**Materials and methods**

Experimental model

Mice and ethical standards

CD1 mice of either sex (P12-P18 for patch-clamp recording, P13-P20 for two-photon Ca2+ imaging, P10, P15 and P20 for EMG recordings and DeepLabCut procedure) were housed under a 12h light/dark cycle in a temperature-controlled area with *ad libitum* access to water and food. *Aldh1L1*-eGFP mice (CD1 background) expressing GFP in astrocytes were kindly provided by Nathalie Rouach (Collège de France, CNRS, INSERM, Labex Memolife, Université PSL, Paris, France). Animals from different litters were used for each experiment. Sample sizes (number of spinal cords and/or cells) for each experiment are indicated in figure legends. All animal care and use were conformed to the French regulations and approved by the local ethics committee (Comité d’Ethique en experimentation animale CE n°071 (INT-Marseille, Nb B 13 014 04), APAFIS authorizations: Nb 17485-2018110819197361 and Nb 44572-2023072115506121).

Experimental procedures

Spinal cord injury (SCI)

The spinal cord transsection was performed on P0-P1 neonatal mice, including both wild-type and *Aldh1L1*-eGFP mice. The animals were anesthetized *via* hypothermia and received a subcutaneous injection of buprenorphine (0.025 mg/kg; Vetergesic, CEVA Santé Animale, France). After a midline skin incision, a laminectomy was performed to expose lower thoracic segments of the spinal cord. The dura was opened and the spinal cord was completely transected at the T8-T9 segmental level. The lesion cavity was filled with sterile absorbable collagen contact hemostat (Pangen, Urgo Medical, France). One drop (ca 20-30 µl) of the antibiotic amoxicillin (Citramox, Axience, France) was subcutaneously applied at the incision site. Finally, the wound was closed with 6-0 absorbable suture (Z1032H, Ethicon, NJ) and covered with adhesive suture (Steri-Strip, R1546, 3M Health Care, MN). Animals were kept in a warm and wet chamber for 2 hr in cotton-wool swab impregnated with their mother smell before they returned to the home cage with their mother. Sham animals were submitted to all procedures except the laminectomy and the spinal cord transection.

Adeno-associated virus (AAV) constructs

AAV2/5-gfaABC1D-cyto-GCaMP6f (52925-AAV5, titer ≥ 1\*1013 vg/mL), AAV2/9-Syn.NES-jRGECO1a.WPRE.SV40 (100854-AAV9, titer ≥ 1\*1013 vg/mL), AAV2/5-gfaABC1D-tdTomato (44332-AAV5, titer ≥ 7\*1012 vg/mL) were sourced from Addgene (https://www.addgene.org/). AAV2/9-gfaABC1D-eGFP-Cre (titer ≥ 2.5\*1010 vg/mL) AAV2/9-gfaABC1D-eGFP-Kir4.1 (titer ≥ 2.3\*1011 vg/mL) were kindly provided by the Hu lab27.

Intrathecal AAV delivery

A minimally invasive technique was employed to micro-inject AAV vectors through the intervertebral space. Briefly, in cryoanesthetized pups, a beveled microcapillary (30-60 µm in diameter) preloaded with the AAV particles was lowered to reaches the subarachnoidal space at the L3-L4 segments. A total volume of 2 µL per animal was then progressively injected (1 µL/5s).

Lumbarslicepreparation and artificial cerebro-spinal fluid (aCSF) solution

Mice were anaesthetized with intraperitoneal injection of a mixture of ketamine/xylazine (100mg/kg and 10 mg/kg, respectively). They were then decapitated, eviscerated and the spinal cord removed by laminectomy, and placed in a Sylgard-lined petri dish with ice-cold (1-2°) aCSF containing (in mM): sucrose (252), KCl (3), NaH2PO4 (1.25), MgSO4 (4), CaCl2 (0.2), NaHCO3 (26), D-glucose (25), pH 7.4. The lumbar spinal cord was mounted on an agar block, embedded in a 4% agarose solution, quickly cooled, and then sliced into 325 µm sections through the L4–L5 lumbar segments using a vibrating microtome (Leica, VT1000S). Slices were immediately transferred into the holding chamber filled with bubbled (95% O2 and 5% CO2) standard aCSF composed of (in mM): NaCl (120), KCl (3), NaH2PO4 (1.25), MgSO4 (1.3), CaCl2 (1.2), NaHCO3 (25), D-glucose (20), pH 7.4, 30-32°C. After a 30-60 min resting period, individual slices were transferred to a recording chamber continuously perfused with standard aCSF heated to 32-34°C.

