM | SAS 1 AMPLITUDE (mV) | SAS 2 AMPLITUDE (mV) | SAS 3 AMPLITUDE (mV) | SAS 4 AMPLITUDE (mV) | SAS 5 AMPLITUDE (mV) | SAS 6 AMPLITUDE (mV) |
|---|---|---|---|---|---|---|
| 1 | 0.04407 | 0.02072 | 0.099545 | 0.10998 | 0.109625 | 0.11856 |
| 2 | 0.001 | 0.00065 | 0.000825 | 0.002235 | 0.006615 | 0.008705 |
| 3 | 0.041085 | 0.02967 | 0.034215 | 0.05234 | 0.01397 | 0.014345 |
| 4 | 0.17374 | 0.14868 | 0.150795 | 0.152305 | 0.1573 | 0.10609 |
| 5 | 0.000955 | 0.00801 | 0.011905 | 0.018445 | 0.043815 | 0.00377 |
| 14 | 0.01947 | 0.053345 | 0.06972 | 0.02215 | 0.13839 | 0.005695 |
| 15 | 0.00656 | 0.002715 | 0.00668 | 0.0036 | 0.014295 | 0.00631 |
| 16 | 0.001875 | 0.050435 | 0.035825 | 0.05153 | 0.03377 | 0.00349 |
| 17 | 0.19126 | 0.1539 | 0.139955 | 0.138515 | 0.151355 | 0.180875 |
| 18 | 0.166825 | 0.204855 | 0.225965 | 0.20944 | 0.134795 | 0.081625 |

| LSOL | SAS 1 AMPLITUDE (mV) | SAS 2 AMPLITUDE (mV) | SAS 3 AMPLITUDE (mV) | SAS 4 AMPLITUDE (mV) | SAS 5 AMPLITUDE (mV) | SAS 6 AMPLITUDE (mV) |
|---|---|---|---|---|---|---|
| 1 | 0.003295 | 0.00463 | 0.04245 | 0.02399 | 0.018115 | 0.02597 |
| 2 | 0.014235 | 0.00404 | 0.009355 | 0.009245 | 0.00491 | 0.010185 |
| 3 | 0.002245 | 0.008205 | 0.06339 | 0.003645 | 0.036975 | 0.004245 |
| 4 | 0.00871 | 0.00778 | 0.018505 | 0.0235 | 0.008245 | 0.01143 |
| 5 | 0.020125 | 0.046895 | 0.02147 | 0.064095 | 0.04702 | 0.02134 |
| 14 | 0.013675 | 0.01463 | 0.017355 | 0.014865 | 0.021085 | 0.014215 |
| 15 | 0.01559 | 0.010335 | 0.006645 | 0.015025 | 0.031695 | 0.012795 |
| 16 | 0.00912 | 0.014155 | 0.00381 | 0.00677 | 0.01278 | 0.021515 |
| 17 | 0.011635 | 0.012775 | 0.010025 | 0.00929 | 0.03525 | 0.010975 |
| 18 | 0.00379 | 0.009165 | 0.006235 | 0.008055 | 0.00765 | 0.03047 |

| RSOL | SAS 1 AMPLITUDE (mV) | SAS 2 AMPLITUDE (mV) | SAS 3 AMPLITUDE (mV) | SAS 4 AMPLITUDE (mV) | SAS 5 AMPLITUDE (mV) | SAS 6 AMPLITUDE (mV) |
|---|---|---|---|---|---|---|
| 1 | 0.002955 | 0.005605 | 0.005085 | 0.01903 | 0.010575 | 0.013275 |
| 2 | 0.00474 | 0.00628 | 0.00746 | 0.001995 | 0.00594 | 0.00559 |
| 3 | 0.007045 | 0.021565 | 0.05613 | 0.0107 | 0.02078 | 0.013245 |
| 4 | 0.012725 | 0.010455 | 0.009925 | 0.00968 | 0.015075 | 0.011635 |
| 5 | 0.015445 | 0.04344 | 0.028255 | 0.040955 | 0.04409 | 0.012095 |
| 14 | 0.005345 | 0.03016 | 0.03161 | 0.01549 | 0.049385 | 0.005965 |
| 15 | 0.015845 | 0.01433 | 0.022385 | 0.00786 | 0.02008 | 0.025085 |
| 16 | 0.019835 | 0.01934 | 0.0338 | 0.01353 | 0.023705 | 0.017705 |
| 17 | 0.02517 | 0.013055 | 0.01365 | 0.02546 | 0.016275 | 0.00952 |
| 18 | 0.001825 | 0.00262 | 0.003165 | 0.007135 | 0.00391 | 0.016375 |

