

Degradation of neuronal function following a spinal cord injury: mechanisms and countermeasures

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Summary

The aim of this study was to evaluate the course of spinal neuronal activity following spinal cord injury (SCI). In patients with a complete SCI, the leg muscle EMG activity early and up to 33 years after an SCI was analysed during locomotor movements induced and assisted by a driven gait orthosis (DGO). Only in chronic SCI patients did a premature exhaustion of neuronal activity occur. This was reflected in a reduced density and fading of leg muscle EMG activity. The early exhaustion of EMG activity was more pronounced in the leg flexor (e.g. biceps femoris) than extensor (e.g. gastrocnemius) muscles. The timing of the leg muscle pattern remained unchanged in the chronic patients. A preserved

amplitude of motor action potentials following repetitive peripheral nerve stimulation and during spasms indicated an interneuronal site of impairment. In patients who participated in a locomotor training programme lasting up to 13 weeks, no positive effect on the slope of exhaustion was seen. It is concluded that a degradation of spinal neuronal activity takes place following an SCI. If in the future regeneration of spinal tract fibres becomes feasible in patients with complete SCI, such an approach can only become functionally successful if neuronal activity below the level of the lesion is maintained. This might be achieved by a continuous training approach starting early after injury.

Keywords: spinal cord injury (SCI); human locomotion; neuronal plasticity; locomotor training; leg muscle activity

Abbreviations: ASIA = American Spinal Injury Association; BF = m. biceps femoris; DGO = driven gait orthosis; GM = m. gastrocnemius medialis; RF = m. rectus femoris; r.m.s. = root mean square; SCI = spinal cord injury; TA = m. tibialis anterior.

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Introduction

During the last years, much progress has been made with respect to the behaviour of the activity within neuronal circuits of the isolated human spinal cord. Our knowledge about this spinal neuronal activity in patients with a spinal cord injury (SCI) became enhanced by a recent approach, which enables the study of the behaviour of locomotor activity in complete paraplegic patients by use of a robotic device to induce controlled stepping movements (Dietz *et al.*, 2002). Evidence arose that for the generation and training of a locomotor pattern, load- and hip joint-related afferent input is essential, while muscle stretch reflex activity plays a minor role. In line with these results, there are observations which suggest that the isolated lumbosacral spinal cord interprets loading during stepping (Harkema *et al.*, 1997). This

knowledge is of basic importance for the optimal exploitation of neuronal plasticity after an SCI for the improvement of function. Indeed, incomplete paraplegic patients profit from locomotor training on a treadmill with partial body unloading for their locomotor ability (Wernig *et al.*, 1992, 1995; Dietz *et al.*, 1995; Kojima *et al.*, 1998; for a review see Barbeau and Rossignol, 1994). Similar effects on leg muscle activation were described for manually and robotic-assisted locomotor movements (Colombo *et al.*, 2001).

In complete or severely affected patients, the level of functional recovery of walking could be improved with a combination of such training approaches and some regeneration of injured spinal tract fibres. Indeed, this seems likely to become feasible in the near future (for reviews see Schwab and

Table 1 Characteristics of subjects with SCI included in the study

Subject	Age (years)	Sex	Level of lesion	ASIA	Duration of lesion (years)	Time of earlier EMG recording (years)	Anti-spastic medication (mg of baclofen/day)
P1	32	M	T 6	A	0.2	–	100
P2	30	M	T 10	A	0.4	–	60
P3	44	M	C 4	B	0.5	–	100
P4	21	M	C 4	A	1.0	–	150
P5	24	F	L 1	B	1.0	–	None
P6	22	F	T 5	A	2.5	–	45
P7	39	M	T 6	A	5.5	–	None
P8	32	M	T 6	A	6.0	0.2	100
P9	34	M	T 5	A	6.5	0.5	100
P10	41	M	C 4	B	7.5	–	10
P11	33	M	C 5	A	9.5	0.3	45
P12	28	F	T 5	A	9.5	0.3	100
P13	52	M	C 7	B	10.5	0.6	45
P14	38	M	T 5	A	11.0	–	None
P15	33	M	T 5	A	12.5	–	None
P16	54	M	T 4	A	33.0	–	50

Neurological level of lesion, C = cervical, T = thoracic; ASIA classification, A = sensorimotor complete, B = motor complete, sensory incomplete; M = male; F = female.

Bartholdi, 1996; Raineteau and Schwab, 2001; Raisman, 2003). However, in these patients, any treatment leading to regeneration only makes sense if the function of neuronal circuits below the level of the lesion is basically preserved. The analysis of spinal reflexes in the isolated human spinal cord and their course over 6 months after SCI indicated, however, that the flexor reflex amplitude becomes smaller over time (Hiersemenzel *et al.*, 2000). This might imply a loss of neuronal activity following an SCI, most probably due to immobility, which is associated with a deprivation from supraspinal more than peripheral inputs. Such a process would be of importance with respect to the progress made to induce regeneration in the rat (for a review see Raineteau and Schwab, 2001), which might become transferable to humans in the near future. However, chronic patients with an SCI could only profit from such a new treatment approach if the functionality of neuronal circuits within the spinal cord is preserved.

Therefore, the aim of this study was to find out: (i) the behaviour of the neuronal activity seen in chronic complete SCI patients during induced locomotor movements and the possible mechanisms underlying any impairment; (ii) the time course of the changes in spinal neuronal activity and; (iii) if the neuronal locomotor activity does indeed become degraded over time, whether this can be stopped, or whether the earlier activity pattern can be restored by appropriate training approaches. That is to say, has some locomotor training an effect on spinal neuronal activity in chronic complete SCI patients?

Methods

Subjects and procedures

With permission from the ethics committee of the canton of Zurich and the informed consent of the volunteers, the leg muscle EMG activity during assisted locomotion was analysed in three healthy subjects as well as in 16 subjects with a complete spastic paraplegia

or tetraplegia. According to the classification of the American Spinal Injury Association (ASIA, see Maynard *et al.*, 1997), these latter subjects were all motor complete and either sensory complete (ASIA A) or sensory incomplete (ASIA B), with a neurological level of lesion (i.e. last intact segment) between C4 and L1 (Table 1). The duration of the lesion lasted from 2 months up to 33 years. For definition, lesion durations up to 1 year were referred to as ‘acute’, whereas ‘chronic’ referred to a duration of >1 year.

