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Changes in spinal reflex and locomotor activity after a complete spinal cord injury: a common mechanism?

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Locomotor activity and spinal reflexes (SRs) show common features in different mammals, including humans. Here we report the time-course of the development of locomotor activity and SRs after a complete spinal cord injury in humans. SRs evoked by tibial nerve stimulation were studied, as was the leg muscle electromyography activity evoked by mechanically assisted locomotion (Lokomat) in biceps femoris, rectus femoris, tibialis anterior and gastrocenmius medialis. Around 8 weeks after the injury, an early SR component (latency 60–120 ms) appeared, as in healthy subjects, and a well-organized leg muscle activity was present during assisted locomotion. At around 6 months after injury an additional, late reflex component (latency 120–450 ms) appeared, which remained even 15 years after the spinal cord injury. In contrast, the early component had markedly decreased at 18 months after injury. These changes in SR were associated with a loss of electromyography activity and a successively stronger electromyography exhaustion (i.e. decline of electromyography amplitude), when comparing the level of electromyography activity at 2 and 10 min, respectively, during assisted locomotion. These changes in electromyography activity affected mainly the biceps femoris, gastrocenmius medialis and tibialis anterior but less so the rectus femoris. When the amplitude relationship of the early to late SR component was calculated, there was a temporal relationship between the decrease of the early component and an increase of the late component and the degree of exhaustion of locomotor activity. In chronic, severely affected but sensori-motor incomplete spinal cord injury subjects a late SR component, associated with an electromyography exhaustion, was present in subjects who did not regularly perform stepping movements. Our data are consistent with the proposal of a common mechanism underlying the changes in SR activity and locomotor activity after spinal cord injury. These findings should be taken into consideration in the development of novel rehabilitation schemes and programs to facilitate regeneration-inducing therapies in spinal cord injury subjects.

Introduction

In subjects with a complete spinal cord injury (SCI), the characteristic pattern of locomotor electromyography (EMG) activity can be induced when the movements are assisted and an appropriate afferent input is provided (Harkema *et al.*, 1997; Dietz, 2002). In such a condition the timing of the leg muscle activity is preserved, while the EMG amplitudes are much reduced as compared with walking in healthy subjects. Recently in chronic (>1 year after injury) complete SCI subjects an exhaustion of locomotor activity, i.e. a decline of EMG amplitude, was reported for assisted stepping [either by the use of a driven gait orthosis (Lokomat) or by therapists], when comparing the level of EMG activity at 10 min versus the onset of a training session (Dietz and Muller, 2004). A possible degradation of spinal neuronal function appeared to occur in chronic complete SCI subjects (Dietz and Muller, 2004).

This phenomenon is of functional importance because chronic spinal cord injury subjects can only profit from regeneration-inducing therapies if spinal neuronal function is preserved below the level of lesion (cf. Curt and Dietz, 2005). For the development of appropriate counter-measures to avoid EMG exhaustion, more information about the pathophysiological basis of changes in neuronal function which occur during the course of a complete spinal cord injury is required.

The progressive changes occurring in spinal reflexes (SRs) after a spinal cord injury might provide additional information about the mechanisms underlying the exhaustion. A close relationship between SRs and spinal locomotor activity in cat and rat has been documented elsewhere (Jankowska *et al.*, 1967; Grillner and Shik, 1973; for review see Pierrot-Deseilligny and Burke, 2005; Lavrov *et al.*, 2006). For example, electrical stimulation of high threshold afferents in peripheral nerves can produce an alternating flexor and extensor activation (Grillner, 1969; Grillner and Zangger, 1979).

The term 'spinal reflex' was chosen here to be defined as a below-nociceptive threshold to tibial nerve stimulation, since a noxious stimulus cannot be determined in complete spinal cord injury subjects. The SR evoked in spinal cord injury subjects is assumed to correspond to the polysynaptic SR associated with the recovery of locomotor function (Lavrov *et al.*, 2006) and the emergence of spastic movements that has been observed in rats with a transected spinal cord (Bennett *et al.*, 2004). It is suggested to be mediated by some of the same neurons that make up the locomotor pattern generator (Bussel *et al.*, 1989; for reviews see Dietz, 2002; Pierrot-Deseilligny and Burke, 2005).

Several studies concerning the behaviour of SR in complete spinal cord injury have been reported (e.g. Schmit *et al.*, 2000; Hornby *et al.*, 2003; Muller and Dietz, 2006). However, no systematic analysis exists on the course of early and late SR components during the course of a complete spinal cord injury.

The aim of this study was to analyse the relationship between the different components of the SR and locomotor activity during the course of a complete human spinal cord injury. We hypothesize that the exhaustion of locomotor activity is associated with changes in the behaviour of SR components.

