Modulation of the Startle Response During Human Gait

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Nieuwenhuijzen, P.H.J.A., A. M. Schillings, G. P. Van Galen, and J. Duysens. Modulation of the startle response during human gait. J Neurophysiol 84: 65–74, 2000. While many studies have shown that there is a phase-dependent modulation of proprioceptive and exteroceptive reflexes during gait, little is known about such modulation for auditory reflexes. To examine how startle reactions are incorporated in an ongoing gait pattern, unexpected auditory stimuli were presented to eight healthy subjects in six phases of the step cycle during walking on a treadmill at 4 km/h. For both legs, electromyographic activity (EMG) was recorded in the biceps femoris (BF), the rectus femoris (RF), the tibialis anterior (TA), and the soleus (SO). In addition, stance and swing phases of both legs, along with knee angles of both legs and the left ankle angle, were measured. All subjects showed various response peaks. Responses with latencies of ~60 ms (F1), ~85 ms (F2), and ~145 ms (F3) were found. The amplitude of the reflex responses was dependent on the timing of the startle stimulus in the step cycle. Although the startle response habituated rapidly, the phase-dependent modulation pattern generally remained the same. The phase-dependent amplitude modulations were not strictly correlated with the modulation of the background activity. The TA even showed a transition from facilitatory F2 responses during stance to suppressive responses during midswing. Responses were observed in both flexors and extensors, often in coactivation, especially during stance. Furthermore, the gait characteristics showed a shortening of the subsequent step cycle and a small decrease in the range of motion of ankle and knees. These results suggest that the responses are adapted to achieve extra stability dependent on the phase of the step cycle. However, even in the first trials, the changes in kinematics were small allowing a smooth progression of gait.

INTRODUCTION

The auditory startle response (ASR) is a generalized motor response caused by a sudden, loud acoustic stimulus. The nucleus reticularis pontis caudalis is known as the last brain stem relay mediating the ASR (Davis 1984; Yeomans and Frankland 1996). The characteristic motor sequence is mostly described as having a rostrocaudal distribution of the muscle responses (Brown et al. 1991b; Davis 1984; Landis and Hunt 1939). Wilkins et al. (1986), for example, recorded auditory startle responses in sitting subjects with latencies of 100–125 ms in the hamstrings and 130–140 ms in the tibialis anterior (TA). Generally the ASR is considered as a response in which flexor activity dominates (Davis 1984; Landis and Hunt 1939; Rossignol 1975), although some authors recorded clear extensor responses (Brown et al. 1991a; b; Delwaide and Schepens 1995). Furthermore the ASR is known for a decline in muscle responses with repeated presentations of the eliciting stimulus (Davis 1984; Landis and Hunt 1939).

Landis and Hunt (1939) described the response as a relatively immutable basic alerting response largely independent of posture. However, several studies recorded twice as many responses in TA and soleus (SO) in subjects who were standing compared with subjects who were sitting (Brown et al. 1991a; Delwaide and Schepens 1995). In addition, the latency of TA and SO responses changed from ~120 ms measured in a sitting position to 70–95 ms measured in a standing position (Brown et al. 1991a; Schepens and Delwaide 1995).

There is some debate about the relation between background activity and amplitude of the startle response. Brown et al. (1991a) and Delwaide and Schepens (1995) did not observe a facilitatory effect of background activity. However, Rossignol (1975) reported a higher incidence of startle responses in SO in the presence of background electromyographic (EMG) activity.

On the basis of the observed effects of posture on the incidence and latency of the ASR, Brown et al. (1991a) suggested that the physiological importance of the ASR lies in the rapid accomplishment of a defensive stance with maximum postural stability. Rossignol (1975) emphasized the necessity of testing ASR during locomotion to acquire full understanding of the functional significance of the ASR.