In some of the chronic patients listed in Table 1, similar EMG recordings also existed from the early acute state (during manually assisted leg movements), i.e. even within 6 months after SCI (see Dietz *et al.*, 1994, 1995). Therefore, we were able to compare the early recordings with the present ones, in order to assess the course of neuronal activity below the level of the lesion over several years. In all SCI subjects, no regular locomotor training was performed during and after rehabilitation.

For assisted walking, the driven gait orthosis (DGO) ‘Lokomat’ (Hocoma AG, Zurich) mounted on a treadmill was used. Briefly, the DGO provides drives for the hip and knee joint movements of each leg, whereas the dorsiflexion of the ankles during the swing phase is achieved by passive foot lifters (elastic straps) (Fig. 1A). The desired angle values for hip and knee are taken from a database of healthy subjects walking within the DGO (Fig. 1B) and are identical for all subjects. Unloading is achieved by a parachute harness connected to counterweights (Fig. 1A). The DGO is fixed to the subjects with straps around the waist, the hip, the thigh and the shank. The DGO can be adjusted in size at the different segments and therefore can be adapted to the different subjects. A detailed description of the device can be found elsewhere (Colombo *et al.*, 2000).

During treadmill walking, speed was kept constant at 2.0 km/h (0.56 m/s). Stepping cadence had to be slightly adjusted according to the leg length of the different subjects. Subjects walked for 10–15 min within the DGO. After an interruption and relaxation of 10 min (sitting down or standing with body suspension, according to the subject’s preference), the subjects walked again for 10 min. The stepping movements of the patients were performed with partial body unloading of 65–80% body weight. Such an unloading is necessary to allow stepping movements to be induced in complete

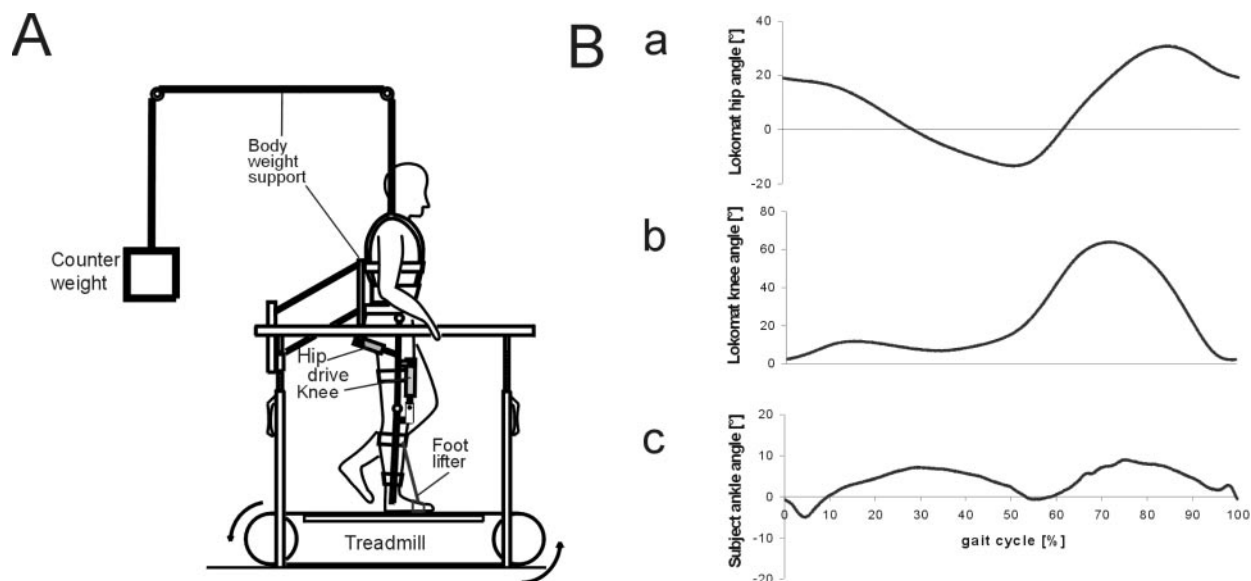


Fig. 1 Experimental set-up. (A) Locomotion on the treadmill within a DGO. (B) Hip (a) and knee (b) joint movement trajectories were imposed by the DGO. For the ankle joint movements (c), support for foot dorsiflexion during the swing phase was provided by passive foot lifters (elastic straps).

paraplegic patients (see Dietz *et al.*, 1995) and to prevent stumbling. The duration of one experiment amounted to ~40 min; subjects did not experience overall fatigue or discomfort within this time.

Data acquisition and analysis

During assisted walking within the DGO, the EMG activity of right and left leg muscles [rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA) and medial gastrocnemius (GM)] was recorded. Surface electrodes were fixed over the muscle belly with an inter-electrode distance of 25 mm.

For data recording and signal analysis, Soleasy Software (ALEA Solutions GmbH, Zurich) was used. EMG recordings were amplified, filtered (bandpass 30–300 Hz) and transferred to a PC via a 12 bit AC/DC converter. In addition, the following signals were recorded: actual hip and knee joint angle trajectories of both orthotic legs as well as force transducer signals measuring the interaction between subject and DGO at hip and knee joints, and two trigger signals identifying the beginning of the right and left stance phases. All data were sampled at 1 kHz.

The EMG activity of each leg muscle was quantified for every gait cycle by calculating the root mean square (r.m.s.) of the EMG signal, representing the mean or effective amplitude (the r.m.s. is the square-root of the mean of the squared voltage values) (De Luca, 1997; Dietz *et al.*, 2002). The resulting data were smoothed using a moving window average (window width: 25 strides, which is equivalent to ~1 min of walking) to account for stride-to-stride variability. The values of each muscle of successive gait cycles were then normalized to the smoothed value of the first gait cycle of walking with constant walking speed.

Statistics

Differences in r.m.s. amplitude between start and end of measurement after 5 and 10 min as well as between BF and GM muscle activity were tested statistically using the Wilcoxon signed-rank test. Correlations between the decline of EMG activity and the duration

or level of lesion, respectively, were statistically tested using the Spearman rank correlation test. Significance level was set at $P < 0.05$.

Training of chronic patients

In three SCI subjects (P9, P12 and P14), a locomotor training within the DGO was performed over 3 months (2–3 times 1 h per week). EMG recordings from the leg muscles were taken before training started, every 4 weeks during the training periods, as well as after the training period.