Methods

General procedures and subjects

The study protocol was approved by the local ethics committee and conformed to the Declaration of Helsinki. All participants gave written informed consent before data collection. In addition, to expand the numbers for our analysis, some earlier recordings of SR activity (four subjects) (Hiersemenzel *et al.*, 2000) and locomotor activity (four subjects) (Dietz and Muller, 2004) in complete spinal cord injury subjects were included. Altogether, 34 subjects with motor complete spinal cord injury (SCI) (ASIA A/B) (Maynard *et al.*, 1997) and 5 subjects with sensori-motor incomplete SCI (ASIA C) were included in this study. Mean age was 37.4 years (SD = 11.9 years) and neurological level of lesion was between C4 and T11 in subjects with motor complete SCI. In subjects with sensori-motor incomplete SCI, mean age was 46.7 years (SD = 18.3 years) and level of lesion was between C5 and L3. The timespan between the SCI and the recordings ranged from 2 months to 15 years in the ASIA A/B subjects, and from 2 to 7 years in the ASIA C subjects. SRs and leg muscle electromyography (EMG) during assisted locomotion were recorded in 28 SCI subjects and 26 SCI subjects, respectively. Clinical data for all of the SCI subjects and the source of the data are given in Table 1. All SCI subjects showed slight to moderate signs of spasticity. About half of the SCI subjects were on anti-spastic medication (usually 20–60 mg Baclofen). The time interval between recordings of both locomotor activity and SRs within one subject was at least 1 month.

Leg movements of SCI subjects were assisted during locomotion by a driven gait orthosis (DGO) Lokomat (Hocoma AG, Volketswil, Switzerland). The DGO controls the patient's leg trajectories in the sagittal plane during walking. The hip and knee joints of the DGO were actuated by linear back-drivable actuators integrated into an exoskeleton structure. The legs of the subjects walking in the DGO moved along a pre-defined trajectory. Subjects wore a harness and were fixed to the DGO by straps around their trunk and pelvis. The legs of the device were attached to the subject's legs with cuffs around the thighs and calves. Proximal and distal leg structures of the DGO were adjusted to align hip and knee joints of the subjects with the joint axes of the DGO. To prevent plantar flexion of the feet, straps were attached around the forefoot and mounted to the DGO. During walking within the DGO, speed was kept constant at 2.0 km/h (0.56 m/s). Cadence had to be slightly adjusted based on the leg length of the subjects. A detailed description of the Lokomat is published elsewhere (Colombo *et al.*, 2000, 2001). Subjects were connected to a body weight support (BWS) system and walked with 65%–75% BWS. Body weight support was adjusted in such a way that subjects were loaded with the maximal tolerable body weight without non-physiological knee flexion during the stance phase or toe dragging during swing phase. Body weight support was not changed during a walking session. Subjects walked for 10–15 min within the DGO.

Leg muscle activity during assisted locomotion

Leg muscle activity during assisted walking within the DGO was analysed. Leg muscle EMGs from biceps femoris (BF), rectus femoris (RF), gastrocnemius medialis (GM) and tibialis anterior (TA) from both legs were recorded using surface electrodes (Noraxon, Cologne, Germany). EMG recordings were amplified, filtered (bandpass 30–300 Hz) and sampled at 1000 Hz via a 12-bit A/D-converter and

Table 1 Characteristics of the SCI subjects included in the study

Subject	Age	Gender	Level of lesion	ASIA	Duration of lesion (months)	SR recording	Locomotor activity recording	Source of data
S1	29.6	M	T4	A	23	+	+	1
S2	38.6	M	T5	A	130	+		1[a]
S3	56.7	M	C7	A	178	+		1
S4	26.6	F	T5	A	74	+	+	1
S5	57.7	M	T4	B	41	+	+	1[a]
S6	50.7	M	T11	A	52	+		1
S7	29.8	M	C7	A	109	+	+	1
S8	43.8	M	T7	A	120	+	+	1[a]
S9	25.8	M	C4	A	62	+	+	1
S10	38.9	M	T9	A	4	+		1
S11	41.9	M	T3	A	6	+	+	1
S12	29.9	M	T10	A	3	+	+	1
S13	45.7	M	T4	A	18	+		1
S14	19.2	M	C5	A	5	+		1
S15	29.7	F	T9	A	131	+	+	1
S16	39.7	M	T5	A	148	+	+	1[a]
S17	33.6	M	T8	A	85	+		1
S18	40.9	M	T7	A	86	+		1
S19	34.9	M	T5	A	165	+		1
S20	59.4	M	T11	A	2	+		3
S21	28.2	M	C7	B	6	+		3[a]
S22	49.1	M	T9	A	6	+		3
S23	28.1	M	T5	A	3	+		3[a]
S24	28.6	M	T10	A	5		+	1
S25	46.5	M	C6	B	32		+	1[a]
S26	30.0	M	T8	A	5		+	2
S27	32.5	M	T6	A	8		+	2
S28	24.6	F	T1	B	34		+	2
S29	25.0	M	T6	A	12		+	1[a]
S30	19.7	M	T5	A	5		+	2
S31	43.3	M	T10	A	11		+	1
S32	67.2	M	T5	A	6		+	1
S33	45.1	M	C7	B	5		+	1[a]
S34	29.5	M	T3	A	3		+	1
S35	34.1	M	L3	C	84	+	+	1
S36	42.6	M	T12	C	67	+	+	1
S37	68.9	M	C5	C	91	+	+	1
S38	33.0	M	C7	C	32	+	+	1
S39	25.7	M	T9	C	29	+	+	1

Source: (1) new data, re-evaluated data from (2) Dietz and Muller (2004) and (3) Hiersemenzel *et al.* (2000).
a more than one recording
Level of lesion: C = cervical; T = thoracic; L = lumbar; ASIA classification, A = sensorimotor complete, B = motor complete, sensory incomplete, C = sensorimotor incomplete; m = male; f = female; SR = spinal reflex.

stored on a standard PC. An additional trigger signal identifying the heel strike was recorded. For data analysis and recording the commercial software Soleasy (ALEA Solution GmbH, Zurich, Switzerland) was used.

