Modulation of cutaneous reflexes from the foot during gait in Parkinson's disease

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abbreviated title for the running head: Cutaneous reflexes in Parkinson’s gait
Abstract

Normal gait is characterized by a phase-dependent modulation of cutaneous reflexes. The role of the basal ganglia in regulating these reflexes is largely unknown. Therefore cutaneous reflex responses from the skin of the foot were studied during walking of patients with mild to moderate Parkinson’s disease. The reflex responses were elicited by stimulation of the sural nerve of the most affected leg. The responses were studied in the biceps femoris (BF) and tibialis anterior (TA) of both legs. The latencies, durations and phase-dependent modulation patterns of the responses were mostly comparable to those observed in healthy subjects. However, on average the amplitude of the responses in the ipsilateral and contralateral BF was respectively 1.4 and 5 times larger for the Parkinson’s disease patients than for the healthy subjects. This increase was mostly seen throughout the whole step cycle. However, in some PD patients the crossed BF responses were very large during the contralateral swing phase. In such cases the increase in crossed reflexes sometimes reflected premotoneuronal gating since it was not always due to increased background activation in that period. Fast activation of contralateral BF reflexes is known to occur in conjunction with ipsilateral perturbations when there is a threat to stability. It is concluded that cutaneous reflexes are facilitated in PD but that some of the increase in reflexes in BF may be indirectly related to unsteady gait and to perceived instability.

Keywords: Parkinson’s disease; gait; cutaneous reflexes; phase-dependent reflex modulation
Introduction

In healthy subjects, a weak (non-nociceptive) electrical stimulus to a cutaneous nerve, innervating the skin of the foot, evokes reflex responses at a latency of about 80 ms in a wide variety of muscles in both the stimulated and the contralateral leg during walking (Yang and Stein 1990; Duysens et al. 1990, 1991, 1996; De Serres et al. 1995; Zehr et al., 1997, 1998a,b). These reflex responses are mediated by the low-threshold Aβ-fibers (Van Wezel et al. 2000). The gain of these responses is modulated according to the phase of the step cycle such that it generally does not vary in parallel with the background activity (and thus presumably not with motoneuronal excitability), indicating premotoneuronal mechanisms that control the gain of the reflex pathways during the course of a step cycle.

This phase-dependent reflex control is specific for the location of the stimulus (Van Wezel et al. 1997; Zehr et al. 1997) and is thought to be mostly due to actions of spinal locomotor central pattern generators (Duysens et al. 2004; Zehr and Duysens, 2004). Indeed, in patients with spinal cord injury (SCI) some elements of the modulation of these responses are largely preserved (Jones and Yang 1994; Knikou et al., 2009). Lesions in the corticospinal tract do not eliminate the phase-dependent modulation but mostly cause an overall reduction in the amplitude of the responses (Duysens et al. 2004b). Inversely, activation of the cortex through TMS (Transcranial Magnetic Stimulation) facilitates the reflex responses, indicating that there is convergence of the corticospinal tract and the reflex pathways involved in these responses, in confirmation of evidence obtained in cat studies (Bretzner and Drew, 2005). These results prompted the suggestion that the cortical input may act to selectively change the gain in these reflex pathways (Haridas et al., 2005, 2008). It was found that conditions which posed a threat to stability during gait (such as walking with arms crossed when surface translations of the belt of the treadmill could occur) selectively increased the amplitude of responses in contralateral muscles (Haridas et al., 2005). Similar results were obtained when an added postural threat was induced by touching the dorsum of the foot unexpectedly during walking (Haridas et al., 2008). It was speculated that these crossed reflexes were facilitated from the motor cortex in order to restore balance in case of instability (Haridas et al., 2005, 2008). Down regulation (decreased response amplitudes) can occur as well. For example, decreased amplitudes of cutaneous reflexes were observed when subjects delivered the stimulation themselves (Baken et al. 2006).

