Stiffness control of the leg in perturbed gait and posture

een wetenschappelijke proeve op het gebied van de Sociale Wetenschappen

Proefschrift

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Statues on cover: adaptation of Piazza by Alberto Giacometti (1948)
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Stiffness control of the leg in perturbed gait and posture
CHAPTER 1
GENERAL INTRODUCTION AND SUMMARY
1.1. Walking and stiffness control

Gait is generally seen as an automatic and seemingly simple motor behavior. Only when a walking impairment arises due to sickness or trauma, we realize that walking is not so simple as we thought. The central nervous system (CNS) is able to coordinate joint movement by sending electrical signals along the nerves to activate the appropriate set of muscles. However, gait is not only about motor activity. The control of movements depends strongly on sensory feedback. During a movement, the nervous system regulates constantly the large flow of sensory input and is able to select the most optimal context-specific information and incorporates this information into the executed movements. This allows a smooth progression of gait even when the body has to react to a perturbation or anticipate an obstacle. Both voluntary movements and reflexes are controlled by the CNS. For instance, if the foot hits an obstacle during the early part of the swing phase of the step cycle it is functional to lift the leg to step over an obstacle. However, if the foot hits the obstacle at the end of the swing phase extra flexion would cause a fall, so the foot must be placed even more quickly (Schillings et al., 2000). Furthermore, in healthy subjects such a phase-dependency could be observed in reflexes evoked by electrical stimulation of cutaneous nerves (Duysens et al., 1990, 1992, 1996; van Wezel et al., 1997; Zehr, 1999). The same stimulation could evoke opposite reaction dependent on the moment of stimulation during the step cycle. Moreover, this phase-dependent behavior was different for the various areas of the foot (Van Wezel et al., 1999). It is essential for proper execution of gait tasks to have the appropriate degree of activity between antagonistic muscle groups. This stiffness control must be regulated throughout the step cycle. Abnormal stiffening can lead to accidents (stumbling, ankle sprains etc.).

Stiffness control is dependent on 3 mechanisms: stiffness caused by reflexes, intrinsic stiffness due to passive structures, and “open loop” stiffness generated by the central nervous system in anticipation of a resistance during the movement. Many studies have examined the modulation of muscle or joint stiffness with activation level (Carter et al. 1990; Hunter and Kearney 1982; Kirsch et al. 1994; Mirbagheri et al., 2000; Nichols and Houk 1976; Sinkjaer et al. 1988; Toft et al. 1991; Weiss et al. 1988; Zhang and Rymer 1997) and joint position (Mirbagheri et al., 2000; Weiss et al. 1986; Zhang et al. 1998). Total joint stiffness is strongly dependent on both the level of voluntary contraction and joint position; it increases with voluntary activation and with movement of the joint from the center of the range of motion (ROM) toward the extremes. Intrinsic and reflex stiffness always appear and change together, so it is difficult to distinguish the mechanical consequences of reflex activity from those of the intrinsic properties of the joint and muscle. Stiffness is an important control parameter of motor behavior. Van Galen and Schomaker (1992) presented evidence that stiffness control optimizes cocontraction and reduces neuro-motor noise, thereby enabling spatial accuracy of aimed movements. It is concluded that in reaching tasks stiffness controls endpoint variability (Van Galen and De Jong, 1995). Furthermore, stress and mental load can
increase stiffness indicators (Van Galen et al., 2002; Van Gemmert and Van Galen, 1997).

It is unclear to what extend each of the stiffness components contribute to gait. During running a peak in EMG activity of the extensors was observed in humans just after foot placement (Dietz et al., 1979). In cat studies also an increase was seen in EMG activity of the extensors with a latency of 10-25 ms. Earlier studies supposed this was due to spinal reflexes (Llewellyn et al., 1989), but recent studies doubt this explanation, because the peak remains after anaesthesia of the foot and the peak remains after stepping on a trap door (i.e. loss of ground support; Gorassini et al., 1994). The latter result suggests a central origin of the peak since no stretch could occur when the animal steps into the “hole”. Similar results have been obtained in studies with monkeys when jumping on a fake paper floor (Laursen et al., 1978). In humans experiments have been done where short jerks were elicited to the muscles during walking to evaluate the increase in the stretch induced stiffness. Yang et al. (1991) estimated in humans a contribution of the stretch reflex to the EMG during stance phase at about 30-60 % (especially at the start of the stance phase. With more refined techniques, Sinkjaer et al. (1996) confirmed this result. Another selective technique is to stretch one particular muscle in several phases of the step cycle. In the quadriceps femoris strongest reflexes were detected just after foot contact (Dietz et al., 1990a). Furthermore, reflex activity in the biceps femoris (BF), an extensor of the hip and flexor of the knee, was strongest at the end of the swing phase (probably in anticipation of foot fall and the following hip extension) (v/d Crommert et al, 1996). The latter example suggests that during walking the EMG activity for muscles like the BF are based on reflex activity rather than a central “program” (Perret and Cabelquen, 1980; Prochazka et al., 1976; Smith, 1986l). An alternative method to study stretch reflexes during walking is by using electrical stimulation (H-reflex) (Capaday and Stein, 1986; Crenna and Frigo 1987; Dietz et al., 1990b; Garrett et al., 1984; Morin et al., 1982). One of the findings was that H-reflexes in the soleus muscle are generally smaller during walking compared to a standing position. This reduction is even more pronounced during walking on a beam (Llewellyn et al., 1990). This suggests that with more difficult tasks the system opts for a voluntary control mode instead of reflex control.

The wider goal of this thesis is to give fundamental insight about stiffness control of the leg in perturbed gait and posture. In early literature reflex stiffness was considered rigid and hard-wired. However, more recent literature showed that reflexes can adapt to the task (Capaday and Stein, 1986, 1987, 1995; Duyssens et al., 1992, 1993,1995, 1996), thereby regulating the amount of stiffness. Stiffness not only adapts to the task but depends on stress factors as well. Mental load increased the level of stiffness (Van Galen et al, 2002). Under psychological stress movement strategies tended to shift towards more constrained trajectories (Higuchi et al, 2002). This stiffness increase could be considered meaningless and inefficient. However, recent studies on movements of the forearm showed that task load had much more pronounced effects on muscle variables and movement kinematics than on performance in terms of successes or failures (Van Galen et al,
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2002). Stiffness reduces trembling in the trajectories or biomechanical noise due to stress and thereby contributing to stable movement trajectories under stress. (Van Galen and De Jong, 1995, Van Galen and Schomaker, 1992). This suggests a system effectively coping with stress rather than a simple physical purpose aimed at stability. Stiffness is part of a complex process adapting to the requirements of the environment, being either physical, psychological, or pathological. Even apparently abnormal motor patterns in patients due to stiffness like observed in Parkinson patients should not be considered pathological but rather adaptive to a primary disorder and may even be viewed as optimal for a given state of the system of movement production (Latash and Anson, 1996). Stiffness increase could increase the accuracy of targeting movements known to be more variable in Parkinson patients (Sheridan and Flowers, 1990).

Stiffness control is a complex and adaptive process. This process is still not well investigated in functional tasks of the lower limb. Therefore in this thesis the way stiffness control adapts to different conditions is investigated. First the startle response known to enlarge stiffness by freezing was investigated during a functional movement (i.e. walking). During the walking task it was investigated if this response would adapt depending on the phase of step cycle in which this response would be elicited. After knowing how these responses are incorporated into the ongoing step cycle of healthy subjects, the startle response was elicited in Parkinson patients known for their increased stiffness. Stiffness resists enforced movement and can thereby cause stability. Therefore, stiffness control in ankle inversions during walking and jumping were studied. Furthermore, a new method was investigated to look at postural perturbations, in order to study stiffness control in postural responses.

In the following sections an introduction on the individual parts of this thesis is given. In the first section, the startle response is discussed, followed by an introduction in Parkinson's disease, and in the third section background information about ankle inversions is presented. Finally, a summary of the thesis is given in the last section.

1.2. Startle

In the first study the startle response was investigated as a model for reflex stiffness during walking. The startle response consists of a characteristic sequence of muscular responses elicited by a sudden intense stimulus (Davis 1984). In humans intense acoustic stimulation is particularly effective in eliciting startle. In humans startle consists of the following set of muscle movements as described by Landis and Hunt (1939): blinking of the eyes, forward head movement, a characteristic facial expression that includes a widening of the mouth and occasional baring of the teeth, raising and drawing forward of the shoulders, abduction of the upper arms, bending of the elbow, pronation of the lower arms, flexion of the fingers, forward movement of the trunk, contraction of the abdomen, and bending of the knees (Fig 1.1). The response is primarily flexion, although extension of various muscles may frequently follow an initial flexion.
Electromyographic (EMG) recordings in leg and ankle muscles show that startle more often occurs in flexors than extensors (Davis, 1984; Landis and Hunt, 1939; Rossignol, 1975), but extensor activity does occur (Delwaide and Schepens, 1995; Brown et al., 1991a, 1991b). The startle response has a very short latency beginning with activation of the jaw (14 ms from the onset of an auditory stimulus), then spreads down the neural axis to reach the leg muscles, with longer latencies associated with longer distances from the ear.

The reticular formation plays a crucial role in mediating the auditory startle response (Davis, 1984; Yeomans and Frankland, 1996). The reticular formation is considered as a sensorimotor interface, where sensory input of different modalities converge to be translated into coordinated reflex activations in the whole body. It is supposed that this reticular formation is involved in coordinating responses evoked by cutane electrical stimulation (Matsumoto et al., 1992) and mechanical perturbations during walking (Forssberg, 1979). The most recent findings on the auditory startle reflex circuit is that the auditory stimulus is received in the ventral cochlear nucleus and projects to the auditory relay neurons in the ventral nucleus of the lateral lemniscus (Yeomans and Frankland, 1996)(see Fig. 1.2). This nucleus in turn projects to the nucleus reticularis pontis caudalis, which serves as the motor effect area for the startle response. From there the medial reticulospinal tract connects to the spinal cord with the alpha motor neurons.

Characteristic of the startle response is that the amplitude of the response is directly related to the intensity of the stimulus. Furthermore, the startle reaction is subjected to habituation. The amplitude of the response rapidly decreases after repeated stimulation. The degree of habituation decreases with the lengthening of time between the stimuli (inter stimulus interval). Both latency as incidence of the response are posture dependent. When a subject is standing the response incidence is twice as high and 30 to 50 ms earlier compared to sitting subjects (Brown et al., 1991a; Schepens and Delwaide, 1995). Until now only Schepens and Delwaide have studied startle responses during walking. A posture/task dependency was found for latency but not for incidence. The tibial muscle showed a phase-dependent modulation of the response amplitude. TA responses were observed during stance but not during swing.
The startle response protects animals from blows or predatory attacks by quickly stiffening the limbs, body wall and dorsal neck in the brief time period before directed evasive or defensive action can be performed (Yeomans and Frankland, 1996). Some diseases know an exaggerated startle response. The most prominent of these is ‘startle disease’ or ‘hyperexplexia’. Clinical features include an enhanced startle response to any stimulus modality characterized by a generalized stiffening and frequently cumulating in a fall to the ground without loss of consciousness.

1.3. Parkinson

In the second study startle responses in Parkinson’s disease were studied to look at the influence of stiffness due to pathological circumstances. Parkinson’s disease is a common progressive neurological disorder characterized by hypokinesia (reduced movement), akinesia (absent movement), tremor (rhythmic, purposeless, quivering movements of a limb, head, mouth, tongue, or other part of the body) rigidity (a condition of hardness, stiffness, or inflexibility of a limb) and postural instability (Morris et al., 2001). Other common symptoms include postural abnormalities, such as stooped posture, a shuffling gait, a mask like face with decreased blinking, and difficulty in writing. The predominant lesion in Parkinson’s disease is cell degeneration and loss of pigmented neurones in the pars compacta of the substantia nigra, causing less of the neurotransmitter dopamine to be made. With less dopamine than normal, there is an imbalance between dopamine, GABA, and acetylcholine. Because of this imbalance the basal ganglia dysfunction and their role in motor control is compromised (Marsden et al. 1976). In gait this means that although most patients can perform simple straight line walking tasks relatively easy, they experience considerable difficulty when walking
and turning, performing simultaneous motor or cognitive tasks (Morris et al., 2001). Furthermore during walking the muscles in the leg are continuously active (there is no complete relaxation), thereby increased stretch reflexes are observed (Murray et al., 1978). The onset of Parkinson's disease is subtle, and individual patients can have significant variations in the symptoms they experience. One of the diagnostic signs of parkinsonism is the glabella reflex: in Parkinson patients no habituation is observed in the blink reflex when tapping on the glabella (Sunohara., 1985). In studies where Parkinson patients were exposed to an auditory stimulus, no difference from healthy subjects was observed in amplitude and pattern of recruitment of the muscles. However, latencies were abnormal (Vidailhet et al., 1992; Kofler et al., 2001). This might be caused by the absence of facilitatory input of the basal ganglia to the brainstem and the reticulo spinal tract. Delwaide et al. (1990, 1991) also suggested an abnormal functioning reticulo spinal tract in the Parkinson patients, when less audio spinal facilitation of the H-reflex in the soleus muscle was observed.

1.4. Ankle inversion

Stiffness can cause stability. Such stability is important for all joints but there is one joint deserving special attention in this respect. The ankle is the most frequently injured joint, both in sports exercise and in daily life. The ankle sprain has a high occurrence in sports. Ten to 25% of injuries in athletics involve the ankle. Up to 85% of these injuries involve sprain of the lateral ligaments (Garrick et al., 1988). These injuries occur predominantly in athletes participating in running and jumping sports (Bahr et al. 1994; Balduini et al. 1987). As many as 10 to 30% of people with acute lateral ligament injuries develop chronic mechanical instability (Peters et al., 1991) Up to 60% of all inversion injuries are recidives (Balduini et al., 1987). It is estimated that, each day, one inversion injury of the ankle occurs for every 10 000 people (Lynch et al., 1999).

According to Kannus et al. (1991), ankle sprains result in various degrees of mechanical damage and can cause instability (see Fig 1.3). This instability can be divided in functional and mechanical instability. Functional instability is the term employed for the recurrent sprains and/or feeling of giving away. It is often seen as a residual disability after ankle joint injuries. It may result from damage to
mechanoreceptors in the lateral ligaments or from muscle/tendons with subsequent partial deafferentation of the proproceptive reflex. Functional instability is a subjective symptom appearing during activity and can be present without mechanical instability (Konradsen et al., 1990; Konradsen et al., 1991). Mechanical instability refers to laxity of a joint due to structural damage to ligamentous tissues, which support the joint. Hertel (2000) states that mechanical instability may affect the talocrural, subtalar and/or inferior tibiofibular joints following lateral ankle sprain resulting in chronic instability. The acute inversion injury results in damage to the lateral structures, which include the lateral ligaments as well as the capsule of the talar and subtalar joints.

When, in a normal situation, an ankle tends to give away, different proprioceptors in the ankle joint are stimulated. The sensory receptors for proprioception provide the major input to the central nervous system. In muscles, the inversion stress results in a physiological neuromuscular stretch response. A failed reaction can lead to an ankle sprain. According to Isakov et al. (1986) especially the peroneal muscles, are considered to provide important protection against lateral ankle injury. The responses that could be evoked in the peroneal muscles cause an appropriate muscular activity to protect the ankle joint (Freeman et al., 1965).

The combined effect of mechanical instability (caused by ligamentous trauma) and proprioceptive deficits (caused by ligamentous injury) and proprioceptive deficits contribute to functional instability, which could lead to further microtrauma and reinjury (see Fig. 1.4 from Lephart et al., 1997).
In addition to a reduction in sensory afferent input for articular mechanoreceptors, diminished postural reflex responses have also been reported following injury. A prolonged peroneal reaction time in response to a sudden inversion was found (Konradsen et al., 1991). A motor output (muscle) is generated by the central nervous system through a neural input that is provided by the peripheral mechanoreceptors as well as the visual and the vestibular receptors that are all integrated in the central nervous system (see Fig. 1.5.). The central nervous system receives input from three main subsystems: the somatosensory system, the vestibular system and the visual system. Information gathered by these subsystems is processed at three distinct levels of motor control: the spinal level, the brain stem and the higher brain centers (Lephart et al., 1997).

At the level of spinal reflexes there are three distinguishable types of responses during ankle inversions. Reflexes can be found with a short (M1), medium (M2) and long (M3) latency. Reflexes with a short latency are supposed to be monosynaptic stretch reflexes. The reflexes with a medium latency are supposed to be polysynaptic with probably a transcortical route. The medium latency reflex is considered to be a voluntary response.

The mechanism of ankle injury is frequently a forced inversion of the ankle. The human body has two kinds of defense mechanisms to prevent the ankle from (lateral) damage. The first one is a passive system. The ankle is protected by the static defense offered by the congruence of bones and the existence of subtalar ligaments and capsule. They give the foot its natural stability. The structure of the ligaments prevents the foot from ‘hyper-inversion’ and acts as a movement restraint.

The second mechanism is a dynamic defense of the ankle joint by the active muscles guided by reflexes and central strategies mediated by the spinal or cortical centers (Konradsen et al., 1997). As a result of the induced stretch some lower leg muscles are activated as result of the stimulation of mechanoreceptors. Most important in this matter are the peroneus longus and brevis muscles. These muscles are the prime evertors of the foot and ankle complex. It has been suggested that they play a role in injury protection as they become active when the ankle is...
being inverted. It is however unclear to what extent this dynamic defense mechanism can protect the ankle joint (Isakov et al., 1986).

1.5. Summary

Stiffness control is a complex and adaptive process and can be divided into three components. The intrinsic stiffness arising from passive structures: the mechanical properties of the joint and passive tissue, the intrinsic stiffness due to active muscle fibers, and finally reflex stiffness. This thesis investigates the adaptability of this mechanism in the leg during different conditions. Three aspects of stiffness control during walking will be addressed. First the reflex stiffness will be studied in chapter two as occurs during the startle response (freezing). Secondly the startle response will be studied in chapter three in Parkinson patients, were rigidity causes an increase in the intrinsic stiffness. And third, a new method to induce ankle inversions will be described in chapter four. The results of this new method inducing these inversions is investigated in chapter five and six, where stiffness is generated in anticipation to a resistance during a movement. These inversions elicited a possible balance correcting response. To further investigate these balance correcting responses a new platform was developed, which is described in chapter seven. The next sections summarize the results and conclusions of all experiments.

Chapter 2: Modulation of the startle response during human gait

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 mid swing. 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 allowing a smooth progression of gait.

**Chapter 3: Startle responses in patients with Parkinson’s disease during treadmill walking**

The auditory startle response during walking was studied in eight patients with Parkinson's disease and in eight healthy subjects. To examine how startle reactions are incorporated in an ongoing gait pattern of these patients, unexpected auditory stimuli were presented in six phases of the step cycle during walking on a treadmill. For both legs electromyographic activity (EMG) was recorded from biceps femoris (BF) and tibialis anterior (TA). In all subjects and all muscles, responses were detected. The pattern of the responses, latency, duration and phase-dependent modulation were similar in both groups. However, the mean response amplitude was larger in patients due to a smaller habituation rate. No correlation was found between the degree of habituation and disease severity. It is discussed that a decreased habituation is already observed in mildly affected patients, indicating that habituation of the startle response is a sensitive measure in these patients. The results on startle complement the earlier findings of reduced habituation of blink responses in Parkinson’s disease.

**Chapter 4: Mechanically induced ankle inversion during human walking and jumping**

A new method to study sudden ankle inversions during human walking and jumping is presented. Ankle inversions of were elicited using a box containing a trap door. During the gait task, subjects walked at a speed of 4 km/h. At a pre-programmed delay after left heel strike an electromagnet released the box on the treadmill. This delay enabled the subject to step on the box without changing without having to change the walking cadence. During the jumping task, subjects jumped from a 30 cm high platform on the box in a standardized way. In both tasks 20 stimulus and 20 control trials were presented randomly. The average tilting velocity of the trap door during the stimulus trials was 403°/s during the walking task and 595°/s during the jumping task. For the control trials a tilting of 0° was used. With this method it is possible to evoke reproducible ankle inversions causing characteristic EMG responses in six lower leg muscles.