| RMGAS | SAS 1 AMPLITUDE (mV) | SAS 2 AMPLITUDE (mV) | SAS 3 AMPLITUDE (mV) | SAS 4 AMPLITUDE (mV) | SAS 5 AMPLITUDE (mV) | SAS 6 AMPLITUDE (mV) |
|---|---|---|---|---|---|---|
| 1 | 0.004843 | 0.015225 | 0.012149 | 0.007044 | 0.017321 | 0.014376 |
| 2 | 0.004094 | 0.001673 | 0.002508 | 0.00191 | 0.001281 | 0.001107 |
| 3 | 0.028397 | 0.016703 | 0.114835 | 0.014328 | 0.005207 | 0.007902 |
| 4 | 0.000609 | 0.001697 | 0.001217 | 0.001518 | 0.002288 | 0.000785 |
| 5 | 0.004521 | 0.005368 | 0.006084 | 0.003547 | 0.01146 | 0.005736 |
| 14 | 0.002943 | 0.004644 | 0.001586 | 0.005557 | 0.009287 | 0.004828 |
| 15 | 0.003118 | 0.003229 | 0.004637 | 0.012921 | 0.008914 | 0.009096 |
| 16 | 0.034008 | 0.007458 | 0.004457 | 0.027346 | 0.036339 | 0.045736 |
| 17 | 0.012432 | 0.00569 | 0.026601 | 0.010711 | 0.006327 | 0.009695 |
| 18 | 0.001638 | 0.005709 | 0.014069 | 0.009527 | 0.004528 | 0.00694 |

# Appendix 4: Repeated Acoustic Stimuli Responses

| RSOL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 30.32 | 111.94 | 0.00251 |
| 2 | 34.72 | 55.18 | 0.00218 |
| 3 | 42 | 114.28 | 0.002475 |
| 4 | 61.24 | 115.32 | 0.005805 |
| 5 | 28.74 | 102.58 | 0.00216 |
| 14 | 49.54 | 104.92 | 0.0039 |
| 15 | 28.22 | 101.8 | 0.002305 |
| 16 | 42.78 | 108.3 | 0.005455 |
| 17 | 50.84 | 91.4 | 0.00412 |
| 18 | | 87.5 | 0.001555 |
| Mean | 40.93333 | 99.322 | 0.003247 |
| stdev | 11.46015 | 18.008093 | 0.001484 |
| sterror | 3.820049 | 5.6946591 | 0.000469 |

| LSOL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 40.44 | 89.28 | 0.00517 |
| 2 | 43.08 | 55.4 | 0.00171 |
| 3 | 48.5 | 109.08 | 0.001215 |
| 4 | 50.32 | 109.08 | 0.002535 |
| 5 | 31.86 | 105.44 | 0.0024 |
| 14 | 88.8 | 106.22 | 0.005845 |
| 15 | 33.68 | 71.9 | 0.001595 |
| 16 | 53.7 | 106.48 | 0.002795 |
| 17 | 39.66 | 124.68 | 0.002145 |
| 18 | 43.04 | 87.24 | 0.001305 |
| Mean | 47.308 | 96.48 | 0.002672 |
| stdev | 16.12125 | 20.59361 | 0.001591 |
| sterror | 5.373751 | 6.51227 | 0.000503 |

| RDEL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | | 110.18 | 0.00082 |
| 2 | | | 0.00032 |
| 3 | | | 0.00015 |
| 4 | | | 0.00003 |
| 5 | | | 0.0002 |
| 14 | | 20.68 | 0.00061 |
| 15 | 44.08 | 141.84 | 0.00045 |
| 16 | | 21.2 | 0.00047 |
| 17 | 55 | 165.5 | 0.00005 |
| 18 | 58.12 | 127.28 | 0.00072 |
| Mean | 52.4 | 97.78 | 0.000382 |
| stdev | 7.372272 | 62.225716 | 0.000278 |
| sterror | 4.256383 | 25.403542 | 8.79E-05 |

| LDEL | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 57.6 | 100.94 | 0.00032 |
| 2 | | 131.74 | 0.00002 |
| 3 | | 24.06 | 0.00045 |
| 4 | 121.3 | 129.88 | 0.00003 |
| 5 | 73.98 | 89.06 | 0.00029 |
| 14 | 77.88 | 88.28 | 0.00056 |
| 15 | 44.86 | 53.96 | 0.00023 |
| 16 | 36.28 | 155.62 | 0.00022 |
| 17 | | 200.08 | 0.00005 |
| 18 | 48.5 | 126.76 | 0.00344 |
| Mean | 65.77143 | 110.038 | 0.000561 |
| stdev | 28.77941 | 50.40151 | 0.001027 |
| sterror | 10.8776 | 15.93836 | 0.000325 |