Protocol of peripheral nerve stimulation

In three SCI subjects, the peroneal nerve and in two SCI subjects the tibial nerve was electrically stimulated with supramaximal intensity at the head of the fibula and the popliteal fossa, respectively (stimulation frequencies 3, 5 and 10 Hz, single stimulus duration 0.1 ms). The motor action potential was recorded over the TA or the GM muscle, respectively. The amplitudes of the motor action potential to 10 successive stimuli were assessed and analysed for any change during the course of stimulation.

Results

The following results show the data of the first bout of 10 min continuous assisted walking within the DGO.

Effect of SCI duration on the locomotor pattern

Figure 2 shows the recordings of the raw EMG from four leg muscles of an acute patient (P3) 2 months after SCI (Fig. 2B), of a chronic patient (P7) 5.5 years after SCI (Fig. 2C) and, for comparison, of a healthy subject (Fig. 2A). The timing of the leg muscle activity pattern with a main activation of the extensors during the stance phase and of the flexor muscles during the swing phase of

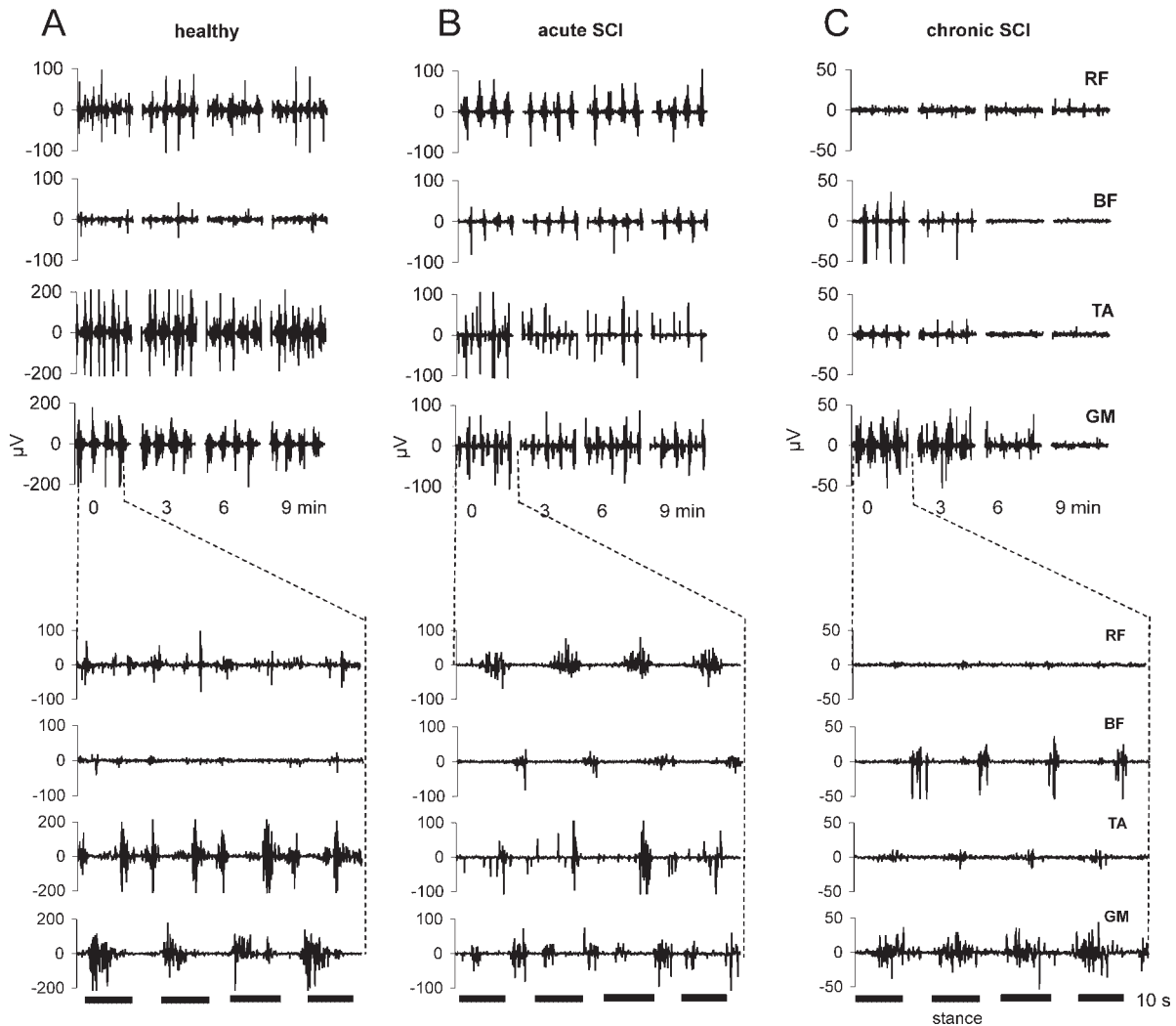


Fig. 2 Raw EMG of four leg muscles of (A) a healthy, (B) an acute (P3) and (C) a chronic (P7) complete SCI subject. The subjects walked continuously over 10 min in a DGO (2 km/h; 65–80% body unloading with SCI subjects). EMG sequences of four steps every 3 min (top); the first four steps with higher time resolution (bottom). Units are given in μV .

gait was preserved with some variability in both the acute and chronic patient and it was similar to that of the healthy subject. In the healthy subject, the locomotor activity was influenced by the unusual slow walking speed. The recordings indicate that in the healthy subject and in the acute patient, the EMG amplitude of the four leg muscles did not change visibly over the recording time of 10 min. However, in the chronic patient, there was a decline in amplitude and a rarefaction of EMG potentials during the course of the recordings. This fading of EMG activity occurred earlier and was more pronounced for the leg flexor (e.g. BF) than for the extensor (e.g. GM) muscle.

After a break of 10 min, the subjects walked again within the DGO. In the acute patients, the pattern and amplitude of leg muscle activation was unchanged with respect to the first recording. However, in the chronic patients, the initial EMG amplitude within this second bout was usually lower than in the first bout and declined more rapidly compared with the first recording (Fig. 3).

In a few patients within the 10 min locomotion task, muscle spasms occurred in leg muscles (due to stumbling or leaning upon the bars). In such instances, the low amplitude locomotor activity was interrupted by large amplitude EMG bursts (Fig. 4). Despite the fading of locomotor activity, the motoneurons themselves obviously could still provide a much stronger activity.