*Ex vivo* electrophysiological recordings

Aldh1L1-eGFP positive astrocytes were visualized in the ventrolateral region of lamina IX in L4-L5 horizontal slices using an Nikon FN1 microscope with appropriate filters. Whole-cell patch-clamp recordings of GFP(+) astrocytes and surrounding large motoneurons (MNs) (soma area >800 µm2)28 were performed using a Multiclamp 700B amplifier (Molecular Devices) with electrodes pulled from borosilicate glass capillaries (1.5 mm OD, 1.12 mm ID; World Precision Instruments) on a Sutter P-97 puller (Sutter Instruments Company). For MN recordings, electrodes (2-4 MΩ) were filled with an intracellular solution containing (in mM): K+-gluconate (140), NaCl (5), MgCl2 (2), HEPES (10), EGTA (0.5), ATP (2), GTP (0.4), pH 7.3. For GFP+ astrocyte recordings, electrodes (6-8 MΩ) were filled with an intracellular solution containing (in mM): K+-gluconate (105), NaCl (10), KCl (20), MgCl2 (0.15), HEPES (10), EGTA (0.5), ATP (4), GTP (0.3), pH 7.3. In some experiments, the intracellular pH was lowered to 6.9 by the addition of HCl. Patch clamp recordings were made using a Multiclamp 700B amplifier driven by PClamp 10 software (Molecular Devices). Recordings were digitized on-line and filtered at 10 kHz (Digidata 1550B, Molecular Devices). Pipette and neuronal capacitive currents were canceled and, after breakthrough, the access resistance was compensated. All experiments were designed to gather data within a stable period (i.e., at least 1-2 min after establishing whole-cell access).

*Ex vivo* two-photon Ca2+ imaging

Two-photon fluorescence measurements were obtained with a dual-scanhead two-photon microscope (FemtoS-Dual, Femtonics Ltd, Budapest, Hungary) and made using an Olympus XLUMPlanFLN 20X, 1.00 NA objective (Olympus America, Melville, NY) or a Nikon LWD 16X/0.80W objective (Nikon Instruments Inc. Melville, NY). For two-photon Ca2+ imaging, excitation of GCaMP6f and jRGECO1a was simultaneously evoked with a femtosecond pulsed laser (Chameleon Ultra II; Coherent, Santa Clara, CA) tuned to 960 nm. Recordings were performed at ~32−34 °C in standard aCSF saturated with 95% O2/5% CO2 (pH 7.4). The microscope system was controlled by MESc acquisition software (https://femtonics.eu/femtosmart-software/, Femtonics Ltd, Budapest, Hungary). A single acquisition plane was selected and full-frame imaging was started in a resonant scanning mode at 30.5202 Hz. Scan parameters were [pixels/line × lines/frame (frame rate in Hz)]: [512 × 519 (30.5202)]. Scanning area was 468 µm \* 474 µm. This microscope was equipped with two detection channels for fluorescence imaging.

*Ex vivo* two-photon imaging of intracellular pH (pHi) in astrocytes

Lumbar slices were incubated in standard aCSF containing BCECF (5 µM) and Pluronic® F-127 (0.02%) for ≃ 45 min followed by a through washing out to allow de-esterification of the dye. Two-photon fluorescence measurements were obtained with a dual-scanhead two-photon microscope (FemtoS-Dual, Femtonics Ltd, Budapest, Hungary) and made using an Olympus XLUMPlanFLN 20X, 1.00 NA objective (Olympus America, Melville, NY) or a Nikon LWD 16X/0.80W objective (Nikon Instruments Inc. Melville, NY). For two-photon BCECF signal imaging, excitation was simultaneously evoked with a femtosecond pulsed laser (Chameleon Ultra II; Coherent, Santa Clara, CA) tuned to 820 nm. Recordings were performed at ~32−34 °C in aCSF saturated with 95% O2/5% CO2 (pH 7.4). Astroglial loading of BCECF was confirmed by analysing the characteristic morphology of the BCECF-stained cells featuring astrocytic phenotype from mice injected at birth with AAV AAV2/5-gfaABC1D-tdTomato. Changes in pHi are expressed as changes in BCECF fluorescence at the maximum of the fluorescent signal over the baseline (ΔF/F0). The microscope system was controlled by MESc acquisition software (https://femtonics.eu/femtosmart-software/, Femtonics Ltd, Budapest, Hungary). A single acquisition plane was selected and full-frame imaging was started in a resonant scanning mode at 30.5202 Hz. Scan parameters were [pixels/line × lines/frame (frame rate in Hz)]: [512 × 519 (30.5202)]. Scanning area was 468 µm \* 474 µm. This microscope was equipped with two detection channels for fluorescence imaging.

