Nogo-A Antibodies and Training Reduce Muscle Spasms in Spinal Cord-Injured Rats

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Objective: Spinal cord injury (SCI) leads to permanent motor and sensory deficits due to the damage of ascending and descending fiber tracts. In addition, malfunctions such as neuropathic pain or muscle spasms develop in many patients, possibly caused by injury-induced plastic changes of neuronal circuits above and below the lesion. New treatment strategies for spinal cord injury aim at enhancing plasticity and neurite growth, for example, by blocking the key neurite growth inhibitor Nogo-A or its downstream effectors. It is therefore crucial to investigate potential effects of such treatments on malfunctions such as muscle spasms. In addition, locomotor training, now a standard therapeutic tool to improve walking ability in incomplete SCI subjects, can be expected to influence the rearrangement of spinal cord circuits and the development of muscle spasms and other malfunctions.
Methods and Results: Here we present and validate a new rat model for muscle spasms after incomplete SCI and show that both intrathecal anti–Nogo-A antibody treatment and locomotor training, started early after injury, permanently reduce the development of muscle spasms.
Interpretation: The results show that an antibody-mediated suppression of the growth inhibitory protein Nogo-A leads to functional recovery and a lower level of malfunctions, suggesting the formation of functionally meaningful connections in the damaged spinal cord. Treadmill training early after SCI also has a beneficial effect.
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Motor and sensory deficits are an immediate consequence of spinal cord injury (SCI), caused by damage to ascending and descending fiber tracts. In addition, signs and symptoms of spasticity, such as muscle hypertonia, hyperreflexia, clonus, and spontaneous muscle spasms, develop gradually over weeks and months in 65 to 78% of patients with spinal cord injury.[1–3] The pathophysiology of spasticity is not well understood, but secondary plastic changes in the spinal cord below the lesion, such as alterations in reflex pathways, changes in the excitability of motoneurons, and formation of inappropriate new connections, have been implicated.[4–6] Painful muscle spasms are a common manifestation of the spastic syndrome.[3] Manifestations of muscle spasms in animal models of SCI have not been analyzed, except for tail spasticity.[7] Methods to test other aspects of spasticity have been used after experimental spinal cord ischemia[8] or contusion injury.[9]

Here, we characterize muscle spasms that readily occur during swimming in spinal cord-injured rats and compare them with spasms described in SCI subjects.

Enhancing neurite growth and circuit plasticity by neutralizing growth-inhibitory factors present in oligodendrocyte myelin has emerged as a novel approach to enhance recovery of motor function after central nervous system injuries.[10–13] A humanized antibody against the key neurite growth inhibitor Nogo-A, as well as Cethrin, a small cell membrane-permeable compound blocking the RhoA pathway on which the signaling pathways of several neurite growth inhibitors converge, are currently being studied in clinical trials for intrathecal and topical epi-

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dural application, respectively, in SCI subjects.[14] However, growth-promoting treatments theoretically might also lead to misguided axon growth and maladaptive plasticity, for example, from sensory afferent fibers or spinal interneurons, and thus could increase secondary complications after a SCI such as chronic pain or spasticity.[5] For example, intraspinal expression of nerve growth factor or implantation of embryonic stem cells after spinal cord lesion triggered extensive sprouting of afferent fibers and induced hyperalgesia in rats.[15,16] Therefore, we studied the impact of a neurite growth-enhancing treatment, that is, anti–Nogo-A antibodies, on the development of muscle spasms after incomplete SCI in adult rats.

Locomotor training, now a standard therapeutic tool to improve walking ability in incomplete SCI subjects, is based on the reactivation of neural circuits underlying locomotion, including spinal pattern generators. Some studies have suggested a positive effect of locomotor training on spasticity in SCI subjects,[17,18] but the extent of this effect, the persistence of the effect beyond the discontinuation of training, and the optimal training onset have not been investigated. Here, we studied the effect of intensive locomotor training on the emergence of muscle spasms in the acute and chronic stage after SCI in a well-defined rat model.

Materials and Methods

Experimental Animals

Adult female Lewis rats (180–300g) were studied. All experiments were approved by the Cantonal Commission for Animal Research and the Veterinary Office of the Canton of Zurich, Switzerland. Except for the experiments that included locomotor training, all rats were housed in groups of 5 in standard cages under a 12 hours light–12 hours dark cycle and had ad libitum access to food and water. For experiments including locomotor training, the animals were single housed.

Spinal Cord Surgery and Postoperative Care

All surgical procedures were performed under Hypnorm/Dormicum anesthesia (Hypnorm: 120µl/200g body weight, Janssen Pharmaceutics, Beerse, Belgium; Dormicum: 0.75mg/200g body weight, Roche Pharmaceuticals, Basel, Switzerland). T-shaped lesions of the thoracic (T8) spinal cord that interrupt the dorsal, dorsolateral, and ventral funiculus were performed on 8- to 10-week-old rats as described previously (Fig 1D).[10] Analgesics (Rimadyl, 5mg/kg, Pfizer AG, Zurich, Switzerland) were given 1 day before and for 2 days after surgery. The antibiotic Baytril (5mg/kg body weight, SC; Bayer AG, Leverkusen, Germany) was administered subcutaneously once a day for 7 days after the operation to prevent bladder infections due to bladder dysfunction. In addition, manual bladder expression was necessary for the first 2 weeks after injury. Thereafter spontaneous voiding recovered. In the chronic phase after injury, bladder infections