| RGAS | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 29 | 76.52 | 0.008585 |
| 2 | 39.78 | 117.28 | 0.00076 |
| 3 | 41.22 | 99.98 | 0.001195 |
| 4 | 52.14 | 105.96 | 0.001175 |
| 5 | 33.16 | 102.06 | 0.00085 |
| 14 | 91.4 | 109.34 | 0.00274 |
| 15 | 33.68 | 102.32 | 0.006515 |
| 16 | 33.16 | 123.38 | 0.00607 |
| 17 | 44.86 | 94.78 | 0.002055 |
| 18 | 39.92 | 79.7 | 0.00492 |
| Mean | 43.832 | 101.132 | 0.003487 |
| stdev | 18.01904 | 14.740123 | 0.002818 |
| sterror | 5.698122 | 4.6612361 | 0.000891 |

| LGAS | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 34.72 | 113.7 | 0.00465 |
| 2 | 36.92 | 54.08 | 0.000385 |
| 3 | 85.42 | 105.44 | 0.002295 |
| 4 | | 104.92 | 0.00104 |
| 5 | 38.88 | 102.06 | 0.002695 |
| 14 | 37.58 | 110.12 | 0.002385 |
| 15 | 24.06 | 102.58 | 0.005745 |
| 16 | 51.1 | 109.86 | 0.001265 |
| 17 | 40.44 | 115.58 | 0.00102 |
| 18 | 33.68 | 73.72 | 0.00312 |
| Mean | 42.53333 | 99.206 | 0.00246 |
| stdev | 17.56104 | 19.6857 | 0.001698 |
| sterror | 5.853681 | 6.225165 | 0.000537 |

| RSCM | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | 14.04 | 65.08 | 0.001495 |
| 2 | 25.26 | 39.12 | 0.000175 |
| 3 | | 37.58 | 0.000265 |
| 4 | | | 0.000075 |
| 5 | | | 0.000255 |
| 14 | | 96.6 | 0.000075 |
| 15 | 20 | 75.28 | 0.000945 |
| 16 | | 105.7 | 0.00008 |
| 17 | 33.68 | 49.02 | 0.0005 |
| 18 | | 98.94 | 0.00071 |
| Mean | 23.22 | 70.915 | 0.000458 |
| stdev | 8.34402 | 27.117495 | 0.000488 |
| sterror | 4.17201 | 9.5874822 | 0.000154 |

| LSCM | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | | 66.4 | 0.0016 |
| 2 | 24.82 | 32.52 | 0.000125 |
| 3 | | 86.72 | 0.00203 |
| 4 | | | 0.00033 |
| 5 | | | 0.000235 |
| 14 | 18.6 | 21.2 | 0.00009 |
| 15 | | 74.5 | 0.000855 |
| 16 | 95.56 | 153.02 | 0.00015 |
| 17 | 16.52 | 111.42 | 0.00017 |
| 18 | 17.56 | 47.2 | 0.000485 |
| Mean | 34.612 | 74.1225 | 0.000607 |
| stdev | 37.95416 | 45.20768 | 0.000723 |
| sterror | 16.97361 | 15.98333 | 0.000229 |

| ROOC | ONSET (ms) | PEAK (ms) | AMPLITUDE (mV) |
|---|---|---|---|
| 1 | | 48.14 | 0.06275 |
| 2 | 20.64 | 51 | 0.00126 |
| 3 | 12.88 | 51.1 | 0.0115 |
| 4 | 11.06 | 53.7 | 0.01254 |
| 5 | 12.88 | 65.4 | 0.02452 |
| 14 | 17.82 | 43.04 | 0.00449 |
| 15 | 23.28 | 46.68 | 0.00762 |
| 16 | 14.7 | 126.5 | 0.00371 |
| 17 | 10.02 | 41.48 | 0.0095 |
| 18 | 12.62 | 60.2 | 0.04431 |
| Mean | 15.1 | 58.724 | 0.01822 |
| stdev | 4.259598 | 23.63003 | 0.01912 |
| sterror | 1.419866 | 7.472471 | 0.006046 |