Puff-application of extracellular **K+**

To measure the astrocytes’ potassium (K+) uptake capacity, a high extracellular potassium concentration ([K+]o, 12mM) was added to standard aCSF and delivered via a pipette similar to the recording pipette, controlled by a Picospritzer (Picrospritzer II, General Valve Corporation) set at 10 psi. The electrode was positioned in the ventro-lateral part (lamina IX) of a lumbar slice, within ~60 μm of a GFP+ astrocyte soma.

**Measurement of extracellular K+ concentration**

K+-sensitive microelectrodes were made from thin-walled borosilicate theta tubes (GC150T-10, Harvard Apparatus, MA) following the procedure described in29. Briefly, the theta tubes were washed in 70% ethanol for 1 hour, then for 1 hour in ultra-pure water, and dried in the oven overnight at 60°C. The double-channel pipettes were pulled to obtain a tip diameter of 3-5 µm. One channel was filled with 150 mM NaCl and the second one with 100 mM KCl. The inside of the tip of the KCl-containing channel was silanized using Silanization Solution I (85126, Merck-Sigma Aldrich) and then filled with Potassium Ionophore I Cocktail A (99311, Merck- Sigma Aldrich). The electrode tip was immersed in a 100 mM KCl solution for 3 hours or longer for stabilization. Before each recording session, the electrode was calibrated using ACSF solutions enriched with gradually decreasing KCl concentrations: 20 mM, 10 mM, 6 mM, 3 mM, 1.25 mM, 0.6 mM, 0.3 mM. The K+ sensor was accepted for the experiment if it generated a stable potential at each K+ concentration and displayed a >540 mV increase at 30 mM K+ drop.

The extracellular potassium concentration ([K+]o) was measured in acute lumbar slices from control and spinal cord injury (SCI) mice. Experiments were conducted in standard aCSF, saturated with 95% O2/5% CO2 (pH 7.4) and heated to 32-34°C. L-glutamate (1 mM, 5 s, at 10 psi) was puffed using a glass pipette connected to a picospritzer (Picrospritzer II, General Valve Corporation). Both the puff electrode and the K+-sensitive microelectrode were aligned and positioned in the ventro-lateral part (lamina IX) of the lumbar slice, within ~50 μm of each other in the z axis. Basal [K+]o was measured after proper positioning and stabilization of the K+-sensitive microelectrode in the lumbar slice.

Data acquisition was performed in current-clamp mode using a Multiclamp 700B amplifier (Molecular Devices), sampled at 10 kHz, filtered at 4 kHz, and digitized with a Digidata 1550B (Molecular Devices). The peak amplitude, area under the curve (AUC) of [K+]o transients, the rise slope from glutamate puff onset to peak amplitude, and decay time from peak amplitude to baseline recovery were measured.

Pharmacological compounds and dyes

All solutions were oxygenated with 95% O2/5% CO2. Barium chloride dihydrate (Ba2+, 100µM; 217565), Pluronic® F-127 (0.02%; P2443), DMSO (0.1%; D4540), L-glutamic acid (L-glutamate, 1 mM; G1251), VU0134992 (30µM; SML2431) and Sodium Propionate (5mM; P1880) were obtained from Merck Sigma-Aldrich. Tetrodotoxin (TTX, 0.5-1µM; 1078) from Tocris Bioscience. S0859 (50-100 µM, M5069) from Abmole Europe Branch (Forlab). 2’,7’-Bis-(2-Carboxyethyl)-5-(and-6)-Carboxyfluorescein, Acetomxymethyl Ester (BCECF-AM, 5 µM) from Invitrogen. All drugs were dissolved in water and added to the standard aCSF except BCECF-AM incubated in Pluronic® F-127 (0.02%) and DMSO (0.2%).