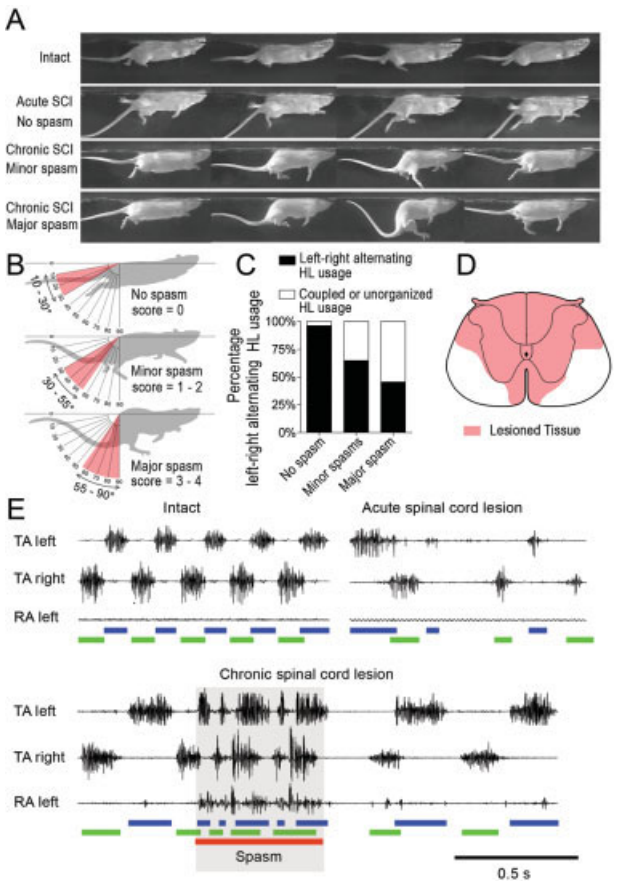

FIGURE 1: Muscle spasms in spinal cord injury (SCI) rats during swimming. (A) Video sequences of swimming intact and spinal cord-lesioned rats in the acute and chronic state after SCI. Upper row: intact rat. Second row: acute SCI, 4 days after injury. Lower 2 rows: chronic SCI (>5 weeks after injury). Normal swimming (1st and 4th photograph in each sequence) is interrupted by short periods of minor or major spasms (2nd and 3rd photograph in each sequence), in which the rat is in a ventroflexed position with the tail erected in major spasms. (B) Spasm score: angle of lower trunk and water surface serves as the measure for spasm severity. Score 1 and 3 are given for rats swimming in the minor or major spasm position, respectively, for less than half a run; score 2 and 4 are give for swimming more than half a run in the minor or major spasm position, respectively. (C) Disturbed hind limb (HL) alternation during spasms in chronically injured rats. Percentage of left–right alternating hind limb swim strokes during normal swimming, minor spasms, and major spasms. (D) Cross section of a spinal cord lesion site reconstructed from sagittal sections. The red area represents lesioned tissue. (E) Electromyographic (EMG) recordings from the tibialis anterior (TA, bilaterally) and the left rectus abdominis (RA) muscle of a swimming rat. The intact rat shows fast left (blue bar)–right (green bar) alternating TA activity and no activity in the RA. Acutely after spinal cord lesion (4 days) the rat shows irregular TA activity and no RA activity. In the chronic rat (3 months after lesion) basic left–right alternating TA muscle activity has recovered but occurs at a lower frequency than in the intact rat. During the spasm (red bar), the RA muscle shows sustained EMG activity, and the TA muscles are active in phase, reflecting the coupled hind limb movements.

were rare. If they occurred, rats were treated with Baytril (5mg/kg) for 3 days, which led to a quick resolution of symptoms (pus or blood in the urine). If rats suffered from bladder infection during testing days, they were not removed from the statistics.

Tissue Preparation and Lesion Size

Animals were perfused with 4% phosphate-buffered paraformaldehyde containing 5% glucose. The tissue was cryoprotected in phosphate-buffered 30% glucose, embedded in Tissue-Tek O.C.T. Compound, and frozen at −40°C. Sagittal sections of 50μm were cut on a cryostat. Lesions were reconstructed for a subset of animals as cross-section projections from sagittal section series (see Fig 1D). The extent of the lesion is reported as percentage of lesioned tissue in the cross-sectional area (Fig 2D).

Motor Behavior Analysis

SCORING OF MUSCLE SPASMS. Rats had to swim in a rectangular Plexiglas basin (150 × 40 × 13cm) filled with water (22°C, unless otherwise stated). The rats were placed at 1 end of the basin and could exit via a ramp on the other end. They were scored while swimming a distance of 0.6m in the middle part of the basin (= 1 run). We first assessed the degree of body flexion during spasms as shown in Figure 1B, and then determined whether the rats remained in the respective flexed position for more or less than half the swimming distance of 0.6m. If several successive spasms occurred in 1 run, they were summated together to determine if they were present for more or less than half the distance. A rat swimming in the slightly flexed position for less or more than half a run scored 1 or 2, respectively. A rat swimming in the strongly flexed position for less or more than half a run scored 3 or 4. For example, a rat with 2 minor spasms in 1 run, each lasting for 0.2m (sum = 0.4m), scored 2; a rat with 2 major spasms in 1 run, each lasting 0.1m (sum = 0.2m), scored 3. Results are the average of 3 separately scored runs. The prevalence of major spasms, that is, the percentage of rats showing major spasms at any investigated time point, and the spasm severity, that is, the average scores of those animals affected by spasms, are reported separately. The testing was carried out at the same time of day within experiments. On testing days, rats swam only 3 runs and were not put into the basin before testing. To analyze the time course of muscle spasm development, spinal cord-injured rats (N = 23) were assessed over a period of 10 weeks after injury (see Fig 2A–C).