BF and GM muscles were taken for further analysis as they showed most consistently EMG activity during the initial steps in nearly all patients, which was not the case for RF and TA. Figure 5 shows a typical example of the successive exhaustion of BF and GM muscle activity during 10 min locomotion within the DGO in a chronic patient (P12). Detailed inspection of the EMG traces shows that the exhaustion is predominantly due to a drop-out of large amplitude EMG potentials. An electrical muscle silence takes place earlier in the BF than in the GM. Nevertheless, fluctuations of EMG activity occurred during the recording time.

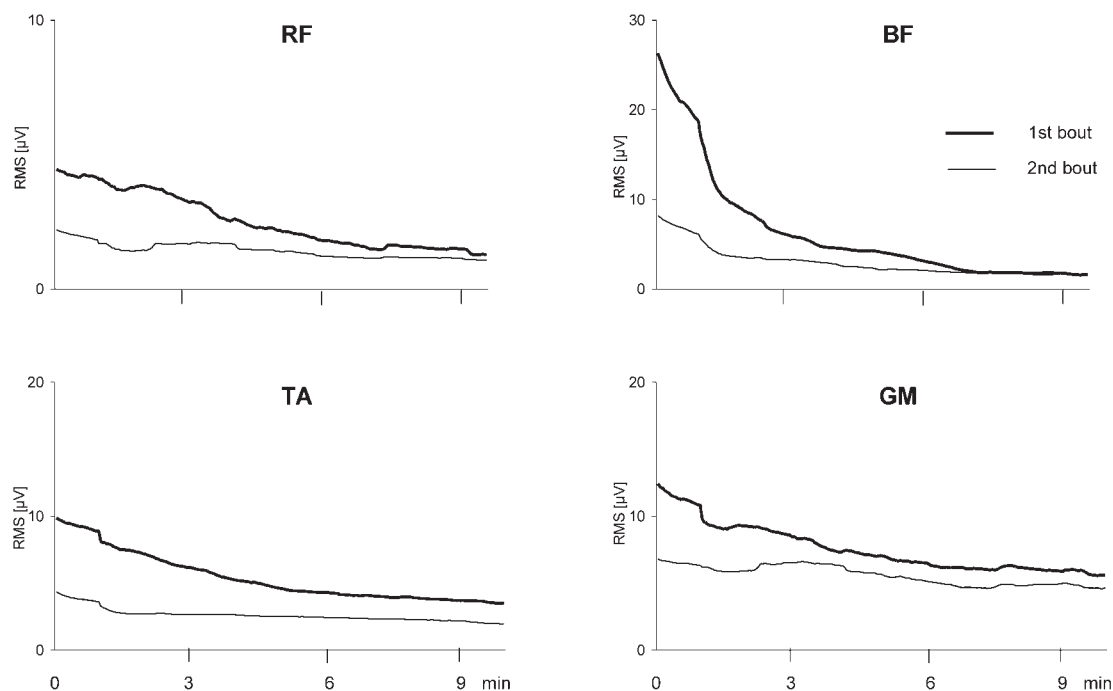


Fig. 3 Typical example (P14) of the decline in leg muscle activity (r.m.s. values) during the first and the second bout of assisted walking within the DGO. The two bouts were interrupted by a break of 10 min. Units are given in μV .

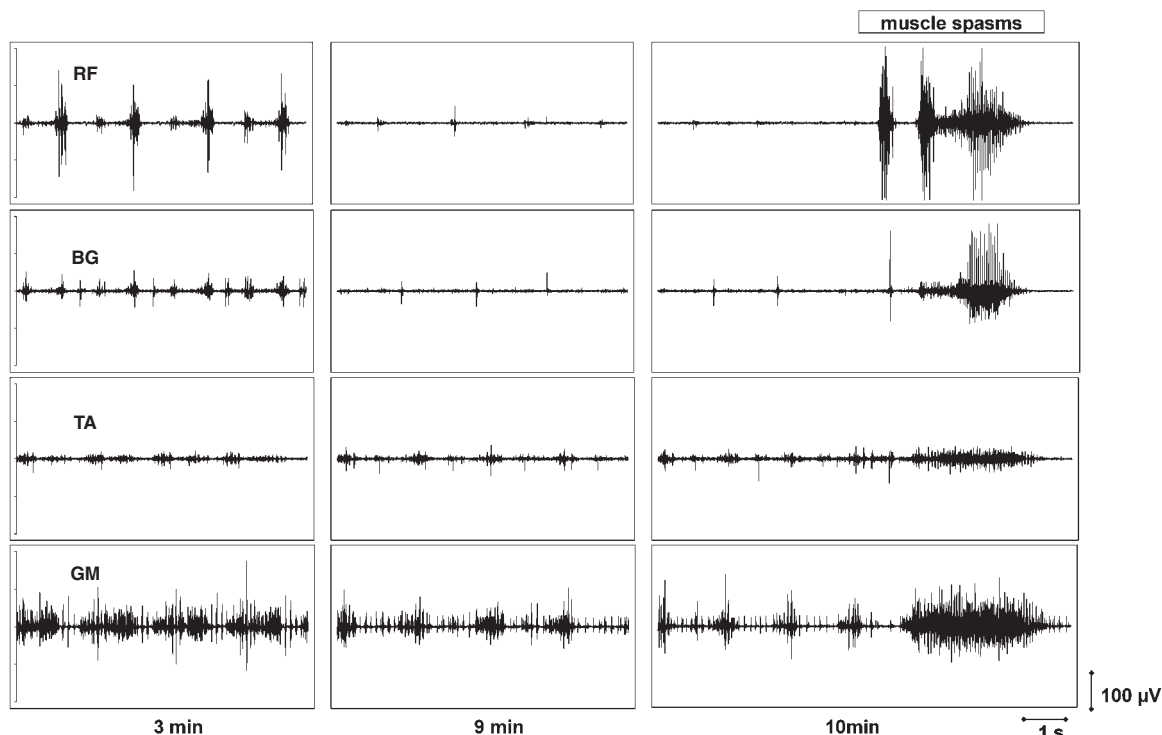


Fig. 4 Raw EMG of the leg muscles of a chronic SCI patient (P16) showing a decrease in EMG activity during the 10 min locomotor task. At the end of recording, muscle spasms occurred in all four leg muscles due to stumbling.

Figure 6 shows the relative decrease of the BF (Fig. 6A) and GM (Fig. 6B) EMG activity as a percentage of their initial r.m.s. value after 5 and 10 min of walking within the DGO for all acute and chronic para-/tetraplegic subjects.