While there is substantial evidence for a role of the motor cortex in the up or down regulation of cutaneous reflexes, the contribution of the basal ganglia in this process is largely unknown. In Parkinson’s disease (PD) there has not yet been a study on the phase-dependent modulation of cutaneous reflexes. However, in a previous study we
investigated the phase-dependent modulation of another type of reflexes (startle) in PD and found very little abnormalities either in the overall amplitude or in the phase-dependent modulation of the responses (Nieuwenhuijzen et al., 2005). This result is consistent with the notion that the substantia nigra is not a major element involved in phase-dependent modulation. It is possible however that abnormalities are present in this patient group since the gait in PD patients is unstable. Walking on a treadmill can be seen by PD patients as a threat to stability. Hence one might expect an exaggeration of responses, especially on the contralateral side (Haridas et al., 2005, 2008).

An overall effect on the amplitude is also possible. For example, at rest conditions there have been indications for some abnormalities of cutaneous reflexes in the arm (Fuhr et al., 1992). Suppressive cutaneous reflexes in the arm are less pronounced in PD subjects. For other reflexes some abnormalities were reported as well. For example, abnormalities in postural reflexes have been observed (Dietz et al. 1988; Schieppati and Nardone 1991; Beckley et al. 1991; Bloem et al. 1995; Dietz et al. 1995; Chong et al. 2000; Carpenter et al. 2004; Dimitrova et al. 2004). However, these abnormalities did not seem to involve the basic synergies (Dimitrova et al., 2004). The latter authors stated that “the direction of maximum activation for each muscle was similar for PD and control subjects, suggesting that the basal ganglia is not critical for programming externally triggered postural synergies”. The present study addresses the same type of question but now in the context of cutaneous reflexes. Are these reflexes abnormal in Parkinson’s disease (either in terms of a general change in amplitude or more specifically with respect to phase-dependent modulation)? To this end, reflex responses to non-nociceptive electrical stimulation of the sural nerve (innervating the lateral side of the foot) were elicited during gait of these patients. Responses were studied in the biceps femoris (BF) and tibialis anterior (TA) of both the stimulated (ipsilateral) and unstimulated (contralateral) legs. The ipsilateral BF (iBF) and TA (iTA) were chosen because these muscles exhibit large facilitatory responses to sural nerve stimulation in normal subjects during gait (Yang and Stein, 1990; Duysens et al. 1990, 1996; Van Wezel et al. 1997, 2000; De Serres et al. 1995; Zehr et al., 1997, 1998b; Baken et al., 2006).
Methods

Subjects

Experiments were performed on a group of 9 patients (8 male, 1 female) with a clinically diagnosed Parkinson's disease (Table 1). The same group participated as described in an earlier study (Nieuwenhuijzen et al. 2006). The average age was 53 years (sd 12, range 42 to 73). The average disease duration was 9 years (sd 4, range 4 to 15).

Motor disability was evaluated using the Hoehn and Yahr score. Due to the nature of the task (treadmill walking) only minor affected patients were included in this study showing in the off stage a Hoehn and Yahr level varying from 3 to 4. The leg function was also evaluated separately by measuring the walking velocity and stride length (velocity range: 1-4 km/h, stride length range: 68 cm – 88 cm).

The data of the patients were compared with those of a group of 10 healthy subjects with no known history of neuromuscular disorder served as control (8 male, 2 female; aged between 19 and 46). The data from the control subjects were previously published in Van Wezel et al. (1997) and in part additionally analyzed. The experiments were carried out in conformity with the declaration of Helsinki for experiments on humans. All subjects had given informed consent and the experiments were performed after approval of the local ethical committee.