QUANTIFICATION OF HIND LIMB ALTERNATION IN CHRONICALLY SPINAL CORD-INJURED RATS DURING SWIMMING. Over 3 videotaped runs, hind limb swim strokes during normal swimming, minor spasms, and major spasms were classified as either left–right alternating, disorganized, or coupled. The percentage of left-right alternating strokes was calculated for each category (see Fig 1C). Chronically SCI rats (10 weeks after injury, n = 20) contributed data.

LOCOMOTOR ASSESSMENT. Deficits in fine motor control were examined with the narrow beam test. Briefly, rats had to cross an elevated, tapered beam (1.4m long) labeled with 24 equally spaced segments, from the wide (6cm) to the narrow (1.5cm) end, and were scored (0–24) according to the segment where they first stepped down onto a ledge fixed underneath. The average of 10 runs is reported (Fig 3D).

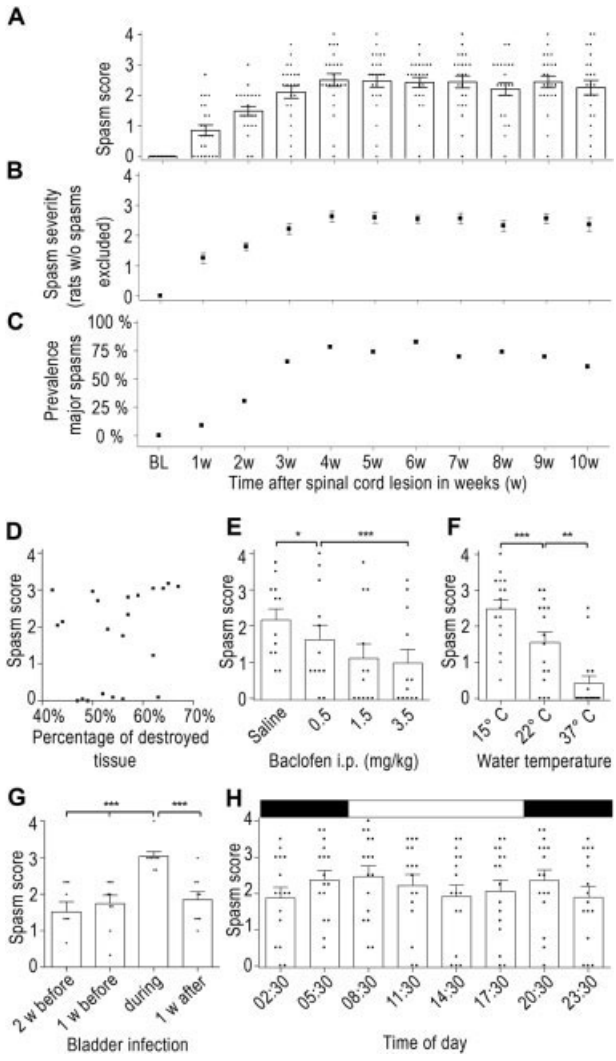

FIGURE 2: Factors influencing spasms. (A) Gradual development of spasms after spinal cord lesion. (B) The severity of spasms in affected animals (rats without spasms are excluded) and the prevalence of major spasms (C) increase gradually after injury, reaching a plateau after 4 weeks (w). (D) The size of the spinal cord lesion, plotted as percentage of lesioned tissue, does not correlate with the average spasm score in the chronic phase after injury. (E) Baclofen reduces muscle spasms in chronically spinal cord-lesioned rats in a dose-dependent manner. (F) Water temperature influences muscle spasms in chronically spinal cord-injured rats. (G) Chronic SCI rats with an incidental bladder infection have increased spasm levels, compared to preinfection and post-treatment spasm levels. (H) Diurnal variation of spasm scores in chronically spinal cord-injured rats ($p < 0.05$). The black/white horizontal bars above the graph indicate dark/light periods. BL = baseline; i.p. = intraperitoneal. Values are means ± standard error of the mean. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Repeated measures analysis of variance (E–H), followed by *t* tests (E–G).

Comparison of Lesion Size and Spasm Score

The lesion size, measured as percentage of lesioned tissue in the cross section, is plotted against the spasm score averaged over week 5 to 11 after spinal cord lesion (= chronic phase, n = 22, see Fig 2D).

Electromyographic Recordings

Bipolar intramuscular electromyographic (EMG) wires were implanted bilaterally into the tibialis anterior (ankle flexor) muscles and unilaterally into the left rectus abdominis muscle as described previously (see Figs 1E, 3G, 4B).[19] The wires were led subcutaneously via the back to a connector fixed on the head. The preamplified signal was digitized at a sampling rate of 1kHz, amplified 1,000×, and high-pass filtered (30Hz).

