The brain integrates proprioceptive information to ensure robust locomotion

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Abstract  Robust locomotion relies on information from proprioceptors: sensory organs that communicate the position of body parts to the spinal cord and brain. Proprioceptive circuits in the spinal cord are known to coarsely regulate locomotion in the presence of perturbations. Yet, the regulatory importance of the brain in maintaining robust locomotion remains less clear. Here,

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through mouse genetic studies and in vivo electrophysiology, we examined the role of the brain in integrating proprioceptive information during perturbed locomotion. The systemic removal of proprioceptors left the mice in a constantly perturbed state, similar to that observed during mechanically perturbed locomotion in wild-type mice and characterised by longer and less accurate synergistic activation patterns. By contrast, after surgically interrupting the ascending proprioceptive projection to the brain through the dorsal column of the spinal cord, wild-type mice showed normal walking behaviour, yet lost the ability to respond to external perturbations. Our findings provide direct evidence of a pivotal role for ascending proprioceptive information in achieving robust, safe locomotion.

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**Abstract figure legend** Wild-type mice with intact muscle spindles and Golgi tendon organs respond to locomotor perturbations by increased kinematic variability and reduced accuracy of muscle activation patterns. When muscle spindles, but not Golgi tendon organs, are genetically removed from birth in Egr3\(^{-/-}\) mice, the animals do not modulate kinematics and muscle activity in response to perturbations and appear to be in a constantly perturbed state. Similarly, but even more dramatically, this happens if both muscle spindles and Golgi tendon organs are acutely ablated in adult PVRc::AvilDTR mice. However, when the ascending proprioceptive pathways in the wild-type dorsal column are interrupted, leaving muscle spindles, Golgi tendon organs and their local spinal circuits intact, any effects of external perturbations on the locomotor output cease to exist, except for often occurring halting behaviour. These findings suggest that supraspinal integration of proprioception is essential to produce robust locomotion.

**Keypoints**

- Whether brain integration of proprioceptive feedback is crucial for coping with perturbed locomotion is not clear.
- We showed a crucial role of the brain for responding to external perturbations and ensure robust locomotion.
- We used mouse genetics to remove proprioceptors and a spinal lesion model to interrupt the flow of proprioceptive information to the brain through the dorsal column in wild-type animals.
- Using a custom-built treadmill, we administered sudden and random mechanical perturbations to mice during walking.
- External perturbations affected locomotion in wild-type mice similar to the absence of proprioceptors in genetically modified mice.
- Proprioceptive feedback from muscle spindles and Golgi tendon organs contributed to locomotor robustness.
- Wild-type mice lost the ability to respond to external perturbations after interruption of the ascending proprioceptive projection to the brainstem.