Experimental set-up

The subjects were asked to walk on a treadmill while wearing a safety-harness which was fastened to an emergency brake at the ceiling. Very thin custom-made insole foot-switch systems were used to detect foot contact. Bipolar electromyographic (EMG) activity was recorded in both legs by means of surface electrodes over the long head of the biceps femoris (BF) and the tibialis anterior (TA) muscles. The EMG signals were (pre-)amplified (total factor ~10^6), high-pass filtered (cut-off frequency at 3 Hz), full-wave rectified and then low-pass filtered (cut-off frequency at 300 Hz). These signals were sampled along with the foot-switch signals. The data were AD-converted (500 Hz) and stored on hard disk. The electrical stimulus (a train of 5 rectangular pulses of 1 ms duration given at 200 Hz) was applied by a custom-made constant-current stimulator through a bipolar stimulation electrode (with poles of 0.5 cm and an inter-pole distance of 2.0 cm) that was positioned over the sural nerve (approximately halfway between the lateral malleolus and the achilles tendon) of the most affected leg. For each subject the exact position of the stimulation electrode was
determined according to the optimal irradiation to the lateral side of the foot (the innervation area of the sural nerve),
similar to previously published methods (Duysens et al., 1996; Van Wezel et al., 1997, 2000; Baken et al., 2006) and
to those used by others (Yang and Stein, 1990; Zehr et al., 1998b).

Experimental procedures

The patients were trained to walk at a comfortable, self-selected speed. Prior to each experimental run, during quiet
standing, the perception threshold (PT) was determined by gradually increasing (to above PT) and decreasing (to
below PT) the stimulus amplitude. Before the start of an experimental run the PT had to be stable before and after a
short walking period. During the experimental runs the intensity of the stimuli was 2 PT (Duysens et al. 1996; Van
Wezel et al. 1997, 2000). This intensity was chosen because it gives a non-nociceptive sensation on the innervation
area of the sural nerve (lateral side of the foot) and reliably elicits reflex responses in healthy subjects. The stimuli
were delivered at 16 preprogrammed and equidistantly distributed points over the whole step cycle. During the same
periods control values (i.e., no stimulus) of the EMG were also measured. Both ipsilateral and contralateral footfall
served as reference points for the time in the step cycle, each for a half of the total step cycle.

There were 10 stimuli presented in each of the 16 positions in the step cycle and there were 10 non-stimulated sweeps
collected for each position in the step cycle, equaling 320 sweeps collected in total. All 320 trials occurred in random
order. To avoid predictability of the stimuli and to reduce habituation of the responses as much as possible, successive
stimulus conditions were separated by a random interval in the range of 3.5 to 6.5 seconds. Hence, two stimuli were
always separated by at least two step cycles without a stimulus. At the end of each experimental run the perception
threshold was determined once more. It was slightly lowered (about 8% on average) as compared to the measurement
taken immediately prior to the experimental run. As this change was small and systematic no further corrections were
deemed essential.

Data analysis

As the present data on PD had to be compared with data from a previous study, a similar analysis to that described in
Van Wezel et al. (1997) was utilized. The overall effect after nerve stimulation was obtained by averaging the 10
trials per condition (16 stimulus phases with and 16 control phases without stimulation) and subsequently by subtracting the resulting control data from the corresponding stimulus data. Hence, for each muscle 16 subtracted responses were obtained, corresponding to the 16 phases in the step cycle (see Fig. 1). Quantification of the responses occurred by determining a time window over the period in which the responses occurred and by calculating the mean of the EMG data within that window. When a muscle showed little or no response no adequate window could be set. In that case an average window was used, calculated from the time windows used to measure responses in (in order of priority) the other muscle in the same leg or the muscles in the other leg (cf. Tax et al., 1995; Van Wezel et al. 1997). For each trial, the mean EMG value was calculated within the applicable window obtained from the above analysis. Subsequently, for all conditions (i.e., for 16 phases both control and stimulus conditions), the average and the standard error were calculated from these window-averaged trials).