**Chapter 5: EMG responses in the lower leg after mechanically induced ankle inversions during human walking**

Ankle inversions have been studied extensively during standing conditions. However, inversion traumas occur during more dynamic conditions, like walking. Therefore, in this chapter sudden ankle inversions were elicited in twelve healthy subjects while walking on a treadmill at 4 km/h. A box with a trap door was
released on the treadmill at a preprogrammed delay so the subjects could step on the box in a natural continuation of the step cycle. Twenty stimulus (25° of rotation) and control trials (0° of rotation) were presented randomly, while EMG recordings were made of six lower leg muscles. In all six lower leg muscles two responses were detected, a short latency response (M1) of ~ 40 ms and a medium latency response (M2) of ~ 90 ms. The peroneal muscles, being evertors and exposed to the largest stretch, showed in both responses the largest amplitudes. The M2 was larger and more consistent than the M1, indicating that this response is functionally the most important response. However, the M2 response was observed after the end of the rotation of the trap door and was thereby too late to resist the induced stretch. The functional relevance of this response must lie in the period after the induced inversion. Furthermore, sequential effects were investigated showing for the M1 no habituation. For the M2 however, the first trials response activity was observed in all muscles, indicating a generally aspecific stiffness was generated. During the experiment the activity distribution of the M2 was adapted to the inversion leaving only the peroneal muscles active. This indicated that these reflexes adapt quickly in order to effectuate a more efficient response pattern.

Chapter 6: Whole body responses after mechanically induced ankle inversions during treadmill walking

As described in the previous chapter, large responses were observed in the peroneal muscles after ankle inversion during walking (Nieuwenhuijzen et al., 2003). These responses however, were too late to resist the induced stretch of the inversion. It was suggested that these responses might be part of a balance correcting response or have a function in protecting the ankle during loading of the leg. Therefore, in the present chapter, trunk and leg kinematics, CoP, and EMG of trunk and leg muscles were studied in twelve healthy subjects after ankle inversions during walking on a treadmill at 4 km/h.

The step cycle showed no significant differences, indicating a smooth progression of gait. During the rotation of the trap door (0 – 60 ms) no kinematic changes were observed (the “neutral phase”). During the next phase, the “initial ipsilateral reaction” (from 70 ms to 150 ms), the knee moved fiercely to the ipsilateral side thereby decreasing the amount of inversion applied to the ankle. In the ensuing phase (the “roll correction response” from 150 ms to 400 ms) during weight acceptance of the ipsilateral leg, EMG responses were detected in the peroneal muscle. Presumably these responses could protect the ankle joint when the leg was fully loaded at toe-off of the contralateral leg. Furthermore, the trunk moved in the opposite direction, along with the contralateral leg. The movement in the trunk (first ipsilateral than contralateral) and the early activation of the contralateral hip and trunk muscles resembled a balance correcting response in the frontal plane as observed by others during roll perturbation in a standing condition. In the final phase (“the recovery phase”: 400-1500 ms) the signals returned
Chapter 1. General introduction and summary

to baseline. Although kinematic changes in the frontal plane during these phases were prominent, the CoP showed only mild deviations, indicating a maintained equilibrium. It is concluded that the reactions following an ankle inversion during the stance phase of gait are well-suited to serve the goals of avoiding excessive stretch of the lateral ligaments and muscles of the lower leg and maintaining balance.

Chapter 7: Dynamic posturography using a new movable multidirectional platform driven by gravity

Human upright balance control can be quantified using movable platforms driven by servo-controlled torque motors (dynamic posturography). We introduce a new movable platform driven by the force of gravity acting upon the platform and the subject standing on it. The platform consists of a 1 m2 metal plate, supported at each of its four corners by a cable and two magnets. Sudden release of the magnets on three sides of the platform (leaving one side attached) induces rotational perturbations in either the pitch or roll plane. Release of all magnets causes a purely vertical displacement. By varying the slack in the supporting cables, the platform can generate small (0.5°) to very destabilizing (19°) rotations. Experiments in healthy subjects showed that the platform generated standardized and reproducible perturbations. The peak rotation velocity well exceeded the threshold required to elicit postural responses in the leg muscles. Onset latencies were comparable to those evoked by torque motor-driven platforms. Randomly mixed multidirectional perturbations of large amplitude forced the subject to use compensatory steps (easily possible on the large support surface), with little confounding influence of habituation. We conclude that this gravity-driven multidirectional platform provides a useful and versatile tool for dynamic posturography.

1.6. Samenvatting

Bij het gepast uitvoeren van loop en posturale taken is het essentieel om de juiste mate van stijfheid te regelen. Deze stijfheidsregulatie is een complex en adaptief proces en kan worden onderverdeeld in drie componenten: 1. de intrinsieke stijfheid, geleverd door passieve structuren zoals het gewrichtskapsel, de banden, de mechanische eigenschappen van het betreffende gewricht en de passieve spiereigenschappen, 2. de intrinsieke stijfheid veroorzaakt door spieractiviteit in anticipatie van een weerstand tijdens een beweging, 3. door reflexen veroorzaakte stijfheid. Dit proefschrift behandelt deze drie aspecten van stijfheidsregulatie en het adaptieve vermogen van dit mechanisme tijdens verschillende condities. Hierbij zal worden gekeken naar de onderste ledematen Als eerste zal in hoofdstuk twee de reflexstijfheid worden bestudeerd in de vorm van auditieve schrikreacties tijdens lopen, die bekend staan om de verstijving die ze geven. Vervolgens wordt in hoofdstuk drie diezelfde reactie bestudeerd in Parkinson patiënten die bekend staan om de rigiditeit (een vergrote intrinsieke stijfheid).
hoofdstuk vier zal een nieuwe methode worden gepresenteerd om enkelinversies uit te lokken tijdens lopen en springen. De resultaten van de enkelinversies uitgelokt tijdens lopen worden gepresenteerd in hoofdstuk vijf en zes, waar stijfheid wordt geleverd in anticipatie van een weerstand tijdens beweging. Deze reacties leveren een balanscorrigerende respons op. Om deze respons verder te onderzoeken werd een nieuw platform ontwikkeld, wat staat beschreven in hoofdstuk zeven. In de volgende paragrafen worden de verschillende hoofdstukken in meer detail samengevat.

**Hoofdstuk 2: Modulatie van de startle respons tijdens menselijk lopen**

Hoewel veel studies fase-afhankelijke modulatie tijdens lopen hebben gevonden in proprioceptieve en exteroceptieve reflexen, is er weinig bekend over deze vorm van modulatie voor auditive reflexen. Om te bestuderen hoe auditive schrikreacties (startle) in het looppatroon worden geïncorporeerd, werden tijdens het lopen op een loopband met 4 km/u, onverwachte auditive stimuli gepresenteerd aan acht gezonde proefpersonen in zes fasen van de stapcyclus. Electromyografische activiteit (EMG) werd gemeten in de biceps femoris, de rectus femoris, de tibialis anterior en de soleus, van beide benen. Bovendien werd ook de stand- en zwaaifase van beide benen, de hoeken van beide knieën, en de linkeroewelkhoek gemeten. Alle subjecten lieten verscheidene responsen zien met een latentietijd van ~ 60 ms (F1), ~ 85 ms (F2), en ~ 145 ms (F3). De amplitudes van de responsen waren afhankelijk van de timing van de stimulus in de stapcyclus. Alhoewel de respons snel habitueerde bleef de fase-afhankelijke modulatie over het algemeen gelijk. Er was geen strikte correlatie tussen de modulatie van de responsen en de modulatie van de achtergrond activiteit. In de tibialis anterior was zelfs een omdraaiing te zien van een facilatoire F2 respons tijdens de standfase naar een suppressieve respons tijdens mid-swing. Responsen werden zowel in flexoren als extensoren gezien, vaak in cocontractie en met name tijdens de standfase. Verder lieten de loopkarakteristieken een verkorting van de volgende stapcyclus zien. En een kleine vermindering van de hoekuitslagen van de knieën en enkel. Deze resultaten laten zien dat de responsen zich aanpassen om extra stabiliteit te verkrijgen afhankelijk van de fase van de stapcyclus. Echter, zelfs in de eerste trials waren de kinematische veranderingen maar klein, waardoor het lopen vloeiend voort kon gaan.

**Hoofdstuk 3: Startle responsen in Parkinson patiënten tijdens lopen op een loopband**
De auditieve startle respons tijdens lopen werd bestudeerd in acht patiënten met de ziekte van Parkinson en acht gezonde proefpersonen. Onverwachte auditieve stimuli werden toegediend in zes fasen van de stapcyclus tijdens lopen op een loopband om te onderzoeken hoe startle-reacties worden ingebouwd bij deze patiënten. In beide benen werd electromyografische activiteit (EMG) gemeten van de biceps femoris (BF) en de tibialis anterior (TA). In alle subjecten en alle spieren werden responsen gevonden. Het responspatroon, de latentie, de duur en de fase-afhankelijke modulatie van beide groepen waren vergelijkbaar. De gemiddelde responsamplitude was echter groter in de patiëntengroep door een verminderde habituatie. Geen correlatie werd gevonden tussen de habituatie en de ernst van de klachten. De verminderde habituatie was al te vinden in patiënten met een lichte mate van de ziekte van Parkinson. Dit suggereert dat deze habituatie een sensitief meetinstrument voor deze patiëntengroep. De resultaten gevonden tijdens startle complementeren de eerder gevonden verminderde habituatie van knipper responsen in patiënten met de ziekte van Parkinson.

Hoofdstuk 4: mechanisch geïnduceerde enkelinversies tijdens menselijk lopen en springen

Een nieuwe methode wordt gepresenteerd om plotse enkelinversies te veroorzaken tijdens menselijk lopen en springen. Enkelinversies van 25° werden uitgelokt door een valluik ingebouwd in een doos. Tijdens de looptaak liepen de proefpersonen met een snelheid van 4 km/u. Na een voorgeprogrammeerd delay na linker hielcontact liet een elektromagneet de doos vallen op de loopband. Dit delay zorgde ervoor dat de subjecten op de doos konden stappen zonder de loop kans te veranderen. Tijdens de springtaak sprongen de subjecten van een 30 cm hoog platform op de doos op een gestandaardiseerde wijze. In beide taken werden 20 stimulus- en 20 controle trials gerandomiseerd uitgevoerd. De gemiddelde kantelsnelheid tijdens de stimulus trials van het valluik was tijdens de looptaak 403°/s en tijdens de springtaak 595°/s. Tijdens de controle trials kantelde het valluik niet. Met deze methode is het mogelijk reproduceerbare enkelinversies uit te lokken waardoor karakteristieke EMG responsen werden veroorzaakt in zes onderbeenspieren.

Hoofdstuk 5: EMG responsen in de onderbenen na mechanisch geïnduceerde enkelinversies tijdens menselijk lopen

Enkelinversies in een staande conditie zijn uitgebreid bestudeerd. Inversietrauma’s ontstaan echter tijdens meer dynamische activiteit, zoals lopen. Daarom werden in dit hoofdstuk plotse enkelinversies uitgelokt bij twaalf subjecten tijdens lopen op een loopband met een snelheid van 4 km/u. Een doos viel op de loopband na een voorgeprogrammeerde vertraging, zodat de proefpersonen
op de doos konden stappen in een natuurlijk voorgang van de stapcyclus. Twintig stimulus trials (25° kanteling) en 20 controle trials (25° kanteling) werden gerandomiseerd aangeboden. EMG activiteit werd gemeten in zes onderbeenspieren. In alle zes spieren werden twee responsen gevonden, een respons met een korte latentietijd (M1) van ~40 ms en een respons met een lange latentietijd (M2) van 90 ms. De peroneus longus en brevis, evertoren die aan de grootste rek worden bootgesteld, lieten bij beide responsen de grootste amplitudes zien. De M2 was algemeen groter en meer consistent dan de M1 en zal daarom waarschijnlijk functioneel de belangrijkste zijn van de twee. Deze M2 werd echter pas gezien nadat de kanteling was afgelopen en dus te laat om de geïnduceerde rek tegen te gaan. De functionele relevantie moet dus na de inversie liggen. Verder werd ook gekeken naar sequentiële effecten. Deze liet geen habituatie zien in de M1 maar wel in de M2. De eerste trial van de M2 liet in alle spieren een duidelijke grote respns zien. Dit wijst op de generatie van een algemene aspecifieke stijfheid. Gedurende de voortgang van het experiment was een adaptatie van de M2 te zien, waarbij op het laatst alleen nog de peroneus spieren actief waren. Dit suggereert dat deze reflexen zich snel aanpassen om een meer efficiënt aanspanningspatroon te bewerkstelligen.

**Hoofdstuk 6: Totale lichaamsresponsen na mechanisch geïnduceerde enkelinversies**

Zoals beschreven in het vorige hoofdstuk geven enkelinversies grote responsen in de peroneï (Nieuwenhuijzen et al, 2003). Deze responsen waren echter te laat om de geïnduceerde rek, ontstaan door de inversie, tegen te gaan. Deze responsen zouden een onderdeel kunnen vormen van een balanscorrigerende respons of een functie kunnen hebben in het beschermen van de enkel tijdens het gewicht zetten op het been. Daarom werd in de huidige studie de romp- en beenkinematics, CoP, en EMG van romp- en beenmusculatuur bestudeerd na enkelinversie in twaalf gezonde proefpersonen tijdens het lopen op een loopband met een snelheid van 4 km/u.

Geen significante verschillen in de stapcyclus werden gezien tussen controle- en stimulus trials. Blijkbaar was er ondanks de verstoring een vloeiende voortgang van het lopen. Tijdens het kantelen van de plank (60 ms) werden geen kinematische veranderingen geobserveerd (de “neutral fase”). Tijdens de volgende fase, de “initiële ipsilaterale reactie” (van 70 ms – 150 ms), bewoog de knie sterk naar de ipsilaterale zijde waardoor de mate van inversie werd beperkt. In de daaropvolgende fase (de “zijwaartse correctieresponse” van 150 ms – 400 ms) werd tijdens het gewicht zetten op het verstoorde beend EMG responsen gevonden in de peroneus longus. Waarschijnlijk kunnen deze responsen de enkel beschermen als het been vol belast wordt na de afzet van het contralaterale been. Tijdens deze fase bewoog de romp naar de contralaterale zijde samen met het contralateral been. Deze beweging en de vroege activatie van de contralateral heup en romp musculatuur zijn vergelijkbaar met een balanscorrigerende respons in het frontale vlak, zoals dat vermeld wordt in andere studies bij verstoringen in het...
frontale vlak tijdens staan. In de laatste fase (de “herstel fase”: 400 ms –1500 ms) kwamen de signalen van de stimulusconditie en de controleconditie weer bij elkaar. Alhoewel kinematische veranderingen in het frontale vlak prominent aanwezig waren, liet het CoP signaal maar kleine uitslagen zien, wat suggereert dat het evenwicht behouden bleef. Er werd geconcludeerd dat de reacties, na een enkelinversie in de standfase tijdens lopen, goed geschikt zijn om excessieve reacties op de laterale ligamenten en onderbeenpieren en bovendien de balans te bewaren.

Hoofdstuk 7: Dynamische posturografie met een nieuw multidirectioneel platform aangedreven door de zwaartekracht

Balanscontrole tijdens staan kan gekwantificeerd worden door gebruik te maken van een beweegbaar platform aangedreven door servomotoren (dynamische posturografie). We introduceren een nieuw platform dat wordt aangedreven door de zwaartekracht die aangrijpt op het platform en de proefpersoon die op het platform staat. Het platform bestaat uit een metalen plaat van 1 m² die op elke hoek vast gehouden wordt door een kabel en twee magneten. Het plots loslaten van de plaat door de magneten aan 3 zijden van het platform (1 zijde blijft vast), veroorzaakt een rotatieperturbatie in het sagittale of frontale vlak. Het loslaten van de plaat door alle magneten veroorzaakt een puur verticale verplaatsing. Door de kabels meer of minder te laten vieren kan het platform kleine (0.5°) tot zeer verstorende (19°) rotaties geven. In experimenten met gezonde proefpersonen waren gestandaardiseerde en reproduceerbare perturbaties te zien. De piesnelheid van de rotatie overschreed de drempel die nodig is voor het uitlokken van posturale responses in de beenspieren. De gemeten latentietijden waren vergelijkbaar met latentietijden gemeten na perturbatie met een platform dat door een koppelmotor wordt aangedreven. Gerandomiseerd gemengde multidirectionele verstoringen met grote amplitude forceerde compensatoire stappen bij de proefpersonen, met weinig invloed van habituatie. Het platform was groot genoeg om deze stappen te maken. Wij concluderen dat dit door zwaartekracht aangedreven multidirectioneel platform een bruikbaar en veelzijdig gereedschap is voor dynamische posturografie.

2.1. Introduction
Stiffness control of the leg in perturbed gait and posture
CHAPTER 2
MODULATION OF THE STARTLE RESPONSE DURING HUMAN GAIT

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 rostro-caudal 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 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, 1991b; 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 SO in subjects who were standing compared to subjects who were sitting (Brown et al. 1991a; Delwaide and Schepens 1995). In addition, the latency of TA and SO responses changed from about 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 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 pretotoneuronal 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. 1998). It is not clear whether such phase dependency also occurs 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).

2.2. Methods

Experimental set-up

Startle reactions were recorded in 8 healthy subjects (4 males and 4 females; age range: 19-27 years) after oral 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 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 in order 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 electromyographic activity (EMG) of the biceps femoris (BF), the rectus femoris (RF), the tibialis anterior (TA), and the soleus (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 (cut-off frequency at 3 Hz), full wave rectified, and then low-pass filtered (cut-off 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 a period starting 100 ms before stimulation and lasting for 2100 ms. To further document the startle induced movements and the gait characteristics, the experimental sessions were videotaped and 3-D measurements were made (Qtrac, Qualisys).

Experimental protocol

The subjects were trained to walk at a comfortable, constant pace with the belt speed set at 4 km/h. Auditory stimuli were given unexpectedly with a random interstimulus interval of 1.5-2.5 min. These relatively long interstimulus periods were necessary to prevent habituation as much as possible. At three moments in the swing phase of the left leg the auditory stimulus was presented. At these moments the left leg was in early, mid, or late swing and the right leg in early, mid, or late stance respectively. There was no asymmetry in walking (see 2.4. Results). Therefore, the stimulation was considered to occur at respective per-
centages of the step cycle after heel strike of 16%, 28%, 38% (based on the left leg), 66%, 78% and 88% (based on the right leg) (see Fig. 2.1). All three stimulus conditions were applied 10 times in a random order. Hence, a total of 30 stimuli were presented. Control trials (i.e., no stimulus) were measured 4 seconds 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. 1998). For each muscle, a single time window was set over 6 phases of the average data. However, when, due to background noise or overlap between responses, these average data showed no clear-cut responses, in one of the phases the individual trials were examined. In this way 60 traces were investigated for each subject (6 phases x 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. Window was set, based on the responses of all 6 phases in the step cycle of both the individual and the averaged trials (cf. Duyssens et al. 1991; Tax et al. 1995; Yang and Stein 1990) (see Fig. 2.2). 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 control activity during the control
Chapter 2. Modulation of the startle response during human gait

2.2 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 (significance level, \( P < 0.05 \)) was used. Phase-dependent modulation was tested by the Kruskall-Wallis one-way ANOVA (significance level, \( P < 0.05 \)). Potential crosstalk 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 (significance level, \( P < 0.05 \)). 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.

2.3 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. 2.3A). Fig. 2.3A and B show 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 SD in Fig. 2.3A), and in agreement with the literature (see, for instance, Inman et al. 1981).
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 2.3 Methods). Three facilitatory responses were detected, which were termed F1 (mean latency 59 ms, SE 7 ms; duration 42 ms, SE 11 ms), F2 (83 ms, SE 8 ms; duration 63 ms, SE 10 ms) and F3 (mean latency 146 ms, SE 8 ms; duration 67 ms, SE 11 ms) (see Fig 2.4A and B). The latencies and durations of the responses are given in Table 2.1.

Very early facilitatory responses (F1 in Fig. 2.4B and Table 2.1) 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 ms, SE 9 ms) was slightly longer than the mean latency of the F2 of the other muscles (especially with respect to the TA, 82 ms, SE 6 ms; see Table 2.1). 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. 2.4B) were seen in the lower leg muscles TA and SO, with mean latency of 101 ms (SE 11 ms) and a duration of 42 ms (SE 7 ms). In all but one subject, these suppressive responses were seen during swing in TA. In five subjects, less clear but consistent suppressive responses were also seen in SO during early/mid stance. 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 2.1). 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

Table 2.1. Startle response characteristics for the whole population

<table>
<thead>
<tr>
<th></th>
<th>Latency ± SE (ms)</th>
<th>Duration ± SE (ms)</th>
<th>Amplitude ± SE</th>
</tr>
</thead>
<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>

Table 2.1. The mean latency and duration was based on the window settings (N=8; one window per muscle per subject). To calculate the mean amplitude all responses (n=480; 8 subjects x 6 phases x 10 responses) were used, expressed as fraction of the maximum background activity (see methods).
(expressed as a fraction of maximum control activity) of the F2 of BF and RF were 0.26 and 0.29, respectively, as compared to 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), which 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 the difference was 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 Fig. 2.5-8 in which for each muscle the average subtracted responses (and SE)

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**Fig. 2.5.** The total responses (sum of all windows) are plotted for all four muscles as a function of the phase in the step cycle. Stick diagrams on top show real position at the start of the response. The solid lines in the figures below the stick diagrams represent the average EMG activity after stimulation, the dashed line the control data and the solid line with the SE (\( n = 8 \) subjects x 10 trials = 80 trials) in the figures below the subtracted data. The data are normalized with respect to the maximum background activity of each muscle (see Methods). Asterisks indicate significant responses (Wilcoxon signed rank test, \( P < 0.05 \)). The dark brown bars at the bottom with the SE (\( n = 480 \)) represent the stance phase. The BF and RF show large responses during the early/mid stance and late swing. The TA and SO shows large responses during stance and small responses during swing.
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. 2.3B), 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. 2.5-8). In other words, for phases 1 to 3 the EMGs of the right leg (stance phase) were used while for phases 4 to 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, 2 and 3 are actually synchronous with phases 4, 5 and 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 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, F2 and F3. The modulation pattern of the total responses showed clear phase-dependent modulation in all muscles (see Fig. 2.5). 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 4-5) and generally largest in early/mid stance (phases 1-2). 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.