Circadian Variance

Chronically spinal cord-injured rats (n = 12, 8–13 weeks after SCI) were assessed for spasms at different time points throughout the day (see Fig 2H). One assessment was done for each time point. All assessments were made within a span of 3 days. Consecutive testing sessions were separated by an interval of 9 hours.

Water Temperature

Chronically spinal cord-injured rats (n = 16, 10–15 weeks after SCI) were assessed for spasms at water temperatures of 15°C, 22°C, and 37°C (see Fig 2F). Spasm assessments for different temperatures were done on 3 successive days.

Bladder Infections

Incidentally occurring bladder infections occurring in the chronic phase after injury (5–10 weeks after injury) were analyzed retrospectively. Nine occasions were identified when bladder infections first occurred at a testing day, that is, before antibiotic treatment was started. Spasm levels 1 and 2 weeks before and 1 week after these incidental infections (ie, after antibiotic treatment) were compared with spasm levels during bladder infections (see Fig 2G).

Drugs and Application

BACLOFEN. Rats (n = 12, 8–13 weeks after spinal cord lesion) were injected intraperitoneally with the gamma-aminobutyric acid $(GABA)_B$ agonist baclofen (Lioresal, Novartis, Basle, Switzerland) at increasing doses (0.5mg/kg, 1.5mg/kg, and 3.5mg/kg body weight), and spasms were assessed 1 hour after drug application (see Fig 2E). General motor performance was assessed by the narrow beam test 90 minutes after drug application (Data not shown).

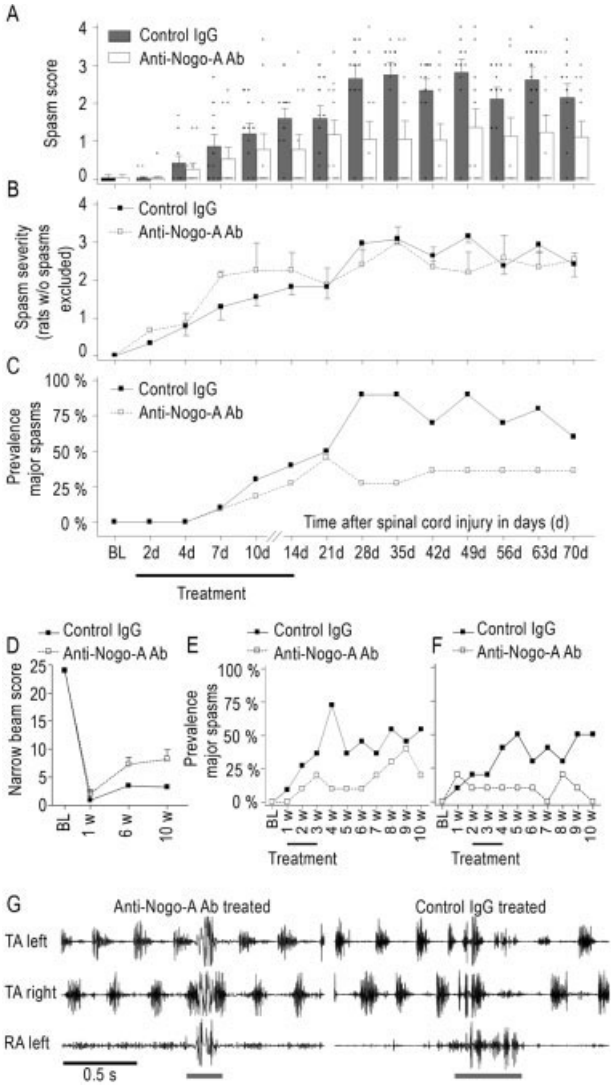

FIGURE 3: Treatment with antibodies (Ab) against the neurite growth inhibitor Nogo-A partially prevents the occurrence of muscle spasms after spinal cord injury (SCI). Intrathecal treatment with monoclonal anti–Nogo-A antibodies for 2 weeks (w) after SCI decreases the overall spasm scores compared to control IgG treated rats (A, $p < 0.05$). The severity of spasms in affected animals is not influenced by anti–Nogo-A antibody (B, rats without spasms excluded). The prevalence of major spasms is much lower in anti–Nogo-A antibody-treated rats than in those treated with control IgG (C, $p < 0.001$). (D) Improved locomotor recovery in the narrow beam test in anti–Nogo-A antibody-treated rats compared to controls ($p < 0.05$). (E, F) Prevalence of major muscle spasms in rats treated with a delay of 1 week or 2 weeks after spinal cord injury. Delayed anti–Nogo-A antibody treatment partially prevents the development of muscle spasms after SCI, irrespective of treatment onset ($p < 0.001$ for both treatment delays). Owing to the higher water temperature at which spasms were assessed (E, F: 25–30°C), the overall prevalence of major spasms was lower compared to testing at 20°C (A–C). For the effect of water temperature (see Fig 2F). (G) Representative electromyogramss of rectus abdominis (RA) and left/right tibialis anterior (TA) muscles during swimming. Both animals had received antibody treatment starting immediately after SCI. Black bars indicate treatment period (A–C, E, F). The gray bar in G indicates spasm duration. Values are means ± standard error of the mean. Repeated measures analysis of variance (A, B, D) and logistic regression analysis (C, E, F). BL = baseline.