**Introduction**

Animals constantly move in complex environments. Locomotor actions often dictate the safe execution of challenging activities such as chasing prey, escaping danger and exploring new territories. In vertebrates, the generation of rhythmic and patterned activities such as those needed for locomotion is partly achieved through neuronal networks located in the spinal cord: the central pattern generators (Brown, 1911). The higher order integration of spatiotemporal information occurring in the cerebral cortex and subcortical regions is known to be important for initiating and halting locomotion or regulating its speed (Cignetti et al., 2017; Karadimas et al., 2019; Roseberry & Kreitzer, 2017; Rossignol et al., 2006). Yet, when somatosensory feedback is removed and supraspinal pathways are left intact, the motor commands generated by the central pattern generators alone are not sufficient to allow for safe, functional locomotion in the presence of external perturbations (Akay, 2020;
Grillner & El Manira, 2020; Santuz et al., 2019). The essential role played by somatosensory feedback in locomotion has been confirmed in studies that took advantage of external perturbations to challenge the locomotor behaviour of various mammal models (Frigon et al., 2021; Pearcey & Zehr, 2019). Early postural responses in standing cats have been shown to depend on somatosensory feedback (Lockhart & Ting, 2007). Moreover, postural responses in the form of stereotyped muscle activation patterns were similarly found in wild-type mice (Mayer & Akay, 2018), intact cats (Macpherson, 1988; Ting & Macpherson, 2005) and healthy humans (Macpherson et al., 1989; Santuz, Brüll et al., 2020) dealing with unpredictable perturbations. Depending on their type and function, various circuits for somatosensory feedback have been shown to contribute differently to perturbation-coping mechanisms (Bolton & Misiaszek, 2009; Ivenenko et al., 2000; Santuz et al., 2019; Stapley et al., 2002) and a crucial supraspinal contribution to postural control has been supported by studies focussing on spinal cord injury or transection, in which perturbations did not produce postural responses as in intact animals (Chvatal et al., 2013; Macpherson & Fung, 1999). In other words, somatosensory feedback is essential for producing robust locomotion, with robustness being the ability to maintain the system’s function in the face of perturbations or errors of execution (Kitano, 2007; Santuz et al., 2018; Stelling et al., 2004). The contribution of supraspinal systems to the generation of postural correction reflexes in mammals has been documented in detail during standing (Deliagina et al., 2008, 2014). In the rabbit, lumbar spinal interneurons controlled by supraspinal systems such as the motor cortex (Beloozerova et al., 2003) and serotonergic descending pathways (Lyalka et al., 2008, 2011) have been shown to be activated during postural correction responses (Zelenin et al., 2015). In the standing cat, the activity of pyramidal tract neurons has been recorded to be strongly modulated after perturbation of the standing surface, indicating an involvement of cortical control for postural corrections (Beloozerova et al., 2005). However, supraspinal mechanisms for postural corrections during locomotion remain largely elusive.

In mammals, information about body position in space and relative to the body itself (i.e. proprioception) is conveyed to the central nervous system by mechanosensory neurons found within muscles, tendons and joints (Dietz, 2002). These neurons bear information from the muscle spindles, Golgi tendon organs (GTOs) and joint receptors. Although not much is known about the function of the latter (Tuthill & Azim, 2018), feedback from muscle spindles (group Ia and II afferent fibres) and GTOs (group Ib) undoubtedly plays a role in mammalian locomotion (Grillner & El Manira, 2020; Pearson, 1995). In cats, mice, humans and other mammals, these proprioceptive afferents are pivotal for setting the timing of the gait cycle and facilitating the switch between different phases, such as propulsion and swing (Rossignol et al., 2006). In mice, the removal of proprioceptive afferents disrupts the kinematic and muscle activity patterns required for locomotion (Akay et al., 2014; Takeoka & Arber, 2019). Muscle spindle-deficient mice present an impaired regulation, in terms of timing and amplitude, of the muscle activity needed to locomote at different speeds (Mayer et al., 2018). Moreover, the orchestrated activations of functionally-related muscle groups known as muscle synergies (Bernstein, 1967; Tresch et al., 1999) undergo profound timing reorganisation in mice lacking muscle spindles (Santuz et al., 2019). The effects of proprioceptive impairment on locomotor patterns are similar to those encountered in wild-type mice (Santuz & Akay, 2020; Santuz et al., 2019), cats (Drew et al., 2008) and healthy humans (Cappellini et al., 2016; Chvatal & Ting, 2012; Martino et al., 2014; Santuz et al., 2018; Santuz, Brüll et al., 2020; Walsh, 2021) locomoting in perturbed environments. This parallelism between the lack of proprioception and perturbations can be leveraged to gain new insight into the role played by sensory feedback in the regulation of the locomotor output. Although the first line of action to sudden perturbations lies in the so-called preflexes (i.e. passive response because of the intrinsic properties of the muscle–tendon unit), sensory feedback from proprioceptors provides excitatory action immediately after (Brown & Loeb, 2000; Grillner, 1972; Grillner & El Manira, 2020). Part of the proprioceptive information is elaborated locally in the spinal cord (spinal sensory processing), although there are three, topographically well distinct, main pathways that convey proprioceptive feedback from the hindlimb to brain structures (supraspinal sensory processing): the dorsal and ventral spinocerebellar tracts (Bosco & Poppele, 2001) and the dorsal column–medial lemniscus (DCML) pathway (Niu et al., 2013). The dorsal spinocerebellar tract originates from Clarke’s column that receives somatosensory information through the dorsal column and conveys it to the cerebellum through the lateral funiculi; it is assumed to be the major carrier of proprioceptive and exteroceptive (i.e. touch and pressure) signals directly to the cerebellum from the hindlimb (Hantman & Jessell, 2010; Lundberg, 1964). Similarly, the ventral spinocerebellar tract relays some proprioceptive information to the cerebellum via the lateral funiculi, but ventral relatively to the dorsal spinocerebellar pathway (Chalif et al., 2022; Jankowska et al., 2010). By contrast to the spinocerebellar tracts, the DCML pathway transmits to the somatosensory cortex via the brainstem and it mostly conveys signals from proprioceptors and touch exteroceptors (Conner et al., 2021; Niu et al., 2013). Although the neural circuitry underlying sensory feedback from muscle spindles and GTOs in the dorsal column is well investigated, the importance of higher
centres in integrating proprioceptive information during locomotion is still obscure (Akay, 2020; Caggiano et al., 2018; Conner et al., 2021).