Secondly the modulation pattern of the individual responses will be dealt with. With respect to the F1 (see Fig. 2.6), 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 reponses in TA were seen during early swing (phase 4) and in SO during early/mid stance (phase 1).

For F2 and F3 (see Figs. 2.7 and 2.8) 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 swing and late swing (phase 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. 2.5), 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. 2.5).

There was no strict correlation between the response modulation and the background modulation. Differences between these modulations were especially

![Fig. 2.7. Phase-dependent modulation of the F2 in all four muscles. Except for the low response activity measured in SO during phase 1, the modulation of the F2 response is comparable to the modulation of the average of all responses seen in Fig. 2.5. TA shows even suppression in the swing phase. The BF and RF show large responses during the early/mid stance and late swing. The same format is used as in Fig. 2.5.](image)
prominent for the F2 responses in TA, which showed clear facilitatory responses in mid stance when TA is normally inactive. Furthermore, there was a reversal to suppressive responses in mid swing when this muscle normally has high background activity.

For the F3 responses such a reflex reversal was absent in TA (see Fig. 2.8). 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/mid stance (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 one-way ANOVA, P > 0.05) was found for all responses in all muscles. As for the bilateral coordination, for all muscles the responses were generally small with respect to the background activity in the leg that was in mid swing. In the same period the opposite leg was in mid stance and generally showed large F2 (e.g. TA), or F3 responses (e.g. SO), or both (e.g. RF).
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. 2.9, there was a slight shortening of the swing phase in the left leg (HO1: first heel-on in Figs. 2.9A and B; Left) and the stance phase in the right leg (TO1: first toe-off in Figs. 2.9A 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 Figs. 2.9A and B; Right, with mean differences
Table 2.2. 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 ± SE (deg)</th>
<th>Angle (deg)</th>
<th>Angle Difference ± SE (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Right</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FlSw</td>
<td>528</td>
<td>- 11 ± 2.4 *</td>
<td>- 43</td>
<td>0.6 ± 0.4 *</td>
</tr>
<tr>
<td>ExSw</td>
<td>805</td>
<td>- 20 ± 2.6 *</td>
<td>9</td>
<td>- 1.0 ± 0.4 *</td>
</tr>
<tr>
<td>Knee Left</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ExSw</td>
<td>240</td>
<td>- 0 ± 1.9</td>
<td>11</td>
<td>- 1.1 ± 0.3 *</td>
</tr>
<tr>
<td>FlSt</td>
<td>388</td>
<td>- 12 ± 3.6 *</td>
<td>2</td>
<td>1.1 ± 0.4 *</td>
</tr>
<tr>
<td>ExSt</td>
<td>726</td>
<td>- 21 ± 3.1 *</td>
<td>13</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>Ankle Left</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ExSt</td>
<td>280</td>
<td>- 2 ± 2.7 *</td>
<td>7</td>
<td>- 0.9 ± 0.2 *</td>
</tr>
<tr>
<td>Flst</td>
<td>721</td>
<td>- 10 ± 4.2 *</td>
<td>12</td>
<td>1.0 ± 0.2 *</td>
</tr>
</tbody>
</table>

Table 2.2. 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 and SE (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 average angle and SE (n=240) of control and stimulus trials. The asterisks indicate significant differences. Abbreviations: FL = maximum flexion, Ex = maximum extension, St = stance, Sw = swing. The abbreviations are illustrated in Fig. 2.10. Values are means ± SE

of 17 and 30 ms, respectively) and the left leg (TO1 and HO2 in Figs. 2.9A 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 2.2 illustrated by Fig. 2.10.

Fig. 2.10. Examples of the averaged angular displacements of all subjects (in degrees). The angular displacements shown are of phase 6 of the right knee, phase 2 of the left knee, and phase 3 of the left ankle. The solid line represents the stimulus data and the dashed line represents the control data. The black bars in the lower part of the figures indicate the stance phases. Generally, a shortening of the step cycle and a small decrease in range of motion was seen. Abbreviations: FL = maximum flexion, Ex = maximum extension, St = stance, Sw = swing.
Inspection of the timing results revealed that in general the maxima occurred earlier in the stimulated cycles as compared to 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 stimulus data increased when more time elapsed (see Table 2.2). 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 2.2) 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 (phase 2) 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

![Fig. 2.11. Sequential effect on the phase-dependent modulation of all four muscles. The same format is used as in Fig. 2.5-8. Normalized subtracted data of the total responses are presented of the average of trial 1 and 2 (thin solid line), the average of trial 9 and 10 (dashed line), and the average off all trials (thick solid line). The asterisks represent significant differences between the first and the last two trials.](image-url)
Chapter 2. Modulation of the startle response during human gait

Comparing the first two trials of each phase with the last two trials (trials 9 and 10).

In Fig. 2.11 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 amplitude of the last trials were 40%, 21%, 27%, and 58% of the first trials for the BF, RF, TA and SO, respectively. Compared with the mean data, the pattern of phase-dependent modulation of all muscles was similar for the first two trials. Comparison between the first and the last trials showed also a similar modulation patterns, except for BF.

![Figure 2.12](image)

Fig. 2.12. Mean changes and SE (n=16) in timing of heel-on and toe-off of trial 1 and 2 (light) versus trial 9 and 10 (dark) of A: the right foot and B: the left foot. The data presented are the averages of all three phases. The asterisks below the bars represent significant differences from the control activity. The asterisks on the X-axis represent significant differences between the first and last two trials. Abbreviations: see Fig. 2.9.

Short-term effects of habituation were hardly present in the kinesiological data. In the foot switch signals of both first and last trials, a comparable shortening was observed in the step cycle duration (see Fig. 2.12). In the first phase transitions (first toe-off (TO1) in the right leg and first heel-on (HO1) in the left leg), 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 to the last trials. Note that Fig. 2.12 shows the average of the 3 phases. Between the 3 phases of each leg, no significant changes were seen.

In general, the gonio signals for both first and last trials showed effects which 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 degrees compared with the control data.
2.4. Discussion

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 versus 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 brainstem 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 to 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 rostro-caudal distribution in amplitude is also present in the lower half of the body. These findings suggest that the rostro-caudal 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, 1991b; 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 monoarticular,
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 (see Table 3). 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 either 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 (see Table 3). When the patients were standing, a third response was seen in TA after ~60 ms. In this way three waves were identified, which 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 clearly two separate responses 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 to 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, which allows 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 (“premotoneural modulation”). Such cases are of interest since it may reveal how the central nervous system actively modulates reflexes in order 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 brainstem, Drew (1991) also observed clear premotoneuronal gating during cat locomotion. He suggested a spinal structure, such as the Central Pattern Generator for locomotion (CPG) 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 motoneurones 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 (Duysens et al. 1996; for a review see Duysens 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 Duysens 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; Duysens et al. 1990-1996; Van Wezel et al. 1997; Yang and Stein 1990; Zehr et al 1997). Furthermore, the presently found suppressive responses in TA had a latency (around 80 to 120 ms) and occurrence (during swing) which was similar to the cutaneously induced suppressions. In contrast, the modulation pattern is very different from the one seen in cutaneous
Chapter 2. Modulation of the startle response during human gait

reflexes. In cutaneous reflexes the largest facilitatory responses in TA are seen at the end of the stance phase and early swing, which 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 is 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. Schepens and Delwaide (1995) also found coactivation in TA and SO when startle stimuli were elicited in standing subjects, and by Delwaide and Schepens (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. 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. These results indicate that the ASR induces a temporary limb stiffening reflecting the large amount of
Cocontraction. 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 in order to brace for action (freezing). During early/mid stance, 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/mid swing 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.
CHAPTER 3
STARTLE RESPONSES IN PATIENTS WITH PARKINSON’S DISEASE DURING TREADMILL WALKING

Adapted from: Nieuwenhuijzen PHJA, Horstink WM, Bloem BR, and Duysens J. Startle responses in patients with Parkinson’s disease during treadmill walking. Submitted for publication.
3.1. Introduction

In a previous paper we reported the phase-dependent modulation of the auditory startle response during walking in healthy subjects (Nieuwenhuijzen et al., 2000). It was found that the amplitude of startle responses in these subjects depended on the timing of the auditory stimulus in the step cycle. Especially during the stance phase, large responses were detected in both flexors and extensors, leading to cocontraction and temporary limb stiffening presumably aimed at stability. These cocontractions could be well integrated into the ongoing step cycle, allowing for a smooth progression of gait (Nieuwenhuijzen et al., 2000).

How is this integration achieved in Parkinson’s disease? There are several reasons to suspect an abnormal startle response during locomotion. The central pattern generator (CPG) is suggested to be the most likely structure to modulate the startle response during locomotion (Drew, 1991; Schepens and Delwaide, 1995; Nieuwenhuijzen et al., 2000). Recent evidence suggested an impaired spinal locomotor pattern generator in parkinsonian gait (Eberschbach et al., 1999). Does this lead to defective phase-dependent modulation of the startle response?

Secondly, Delwaide et al. (1990, 1991, 1993) found evidence for an abnormal functioning of the reticulospinal pathways in Parkinson’s disease. The auditory startle response, originating in the nucleus reticularis pontis caudalis, is considered to connect with efferents in this reticulospinal pathway (Davis, 1984; Yeomans and Frankland, 1996). Moreover, connects with afferents originating in the basal ganglia (Kofler et al., 2001). Basal ganglia show an abnormal output in Parkinson patients (Alexander and Crutcher, 1990 (uit Vidailhet ‘92). Therefore, this abnormal output might influence the startle response. This influence on startle is of special interest in the context of gait, since it has been proposed that the basal ganglia output to brainstem nuclei is involved in the control of normal posture and locomotion (Coles et al., 1989; Garcia-Rill, 1991) and that disturbances of this projection contribute to postural instability in Parkinson’s disease.

Several studies have investigated startle reactions in Parkinson patients with contrasting results. Compared with healthy subjects, either prolonged (Vidailhet et al., 1992), normal (Bisdorff et al, 1999), or reduced (Kofler et al., 2001) response latencies have been reported in patients with Parkinson’s disease. Also the amount of observed habituation differed, with a normal pattern of habituation in the study of Koffler et al. (2001) and no habituation in the study of Vidailhet et al. (1992).

Therefore, investigating the auditory startle response during walking in patients with Parkinson’s disease might give more insight in the functioning of the CPG and reticulospinal pathway in these patients, and provide better understanding of the nature of their gait abnormality. Furthermore, studying these responses might clarify the occurrence of falls in these patients while walking when startled (Bloem and Bhatia, in press). To answer these questions, we investigated the startle response in Parkinson patients during walking.
3.2. Methods

A detailed description of the methods, performed on the healthy subjects mentioned in the present study, has been given elsewhere (Nieuwenhuijzen et al., 2000). Eight Parkinson patients (6 males and 2 females; age range 43–74 yr) and eight healthy subjects (4 males and 4 females; age range 19–27) participated after given informed consent. The experiments were performed in conformity with the declaration of Helsinki for experiments on humans. None of the subjects had a known hearing disorder. Furthermore, none of the healthy subjects had a neurological or motor disorder. The patients were on normal medication. Motor disability was evaluated using the UPDRS (Fahn et al., 1987). The patients were only mildly affected showing a UPDRS score varying from 2 to 13. Since arm function and leg function correlate moderately, the leg function was also evaluated separately by the walking velocity and stride length (velocity range: 1–4 km/h, stride length range: 68 cm–88 cm).

The healthy subjects walked on a treadmill at a comfortable speed of 4 km/h and the comfortable velocity of the patient group varied between 1 and 4 km/h. During the experiment subjects wore a safety harness fastened to an emergency brake at the ceiling. Furthermore, an additional emergency brake was attached to the handrail of the treadmill. Startle responses were elicited using a stimulus of 50 ms white noise with an intensity of 110 dB. Bipolar electromyographic (EMG) activity was recorded from the biceps femoris (BF) and the tibialis anterior (TA) of both legs. Placement of the electrodes was standardized according to Basmajian (1989). These 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). Thin insole footswitches 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 a period starting 100 ms before stimulation and lasting for 2,100 ms. To further document the startle-induced movements and the gait characteristics, the experimental sessions were videotaped.

Experimental Protocol

The auditory stimuli were given unexpectedly with a random interstimulus interval of 1.5–2.5 min. At three moments in the swing phase (early, mid, or late swing) of the left leg, the stimuli were presented. At these moments the right leg was in early, mid or late stance phase. Therefore, the simulation was considered to occur at 6 different percentages during the step cycle (16, 28, 38, 66, 78, and 88%). All stimulus conditions were applied 5 times in random order. Control trials (i.e. no stimulus) were measured in the same phase of the step cycle ~ 4 s prior to the stimulus trial.
Data analysis

For each phase the control data were averaged and subtracted from both individual and averaged stimulus trials. A single time window was set over the 6 phases of the individual trials. In this way 30 traces were investigated for each subject (6 phases x 5 trials). Latency and duration was defined as the onset and duration of this time window. The response amplitudes were calculated by averaging the rectified EMG signal within the time window. To enable a proper comparison between subjects and muscles the data were normalized with respect to the maximum EMG activity during unperturbed walking. To determine whether the responses were significantly different from the background activity 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. Sequential effects in EMG were also examined by the Wilcoxon signed-rank test. Correlation between the severity of the disease and the degree of habituation was tested using the Spearman's rho. The same test was used to test for correlations between the response amplitude and background activity. In all statistical tests a significance level of $P < 0.05$ was used.

3.3. Results

The results for the healthy subjects have been given elsewhere (Nieuwenhuijzen et al. 2000). The results of the patients will be discussed mainly in relation to these healthy subjects.

![Fig. 3.1. Typical EMG response patterns of the tibialis anterior to auditory stimulation during locomotion (single trials of a single representative patient). A: 2 facilitory responses found in both BF and TA (F2 with a latency of ~80 ms and F3 with a latency of ~145 ms). B: 3 responses recorded only in TA (F1, a facilitory response with a latency of ~60 ms and S a suppressive response with a latency of ~100 ms). Furthermore a long latency response was found with a latency of ~145 ms corresponding in latency to the F3 shown in A.](image-url)
### Table 3.1. Startle response characteristics.

<table>
<thead>
<tr>
<th>Latency (ms) N=8</th>
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<th>Amplitude N=240</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parkinson</td>
<td>Healthy</td>
</tr>
<tr>
<td>TA: F1</td>
<td>62 ± 3</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>F2</td>
<td>77 ± 3</td>
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</tbody>
</table>

Table 3.1. 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 = 240; 8 subjects x 6 phases x 5 responses) were used, expressed as a fraction of the maximum background activity (see Methods). Values are means ± SE. The asterisks indicate significant higher amplitudes of the patients compared to the healthy subjects (Wilcoxon signed-rank test: P < 0.02).

### Response characteristics

In all muscles and all subjects from both groups significant responses were found. Similar to the healthy subjects three facilitatory responses were detected in patients (see Table 3.1 and Fig. 3.1). For both onset latency and duration, no significant differences were found between the two groups (Wilcoxon signed-rank test: P > 0.05). Early responses were detected only in TA and were termed F1 responses with a latency of ~60 ms and a duration of ~40 ms. Furthermore, in both TA and BF a mid latency response was found, called F2, with a latency of ~80 ms and a duration of ~65 ms. In addition, in both muscles a late latency response was observed, called F3, with a latency of ~145 ms and a duration of ~70 ms. During swing a suppressive response, called S response, was observed instead of the facilitatory F2 response in all but one healthy subject. However, in the Parkinson group clear suppressive responses were observed in only 4 patients.

In contrast to the latency and duration, the amplitude of the responses differed between both groups. Except for the F3 of the TA, the patients showed significantly larger mean responses compared to the healthy subjects (Wilcoxon signed-rank test: P < 0.02). Especially the F1 of the TA and the F2 and F3 of the BF showed 1.5 to 2 times higher responses in the Parkinson patients.

### Habituation

One possible explanation for the observed difference in amplitude is a difference in habituation. The startle response is known for a rapid habituation. To study the habituation, the overall response activity of all muscles of all subjects and all 3 phases was averaged for each of the 5 trials (see Fig. 3.2). The averaged
amplitude of the first trial was set to 100%. The absolute amplitude of the first trial showed no significant difference between both groups (mean amplitude of 0.45 ± SE 0.04 for the healthy subjects and 0.46 ± SE 0.04 for the patients) (Wilcoxon signed-rank test: P < 0.05). After 5 trials the healthy subjects showed a significantly larger response decrease compared to the patient group (50% and 76% respectively). Significant difference in the amount of response decrease between patients and controls was already observed at the second trial (Wilcoxon signed-rank test: P < 0.05). To examine if the degree of habituation was correlated with the severity of the disease, as assessed by the UPDRS, walking velocity, and stride length, two methods were used. The habituation was expressed as a ratio of the first and the last trial, or as a ratio of the average of the first two trials and the average of the last two trials. Both methods showed no significant correlation between the habituation and the severity of the disease.

**Phase dependent reflex modulation**

In both groups the response amplitude depended on the timing of stimulation during the step cycle. This so-called “phase-dependent modulation” can be observed in Fig. 3. Except for the larger decrease in the F3 of the TA of the patients group during the swing phase, the response amplitude pattern during the 6 phases of the step cycle showed in both groups a similar modulation, with generally large responses during the stance phase and smaller responses during the early/mid swing (phase 4 and 5). For both groups all responses, except the F1 of the TA and the F3 of the BF, showed a significant effect of phase (Kruskall-Wallis 1-way ANOVA, P < 0.05). Between patients and healthy subjects no significant difference in phase-dependent modulation could be detected (Kruskall-Wallis 1-way ANOVA, P < 0.05).
ANOVA, $P < 0.05$). However, as observed earlier, in patients the mean response amplitude was mostly larger, as was the background activity (up to twice as large). In both groups, there was no strict correlation between the response amplitude modulation and the background modulation. Especially the F2 of the TA showed in both groups an almost opposite modulation pattern in the subtracted amplitudes compared to the EMG activity during normal walking in the same phase. Furthermore, there was no correlation between the difference in background activity of both groups and the difference in response amplitude (Spearman’s rho, $P > 0.05$).

![Fig. 3.3. Phase dependent modulation of the responses found in the TA and the BF after auditory stimulation. The amplitudes of the responses are plotted as a function of the phase in the step cycle. Top: stick diagrams indicate the position at the start of the response. Middle: average EMG activity of the patients after stimulation (-) and during normal walking in the same phase (---). Bottom: subtracted EMG activity and SE (n = 8 subjects x 5 trials = 40 trials) of healthy subjects (thin line) and patients (thick line). Data are normalized with respect to the maximum background activity of each muscle (see Methods). The dark brown bars at the bottom left corner represent the stance phase. Generally higher responses are observed in the Parkinson group but with a similar phase dependent modulation.](image)
3.4. Discussion

The main finding of the present study is that startle responses evoked during walking showed similar latencies, durations, and phase-dependent modulations between these patients and healthy subjects, but the amplitude and habituation differed between these groups.

Response characteristics

There were hardly differences found between patients and healthy subjects for the response latency and duration. In the literature there is no general consensus about changes in latency between the two groups. Vidailhet et al. (1992) found a delayed response latency of the startle response was in the Parkinson group. Kofler et al. (2001), however, found shorter latencies compared to healthy subjects. The latency of startle responses is known to be task and posture dependent (Brown et al. 1991, Delwaide et al. 1995, Schepens et al. 1995, Nieuwenhuijzen et al. 2000), which might explain the difference found between the present study and some of the other studies. In these studies, subjects where either lying down (Kofler et al., 1992) or sitting (Vidailhet et al., 1992). Another explanation might lie in the larger amplitude of the response in the Parkinson group in the present study as compared to the previous ones, as it is well known that larger responses can lead to smaller latencies. The mean response amplitude was indeed found to be up to 2 times higher in the Parkinson group compared to the healthy subjects.