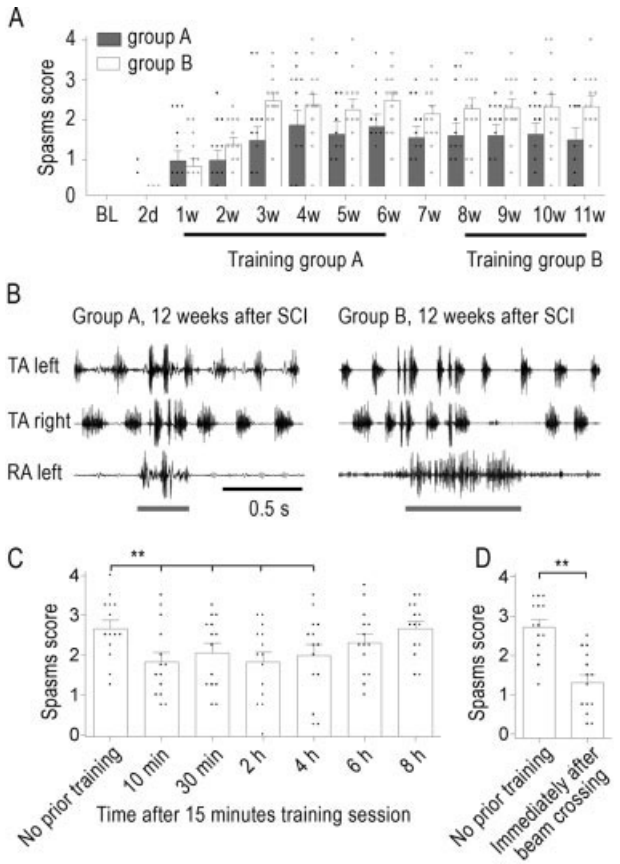

FIGURE 4: Locomotor training attenuates muscle spasms. (A) Intensive locomotor training after spinal cord injury (SCI) permanently reduces muscle spasms in group A, which receives early training (weeks 2–6 after spinal cord lesion) compared to group B, which receives no training at this stage ($p < 0.05$, repeated measures analysis of variance [ANOVA]). The same training paradigm has no permanent effect in chronically spinal cord-injured rats (group B) trained during weeks 9 to 11 after spinal cord lesion ($p > 0.05$, within-subject effect during weeks 8–11 postlesion, repeated measures ANOVA, spasms assessed 6 hours after training sessions). (B) Representative electromyograms (EMGs) of rectus abdominis (RA) and tibialis anterior (TA) muscles during swimming of rats that had undergone locomotor training in the early and chronic postinjury phase. The EMGs were recorded 12 weeks (w) after SCI, that is, after the training phase. (C) A 15-minute locomotor training session in chronically spinal cord-injured rats (6 months after SCI) transiently attenuated spasms for up to 4 hours compared to spasms without previous training (repeated measures ANOVA followed by post hoc paired *t* tests). (D) Repeated narrow beam crossing (25×, spasms assessed immediately afterwards) of the same animals reduced spasms similar to wheel running (2-sided paired *t* test). Values are mean ± standard error of the mean; **$p < 0.01$. BL = baseline.

ANTI–NOGO-A ANTIBODIES. Purified monoclonal function-blocking mouse anti–Nogo-A antibody 7B12[10] or isotype control antibody directed against wheat auxin were continuously delivered into the CSF over 2 weeks through an intrathecally implanted catheter (32ga, Recatho, Allison Park, PA), with the catheter's tip positioned immediately below the injury site as described previously (see Fig 3A–D, G).[10] The antibodies were dissolved in 2ml of phosphate-buffered saline at a concentration of 3mg/ml. The antibody solution was infused at a rate of 360μg/d (5μl/h) using osmotic minipumps (Alzet, Charles River Laboratoires, Les Oncins, France).

We reanalyzed recorded swim tests of experiments designed to study the effect of delayed anti–Nogo-A antibody treatment (see Fig 3E, F). Due to availability, the monoclonal anti–Nogo-A antibody 11C7[10] was used instead. An identical antibody application protocol was used. The antibody treatment was delayed for 1 week (antibody infusion over 2 weeks, starting 7 days after injury; anti–Nogo-A antibody: n = 10; control-IgG: n = 11) or for 2 weeks (antibody infusion over 2 weeks, starting 14 days after injury; anti–Nogo-A antibody: n = 10; control-IgG: n = 10). Unlike in the experiment presented above, the swim test was performed after a series of locomotor tests, and the water temperature was adjusted at 25 to 30°C.