So far, only Schepens and Delwaide (1995) studied the ASR during human gait. When auditory stimuli were delivered at the start of SO and TA activity, they found responses to be absent in SO during both periods in the step cycle but present in TA in periods when that muscle is normally inactive. Apparently, the modulation of the ASR in TA differs from the modulation of the background activity. This suggests that the responses during gait are actively modulated at a premotoneuronal level. Such modulation is well known for other types of reflexes, such as cutaneous and proprioceptive reflexes, where the amplitude of responses depends heavily on the phase in which the stimulation is applied (Capaday and Stein 1986; Duysens et al. 1990; Van Wezel et al. 1997; Yang and Stein 1990; Zehr et al. 1997). It is not clear whether such phase dependency also...
occur for the ASR. In the Schepens and Delwaide study (1995), auditory stimulation was limited to the period of the onset of SO and TA activity, and there was no systematic investigation of response modulation using several phases. Furthermore, startle responses are known to occur in a wide variety of leg muscles during sitting and standing while in the Schepens and Delwaide (1995) study only the TA and SO were investigated. Therefore the aim of the present study was to investigate the auditory startle response in both upper and lower leg muscles, during human walking, in different periods of the step cycle. A preliminary account of the results has been given (Nieuwenhuijzen et al. 1997).

METHODS

Experimental setup

Startle reactions were recorded in eight healthy subjects (4 males and 4 females; age range: 19–27 yr) after informed consent had been obtained. The experiments were performed in conformity with the declaration of Helsinki for experiments on humans. None of the subjects had a known hearing, neurological or motor disorder. The subjects were asked to walk on a treadmill at 4 km/h while wearing a safety harness that was fastened to an emergency brake at the ceiling. An additional emergency brake was attached to the handrail of the treadmill to make sure that the subject could stop the treadmill at any moment.

A custom-made noise generator delivered auditory stimuli through binaural earphones. The stimulus consisted of 50-ms white noise with an intensity of 110 dB. Bipolar surface electrodes measured EMG of the biceps femoris (BF), the rectus femoris (RF), the TA, and the SO muscles of both legs. The EMG signals were (pre-) amplified (by a factor in the order of $10^4$–$10^5$), high-pass filtered (cutoff frequency at 3 Hz), full wave rectified, and then low-pass filtered (cutoff frequency at 300 Hz). The activation pattern during gait of all muscles was visually inspected to test for possible cross-talk. Kinematic measurements were made by laterally placed goniometers on both knees and the left ankle. Thin insole footswitches (designed in collaboration with Algra Fotometaal b.v., Wormerveer, The Netherlands) were used to detect foot contact and to deliver a trigger signal for the timing of the stimulus. The data were sampled at 500 Hz and stored on hard disk in individual trials (i.e., no stimulus) were measured 4 s prior to stimulus trials. The data were presented in a random order. Hence, a total of 30 stimuli were presented. Control trials (i.e., no stimulus) were measured 4 s prior to stimulus trials. The signals were visually inspected by on-line monitoring on an oscilloscope and on a computer display.

Data analysis

For each phase, the control data were averaged and subtracted from both individual and averaged stimulus trials. This subtraction method enables one to look at the net effect of the stimulus. Hence both facilitatory and suppressive responses can be observed (Duyssens et al. 1990; Van Wezel et al. 1997; Yang and Stein 1990; Zehr et al. 1997). For each muscle, a single time window was set over six phases of the step cycle. These responses were used to estimate the overall window setting. In this way, 60 traces were investigated for each subject (6 phases $\times$ 10 trials). In these 60 traces, several well-defined responses were always found. These responses were used to estimate the overall window setting. In
this way, a window could be set for all the muscles of all subjects (cf. Duysens et al. 1991; Tax et al. 1995; Yang and Stein 1990) (see Fig. 2). Latency and duration was defined as the onset and duration of the time window. The response amplitudes were calculated by averaging the rectified EMG within the time window. To enable a proper comparison between the different muscles and subjects, the response amplitudes were normalized with respect to the maximum EMG activity during the control step cycles. To determine whether the responses observed were statistically significant and to compare mean response amplitude, latency, and duration between the different muscles, the Wilcoxon signed-rank test was used. Phase-dependent modulation was tested by the Kruskall-Wallis one-way ANOVA. Potential cross-talk was investigated by the Spearman correlation test. The difference in stance and swing phase duration between control and stimulus trials was analyzed by the Wilcoxon signed-rank test. The same test was used to evaluate stimulus-induced changes in joint angles (as measured by goniometers). Sequential effects in EMG, joint angles, and stance and swing duration were also examined by the Wilcoxon signed-rank test. In all statistical tests a significance level of $P < 0.05$ was used.

**RESULTS**

To be able to compare reflex responses between left and right leg (see further), we first had to ensure that the gait activation patterns in the muscles of both legs were comparable. For this purpose, the background locomotor patterns of each muscle and each subject were normalized and then averaged for the whole population (see Fig. 3A). Figure 3, A and B, shows that the EMG patterns of the muscles were almost identical for the two legs. In addition, as expected, the EMG variability between subjects was small (see SE in Fig. 3A), and in agreement with the literature (see, for instance, Rose and Gamble 1994).