In the chronic patients, the r.m.s. values of both muscles declined significantly ($P < 0.01$) within 5 and 10 min, respectively. The drop in EMG activity over time was significantly ($P < 0.05$) more pronounced for the BF than for the GM

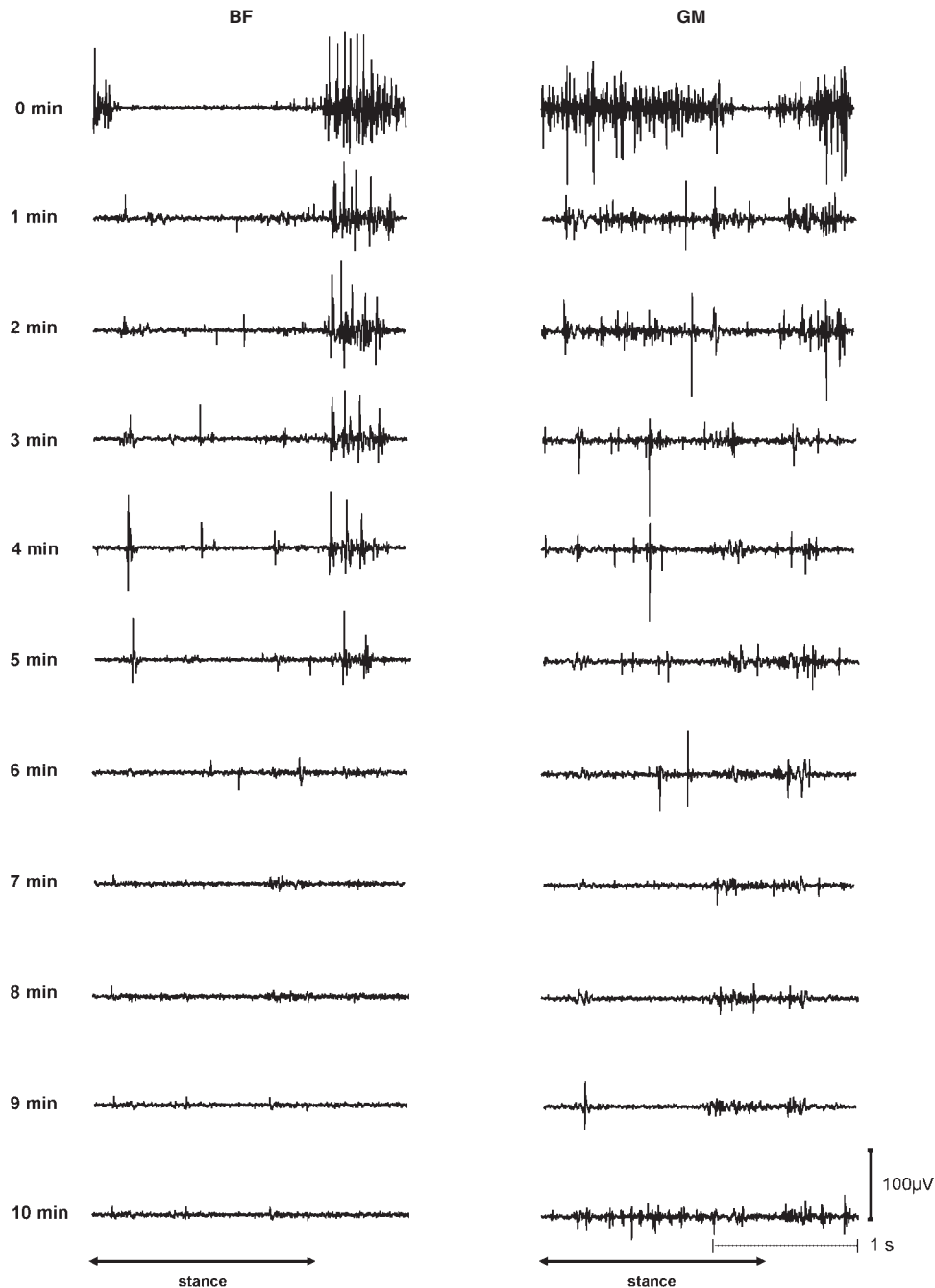


Fig. 5 Successive change in BF and GM muscle activity during continuous walking within the DGO (P12, body unloading 80%). One gait cycle is plotted every minute over 10 min of locomotion.

muscle. There was no correlation between level or duration of lesion and the drop in EMG activity after 5 and 10 min, respectively, in any one of the recorded muscles in the chronic subjects. We are aware that an arbitrary *post hoc* separation was made between acute and chronic SCI patients. This grouping was based on the occurrence of an EMG exhaustion which usually was seen in patients >1 year after SCI. This does of course not exclude some variability, for example reflected in patients P4 and P5, both being 1 year after injury, but only P5 shows exhaustion. In the four acute

patients, no decline in EMG amplitude occurred over the time of recording.

Individual course and training effect

Figure 7 shows the raw EMG recordings of five patients early and late after SCI. In the earlier recordings, leg EMG activity was usually recorded over ~20 gait cycles only. Therefore, for comparison, only the corresponding parts of four steps are displayed. In all subjects, the BF showed a reduced density of

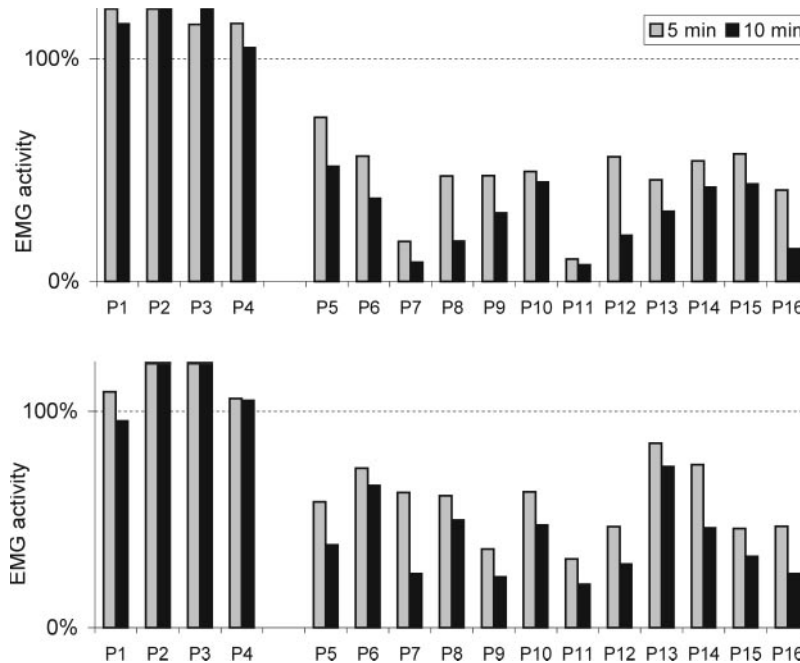


Fig. 6 Relative decrease of RMS value (in % of the initial value) per gait cycle of (A) BF and (B) GM muscle activity from all 16 acute and chronic complete SCI subjects during the first 5 and 10 min, respectively, of continuous walking within the DGO (body unloading 65–80%). The values of the four acute SCI subjects (<1 year) are plotted on the left side of the figure; the values of the chronic SCI subjects are plotted in ascending chronicity on the right side.