Running Wheel Training

In a first experiment, the effect of intensive running wheel training for several weeks was assessed in the acute and chronic phase after SCI (see Fig 4A). All animals were single-housed throughout the experiment. For the acute phase after injury, rats were divided into 2 groups with equal functional deficits, matched according to their performance in the swim test and the narrow beam test 6 days after a T-lesion at T8. One group (group B, n = 13) received no training at this stage, whereas the other group (group A, n = 13) received an intensive daily training for 6 weeks according to the following protocol. The rats were trained on a motorized running wheel (diameter 82cm; track width: 9cm) 7 d/wk, 2 × 30 min/d (at 8 AM and 8 PM) on 5 days and 1 × 30 min/d (at 8 AM) on 2 days per week. During assessment days, the rats were assessed for muscle spasms and locomotor performance 6 hours after the first training session (ie, at 2 PM). The second training session was done 6 hours after the spasm assessment. For the chronic phase after injury, the previously untrained group B (n = 13) underwent the identical training regime as described above, starting 8 weeks after injury. The animals were trained for 4 weeks. During all training sessions, the rats were carefully observed, and the running wheel speed was adapted to the animals' walking ability (0.07–0.2m/s in the early training group and 0.1–0.2m/s in the late training group).

In a second experiment (see Fig 4C), the effect of a single training session in chronically injured rats was assessed. Chronically injured rats (n = 14, 6 months after injury) were trained for 15 minutes on the running wheel. The running wheel speed was adapted to the animals' walking ability (0.1–0.2 m/s). Spasms were assessed at different intervals (10 min, 30 min, 2 hours, 4 hours, 6 hours, and 8 hours) after the training session. Each interval was tested on different days, with at least 3 days between testing days. In the same animals, we also assessed the effect of repeated narrow beam crossing; rats had to cross the narrow beam 10× and were assessed for spasms immediately afterward (see Fig 4D).

Statistical Analysis

Statistical analysis was performed with SPSS software (SPSS 14, SPSS Inc., Chicago, Ill). To assess treatment effects (anti–Nogo-A antibody treatment and early locomotor training of group A), a 2-way repeated measures analysis of variance (ANOVA) was performed. One-way repeated measures ANOVA was performed to assess the effect of locomotor training in chronically lesioned rats (week 8–11, group B) and to assess diurnal variation of muscle spasms. Repeated measures ANOVA followed by post hoc paired *t* tests (2-sided) were performed to assess the effect of baclofen treatment, water temperature, and bladder infections. To assess the effect of narrow beam crossing on muscle spasms, a 2-sided paired *t* test was performed. Logistic regression analysis was performed to compare the prevalence of major spasms in treated (trained and anti–Nogo-A antibody-treated) and untreated (no training and control antibody-treated) animals.

Results

Spinal Cord Lesion

Despite standardized surgery techniques, the percentage of tissue destruction ranged between 40 and 70% of the spinal cord cross section. This considerable variability of lesion size is mainly due to secondary tissue damage, such as bleeding, ischemia, and inflammation. Importantly, however, the percentage of tissue destruction was similar in the different treatment groups. The dorsal, dorsolateral, and ventromedial funiculi were interrupted in all animals. For quantitative analysis, the lesion size was reconstructed in a subset of animals.

General Locomotor Performance after Spinal Cord Lesion

OPENFIELD LOCOMOTION.

Two days after spinal cord lesion, most rats were dragging their hind limbs with extensive movements in the hip, knee, and ankle. One week after the lesions, rats scored 8 to 12 points in the Basso, Beattie, and Breshnahan (BBB) open field locomotor score, indicating that some animals were able to make weight-supporting steps, whereas others showed sweeping movements with their hind limbs without weight-supporting steps.

SWIMMING. Intact rats swim by using the hind limbs and tail. The forelimbs are mostly held under the chin and only occasionally used for steering. After the spinal cord lesion, the swimming pattern had changed. Initially (1–3 days after spinal cord lesion), rats used mostly their forelimbs for propulsion and only occasionally made uncoordinated hind limb strokes. Within 1 to 2 weeks after injury, most rats had recovered alternating hind limb usage but continued to use their forelimbs.

Spasms in Spinal Cord-Injured Rats during Swimming

Several days after an incomplete lesion of the thoracic spinal cord (T8), spasms developed progressively during swimming in SCI rats. Spasms were characterized by a bent, ventroflexed posture and an erected tail (see Fig 1A, 3rd and 4th row). In a single run over 0.6m, rats exhibited several short spasms, longer lasting spasms over a fraction of the 0.6m distance, or spasms that lasted throughout the 0.6m stretch. Occasionally, rats showed no spasms at all.

Along with spasm onset, there was a sudden conversion from the normal, alternating hind limb paddle motions to coupled, hopping-like hind limb strokes (see Fig 1C, E). Intramuscular electromyographic (EMG) recordings during swimming showed muscle activity in the rectus abdominis muscle during spasms but not silent during normal swimming without spasms (see Fig 1E).

Course of Spasms after SCI

Based on a score reflecting the severity and the duration of the spasms (see Fig 1B), we observed a gradual increase in the number of animals affected by spasms (ie, the prevalence of spasms), an increasing occurrence of major spasms, and a progression of spasm severity within affected animals (see Fig 2A–C). These parameters reached a plateau at 3 to 4 weeks after SCI. Spasms were rare and mild in the first few days after the lesion. The order of runs (1–3) at 1 assessment day did not affect the severity of muscle spasms.

No Effect of Lesion Size on Spasm Score

The lesion size was reconstructed in a subset of animals. Lesion size, reported as percentage of destroyed tissue in the cross-sectional area, had no influence on muscle spasms (see Fig 2D).