**Response latency and duration**

Responses were found in all subjects ($n = 8$) and all muscles. Quantification of these responses was done by setting a time window around the responses (see METHODS). Three facilitatory responses were detected, which were termed F1 [mean latency, 59 ± 7 (SE) ms; duration, 42 ± 11 (SE) ms], F2 (latency, 83 ± 8 ms; duration, 63 ± 10 ms), and F3 (mean latency, 146 ± 8 ms; duration, 67 ± 11 ms) (see Fig. 4, A and B). The latencies and durations of the responses are given in Table 1.

Very early facilitatory responses (F1 in Fig. 4B and Table 1) were observed in TA and SO especially during early stance and early swing. These responses were small but distinct and were observed in TA in four subjects and in SO in five subjects. The F2 and F3 responses, in contrast, were seen in all subjects and all muscles. The mean latency of the F2 of the BF (86 ± 9 ms) was slightly longer than the mean latency of the F2 of the other muscles (especially with respect to the TA, 82 ± 6 ms; see Table 1). However, this difference was not statistically significant (Wilcoxon signed-rank test: $P > 0.05$). In addition, the F3 response latencies also showed no significant differences. Instead of a facilitatory F2 response, short suppressive responses (S in Fig. 4B) were seen in the lower leg muscles TA and SO, with mean a latency of 101 ± 11 ms and a duration of 42 ± 7 ms. In all but one subject, these suppressive responses were seen during swing in TA. In five subjects, less clear but

![Figure 3](http://jn.physiology.org/Downloaded from 10.220.32.246 on September 14, 2016)

**FIG. 3.** Averaged and normalized electromyographic (EMG) data of all trials and all subjects of the background activity for each muscle. A: averaged EMG data (white) of each muscle with standard error (black). B: averaged EMG data of the left leg (solid line) and the right leg (dotted line). The vertical lines in both A and B indicate the transitions between stance and swing. The biceps femoris (BF), a flexor of the knee and an extensor of the hip, is active at the end of the swing phase, decelerating the forward swinging leg. Activity remains till early stance to assist in extending the hip. At the end of the stance phase, a 2nd smaller burst is seen that may be involved in bending the knee and extending the hip when the foot comes off the ground (toe-off). The rectus femoris (RF), a flexor of the hip and an extensor of the knee, is active in early stance to restrict knee flexion. A 2nd activity period occurs after toe-off to prevent further knee flexion and initiate knee extension. The tibialis anterior (TA), a dorsal flexor of the foot, is maximally active just after the foot touches the ground (heel-strike) to decelerate the plantar flexion preventing the foot from slapping down on the floor. During midstance, TA activity is small or absent. Activity increases after toe-off to lift the foot up against gravity. The soleus (SO), a plantar flexor of the foot, is mainly active in the stance phase with a maximum at end stance to achieve heel rise. The activity of the SO is largely antagonistic to the activity of the TA.
consistent suppressive responses were also seen in SO during early/midstance. However, in the mean of all subjects, these suppressive responses in SO were not significant (Wilcoxon signed-rank test: \( P < 0.05 \)). These responses were enclosed by the window of the F2 responses and were therefore seen as a suppressive part of the F2 response.

**Amplitude**

For each subject the amplitudes of the responses were normalized (see METHODS) and averaged over all phases (see Table 1). In the upper leg muscles significant larger response activity (Wilcoxon signed-rank test: \( P < 0.05 \)) was measured than in the lower leg muscles. For example, the mean values (expressed as a fraction of maximum control activity) of the F2 of BF and RF were 0.26 and 0.29, respectively, as compared with TA and SO with mean values of 0.14 and 0.10 respectively. Especially RF showed large responses in both F2 (0.29) and F3 (0.28) that were significantly larger (Wilcoxon signed-rank test: \( P < 0.05 \)) than the F2 and F3 responses in the other muscles. In the lower leg, F2 and F3 of the TA (mean of 0.14 and 0.20, respectively) were larger than the equivalent responses of the SO (mean of 0.10 and 0.16), although only for F3 was the difference significant (Wilcoxon signed-rank test: \( P < 0.05 \)). With respect to the F1 responses in TA (mean of 0.04) and SO (mean of 0.03), these responses were much smaller than the other facilitatory responses in all other muscles.