Here, using a combination of mouse genetics, in vivo electrophysiology, spinal lesion models and computational neuroscience, we set out to explore the relevance of the supraspinal integration of proprioceptive feedback via the DCML pathway during murine locomotion. During walking, we administered random mediolateral and anteroposterior perturbations to four groups of animals by means of sudden lateral displacement or acceleration of the treadmill belt. A first group of wild-type mice served as control. A genetically modified strain (Egr3+/−) in which muscle spindles regress immediately after birth was used to assess the consequences of missing feedback from muscle spindles on unperturbed and perturbed locomotion, when GTOs are left intact. To investigate the effects of the concurrent lack of feedback from spindles and GTOs and to control for potential adaptation to the missing sensory information during development, we included a third group in which both proprioceptor classes could be ablated systemically and acutely in adult age (PVCre::AvilDTR). Lastly, we surgically lesioned the dorsal column to disrupt proprioceptive information reaching the brain through the DCML and partly through the dorsal spinocerebellar pathways in wild-type animals.

This was performed to isolate a major portion of proprioceptive information travelling from the hindlimb to the brain, leaving the local spinal circuitry intact.

Based on the notion that the supraspinal integration of somatosensory feedback is essential to postural control (Frigon et al., 2021), we hypothesised that not only spinal, but also supraspinal processing of proprioception must be involved in the tuning of motor output when locomotion becomes challenging. Specifically, we anticipated that: (a) only wild-type animals would be able to modulate kinematics and muscle activation patterns in response to perturbations; (b) the lack of feedback from proprioceptors would leave the animals in a state similar to that encountered in wild-type when locomoting in perturbed conditions; (c) the kinematics and the temporal characteristics of muscle activation patterns would be more seriously affected in PVCre::AvilDTR than in Egr3+/− mice; and (d) leaving local proprioceptive circuits intact at the same time as interrupting ascending proprioceptive information through the DCML pathway would reduce the capabilities of wild-type mice to tune kinematics and muscle activation patterns in response to perturbations. By means of high-speed video recordings, EMG and an analysis framework based on linear and nonlinear tools, we show that supraspinal processing of proprioceptive feedback becomes crucial whether locomotion is challenged by external perturbations.

Methods

Ethical approval

All procedures were performed according to the guidelines of the Canadian Council on Animal Care and approved by the local councils on animal care of Dalhousie University with protocols #19-014 and #19-021. The experiments conformed to the principles and regulations described in the editorial by Grundy (2015).