The EMG amplitude of leg muscles during locomotion was analysed using the root mean square (RMS) value per stride. This value represents the mean or effective amplitude per stride (Dietz and Muller, 2004). The resulting data were smoothed using a moving average (window width: 25 strides equivalent to ∼1 min of walking) to compensate for stride-to-stride variability. Data were screened for outliers (mean ± 3 SD). Some of the subjects showed some clonus jerks in the GM at the beginning, but these usually disappeared after ∼2 min of assisted walking. Therefore, smoothed RMS values of all leg muscles of the first gait cycle after 10 min of walking were normalized to the RMS values of the first gait cycle after 2 min of walking. This should

ensure that only the drop of muscle activity occurring during 10 min of assisted locomotion was assessed. These values were also screened for artefacts in signal strength. Quantified EMG values of both legs of one subject were taken together. Some of the SCI subjects were measured at different timepoints after injury (including those from an earlier study, Dietz and Muller, 2004), resulting in a total of 36 EMG measurements from 21 motor complete and 5 incomplete SCI subjects during assisted locomotion. All data were recorded under similar conditions.

Spinal reflexes

To assess changes in SRs during the course of a SCI, 23 motor complete and 5 incomplete SCI subjects were examined. Subjects were classified as ASIA A, B or C (Maynard *et al.*, 1997) and all

showed signs of spasticity with exaggerated reflexes. As a control group, 10 healthy subjects (mean age: 38.2 years; SD: 12.8 years) were recorded under similar conditions. The SR was elicited by electrical stimulation of the distal tibial nerve at the dorsal aspect of the medial malleolus with the electrical stimulator AS 100 constant-current source (ALEA Solutions GmbH, Zurich, Switzerland). The electrical stimulus consisted of a train of eight biphasic rectangular pulses with single stimulus duration of 2 ms and a frequency of 200 Hz (Muller and Dietz, 2006). The total stimulation duration amounted to 40 ms. The stimulation intensity used was set at two times motor threshold (first visible contraction of the abductor hallucis muscle) (Hiersemenzel et al., 2000). In the healthy subjects, this stimulation strength was experienced as non-noxious. During the measurements subjects were in an upright position, fixed within a harness and completely unloaded from their body weight. Stimulation was elicited 10 times and randomly released every 30–45 s to minimize habituation (Shahani and Young, 1971; Fuhrer, 1976). The SR recordings were performed in all subjects before, and in two subjects also after, the locomotion session.

In some of the SCI subjects listed in Table 1, SR recordings from an acute state (within 3–6 months after injury) were included in an earlier study (see Hiersemenzel et al., 2000) using a similar stimulation approach (stimulation frequency of 100 Hz). The measurements were performed in a recumbent position. The SR showed no difference between an upright and supine (Hiersemenzel et al., 2000) body position.

SR activity was analysed only in the TA muscle of the stimulated leg, as in earlier recordings (Muller and Dietz, 2006) reflex responses could only rarely be separated in the BF. SR responses were analysed for presence of early and late components separately. Time windows were set from 60 to 120 ms for the early component and from 120 to 450 ms for the late component after stimulation onset. The presence of reflex responses within these time windows was determined by an increase of EMG activity three times above standard deviation from the mean baseline. If a reflex response was detected, the highest peak amplitude within the corresponding time window was determined, and the RMS value of 25 ms before and 25 ms after the peak amplitude was calculated. If no response was detectable the value was set to zero. Because of the large inter-individual variability of the reflex amplitudes, we additionally calculated the relationship between the two components over the time after injury. For this, the two reflex components were compared with each other and the greater component was set to 1 and was used for normalization of the other component. This procedure was chosen because the amplitude relationship between the early and late SR components within a subject was the focus of interest. The normalized values of each subject were averaged and subsequently the difference between the early and late SR component was calculated to assess the relationship between the two responses.

Statistics

The course of the leg muscle activity during assisted locomotion early and late (chronic) after SCI was calculated. Correlation between exhaustion and duration of lesion was tested using the Spearman rank correlation test. Significance level was set at $P < 0.05$. The correlation between the presence of early and late SR components and the duration of SCI was tested statistically using the Spearman rank correlation test. Significance level was set at $P < 0.05$. The correlation analysis over the course after injury was only performed in the group of motor complete SCI subjects.

The course over time of EMG exhaustion and SR components was quantified using a linear regression coefficient. In order to compare the differentially scaled measures, data were z-transformed and the 95% CIs were compared around the regression coefficients.

Results

In the groups of healthy and SCI subjects the SR was evoked by a similar stimulus intensity at the right tibial nerve (see Methods). In the group of healthy subjects, the tibial nerve stimulation resulted in a single SR response in the TA muscle (mean latency = 78 ms; SD = 12.7 ms). In one subject, inconsistent small late responses were observed (around 170 ms). The stimulus was reported by the subjects to be non-noxious.