**Phase-dependent reflex modulation**

The responses of the muscles generally depended on the timing of stimulation in the step cycle. This so-called “phase-dependent modulation” can be observed in Figs. 5–8 in which for each muscle the average subtracted responses (and SE) are shown with respect to their appearance in the step cycle. Responses were observed both in the leg that was in stance and in the leg that was in swing. Since there was no basic difference between the EMG activities of the two sides (see Fig. 3B), the three phases during stance from one leg and the three phases during swing from the other leg were taken to describe the whole step cycle (see Figs. 5–8). In other words, for phases 1–3, the EMGs of the right leg (stance phase) were used, whereas for phases 4–6, the EMGs of the equivalent muscle of the left leg (swing phase) was taken. Hence these plots can be used in two ways, either to study the phase dependency over the whole cycle or to evaluate the bilateral responses (in which case one has to consider that phases 1–3 are actually synchronous with phases 4–6 from the opposite leg).

Some groups, working on phase-dependent modulation of cutaneous responses, have argued that from a functional point of view the whole response should be considered rather than the individual components (Zehr et al. 1997). To examine whether this would be a valid approach for the presently

**TABLE 1. Startle response characteristics for the whole population**

<table>
<thead>
<tr>
<th></th>
<th>Latency, ms</th>
<th>Duration, ms</th>
<th>Amplitude</th>
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<tbody>
<tr>
<td>BF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>86 ± 3</td>
<td>62 ± 3</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>F3</td>
<td>148 ± 2</td>
<td>67 ± 4</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>RF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>82 ± 3</td>
<td>62 ± 4</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>F3</td>
<td>145 ± 3</td>
<td>73 ± 4</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>TA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>57 ± 2</td>
<td>43 ± 5</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>F2</td>
<td>82 ± 2</td>
<td>66 ± 3</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>F3</td>
<td>147 ± 3</td>
<td>62 ± 2</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>SO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>61 ± 3</td>
<td>41 ± 3</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>F2</td>
<td>83 ± 4</td>
<td>61 ± 5</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>F3</td>
<td>144 ± 3</td>
<td>65 ± 5</td>
<td>0.16 ± 0.01</td>
</tr>
</tbody>
</table>

The mean latency and duration was based on the window settings (\( n = 8 \); 1 window per muscle per subject). To calculate the mean amplitude, all responses (\( n = 480 \); 8 subjects \( \times \) 6 phases \( \times \) 10 responses) were used, expressed as fraction of the maximum background activity (see METHODS). Values are means ± SE.
studied startle responses as well, an analysis was made of the total responses. In the upper leg muscles the amplitude of the total response is the mean amplitude of the combined windows set for F2 and F3 and in the lower leg muscles the mean amplitude of F1–F3. The modulation pattern of the total responses showed clear phase-dependent modulation in all muscles (see Fig. 5). Except for phase 5 of the TA, all responses of all muscles were statistically significant (Wilcoxon signed-rank test: \( P < 0.05 \)). Responses were smallest around early/mid-swing (phases 4 and 5) and generally largest in early/mid-stance (phases 1 and 2). In addition, during late swing (phase 6), BF and RF showed large responses while TA and SO showed moderate responses. Agonistic muscles showed a similar modulation. To investigate possible contamination of cross-talk, a Spearman correlation test was performed on the amplitudes of all total responses. A weak correlation was found for the BF and RF (\( r = 0.36, P < 0.05 \)), and an even smaller correlation was found for the TA and SO (\( r = 0.21, P < 0.05 \)). The existing weak correlation can be explained by the agonistic coactivation. Therefore cross-talk is unlikely to account for the observed similarity in modulation.

Second, the modulation pattern of the individual responses will be dealt with. With respect to the F1 (see Fig. 6), the responses were only slightly modulated. Significant F1 responses (Wilcoxon signed-rank test: \( P < 0.05 \)) were recorded during phases 1, 2, and 4 in TA and 1, 4, and 6 in SO. Clearest responses in TA were seen during early swing (phase 4) and in SO during early/mid-stance (phase 1).