Mouse lines

Recordings were conducted on 18 adult (age 89 ± 20 days; mass 25.9 ± 3.9 g) male mice on C57BL/6 background fed ad libitum. Five C57BL/6 wild-type mice were used for the DCML lesion protocol. To investigate the role of muscle spindles, we performed experiments with five Egr3+/− mice (Tourtellotte & Milbrandt, 1998). To investigate the role of proprioceptive feedback from muscle spindles and GTO, we used three PVCre::AvilDTR and two PVCre::AvilDTR::RosaEGFP mice (Takeoka & Arber, 2019). Three PVCre::RosaEGFP mice were used for the sham experiments. For the recordings, mice of different litters but same genotype were allocated to the relevant experimental group using as the only inclusion criteria: (a) the genotyping; (b) a minimum mass of 22 g; and (c) a minimum age of 60 days at the time of recordings.

The mice used to breed the experimental mice were obtained from: PVCre: Jackson Laboratories, Bar Harbor, ME, USA (01 7320) (Hippenmeyer et al., 2005); AvilDTR: European Mouse Mutant Archives (EM:10 409) (Stantcheva et al., 2016); RosaEGFP: Jackson Laboratories (01 0701) (Sousa et al., 2009); Egr3+/−: courtesy of Dr Warren Tourtellotte (Northwestern University, Chicago, IL, USA; currently Cedars-Sinai Medical Centre, Los Angeles, CA, USA); Wild-type: littermates of different breeding colonies in the lab with C57BL6 background.

EMG implantation surgeries

Each mouse received an electrode implantation surgery as previously described (Akay et al., 2014; Santuz & Akay, 2020; Santuz et al., 2019). Eight bipolar EMG electrodes were implanted (Akay et al., 2006; Pearson et al., 2005) in as many muscles of the right hindlimb. The muscles that were implanted were: gluteus maximus (MA), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), tibialis anterior (TA), gastrocnemius medialis (GM) and gastrocnemius lateralis (GL). Briefly, mice were anaesthetized with isoflurane at 2% to 3% concentration through inhalation at 1 l min−1 and opthalmic eye ointment was applied to the eyes. The skin was sterilized using a three-part skin
scrub by means of Hibitane (Derma UK Ltd, Newcastle upon Tyne, UK) (chlorhexidine gluconate 4%), alcohol and povidone–iodine. Two small midline skin incisions on the back and one on the posterior part of the right hind leg were made to expose the target muscles and position the custom 3D-printed back-mounted connector cap. The electrodes were then drawn from the most anterior neck incision to the leg incisions s.c. and the EMG connector cap was sutured to the skin. Afterwards, each electrode was implanted in the muscles as described before (Pearson et al., 2005), the incisions were closed, anaesthetic was discontinued and buprenorphine (0.03 mg kg\(^{-1}\)) and ketoprofen (5.00 mg kg\(^{-1}\)) were injected s.c. The mice were then placed in a heated cage for at least 6 days and returned to their regular mouse rack once recovered. Food mash and hydrogel were provided for the first 3 days after the surgery multiple times a day and subsequently once a day until the animal returned to the rack. Handling of the mice was mostly avoided until they were fully recovered and at least for 1 week. Additional injections of buprenorphine and ketoprofen were performed at 24 and 12 h intervals, respectively, for at least 48 h after surgery.

**Spinal lesion surgeries**

The DCML pathway lesion surgeries that were conducted after the first EMG recordings followed the same anaesthesia and care protocols as the EMG implantation surgeries. After preparation and anaesthesia, one midline skin incision of around 10 mm was made to expose the back muscles that were then separated with a scalpel to reveal the seventh or eighth thoracic vertebral body (Harrison et al., 2013). A laminectomy was then performed, the dura mater opened and the spinal cord lesioned at the ninth (three mice) or tenth (two mice) thoracic spinal segment using tungsten steel micro scissors (Fig. 1). Muscles were then stitched over the missing spinous process using absorbable suture, the skin closed and the animals placed in their recovery cage for at least 3 days.