The maximum of the 16 averaged control values was used to normalize the EMG data so that a proper intersubject comparison could be made. The response amplitudes to cutaneous stimulation for a given condition are not normally distributed (as described in Duysens et al., 1996) and therefore it is common in this field to use non-parametric statistics. The same approach was taken here, especially since there was a need to compare the results with previous work on which the present study was built (Van Wezel et al., 1997). To determine whether any given mean subtracted response at a given phase was significantly different from zero the Wilcoxon signed-rank test (significance level $p<0.05$) was used. The differences in subtracted responses between patients and control subjects were tested using Independent-Samples Mann-Whitney U Tests. To look for similarities in the phase dependency between the 2 groups, non-parametric correlation coefficients between the phase pattern of the controls and PD groups were calculated.

Results

The results of the patients will be presented in relation to the results of the healthy subjects, which have been published elsewhere (Van Wezel et al., 1997). The control subjects were studied at 4 km/h. The patients were asked to
determine their preferred walking velocity during a short walking period on the treadmill. On average, the preferred velocity for the patients was 2.8 km/h (SD 0.9; range 1.3-4.0 km/h). An example of the BF EMG and foot contact data during a single step cycle are given in Fig. 1A for single healthy subject and a patient with PD.

The healthy subjects showed two EMG activity bursts in biceps femoris during the step cycle. The largest activity burst was observed during the late swing phase to decelerate the leg in preparation for the stance phase. This activity gradually declined during the stance phase. A second, considerably smaller activity was often observed during the late stance phase. In the patient the activity in BF lasted longer in the stance phase, a change that was consistently observed in the whole population (see further).

Reflex responses: latency and duration

Results of typical reflex responses are shown in Figure 1B for iBF of a healthy subject and a patient with Parkinson’s disease. In both subjects large facilitatory reflex responses were observed at a latency of about 80-90 ms. The step cycle was subdivided in 16 parts (as described in Van Wezel et al., 1997) with phase 1 being the phase that the response occurred just after touchdown. The latency of these responses was rather constant over the 16 phases of the step cycle. On average for all subjects, this time window started at 82 ms (SD 9), and ended at 116 ms (SD 12). On average for all subjects, this time window started at 82 ms (SD 9), and ended at 116 ms (SD 12). The window settings were very similar for the 2 groups. For the control subjects the onset was 84 ms (sd 8) to 115 (sd 7) ms while for the patients it was 81 (sd 6) ms to 117 (sd 5) ms.

Using these windows, a considerable phase-dependent effect on the magnitude of these responses could be observed. For example, both patient and healthy subject exhibited large responses during phases 7 to 11, while considerably smaller responses were elicited during the succeeding phases 12 to 14. While phase-dependency appeared similar in patients and control subjects there was a tendency for the responses to be larger in the patient group. To quantify the responses a time window was set around the responses in which the mean EMG value was
calculated (see Methods). To examine the overall difference in amplitude the mean amplitude was calculated over the whole step cycle for each subject and then the overall means were calculated for the 2 populations. The results are shown in Fig. 2.

Figure 2 near here.

In all muscles except iTA the responses were larger in the PD than in the control group. This was verified using Independent-Samples Mann-Whitney U Tests. The differences were significant for all muscles (iBF, p=0.003; cBF, p<0.001; cTA, p=0.032) except iTA (p=0.61).

Habituation

In a previous study (Nieuwenhuijzen et al., 2006) it was found that startle responses were larger in PD than in control subjects but this effect was due to a decrease in habituation in the PD patients. Since the present cutaneous reflex responses were also found to be exaggerated in PD the question arose whether this differences was also due to a difference in habituations. To examine this, the degree of habituation of the responses was checked for differences between patients and control subjects. For all subjects the subtracted trials of all stimulus phases were averaged in order of delivery and the responses were then normalized to their mean. Subsequently, they were averaged for both patients and healthy subjects, and a regression analysis was performed. The habituation did not differ significantly between the two groups. The amount of habituation was 26% for the group of patients, and 23% for the group of healthy subjects. These results are consistent with previously reported habituation in comparable experiments on healthy subjects (25%, Tax et al. 1995).

Phase-dependent reflex responses

The overall increase in reflex responses could have been due to an overall increase at all phases or it could be related
to the responses being selectively larger in given parts of the step cycle. To examine this question the phase-
dependency of the responses was examined in detail for the various muscles investigated.