According to the automatic gain principle (Bloem et al., 1993; Matthews, 1986), the higher response amplitude could be explained by the higher background activity in Parkinson patients. Indeed, the background activity in some phases was almost twice as high in the Parkinson group. However, there was no correlation between the difference in background activity of both groups and the difference in response amplitude. Another explanation for the larger response amplitudes in the patients might lie in their fear of falling. Parkinson patients suffer frequently from falls (Bloem et al. 2001) resulting in fear of falling during walking (Bloem et al., 1999). Fear potentiates the startle response and could thereby increase the amplitude of the response. Finally, the patients showed a decreased habituation, thereby increasing the average amplitude (see further).

Phase-dependent modulation

The phase-dependent modulation of the startle response was similar in both groups. We suggested earlier (Nieuwenhuijzen et al., 2000) that the central pattern generator (CPG) is the most likely structure for regulating the phase-dependent modulation. Patients were tested during their ON state, hence medication might have masked more prominent abnormalities. Ebersbach et al. (1999) found in Parkinson patients some irregular timing of steps when they adapted to a rhythmic constraint in their cadence, suggesting impaired locomotion pattern generation. The authors imply a defective supraspinal control on the CPG to account
for the irregular timing. The present results do not necessarily undermine their conclusion but instead indicate that the abnormalities do not affect the normal phase-dependent modulation of startle responses.

**Habituation**

The Parkinson patients in the present study showed less habituation of the startle response compared to the healthy subjects. The question whether startle responses show a different habituation in Parkinson's disease is still unresolved. A normal pattern of habituation was observed in the study of Koffler et al. (2001) and no habituation in the study of Vidailhet et al. (1992). However, Vidailhet et al. (1992) studied only three stimuli while Kofler et al. (2001) elicited eight stimuli. Although normal habituation was observed in postural stretch reflexes (Bloem et al. 1998), studies on other reflexes like cutaneous reflexes (Delwaide et al., 1974), P1 or P50 midlatency auditory evoked potentials (Teo et al., 1997) and blink reflexes (Kimura, 1973; Messina et al., 1970; Penders and Delwaide, 1971; Sandrini et al., 1985) all showed a decreased habituation in Parkinson patients. The observation on the blink reflex is of special interest since this reflex is associated with startle. Furthermore, startle responses are known to show reduced habituation when preparing for a motor task (Valls-Solé et al., 1997). The attention needed for the motor preparation might partially block the input from other sensory signals to the sensorimotor cortex (Brunia, 1993). In contrast to the largely automatic gait control in healthy subjects, gait in Parkinson's disease is more conscious (Bloem and Bhatia, 2003), thereby decreasing the cortical control of startle reactions. Evidence gathered from humans with “brain pathology” suggests that the cerebral cortex inhibits the startle reaction (Liégeois-Chauvel et al. 1989). However, cerebral cortical projections are not essential for startle responses. Startle responses have been obtained in choralose anaesthetized or in decerebrated animals (see Davis et al. 1982 for a review). Delwaide et al. (1993) postulate that the nucleus reticularis gigantocellularis is functionally modified in Parkinson's disease and contributes to the rigidity found in these patients.

In addition, a positive correlation was found between the rate of habituation of the blink reflex and disease severity (Matsumoto et al. 1992). Other studies in Parkinson patients found a similarly positive correlation when studying P1 or P50 midlatency auditory evoked potentials (Teo et al., 1997). In contrast, we found no correlation for the severity of the disease and the degree of decrease of habituation. This may be due to a relatively larger homogeneity of the present group as compared to these previous studies.

In conclusion the present study showed that although these patients where mildly affected (UPDRS range: 1.2 – 12.5), the data clearly indicate that habituation is affected while other reflex abnormalities were absent. This indicates that habituation of startle is a very sensitive measure in these patients. The question remains what happens in more severe cases of Parkinson's disease. Patients who suffer from freezing would be of special interest, since startle responses during gait show a stiffness increase.
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CHAPTER 4

MECHANICALLY INDUCED ANKLE INVERSION DURING HUMAN WALKING AND JUMPING

4.1. Introduction

Ankle inversion injuries are a common trauma. Particularly sports involving running and jumping are known for the high incidence of inversion traumata (Bahr et al. 1994; Balduini et al. 1987). It is estimated that, each day, one inversion injury of the ankle occurs for every 10,000 people (Lynch and Renström, 1999). Furthermore, ankle sprains constitute 7-10% of all admissions to hospital emergency departments (Lynch and Renström, 1999). The injury results in various degrees of mechanical damage and can cause instability (Kannus et al., 1991). Furthermore, up to 60% of the ankle sprains are recurrent sprains (Balduini et al., 1987).

A number of studies has investigated sudden inversion in standing conditions using a trap door (see for example: Ebig et al., 1997; Johnson and Johnson, 1993; Konradsen et al., 1991; Lynch et al., 1996; Podzielny et al., 1997). However, in every day life injuries rarely occur with a person standing at rest. Little is known about muscle responses after inversions under more natural conditions such as walking and jumping. One study of Konradsen et al. (1997) mentions some experiments of inversion after stepping on a trap door, but no full description of the method is provided. Nobody has made an attempt to develop a method evoking inversions using a treadmill. The use of a treadmill has the advantage of controlling the timing of the perturbation. As far as jumping is concerned to our knowledge there are no previous studies.

The results of the inversion studies during standing cannot a priori be extrapolated to more dynamic conditions, since various reflexes are known to be task-dependent (for review see Zehr and Stein, 1999). For instance, Capaday and Stein (1986) described smaller H-reflexes during walking than during standing. Even smaller responses were found during running (Capaday and Stein, 1987). In addition, cutaneous reflexes elicited during running were larger than when these responses were elicited during walking (Duysens et al. 1993).

A second argument for studying inversion perturbations during walking and jumping rather than in standing is given by A second argument for distinguishing between responses found after inversions during standing conditions and during walking or after jumping is given by Stormont et al. (1985). These authors found in an in vitro study that when the ankle was physiologically loaded, as occurs during standing at rest, inversion and eversion stability is high and solely accounted for by the articular surface. Other in vitro studies also demonstrated that loading increases the stability of the ankle (McCullough et al., 1980; Sammarco, 1977). Although in vitro studies can not determine the role of the muscles in stabilizing and protecting the ankle joint, these studies do suggest as Stormont et al. (1985) mentioned that ankle instability occurs during loading and unloading, as occurs during walking or jumping, but not once the ankle is fully loaded. Similarly, in vivo an increase of the stability was found during loading of the ankle (Sheuffelen et al., 1993). Studying ankle inversions during the loading acceptance of the stance phase of walking or during the landing phase of jumping might therefore give new insights in the control of ankle or ankle stability.
According to Lynch et al. (1996), increased speed of inversion causes a shorter response latencies in the peroneus longus. To study muscle responses after inversion, the speed of inversion must therefore be constant during the experiment. Furthermore, the speed must be high enough to evoke muscle responses at all.

Therefore, the method described in the present study investigates rapid ankle inversion, elicited during the loading part of walking and jumping. It will be shown that with this method it is possible to evoke reproducible sudden ankle inversions with an eliciting characteristic lower leg responses under controlled laboratory settings.

4.2. Methods

Ankle inversions were recorded during the walking task in 12 healthy subjects (6 males and 6 females; age range: 22-28 years) and during the jumping task in 11 healthy subjects (5 males and 6 females; age range: 22-28 years). The experiments were performed after informed consent had been obtained and in conformity with the declaration of Helsinki for experiments on humans. None of the subjects had a known ankle instability or weakness, or a neurological or motor disorder.

A trap door box produced the mechanical induced ankle inversions. This box, consisting of PVC plastic and metal, had a length, width, and height of 35, 20, and 10 cm, respectively (see Fig. 4.1). A spiral spring kept the trap door on top of the box in neutral position (i.e. 0° tilting). Except for the resistance of the spiral spring, the trap door was controlled by gravity. To overcome the initial resistance
of the spring only 200 gram was needed for the first visible rotation (of 0.1°) of the trap door and 2300 gram was needed for a rotation of 25°. Therefore, the delay between first contact of the foot on the trap door and the start of the inversion is negligible. The trap door could tilt up to 30°. However, pilot studies showed reproducible and clearly detectable responses using only 25°. To minimize the risk of injury, the present study therefore used this 25° for the stimulus trials. For the control trials a tilting of 0° was used. This restriction of rotation could mechanically be established through inserting a wedge in a slit. A build-in potentiometer was used to measure the amount of rotation of the trap door (KMA 10/70, Philips Semiconductors and AV Electronics). To prevent that the subjects could see whether the next trial a stimulation trial or a control trial was going to be, the box was covered with a black cover to hide the wedge in the slit from the view of the subject.

Kinematic measurements were made of the left limb and trunk using a 3D infrared motion analysis system (Qualisys, MacReflex system) with a sample rate of 50 Hz and passive markers. The markers were placed on the left shoulder, crista iliaca, trochantor major, lateral epicondyl of the femur, fibular head, lateral malleolus, heel, metatarsal bone I. In this way, angles of the hip, the knee, and the ankle could be obtained.

Bipolar surface electrodes, with an inter electrode distance of 30 mm, measured electromyographic activity (EMG) of the tibialis anterior (TA), the peroneus longus (PL), the peroneus brevis (PB), the soleus (SO), the gastrocnemicus lateralis (GL) and the gastrocnemicus medialis (GM) muscles of the left leg (the leg that stepped on the trap door). The placement of the EMG electrodes for the TA, SO, GM, GL and PL was standardized according to Basmajian. (1989). For the PB, electrode placement was used according to Lynch et al. (1996). Skin impedance was reduced to less than 5 kW by cleaning the electrode sites with alcohol and rubbing with sandpaper. Cross-talk was examined by online monitoring using muscle specific tests. After the experiment, cross-talk between the measured muscles was tested using a Spearman correlation test. No statistically significant correlation was observed (P > 0.05).

Thin insole footswitches (designed in collaboration with Algra Fotometaal b.v., Wormerveer, The Netherlands) were used to detect foot contact during the walking task and to deliver a trigger signal for the release of the trap door box on the treadmill. The EMG and footswitch data were sampled at 500 Hz. The EMG signals were (pre-) amplified (by a factor in the order of 10^4-10^5), high-pass filtered (cut-off frequency at 3 Hz), full wave rectified, and then low-pass filtered (cut-off frequency at 300 Hz). During the experiment the signals were visually inspected by on-line monitoring on an oscilloscope and on a computer display.
Experimental protocol

Inversion during gait (see Fig. 4.2)

The subjects were asked to walk on a treadmill (Woodway type ERGO EL2; walking surface: length x width = 2.0 x 0.7 m) at a speed of 4 km/h wearing a safety harness which was fastened to an emergency brake at the ceiling.

An additional emergency brake was attached to the handrail of the treadmill so that the subject could stop the treadmill at any moment. Before the actual experiment started, the subjects were trained to walk on the treadmill at a comfortable, constant pace. During the experiment, an electromagnet held the box by a metal circular plate (of 7 cm diameter) sunk into the middle of the trap door. The hanging box was positioned approximately 1 meter in front of the location of the left heel strike. The bottom of the box was 11 mm above the treadmill surface. At a preprogrammed delay (dependent on the step cycle time) after heel strike of the left foot, the computer triggered the electromagnet to release the box on the treadmill. To program the delay, the step cycle time was, prior to the experiment,
calculated over 20 strides. A trigger signal was given to the computer by the insole footswitch of the left foot. At the time of the next heel strike, the box should be at the location of foot placement. Therefore, the magnet should release the box a specific time earlier to cover the distance (kept constant at 1 m) from the electromagnet to the location of heel strike. This time was calculated by dividing the distance by the velocity of the treadmill (4 km/h = 1.11 m/s).

Furthermore, pilot studies showed that the magnet had a small delay, after triggering the computer, before dropping the box (mean 169 ms; SE=1.0 ms; n=60). Consequently, this magnet release time was also included into the formula for the preprogrammed delay: Delay = step cycle time / velocity - magnet release time.

The subjects wore headphones through which loud music was played, to prevent them from hearing the box fall on the treadmill and from getting any cues about the type of trial condition. To ensure that the subject could step on the trap door without changing the cadence of the step cycle, it was important that the subject kept the same (anterior-posterior) position on the treadmill during the experiment. Furthermore, the actual position of the subject was such that left heel strike on the box was always one meter in front of the electromagnet. In this way reaction time between subjects and trials was kept as constant as possible in order to minimize differences in the amount of anticipation. To help subjects remaining this position, visual feedback was given using a series of light emitted diodes that was connected to position measuring device based on sonar. This sonar was aimed at the thorax and every next diode stood for 10 cm of displacement. Subjects could walk relatively comfortably within the boundaries of one diode (i.e. 10 cm). To control medial-lateral placement on the trap door and consequently equal vertical drop for all subjects and trials, the subjects were instructed to step on the circular metal plate of the trap door. The center of this metal plate was located at 7 cm from the fulcrum, causing a maximum vertical drop of 3.0 cm.

The experiment consisted of 20 stimulus trials and 20 control trials. These 40 trials were presented randomly. The signals were stored on hard disk in a period starting from the moment of the release of the box and lasting for 4000 ms.

*Inversion during the landing phase of jumping (Fig 4.3).*

Subjects had to jump from a platform 30 cm above the landing surface. This landing surface consisted of the trap door for the left foot and a solid box with equal dimensions and equal material for the right foot. The two boxes were positioned 5 cm in front of the platform. A pressure sensitive strip was attached on the surface of the trap door to detect foot contact. The subject initiated the drop by positioning the left leg slightly forward and jumping from the platform by pushing off with an almost straight right leg. In this way the jumping task was standardized and jumping upwards or decreasing the speed of landing by lowering on the platform, was restricted. The subjects were instructed to land with both feet simultaneously on the circular plates located in the middle of each of
the two boxes. Before data collection, each subject practiced the technique without rotation of the trap door. The subjects wore headphones with loud music and the wedges were replaced outside the field of vision of the subject to prevent any pre-knowledge of the type of trial. Both stimulus and control conditions were applied 20 times in a random order. Measurements started 1000 ms prior before the landing and lasted 2000 ms.

Statistical Data analysis

Zero time was defined as the moment the foot touched the box. The average control EMG (stepping on the box without rotation of the trap door) was subtracted from the individual stimulus EMG (stepping on the box with rotation of the
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This subtraction method enables one to look at the net effect of the stimulus. (Duysens et al. 1990; Van Wezel et al. 1997; Yang and Stein 1990; Zehr et al. 1997). For each response peak, a time window was set by visual judgment on the average EMG data of all trials for all muscles of each subject. To examine intra subject variations of the EMG responses, time windows were also set on the individual subtracted trials of the PL for each subject. Latency and duration was defined as the onset and duration of the time window after the start of rotation of the trap door. To determine whether the responses observed were statistically significant, the Wilcoxon signed-rank test was used. Responses that were not significant were excluded.

Possible difference in step cycle duration between normal walking control trials and stimulus trials was tested by the Wilcoxon signed-rank test. In all statistical tests a significance level of $P < 0.05$ was used.

4.3. Results

Stepping on a box versus normal walking

To verify whether the subjects stepped on the box in a natural continuation of the step cycle, the timing of heel strike during normal walking, control trials (i.e. stepping on the box without rotation of the trap door), and stimulus trials (i.e. stepping on the box with rotation of the trapdoor) was compared. Although significant (Wilcoxon signed-rank test: $P < 0.05$) only a small average decrease of 1.8%

<table>
<thead>
<tr>
<th>Subject</th>
<th>Duration of trap door rotation during walking ± S.E. [ms]</th>
<th>Successful trials</th>
<th>Duration of trap door rotation during jumping ± S.E. [ms]</th>
<th>Successful trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51 ± 0.5</td>
<td>16</td>
<td>44 ± 0.7</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>55 ± 1.3</td>
<td>19</td>
<td>48 ± 1.1</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>65 ± 3.3</td>
<td>13</td>
<td>40 ± 0.9</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>64 ± 2.2</td>
<td>16</td>
<td>38 ± 0.3</td>
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<tr>
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</tr>
<tr>
<td>6</td>
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<tr>
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<tr>
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<td>62 ± 1.7</td>
<td>17</td>
<td>42 ± 1.2</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 4.1. Duration of the trap door rotation with standard error and number of successful trials of all subjects during walking and jumping.
(i.e. a decrease of approximately 20 ms) of the step cycle time was found in the control trials as compared to normal walking (based on n=20x12=240 trials). The stimulus trials (n=240) showed an even smaller decrease of 0.9% compared with normal walking.

**Incidence of successful trials**

Both during the walking task and the jumping task, successful inversions could be elicited in all subjects and in the majority of the trials. During the walking task, at least 13 out of 20 stimulus trials were performed correctly by the subjects (see Table 4.1). The average percentage of successful trials was 85 % (SE: 4.0%; n=12). A trial was considered successful when the foot was placed correctly in the middle of the trap door and no extra short step was made prior to the step on the trap door.

During the jumping task an even higher rate of successful stimulus trials was achieved. At least 17 out of 20 stimulus trials were performed correctly (see Table 4.1). The average percentage of successful trials was 95 % (SE: 1.5%; n=11). A trial was considered successful when the subject landed correctly on the middle of the trap door.

**Angular velocity of the trapdoor during inversion**

When ankle inversions were elicited during walking, the average duration of the 25° rotation of the trap door was 62ms with a standard error for 12 subjects of 1.7ms. Converted to average angular velocity this means 403°/s (SE: 18°/s; n=12). An example to illustrate the consistency of these inversions can be seen in Fig. 4.4. The average duration varied between 51 ms and 70 ms with a maximum standard error of 3.6 ms (see Table 1). The duration of the rotation did not correlate with the weight of the subject (Spearman’s rank correlation: $r = 0.15$, $P=0.64$). An example of the successful inversions of a single subject can be seen in Fig. 4.4.

During the jumping task, 25° of rotation was completed in 42 ms with a standard error for 11 subjects of 1.2ms. This duration corresponds with an angular
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velocity of 595°/s (SE: 27°/s; n=11). Over all subjects the mean duration varied between 37 and 48 ms with a maximum standard error of 1.7 ms (see Table 4.1). In contrast to the walking task, during the jumping task duration of the rotation did correlate with the weight of the subject (Spearman’s rank correlation: r=-0.644, P=0.32).

**Responses**

In both tasks, reflex responses could be elicited in all subjects and all muscles. An example of EMG activity after an inversion during gait is illustrated in Fig. 4.5. During gait, in all muscles, a small response (M1) could be observed ~40 ms after the start of the inversion and this response had a duration of ~25 ms. A second response (M2) was seen after ~100 ms with a duration of ~35 ms. During the jumping task also an early response was found, in all muscles, ~35 ms after the onset of rotation. This response had a duration of ~20 ms. A second response was found with a latency of ~90 ms and a duration of ~30 ms.

For a more detailed description of the reproducibility of the latency and the duration of the EMG responses, the PL (peroneus longus) was examined in more detail for both tasks by setting windows on individual trials. To indicate the variation within subjects (intra subject), the standard error that was found for the response latency and duration of each of the subjects was averaged and expressed in the mean individual SE. Variation between subjects was evaluated by the standard error (SE) of the mean values of the two groups (n=12 for the walking task and n=11 for the jumping task). During walking, the M1 response showed a mean latency of 42 ms ± 1.0 ms (SE) (mean individual SE: 2.8 ms) with a mean duration of 21 ms ± 0.7 ms (mean individual SE: 1.9 ms). The M2 response during walking showed a mean latency of 93 ms ± 1.0 ms (mean individual SE: 1.7 ms) with a mean duration of 35 ms ± 1.0 ms (mean individual SE: 1.9 ms). A significant M1 could be observed in 52

![Fig. 4.5. Typical subtracted and normalized EMG responses of a single subject of 15 successful trials of the peroneus longus measured during the walking task. Zero time is the moment the foot touches the trap door. Two responses were found (M1 and M2).](image-url)
% of all trials. The M2 showed a much higher incidence of significant responses (96% of all trials).

During the jumping task, a mean M1 latency was found of 41 ms ± 1.1 ms (mean individual SE: 1.7 ms) with a mean duration of 18 ms ± 0.8 ms (mean individual SE: 2.8 ms). The M2 response showed during this task a mean latency of 87 ms ± 0.6 ms (mean individual SE: 2.3 ms) and a mean duration of 27 ms ± 1.0 (mean individual SE: 2.0 ms). The M1 response could be observed in only 17% and the M2 in 61% of all successful trials of all subjects.

4.4. Discussion

Ankle sprain is considered to be the most common sports injuries among athletes from different disciplines. The possible role of neural mechanisms behind the ankle sprains remains unresolved. We have designed the current method to be able to evaluate the contribution of the lower leg muscle reflexes in protecting the ankle joint during sudden inversion motion under natural conditions.