For F2 and F3 (see Figs. 7 and 8), it can be seen that in the upper leg, all responses were significant (Wilcoxon signed-rank test: \( P < 0.05 \)). In the lower leg muscles, most responses were significant except for the F2 in early and late swing (phases 4 and 6) in TA and 1, 4, and 6 in SO. Clearest responses in TA were seen during early swing (phase 4) and in SO during early/mid-stance (phase 1). For F2 and F3 (see Figs. 7 and 8), it can be seen that in the upper leg, all responses were significant (Wilcoxon signed-rank test: \( P < 0.05 \)). In the lower leg muscles, most responses were significant except for the F2 in early and late swing (phases 4 and 6) in TA and for the F2 in early/mid-stance (phase 1) in SO. The modulation pattern for the F2 responses was similar to the one seen for the total responses (see Fig. 5), although the modulation depth (difference between maximum and minimum) was more pronounced in BF and TA for F2. However, unlike the average of all responses, the F2 in SO showed small responses during phase 1 (see Fig. 5).

There was no strict correlation between the response modulation and the background modulation. Differences between these modulations were especially prominent for the F2 responses in TA, which showed clear facilitatory responses in midstance when TA is normally inactive. Furthermore there...
was a reversal to suppressive responses in midswing when this muscle normally has high background activity.

For the F3 responses, such a reflex reversal was absent in TA (see Fig. 8). Moreover both the TA and the BF showed little variation in F3 response activity except for the peak in the TA at the transition point from swing to stance (phase 6). In RF and SO, the amplitudes of the F3 reflexes followed the background rather closely with the exception of the larger than expected RF and SO responses in early/midstance (phases 6 and 1 for RF and phase 1 for SO).

In conclusion, with the exception of the F3 of the BF and the F1 of the TA, a significant effect of phase (Kruskall-Wallis and 1 for RF and phase 1 for SO).

Effects on the kinesiology

The footswitches were used to indicate the onset and duration of the stance and swing phases. In general, as can be observed in Fig. 9, there was a slight shortening of the swing phase in the left leg (HO1: 1st heel-on in Fig. 9, A and B, left) and the stance phase in the right leg (TO1: 1st toe-off in Fig. 9, A and B, right) during which the stimulation was given, but in both cases this was not significant (Wilcoxon signed-rank test: \( P > 0.05 \)). However, subsequent phases were also affected and this introduced statistically significant changes in both the right leg (HO1 and TO2 in Fig. 9, A and B, right, with mean differences of 17 and 30 ms, respectively) and the left leg (TO1 and HO2 in Fig. 9, A and B, left, with mean differences of 20 and 25 ms, respectively).

Changes in joint angles were evaluated from the goniometers recordings. Behavioral changes were demonstrated by considering both timing and joint angle of the local maximum excursions in flexion or extension directions during stance and swing in an 800-ms period following stimulation. These values were compared with the timing and angle values of the control trials. The mean results for all subjects and all phases were pooled and are given in Table 2 and illustrated by Fig. 10.

Inspection of the timing results revealed that in general the maxima occurred earlier in the stimulated cycles as compared with the control cycles. This basically complements the earlier results derived from the footswitch data. In accordance with the footswitch data, the time difference between control and

### Table 2. Timing and amplitude for the whole population of the local joint angle maxima

<table>
<thead>
<tr>
<th></th>
<th>Delay, ms</th>
<th>Time Difference, ms</th>
<th>Angle, °</th>
<th>Angle Difference, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee right</td>
<td>FlSw</td>
<td>528</td>
<td>-11 ± 2.4*</td>
<td>-43</td>
</tr>
<tr>
<td></td>
<td>ExSw</td>
<td>805</td>
<td>-20 ± 2.6*</td>
<td>9</td>
</tr>
<tr>
<td>Knee left</td>
<td>ExSw</td>
<td>240</td>
<td>0 ± 1.9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>F1St</td>
<td>388</td>
<td>-12 ± 3.6*</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>ExSt</td>
<td>726</td>
<td>-21 ± 3.1*</td>
<td>13</td>
</tr>
<tr>
<td>Ankle left</td>
<td>ExSt</td>
<td>280</td>
<td>-2 ± 2.7*</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>F1st</td>
<td>721</td>
<td>-10 ± 4.2*</td>
<td>-12</td>
</tr>
</tbody>
</table>

* Stimulus-induced changes in timing and excursion of local maximum flexion and extension during stance and swing in the left and right knee and the left ankle in all subjects. The values are averages of the three phases. Delay: the average time from the stimulus to the respective local maximum. Time difference: the difference in time (\( n = 240 \)) between the local maxima of the control and the stimulus trials. Angle: the average angle at the local maximum. Angle difference: the difference in angle (\( n = 240 \)) of control and stimulus trials. The asterisks indicate significant differences. Fl, maximum flexion; Ex, maximum extension; St, stance; Sw, swing. The abbreviations are illustrated in Fig. 10. Values are means ± SE.
stimulus data increased when more time elapsed (see Table 2). The earliest significant change in the timing (16 ms) of the maxima in the average of all subjects was seen in phase 3 of the left knee at ∼275 ms after the stimulus was given.