*Ipsilateral biceps femoris*

The average EMG activation profiles for the whole population of PD patients are shown at the top of all plots in
Figures 3-6 so that an easy comparison can be made with the reflex responses (to judge the presence of
premotoneuronal activation present in periods where reflexes are large despite the background activity being low or
absent). In Figure 3 the results are given for the reflexes in iBF following a format as used in van Wezel et al. (1997).
In this and following figures the significant responses (subtracted responses different from zero) are indicated by bold data
points. The timing can be evaluated by the bars underneath the figures (top is stance ipsilaterally, bottom
contralaterally). The method is first illustrated based on the data of a single subject (Fig. 3 top).

Figure 3 near here.

The reflex responses were mostly facilitatory over the whole step cycle in iBF. In patient 5 (the same as used for Fig.
1) the responses were large from late stance to early swing (phases 7-11), smaller during mid swing (phases 12-15)
and again large from late swing into the stance phase (phases 16-4; Fig. 3 top, based on same data as Fig. 1). The
reflex responses were clearly not strictly related to the background activity in this muscle. For example, the reflex
responses from late stance to early swing (phases 7-11) were larger in amplitude than the responses from late swing
into the stance phase (phases 16-4), whereas the opposite was observed for the background activity (Fig. 3, top panel).
For the whole population of patients the phase dependency was comparable to the example shown at the top of Fig.3,
although less pronounced (Fig. 3, middle panel). At the bottom of Fig. 3 the data of the control subjects are shown.
The overall amplitude was somewhat smaller but the phase-dependency of the modulation was not basically different
between patients and control subjects. Hence the average increased response amplitude that was observed in this
muscle (Fig. 2) was not restricted to specific phases. Rather, the average increase of response amplitude was observed
across the whole step cycle (Fig. 3; middle panel versus bottom panel).
Ipsilateral tibialis anterior

In tibialis anterior of the healthy subjects most activity was observed during the swing phase (Fig. 4 for iTA).

The largest activity occurred during the end of the swing phase to prepare the foot for the stance phase. After the foot was set on the ground (phase 1), the activity in TA declined rapidly and during most of the stance phase TA activity was absent. In the patients comparable TA activity was observed during the swing phase. However, in stance there was a clear difference. In three patients (including patient 5 from the top of the figure), activity was also observed during most of the stance phase (as also seen in the average on top of the population plot, Fig. 4 middle).

The reflex modulation was quite similar in patients and control subjects. During the first half of the stance phase no significant responses were observed for iTA in the patients of control subjects. The responses were on average facilitatory during the second half of stance and most of the swing phase while they were suppressive during end swing. Such a combination of facilitations and suppressions was observed in 6 patients (a typical example is shown in Fig. 4, top panel). Three patients primarily showed suppressions in these phases. This intersubject variability in the iTA responses during the swing phase is normal for the sural nerve (see also Yang and Stein 1990; Duysens et al., 1990; Duysens et al. 1996; Van Wezel et al. 1997; Zehr et al., 1997; Baken et al. 2006). The main difference in reflex modulation was that significant responses were observed already at mid stance (phase 6). In two patients the responses were observed even in the first half of the stance phase (e.g., Fig. 4, top panel). Note, however, that these patients also tended to show more background TA activity in these periods as compared to control subjects. Hence the increased responses can be attributed to increased background activity. Overall the patients and the control subjects showed many similarities in phase dependency despite the variability within the populations. This was further quantified by correlating the mean subtracted responses for all phases for the 2 populations. This yielded a Spearman correlation coefficient of 0.88, well-above the correlations found for other muscles (0.36 for iBF; 0.29 for cBF and 0.02 for cTA).
Contralateral biceps femoris

In cBF, the difference between patients and control subjects was most obvious. In fact, the y-scale of the plots in Fig. 5 had to be adjusted to allow for the much larger responses in the patients.