Immunohistofluorescence

Spinal cords of 14-16 days-old mice were.dissected out and fixed for 5-6 h in 4% paraformaldehyde (PFA), then rinsed in phosphate buffered saline (PBS) and cryoprotected overnight in 20% sucrose at 4°C. Spinal cords were frozen in OCT medium (Tissue Tek) and 30 μm cryosections were collected from the L4-L5 segments. After having been washed in PBS 3×5 min, the slides were incubated for 1 h in a blocking solution (BSA 1%, Normal Donkey Serum 3% in PBS) with 0.2% triton X-100 and for 24 h at 4 °C in a humidified chamber with the following primary antibodies diluted in the blocking solution with triton x-100: rabbit anti-Kir4.1 (1/1000; APC-035, Alomone, Israel), mouse anti-NeuN (1/1000; MAB377, Merck Millipore, MA), rabbit anti-GFAP (1/1000; Z0334, Dako, Agilent, CA) and/or goat anti-C3d (1/40; AF2655, R&D Systems, MN). For STAT-3 staining, slices were pretreated with a drop of MetOH 100% (34860-1L-R; Merck Sigma-Aldrich, MA) for 10 minutes at -20°C. After 3 washes of 5 min in PBS, they were incubated for 1h in a blocking solution (Normal Donkey Serum 5% in PBS 0.1M) with 0.3% triton X-100. The primary antibody anti-STAT3 (1/300; 8768, Cell Signaling Technology, MA) was incubated with Cell signal blocking solution (8112, Cell Signaling Technology, MA) for 48 h at 4°C. Slides were then washed 3×5 min in PBS and incubated for 2 h with the appropriate secondary antibodies diluted in the blocking solution: Alexa Fluor® Plus 555- donkey anti-rabbit (1/400; A32794, Invitrogen, MA), Cy5- donkey anti-mouse (1/400; 715-175-151, Jackson ImmunoResearch, PA) and/or Alexa Fluor® Plus 555- donkey anti-goat (1/400; A32816, Invitrogen, MA). After 3 washes of 5 min in PBS, slides were incubated with Hoechst 33342 (2 µM in PBS; 62249, Thermo Fisher Scientific, MA) for 5 min and washed again 3x5 min before being mounted with a homemade gelatinous aqueous medium. Images were acquired using a confocal microscope (LSM700, Zeiss) equipped with either a 20x air objective, a 40x oil objective or a 63x oil objective and processed with the Zen software (Zeiss).

Protein analysis by capillary Western Blot

The lumbar parts of the spinal cords were dissected in aCSF at 4°C and conserved at -80°C until protein extraction. Tissues were homogenized in ice-cold lysis buffer (250 mM sucrose, 3.9 mM Tris pH 7.5, 10 mM iodoacetamide) supplemented with protease inhibitors (cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail, 11836170001, Roche Diagnostics, Germany). Unsolubilized material was pelleted by centrifugation at 7,000 x g for 5 min at 4°C and discarded. The supernatants were subjected to an additional centrifugation step at 19,000 x g for 70 min at 4°C. The resulting pellets corresponding to the membrane-enriched fraction were resuspended in ice-cold lysis buffer (PBS 1X, IGEPAL® CA-630 1%, SDS 0.1%) supplemented with protease inhibitors (cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail, 11836170001, Roche Diagnostics, Germany). Protein concentrations were determined using the Pierce™ BCA Protein Assay Kit (23227, Thermo Fisher Scientific, MA). Kir4.1 and NBCe1 expressions were then analyzed using the 12-230 kDa and the 66-440 kDa separation modules respectively (SM-W004 and SM-W008, ProteinSimple, Bio-Techne, MN) on an automated capillary western blotting system (‘Jess’, ProteinSimple, Bio-Techne, MN) according to the manufacturer’s protocol with small modifications. Samples were not implemented with DTT and they were not submitted to heat denaturation in order to keep proteins in a more ‘native’ form. Total protein concentrations of 0.1 mg/mL and 0.5 mg/mL were used for Kir4.1 and NBCe1 respectively. Samples were probed with a rabbit polyclonal Kir4.1 antibody (1:60; APC-035, Alomone, Israel) or a rabbit polyclonal NBCe1 antibody (1:90; ANT-075, Alomone, Israel) and revealed with the appropriate detection module (DM-001, ProteinSimple, Bio-Techne, MN). Loaded samples were normalized to their own total protein content using the ‘total protein detection module’ (DM-TP01, ProteinSimple, Bio-Techne, MN).

