### LOADING ENHANCES THE OCCURRENCE OF STARTLE RESPONSES IN LEG MUSCLES

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Abstract—Introduction: The startle reflex is an involuntary reaction to sudden sensory input and consists of a generalized flexion response. Startle responses in distal leg muscles occur more frequently during standing compared to sitting. We hypothesized that sensory input from load receptors modulates the occurrence of startle responses in leg muscles.

Methods: We administered sudden startling auditory stimuli (SAS) to 11 healthy subjects while (1) sitting relaxed, (2) standing relaxed, (3) standing while bearing 60% of their weight on the right leg, (4) standing while bearing 60% of their weight on the left leg, and (5) standing with 30% body weight support ('bilateral unloaded'). The requested weight distribution for each condition was verified using force plates. Electromyography data were collected from both tibialis anterior (TA) and the left sternocleidomastoid muscles. Results: In the TA, startle responses occurred much more frequently during normal standing (26% of trials) compared to both sitting (6% of trials, p < 0.01) and bilateral unloading (3% of trials, p < 0.01). In the asymmetrical stance conditions, startle responses in the TA were more common in the loaded leg (21% of trials) compared to the unloaded leg (10% of trials, p < 0.05).

*Discussion:* The occurrence of startle responses in the leg muscles was strongly influenced by load. Hence, it is likely that information from load receptors influences startle response activity. We suggest that, in a stationary position, startling stimuli result in a descending volley from brainstem circuits, which is gated at the spinal level by afferent input from load receptors. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: startle reflex, postural control, load receptors, reticulospinal tract.

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Abbreviations: EMG, electromyography; pmr, pontomedullary reticular formation; SAS, startling auditory stimuli; SCM, sternocleidomastoid; SPL, sound pressure level; TA, tibialis anterior.

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#### INTRODUCTION

The startle reflex is a generalized flexion response to sudden sensory input and is the fastest generalized motor reaction in humans and animals (Valls-Sole et al., 2008). The startle reflex arises from the pontomedullary reticular formation (Davis et al., 1982; Yeomans and Frankland, 1995) and the latencies of the muscle responses increase with the distance of the respective muscle from the caudal brainstem (Brown et al., 1991b). Interestingly, startle responses in distal leg muscles occur more frequently in a standing position compared to sitting relaxed (Brown et al., 1991a; Delwaide and Schepens, 1995). Therefore, it has been suggested that the function of the startle responses in distal leg muscles lies in rapidly accomplishing a defensive stance with maximum postural stability (Brown et al., 1991a). However, it is unknown how the startle reflex is modified during various postures.

We hypothesized that the amount of loading of a leg contributes to the occurrence of startle responses in leg muscles. A large body of evidence exists that leg muscle activity can be modulated by various loading conditions (Dietz and Duysens, 2000; Duysens et al., 2000). For example, an increase in loading yielded larger amplitudes of postural responses (Dietz et al., 1989; Horstmann and Dietz, 1990). Furthermore, the observation that startle responses during gait are larger during the stance compared to the swing phase may also be suggestive of load-induced modulation (Schepens and Delwaide, 1995; Nieuwenhuijzen et al., 2000). We aimed to seek further evidence for this hypothesis by investigating startle reflexes during various loading conditions of the legs.

#### **EXPERIMENTAL PROCEDURES**

#### **Participants**

Participants in this study were 11 healthy adults (seven women, four men; mean 24 years, range 20–28 years). None of them suffered from any hearing, neurological or motor disorder that could interfere with their performance during the experiments. All subjects gave written informed consent prior to the experiment. The experiments conformed with the standards of the Declaration of Helsinki and with local ethical guidelines.

#### Experimental setup and protocol

Startling auditory stimuli (SAS) were given in five different body positions ('conditions'). In each condition four SAS were delivered. Subjects were either (1) sitting quietly (in a chair with

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backrest), (2) standing quietly (with equal weight distribution), (3) standing while bearing 60% of their weight on the right leg (40% on the left leg), (4) standing while bearing 60% of their weight on the left leg (40% on the right leg) or, (5) standing symmetrically with 30% body weight support ('bilateral unloaded'). Bilateral unloading was achieved by suspending the participants from a parachute harness connected to an overhead crane. Conditions 3 and 4 are referred to as 'unilateral loaded'. We chose for the subtle 60-40% weight distribution in these unilateral loaded conditions, to ensure that both legs were still involved in the regulation of posture (Anker et al., 2008). During conditions 2-5, weight distribution was monitored using two force plates and feedback was given to the participants. Acceptable deviations were within  $\pm 5\%$ .