In this study it was shown that with this method it is possible to successfully reproduce ankle inversions during walking on a treadmill and during the landing phase of jumping. The singularity of the used method makes it difficult to compare the rate of successful trials with other studies. However, the present rate of 75% and 95% successful perturbation trials during the walking and jumping task is comparable with mechanical perturbations of the lower leg during human walking in previous studies. For example, in the study of Schillings et al. (1996), who studied stumbling reactions during human walking, 61% of all perturbations was successful.

In previous studies the ankle inversions were elicited almost exclusively in a standing position. However, inversion injuries occur most frequently during locomotion and jumping. Furthermore, inversion injuries seem to occur in real life mainly during loading of the ankle (Stormont et al., 1985). In the current set-up the trap door starts to tilt the moment the foot touches the box. Therefore, the trap door tilts when the ankle starts to be loaded. Compared to other studies the current approach is the only method that enables investigating sudden ankle inversions during this hazardous part of the step cycle during gait and during the landing phase after jumping.

To mimic the natural situation of inversions occurring during walking and to ensure stimulus constancy, the step cycle was evaluated during normal walking and stepping on the box in both control and stimulus trails. The step cycle time was hardly different during the control and stimulus trails as compared to normal gait, indicating the subjects could step on the box without changing their walking cadence. Only a small difference (10-20 ms) was found between the different conditions, which can be explained by the height of the box causing an slightly earlier heel strike.

As was stated in the introduction the speed of inversion must be high enough to elicit responses. Lynch et al. (1996) already found responses after inversions of 50°/s. The inversion speed used in the present study was 403°/s during walking and 595°/s in the jumping task. The difference in velocity between both tasks can be explained by the higher impact on the trap door in the jumping task compared to the walking task. Furthermore, the velocity of the trap door depended on the weight of the subject dur-
ing the jumping task. This was expected since the primary force during this task is gravity. However, a perfect correlation had to be found if gravity was the only factor determining the velocity of the trap door. This perfect correlation was not found, because subjects had to land on both feet simultaneously, thereby allowing them to put a different load on each leg. Preference for the left or right leg, or being somewhat anxious for the inversion could account for a weight transfer. In contrast to the jumping task, during the walking task no significant correlation was found between the weight of the subject and the velocity of the trap door. During this task, rather than gravity, the speed of loading of the foot determines the velocity of the trap door. However, as can be seen in table 1 these differences between subjects influenced the velocity in both tasks only slightly and compared to the study of Isakov et al. (1986) a similar range in duration of the inversion was found during the walking task (51-70 ms) and an even smaller range was found during the jumping task (37-48 ms). This small variation in inversion duration underlines the reproducibility of the stimulus. The standard error of the duration of rotation never exceeded the 3.7 ms, indicating a small intra subject variation.

Although the trap door delivered a constant external stimulus, anatomical variance of the subtalar joint axis can provide inter subject variation in the movements of the joints involved in inversion. Consequently, no assertions can be made about movements in the separate joints in the ankle. To improve on this, it would be interesting to study the inversions with a goniometer (see for instance Ebig et al., 1997; Konradsen et al., 1997; Podzielny et al., 1997; Scheuffelen et al., 1993).

The responses in the PL showed little variation in latency and duration, both within and between subjects. While most of the inversion studies on standing subjects gave data on variations between subjects, Isakov et al. (1986) also mentioned the variability within subjects. They reported one response with an average SE of the latency of 1.1 ms. The responses found in the present study showed slightly larger variations in latency within subjects. The average SE of the latency in the present study was 1.7 ms (for the M2 during walking) and 2.7 ms (for the M1 during jumping). These slightly larger standard errors in the present study as compared to the one by Isakov et al. (1986) are probably caused by the higher background activity that exist during these tasks and which is negligible during standing. Between subjects, the variation in the present study was small (the latency of both responses in both tasks showed a SE of ~ 1 ms) and was comparable to the studies during standing (Isakov et al., 1986; Karlsson et al., 1992a; Karlsson et al. 1992b) or even smaller (Ebig et al., 1997; Johnson et al., 1993; Konradsen et al., 1998; Lynch et al., 1996). The lower occurrence of significant responses during the jumping task compared to the walking task was probably caused by the higher amount of background activity in this task, thereby masking the responses (especially the small M1).

This study shows that with the current method, reproducible natural perturbations can be presented, eliciting two EMG responses. An early small and inconsistent response and a mid latency larger and more consistent one. Future studies will further examine the role of the lower leg muscles in stabilizing and protecting the ankle joint during sudden inversion in gait and the jumping task.
CHAPTER 5
EMG responses in the lower leg after mechanically induced ankle inversions during human walking

Adapted from: Nieuwenhuijzen PHJA, Grüneberg C, Van Galen GP, and Duysens J.
EMG responses in the lower leg after mechanically induced ankle inversion during human walking. Submitted for publication
5.1. Introduction

Injuries at the ankle due to inversion happen in daily live situations like walking and especially in sport. In particular athletes participating in running and jumping sports get injured at the ankle due to sudden uncontrolled inversions of the ankle (Bahr et al., 1994; Balduini et al., 1987). Ankle sprains constitute 7 - 10% of all admissions to hospital emergency departments (Lynch et al., 1999) of which up to 60% are recurrent ankle sprains. Researchers found that 38-45% of all injuries in sports are ankle injuries, 85% of these injuries are ankle sprains. The injury results in various degrees of mechanical damage of the ankle that often causes mechanical ankle instability and/ or in functional ankle instability (Kannus et al., 1991). The inversion stress results in a physiological neuromuscular stretch response. According to Freeman et al., (1965) this response causes an appropriate muscular activity to protect the ankle joint. A failed reaction can lead to an ankle sprain. Especially the peroneal muscles, which give eversion of the ankle, are considered to provide an important protection against lateral ankle injury (Isakov, 1986). The coupling effect of ligamentous trauma, resulting in mechanically instability and proprioceptive deficits, contributes to functional instability, which could lead to further microtrauma and reinjury (Lephart et al., 1997). The neural input that is provided by the peripheral mechanoreceptors as well as the visual and vestibular receptors are all integrated by the CNS to generate a motor response. These responses generally dispose under three levels of motor control: a) reflexes b) cognitive programming and c) brainstem activity (Lephart et al., 1997). From reflex studies it is well known that responses after sudden perturbations can be found with a short (M1), medium (M2) and a late (M3) latency (Diener et al., 1991; Toft et al., 1991; Brooke et al., 1997). Reflexes with a short latency are supposed to represent the monosynaptic stretch reflex. Reflexes with a medium latency are polysynaptic, possibly with a trancortical route. Reflexes with a late latency are considered to be voluntary responses.

Most studies have investigated responses after sudden induced ankle inversion in subjects who were standing (Ebig et al., 1997; Johnson and Johnson, 1993; Konradsen et al., 1991; Lynch et al., 1996; Podzielny et al., 1997). Mostly the peroneal muscles have been studied and the latencies varied between very early responses of ~50 ms (Konradsen et al., 1997) and later responses who had a latency of up to 100 ms (Lynch et al., 1996). However, inversion traumas do not occur during standing at rest (Lynch et al., 1996) but during more dynamic conditions like walking, running or jumping. Little is known about ankle inversions under these circumstances. Since responses are known to be task dependent (for review see Zehr and Stein, 1999), the responses found during standing can differ from the responses found during more dynamic conditions. Furthermore, both in vitro as in vivo studies have found evidence that the ankle stability increases with loading of the ankle (McCullough et al., 1980; Sammarco, 1977; Schueffelen et al., 1993; Stormont et al., 1985). According to Stormont et al. (1985) ankle instability occurs during the loading and unloading (as occurs during walking) and not once the ankle is fully loaded. Studying ankle instability during the loading acceptance
of the stance phase of walking might give new insight in the control of ankle instability. Most studies have focused on the peroneal muscles and few data are available about the role of the other lower leg muscles in ankle inversions. Therefore, the present study concerns EMG responses in six lower leg muscles after ankle inversions during walking.

5.2. Methods

Ankle inversions were measured in twelve young healthy subjects (six males and six females; age range between 22 and 28 years) after written consent had been obtained. The experiments performed here received prior approval from the ethics committee at the University of Nijmegen and conformed to the standards of the 1964 Declaration of Helsinki. None of the subjects had a history of ankle instability or weakness or a neurological or motor disorder. During the experiments subjects had to walk on a treadmill, kept at a constant speed of 4 km/h while wearing a safety harness that was fastened to an emergency break at the ceiling. Furthermore, an emergency break was attached on the handrail, so the subjects could stop the experiment at any moment.

The method used to elicit the inversion is extensively discussed in Nieuwenhuijzen et al. (2002). A summary of this method will be discussed in this paper. At a preprogrammed delay after left heel strike, an electromagnet released a box on the treadmill in front of the left foot of the subject. The delay ensured that the subjects could step on the box without changing their cadence of walking. To help subjects maintain the same anterior-posterior position, visual feedback about the position was given using a series of light emitted diodes connected to a position measuring device based on sonar. The top of the box contained a trap door that could tilt 25° (during stimulus trials) or did not tilt (during control trials). Twenty stimulus trials and twenty control trials were presented randomly. Surface electromyography (EMG) was recorded of the tibialis anterior (TA), the peroneus brevis (PB), the peroneus longus (PL), the soleus (SO), the gastrocnemius lateralis (GL), and the gastrocnemius medialis (GM) of the left leg. Furthermore, the trap door was connected to a goniometer to record the tilting of the trap door. Thin insole foot switches detected contact with the treadmill and the left foot switch was used to trigger the electromagnet. The subjects wore headphones through which loud music was playing, to prevent them from hearing the box fall on the treadmill and thereby get any cues about the type of trial condition.

All signals were sampled at 500 Hz. The EMG signals were (pre-) amplified (by a factor in the order of $10^4$–$10^5$), high-pass filtered with a cut-off frequency at 3 Hz, than full wave rectified, and low-pass filtered with a cut-off frequency at 300Hz. During the experiments the signals were visually inspected by on-line monitoring on an oscilloscope and on a computer display.

Zero time was defined as the onset of the rotation of the trap door. This was calculated by a difference more than 1 x the standard deviation of the average
signal before the inversion. To study the net effect of the stimulus the average control EMG data was subtracted from the individual stimulus EMG. For each response peak a time window was set on the average EMG data of all 6 muscles of each subject. Latency and duration was defined as the onset and duration of the time window. The response amplitude was calculated by averaging the rectified EMG within the time window. To enable a proper comparison of the response amplitude between the different muscles and subjects, the response amplitudes were normalized with respect to the maximum EMG activity during normal walking (measured before each trial and averaged). To determine whether the responses observed were statistically significant and to compare mean response amplitude, incidence, and latency between the different muscles, the Wilcoxon signed rank test was used. In all statistical tests a significance level of $P > 0.05$ was used.

5.3. Results

Rotation of the trap door and reproducibility of the experiment

The $25^\circ$ tilting of the trap door, causing the ankle inversion in the subjects, had an average duration of 62 ms with a standard error (SE) of 1.7 ms. This means an angular velocity of $403^\circ/s$ with a SE of $18^\circ/s$ ($n=12$). No correlation was found between the velocity and the weight of the subjects. The average of the individual standard errors of the tilting duration of all subjects was 2.0 ms. For the intra subject reproducibility of the EMG responses the latency and duration of the individual trials of the PL were analyzed. For the inter subject variation we refer to Table 5.1. The latency of M1 showed an average individual SE of 2.8 ms and M2 showed an SE of 1.7 ms. An average individual SE of 1.9 ms was observed for both M1 and M2.
Table 5.1. Ankle inversion characteristics for the whole population

<table>
<thead>
<tr>
<th></th>
<th>Occurrence, %</th>
<th>Latency, ms</th>
<th>Duration, ms</th>
<th>Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
<td>M1</td>
<td>M2</td>
</tr>
<tr>
<td>TA</td>
<td>39 ± 3.6</td>
<td>29 ± 6.5</td>
<td>36 ± 1.9</td>
<td>21 ± 3.2</td>
</tr>
<tr>
<td>PL</td>
<td>63 ± 4.9</td>
<td>92 ± 2.2</td>
<td>40 ± 2.0</td>
<td>85 ± 1.6</td>
</tr>
<tr>
<td>PB</td>
<td>60 ± 4.7</td>
<td>68 ± 2.0</td>
<td>42 ± 2.9</td>
<td>87 ± 2.0</td>
</tr>
<tr>
<td>SO</td>
<td>30 ± 3.2</td>
<td>76 ± 5.8</td>
<td>42 ± 2.2</td>
<td>97 ± 2.4</td>
</tr>
<tr>
<td>GL</td>
<td>44 ± 4.6</td>
<td>68 ± 3.2</td>
<td>35 ± 1.1</td>
<td>88 ± 2.0</td>
</tr>
<tr>
<td>GM</td>
<td>34 ± 4.1</td>
<td>60 ± 5.2</td>
<td>36 ± 1.5</td>
<td>98 ± 2.1</td>
</tr>
<tr>
<td>Mean</td>
<td>39 ± 0.9</td>
<td>92 ± 1.0</td>
<td>24 ± 1.2</td>
<td>50 ± 2.2</td>
</tr>
</tbody>
</table>

Table 5.1. The mean percentage of occurrence, latency, duration, and amplitude (n=12). Latency and duration was based on the window settings. The amplitude was calculated as the mean EMG activity within the time window, and then expressed as a fraction of the maximum background activity (see 5.2. Methods). Values are means ± SE.

Response characteristics

In most muscles and all subjects two responses were detected (see Fig. 5.1). An early response called M1 was observed after ~ 40 ms and had a duration of ~ 25 ms. These responses were small but distinct. A larger response called M2, was observed after ~100 ms and had a duration of ~35 ms. Only TA showed hardly any M2 activity. The frequency of the response occurrence varied depending on the muscle and type of the response (M1 or M2) (see Table 5.1). Except for the TA all muscles showed a higher incidence of the M2 compared to the M1 (on average 96 % and 52 %, respectively). The highest incidence of both M1 and M2 was found in PL and PB. Compared to the peroneal muscles, the GL showed less responses but still significantly more than the other muscles (Wilcoxon signed-rank test: P > 0.05). The SO showed the smallest incidence in the M1 and the TA showed the smallest incidence of the M2.

The average response latency of each muscle of all subjects can be observed in Table 5.1. The M1 response had an average latency of 39 ms (± 0.9 ms, SE) and showed no significant difference between the six muscles (Wilcoxon signed-rank test: P > 0.05). The M2 response however, showed larger differences. The PL showed the shortest latency (85 ms ± 1.6 ms, SE), although not significantly shorter than the PB (87 m ± 2.0 ms, SE) and the GL (88ms ± 2.0). Note that the GL had the shortest latency of the triceps surae. No significant difference was found between the SO and the GM (~100 ms).

The response amplitude depended on the type of the response (M1 or M2). The M2 showed higher EMG activity than the M1. Comparing the various muscles, the peroneal muscles showed the highest M1 response activity (Wilcoxon signed-rank test: P < 0.05), followed by the TA and then the GL (although the latter was not significantly different from the other triceps surae muscles). The GM showed small but significant M1 response activity. For the M2, again the peroneal muscles (especially the PB) showed significantly larger response activity than the other muscles. The amplitudes of the peroneal muscles were more than three times...
higher than the GL, which had the largest amplitude of the triceps surae muscles. The TA showed no significant M2 response activity.

Response latency and tilting duration

The M2 showed the largest and most consistent responses compared to the M1. Furthermore, the peroneal muscles showed the shortest M2 latency of ~ 85 ms. Comparing the latency of the M2 peroneal muscles and the inversion duration of the trap door (~60 ms), it is clear that the M2 response started after the trap door fully tilted (Fig 5.2).

Habituation

To study sequential effects, the mean response amplitudes of all first trials and thirteenth trial of each muscle were compared (thirteen was the minimum number of successful trials of a subject). The M1 did not show any significant decrease in response amplitude. In M2 however, except for the PB, in all muscles a significant decrease in amplitude was observed (Wilcoxon signed-rank test: $P < 0.05$, n=12).
Chapter 5. EMG responses in the lower leg after mechanically induced ankle inversions during human walking

(see Fig. 5.3). Especially the TA, SO and the GM decreased to practically zero. Of these muscles the TA already showed no significant difference with the baseline activity after 5 trials, while the SO and GM demonstrated this effect after 10 trials. The GL showed a decrease of ~ 80% and the PL of ~ 40%. Anticipation could account for the difference in amplitude. However, the EMG activity in the first 30 ms, just before the first responses were observed, was not significantly different between these trials (Wilcoxon signed-rank test: $P > 0.05$, $n=12$). Although after the first inversion the peroneal muscles showed the largest normalized response amplitude (1.7 times the maximum activity during normal walking), the other muscles still showed up to half of this amount of normalized EMG activity (0.4-0.9 times the maximum background activity). Between the other muscles there were no significant differences. After the thirteenth inversion only the peroneal muscles and the GL showed significant EMG activity.
5.4. Discussion

In the present study for the first time EMG responses have been studied after ankle inversions during human treadmill walking. In a previous study we showed that with this method it is possible to evoke reproducible ankle inversions. In all subjects and most muscles two responses were detected (M1 and M2). The late response with an average latency of ~100 ms was larger and more consistent than the M1 with a latency of ~ 40 ms. The latency of the peroneal M2 response in the present study (80-90 ms) is comparable with other studies (Lynch et al., 1996; Konradsen et al., 1991, 1992). However, the response latency of the M1 is slightly shorter than observed in other studies. These differences might be caused by the difference in test conditions, while in other studies the subjects were standing, in this study the subjects were walking. Many responses are known to be task dependent (for review see Zehr and Stein, 1999). Furthermore, the onset of the rotation is often difficult to judge. In the present experiment the onset of the rotation was set at difference of 1 x the standard deviation of the mean signal before the rotation. Previous studies (during standing) do not mention how the onset of the rotation was determined. The short latency of the M1 suggests this response is a stretch response. Short-loop reflexes are known to be dependent on the velocity of stretch (Kearney and Hunter 1982; Matthews 1972). The inversion velocity in the present study (403°/s) was higher compared with other studies (varying from 50°/s (Lynch et al., 1996) up to 375°/s (Konradsen et al., 1997)). Lynch et al. (1996) eliciting inversions up to 200°/s did find a short latency response, while Isakov et al. (1986) found response latencies of ~ 60 ms using inversions with a velocity of 250 °/s – 333 °/s. Konradsen et al. (1997), using a faster inversion of 375°/s, indeed found response latencies that were slightly shorter (~ 50 ms). These findings are in conformity with the study of Lynch et al. (1996), who observed an effect of inversion speed on response latency. Grey et al. (2001) also found a modulation of the stretch reflex due to stretch velocity changes. Furthermore, that study found, like the present study, no such dependency for the medium latency response.

The M2 was generally larger and more consistent then the M1 response, indicating that this medium latency response is functionally more important than the short latency response. Indeed, literature more often reports medium latency responses after a sudden ankle inversion compared to short latency responses (Ebig 1997; Isakov et al. 1986; Johnson and Johnson, 1993; Karlsson et al. 1992; Lynch et al., 1996; Sheth et al. 1997). Furthermore, larger responses in the medium latency response have been obtained in other perturbations during walking (Schillings et al., 2000; Van Wezel et al., 1997; Zehr et al. 1997). Based on the latency for the M1, that is compatible with the delay involved in the monosynaptic activation by Group la spindle afferents, and the induced stretch it is generally agreed to be a spinal stretch reflex. The medium latency response is thought to represent a polysynaptic reflex arc with some probable supraspinal control (Lynch et al., 1996). During an ankle inversion several structures could be stimulated, leaving the origin of this response open for debate. Proprioceptive afferents might contribute to the M2 responses observed. Similar latencies have been reported by many authors after joint rotation during various conditions (Fellows et al., 1993; Schieppatti and Nardone, 1997; Schieppatti et al., 1995; Schillings et al. 2000;
Sinkjaer et al., 1988; Toft et al, 1989). Although some authors suggest Ia afferents could mediate this response (Berardelli et al., 1982; Fellows et al., 1993), group II afferents seems to be most important for this type of response (see Corna et al 1995; Dietz 1992; Nardone et al. 1996; Schiepatti et al, 1995). Furthermore, the inversion causes for body movements in the frontal and sagittal plane (Karlsson et al., 1992; Konradsen and Ravn, 1990; Konradsen et al, 1997), thereby stimulating vestibular afferents. Alternatively, cutaneous afferents could be stimulated since the skin stretches. Cutaneous afferents are assumed to be involved in several perturbations during walking (Van Wezel et al. 1997; Zehr et al. 1997). Recently, evidence was delivered by Corden et al. (2000), that the late component of the stretch reflex is not mediated by intramuscular stretch receptor, but by (sub)cutaneous receptors. By comparing the M1 and M2 observed after stretch, with selectively stimulating the skin (abolishing the M1 and leaving the M2 unchanged) or selectively stimulating muscle stretch receptors (abolishing the M2 and leaving the M1 unchanged). However, experiments with anesthesia of cutaneous receptors failed to eliminate this medium latency response (Bawa and McKenzie, 1981; Grey et al, 2001; Wu and Chiang, 1997). A different origin of the M1 and M2 could account for the different behavior (like velocity dependency) of these responses.