EMG potentials and a predominance of mono-morphological large amplitude potentials in the chronic compared with the early stage of SCI of each subject. In contrast, by visual inspection, there was no systematic change in GM activation.

In three chronic subjects with SCI, locomotor training (2–3 times 1 h per week, i.e. 26–33 training sessions with 30–40 min of effective DGO walking) was performed over 3 months. Figure 8 shows the EMG recordings made in one subject (P12) at the beginning of the training (Fig. 8A) and after 3 months of training (Fig. 8B). There was no visible effect of the training on the pattern of leg muscle EMG activity. In particular, the drop in EMG activity was still present and not significantly changed in all three patients (Table 2).

Effect of repetitive nerve stimulation

The rapid exhaustion of spinal neuronal activity in chronic patients could occur at either a central or a peripheral site. The peripheral site can be tested by repetitive electrical nerve stimulation, as has been performed in five chronic SCI subjects (see Methods). In none of the subjects did an eventual decline of the muscle action potential exceed 10% of the initial value at any of the stimulation frequencies.

Discussion

Today, there exist several promising approaches to induce regeneration of spinal tract fibres in the rat (Schwab and Bartholdi, 1996; Raineteau and Schwab, 2001; for a review see Raisman, 2003). Hence, a partial repair of human SCI

seems also to be feasible in the near future. However, such a novel treatment approach can only be successful if the target neurons that become reconnected by the regenerating tract fibres are functionally intact. The aim of this study was to assess the spontaneous course of spinal neuronal activity after a complete SCI and the influence of training in chronic patients. The main observations were the following. (i) Around 1 year after an SCI, an early exhaustion of neuronal activity underlying locomotor movements occurs whose strength is independent of the duration of the SCI; exhaustion in this context is used to describe the phenomenon of a decline in EMG activity during the course of locomotor movements. From the present experiments, little can be said about the underlying pathophysiology, e.g. premature fatigue or habituation of spinal neuronal circuits. (ii) The exhaustion of neuronal activity most probably takes place at a pre-motoneuronal site and is assumed to be predominantly due to an impaired function and/or loss of interneurons underlying locomotor activity. (iii) Training has no effect on neuronal activity in the chronic patients, while a positive effect was described early after SCI (Dietz *et al.*, 1995). These findings are discussed in relation to their clinical relevance and their consequences for appropriate countermeasures.

Mechanisms involved in the course of the neuronal activity after SCI

The first systematic analysis of leg muscle activation during assisted locomotor movements in complete paraplegic patients showed a well organized EMG pattern which changed little