Assessement of electromyography (EMG) recordings

Mice were tested at 10, 15, and 20 days post-SCI, when signs of spastic motor behaviors, such as excessive involuntary twitches/movements and exaggerated reflexes, were evident. For electrophysiological assessment of spasticity, a stainless steel needle electrode was transcutaneously inserted into the lateral gastrocnemius muscle (ankle extensor). Two reference electrodes were placed subcutaneously on the back and in contact with the patellar tendon. Because mice were spinalized, they did not feel any pain during this procedure. Following a 30-minute acclimation period to the recording environment, mice were immobilized with tape. Spontaneous motor responses were recorded for 15 minutes. Motor responses evoked by tail pinch (50.5 ± 5.6 g, 0.5 ± 0.2 s) were recorded until baseline activity returned. Each mouse experienced five consecutive sensory stimuli, each separated by a resting period of at least 30 seconds. The proximal ends of the recording wires were connected to a A-M Systems Amplifier (Model 1700, Everett, WA). EMG signals were amplified (1000x) and bandpass filtered (100 Hz to 5 kHz), then sampled at 13.5 kHz (Digidata 1440A, Molecular Devices). The applied pressure (measured in grams) to the distal third of the tail was recorded by a miniature pressure sensor (#FS1901-000-0500-G, Farnell) placed between the thumb and the tail, and monitored in real-time.

Assessment of DeepLabCut video acquisition

Animals were tested at P10, P15, and P20, when signs of a spastic motor phenotype, such as excessive involuntary hindlimb twitches and exaggerated reflexes, were visible.

**Assessment of mice hindlimb spontaneous activity:** Animals were removed from their home cages, weighted, and individually subjected to a single 20-second video recording in a standardized homemade setup. Video acquisition was performed at 240 frames per second (FPS) to allow complete visualization of hindlimb spasms from a lateral view, at a distance of 10 cm from the mouse.

**Assessment of mice hindlimb induced activity:** For the behavioral assessment of induced activity, the same procedure was applied as previously described. Induced activity was initiated by gently pinching the tail (without causing discomfort or signs of physical pain) with the thumb. Hindlimb motor responses following tail pinching were recorded by a miniature pressure sensor placed between the thumb and the tail and monitored online. Each animal underwent three consecutive pinching stimulations, separated by 10 seconds, through a single video recording

### Experimental design and statistical analysis

No statistical method was used to predetermine sample size. Group measurements are expressed as means ± S.E.M. The Mann-Whitney test was used for comparisons between two groups, while the Fisher’s test was employed to compare percentages. For comparisons between two conditions, the Wilcoxon matched pairs test was utilized. One-way or two-way ANOVA test was applied for multiple comparisons. Normality of the data sets was evaluated for all statistical analyses, and the significance level was set at p < 0.05. Statistical analyses were conducted using GraphPad Prism 9 software. Details of the analyses are provided in each figure legend.