In complete SCI subjects, SR could not be evoked during spinal shock and appeared only around 2 months after SCI. Similarly, locomotor activity during assisted locomotion could only be recorded after the period of spinal shock. Except for a higher level of co-contraction of the antagonistic leg muscles and an amplitude reduction of EMG signals in SCI subjects, the locomotor pattern was similar in SCI and healthy subjects. An effect of the anti-spastic medication could not be detected in either the behaviour of SR or in the locomotor activity.

Figure 1 shows representative recordings of right leg muscle EMG activity at 2 and 10 min of assisted locomotion in three subjects, in the early (Fig. 1A), intermediate (Fig. 1B) and later (Fig. 1C) stages after a motor complete SCI. After the spinal shock, leg muscle activity re-appeared and increased progressively up to 3–4 months after SCI (Fig. 1A) while little EMG exhaustion (i.e. decline of EMG amplitude) occurred during assisted locomotion over 10–15 min in BF, GM and RF (except for the TA, see below). Around 6 months after SCI the leg muscle EMG already showed some degree of exhaustion during assisted locomotion over 10 min (Fig. 1B). During the later period after the SCI (>12 months after SCI), a pronounced degree of EMG exhaustion and loss of EMG activity was present during assisted locomotion (Fig. 1C).

Figure 2A shows the mean values obtained from all subjects with regard to the changes in locomotor activity occurring after a complete SCI. There was a significant degree of exhaustion in BF [Fig. 2A(i)] and GM [Fig. 2A(ii)], while there was no significant depression of EMG amplitude in the RF [Fig. 2A(iii)].

Figure 1 also shows (uppermost records) representative recordings of SR (TA muscle) at early (Fig. 1A), intermediate (Fig. 1B) and later (Fig. 1C) stages after a motor complete SCI. At early stages after SCI (Fig. 1A: 3 months) only a short latency SR component was present (latency 85 ms). Several months after the SCI (Fig. 1B: 6 months) a second late SR component appeared (latency 220 ms), and in the chronic SCI state (Fig. 1C: 41 months) only a late SR component (latency 220 ms) was present. There was no difference in the SR behaviour when it was recorded before and after the locomotor session (two complete SCI subjects).

Figure 2B shows the mean values obtained from all subjects with regard to the development of early and late SR components. The window for the analysis of the early component was set to
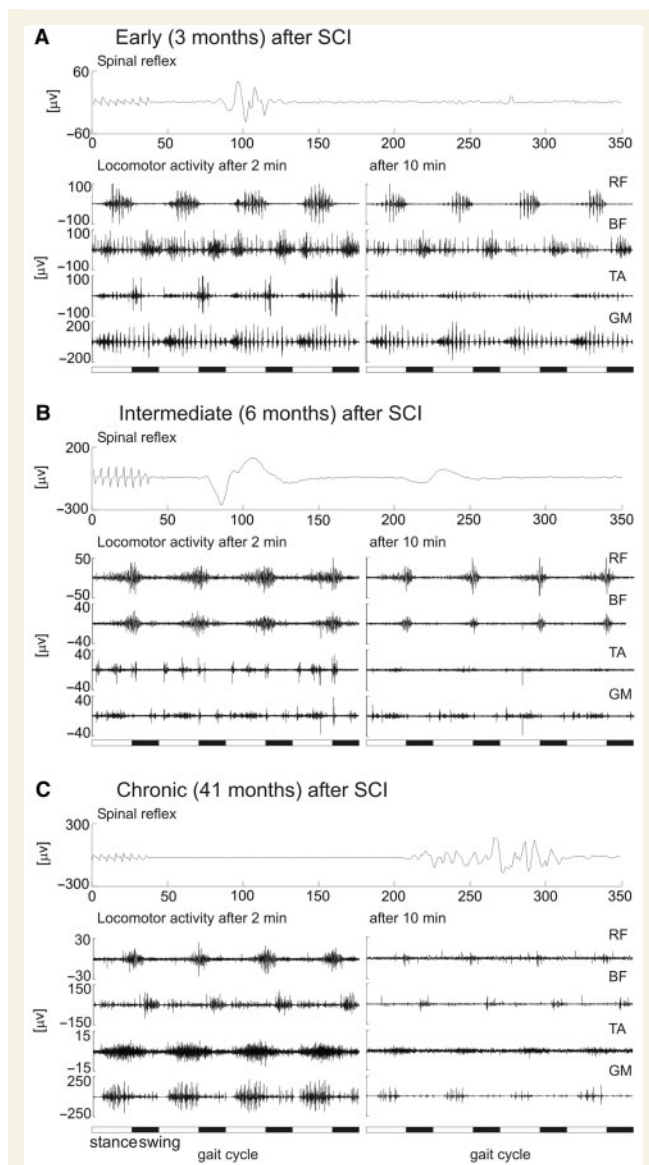
Figure 1 SR and locomotor activity. Representative examples of the two components of SR and locomotor activity at (**A**) early (S12), (**B**) intermediate (S11) and (**C**) late (S5) stages after a complete SCI. The SR was evoked by tibial nerve stimulation (see Methods section) and recorded over the ipsilateral TA muscle. The leg muscle activity is shown at the beginning (left side) and after 10 min (right side) of assisted locomotion.