Figure 5 near here.

In the control subjects (Fig. 5 bottom) most significant cBF responses occurred in the contralateral stance phase. In the patients the most significant responses occurred in the same period but the responses were considerably larger. Furthermore, in the patients some large responses were present during contralateral swing while such responses were absent in the control subjects. In the patient group, the average response amplitude in this part of the step cycle was comparable to the one seen during contralateral mid to late stance, but the variability was considerably larger. This was due to large responses being present in patients 4, 6, and 9 but not in the other patients. In patient 6 the amplitude of the responses reached levels of more than 2 times the maximum background activity and in patient 4 the amplitude even reached levels of more than 4 times this level. The latter patient is therefore illustrated separately (Fig 5, top panel; note that an adjustment of the Y-scale was needed). In these two patients these responses were larger for cBF than for iBF, whereas in the other patients and all healthy subjects the opposite was the case. The large responses were not correlated with elevated background activity (as is particularly clear for the example of patient 4, illustrated at the top of Fig. 5).

Contralateral tibialis anterior

In cTA no clear differences were observed for the responses of the patients as compared to those of healthy subjects. The response pattern consisted basically of small facilitations during the contralateral stance phase (phases 1 and 9-16), while on average the responses were smaller or absent during contralateral swing (Fig. 6).
A major result of the present study was that the responses to sural nerve stimulation are selectively increased in some muscles such as BF in PD patients. Two types of increases were observed. The first was an increase over the whole step cycle, as was found on average over the whole population. The second was a phase dependent increase in cBF at a specific time of the step cycle and this was only present in a subset of the patients.

Overall there was some increase in reflex amplitude but there was no basic difference in phase-dependent modulation in patients versus control subjects. In iTA the typical phase-dependent reversal was present in both groups. This indicates that deficits in the substantia nigra have little or no effect on this modulation. This is consistent with the idea that such modulation is regulated at other levels of the central nervous system. Phase-dependent modulation of cutaneous reflexes persist in patients with a damaged corticospinal tract (Duysens et al., 2004a) and it was argued that most of the phase-dependent modulation of cutaneous reflexes could be due to spinal mechanisms supplemented by cortical input (Duysens et al. 2004b). If so one would expect little change in patients with intact spinal cord and motor cortex but deficient substantia nigra (such as seen in PD).

While the amplitude of the responses was affected in PD there was no significant difference for the latency and the duration of the reflex responses. The absence of a change in latency agrees with earlier findings on sural nerve reflexes in Parkinson patients at rest (Delwaide et al. 1974) but the effect on amplitude during gait is a new finding for this type of reflexes. The average increase was not due to a difference in habituation since habituation was of the same magnitude as compared to healthy subjects. The increase was also not related to the average age difference between the Parkinson’s disease patients and control subjects, since the largest cBF responses were observed in the youngest patient (see results). Furthermore the average age was 53, which does not place these subjects in the group of “elderly”. If anything, one would expect responses to decrease with age since somatosensory medium-latency reflex responses were decreased in healthy elderly as compared to younger subjects (Tang and Woollacott 1998, 1999; Pijnappels et al. 2005b; Schillings et al. 2005). Another possible confound is the difference is walking speed. The patients walked at a comfortable self-selected speed that was on average lower than the speed of the control subjects.
Potentially, this could influence the results on the reflex responses. However, previously published studies indicate that there is little effect of speed on these reflex responses. The phase dependency of the medium-latency cutaneous reflexes at 2.5 km/h walking (Van Wezel et al. 2000), 4 km/h walking (Duysens et al. 1996; Van Wezel et al. 1997) and 8 km/h running (Tax et al. 1995) are completely comparable. Other studies confirm the low speed-dependency on cutaneous reflexes during human gait (Zehr et al. 1997; 1998a, b).