The analysis of the average amplitude changes (“angle difference” in Table 2) showed generally a small (∼1°) but significant (Wilcoxon signed-rank test: P < 0.05) decrease in both maximum flexion and extension in both knees and ankle after stimulation. The first significant deviations of the amplitudes of the peaks were seen after ∼230 ms in the left knee (phase2) and as early as 195 ms in the left ankle (phase 1).

Habituation

The startle response is known for a rapid habituation. The first two trials of each phase showed the highest responses and after the third trial a more or less stable situation was found. Therefore sequential effects were investigated by comparing the first two trials of each phase with the last two trials (trials 9 and 10).

In Fig. 11 the influence of habituation is seen on the amplitude modulation over the step cycle. In all muscles, a decrease of amplitude was observed as a function of time although the amount of decrease varied between the muscles. The mean amplitudes of the last trials were 40, 21, 27, and 58% of the maximum amplitude decrease was never more than 3.6° compared with the control data. No significant sequential effects were observed (Wilcoxon signed-rank test: P > 0.05). However, sequential effects were observed in the long-term effects. The subsequent phase transition of the left leg (TO1) and the next phase transition in both legs (TO2 in the right leg and HO2 in the left leg), showed a significantly larger shortening in the first trials compared with the last trials. Note that Fig. 12 shows the average of the three phases. Among the three phases of each leg, no significant changes were seen.

In general, the gonio signals for both first and last trials showed effects that were similar to those seen in the average of all trials with a small decrease in range of motion and a shortening of the step cycle. However, no significant differences (Wilcoxon signed-rank test: P > 0.05) were observed between the first and the last trials. Even in the first trial the maximum amplitude decrease was never more than 3.6° compared with the control data.

D I S C U S S I O N

Incidence of startle responses during walking

In the present study, startle responses to an auditory stimulus during walking were found in all subjects and in all muscles. Auditory startle stimuli delivered to subjects who were standing, elicited a response in TA and SO in ∼65% of the subjects (Schepens and Delwaide 1995) and in ∼40% of the subjects during sitting (Delwaide and Schepens 1995). The present incidence of 100% might be related to the higher intensity of the sound used (50 ms of 110 dB vs. 30 ms of 90 dB in the Delwaide and Schepens study and the Schepens and Delwaide study). However, in a study on standing and sitting subjects, Brown et al. (1991a) did not find responses in all their subjects, despite the use of an auditory stimulus that was even higher than the stimulus used in the present study (124 dB during 50 ms). It is likely that the task of walking itself contributed to the high incidence found. Support for the task dependency of startle was given by Brown et al. (1991a), who recorded about twice as many responses in standing subjects as in sitting subjects. The difference in incidence between the two tasks was not due to the increased level of background activity occurring in a standing position since augmented isometric activity did not increase the incidence of responses (Brown et al. 1991a; Delwaide and Schepens 1995). Task-dependent
changes of reflexes are also found in other reflex studies. In cats, Drew (1991) found that electrical stimulation of brain stem areas, thought to be involved in startle responses, generally had larger effects during walking than during standing. In humans, similar task-dependent facilitation of responses has been reported by Duysens et al. (1993) for cutaneous reflexes during running as compared with standing.

**Amplitude of startle responses in various muscles during walking**

The present data showed that large responses are seen in the upper leg muscles BF and RF. Since previous work has concentrated on responses evoked during walking in lower leg muscles such as TA and SO (Schepens and Delwaide 1995), it is especially important to note that the response amplitudes of the upper leg muscles presently observed were significantly larger than the amplitudes of the lower leg muscles. Brown et al. (1991b) observed that the startle reaction is most prominent in the upper body and less marked in the lower half of the body. The present results show that the rostrocaudal distribution in amplitude is also present in the lower half of the body. These findings suggest that the rostrocaudal gradient might not only apply to latencies (see INTRODUCTION) but also to amplitudes of the startle responses.