60–120 ms and that of the late to 120–450 ms (see Methods section). Figure 2B(ii) shows that there was a significant decrease of the early SR component ($P < 0.01$) to a very low level after 100 months. The late SR component was more variable and instead increased significantly ($P < 0.01$) from a low level during the first few months (starting around 2 months after SCI) to a higher value after 100 months [Fig. 2B(iii)]. Figure 2B(i) shows the normalized difference between the early and the late response. Except for the first few months the late SR response dominated as presented here. The characteristics of the SR did not differ between ASIA A ($n = 21$) and ASIA B ($n = 2$) subjects.

The leg flexor muscle TA showed a high degree of exhaustion at 3 months (Fig. 1A), which became more pronounced at later stages (Fig. 1B and C). Around 1–1.5 years after SCI little or no TA EMG activity ($<5\,\mu V$) could be recorded during assisted locomotion in most subjects (Fig. 3A and B). However, a late SR component could still be evoked in the TA at this stage. The GM, antagonist of TA at the ankle joint, showed a significant exhaustion [Fig. 2A(ii)].

In the group of five severely affected, but sensori-motor incomplete, chronic (>1 year) SCI subjects, three regularly walked at home with external support ('therapeutic stepping'), while two subjects remained wheelchair bound. In the walking subjects, the presence of an early SR component was associated with no exhaustion of leg muscle EMG activity during assisted locomotion (Fig. 4A). In contrast, in the two wheelchair-bound subjects, the presence of only a late SR component was associated with a loss and a pronounced exhaustion of EMG activity after 10 min of assisted stepping (Fig. 4B).

There was a similar course of the BF exhaustion and SR components over time: the 95% CI of the regression coefficient calculated for BF exhaustion (-0.357) amounted to (-0.520 to -0.193), and paralleled the course of the SR components [regression coefficient: -0.416; 95% CI: (-0.631 to -0.201)].

Discussion

The aim of this study was to analyse the changes in SR during the course of a complete SCI in relation to changes in locomotor activity. The main observations were:

(i) After recovery from spinal shock, an early SR component and a weak locomotor pattern can be evoked. The pattern becomes strengthened up to 3–4 months after SCI. After around 5–6 months, both an early and a late SR component can usually be distinguished and the locomotor activity shows some exhaustion towards the end of a 10 min training period;

(ii) After around 1 year, a high level of EMG activity is present initially, but the amplitude of the locomotor activity decreases markedly towards the end of a training session. The exhaustion of the locomotor activity occurs in parallel with an enhanced late and a diminished early SR component;

(iii) From 2 years and up to 15 years after injury the loss of the early component is associated with a prominent exhaustion during a training period and a loss of locomotor EMG activity. The decrease is more pronounced in the BF and TA than in the GM and is almost non-existent in the RF; and

(iv) In severely affected but sensori-motor incomplete, chronic SCI subjects, the early SR component remains preserved and no EMG exhaustion occurs only if they regularly perform stepping movements.

These observations will be discussed below with regard to their pathophysiological relevance and possible therapeutic consequences.
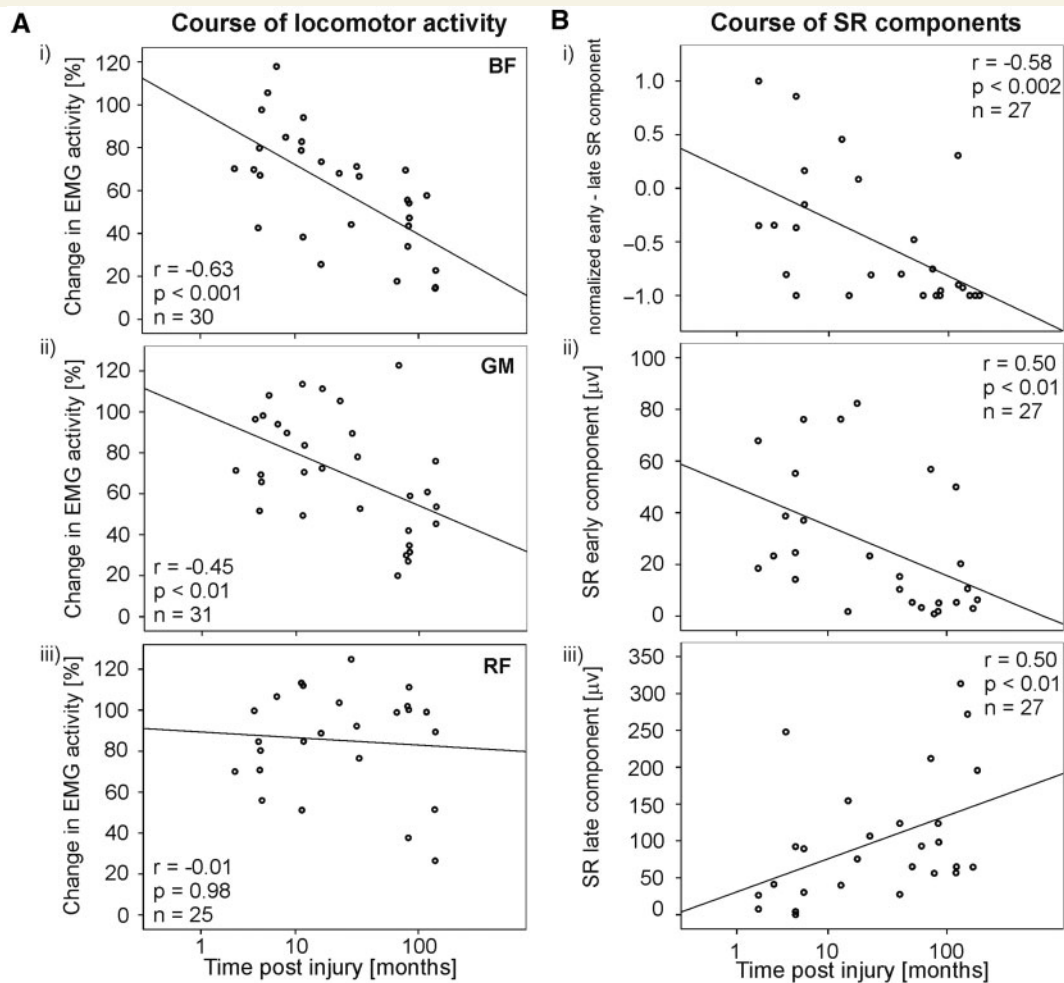
Figure 2 Course of locomotor activity and SR. (**A**) The change in BF (i), GM (ii) and RF (iii) EMG activity during 10 min of assisted locomotion (in percentage of the initial value) and (**B**) the course of the two SR components (in the TA) is shown for all motor complete SCI subjects during the time course after injury (for each graph 25–31 measurements). The changes in the SR components are presented as normalized [(i) early minus late component] and absolute [(ii) early and (iii) late component] values. The time after SCI is displayed on a logarithmic scale.