**Diphtheria toxin (DTX) delivery**

Intraperitoneal DTX (D0654 lyophilized; Sigma-Aldrich, St Louis, MO, USA) injection was carried out on three \(Pv^{Cre}::Avil^{DTR}\) and two \(Pv^{Cre}::Avil^{DTR}::Rosa^{EGFP}\) experimental mice and on \(Pv^{Cre}::Rosa^{EGFP}\) mice for the sham experiments. We injected each mouse with 100 \(\mu\)g kg\(^{-1}\) toxin (Takeoka & Arber, 2019) diluted in ultrapure water (TMS-006-C; Millipore, Burlington, MA, USA) and let the animals in a confined cage for 3 days, when they could return to their rack. Recordings were conducted on day 7 after injection.

**Immunohistochemistry**

To assess the success of the DCML pathway lesion at the ninth/tenth spinal segment (Fig. 1A), we perfused the animals immediately after death. After thoracotomy, the mice were perfused with 20 mL of saline solution followed by 10 mL of 4% paraformaldehyde solution (PFA) through the left cardiac ventricle. The spinal cord

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![Figure 1. Lesion of the dorsal column-medial lemniscus (DCML) pathway leaves the corticospinal tract intact](image)

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was dissected and immersed in PFA for at least 24 h. The fourth lumbar and first thoracic spinal segments were then dissected and cryoprotected by immersion in 30% sucrose-PBS solution for at least 24 h or until sunk. After cryoprotection, the spinal cord sections were embedded in optimal cutting temperature (OCT) mounting medium, flash frozen on dry ice and stored at −80°C. The tissue was then sectioned transversally at 30 μm by means of a cryostat (Leica CM3050 S; Leica Biosystems AG, Muttenz, Switzerland) and placed on microscope slides to dry for at least 1 h. Half of the sections were then prepared for Toluidine Blue O (TBO) staining and the remaining half for Protein Kinase C Gamma (PKCG) staining. For TBO (T3260; Sigma-Aldrich) staining, slides were washed three times for 5 min in 1xPBS to remove OCT and then immersed in 1:1000 TBO-1 × PBS solution for 5 min. Slides were then washed once for 5 min in 1 × PBS and mounted on coverslips using PermaFluor Aqueous mounting medium (Thermo Fisher Scientific, Waltham, MA, USA). For PKCG staining of the corticospinal tract, after the three initial washes in 1 × PBS, the slides were incubated for 60 min in 5% blocking solution (5% bovine serum albumin in 1 × PBS + 0.3% Triton) at room temperature. Subsequently, the sections were incubated overnight at 4°C in a primary antibody solution consisting of 1:1000 rabbit-anti-PKCG (ab71558 rabbit pAB to PKCG; Abcam, Cambridge, UK) in 1% blocking solution (1% bovine serum albumin in 1 × PBS + 0.3% Triton). The next day, the tissue received three 10 min washes in 1 × PBS and a secondary incubation of 90 min at room temperature. For markerless body part tracking we used, a high-speed camera operating at 500 Hz with resolution 1280 × 800 pixels (IL3; Fastec Imaging, San Diego, CA, USA). Kinematics

The gait cycle segmentation (paw touchdown and lift-off timing) was obtained by the elaboration of high-speed videos. The kinematics of the hindlimb was acquired with a high-speed camera operating at 500 Hz with resolution 1280 × 800 pixels (IL3; Fastec Imaging, San Diego, CA, USA). For markerless body part tracking we used, DeepLabCut, v2.1.10 (Mathis et al., 2018). We labelled 16 landmarks on 297 frames taken from 30 videos of 15
different animals, assigning 95% of those images to the training set without cropping. Namely, we labelled six calibration markers, iliac crest and hip (highlighted by two white dots placed with an oil-based marker under brief 3% isoflurane anaesthesia through inhalation at 1 l min$^{-1}$), knee, ankle, fifth metatarsal, toe tip of the rear paw, toe tip of the front paw and the four paw reflections on the mirror placed under the treadmill at an angle of 45°. We used a ResNet-50-based neural network (He et al., 2016; Insafutdinov et al., 2016) with default parameters for 1 030 000 training iterations and two refinements of 100 000 and 300 000 iterations, respectively. We validated with one shuffle and found the test error was 2.75 pixels and the train error 2.57 pixels.