Phase-dependent increase in cBF in some PD patients

While generally the phase-dependent modulation of reflexes was maintained in PD, an additional effect appeared in 3 of them since the amplitude of the cBF reflex responses was selectively increased during the contralateral swing phase. In such cases the crossed responses were considerably larger than the amplitude of the responses in the iBF. In patient 4 (illustrated in Fig. 5) the amplitude of the subtracted responses were often larger than 4 times the maximum background activation encountered during gait. In control subjects the contralateral medium-latency cutaneous reflex responses are normally smaller than the corresponding ipsilateral responses during gait (Van Wezel et al. 1997) and during non-locomotor tasks (Burke et al. 1991). Furthermore, in our laboratory such high amplitudes of contralateral reflex responses were never observed in healthy subjects during normal gait (Duysens et al. 1990; Duysens et al. 1991; Van Wezel et al. 1997).

In principle these large responses could have been due a related increase in background activation. Indeed, for particular muscles such as cBF an increase in response amplitude in some phases of the step cycle (second half of the contralateral step cycle) could be partly related to increased background EMG activity in PD subjects. This additional background activity is consistent with earlier findings since increased EMG activity patterns during Parkinsonian gait has been reported for BF by several authors (Cioni et al. 1997; Dietz et al. 1997; Mitoma et al. 2000). However, the changes in reflex amplitudes in cBF were not limited to the phases with increased background. In the beginning of the contralateral swing phase there was no extra background EMG activity in the cBF, yet in 3 patients very large reflex activations occurred in that period. The fact that these responses occurred without concomitant increase in background EMG indicates involvement of premotoneuronal gain control of the pathways that mediate these reflexes.

Hence, to understand these extremely large cBF reflexes other factors have to be considered. These responses occurred in the contralateral leg which was the least affected one and therefore the limb that is most important in
making compensatory movements after a perturbation to the most affected leg. In case of an ipsilateral perturbations
(such as stumbling) it is well-known that contralateral BF is strongly activated with a latency which is comparable to
the one observed for the present responses (Pijnappels et al. 2005a, b). In such instances the cBF responses occur at
almost the same latency as ipsilateral BF activations (Schillings et al. 2000, 2005). During the crossed stance phase
the cBF responses are thought to be important in braking the forward momentum of the upper body (BF being a hip
extensor which can tilt the upper body backwards; see Pijnappels et al. 2005a, b). During contralateral swing, the cBF
may be important as a knee flexor since stumbling (or slipping) is likely to lower the body mass towards the ground
surface and the contralateral limb has to flex to make a compensatory step if required. Large crossed responses have
been observed in other situations as well. When the support surface is suddenly translated on one side the amplitude of
medium-latency responses in the unperturbed leg occurs in contralateral swing and these responses can exceed the
responses in the perturbed leg (Berger et al. 1984; Dietz et al. 1984). Similar results can be obtained with mechanical
perturbation of the swing phase (Dietz et al. 1986; Schillings et al. 2000). Perceived threat to stability has been shown
to affect crossed reflex responses (Haridas et al. 2005, 2008). Hence, in agreement with Haridas et al. (2005,2008) it is
proposed that the currently described cBF activations reflect the facilitation of reflex pathways which are commonly
recruited in cases of perceived instability (for example when ipsilateral perturbations threaten stability). In other
words, the results as presented, could be either due to the direct effect of the neuronal deficit (e.g. in substantia nigra)
or to the indirect effect of having an unstable gait (as a result of the neuronal loss).