Systemic Baclofen Treatment Reduces Muscle Spasms

To further characterize the muscle spasms, we treated chronic SCI rats with the $GABA_B$ agonist baclofen, a potent antispastic drug used widely in humans with SCI.[3,20] Systemic application substantially reduced muscle spasms during swimming in the rats in a dose-dependent manner (see Fig 2E). Locomotor performance as assessed by the narrow beam test was not impaired after a low and intermediate dosage (0.5mg/kg or 1.5mg/kg, data not shown).

Spasm Severity Depends on Water Temperature

In SCI subjects, the spastic muscle tone is influenced by temperature.[21] Severity and frequency of muscle spasms in chronically injured rats were markedly increased in

cold water (15°C) and substantially reduced in warm water (37°C) compared to intermediate water temperature (22°C) (see Fig 2F).

Effect of Urinary Tract Infections

In human SCI subjects, urinary tract infections are frequently accompanied by increased symptoms of spasticity.[22] Similarly, incidentally occurring bladder infections in chronically injured rats led to transiently elevated spasm levels during swimming compared to spasm levels before infection or after treatment (see Fig 2G).

Circadian Variation of Spasm Severity

In SCI subjects, the spastic movement disorder is known to fluctuate during the day, and is usually worst in the morning and afternoon.[23] Similarly, severity of muscle spasms in chronically spinal cord-injured rats showed a diurnal fluctuation which peaks in the early morning and in the early evening (see Fig 2H).

Anti–Nogo-A Antibody Treatment after Spinal Cord Injury Reduces the Emergence of Muscle Spasms

To examine the effect of neurite growth-enhancing treatment on the development of muscle spasms, we infused monoclonal anti–Nogo-A or IgG control antibodies continuously into the cerebrospinal fluid (CSF) for 2 weeks, starting immediately after the spinal cord lesion. Treatment with anti–Nogo-A antibodies significantly decreased the number of animals affected by spasms. Whereas up to 90 % of IgG control antibody-treated animals developed spasms, only 36 to 63% of anti–Nogo-A antibody-treated animals showed spasms (see Fig 3A). Similarly, severe (major) spasms developed in most control animals, but only in less than half of the anti–Nogo-A antibody-treated animals. However, the severity of spasms in affected animals was similar in both treatment groups. Representative EMG recordings of an anti–Nogo-A and control antibody-treated rat are shown in Figure 3G. Similar results were obtained if the anti–Nogo-A antibody treatment was delayed for 1 or 2 weeks. Anti–Nogo-A antibody treatment also improved motor function, assessed in the narrow beam test.

Locomotor Training Reduces Muscle Spasms

We examined the short- and long-term effects of different locomotor training protocols on muscle spasms in the acute and chronic stage after SCI: (1) the long-term effect of daily training started early or, alternatively, with a delay of 8 weeks after SCI; and (2) the short-term effect of a single training session in chronically spinal cord-injured rats (8–13 weeks after SCI).

First, we examined the effect of early locomotor training on the development of muscle spasms. Locomotor training, starting 1 week after SCI, reduced muscle spasms by up to 25% compared to untrained SCI rats (Fig 4A, group A = trained, group B = untrained, $p < 0.05$, repeated measures ANOVA). This effect persisted for at least 5 weeks (ie, until the end of the observation period) after the cessation of daily training sessions. Locomotor performance assessed with the narrow beam test showed no significant difference between the groups (data not shown).

Second, we examined whether the same training paradigm could reduce spasms in chronically injured rats that had reached a constant level of muscle spasms. Intensive locomotor training, starting 8 weeks after SCI in previously untrained rats, had no lasting effects on muscle spasms (group B in Fig 4A; within-subject effect week 8–11 of group B animals, repeated measures ANOVA).

A single 15-minute training session in chronically spinal cord-injured rats transiently reduced muscle spasms (see Fig 4C). Compared to baseline without training, spasms were reduced by 23 to 32% during the first 4 hours after training (repeated measures ANOVA, followed by post hoc paired *t* tests), but returned to baseline levels thereafter. Comparable spasm-reducing effects were observed when the rats repeatedly crossed a narrow beam before being assessed for spasms (see Fig 4D, paired *t* test).

Discussion

Adult rats with incomplete SCI roughly corresponding to incomplete SCI subjects (American Spinal Injury Association C level of impairment) develop mild to severe muscle spasms over 1 to 4 weeks after injury. The spasms are easily observed during swimming. Interestingly, they resembled spasms described for human SCI subjects in several aspects; they typically develop with a delay (several days in rats, several weeks in humans), gradually increase in frequency and severity, and are influenced by the water temperature and by the circadian rhythm. They are efficiently reduced by the $GABA_B$ agonist baclofen. Growth-enhancing treatment of SCI rats with anti–Nogo-A antibodies reduced the number of animals affected with spasms. Likewise, treadmill training applied early, but not late, after the injury reduced the occurrence of muscle spasms during swimming.

An Animal Model for Muscle Spasms after SCI

The occurrence of spasms in SCI rats was characterized by a delayed onset, similar to the situation in humans. Frequency and severity of spasms reached a stable plateau at

4 to 5 weeks, compared to 2 to 6 months in humans.[2] About 75% of SCI rats developed severe spasms. This is in line with a reported percentage of 65 to 78% in humans with incomplete SCI.[3] Lesion size did not correlate with the severity of muscle spasms. This is similar in humans, where the extent of the spinal cord destruction does not predict the occurrence of spasms.