Of the 16 landmarks, we used eight for the segmentation of the gait cycle: the six calibration, the metatarsal and the rear toe tip markers. Following a procedure extensively reported previously (Santuz & Akay, 2020; Santuz et al., 2018), we processed the data to detect touchdown and lift-off of the right-side hindlimb. For touchdown estimation, we used the modified foot contact algorithm developed by Maiwald et al. (2009). For estimating lift-off, we used the paw acceleration and jerk algorithm (Santuz & Akay, 2020; Santuz et al., 2018). We found [LOe – 20 ms, LOe + 20 ms] to be the sufficiently narrow interval needed to make the initial lift-off estimation. Both approaches have been validated against a set of 104 manually-labelled touchdown/lift-off events from four videos of two animals at different speeds (0.2 and 0.3 m s$^{-1}$) and showed a true error within the frame rate of the camera (i.e. $\leq$ 2 ms).

Poincaré maps were produced by mapping the hip-joint angle $\theta_{\text{hip, norm}}$, normalized between the minimum and the maximum of each gait cycle, at touchdown of the gait cycle

![Figure 2. Schematic representation of the perturbation protocol](image-url)

A, the perturbation protocol of 30 s that would repeat itself indefinitely at need. Sudden accelerations (top) and mediolateral displacements (bottom) of the belt are presented as shaded areas. B, detail of the first 5.2 s of the perturbation protocol.
n (x-axis) and at touchdown of the following gait cycle (y-axis). To quantify the dispersion of the obtained map, we used the sum of the descriptors (Brennan et al., 2001; Golińska, 2013) SD1 and SD2, each calculated as:

\[
SD1 = SD\left[\theta_{\text{hip,norm}}(n) - \theta_{\text{hip,norm}}(n + 1)\right] / \sqrt{2}
\]

\[
SD2 = \sqrt{2 \cdot SD\left[\theta_{\text{hip,norm}}(n)\right]^2 - 0.5 \cdot SD\left[\theta_{\text{hip,norm}}(n) - \theta_{\text{hip,norm}}(n + 1)\right]^2}
\]

where SD is the standard deviation of the time series, SD1 is the width of the Poincaré cloud and SD2 is its length.

**EMG resampling**

Given the challenges imposed by the implantation of many muscles (eight), some EMG channels suffered degradation of the signal between different sessions or right after surgery. To avoid issues in the extraction of muscle synergies originating from missing data, we used a bootstrap-like resampling approach as reported previously (Santuz et al., 2019). The most obvious advantage of this approach lies in the substantial reduction of the number of animals that are killed, in accordance with the principles of humane animal research. Moreover, it allows for a broader representation of the mouse system and less of the individual animals because recordings from different mice are pooled together and analysed as if they were acquired on the same animal. Briefly, for each group and condition, we created 1000 resampled data sets each containing eight-muscle EMG data for 30 gait cycles. Each muscle activity in every gait cycle was randomly selected, without replacement, from all the available recorded activities for that group of animals and condition. In the case of perturbed locomotion, we followed the random perturbation protocol and inserted a perturbation every five steps or less (choosing randomly), picking each time from the relevant data set (i.e. if the selected perturbation was a mediolateral displacement to the left, we would only pick from the EMG data sets that contained a mediolateral perturbation to the left for that cycle, etc.). Accordingly, we obtained 1000 trials of 30 gait cycles each for wild-type, unperturbed walking, pre-DCML pathway lesion; another 1000 for Egr3−/−, perturbed walking; and so on, for a total of 12 000 trials, given the 12 analysed conditions.