# Appendix 5: SAS Responses and SAS with Pre-Pulses Responses

| RSOL | SAS | | | Pre-pulse and SAS | | | % Pre-Pulse inhibition (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| Participant | Onset (ms) | Peak (ms) | Amplitude (mV) | Onset (ms) | Peak (ms) | Amplitude (mV) | |
| 6 | 135.34 | 149.38 | 0.004 | 111.42 | 119.48 | 0.002 | 41.5 |
| 7 | 19.64 | 199.82 | 0.015 | 181.1 | 186.04 | 0.008 | 53.7 |
| 8 | 16.26 | 140.02 | 0.069 | 87.24 | 115.06 | 0.022 | 31.3 |
| 9 | 183.44 | 186.82 | 0.004 | 214.38 | 74.76 | 0.004 | 104.3 |
| 10 | 87.24 | 186.82 | 0.04 | 82.04 | 118.96 | 0.075 | 185.8 |
| 11 | 93.22 | 101.8 | 0.004 | 90.1 | 102.06 | 0.019 | 428.1 |
| 12 | 40.7 | 88.28 | 0.013 | 121.82 | 130.66 | 0.007 | 51.7 |
| 13 | 194.88 | 200.08 | 0.009 | 91.92 | 110.38 | 0.037 | 412.3 |
| Mean | 96.34 | 156.63 | 0.02 | 122.5 | 119.68 | 0.022 | 109.3 |

| LSOL | SAS | | | Pre-pulse and SAS | | | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| Participant | Onset (ms) | Peak (ms) | Amplitude (mV) | Onset (ms) | Peak (ms) | Amplitude (mV) | |
| 6 | 133.26 | 149.6 | 0.006 | 644.42 | 47.2 | 0.004 | 60.9 |
| 7 | 162.64 | 164.5 | 0.007 | 477.24 | 112.98 | 0.003 | 44.4 |
| 8 | 128.58 | 138.5 | 0.037 | 4.56 | 133.78 | 0.011 | 30.8 |
| 9 | 268.72 | 190 | 0.004 | 105.96 | 107.26 | 0.003 | 70.5 |
| 10 | 26.14 | 98.68 | 0.033 | 74.5 | 102.06 | 0.066 | 201.5 |
| 11 | 135.86 | 141.1 | 0.007 | 47.72 | 110.9 | 0.039 | 539.6 |
| 12 | 41.22 | 198 | 0.012 | 56.56 | 56.82 | 0.006 | 48.9 |
| 13 | 19.64 | 123.1 | 0.07 | 88.54 | 112.2 | 0.051 | 72.5 |
| Mean | 114.508 | 150.4 | 0.022 | 187.44 | 97.9 | 0.023 | 104.0 |

| RDEL | SAS | | | Pre-pulse and SAS | | | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| Participant | Onset (ms) | Peak (ms) | Amplitude (mV) | Onset (ms) | Peak (ms) | Amplitude (mV) | |
| 6 | 62.5 | 143.1 | 0.03 | 0.4 | 16.52 | 3E-04 | 1.1 |
| 7 | 26.14 | 147.8 | 5E-04 | 279.38 | 146.26 | 1E-04 | 31.1 |
| 8 | 25.36 | 121 | 0.12 | 51.62 | 53.7 | 2E-04 | 0.2 |
| 9 | 208.92 | 1.44 | 0.001 | 166.02 | 174.6 | 0.001 | 83.8 |
| 10 | 41.24 | 149.6 | 0.074 | 65.74 | 175.12 | 0.123 | 167.3 |
| 11 | 175 | 0.66 | 4E-04 | 82.5 | 130.66 | 0.001 | 297.7 |
| 12 | 57.98 | 153.3 | 0.03 | 0.4 | 22.24 | 9E-04 | 3.1 |
| 13 | 56.46 | 147 | 0.273 | 0.4 | 177.2 | 0.089 | 32.7 |
| Mean | 81.7 | 108 | 0.066 | 80.808 | 112.04 | 0.027 | 40.9 |

| LDEL | SAS Onset | Peak | Amplitude | Pre-pulse and SAS Onset | Peak | Amplitude | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| Participant | (ms) | (ms) | (mV) | (ms) | (ms) | (mV) | |
| 6 | 43.56 | 148.1 | 0.057 | 26.92 | 59.94 | 0.002 | 2.8 |
| 7 | 21.2 | 133.8 | 2E-04 | 0.92 | 23.28 | 2E-04 | 123.5 |
| 8 | 44.34 | 163.4 | 0.014 | 19.64 | 145.74 | 0.003 | 19.1 |
| 9 | 62.02 | 101.3 | 0.018 | 51.1 | 172.78 | 0.003 | 14.2 |
| 10 | 44.86 | 140.5 | 0.083 | 77.88 | 159.52 | 0.184 | 220.7 |
| 11 | 83.86 | 200.1 | 9E-04 | 234.66 | 163.16 | 1E-04 | 13.8 |
| 12 | 16.52 | 125.7 | 0.005 | 692.26 | 34.46 | 1E-03 | 18.5 |
| 13 | 44.86 | 144.2 | 0.225 | 54.48 | 165.76 | 0.178 | 79.0 |
| Mean | 45.1525 | 144.6 | 0.05 | 144.73 | 115.58 | 0.046 | 91.5 |