Furthermore EMG responses were observed in both flexors and extensors. Several authors (Brown et al. 1991a,b; Delwaide and Schepens 1995) reported clear responses in the ankle extensor soleus. In general, however, the startle response is described as a reaction where flexor activity dominates (Davis 1984; Landis and Hunt 1939; Rossignol 1975). Because the TA and SO are monarticular, they are the only pure flexor and extensor muscles in the present study. The average responses of all phases showed indeed a larger mean response amplitude in the flexor TA than in the extensor SO. However, in late stance of the F2 and in early and late stance of the F3, the response amplitude of the SO was larger than the response amplitude of the TA. Hence it is oversimplified to state that the startle reaction is mainly a flexion reaction.

**Latency of startle responses during walking**

Several studies (Brown et al. 1991a; Delwaide and Schepens 1995; Schepens and Delwaide 1995) reported an effect of posture on latency and duration of the startle response. Two different latencies were found between standing/walking (80–95 ms) and sitting (120 ms). Delwaide and Schepens (1995) suggested the existence of two descending waves of bulbo-spinal activity. Brown et al. (1991a) even mentioned three bulbo-spinal waves with any one of these waves being present depending on the posture used. The existence of three waves was based on results from hyperekplexia patients. In these patients, who exhibit exaggerated startle responses, three response peaks were observed. In accordance with the healthy subjects, a response peak with a latency of 120 ms was seen in TA when sitting relaxed. However, in addition an earlier response was observed with a latency of 80 ms corresponding to latencies found in healthy subjects stimulated in a standing position. When the patients were standing, a third response was seen in TA after 60 ms. In this way, three waves were identified that, for the TA, gave responses with latencies of 60, 80, and 120 ms.

The three responses have similar latencies as the three responses found in the present study (60, 80, and 145 ms). Note that although in the present study two separate responses clearly were observed in the individual trials, in the average data, the transition of F2 to F3 was often blurred. This may account for the larger latency of F3 found here compared with the latency found when only one response was measured when subjects were sitting. No mention is made of the early F1 in the study of Schepens and Delwaide (1995) when the ASR was elicited in walking subjects. An explanation for this might be the higher stimulus intensities used in the present study. Alternatively, it is possible that the analysis method is critical. Schepens and Delwaide (1995) did not use the present subtraction method that enables one to filter out background activity, allowing detection of small responses such as the F1. The present data show that locomotion is a task that can reveal the existence of this early response in healthy subjects. As for the F3, the duration of the response (120 ms) recorded by Schepens and Delwaide (1995) suggests that no distinction was made between F2 and F3 (both ~60-ms duration), which may explain the absence of the F3.

In conclusion, in healthy subjects loud acoustic stimuli during locomotion induces all the startle response peaks observed in hyperekplexia patients. This is in accordance with the theory of Brown et al. (1991a) that the normal and pathological startle responses share the same neural pathway.

**Phase-dependent modulation**

In the present study, all muscles showed a clear phase-dependent modulation in the total responses. As for the individual responses, the present data showed that for some muscles the phase-dependent modulation is somewhat different for the three responses, thereby lending further support to the contention that these are independently controlled responses. Variations in the amplitudes of responses in different phases of the step cycle can be expected on the basis of changes in background activity (Matthews 1986). However, responses can also be modulated differently from the background ("preamotoneural modulation"). Such cases are of interest since it may reveal how the CNS actively modulates reflexes to accommodate to the requirements of particular phases of the movement (for a review, see Duysens and Tax 1994). The question is whether such premotoneural modulation also occurs in startle during gait. Schepens and Delwaide (1995) already showed that large startle responses were seen in TA during the stance phase when this muscle is normally inactive. Regarding the total responses, the present findings support this result and extend the observations to more phases of the step cycle and to more muscles. In his study on the phase-dependent gating of responses elicited after electrical stimulation of startle circuits in the brain stem, Drew (1991) also observed clear premotoneuronal gating during cat locomotion. He suggested a spinal structure, such as the central pattern generator (CPG) for locomotion to be the most likely structure for regulating the phase-dependent modulation. Activation of the medullary reticular formation would lead to a rather specific descending volley (Drew 1991). However, at the spinal level, this descending activity could be manipulated by the CPG, thus providing activation or suppression of given motoneurons depending on the phase requirements. Schepens and Delwaide (1995) favor a
similar explanation for their human data. Furthermore they argue that such a CPG modulation is made more likely by the observation that a similar phase-dependent modulation was not seen in muscles which did not participate in the locomotion (e.g., the shoulder muscle trapezius). Our own laboratory work further found indications for a role of a CPG-like structure in the phase-dependent reversal of other types of reflexes during human gait (Duyens et al. 1996; for a review, see Duyens and Van de Crommert 1998).