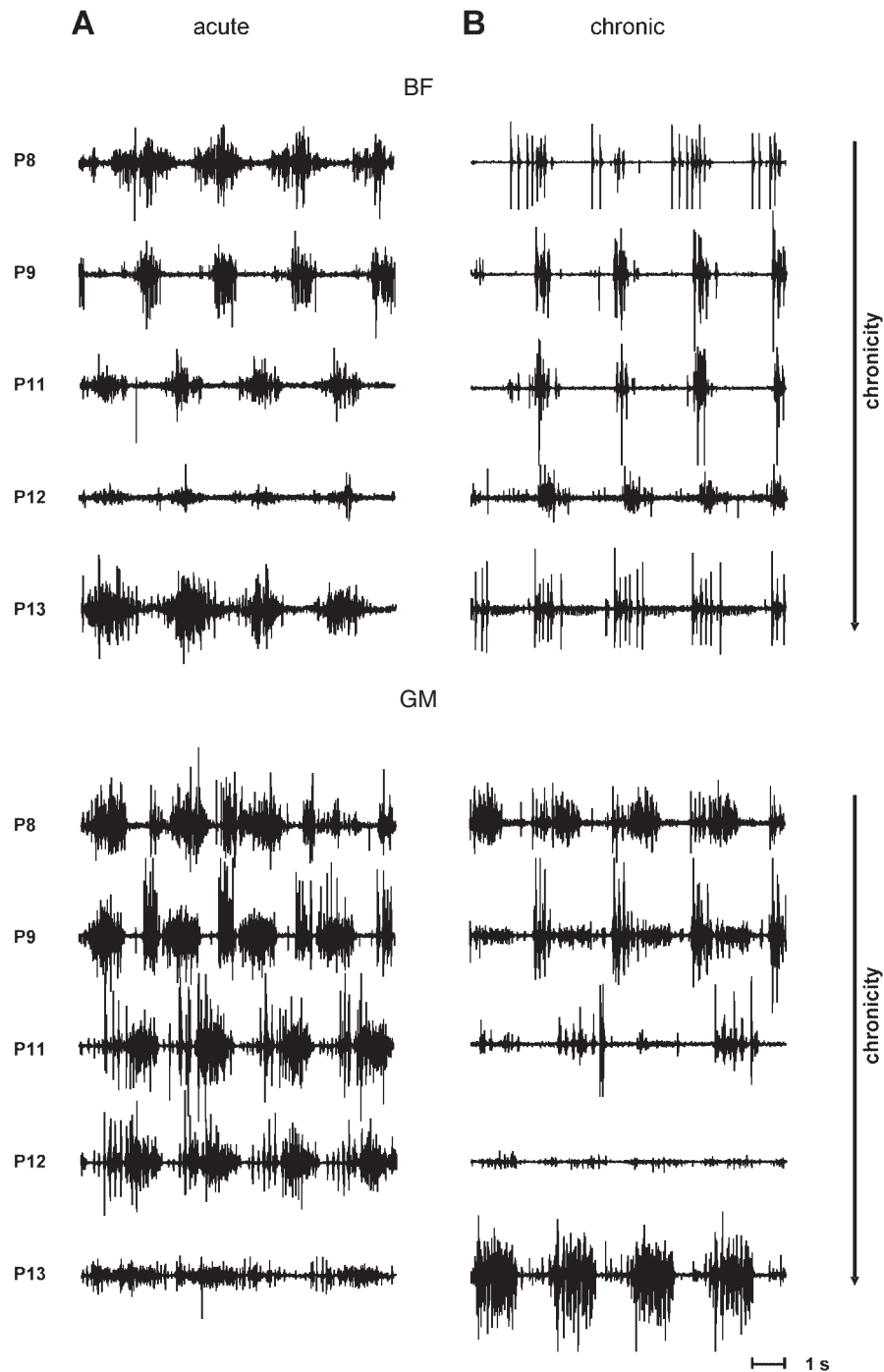


Fig. 7 EMG recordings of left leg muscles (BF and GM) of five complete SCI subjects early and late after SCI. **(A)** Manually assisted stepping (40–70% body unloading, 1.5 km/h) early after injury (i.e. 2–7 months); **(B)** stepping within a DGO (65–80% body unloading, 2.0 km/h) late after injury (i.e. 6–11 years). EMG sequences of four consecutive steps are plotted. Absolute EMG amplitudes cannot be compared between the acute and chronic stages due to technical reasons; therefore, no amplitude calibration is given.

during the time of recordings (Dietz *et al.*, 1994, 1995). These recordings were done quite early, i.e. 2–6 months after SCI. Now, several years later, we were able to compare these early recordings with recent recordings from these same chronic patients. Of course we have to remain cautious with the interpretation of the data as early and late recordings—only

available from five patients—were obtained by different measurement techniques. Nevertheless, the present recordings indicate that the neuronal activity underlying stepping movements has changed over time, with reduced density of EMG potentials especially in the BF. Furthermore, the recordings done in chronic patients show, in contrast to the acute patients,

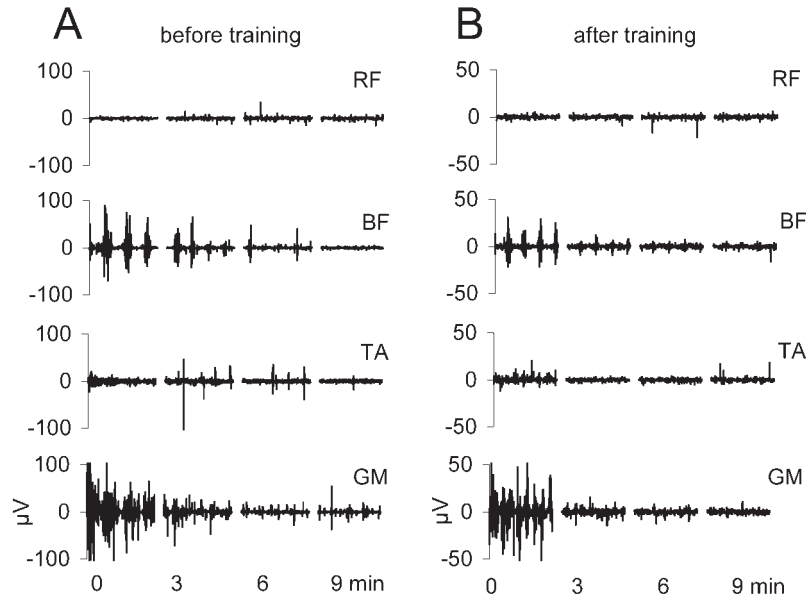


Fig. 8 Leg muscle EMG activity of a chronic complete SCI subject (P12) (A) before and (B) after a locomotor training period of 3 months. Sequences of four steps every 3 min of continuous walking within a DGO (80% body unloading) are displayed. Units are given in μV ; four steps correspond to ~ 10 s (see Fig. 2).

Table 2 Effect of training on exhaustion of EMG

Subject	EMG activity after 10 min walking			
	BF		GM	
	Before training	After training	Before training	After training
P9	39%	40%	15%	36%
P12	23%	32%	39%	31%
P14	13%	11%	35%	30%

Drop of EMG activity after 10 min of assisted walking in three chronic SCI patients before and after locomotor training over 13 weeks. Data are given as a percentage of initial r.m.s. value of EMG activity.

a premature exhaustion of EMG activity in all leg muscles which rapidly leads to an electrical silence of leg muscle EMG during locomotion. Repetitive peripheral nerve stimulation performed in five chronic patients did not lead to a change in the amplitude of muscle action potentials. Therefore, it is assumed that the decline in EMG activity takes place at a pre-motoneuronal (spinal) site. The fact that this exhaustion concerns the flexor (BF) more than the extensor (GM) muscles may be attributed to a differential neuronal control of the antagonistic leg muscles (for a review see Dietz, 2002). The activation of leg extensors is achieved predominantly by proprioceptive afferent input, which in some part is still present after SCI, while in complete SCI patients central control of the flexors becomes lost. More than 1 year after SCI there was no correlation between the relative decline of EMG activity and the duration or the level of SCI. This might be due to the small number of patients.

The observations made here are in line with the course of spinal reflexes in complete para-/tetraplegic patients. Early after SCI, when spasticity develops (~ 4 weeks after SCI), the reappearance of tendon reflexes and of muscle tone is associated with a recovery of flexor reflex excitability (Hiersemenzel *et al.*, 2000). However, during the later course (i.e. ~ 6 months after SCI), despite clinical signs of spasticity, M-wave and flexor reflex amplitudes decrease, while H-reflex amplitude remains roughly constant (Hiersemenzel *et al.*, 2000). In line with this, a ‘habituation’ of flexor reflexes to repeated electrical skin stimulation was described to occur in chronic complete SCI patients (Dimitrijevic and Nathan 1970). These results are of interest as the flexor reflex is suggested to be part of the spinal neuronal circuits underlying locomotion (Roby-Brami and Bussel, 1987; Parise *et al.*, 1997; Dietz, 2002). In contrast, stretch reflex activity seems to be of minor importance during locomotion of paraplegic subjects (Dietz *et al.*, 1997, 2002; Harkema *et al.*, 1997). The flexor reflex studies as well as the observations made here suggest a degradation of interneuronal circuit function below the level of the lesion during the course of an SCI which is reflected in an exhaustion of locomotor activity. Although we assume that mainly the loss of neuronal activity leads to a degraded function of interneuronal circuits, a contribution of external factors (e.g. antispastic medication) cannot be excluded.