| RGAS | SAS Onset | Peak | Amplitude | Pre-pulse and SAS Onset | Peak | Amplitude | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| Participant | (ms) | (ms) | (mV) | (ms) | (ms) | (mV) | |
| 6 | 142.1 | 143.4 | 0.001 | 233.1 | 12.1 | 9E-04 | 70.9 |
| 7 | 97.12 | 163.9 | 0.002 | 37.06 | 38.36 | 0.001 | 43.7 |
| 8 | 66.44 | 87.76 | 0.02 | 30.82 | 32.64 | 0.011 | 54.6 |
| 9 | 66.7 | 75.54 | 0.007 | 1.96 | 2.48 | 0.004 | 52.0 |
| 10 | 41.48 | 106 | 0.058 | 68.78 | 113.76 | 0.092 | 158.9 |
| 11 | 484.26 | 29 | 0.005 | 85.42 | 106.22 | 0.019 | 371.2 |
| 12 | 38.1 | 136.1 | 0.01 | 47.98 | 127.28 | 0.001 | 10.0 |
| 13 | 98.47 | 156.1 | 0.008 | 89.32 | 111.16 | 0.01 | 127.5 |
| Mean | 129.334 | 112.2 | 0.014 | 74.305 | 68 | 0.017 | 123.9 |

| LGAS | SAS Onset | Peak | Amplitude | Pre-pulse and SAS Onset | Peak | Amplitude | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| Participant | (ms) | (ms) | (mV) | (ms) | (ms) | (mV) | |
| 6 | 41.22 | 166.8 | 0.002 | 120 | 133 | 0.002 | 90.6 |
| 7 | 218.54 | 83.08 | 0.002 | 224.78 | 41.74 | 0.004 | 177.8 |
| 8 | 50.32 | 122.3 | 0.018 | 92.44 | 111.68 | 0.02 | 113.4 |
| 9 | 70.34 | 111.4 | 0.013 | 101.02 | 102.84 | 0.004 | 27.8 |
| 10 | 61.76 | 135.9 | 0.074 | 68.52 | 106.22 | 0.083 | 111.5 |
| 11 | 131.96 | 199.3 | 0.002 | 38.1 | 107.26 | 0.008 | 454.0 |
| 12 | 77.36 | 97.12 | 0.003 | 14.44 | 123.64 | 6E-04 | 22.2 |
| 13 | 92.44 | 136.1 | 0.095 | 32.9 | 112.7 | 0.044 | 46.8 |
| Mean | 92.9925 | 131.5 | 0.026 | 86.525 | 104.89 | 0.021 | 79.2 |

| ROOC<br>Participant | SAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and SAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 13.4 | 53.18 | 0.039 | 47.46 | 49.28 | 0.02 | 51.8 |
| 7 | 15.48 | 116.1 | 0.174 | 42 | 149.12 | 0.049 | 28.3 |
| 8 | 15.22 | 79.18 | 0.228 | 33.94 | 139.76 | 0.013 | 5.6 |
| 9 | 12.88 | 62.8 | 0.083 | 22.24 | 54.74 | 0.023 | 27.5 |
| 10 | 12.1 | 54.22 | 0.105 | 33.16 | 180.84 | 0.08 | 76.2 |
| 11 | 40.48 | 59.68 | 0.015 | 17.82 | 46.42 | 0.014 | 95.2 |
| 12 | 20.06 | 75.28 | 0.056 | | 74.5 | 0.025 | 44.2 |
| 13 | 18.86 | 125.2 | 0.247 | 19.9 | 173.82 | 0.159 | 64.3 |
| Mean | 18.56 | 78.21 | 0.119 | 30.931 | 108.56 | 0.048 | 40.4 |