**Muscle synergies extraction**

Muscle synergies were extracted from the bootstrapped EMG activity through a custom script (R, v4.0.4; R Core Team, 2021; R Foundation for Statistical Computing, Vienna, Austria) based on the R package ‘musclesyneRgies’ (Santuz, 2022) using the classical Gaussian non-negative matrix factorization algorithm as extensively reported previously (Lee & Seung, 1999; Santuz et al., 2017). A schematic representation of the procedure is shown in Fig. 3. The main technical advantage of this linear decomposition approach, as compared to more traditional analysis frameworks, is the compact representation of large amounts of EMG data. Moreover, it is a well-accepted concept in motor control and builds on the seminal ideas of Bernstein (1967), as well as on more recently developed ideas (Tresch et al., 1999), based on the modularity of the central nervous system, which might benefit from strategies similar to linear decomposition to simplify the control over hundreds of muscles and joints via a few common commands (i.e. the muscle synergies). The R package ‘musclesyneRgies’ for the pre-processing of raw EMG data and for the extraction and classification of muscle synergies is available on CRAN – The Comprehensive R Archive Network, together with extensive documentation and examples (Santuz, 2022). The source code version 0.7.1-alpha, as used in the present study, is archived at Zenodo (Santuz et al., 2021).

**Fractal analysis of activation patterns**

To assess the local and global complexity of the bootstrapped activation patterns (Fig. 3D), we calculated the Higuchi’s fractal dimension and Hurst exponent, respectively (Higuchi, 1988; Hurst, 1951). The main advantage of using this kind of metrics lies in their capabilities of (a) explaining features in biosignals that might not be visible to the naked eye and (b) being extremely sensitive to many kinds of internal or external perturbations. The numerical procedures for obtaining both metrics were recently reported in detail (Santuz & Akay, 2020). The Higuchi’s fractal dimension was calculated with a maximum window size of 10 points. The Hurst exponent was calculated following the rescaled range approach (Mandelbrot & Wallis, 1969) with a minimum window length of 200 points. Higuchi’s fractal dimension values range from 1 to 2, with increasing values correlating to increasingly complex data and Higuchi’s fractal dimension \( n = 1.5 \) indicating random Gaussian noise (Anmuth et al., 1994; Higuchi, 1988; Kesić & Spasić, 2016). The Hurst exponent can vary between 0 and 1.
A, eight muscles were implanted in the right hindlimb: MA, gluteus maximus; VM, vastus medialis; VL, vastus lateralis; ST, semitendinosus; BF, biceps femoris; TA, tibialis anterior; GM, gastrocnemius medialis; and GL, gastrocnemius lateralis. B, raw EMG was filtered and time- and amplitude-normalized. C, filtered and normalized.

Figure 3. Schematic representation of the muscle synergies approach and following fractal analysis. A, Cycle A is smoother, locally less complex than B. Activation pattern A is more accurate and globally complex than B. B, Cycle 1 is smoother, locally less complex than B. HFD-cycle A is lower than HFD-cycle B. Activation pattern A is more accurate and globally complex than B. Cycle 1 is smoother, locally less complex than B. HFD-pattern A is lower than HFD-pattern B.
For $0.5 < \text{Hurst exponent} < 1$, in the long-term, high values in the time series (the activation pattern in our case) will be probably followed by other high values and a positive or negative trend is visible (Gneiting & Schlather, 2004; Mandelbrot, 1983). For $0 < \text{Hurst exponent} < 0.5$, in the long term, high values in the series will be probably followed by low values, with a frequent switch between high and low values (Gneiting & Schlather, 2004; Mandelbrot, 1983). Hurst exponent $\approx 0.5$ corresponds to a completely random series (Mandelbrot, 1983; Qian & Rasheed, 2004). In other words, values of Hurst exponent approaching 0.5 from both ends indicate the more complex (or random) behaviour of the time series (Hurst, 1951).

Sham experiments

Three $\text{PV}^{\text{Cre-}}:\text{Rosa}^{\text{EGFP}}$ mice underwent electrode implantation as described above. After recovery, the same recording protocol used for the other mice was performed. Then, the three animals were injected with DTX and, after 7 days, another recording session was completed. Finally, the mice underwent the same surgery as the five lesioned animals, only this time without the actual lesion. The last recording session was then performed after recovery from surgery and the animals were perfused and dissected.