For both M1 and M2, the peroneal muscles showed the largest and most consistent responses. Not surprisingly, since these muscles receive the largest stress. In the average EMG data hardly any M2 response was detected in the TA. Few studies have investigated the TA during ankle inversion. Some found an M1 response (Löfvenberg et al., 1995), but in contrast to the present study, some authors did found M2 responses in the TA (Lynch et al., 1996; Sheth et al., 1997). The latter study did mention a lower success rate of occurrence (72 % in TA compared to 97 % in PB and 88 % in PL). Again the difference could be caused by the difference in task (standing versus walking). In contrast to the TA the triceps surae did show activation after inversion. Konradsen et al. (1997) indicated that inversion during quiet standing induced a dorsal flexion of the ankle and leaning forward of the body, thereby inducing stretch to the triceps. Of the triceps surae the GL showed more consistent and larger response than the SO and GM. This suggests that the GL has a slightly different function in the inversion. This might be related to the lateral position of the muscle, thereby having some effect as an eveter, protecting the ankle against inversion.

The data on sequential effects of the M2 shows that an activation of all muscles was measured in the first trial. This indicates a stiffening of the lower leg. Further in the experiment only the evertors staid active, indicating a more directed body response. Several authors found in inversion experiments with healthy subjects a longer latency in the TA after ankle disk training and no difference in the peroneal muscles (Osborne et al., 2001; Sheth et al., 2000). Sheth et al. (2000), suggested the ankle disk training was responsible for the longer latency of the TA, thereby improving the efficacy. However, the same result was observed in patients with a history of ankle sprain showed the same result, thereby questioning as to whether a proprioceptic cross-training effect occurred. Furthermore, this study showed that a sequential effect on the response characteristics. Comparing the first trial with the thirteenth trial a strong decrease in response amplitude was observed in the triceps surae and the TA. During postural perturbations of quiet standing reports have been made about an excessively
large postural response, followed by large reduction in the subsequent trial (Hansen et al., 1988; Timmann and Horak 1997). The authors explained the modification as habituation of a “startle-like” response. Indeed, startle responses elicited during walking evoke cocontractions with similar latencies in the lower leg especially during the stance phase (Nieuwenhuijzen et al. 2000). However, the habituation rate in the present study was even more rapid. In the present study the amplitudes decreased to baseline value after 10 trials compared to a decrease of 42% during auditory startle. Furthermore, a large long latency response was detected during the auditory startle response at ~145 ms that was not detected in the first trials. However, on account of the great similarities we cannot rule out that startle habituation might play a role in the observed response decrease. Nevertheless, we recommend examining habituation effects when evaluating a repetitive study with inversion experiments. The strong habituation found in the TA (after 5 trials a decrease to baseline level) explains the low amplitude and occurrence in the average data. The ability to change to more appropriate response after repeated exposures of the same type is mentioned in a number of studies (Buchanan and Horak 1999; Keshner et al. 1987; Nashner 1976). Not many studies describe this type of sequential effects during walking. Only recently, Marigold and Patla (2002) observed a change of motor responses to a more effective muscle distribution in subjects stepping on a slippery surface. These findings indicate that the central nervous system is able to react quickly to adapt motor programs to a more efficient response. Furthermore in patients with a recent ankle sprain, the coactivation pattern between PL and TA was observed not only in the first trials but throughout the experiment (Brunt et al., 1992). Similarly, in patients with bilateral chronic ankle instability, a significant increase was observed in TA activity during normal walking compared to healthy subjects (Louwerens et al, 1995). Through cocontraction the amount of intrinsic and reflex stiffness in the ankle is increased (Nichols and Houk, 1976). This seems to be favorable for protection of the joint. However, higher activation of the TA could create an extra inversion moment thereby, increasing the risk of a re-injury known to happen in these subjects (Balduini et al., 1987).

As mentioned earlier the M2 shows the largest and most consistent responses and is therefore probably functionally more important than the M1. However, the earliest M2 responses (in PL) were observed after ~ 85 ms and the rotation of the platform ended after ~ 60 ms. These findings are in agreement with other authors who found responses after the tilting of the trap door in standing subjects (Isakov et al. 1986; Johnson and Johnson 1993) The responses in the evertors are too late to resist the induced stretch that is applied to the ankle joint and can not directly prevent the ankle from being damaged. The question arises what purpose this response serves in protecting the ankle. Several studies have detected after inversion elicited during quiet standing, both in the frontal and the sagittal plane a disturbance in body posture (Karlsson et al., 1992; Konradsen and Ravn, 1990; Konradsen et al, 1997). Furthermore, damage can only occur when force is applied on the induced stretch, i.e. when weight is put on the leg after the inversion. Therefore, the function of the M2 might lie in balance control and / or in reducing the loading of the ankle. Further study is necessary to produce evidence for this theory.
CHAPTER 6

WHOLE RESPONSES AFTER MECHANICALLY INDUCED ANKLE INVERSIONS DURING TREADMILL WALKING

Adapted from: Nieuwenhuijzen PHJA, Smits MJAW, and Duysens J. Whole body responses after mechanically induced ankle inversions during treadmill walking. Submitted for publication
6.1. Introduction

Of all sports injuries, ankle traumas occur most frequently (Glick et al., 1976). Most studies have investigated ankle inversions during quiet standing. However, ankle inversions do not occur during such static conditions (Lynch et al., 1996), but during more dynamic conditions like walking and jumping. We developed a new method to induce ankle inversions during walking and jumping (Nieuwenhuijzen et al., 2002). During these tasks ankle inversions elicited two EMG responses namely a short latency response with a latency of ~40 ms (most likely a stretch reflex), and a medium latency response of ~90 ms (Grüneberg et al., 2003; Nieuwenhuijzen et al, 2004). The short latency response (M1) was small and inconsistent compared to the medium latency response (M2), suggesting the M2 being functionally more important. However, comparing the latency of the M2 with the duration of the rotation of the trap door causing the inversion, the M2 (in the evertors) is too late to resist the induced stretch applied to the ankle. This finding is in agreement with literature (Isakov et al., 1986; Johnson and Johnson, 1993). Therefore, this response cannot directly protect the ankle from being damaged. Several indications from the literature suggest that instead the M2 responses could be part of a much broader balance control reaction. For example, during slips in mid-stance of the step cycle Tang et al (1999) showed that erector trunci was one of the first muscles to be recruited. Similarly, studies with multidirectional platform perturbations showed early activations of the paraspinals (Carpenter et al, 1999a, Commissaris et al, 2002). Studies which focussed specifically on ankle inversions were almost exclusively done on standing subjects. Konradsen et al. (1990), found that the whole body was involved in the correcting reactions. Ankle inversion elicited an adduction of 5° of the hip and a large shift of the CoP in the anterior lateral direction. Furthermore, the vertical force showed an M-shaped curve, indicating an intermediate relief of pressure.

How do these data compare to ankle inversions during gait? Differences can be expected since gait requires dynamic balance control based on alternating support on the two legs.

To study the body reactions to an inversion perturbation during walking, we measured kinematics, CoP, and EMG. Because inversions are mainly in the medial-lateral direction, we focused on the frontal plane in the present study.

6.2. Methods

Twelve healthy subjects (8 males and 4 females; age range between 18 and 27 years, mean 23.58 SD= +/- 2.47) participated in the experiment. None of the subjects had a history of ankle instability or weakness or a neurological or motor disorder. The experiments were carried out in conformity with the declaration of Helsinki. All subjects gave informed consent, and the study was approved by the local ethical committee.
Chapter 6. Whole responses after mechanically induced ankle inversions during treadmill walking

Fig. 6.1. Schematic representation of the experimental set-up. At a pre-programmed delay after left heel strike an electromagnet released a box containing the trap door on the treadmill. When the subjects stepped on the box the trap door could tilt, eliciting an inversion movement of the left ankle. To calculate the CoP, a force plate was placed under each corner of the treadmill (pointed out by the arrows at the bottom of the picture). The 3D axis indicate the X, Y and Z direction for the CoP.

Experimental set-up (see Fig. 6.1)

A detailed account of the experimental setup can be found in Nieuwenhuijzen et al. (2000). While subjects walked on a treadmill at a speed of 4 km/h, a box containing a trap door was released by an electromagnet on the belt. The timing of release was triggered by left heel contact and a predetermined delay ensured that the subjects could step on the box without changing their cadence of walking. For the same purpose, position feedback was given based on sonar so the subjects could maintain the same position on the treadmill. When the subjects stepped on the box the trap door tilted 25° during a stimulus trial and 0° during a control trial. The tilting of the trap door lowered the subject 3 cm. This small drop by itself is not expected to cause major kinematic changes. Literature comparing stair climbing with level walking showed hardly any effect in hip and knee kinetics in the frontal plan (Costigan et al., 2002). The subjects wore headphones...
with loud music to assure they did not get any auditory clues on the type of trial condition when the box was dropped on the treadmill. Furthermore, the magnet and box were covered from view, and thus blocked the view of the obstacle. The subjects wore a safety harness, fixed to a safety break on the ceiling that would stop the experiment in case the subjects would start to fall. The harness was loosely suspended and did not provide extra stability during the experiment. In addition, an emergency break was attached on the handrail, so the subjects could stop the experiment at any moment. In practice, both breaks never needed to be utilized. Twenty stimulus trials and twenty control trials were presented randomly in each subject.

![Fig. 6.2. Placement of the surface electrodes (A) and light reflecting markers (B).](image-url)
Chapter 6. Whole responses after mechanically induced ankle inversions during treadmill walking

**Data Sampling**

Bipolar surface electrodes were used to measure electromyographic (EMG) activity of the peroneus longus (PL) of the ipsilateral leg, and the gluteus medius (GM) and the erector trunci (ET) of both legs (see Fig.6.2). These signals were sampled at 500 Hz, (pre-) amplified (by a factor in the order of \(10^4\) to \(10^5\)), high-pass filtered (cut-off frequency at 3Hz), full wave rectified, and then low-pass filtered (cut-off frequency at 300Hz). From previous publications from this group and others, the sampling rate was shown to be basically sufficient for the present type of research (Jeffrey et al., 2003; Duysens et al., 1991; Van Wezel et al., 1997; Schillings et al., 2000). During the experiments the signals were visually inspected by online monitoring on an oscilloscope and computer display. 3D-video recordings were made (sample frequency of 60 Hz) using a passive marker system (Qtrac®, Qualisys Sweden) to record movements of the lower limb and trunk during the experiments. The markers were positioned (bilaterally) on metatarsal V, tuber calcaneus, caput fibulae, lateral condylus femoris, spina iliaca posterior superior (SIPS), trochantor major and acromion of the shoulder (Fig 6.2).

Subjects wore thin insole foot switches to detect foot contact with the treadmill and to deliver the trigger for the electromagnet.

To measure the Center of Pressure (CoP), force plates were placed under each corner of the treadmill. Pilot studies showed that the offset of the force plates depended on the temperature of the environment and the temperature of the force plates. Therefore, the environment temperature was kept constant and the force plates were activated at least 3 hours in advance of the experiments. These pilot studies showed no changes in the offset after these 3 hours.

To calibrate the force plates, a weight of 420 N was placed on 6 different places on the treadmill. A constant had to be determined for each force plate \((C_1 - C_4)\) to calculate the force applied on the treadmill \((F_m)\) from the force plates signals \((S_1 - S_4)\) (Formula I).

\[
I: \sum_{s=1}^{4} S_s \times C_s = F_m
\]

The moment of the weight relative to force plate one (in the walking direction: \(Y_m \times F_m\), and perpendicular to the walking direction: \(X_m \times F_m\) (see Fig. 6.1) should be equal to the sum of the force plates signals, times the constant, times the distance of each force plate relative to force plate one \((X_1 - X_4)\) (Formulas II, and III). Since the origin is placed above force plate one, the moment around this force plate is zero, therefore the contribution of this force plate can be omitted in the formulas II and III. The \(X\)-coordinate of the weight relative to force plate 1 is known.

\[
II: X_m \times F_m = S_2C_2X_2 + S_3C_3X_3 + S_4C_4X_4
\]
The Y-coordinate can be calculated in the same way. The X-axis was defined as a line through force plate one and three (see Fig. 6.1). Therefore, the moment in the y direction for force plate three is zero and can therefore be omitted.

III: \[ Y_m \times F_m = S_2C_2Y_2 + S_4C_4Y_4 \]

Since only the four calibration factors were unknown, two measurements are sufficient to calculate the calibration factors. However, the weight has been placed on six positions. Therefore, the optimum value for the calibration factors has been calculated using the least mean square method. The average error for the x-position was 3.1 mm (SD 3.1 mm) and for the y-position 5.1 mm (SD 3.2 mm). The same formulas described above were used to determine the location of the CoP after the experiment. To determine the amount of noise in the force plate signals, due to the running of the belt of the treadmill at 4 km/h, a fourier analysis was executed. The power spectrum showed peaks at \~18 Hz. During normal walking no higher frequencies than 6 Hz occur in walking movement and ground reaction force signals (Winter et al., 1974). Therefore a 4th order low-pass digital Butterworth filter was used with a cut off frequency of 6 Hz.

Data analysis

A trial was considered unsuccessful in case the subjects did not achieve a correct landing on the trap door. That could mean that the foot was placed too near to the fulcrum of the trap door, causing a decrease in the velocity of rotation. Furthermore, stepping over the trap door or making an extra short step prior to stepping on the box were considered as “unsuccessful”. These trials were excluded from the data. The moment the foot touched the box was defined as zero time. To study the net effect of the stimulus, the average control EMG was subtracted from the individual stimulus EMG. The response amplitude was quantified as the mean EMG activity of the period between onset and end of the response. For this purpose, windows were set around the individual response peaks. Latencies were defined as the onset of the time window (see Nieuwenhuijzen et al., 2002 for details). To enable a proper response amplitude comparison between subjects and muscles, the amplitudes were normalized with respect to the maximal EMG activity during normal walking.

To determine if a response was significant the Wilcoxon signed-rank test was used. Furthermore, the same test was used to evaluate differences between stimulus and control trials for the kinematics, the CoP and EMG response activity. For all statistical tests a significance level of \( P > 0.05 \) was used.
### 6.3. Results

**Step cycle structure and kinematics**

The signals of the foot switches were analyzed in order to check whether there were differences in the course of the gait cycle between control and stimulus trials (Fig 6.3). No significant differences were observed between both conditions (Wilcoxon signed-rank test $P < 0.05$).

Despite the absence of gross perturbations in the step cycle there were clear kinematic adjustments. Inversion is primarily a rotation in the frontal plane, therefore shifts of the markers and CoP were studied in the medio-lateral direction. We first focused on the markers placed on the ipsilateral femur condyl, the ipsilateral spina iliaca posterior superior (SIPS), and the ipsilateral acromion to represent the movements of the trunk and ipsilateral leg (see Fig. 6.4). These markers formed the hip angle $\alpha$, indicative of the movements of the leg in relation to the trunk.

To obtain an overview of what happened with the body during the control and stimulus trials the stick figures of Fig. 6.5 are presented. These stick figures are based on the 3D data of a typical subject. It shows the body in the frontal plane in an

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**Fig. 6.3.** Representation of treadmill contact of the ipsilateral and contralateral foot. The printed signals are the mean signals and standard deviation for all subjects divided in control (light bars) and stimulus (dark bars) signal. Zero time is the moment the foot touches the trap door.

**Fig. 6.4.** Posterior view of the placement of the three markers forming the hip angle $\alpha$ and the marker on the calcaneus of the contralateral leg.
anterior view during the control trials and the stimulus trials. After the inversion several changes were observed compared with the control trial at the same time after ipsilateral heel-strike. The first visible difference (second stick diagram in Fig. 6.5) was seen in the ipsilateral knee, which shifted to the ipsilateral side (at a time when the inversion movement was just completed during the double support phase). It should be emphasized that this figure represents a projection in the frontal plane but that movement was not restricted to that plane. In fact, in this case the outwards movement of the knee was probably a combination of flexion of the knee and exorotation of the hip rather than to an exaggerated valgus motion.

Fig 6.5. Typical example of the body motions in the frontal plane during a control trial without inversion (upper panel) and stimulus trial with inversion (lower panel). The stick figures are presented in anterior view.

In the next phase (third stick diagram in Fig 6.5, bottom) the contralateral leg swings forward and laterally (to avoid the obstacle). At the same time the trunk is
tilted contralaterally and the angle alpha is increased. Finally (fourth stick diagram in Fig. 6.5) the contralateral foot is placed and the whole body returns to an upright position.

These changes are shown in more detail in Fig. 6.6. The mean control and stimulus position and rotation signals for all subjects in medio-lateral direction are presented for the markers described above. The position displacements of these markers can be observed in the upper 4 traces. The angle α is plotted in the lower figure. To study the net effect of the stimulus, the subtracted signal is printed next to the mean control and stimulus. Furthermore, the subtracted data of the first 500 ms is displayed in the right panel to show the first changes in more detail.

The reaction to the inversion can be divided in 4 parts. In the first part, during the platform rotation (from 0 ms to 60 ms), no significant changes were observed in the kinematic data between both conditions. Therefore this phase will be referred to as the “neutral phase”. At the start of the second phase, from 70 ms to 120 ms, the stimulus trials showed a fast outward movement of the knee marker, with a maximum difference between control and stimulus condition of 45 mm.
This outward knee movement correlated with an increase in the frontal plane hip angle alpha (maximum subtracted deviation of $4.6^\circ \pm 0.41^\circ$ SE; n=12). The ensuing outward movement of the knee marker is then primarily due to exorotation at the hip while the knee is flexed from stepping on the box. During this period, in the subtracted data a small deviation to the ipsilateral side is also observed in the ipsilateral SIPS and shoulder. This phase will be termed the “initial ipsilateral reaction”. In the third phase, starting just before contralateral toe-off (from 120 ms to 400 ms), the subtracted data showed that the ipsilateral movement of the knee is counteracted by a relatively contralaterally directed movement of first the SIPS (at $\sim 130$ ms), then the ipsilateral shoulder and the opposite leg (both at $\sim 180$ ms). As a result of the contralateral trunk movement in combination with the increase of the outward shift of ipsilateral knee, the subtracted signal of the ipsilateral hip angle showed no further increase in that period. This phase will be referred to as the “roll correction response”. In the final fourth phase, from 400 ms to 1500 ms, the contralateral leg in the stimulus condition changed direction and moved towards the ipsilateral side as it prepared for landing behind the trapdoor with a more ipsilateral placement, compared to the control condition. During this period, the relative outward movement of the ipsilateral knee decreased, while the ipsilateral shoulder and SIPS moved relatively to the ipsilateral side. At the end of this period the trajectories of stimulus and control cycles overlapped again, therefore this phase will be called the “recovery phase”.

Fig. 6.7. The mean behaviour of the CoP during the control and stimulus trials. The left upper figure shows the signal for 1600 ms. The lower figure represents the corresponding subtracted signal (light brown line) with the standard error (dark). The colored bars at the bottom represent the stance phase: ipsilateral (dark bar) and contralateral (light bar). Zero time is the moment the foot touches the trap door. The numbers at the bottom indicate the phases as discussed in the text: 1. the neutral phase; 2. initial ipsilateral phase; 3. the roll correction response; 4. the recovery phase.
Chapter 6. Whole responses after mechanically induced ankle inversions during treadmill walking

Center of pressure (see Fig. 6.7)

Overall the stimulus induced changes in CoP were relatively minor (maximum 41 mm). The CoP during the stimulus condition deviated to the ipsilateral direction mainly during both single stance periods. During the platform rotation and the “initial ipsilateral reaction”, from 0 ms to 150 ms, the CoP traces differed very little between the stimulus and the control condition (see Fig. 6.7). During the “roll correction response” (150-400 ms), the CoP moved slightly more ipsilaterally during the stimulus condition as compared to the control but this ipsilateral movement was halted near the end of this period and further counteracted early in the final recovery phase. During the stance phase of the contralateral leg (from 650 ms to 1250 ms) the CoP moved again more towards the ipsilateral side because the contralateral foot is placed more in that direction in the stimulus condition.