| RSCM<br>Participant | SAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and SAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 25.62 | 72.16 | 0.004 | 28.74 | 131.44 | 0.002 | 56.8 |
| 7 | 51.88 | 70.86 | 0.021 | 121.04 | 139.5 | 0.007 | 33.0 |
| 8 | 7.68 | 67.22 | 0.109 | 20.94 | 70.6 | 0.068 | 62.0 |
| 9 | 15.74 | 108.8 | 0.036 | 30.04 | 78.92 | 0.004 | 10.1 |
| 10 | 23.28 | 122.1 | 0.009 | 69.04 | 163.94 | 0.013 | 144.7 |
| 11 | 77.1 | 197.5 | 0.012 | 55.52 | 139.24 | 0.012 | 104.0 |
| 12 | 49.54 | 76.06 | 0.12 | 39.66 | 185.24 | 0.014 | 11.8 |
| 13 | 18.6 | 178 | 0.121 | 24.32 | 183.18 | 0.136 | 113.0 |
| Mean | 33.68 | 111.6 | 0.054 | 48.663 | 136.51 | 0.032 | 59.4 |

| LSCM<br>Participant | SAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and SAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PSAS/SAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 34.72 | 67.22 | 0.008 | 0.4 | 136.12 | 0.009 | 115.8 |
| 7 | 41.74 | 75.54 | 0.004 | 121.04 | 130.14 | 0.001 | 32.7 |
| 8 | 24.06 | 65.4 | 0.027 | 20.94 | 73.2 | 0.01 | 36.4 |
| 9 | 22.5 | 100.2 | 0.112 | 30.04 | 136.12 | 0.028 | 24.6 |
| 10 | 10.8 | 71.38 | 0.045 | 69.04 | 157.44 | 0.043 | 95.5 |
| 11 | 90.88 | 200.1 | 0.004 | 55.52 | 165.24 | 0.009 | 204.9 |
| 12 | 48.24 | 76.32 | 0.157 | 39.66 | 80.22 | 0.019 | 12.3 |
| 13 | 15.48 | 105.2 | 0.203 | 24.32 | 139.5 | 0.156 | 76.8 |
| Mean | 36.0525 | 95.17 | 0.07 | 45.12 | 127.25 | 0.034 | 49.0 |

# Appendix 6: RAS Responses and RAS with Pre-Pulses Responses

| RSOL | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 66.7 | 90.1 | 0.00066 | 80.74 | 88.02 | 0.000375 | 56.8 |
| 7 | 40.96 | 104.14 | 0.00342 | 34.72 | 82.3 | 0.003905 | 114.2 |
| 8 | 75.8 | 102.32 | 0.009175 | 63.06 | 84.12 | 0.00698 | 76.1 |
| 9 | 31.86 | 115.32 | 0.00188 | 52.66 | 111.42 | 0.00074 | 39.4 |
| 10 | 72.42 | 109.86 | 0.006605 | 62.02 | 111.94 | 0.00515 | 78.0 |
| 11 | 115.84 | 127.02 | 0.001005 | 97.9 | 109.6 | 0.00054 | 53.7 |
| 12 | 43.3 | 79.44 | 0.003395 | 48.5 | 81 | 0.003015 | 88.8 |
| 13 | 70.6 | 104.14 | 0.004865 | 76.84 | 92.7 | 0.00329 | 67.6 |
| Avg | 64.685 | 104.0425 | 0.003876 | 64.555 | 95.1375 | 0.002999 | |

| LSOL | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 72.94 | 90.1 | 0.00102 | 76.32 | 90.1 | 0.00096 | 94.1 |
| 7 | 50.32 | 88.54 | 0.002065 | 38.88 | 79.7 | 0.002415 | 116.9 |
| 8 | 43.56 | 105.44 | 0.00682 | 58.12 | 83.08 | 0.005695 | 83.5 |
| 9 | 37.58 | 117.92 | 0.00156 | 48.24 | 112.72 | 0.00125 | 80.1 |
| 10 | 39.92 | 112.98 | 0.00679 | 83.08 | 103.88 | 0.005475 | 80.6 |
| 11 | 56.3 | 129.1 | 0.00116 | 56.3 | 125.98 | 0.001905 | 164.2 |
| 12 | 37.58 | 55 | 0.002875 | 47.98 | 100.76 | 0.00174 | 60.5 |
| 13 | 60.46 | 101.02 | 0.005375 | 63.84 | 101.54 | 0.003695 | 68.7 |
| Avg | 49.8325 | 100.0125 | 0.003458 | 59.095 | 99.72 | 0.002892 | |