Data analysis

Assessment of astrocyte reactivity

1. ***Morphometric analyses.*** Confocal images at 63X magnification, immunostained with GFAP antibody in Aldh1L1-eGFP mice, were serially stacked and maximally projected. The maximal projection image of the GFAP signal in the ventral horn of the spinal cord was used for analysis. The maximal projection image of the GFAP signal in the ventral horn of the spinal cord was used for analysis. The Sholl analysis plugin in ImageJ automatically drew serial concentric circles at 5 μm intervals from the center of the nucleus (Hoechst signal) to the end of the longest process in each individual astrocyte. This plugin provided key morphometric parameters such as the number of intercepts of GFAP processes with each circle, the ramification index, and the ending radius (longest process). Four different fields from the left and right parts of the ventral spinal cord were analyzed in at least five different slices from the same mouse, evaluating all astrocytes within the field. For illustrative purposes, a few representative astrocytes were fully reconstructed in 3D using Chimera software.
2. **Evaluation of C3d positive astrocytes:** We quantified the proportion of C3d(+) versus C3d(–) astrocytes. Immunofluorescence staining was analyzed from stacked confocal images (15 steps; Z-step, 1 μm, maximum intensity stack) acquired with a 40× objective on five slices per mouse, with four different fields on each slice. The mean gray value for C3d staining was measured using Fiji, and we determined the number of cells exceeding a fixed cutoff threshold (with background subtracted) as C3d(+) in each experiment. All Aldh1L1-eGFP positive astrocytes in the field, were evaluated. We also quantified C3d immunofluorescence intensity (comparing control and SCI conditions) on a z-projection generated from 3 stacks acquired in the lumbar ventrolateral region (lamina IX). This region was defined by a square of approximately 250 μm by 250 μm centered on the motoneuron pool.
3. **Quantification of astrocytic STAT3 nuclear fraction.** To evaluate the nuclear fraction of the STAT3 signal, we measured the STAT3 signal intensity within the Hoechst (nuclei) signal. Immunofluorescence staining was analyzed from stacked confocal images (15 steps; Z-step, 1 μm, maximum intensity stack) acquired with a 40× objective on five slices per mouse, with four different fields on each slice. All Aldh1L1-eGFP positive astrocytes in the field with a clear Hoechst signal were included. Nuclear masks were manually segmented using the Hoechst signal, applied to the STAT3 channel, and the mean fluorescence intensity was measured (see 30).

Analysis of *ex vivo* electrophysiological (Patch-clamp) recordings

Electrophysiological data were analyzed offline using Clampfit 10.7 software (Molecular Devices). To ensure optimum quality of intracellular recordings, several criteria were established: only cells exhibiting a stable resting membrane potential (RMP), access resistance with less than 20% variation, and an action potential amplitude larger than 40 mV under standard aCSF were considered.

**Passive Membrane Properties:** Passive membrane properties of astrocytes and motoneurons (MNs) were measured by determining the largest voltage deflections induced by small current pulses from the holding potential, avoiding activation of voltage-sensitive currents. Input resistance was determined by the slope of linear fits to voltage responses evoked by small positive and negative current injections.

**Recording Kir4.1-Currents in GFP (+) Astrocytes.** Voltages were repeatedly stepped for 2.5s from -140 to 0 mV in 10 mV increments, with astrocytes initially clamped at their RMP. Approximately 5 minutes after the start of the recording, barium chloride (Ba2+, 100 µM) was bath-applied to the slice. The current amplitude was measured as the difference between the baseline level before the initial voltage step and the mean amplitude over a 10 ms window starting 10 ms after the onset of the hyperpolarizing voltage step. The Ba2+-sensitive current was obtained by subtracting the current after Ba2+ application from the current before application.

**MNs excitability.** For MNs, firing properties were measured from depolarizing current pulses of varying amplitudes. The rheobase was defined as the minimum step current intensity required to induce an action potential from the membrane potential held at RMP. The instantaneous discharge frequency was determined as the inverse of the interspike interval. The firing gain refers to the ratio between the measured instantaneous firing frequency (Hz) and the injected current (pA).

Analysis of two-photon Ca2+ and BCECF pHi changes imaging

The fluorescent time series depicting Ca2+ and BCECF changes in ex vivo neurons and/or astrocytes were analyzed using MESc data acquisition software (Femtonics Ltd, Budapest, Hungary) and the MES curve analyzer tool. Regions of interest (ROIs) for neurons and astrocytic soma were selected manually, while ROIs for astrocytic processes and microdomains were selected automatically using a custom script in ImageJ (Fiji). A signal was declared as a Ca2+ transient if it exceeded the baseline by more than twice the baseline noise (standard deviation, SD). We defined active astrocytes as astrocytes displaying at least one Ca2+ transient during the recording session.

In Ca2+ imaging experiments, ROIs included : (i) active soma for neurons (jRGECO1a), (ii) active soma, processes and microdomains for astrocytes (GCaMP6f). In BCECF experiments, ROIs corresopnded to astrocytic soma double-labeled with Td-tomato (AAV2/5-gfaABC1D-TdTomato).