Treatment with the $GABA_B$ agonist baclofen, a potent antispastic drug used widely in spastic humans,[3,20] substantially reduced muscle spasms during swimming in SCI rats in a dose-dependent manner. This antispastic effect was unlikely due to an unspecific inhibition of normal motor activity, as performance in the narrow beam test was unchanged.

In addition, spastic symptoms in SCI subjects are worst in the morning and afternoon.[23] Similarly, muscle spasm severity in chronically spinal cord-injured rats showed a circadian fluctuation, with which peaks in the early morning and in the early evening.

Ambient temperature affects muscle spasms in spastic humans[21]: many patients report relief from spasms by thermotherapy.[24] Moreover, patients frequently suffer muscle spasms in cold weather.[25] Swimming in cold water, for instance, during aquatic physical training, can also trigger spasms. This is in line with spasms in SCI rats shown here; swimming at a low water temperature increased the spasm severity compared to swimming at moderate temperature. Conversely, at warm temperature muscle spasms were either completely absent or substantially reduced.

Finally, in SCI subjects urinary tract infections are often accompanied by an increased occurrence of spasms.[22] Similarly, incidentally occurring bladder infections in chronically injured rats led to more spasms during swimming.

The majority of motoneurons innervating the rectus abdominis muscle are located at T10–T14,[26] i.e. below the spinal cord lesion site at T8. Here we focused on behavioral aspects after T8 lesions. This lesion paradigm will allow examining changes in intraspinal neuronal circuitries and their correlation to muscle spasms in future studies. Possible mechanism that might underlie the effects of locomotor training or anti–Nogo-A antibody treatments are discussed below.

Anti–Nogo-A Antibody Treatment Reduces the Development of Muscle Spasms

Anti–Nogo-A antibody treatment enhances regeneration of lesioned axons as well as compensatory sprouting and fiber growth.[12] As a consequence, inactivation of Nogo-A improves the recovery of locomotor function after SCI in adult rats and the recovery of skilled hand function in spinal cord-lesioned macaque monkeys.[11] The increased regeneration and sprouting of nerve fibers could affect the development of secondary malfunctions such as muscle spasms. Here, we show that anti–Nogo-A antibody treatment had a preventive effect on the development of muscle spasms in the majority of SCI rats; animals treated with anti–Nogo-A antibodies less often developed major spasms compared to control antibody treated animals. Interestingly, the spasm severity of affected rats did not differ between the treatment groups, suggesting that a minority of rats did not respond to the treatment. We could not identify any differences between responders and nonresponders.

Several mechanisms may account for the spasm-preventing effect of neurite growth-enhancing treatment. A partial reconstitution of descending projections via regenerated axons[10] or collateral sprouts of spared fibers into denervated spinal cord segments, as observed after anti–Nogo-A antibody treatment,[12,27,28] may restore supraspinal control over spinal pattern generators and reflex circuits mediating muscle spasms. Furthermore, the enhanced recovery of motor function in anti–Nogo-A antibody-treated rats after SCI[10] may result in more physiological movement patterns and higher levels of motor activity early after injury. Such an increased level of motor activity may lead to an enhanced entrainment of spinal neuronal circuits, which has been shown to influence spinal reflex behavior.[29,30] A neuroprotective effect of anti–Nogo-A antibody treatment was not observed, as described before.[10]

Locomotor Training Reduces Spasms

Our results show that locomotor training can reduce muscle spasms. The training effect, however, crucially depends on the timely initiation of training after SCI. Early onset of locomotor training, that is, 1 week after SCI, permanently reduced muscle spasms by up to 25%, whereas onset of locomotor training in the chronic stage had only a transient effect on muscle spasms. Comparable short-term spasm-reducing effects in chronically injured animals were observed when the rats repeatedly crossed a narrow beam.

Several mechanisms may explain the spasm-reducing effect of locomotor training. After SCI, an excess of new axon collaterals and synaptic connections may be formed.[31,32] Inappropriate synapses may then be eliminated or weakened in an activity-dependent manner.[32,33] The persistent spasm reducing effect of early locomotor training may reflect this process of network rearrangement during the early postinjury phase. In addition, increased levels of neuronal activity might protect the intraspinal neuronal networks from degradation.[34] The training-

induced temporary reduction of muscle spasms in the chronic stage might reflect short-term facilitation in stable spinal neuronal circuits. Training-induced modifications of proprioceptive and cutaneous afferent pathways may also play a role.[35–38]

In conclusion, we show that the SCI rat is a useful model to study the development of muscle spasms. Our findings have several clinical implications. First, we show that treatment with anti–Nogo-A antibodies has the potential to reduce muscle spasms. This would be a highly desirable outcome in SCI subjects. Spasticity assessments should therefore be included in the clinical trial protocols. Our results also provide a rationale for locomotor training after SCI, as we demonstrate that training, in addition to improving locomotor function,[30] also reduces muscle spasms. Patients should start with locomotor training as early as possible after SCI for it to achieve permanent effects. In the chronic phase of injury, short training sessions may alleviate muscle spasms for several hours. Clinical studies are needed to determine the optimal onset, intensity, and frequency of locomotor training, and its combination with regeneration-enhancing treatments like anti–Nogo-A antibodies.

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Potential Conflicts of Interest

Nothing to report.

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