| RDEL | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 0.4 | 3.26 | 0.00037 | 0.4 | 20.68 | 0.00028 | 75.7 |
| 7 | 153.02 | 162.12 | 0.00008 | 165.24 | 175.12 | 0.00009 | 112.5 |
| 8 | 74.5 | 182.4 | 0.00005 | 3.26 | 146.26 | 0.00005 | 100.0 |
| 9 | 0.4 | 2.74 | 0.00014 | 0.4 | 0.4 | 0.0001 | 71.4 |
| 10 | 0.4 | 4.82 | 0.00136 | 0.4 | 2.48 | 0.00135 | 99.3 |
| 11 | 9.76 | 19.64 | 0.00028 | 0 | 69.3 | 0.00035 | 125.0 |
| 12 | 0.4 | 17.82 | 0.00094 | 0.4 | 17.56 | 0.00092 | 97.9 |
| 13 | 0.4 | 12.62 | 0.00081 | 0.4 | 20.94 | 0.00077 | 95.1 |
| Avg | 29.91 | 50.6775 | 0.000504 | 21.3125 | 56.5925 | 0.000489 | |

| LDEL | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 0 | 5.34 | 0.00017 | 0 | 13.4 | 0.00008 | 47.1 |
| 7 | 0.4 | 24.84 | 0.00006 | 0.4 | 23.8 | 0.00007 | 116.7 |
| 8 | 0.4 | 134.04 | 0.00014 | 0.4 | 23.54 | 0.00009 | 64.3 |
| 9 | 79.18 | 93.22 | 0.00058 | 47.72 | 118.44 | 0.00025 | 43.1 |
| 10 | 22.24 | 159.26 | 0.00034 | 91.66 | 108.82 | 0.00017 | 50.0 |
| 11 | 99.46 | 125.72 | 0.00017 | 55.78 | 59.94 | 0.0001 | 58.8 |
| 12 | 0 | 138.72 | 0.00011 | 297.32 | 0.14 | 0.00006 | 54.5 |
| 13 | 0.92 | 148.86 | 0.00024 | 444.22 | 65.14 | 0.00002 | 8.3 |
| Avg | 25.325 | 103.75 | 0.000226 | 117.1875 | 51.6525 | 0.000105 | |

| RGAS | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 79.44 | 92.96 | 0.000385 | 72.94 | 89.58 | 0.00042 | 109.1 |
| 7 | 39.14 | 84.64 | 0.00063 | 32.64 | 80.22 | 0.00057 | 90.5 |
| 8 | 70.08 | 96.08 | 0.002685 | 59.68 | 82.3 | 0.002075 | 77.3 |
| 9 | 42.52 | 112.46 | 0.001815 | 39.4 | 47.72 | 0.001035 | 57.0 |
| 10 | 53.96 | 113.24 | 0.00424 | 50.58 | 111.42 | 0.00321 | 75.7 |
| 11 | 114.02 | 122.6 | 0.002115 | 49.54 | 124.16 | 0.000925 | 43.7 |
| 12 | 31.94 | 60.2 | 0.000535 | 37.58 | 178.76 | 0.000355 | 66.4 |
| 13 | 67.22 | 94.26 | 0.001045 | 73.72 | 83.34 | 0.000675 | 64.6 |
| Avg | 62.29 | 97.055 | 0.001681 | 52.01 | 99.6875 | 0.001158 | |

| LGAS | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 33.16 | 83.6 | 0.000675 | 73.46 | 87.5 | 0.000355 | 52.6 |
| 7 | 50.84 | 83.6 | 0.001845 | 44.34 | 77.1 | 0.002065 | 111.9 |
| 8 | 38.88 | 105.7 | 0.00132 | 53.58 | 80.22 | 0.001335 | 101.1 |
| 9 | 9.5 | 110.38 | 0.004975 | 33.94 | 108.82 | 0.003465 | 69.6 |
| 10 | 83.08 | 113.5 | 0.003925 | 67.48 | 99.98 | 0.003215 | 81.9 |
| 11 | 56.3 | 132.22 | 0.001105 | 40.44 | 83.86 | 0.00089 | 80.5 |
| 12 | 43.3 | 113.5 | 0.000525 | 41.22 | 87.76 | 0.000575 | 109.5 |
| 13 | 55.52 | 103.62 | 0.00203 | 70.86 | 85.16 | 0.00112 | 55.2 |
| Avg | 46.3225 | 105.765 | 0.00205 | 53.165 | 88.8 | 0.001628 | |