Behaviour of the SRs

Most of the earlier studies on SRs have been performed at different stages of SCI with recordings of either the early or late, or both components of the SR. A great variability has been noted (Dimitrijevic and Nathan, 1970; Andersen *et al.*, 2004). Little attention has been paid to the time course following the SCI with regard to the early and late components of the SR (Schmit *et al.*, 2000; Hornby *et al.*, 2003; Muller and Dietz, 2006).

Several SR studies on SCI subjects have been focused on the relationship with muscle spasms. It has been suggested that a 'wind-up' of these reflexes (Hornby *et al.*, 2003) would occur, with a hypersensitivity to input from force-sensitive muscle afferents (Schmit *et al.*, 2000; Conway and Knikou, 2008), or alternatively, that an expansion of the receptive fields would contribute to the spasms (Andersen *et al.*, 2004). This hyperexcitability has been assumed to be due to a lack of descending inhibitory control and/or increased sensitivity of the SR.

Corresponding to human SCI (Hiersemenzel *et al.*, 2000), the polysynaptic SR is lost after a spinal cord transection in the rat, but becomes restored several weeks later (Valero-Cabre *et al.*, 2004; Lavrov *et al.*, 2006). Also the direct leg muscle EMG and the H-reflex responses show a similar course after an acute complete SCI in humans (Hiersemenzel *et al.*, 2000) and rats (Lavrov *et al.*, 2006). Here we found that 6–12 months after complete SCI, the early SR component becomes successively smaller, while the late component increases. Between 2 and 15 years after a SCI, the late component was preferentially present. At this stage, a SR could also be recorded in the contralateral GM (Muller and Dietz, 2006). SR did not change within a walking session.

In the spinal cat an early and a late reflex was evoked, mediated through different pathways (Jankowska *et al.*, 1967). A reciprocal relationship of the two reflexes was shown to occur. The early short-latency flexion reflex (central latency 2–3 ms) was elicited in the acute spinal state. If L-DOPA was administered (acting through release of noradrenaline in the spinal cord), the early
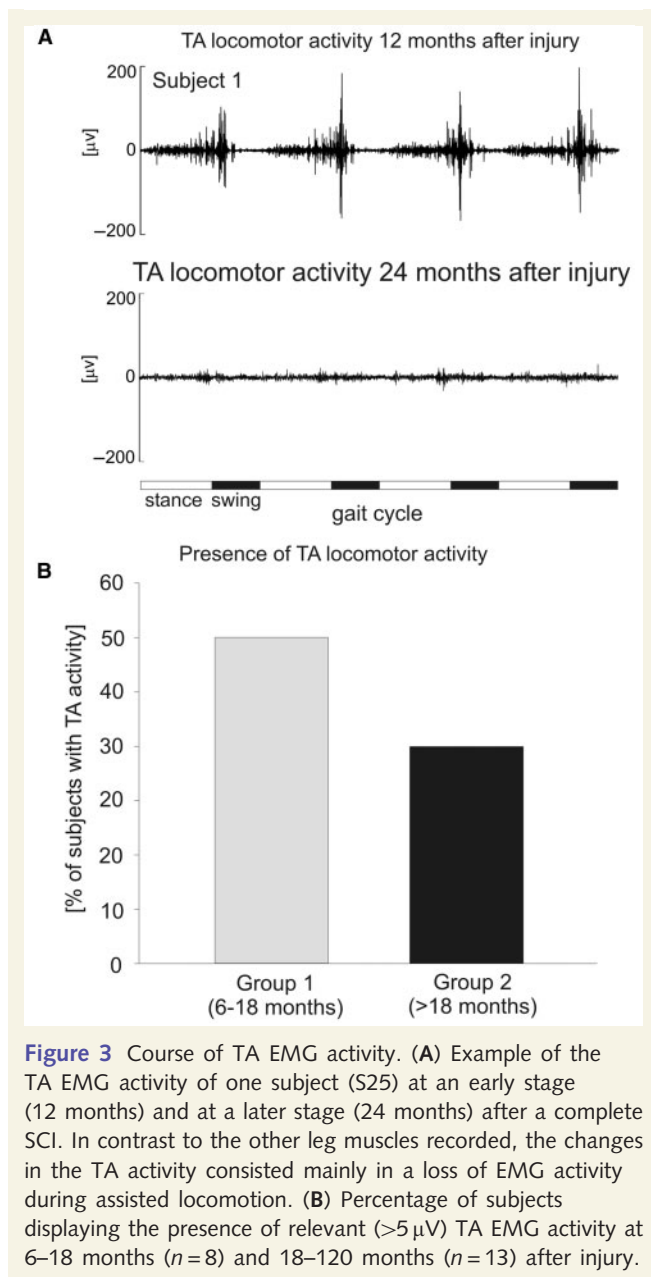
Figure 3 Course of TA EMG activity. (**A**) Example of the TA EMG activity of one subject (S25) at an early stage (12 months) and at a later stage (24 months) after a complete SCI. In contrast to the other leg muscles recorded, the changes in the TA activity consisted mainly in a loss of EMG activity during assisted locomotion. (**B**) Percentage of subjects displaying the presence of relevant (>5 μV) TA EMG activity at 6–18 months (*n* = 8) and 18–120 months (*n* = 13) after injury.