EMG analysis

Since our primary goal was to study body responses in the frontal plane, we investigated a number of muscles known to elicit movements in this plane (see Fig. 6.8). In chronological order we detected latencies in the contralateral ET of ~ 70 ms, in the contralateral GM and the peroneus longus of ~ 80 ms. In the ipsilateral GM and ipsilateral ET facilitory responses with a latency of ~ 100 ms were observed. However, these responses were preceded by an inhibitory response with a latency of ~50 ms. The largest responses were observed in the ipsilateral GM and ipsilateral PL. Smaller responses were observed in the ipsilateral ET and the contralateral GM and ET.
6.4. Discussion

The first important result of the present study is that the reaction to an inversion perturbation during gait is not restricted to the ankle but consist of an orderly and reproducible sequence of whole body reactions. Furthermore, the reactions observed occurred after the rotation of the trap door was completed. Therefore, these reactions were not based on restricting the elicited inversion during the trap door tilt, but their relevance lies in the period after the tilt. The first reaction after the tilt, “the initial ipsilateral reaction”, occurred during weight acceptance (70 – 150 ms). The reaction consisted of an outward movement of the knee (based on a exorotation at the hip with the knee in flexion), and to a lesser extend an outward movement of the trunk. This outward movement was expressed as a small ipsilateral deviation of the CoP. Clearly this movement limits the amount of ankle inversion needed. With an average length of the knee marker to the subtalar joint of 47 cm, it was calculated that this inversion decrease was 6.1°. During static experiments involving inversion perturbations a similar decrease of inversion of 5° was detected (Konradsen and Ravn, 1990). However, this decrease was due to an adduction of the hip of 5°. This difference in the latter experiment compared to the present study is probably caused by the standing condition, with (initially) extended knees, allowing only minimal displacement of the knee in the static experiment. Furthermore in the present conditions, the subjects placed all their weight on the perturbed limb during the stance phase while in the standing experiments the weight was distributed over the two legs. Finally, in the present study the perturbation was during weight acceptance, while the static experiments were executed with continuous full body weight on both legs. This difference in dynamic and passive loading could account for differences found between both conditions. Both in vitro experiments (McCullough et al., 1980; Sammarco, 1977; Stormont et al., 1985) and in vivo experiments (Scheuffelen et al., 1993) showed that loading of the ankle before the perturbation increases the stability against inversion.

Why did the ipsilateral knee move outward and why did the subjects not simply use ankle inversion to follow the inversion of the supporting surface? The inversion in the present study of 25° is well within the range of motion of the ankle (Nawoczenski et al, 1985). However, it may be wise not to use this range, especially not during the loading phase of gait since strain on the lateral ligaments and muscles is at a peak under these conditions. Furthermore this outward movement seems useful also at later stages of the reaction since the lateral movement of the knee continued well after the inversion perturbation ended.

How was the knee movement performed. A first possibility is that this movement could have been achieved by passive biomechanics. In that case, gravity in conjunction with the shape of the bones and the articular surface of the joints would be sufficient to move the knee outward after an inversion, without interference of both passive and active structures. Another possibility is that the stiffness in the ankle, either passive or (pro-)active, forced the knee to move outward. In case of passive stiffness, structures like joint capsule, ligaments and the passive
properties of the muscles would resist the inversion enough to elicit the knee movement. Damage to these structures might influence the proximal joint strategy, thereby endangering this first line of defense against injury due to the inversion. An inversion trauma can cause damage to the joint capsule and ligaments, thereby making the joint instable and hypermobile (Karlsson et al., 1992). Due to this mechanical instability, the intrinsic stiffness of the joint could decrease and diminish the force for the outward movement of the knee. This could explain the high rate of recurrence known for inversion injuries.

The ankle could also actively be held stiff during the inversion. Subjects did not know whether the trap door would rotate or not but they knew that a perturbation was possible. Therefore they could increase the muscle activity of the ankle in anticipation of a possible perturbation. By this muscle activity the intrinsic stiffness of the joint could be increased (Hunter and Kearney, 1982). During landing on an inverting platform after a jump significant anticipatory effects were observed (Grüneberg et al. in press). However, these effects consisted of changes in the ipsilateral soleus muscle and not in the peroneus longus.

Finally, the knee could (re-)actively have been moved outward, either by stretch reflexes in the exorotators/adductors of the hip or in the peroneal muscles. In principle, the early shift of the knee to the ipsilateral side could have been evoked by stretch reflexes in the abductors and exorotators of the ipsilateral hip, since these muscles could be stretched during an inversion moment of the ankle. However, no stretch reflexes were detected in the ipsilateral gluteus medius. Instead, even suppressive responses were observed. Furthermore, stretch reflexes in the peroneal muscles are probably to small and insufficient to produce enough force to resist the induced stretch during the inversion movement (Grüneberg et al., 2003; Nieuwenhuijzen et al. 2002, 2004). This is further confirmed in the present study in which early stretch reflexes were either small or absent (see Fig. 6.8).

Although only small and inconsistent stretch reflexes were detected in the peroneal muscles, larger and more consistent responses were observed during the “initial reaction” from 70 to 150 ms. From related studies (Nieuwenhuijzen et al., 2004; Grueneberg et al., 2003) it is known that these responses are specific for these stretched muscles since other lower leg muscles are much less activated in these inverting responses as compared to controls. In this period the stretch responses of the peroneal muscles are highly functional. Indeed, it is known that damage to the structures, stretched by the inversion, occurs when force is applied to the stretched ligaments and muscles, for example because weight is put on the leg. This loading of the ankle occurs at the end of the rotation at the start of the stance phase (at ~ 60 ms). The increase in loading of the ankle proceeds until toe off of the contralateral leg (~150 ms). During that time the peroneal muscles become active, possibly to protect the ankle from damage during the “initial ipsilateral reaction”. At the same time the contraction in the peroneal muscles ensures ankle stability that is essential to prepare the limb for the ensuing roll correction reaction.
Roll correction responses

Another major result of the present study is that these responses in the peroneal muscles occur more of less synchronously with responses in paraspinal and hip muscles. These latter responses closely resemble the balance corrective responses described by others following inversion perturbations during standing. The knee, SIPS and, shoulder marker moved to the ipsilateral side in the presently described initial reaction. Indeed, the CoP showed a shift to this side of ~ 2 cm. To maintain balance the subject used a balance corrective response resulting in a movement of the SIPS (at ~120 ms) and the shoulder (at ~140 ms) to the contralateral side. Furthermore, the contralateral leg started abducting at toe off. This counter reaction avoids an unstable situation, ensuring a moderate deflection of the CoP in the frontal plane. Although, the abduction of the contralateral leg is favourable for maintaining balance the abduction movement also could have been caused by the slight lowering (3 cm) of the body due to the tilt (the box could then present an obstacle for this leg).

Studies on balance correcting responses and compensatory strategies of subjects standing on a platform delivering rotational perturbations in the roll plane, showed similar results compared with the present study (Bloem et al., 2001; Bloem et al., 2002; Carpenter et al., 1999a,b; Commissaris et al. 2002). They found that initially the lower and upper leg segments rotated in the same direction as platform rotation. The trunk segment thereafter demonstrated consistent early roll velocities in the opposite direction. The present study shows the same ipsilateral movement of the leg and, although less clear, of the trunk as well. Comparable to the balance studies a movement to the contralateral side was observed after this intial movement to the ipsilateral side. EMG responses in these platform studies showed an early reflex in the contralateral GM and ER. At that moment they observed an unloading response on the ipsilateral side. In the present study the contralateral GM and ER did show an earlier response and a suppression was observed in the ipsilateral GM and ER. Similar to the postural balance correcting responses in the roll plane the activation of these contralateral muscles was earlier than observed in the lower leg muscles. Furthermore, in agreement with those studies, the early suppressive responses in the ipsilateral GM were followed by facilitatory responses (with a latency of about 100 ms, see Fig. 8), the amplitude of which was higher than the one seen in the contralateral GM and paraspinals. These balance correcting responses might be important in preventing ankle injuries and a disturbed balance reaction might play a role in the high rate of recurrence of the inversion trauma. Indeed, subjects with recurrent ankle sprains often show a larger postural sway compared to healthy subjects, indicating that they are possibly defective in balance restoring activities (Cornwall and Murrell, 1991; Freeman, 1965; Fridén et al., 1989; Leanderson et al., 1999, 1996; Tropp et al., 1984). Such deficits may be due to the inability to hold the ankle stiff during these reactions. The alternative explanation is that these patients have defective proprioceptive feedback because of the damage to the ligaments. Although these balance corrective responses were long considered to be
triggered and modulated by lower leg proprioceptive feedback (Diener et al. 1985; Nasher, 1976; Horak et al., 1994; Dietz et al., 1989; Schiepatti and Nardone, 1997), there is growing evidence that more proximal (hip and trunk) proprioceptive input is critical for these responses (Allum et al., 1995). Several authors showed prominent balance correcting responses in subjects where the proprioceptive input of the ankle was removed or reduced by fixation (Gurfinkel et al., 1979) or by disease (Bloem et al. 2000). An alternative explanation is that the EMG activations during the initial response are stretch reflexes. This would explain not only the responses in the hip and trunk muscles, but also in the peroneal muscles.

Responses beyond 150 ms (voluntary reaction time)

In comparison with the balance studies mentioned above, some differences in the roll correction response were observed as well. The duration of the contralateral GM activity was longer than the one found in other muscles, probably because this extra activity is needed to abduct the contralateral leg during its swing phase. The latencies of these reactions are very long and therefore could constitute voluntary reactions. The same is true for the next phase when a contralateral foot placement is observed which is in a more ipsilateral position than during the control trials, indicating a slight perturbation of gait. This ipsilateral movement, just before contralateral foot placement, is also observed in the trunk (SIPS and shoulder marker). After contralateral foot placement, the ipsilateral knee that was in a lateral position during the whole single stance phase, moves first to a more equal position as the control trial, followed by the trunk and contralateral foot. Finally, at approximately the next double support phase the stimulus and control situation are more or less the same.

In summary, after an inversion perturbation a whole body reaction is observed. The first reaction consisted of a lateral shift of the knee, thereby decreasing the amount of inversion at the ankle level. This roll movement forces the body to make a response comparable with balance correcting responses after a perturbation in the roll plane during standing. The activation of the peroneal muscles occurs mainly in the “initial response” during loading of the ankle in the early stance phase. Conducting a similar study on subjects with recurrent ankle sprains might give more insight in the mechanism behind these reactions. It is speculated that these subjects use more ankle inversion in the initial response rather than moving the knee outward because they are less able to hold the ankle stiffly. Furthermore it is speculated that these patient could have an inadequate balance correction response. These differences to healthy subjects would lead more easily to a recurrence of the injury. Further experiments are needed to test these hypotheses.
Stiffness control of the leg in perturbed gait and posture
Chapter 7
Dynamic posturography using a new movable multidirectional platform driven by gravity

7.1. Introduction

Maintaining balance while standing upright is a prerequisite for successful performance of many daily activities. Recognising the essential role of human upright stance, many studies have been carried out to elucidate the physiological mechanisms underlying normal postural control (Diener et al., 1984; Dietz et al., 1989; Gollhofer et al., 1989; Nashner, 1976) and the pathophysiology of balance disorders (Allum and Pfaltz, 1985; Horak et al., 1992; Nashner et al., 1982; Schieppati and Nardone, 1991). These insights have increased noticeably with the advent of dynamic posturography: the assessment of balance correcting responses following controlled postural perturbations. A common type of dynamic posturography is to perturb upright stance by sudden movements of a supporting platform upon which the subject is standing (Nashner, 1983). Examples of commonly used platform movements include horizontal translations (Horak et al., 1989) and dorsiflexion or plantarflexion rotations about the ankle joint (Allum, 1983; Bloem et al., 1993; Diener et al., 1984).

Studies using these movable platforms have revealed valuable information about postural control mechanisms in healthy subjects and patients with various balance disorders (Allum and Pfaltz, 1985; Bloem et al., 1992; Horak et al., 1990). However, most currently available platforms have shortcomings. The first drawback relates to the size of the support surface, which for some platforms is too small to allow subjects to take corrective steps. For example, Allum and colleagues use a relatively small-sized platform to which the feet of their subjects are strapped to prevent them from stepping off the platform (Allum et al., 1998; Bloem et al., 2000). Studies that used larger support surfaces have stressed the importance of compensatory stepping responses, not only when balance is truly jeopardised but also under less threatening conditions (McIlroy and Maki, 1993; McIlroy and Maki, 1996). Second, for some platforms, even the largest or fastest motions generated are insufficiently destabilising to actually bring subjects beyond their stability limits. For example, the most destabilising rotations supplied by the commonly used NeuroCom platform (10 degrees rotation amplitude, 50 degrees/s rotation velocity) rarely cause serious balance problems for young persons (Beckley et al., 1991), while they bring about moderate balance problems in elderly persons and patients with Parkinson's disease (Beckley et al., 1993). We speculate that bringing subjects beyond their stability limits is required to further increase insights into the (patho-) physiological processes causing falls in daily life. Third, many movable platforms can only produce perturbations in a single direction, typically the pitch plane. Multidirectional perturbations may be more informative, because in daily life falls may occur in any given direction. Indeed, studies using multidirectional perturbations have unveiled abnormalities in patients that would have been missed using strictly unidirectional perturbations (Carpenter et al., 1999). Furthermore, a multidirectional protocol reduces habituation effects by diminishing the predictability of the upcoming perturbation direction (Bloem et al., 1998). Multidirectional perturbations thus correspond better to falls in daily life, which are predominantly unexpected events. A final drawback relates to the high costs,
mainly due to the often expensive servo-controlled torque motors that deliver the platform movements. This precludes more widespread use in clinical settings.

In view of these considerations, we sought to develop a less expensive movable platform that could produce destabilising, multidirectional postural perturbations. Furthermore, the platform surface should be sufficiently large to allow subjects to take a corrective step. In all, the set-up should more validly simulate falls and postural reactions in daily life.

The goals of this paper are threefold. First, we describe the perturbation characteristics of the newly developed gravity-driven platform for various perturbation amplitudes and directions. Second, we describe the patterns of postural responses evoked by these multidirectional perturbations. Finally, we evaluate whether multidirectional perturbations beyond the limits of stability could be used to evoke compensatory stepping responses, with little confounding influence of habituation.

7.2. Methods

Platform characteristics

A photograph and schematic illustrations of the movable platform are shown in Fig. 7.1 and 7.2. The platform consists of a 1-m² metal plate (mass 38 kg) which is supported at each of its four corners by a cable and two magnets (see Fig. 7.1A). Sudden release of the platform by switching off the magnets on three of the four sides of the platform (leaving one side attached) induces platform rotations in either the pitch or roll plane. Thus, the rotation axis is located on the side of the platform where the magnets remain attached, and it runs through the centres of the bottom of those magnets. This differs from most other platforms where the rotation axis is along the ankle joints of the perturbed subject. The force of gravity, which acts upon the standing subject and the metal plate, provides the driving force. Release of all magnets except those on the ventral side of the subject causes a “heels down” (see Fig. 7.1A) motion about the ankle joints, resulting in a backward directed fall (see Fig. 7.1B). A comparable fall is induced by the commonly used “toes up” rotations (Scholz et al., 1987; Allum et al., 1989; Schieppati and Nardone, 1991). A notable difference is the downward body motion associated with “heels down” rotations on our platform versus the upward head acceleration following “toes up” rotations on a platform with a rotation axis along the ankle joints (Carpenter et al., 1999). The magnitude of this downward displacement is identical for both feet and depends on the rotation angle, as well as the horizontal distance between the feet and the rotation axis. Release of all magnets except those on the dorsal side of the subject causes a “toes down” movement. Release of all magnets except those on the left side of the subject causes a “right foot down” movement, and vice versa. Again, the subject is also displaced downwards, with an asymmetrical displacement of the feet (largest for the “downhill” foot farthest away from the rotation axis). The magnitude of this displacement again depends upon the rotation angle and the subject’s position relative to
the rotation axis. Finally, release of the platform by switching off all eight magnets causes a purely vertical downward motion (“all down”). Fig. 7.2 illustrates the perturbation types described above.

Before each trial, the experimenter determines the perturbation characteristics using a customised computer program. The rotation amplitude is predefined with steps of 0.5° (between 0.5°-19°) by varying the amount of slack in the supporting cables. This is achieved by a simple motor (180 W 3-phase motor, ZAE Hamburg, Germany, GM63/71S/6D) attached to each of the four cables (see Fig. 7.1A). For purely downward displacements, the amplitude is predefined with steps of 0.5 cm (between 0.5-9.5 cm). Finally, the perturbation direction is predefined by a specific combination of magnets to be released. The onset of platform movement is detected by a motion sensor (MMB Gelma, LG 433-410-360, 38 pulses per degree
platform rotation) attached beneath the centre of the support surface. After each platform rotation, the support plate is brought back to its initial horizontal position by the motor, allowing the magnets to restore their contacts. During repositioning, the subject remains standing on the platform.

During the experiments reported here, subjects wore a lightweight safety harness attached with straps to the ceiling overhead. This allowed body sway beyond the limits of stability, but prevented actual falls. All subjects gave informed consent, as approved by the local ethical committee.

**Experiment A: perturbation characteristics of the platform**

Two healthy male subjects, aged 32 and 30 years, participated. Their respective body mass was 67 and 72 kg, but the mass of the latter person was artificially increased to 90 kg with several weights attached to a waist belt (10 kg) and to the safety harness (8 kg) to obtain a larger difference between both masses applied.
Since the platform motion is gravity-driven, the perturbation characteristics of the platform depend upon the mass of the subject (\(F_{\text{down}} = F_{\text{gravity}} = \text{mass} \times \text{gravitational acceleration}\)) and the position of the feet relative to the rotation axis. We therefore determined how subject mass (67 versus 90 kg) and feet position (two different ones) affected the perturbation characteristics of the platform. This was examined for three different rotation directions (“toes down”, “heels down” and “left foot down”) and three different rotation angles (4°, 10° and 19°). The two different feet positions included (a) close to the rotation axis, and (b) in the centre of the platform. The lateral distance between the metatarsal-phalangeal joints of the toes of both feet was always 10 cm. The actual feet position for each individual was standardised by measuring, for pitch plane perturbations, the horizontal distance between the right malleolus and the rotation axis. For roll plane perturbations, feet position was standardised by measuring the horizontal distance between the centre of the medio-lateral base of support and the rotation axis. All distances were measured while the support plate was in its original horizontal position. The actual distances were 15 cm (close to rotation axis) and 48 cm (platform centre) for the “toes down” condition, 29 and 62 cm for the “heels down” condition and 23 and 55 cm for the “left foot down” condition. To avoid changes in subject position across trials, stickers (in the shape of human feet) precisely indicated all different positions on the platform.

Prior to the actual experiment, subjects were informed about the goal of the experiments and they received several platform rotations of various directions and angles. The actual test protocol consisted of 126 trials: 7 successive trials in each condition (3 rotation directions x 3 rotation angles x 2 feet positions, nested in this order). The perturbations were offered in blocks of 21 trials (7 trials x 3 rotation angles). Within each block the rotation direction and feet position were kept constant. A pause of two minutes separated each block. Prior to each new condition, the subject received information about the upcoming direction, angle and feet position. The total duration of the protocol was about 90 minutes for each subject.

The rotation angle of the platform was measured by a goniometer (Penny & Giles M180, twin-axis) attached (in the sagittal plane) to the cover of one of the magnets that remained attached (fixed end) and to the metal plate (moving end). The data were low-pass filtered (cut-off frequency at 8 Hz, time delay 5 ms), sampled at 1000 Hz and stored on hard disk for a period of 2000 ms, starting 500 ms before the rising edge of the trigger signal provided by the motion sensor. The delay of 5 ms was accounted for in all onsets that were deduced from the goniometer data. We determined the following perturbation characteristics: rotation angle (peak rotation angle relative to baseline), rotation duration (time between onset of platform motion and instant of peak rotation), mean rotation velocity (rotation angle divided by rotation duration), peak rotation velocity (peak in the first derivative of the rotation angle), time-to-peak velocity (relative to the onset of platform motion) and the instant (relative to the onset of platform motion) at which the rotation velocity exceeded 20 deg/s (the approximate threshold to elicit stretch responses (Nashner and Cordo, 1981)). We also calculated the
downward displacement of the feet (tangent of rotation angle x horizontal distance between rotation axis and subject’s feet) and we evaluated the compensatory stepping responses (using video recordings). The perturbation characteristics were first determined for each single trial and then averaged for each of the 36 conditions. The main effects and interaction effects of rotation direction (three levels) and –angle (three levels) on the perturbation characteristics were evaluated with a two-way MANOVA. Likewise, the effects of feet position (two levels), body mass (two levels) and rotation angle (three levels) were assessed with a three-way MANOVA. Effects were considered significant in case of P<0.05.

Experiment B: postural responses to various perturbations

One female subject (26 years, 56 kg) participated. She was informed about the goal of the experiment and the number of trials, but did not receive any ‘practice’ platform rotations before the actual tests to avoid habituation effects. Theoretically, it would be possible to predict the perturbation size by listening to the sound of the motor (for larger perturbation angles, the motor would be active for a proportionally longer period of time to reach the required amount of slack in the cables). To prevent this, the subject wore earplugs and headphones that produced white noise.