| RSCM | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 14.7 | 29 | 0.000115 | 21.72 | 30.56 | 0.00008 | 69.6 |
| 7 | 13.14 | 26.66 | 0.00073 | 81.52 | 95.3 | 0.000555 | 76.0 |
| 8 | 37.84 | 76.06 | 0.00104 | 9.5 | 67.74 | 0.000575 | 55.3 |
| 9 | 14.18 | 25.88 | 0.000445 | 12.88 | 31.08 | 0.000475 | 106.7 |
| 10 | 249.48 | 88.02 | 0.00001 | 0 | 46.42 | 0.00001 | 100.0 |
| 11 | 13.14 | 51.36 | 0.000125 | 38.62 | 86.72 | 0.000205 | 164.0 |
| 12 | 57.6 | 63.32 | 0.00049 | 18.62 | 26.14 | 0.000485 | 99.0 |
| 13 | 30.82 | 75.8 | 0.000595 | 0 | 7.68 | 0.000045 | 7.6 |
| Avg | 53.8625 | 54.5125 | 0.000444 | 22.8575 | 48.955 | 0.000304 | |

| LSCM | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 0.4 | 29 | 0.000425 | 0.4 | 24.58 | 0.000405 | 95.3 |
| 7 | 9.76 | 128.84 | 0.000115 | 74.76 | 83.6 | 0.000095 | 82.6 |
| 8 | 49.54 | 69.04 | 0.00013 | 13.92 | 59.94 | 0.000055 | 42.3 |
| 9 | 26.14 | 48.76 | 0.000505 | 476.2 | 28.74 | 0.000205 | 40.6 |
| 10 | 0.4 | 4.56 | 0.000145 | 0.4 | 0.92 | 0.00017 | 117.2 |
| 11 | 10.02 | 33.68 | 0.000255 | 21.2 | 37.58 | 0.000235 | 92.2 |
| 12 | 133.52 | 166.8 | 0.0002 | 1.96 | 1.96 | 0.00007 | 35.0 |
| 13 | 0.4 | 72.42 | 0.000585 | 60.72 | 62.8 | 0.000085 | 14.5 |
| Avg | 28.7725 | 69.1375 | 0.000295 | 81.195 | 37.515 | 0.000165 | |

| ROOc | RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Pre-pulse and RAS Onset (ms) | Peak (ms) | Amplitude (mV) | Percent Amplitude of PP (PRAS/RAS*100) |
|---|---|---|---|---|---|---|---|
| 6 | 20.42 | 60.46046 | 0.012058 | 14.18 | 62.80046 | 0.006909 | 57.3 |
| 7 | . | 58.12046 | 0.042332 | . | 37.32044 | 0.027316 | 64.5 |
| 8 | 13.92 | 56.04046 | 0.052984 | 9.5 | 51.10045 | 0.033371 | 63.0 |
| 9 | 25.62 | 48.76045 | 0.002721 | 24.32 | 57.60046 | 0.001583 | 58.2 |
| 10 | 12.1 | 57.60046 | 0.065219 | . | 48.76045 | 0.05454 | 83.6 |
| 11 | 19.6 | 59.94046 | 0.001136 | . | 38.88044 | 0.001318 | 116.0 |
| 12 | . | 73.46047 | 0.007805 | . | 63.84046 | 0.010458 | 134.0 |
| 13 | 3 | 50.58045 | 0.026367 | 3 | 43.04044 | 0.011954 | 45.3 |
| Avg | 15.77667 | 58.12046 | 0.026328 | 12.75 | 50.41795 | 0.018431 | 77.7 |

## Appendix 7: UBC Research Ethics Board Certificate of Approval

*The University of British Columbia*
*Office of Research Services*
*Clinical Research Ethics Board – Room 210,*
*828 West 10th Avenue, Vancouver, BC V5Z 1L8*

# ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL WITH AMENDMENTS TO THE STUDY

| PRINCIPAL INVESTIGATOR: | DEPARTMENT: | UBC CREB NUMBER: |
|---|---|---|
| J.Timothy Inglis | | H05-70609 |

**INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:**

| Institution | Site |
|---|---|
| UBC | Vancouver (excludes UBC Hospital) |

**Other locations where the research will be conducted:**
Not applicable

**CO-INVESTIGATOR(S):**
Brynne Elliott
Melanie G. Roskell
Jean-Sébastien Blouin
Dave Nichol

**SPONSORING AGENCIES:**
- Natural Sciences and Engineering Research Council of Canada (NSERC) - "Sensory contributions to human movement and balance"

**PROJECT TITLE:**
Sensory contributions to human movement and balance

**The current UBC CREB approval for this study expires: April 24, 2009**

**AMENDMENT(S):**

| Document Name | Version | Date |
|---|---|---|
| **Consent Forms:** | | |
| Sensory Posture consent form | version 9 | April 9, 2008 |

**AMENDMENT APPROVAL DATE:**
**April 24, 2008**

CERTIFICATION:
In **respect of clinical trials:**
*1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.*
*2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.*
*3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.*

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.