In the most extreme case of phase-dependent modulation, a given stimulus can yield facilitatory responses in one phase but suppressive ones in the other (phase-dependent reflex reversal of cutaneous reflexes) (see Duyens et al. 1990; Yang and Stein 1990). The subtraction technique presently used allowed to demonstrate that a reflex reversal occurs in startle responses during gait. It is striking that this reversal was mainly seen in the TA (F2) and hardly in the other muscles investigated, since this is very similar to the situation observed for cutaneous reflexes (De Serres et al. 1995; Duyens et al. 1990, 1991, 1993, 1996; Van Wezel et al. 1997; Yang and Stein 1990; Zehr et al. 1997). Furthermore the presently found suppressive responses in TA had a latency (80–120 ms) and occurrence (during swing) that were similar to the cutaneously induced suppressions. In contrast, the modulation pattern is very different from the one seen in cutaneous reflexes. In cutaneous reflexes, the largest facilitatory responses in TA are seen at the end of the stance phase and early swing; this is meaningful since extra flexion can help in stepping over an obstacle that is touched by the foot, for example. In startle reactions, on the other hand, there are no obstacles and small responses are seen in the equivalent period.

When the total responses are considered, a cocontraction of opposing muscles is the rule for both the upper and the lower leg. This cocontraction reaches a maximum in the period surrounding foot placement (end swing and early stance) but continues throughout stance. During most of the swing phase, the responses are small. In terms of bilateral coordination, it follows that the supporting leg receives extra stiffening. Scheepens and Delwaide (1995) also found coactivation in TA and SO when startle stimuli were elicited in standing subjects and by Delwaide and Scheepens (1995) in sitting subjects. When a response appeared in one muscle of the leg a concomitant response was observed in the antagonist in 85% of the cases in standing subjects and in 74% of the cases in sitting subjects.

Habituation

While habituation was clearly present in the amplitude of the responses, the phase-dependent modulation pattern generally remained the same. The kinesiologic data also showed hardly any sequential changes. One might expect that the stronger EMG responses during the first trials would also evoke larger changes in the kinesiologic data. In the first 800 ms, this is not seen presumably because many of these responses evoke cocontraction of antagonistic muscles, irrespective of the sequence of stimulation. However, sequential changes were observed after ~800 ms. Compared with the first trials, the last trials showed a smaller decrease in step cycle time, indicating a faster recovery from the small perturbation.

In summary, although the startle response habituates rapidly, the phase-dependent modulation seems to be robust, and hardly changes the ongoing locomotor pattern.

Functional considerations

The EMG pattern of the ASR during human locomotion shows a typical robust modulation pattern dominated by cocontraction. Such cocontraction might lead to a decrease in range of motion, as was indeed observed in the changes (although small) of the gonio signals of the knees and ankle. It is likely that when walking on a treadmill with a constant velocity, a decrease in range of motion of knee and ankle will lead to a shortening of the step cycle as was seen in the footswitch signals. Cocontraction often indicates a search for stability and is typical in stress related instances (Van Gemmert and Van Galen 1997, 1998). The way the cocontraction is modulated in the present data may be functional in terms of stability. Building up stability is functional as an adaptive defensive behavior that is expressed in response to an imminent threat to brace for action (freezing). During early/midstance, when the foot is firmly on the ground, there is a maximum chance to build up stability and indeed large mean responses are seen in both antagonistic muscles. Later on during the stance phase cocontraction is still needed but should not hold back the center of mass and thus prevent the opposite leg to swing forward since this would lead to an unstable situation. During early/midswing, a cocontraction has no function. In late swing, cocontraction in the upper leg muscles is large to prepare for a stable foot placement.

In conclusion, our study shows that the ASR is not an immutable flexor response but adapts to the movement context. The ASR consists of a complex pattern of responses in both flexors and extensors, often in cocontraction, which depends on the phase of the step cycle. These cocontractions only mildly affected the walking behavior even in the first trials when large responses were observed, indicating that a temporary limb stiffening, aimed at stability, could be well integrated into the ongoing step cycle, allowing for a smooth progression of gait.

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