An argument in favor of this explanation is that in PD patients there were clear signs of compensation for
instability, which were not present in the control subjects. In the present study a clear sign of perceived threat is the
iTA background activations in the first part of the stance phase. This TA activity is part of the ankle muscle
coactivation frequently seen when subject are walking while feeling unstable. For example, under conditions that
resemble limping, TA activity was observed during the stance phase of the more stable leg in both healthy subjects
(Dietz et al. 1994; Duysens et al. 2004b) and patients with Parkinson’s disease (Dietz et al. 1995). Under stable
conditions however, there is usually no extra TA activity during the stance phase (Dietz et al. 1981, 1995, 1997; Cioni
et al. 1997; Lewis et al. 2000; Mitoma et al. 2000; for exceptions see Dietz et al. 1995; Cioni et al. 1997). This extra
TA activity is not due to the slower speed since the TA does not show extra activity during the stance phase in slow
gait (Den Otter et al. 2004). Rather, this activity may reflect the increased instability that is characteristic for patients
with Parkinson’s disease. On the basis of the present experiments it is reasonable to assume that this TA activation in
stance is related to perceived instability. An age effect can be excluded since ankle coactivation is not normally
present during walking of this age group (Lamontagne et al., 2000). It follows that one likely explanation for the
currently observed increase in facilitatory responses in PD is the instability of the gait, presumably accompanied by
feelings of fear. To distinguish between this “fear” effect and a direct contribution of the disease some future
experiments are needed with age matched control subjects walking under “threatening” conditions (imitating
situations that produce some threat as experienced by PD patients).

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**Figure Legends**

**Figure 1.**
Example data both of a typical unperturbed step cycle (A) and of reflex responses (B) of the ipsilateral biceps femoris (iBF) of a healthy subject and a patient with Parkinson’s disease. (A) iBF background locomotor activity in relation to the stance (signal up) and swing phase of the ipsilateral leg. (B) For the iBF a typical set of 16 average (n = 10 trials) subtraction traces is shown for the same subjects. The solid vertical lines indicate the time of stimulation of the sural nerve at 2 times perception threshold. The dotted lines refer to a delay of 100 msec with respect to the onset of stimulation. The number on the left side of each subtraction trace indicates in which of the 16 step cycle intervals the response (with a latency of about 80 msec) occurs. Phase 1 coincides with a response (onset of response window) coinciding with ipsilateral footfall. In all panels: time calibration, 100 msec; EMG calibration, 1 mV. Data of the healthy subject adapted from Van Wezel et al. (1997).

**Figure 2.**
Overall amplitude of the reflex responses. Step-cycle averages of the absolute value of the subtracted responses (± SE) are plotted for all muscles, averaged for the group of healthy subjects and the group of patients with Parkinson’s disease. The data are normalized with respect to the maximum background locomotor activity of each muscle in each subject (see Methods). The symbols (*) indicate significant differences according to the Independent-Samples Mann-Whitney U Tests.

**Figure 3.**
Window-averaged and normalized background activity and subtracted responses of the iBF. Within the three panels subtracted data (± SE) are plotted as a function of their appearance in the 16 phases of the step cycle. On top of the panels the background activity is shown. Top panel: window-averaged data of the same patient
as in Figure 1. Middle panel: average of the population of patients. Bottom panel: average of the population of healthy subjects (adapted from Van Wezel et al. 1997). The statistical significance of the reflexes (Wilcoxon signed rank test, \( p < 0.05 \)) is indicated by the black dots. Phases 1 and 9 correspond to the onset of ipsilateral and contralateral stance, respectively. The ipsilateral (i) and contralateral (c) stance phases are shown at the bottom (as upper and lower sets of lines respectively). Note the different vertical scales for the different panels.

**Figure 4.** Window-averaged and normalized background activity and subtracted responses of the iTA. The same format is used as in Figure 3.

**Figure 5.** Window-averaged and normalized background activity and subtracted responses of the cBF. The same format is used as in Figure 3.

**Figure 6.** Window-averaged and normalized background activity and subtracted responses of the cTA. The same format is used as in Figure 3.
References


Cioni M, Richards CL, Malouin F, Bedard PJ, Lemieux R. Characteristics of the electromyographic patterns of lower limb muscles during gait in patients with Parkinson's disease when OFF and ON L-Dopa treatment. *Ital J*


Haridas C, Zehr EP, Misiaszek JE. Postural uncertainty leads to dynamic control of cutaneous reflexes from the


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IPSILATERAL BICEPS FEMORIS

Subtraction (norm.)

Mean patients

Mean healthy subjects

Phase in step cycle