20 SAS were given divided over three sessions (six or seven stimuli per session) on separate days, to prevent habituation of the startle reflex. Each session comprised a maximum of two trials of the same conditions and the order of the conditions was varied between sessions. Within one session, the period between two subsequent SAS was approximately 5 min. The SAS were given through binaural earphones and consisted of 50 ms of white noise with an intensity of 117 dB sound pressure level (SPL), generated by a custom-made noise generator. Acoustic stimuli are commonly used to evoke startle response (Brown et al., 1991a; Valls-Sole et al., 1999; Carlsen et al., 2004).

Data collection. Electromyography (EMG) data were collected from bilateral tibialis anterior (TA) and left sternocleidomastoid (SCM) muscles. Self-adhesive Ag–AgCl electrodes (Tyco Arbo ECG) were placed approximately 2 cm apart and longitudinally on the belly of each muscle, according to Seniam guidelines (Hermens et al., 1999). EMG signals were sampled at 2000 Hz and band-pass filtered at 10 and 500 Hz.

Data analysis. Two observers, who were blinded for the conditions, independently identified startle-induced responses from the EMG signals and, if present, they determined the onset latencies. A response had to occur within 100 ms after SAS for the SCM (Brown et al., 1991b; Thevathasan et al.,

2011) and within 180 ms for the TA (Brown et al., 1991a). For each muscle, the rate of occurrence of startle-induced activity was determined as the percentage of trials in which a response could be identified. The rates of occurrence and onset latencies were averaged for both raters; the rate of occurrence did not differ between the two raters, the maximum deviation of latencies was 4 ms. In addition, for each condition the mean background EMG activity in the TA was determined over a period of 500 ms before the SAS.

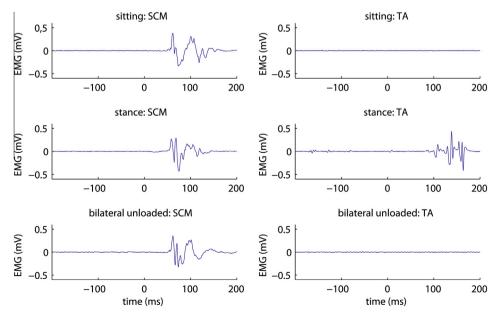
Statistical analysis. The occurrences of startle responses were analyzed using Pearson's Chi-square tests. The onset of SCM responses and background EMG activity in TA were compared between the various conditions using a repeated measures analysis of variance (ANOVA) with 'Condition' as a within-subjects factor. The alpha level was set at 0.05.

#### RESULTS

#### Occurrence of startle responses

The SAS resulted in startle responses in the SCM in 77% (bilateral unloaded) to 93% (quiet sitting and standing) of the trials (Fig. 2a). The occurrence of startle responses in the SCM did not differ between the quiet sitting, quiet standing and unilateral loaded conditions (p > 0.18). However, the occurrence of the startle responses in the SCM was less frequent in the bilateral unloaded condition compared to quiet standing (p = 0.034).

There was an absence of background activity in the TA in all conditions (see Fig. 1); the mean activity varied between 0.0045 and 0.0059 mV (no significant differences between conditions:  $F_{4,7} = 2.107;$ p = 0.183). TA responses occurred much more frequently during quiet standing (26%) compared to sitting (6%) (p < 0.010) and bilateral unloading (3%) (p < 0.004; Fig. 2b). In the symmetrical standing condition, the rates of occurrence of TA responses did not differ between the left (27%) and right leg (25%;



**Fig. 1.** EMG traces of a representative subject during the sitting, stance and bilateral unloaded conditions, for both the sternocleidomastoid (SCM, left panels) and tibialis anterior muscle (TA, right panels). For these trials the latencies were determined as follows: sitting SCM = 50 ms; stance SCM = 53 ms; bilateral unloaded SCM = 54 ms and stance TA = 100 ms.

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