For the two-photon Ca2+ imaging analysis, raw fluorescence traces were extracted to Excel and analyzed using a custom Matlab script. These traces were converted to show fluorescence changes according to Equation (1):

ΔF/F=(F1-F0)/F0 (1)

where *F* is the fluorescence at any given time, and F0is the mean fluorescence value for the 5 to 10 s range, preceding the bath perfusion of L-Glutamate (1 mM). The signal-to-noise ratio (SNR) was calculated as:

SNR = (ΔF/F)peak/ σF

where σF is the SD of the baseline period. Events were considered as Ca2+ response if they exceeded twice the SD of the baseline period. The peak amplitude, duration and frequency of both spontaneous and glutamate-evoked Ca2+ transients were analysed by using a Matlab script.

For the two-photon pHi imaging analysis, raw fluorescence traces were extracted to Excel. The proportion of Tdtomato (+) astrocytes showing increased or decreased fluorescence (ΔF/F) and the peak amplitude of acidic pHi reponses to L-glutamate were analyzed from both control and SCI mice.

Analysis of *in vivo* electromyography (EMG) recordings

Custom-built amplifiers enabled simultaneous online rectification and integration (100ms time constant) of the raw AC-mode signals. Data analysis was performed offline with the automatic event detection plugin in Clampfit 11 software. Spontaneous muscle contractions were identified when the signal envelope exceeded a predetermined threshold, and the contraction was considered to end when the signal fell below this threshold. This threshold was adjusted for each signal, typically set at 1.3 times the background noise level. For an event to be classified as a valid muscle spasm, it needed to remain above the threshold for at least 250 ms. We quantified both the average and cumulative distribution of the duration and frequency of these muscle events over a 15 min intervals. For responses triggered by tail stimulation, spasm duration was measured from the stimulus artefact until the recovery of a stable baseline. The experimenter was blinded during both the procedure and the data analysis of the electrophysiological experiments.

Analysis of mice spastic motor behaviors with DeepLabCut

**Analysis of kinematics hindlimbs spontaneous activity.** For hindlimb spams analysis, videos captured during experiments were analyzed using the markerless pose estimation software DeepLabCut 2.2 (DLC)31 to extract tracks based on digital markers placed on the mice (Figure 7). For tracking DeepLabCut was installed on a PC equipped with a NVIDIA Ge-Force RTX 3060 12Go graphics card. DeepLabCut was used to identify four reference points among the mouse body (hip, knee, ankle and foot) on a side view (Figure 7) for kinematic analysis. The ResNet-50 based neural network (imgaug augmentation, 500,000 iterations) was used to identify points of interest on the mice. The training was performed on a set of 800 frames randomly selected by DeepLabCut from 40 videos representatives of experimentation conditions (same background, luminosity, distance from the mouse and the camera).

Note that two separate training has been performed between the first post-natal and second post-natal week face to the very fast morpho-physical changes occurring in mice at this period. Network was trained until it reaches a stable error rate plateau (> 400,000 iterations, 0.0005 error per frame). Next network was evaluated (test error = 2.89 pixels 0,19 mm), and videos analysis was consecutively performed.Raw data provide by DeepLabCut was extracted and proceeded to collect key parameters such as the number of events, acceleration of the spasms, maximum angle of the ankle and the mean ankle angle using custom scripts in Python (see below).

1. *Acceleration*. Sudden increase in the speed of the hindlimb was quantify by estimating the instantaneous acceleration derived from the instantaneous speed estimates:

𝑎𝑡=𝛥𝑣𝑡/𝛥𝑡(cms−2)

1. *Number of events.* Hindlimbs motion event was considered as ankle angle variation of more than 10° degree from the initial position through the entire video recording

**Analysis of hindlimb evoked activity.**

1. *Measure of the ankle angle variation following stimulation.* Angle of the ankle was measured using the angle tool of Fiji (ImageJ) before stimulation and immediately following the stimulation. Each measure is performed for the 3 consecutives stimulation for each animal.
2. *Measure of spasms duration.* Spasms duration was measured from the end of the stimulation until the recovery of a stable hindlimb motion activity (e.g no hindlimb twitches) using the free and open source cross-platform multimedia player and framework, VLC.
3. *Measure of tail pinch force*. Stimulation intensity was recorded by a miniature pressure sensor (#FS1901-000-0500-G, Farnell) placed between the thumb and the tail, and monitored in real-time. The applied pressure measurements were compared between the groups (SCI + control-GFP *vs* SCI + Kir4.1-GFP) and were similar.