Such a mechanism would fit with the observation of a trans-synaptic degeneration of neuronal systems in the rat that strongly depends on the input which is lost (Wu and Ling, 1998; Ginsberg and Martin, 2002). Also, in humans, some indirect evidence exists for a trans-synaptic degeneration of spinal neurons following SCI (Chang, 1998) or stroke (Hara *et al.*, 2000). After the loss of supraspinal control in an SCI,

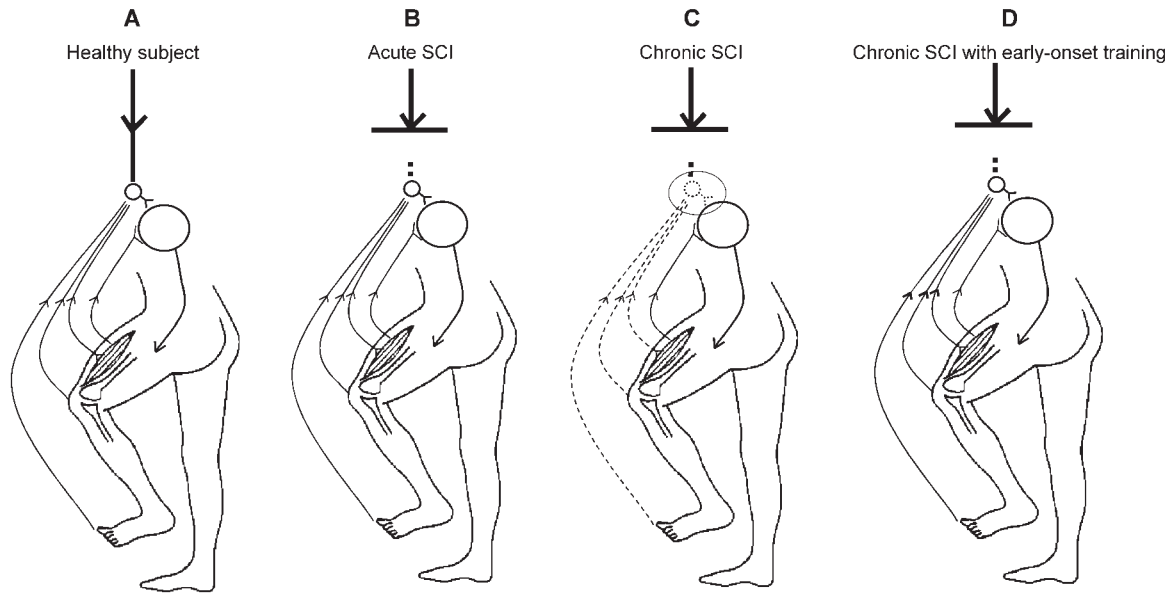


Fig. 9 Schematic illustration of the behaviour of spinal neuronal circuits underlying locomotion that might occur after a SCI and possible countermeasures. Neuronal behaviour (A) in a healthy subject, (B) early (<1 year) after injury, (C) in a chronic SCI (>1 year, not trained) and (D) in a chronic SCI with an early onset of training (modified from Jankowska and Lundberg, 1981).

the remaining input from peripheral receptors may differentially affect the function of motoneurons and interneurons. It is supposed that the exhaustion of locomotor activity may be due predominantly to an impaired function of interneurons, as motoneurons could still be strongly activated when muscle spasms occurred (see Fig. 9). Such a process of degradation of neuronal activities with time has clinical consequences as it questions the beneficial effect of future regeneration inducing treatments in chronic patients with SCI.

Target for countermeasures: plasticity of neuronal circuits

What is the therapeutic consequence of such behaviour of neuronal activity after SCI, i.e. can it be maintained by a specific training programme? There is convincing evidence in spinal animals that a use-dependent plasticity of spinal neuronal circuits exists (for reviews see Edgerton *et al.*, 1997; Pearson, 2000b). The plasticity and modifiability of the sensory-motor network function of the lesioned adult mammalian lumbo-sacral spinal cord has been studied in detail in rats and cats (Harris-Warrick, 1991; Edgerton *et al.*, 1992; Dickinson, 1995; Katz, 1995; Pearson, 2000a; for reviews see Edgerton *et al.*, 1997; Pearson, 2000b). When stepping is trained in spinalized cats, this task can be performed more successfully than when it is not practised (Lovely *et al.*, 1986, 1990). The training of any motor task provides sufficient stimulation to initiate a reorganization of neural networks within the spinal cord and, for example, to generate locomotion. The neuronal networks below the level of the lesion adapt to generate locomotor activity even in the absence of supraspinal input in the cat (De Leon

et al., 1998a, b). In adult rats, Skinner *et al.* (1996) have shown that exercise helps to normalize the excitability of spinal reflexes following spinal cord transection.

Similarly to the cat and rat, training effects with an enhancement of locomotor EMG activity have also been observed in acute complete paraplegic patients (Wirz *et al.*, 2001). However, these effects become lost over years after the completion of the training period. Observations in spinalized cats also indicate that if the training of a motor task is discontinued, the performance of this task is degraded (Edgerton *et al.*, 1997).

Furthermore, in chronic incomplete patients, an improvement of locomotor function can be achieved by training (Wernig *et al.*, 1995). This effect was described for a manually assisted training. However, the training by a robotic device (Lokomat) as used in this study was also shown recently to improve locomotor function in chronic incomplete SCI patients (Wirz *et al.*, 2004). In contrast, our study shows no training effects in chronic complete SCI patients. The early exhaustion of EMG activity remains unchanged after a locomotor training programme in three chronic patients studied for up to 13 weeks. Therefore, the present experiments suggest that in these patients, spinal (inter-)neuronal function underlying locomotion is degraded (cf. Fig. 9). Of course, our observations cannot exclude that some neuronal plasticity could be achieved by other approaches. The training applied here, its intensity and duration might have been insufficient to achieve an effect on locomotor activity. Nevertheless, in acute SCI patients after such a period, training effects can usually be seen (Dietz *et al.*, 1995). Spinal neuronal activity most probably can best be maintained if the training starts (i) early after SCI; (ii) provides an appropriate afferent input; and (iii) is

performed with some continuity over time (Dietz *et al.*, 1995, 2002). How intensive such a training must be for the maintenance of neuronal function after SCI currently is unknown, and will provide a basis for future studies.

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