To compare the automatic postural responses elicited by our new platform with analogous responses reported in the literature, we used a series of 4° “heels down” rotations that resemble the commonly applied 4° “toes up” rotations (rotation axis about the ankle joint). Furthermore, to describe the postural reactions to less common perturbations, we also used a series of “heels down” rotations with a much larger amplitude (15°), as well as “right foot down” rotations of 4° and 15° amplitude. Feet position was always in the centre of the platform. Each condition was repeated 10 times and the complete series of 40 trials was offered in random order to avoid habituation effects. A random inter-stimulus interval of 45-75 seconds was applied. The total duration of the protocol was about 40 minutes.

Following these 40 trials, the characteristics of the platform movements were determined for this particular subject’s mass and feet position. To this end, three successive trials in each of the four conditions were performed while the platform rotation angle was measured. The perturbation characteristics were measured as in experiment A.

Bipolar surface electrodes measured muscle activity (EMG) of the right tibialis anterior and medial gastrocnemius muscles, and bilaterally the gluteus medius, lumbar erector spinae (level L3), and deltoid (caput mediale) muscles. The EMG signals were amplified (by a factor in the order of 10^4-10^5), high-pass filtered (cut-off frequency at 3 Hz), full wave rectified and then low-pass filtered (cut-off frequency at 300 Hz). The data were sampled at 1000 Hz for a period of 1250 ms, starting 250 ms before the rising edge of the trigger signal provided by the
motion sensor. All data were stored on hard disk. In addition to the muscle activity, we evaluated compensatory stepping reactions using video recordings.

Short latency (SL), medium latency (ML) and long latency (LL) automatic postural responses were visually identified in the EMG traces using the following criteria: 1) occurrence in pre-determined reference windows (Bloem et al., 1995), 2) burst activity more than 1 SD above background activity (Carpenter et al., 1999), and 3) response present in at least 6 out of 10 trials (Henry et al., 1998). If all criteria were met, a time window that optimally captured the overall response in each rotation direction was set for each individual muscle (procedure described in Duysens et al. (1991) and Nieuwenhuijzen et al. (2000)). The left line of this window indicated the latency of the overall response relative to the onset of platform motion (detected by the motion sensor and set at 0 ms).

### 7.3. Results

**Perturbation characteristics of the platform**

The rotation angle and angular velocity patterns of seven successive trials indicate that the platform generated reproducible postural perturbations. Fig. 7.3 shows these patterns for the smallest perturbation (i.e. 4° rotation amplitude, 67 kg subject standing close to the rotation axis; left panels) versus the largest one (i.e. 19° rotation amplitude, 90 kg subject standing at the platform centre; right panels). Particularly between the onset of platform motion and the moment of peak rotation the rotation characteristics were similar.

Table 7.1 presents an overview of the quantitative measures of the perturbation. These perturbation characteristics were significantly affected by the rotation angle (Wilks’ $\lambda=0.002$, $F(10,46)=97.70$, $P<0.001$). A larger angle (i.e. 4° vs. 10° vs. 19°) resulted in a longer rotation duration (173 - 231 - 304 ms), a higher mean rotation velocity (27.8 - 48.7 - 67.8 deg/s), a higher peak rotation velocity (52.3 - 91.9 - 124.1 deg/s) and a longer time-to-peak velocity (116 - 177 - 244 ms). The time-to-threshold velocity was not affected by the rotation angle. Contrary to the rotation angle, the rotation direction did not influence the perturbation characteristics (Wilks’ $\lambda=0.648$, $F(10,46)=1.12$, $P=0.371$). The interaction between rotation direction and angle was not significant either (Wilks’ $\lambda=0.733$, $F(20,77)=0.38$, $P=0.992$). Both subject mass and feet position showed a significant main effect on the perturbation characteristics (mass: Wilks’ $\lambda=0.403$, $F(5,20)=5.92$, $P=0.002$; position: Wilks’ $\lambda=0.105$, $F(5,20)=33.92$, $P<0.001$), while no interaction effect was found (Wilks’ $\lambda=0.842$, $F(5,20)=0.75$, $P=0.595$). The larger subject mass (i.e. 90 vs. 67 kg) resulted in a shorter rotation duration (229 - 242 ms), a higher peak velocity (91.5 - 87.4 deg/s) and a shorter time-to-threshold.

Velocity (46 - 52 ms). Standing in the platform centre yielded (compared to standing close to the rotation axis) a shorter rotation duration (225 - 247 ms), a higher mean velocity (51.4 - 44.8 deg/s), a higher peak velocity (96.4 - 82.4 deg/s), a shorter time-to-peak velocity (164 - 194 ms) and a shorter time-to-thresh-
old velocity (41 - 57 ms).

After reaching the peak rotation amplitude, the platform bounced back slightly to reach the final rotation angle set by the experimenter (see Fig. 7.3A, both panels). This overshoot in peak rotation amplitude and the resulting rebound occurred in all trials and were due to the elastic properties of the platform elements braking the fall of the metal plate. The mean onset of this platform rebound occurred 173 ms (SD 9, n=12) after the onset of platform motion for rotations of 4°, 231 ms (SD 17, n=12) for 10° rotations and 304 ms (SD 27, n=12) for 19° rotations. The mean rebound angles were 0.6° (SD 0.3) for 4° rotations, 0.6° (SD 0.2) for 10° rotations and 0.7° (SD 0.1) for 19° rotations. These rebound characteristics were significantly affected by the angle of platform rotation (Wilks’ l=0.101, F(4,52)=27.85, P<0.001), but not by the direction of rotation (Wilks’ l=0.960, F(4,52)=0.26, P=0.899). There was no interaction between rotation angle and direction (Wilks’ l=0.971, F(8,52)=0.10, P=0.999).

Due to the distance between the axis of rotation and the subjects’ feet the platform rotations also displaced the subjects downward. For pitch plane rotations, the magnitude of this displacement was identical for both feet, ranging
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Table 7.1.

<table>
<thead>
<tr>
<th></th>
<th>4° (n=12)</th>
<th>10° (n=12)</th>
<th>19° (n=12)</th>
<th>close to axis (n=18)</th>
<th>platform centre (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rotation duration (ms)</strong></td>
<td>173 (9)</td>
<td>231 (17)</td>
<td>304 (28) *</td>
<td>247 (66)</td>
<td>225 (47) †</td>
</tr>
<tr>
<td><strong>Mean rotation velocity (deg/s)</strong></td>
<td>27.8 (2.9)</td>
<td>48.7 (3.7)</td>
<td>67.8 (5.7) *</td>
<td>44.8 (15.8)</td>
<td>51.4 (18.1) †</td>
</tr>
<tr>
<td><strong>Peak rotation velocity (deg/s)</strong></td>
<td>52.3 (5.4)</td>
<td>91.9 (7.0)</td>
<td>124.1 (14.5) *</td>
<td>82.4 (28.4)</td>
<td>96.4 (33.2) †</td>
</tr>
<tr>
<td><strong>Time-to-peak velocity (ms)</strong></td>
<td>116 (15)</td>
<td>177 (20)</td>
<td>244 (25) *</td>
<td>194 (59)</td>
<td>164 (52) †</td>
</tr>
<tr>
<td><strong>Time-to-threshold (20 deg/s velocity (ms))</strong></td>
<td>51 (12)</td>
<td>48 (9)</td>
<td>47 (9)</td>
<td>57 (6)</td>
<td>41 (5) †</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>“heels down” (n=12)</th>
<th>“toes down” (n=12)</th>
<th>“left foot down” (n=12)</th>
<th>67 kg (n=18)</th>
<th>90 kg (n=18)</th>
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</thead>
<tbody>
<tr>
<td><strong>Rotation duration (ms)</strong></td>
<td>239 (60)</td>
<td>232 (57)</td>
<td>236 (61)</td>
<td>242 (60)</td>
<td>229 (55) †</td>
</tr>
<tr>
<td><strong>Mean rotation velocity (deg/s)</strong></td>
<td>47.1 (17.5)</td>
<td>49.8 (17.5)</td>
<td>47.4 (17.6)</td>
<td>47.5 (16.9)</td>
<td>48.8 (17.8)</td>
</tr>
<tr>
<td><strong>Peak rotation velocity (deg/s)</strong></td>
<td>88.7 (32.0)</td>
<td>91.7 (33.1)</td>
<td>87.9 (31.3)</td>
<td>87.4 (29.2)</td>
<td>91.5 (33.9) †</td>
</tr>
<tr>
<td><strong>Time-to-peak velocity (ms)</strong></td>
<td>181 (63)</td>
<td>179 (56)</td>
<td>177 (56)</td>
<td>184 (55)</td>
<td>174 (60)</td>
</tr>
<tr>
<td><strong>Time-to-threshold (20 deg/s velocity (ms))</strong></td>
<td>51 (9)</td>
<td>45 (11)</td>
<td>50 (8)</td>
<td>52 (11)</td>
<td>46 (8) †</td>
</tr>
</tbody>
</table>

Table 7.1: Mean values (± 1 standard deviation) of the perturbation characteristics in three platform rotation angle conditions (4°, 10° and 19°), in two subject feet position conditions (close to the rotation axis and in the centre of the platform), in three platform rotation direction conditions (“heels down”, “toes down” and “left foot down”), and in two subject mass conditions (67 kg and 90 kg). The number of trials (n) is indicated in the second row. The symbols denote significant (P < 0.05) main effects of rotation angle (*), feet position (‡) and mass (†).

from 1 cm (4° “toes down” rotation, subject standing close to the axis) to 21 cm (19° “heels down” rotation, subject standing in the platform centre). In case of leftward roll plane rotations, an asymmetrical downward displacement of the feet occurred that was largest for the left foot. For this foot, the magnitudes ranged from 2 cm (4° rotation, subject standing close to the axis) to 22 cm (19° rotation, subject standing in the platform centre). For the right foot, the magni-
tudes ranged from 1 cm (4° rotation, subject standing close to the axis) to 15 cm (19° rotation, subject standing in the platform centre).

4° heels down

A: tibialis anterior

B: medial gastrocnemius

Fig. 7.4. EMG activity (in arbitrary units, a.u.) of the tibialis anterior (A) and medial gastrocnemius (B) recorded in 10 trials in the 4° “heels down” condition. The 10 trials were part of a random series of 40 trials (4 different conditions of 10 trials each). The solid vertical line indicates the onset of platform motion. The dashed vertical lines mark the single optimal time windows set for the LL response in the tibialis anterior and the ML response in the medial gastrocnemius.

Postural responses to various perturbations

The 4° “heels down” rotations elicited automatic postural responses in the tibialis anterior (onset latency 117 ms) and medial gastrocnemius (onset latency 71 ms), indicative of LL and ML responses (see Fig. 7.4). In some trials (e.g. 6 and 7) the medial gastrocnemius also showed a SL response around 30 ms, but this was not a consistent observation. Likewise, a ML response in the tibialis anterior around 70 ms (e.g. trial 7) was inconsistently present. Whereas muscle activity returned to base line after about ±200 ms in the medial gastrocnemius, tibialis anterior remained active in most trials. The mean rotation duration in this condition was ~160 ms, the mean rotation velocity was ~30 deg/s, while the peak rotation velocity (~55 deg/s) occurred ~105 ms after onset of platform motion.

Both rotation directions and both rotation amplitudes evoked postural responses in the proximal muscles recorded (see Fig. 7.5). ML responses were identified in the left gluteus medius EMG for both rotation directions (latency ~75 ms), and bilaterally in the medial deltoid EMG when rotating “heels down” (latency ~60 ms). LL responses were identified in each muscle and in most conditions. For example, clear responses were observed bilaterally in the lumbar erector spinae (“heels down” latency ~120 ms for both sides; “right foot down” latency ~130 ms for the right side and ~160 ms for the left side). Also, the “right foot down”
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Fig. 7.5. Mean EMG activity (in arbitrary units, a.u.; 10 trials) of the gluteus medius (A), the lumbar erector spinae (B) and the medial deltoid (C). The left panels show EMG activity in the “heels down” (hd) conditions, the right panels show EMG activity in the “right foot down” (rfd) conditions. For each muscle, the scales of EMG activity were similar for both recording sides and all perturbation conditions. The solid vertical line indicates the onset of platform motion.
rotations elicited LL responses in the tibialis anterior (latency ~105 ms) and in the medial gastrocnemius (latency ~115 ms).

Compensatory stepping responses

The subjects successfully maintained upright stance in all experimental conditions, although some perturbations were sufficiently destabilising to evoke stepping responses. In experiment A, where a predictable series of 7 identical stimuli was given, stepping occurred in 7 out of 252 trials (3%), while in experiment B, which comprised a random mix of non-predictable stimuli, corrective steps were observed in 12 out of 40 trials (30%). Compensatory stepping was only observed following the largest perturbations, i.e. 15° in the random protocol and 19° in the serial one. After the largest amplitude “heels down” rotations, compensatory steps were much more common in the random protocol than in the serial one: 100% versus 21% (Pearson Chi2=18.66, P<0.001). This difference was present even though the rotation angle was 4° smaller in the random protocol compared to the serial one. In all trials where stepping occurred, subjects made one, two or three steps within the limits of the support surface.

7.4. Discussion

The perturbation characteristics of the platform

The present study showed that our newly developed, gravity-driven movable platform could generate standardised and reproducible postural perturbations in both the pitch and roll planes. Standardised and reproducible stimuli are essential to reliably evoke stretch reflexes and automatic balance corrections, such that interindividual differences are attributable to different characteristics of the subjects under study, rather than to different stimulus characteristics. Because our platform is gravity-driven, several perturbation characteristics (including peak rotation velocity) depended upon the subject’s mass and feet position relative to the rotation axis. Because perturbation velocity influences automatic postural responses (Beckley et al., 1993; Diener et al., 1988), feet position should be standardised (as was done in this study) and body weight must be taken into account by matching subjects or by including it as a covariate in statistical analyses.

The perturbations, even the 4° rotations, were sufficiently large and rapid to elicit postural responses in various muscles throughout the body. The platform is also capable of delivering much larger perturbations (up to 19°) than commonly used in the literature. These large amplitude perturbations proved particularly destabilising, not only because of the increased stimulus angle, but also because increasingly large perturbations yielded progressively higher mean and peak rotation velocities. The platform thus fulfilled one of our primary goals, i.e. to test subjects around the limits of stability. The perturbation characteristics did not
depend upon the direction of platform rotation (i.e. “heels down”, “toes down” or “left foot down”), allowing direct comparisons of postural responses across different perturbation directions. Furthermore, the time period to reach the threshold for eliciting stretch responses (about 20 deg/s, (Nashner and Cordo, 1981)) did not differ for a wide range of perturbation amplitudes. However, differences in rotation velocity and acceleration did appear after about 115 ms, and such differences may well affect secondary balance corrections and voluntary actions, which occur more than 250 ms post-stimulus (Carpenter et al., 1999). As compared to torque motor-driven platforms, the present platform introduces perturbations which are closer to natural perturbations (i.e. standing on a support which gives way).

The performance of our gravity-driven platform differs in some ways from that of torque motor-driven platforms. First, the platform is not decelerated by a torque motor, but abruptly stops when the cables, attached to each of its corners, reach the maximum slack. The stop was found to be followed by a small rebound of the metal plate. This rebound did not modify the stretch reflexes and early balance corrections because the earliest onset of rebound arose around 160 ms, i.e. well after appearance of ML and LL responses. Second, a downward body displacement accompanied the rotatory stimulus, since the axis of platform rotation was not aligned with the ankle joints. Such vertical displacements could reach more than 20 cm for the largest rotatory stimuli, which is probably sufficient to elicit vestibulospinal responses (Allum and Pfaltz, 1985; Allum et al., 1989; Horak et al., 1994). Using torque motor-driven platforms with the axis of rotation along the ankle joints, the vestibular system contributes in a different way because the head is accelerated upward following the traditional “toes up” stimuli (Carpenter et al., 1999). Sudden downward displacements can also elicit startle responses in trunk and limb muscles with onset latencies between 50 and 90 ms (Bisdorff et al., 1994). In our study, early activity in deltoid muscles (around 60 ms) would be appropriately timed to represent such a startle response. Both the vestibular and startle effects must be taken into account to fully understand the balance corrections evoked by the rotatory and vertical perturbations applied by our new platform. In fact, these perturbation characteristics of our new platform increase its versatility and render it an excellent tool for probing the role of vestibular influences and startle responses in health and disease.

Unlike the commonly used NeuroCom platforms, our platform does not provide force or centre of foot pressure data in the current experimental set-up. It’s main value is in providing reliable EMG data. However, it is possible to add force transducers and a motion analysis system to the current set-up, thus providing force and motion data, and enabling inverse dynamic calculations.

The postural responses to various perturbations

The 4° “heels down” rotations elicited automatic postural responses in the lower legs. The onset latencies of ML responses in the medial gastrocnemius (~70 ms post-stimulus) and LL responses in the tibialis anterior (~120 ms post-stimu-
lus) corresponded well to latencies of comparable responses evoked by the more traditional 4° “toes up” rotations (Bloem et al., 1992; Gollhofer et al., 1989). The “heels down” rotations also evoked postural responses in proximal muscles. For example, early responses were observed in the left gluteus medius (~75 ms) and both medial deltoid muscles (~60 ms), while later responses were seen in both lumbar erector spinae (~120 ms). These paraspinal responses might reflect stretch responses, in reaction to the forward trunk flexion that followed the “heels down” rotation (Allum et al., 1998). Furthermore, the paraspinal onset latencies are in line with previous reports (~70-120 ms following 4°-7.5° rotational perturbations (Bloem et al., 2000; Carpenter et al., 1999)). The postural reactions to the less commonly used “right foot down” rotations involved responses in both distal and proximal muscles. The earliest response occurred in the contralateral gluteus medius muscle (~75 ms), thus preceding the responses in the ipsilateral tibialis anterior and medial gastrocnemius (~105-115 ms). In the ipsilateral erector spinae a response occurred at ~130 ms, while for the contralateral muscle a response latency of ~160 ms was observed. This activation pattern is similar to what is seen following roll plane rotational perturbations about the ankle joint (Carpenter et al., 1999). We conclude that both the pitch and roll plane perturbations delivered by our new platform could elicit automatic postural responses in muscles throughout the body.

The compensatory stepping responses

“Heels down” perturbations of ≥15° amplitude were sufficiently large and fast to bring the subjects beyond their limits of stability, as reflected by the clear presence of ‘rescue reactions’ (Nutt et al., 1993). These compensatory stepping responses were seen both following the predictable series of identical trials, but especially following the randomised mix of different perturbations. Allowing (or, indeed, forcing) subjects to step following an induced postural perturbation may be a more valid simulation of falls in daily life than constraining subjects to ‘feet in place behaviour’. Certainly, stepping responses are an important element in the movement repertoire to maintain upright stance in response to external perturbations (Burleigh et al., 1994; McIlroy and Maki, 1993). Interestingly, stepping was much more common following the randomised mix of different perturbations. This suggests that randomising different multidirectional perturbations can be used to diminish habituation of the stepping response. Such habituation effects following series of identical postural perturbations have previously been described to affect automatic postural responses in lower leg muscles (Bloem et al., 1998; Hansen et al., 1988). Habituation is unwanted as it obscures proper insight into the mechanisms underlying falls in daily life, which are usually unexpected single events. For example, postural responses of patients with Parkinson’s disease are particularly abnormal when a novel postural perturbation is first met, but habituation following repeated exposure to identical stimuli conceals these abnormalities (Bloem et al., 1998).
A unique feature of our new platform is the vertical (and rather startling) displacement that accompanies the rotational perturbation, and may be particularly suitable to minimise habituation effects. An additional advantage is that use of large and quite destabilising perturbations may, via anxiety and cognitive set, further reduce habituation. Application of multidirectional perturbations has other benefits. First, they may be particularly informative about daily life performance where falls and perturbations also occur in any given direction. Second, postural abnormalities can be found that would have been missed using strictly unidirectional perturbations (Carpenter et al., 1999).

In all, we conclude that our new gravity-driven multidirectional platform provides a useful and versatile tool for dynamic posturography.
CHAPTER 8
REFERENCES AND FINAL WORDS
References


Stiffness control of the leg in perturbed gait and posture


Publications

Articles


Abstracts


Duysens J, Grüneberg C, Bastiaanse C, Nieuwenhuijzen PHJA and Dietz V. To load or not to load that is the question. Motor Control Conference III MCC2003, Varna, 2003


Nieuwenhuijzen PHJA. Phase-dependent modulation of startle responses during human walking. Proceedings of the Dutch-Flemish Meeting on Movement Control and Coordination, Leuven, October 7-8, 1999

Stiffness control of the leg in perturbed gait and posture


Dankwoord

Zo door de jaren heen dat ik op de vakgroep rondliep, verzamelde ik een hoop mensen aan wie ik mijn dank verschuldigd ben. Op deze plek wil ik daarom graag van de gelegenheid gebruik maken om een aantal van die mensen te bedanken voor alle directe of indirecte hulp, inspiratie en geduld bij de totstandkoming van dit proefschrift.

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Curriculum Vitae

Stiffness control of the leg in perturbed gait and posture