reflex was depressed, while a long-lasting, long-latency reflex discharge was released instead (Jankowska *et al*., 1967). This long-latency discharge was considered to represent an activation of the spinal locomotor circuitry (Forssberg and Grillner, 1973; Grillner and Zangger, 1979). Although the mode of stimulation was different, it is assumed that this long-latency discharge corresponds to the SR associated with the recovery of locomotor function in the rat with a transected spinal cord (Lavrov *et al*., 2006) and with the appearance of the early component of the SR described here. For the late component, a chronic SCI animal is not yet available.

Here we have observed first, that the early component of the SR dominates in healthy subjects and during the first months after a complete SCI and second, that during the later course of a SCI the early component becomes successively depressed while

the late SR component increases. This was the case under the condition that a similar stimulus intensity was applied in all subjects. By an increase of stimulus intensity an early SR component might also be evoked in some chronic SCI subjects (Shahani and Young, 1971) which is the change that might just represent a threshold effect.

Behaviour of locomotor activity

In humans, around 2–3 months after a complete SCI, a locomotor pattern can be recorded with a normal timing but with a strongly reduced EMG amplitude (Dietz, 2002; Dietz and Muller, 2004). On the basis of this low level amplitude, no further relevant drop in EMG amplitude occurred during assisted locomotion.

Around 6–12 months after the SCI a progressive drop and a loss of EMG amplitude was observed during the walking episodes. This phenomenon mainly occurred in leg flexor muscles. The EMG exhaustion has been assumed to occur on a pre-motoneuronal level (Dietz and Muller, 2004; Muller and Dietz, 2006). The loss of TA EMG activity has been suggested to be due to a reduced common synaptic drive to motoneurons (Hansen *et al*., 2005). In addition, axonal changes were assumed to be responsible for the loss of TA contraction to nerve stimulation below the level of a SCI (Lin *et al*., 2007).

It is important to emphasize that an appropriate locomotor pattern could be elicited during the first minutes of a walking episode, even in chronic SCI subjects. However, the motor pattern deteriorated during the 10-min walking period in chronic motor complete SCI subjects to an extent which was neither seen in these subjects early after injury nor in ambulatory ASIA C subjects during assisted locomotion. Thus the appropriate motor program is available initially and can be generated without exhaustion only in the early period after a complete SCI. The reason for the exhaustion remains unclear. It can possibly be ascribed to a synaptic fatigue within the spinal locomotor network and be due to disuse as a result of the motor complete SCI. In such a condition, leg extensors, in contrast to the leg flexors, become continuously more activated by proprioceptive input evoked by external stimuli, i.e. are less deprived from afferent input.

Relationship between SR and locomotor activity

A close relationship between polysynaptic SRs and the generation of a locomotor pattern has been emphasized for rats (Schouenborg, 2002) and cats (Jankowska and Riddell, 1995). The modulation of SR was shown to be related to specific phases of the step cycle in normal rats and cats and when the spinal cord has been transected (Gerasimenko *et al*., 2006; Frigon and Rossignol, 2008; for review see Rossignol *et al*., 2008). Group II and III muscle afferents and cutaneous afferents were shown to be responsible for the SR responses (Jankowska *et al*., 1967; Gerasimenko *et al*., 2006; Lavrov *et al*., 2006). Consequently, the neurons responsible for the SR in SCI subjects were suggested to be part of the spinal networks generating locomotion, i.e. to share the same spinal circuitry (Bussel *et al*., 1989; Nicol *et al*., 1995; Parise *et al*., 1997).
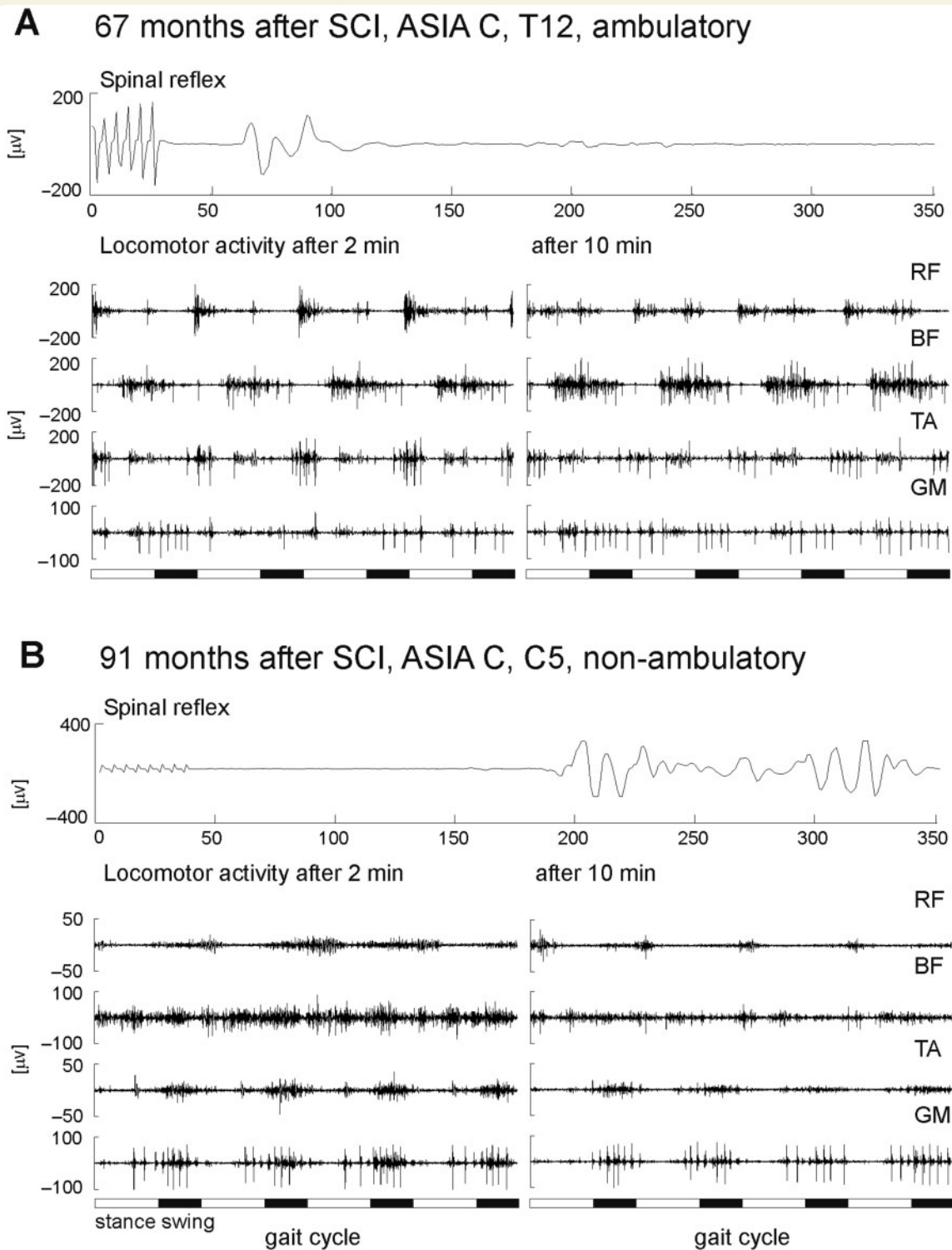
Figure 4 SR and locomotor activity in chronic sensori-motor incomplete SCI subjects. Representative examples of SR and locomotor activity in (**A**) a subject (S36) who regularly walked at home with external support ('therapeutic stepping') and (**B**) a subject (S37) who remained wheelchair bound (note the different calibration in **A** and **B**).

SRs were suggested to provide a measure for the restoration of spinal neuronal circuits responsible for locomotion after transection of the spinal cord in rats (Lavrov *et al.*, 2006). Correspondingly, in humans early after a motor complete SCI as well as in chronic ambulatory incomplete SCI subjects, the presence of an early SR component was associated with a preserved locomotor activity, as is the case in healthy subjects. Vice versa, the presence of an EMG exhaustion was associated with the dominance of a late SR

component. Despite the pronounced exhaustion and loss of TA EMG in chronic complete SCI subjects, a SR could always be evoked in the TA (cf. also Muller and Dietz, 2006). The observations made in chronic incomplete (ASIA C) SCI subjects suggest that the presence of an early SR component, associated with no EMG exhaustion, depended more on the regular performance of stepping movements than on the 'incompleteness' of the SCI.

Although there is ample evidence for a temporal relationship of the changes in SR behaviour and locomotor activity over time this is not conclusive proof that they necessarily depend on the same underlying mechanisms.

It will be important to further analyse if changes in the locomotor training methods used for SCI subjects will reduce the exhaustion of the locomotor pattern and if this is associated with a persistence of the early SR component. This would represent a prerequisite for a successful regeneration-inducing therapy. It is important to note that the appropriate pattern is available initially during a walking period and that the locomotor network can thus operate, although it has become more fragile.

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