Statistical analysis

To investigate the effects of perturbations and sensory ablation on the kinematics, gait temporal parameters, EMG activity and muscle synergy-related metrics, we followed a Bayesian multilevel modelling approach implemented in the R package $\text{brms}$ 2.17.0 (Bürkner, 2017; Carpenter et al., 2017). For each variable of interest, we built a mixed effects model containing both ‘fixed’ and ‘random’ effects. The constant effects analysed were the locomotion condition (i.e. walking with or without perturbations), the animal group (with pre/post conditions where relevant) and their interaction. In all groups, we added the random effects as a by-animal varying intercept. Each model was run with five independent Markov chains of 10 000 iterations, with the first 4000 warm-up iterations used only for calibration and then discarded, thus resulting in 30 000 post-warm-up samples. Convergence of the chains and sufficient sampling of posterior distributions were confirmed by ensuring a potential scale reduction factor $R^\ast < 1.01$ and an effective sample size of at least 20% of the number of iterations. Accordingly, we obtained the 95% credible intervals for the differences (CrI95%), defined as those intervals around the estimated differences having a 95% probability of encompassing the population value, given the data and the prior assumptions (Nalborczyk et al., 2019). Effect size in the style of Hedges (i.e. considering all the variance sources in the model) were also calculated and reported as $h$. Post hoc effects and the highest posterior density (HPD) intervals were calculated using the R package $\text{emmeans}$ 1.7.3 (Lenth, 2022). We used different priors depending on the investigated parameters and based on the mean and two SDs of previously recorded data. Specifically, we used normal priors with the following means and standard deviations: stance duration $\sim N(115 \text{ ms}, 55 \text{ ms})$; swing duration $\sim N(95 \text{ ms}, 30 \text{ ms})$; cadence $\sim N(625 \text{ steps/min}, 160 \text{ steps/min})$; sum of Poincaré descriptors $\sim N(0.2, 0.2)$; full width at half-maximum $\sim N(65, 17)$; centre of activity $\sim N(85, 30)$; Hurst exponent $\sim N(0.55, 0.15)$. Higuchi’s fractal dimension values often produce target distributions demonstrating features with a resolution that cannot be grasped by the Hamiltonian model used by $\text{brms}$. Accordingly, we used flat priors for the Higuchi’s fractal dimension to avoid getting biased estimates. Because the EMG-related parameters (i.e. $\text{RMS}_{\text{EMG}}$, full width at half-maximum, centre of activity, Higuchi’s fractal dimension and Hurst exponent) were obtained by resampling of the EMG signals as described above, the mixed effects models for these variables were obtained by sampling without replacement 10 random values from the 1000 obtained with the bootstrap-like procedure. This was carried out to calculate the posterior distributions based on samples having the same size as the number of animals (five, two trials each) assigned to each group. Moreover, hindlimb joint angles were compared by evaluating the one-dimensional statistical parametric mapping (Pataky, 2012). All calculations were performed in R, v4.2.0.

Data availability

The datasets generated and analysed during the present study and an exhaustive explanation of their structure are available at the Zenodo repository, together with the code for muscle synergy extraction (Santuz et al., 2021).
Results

Wild-type mice show robust locomotion despite perturbations

First, we investigated the effects of external perturbations on locomotion in wild-type mice (see Supporting information, Movie S1). Typical hindlimb joint angles and muscle activities are presented in Fig. 4. The perturbation protocol detailed in the methods increased the cadence, or number of steps per minute (CrI\textsubscript{95%} = 39–140, \(\delta_1 = 1.86\)) (Fig. 5) and the step-to-step variability of the hip joint angle at touchdown, as shown by the increased spread in the Poincaré maps (CrI\textsubscript{95%} = 0.01–0.17, \(\delta_1 = 0.87\)) (Fig. 6A). Additionally, perturbations reduced the amount of flexion of the knee- and ankle-joints in the early stages of the swing phase (Fig. 6B). The effect of perturbations was not as large on the stance (CrI\textsubscript{95%} = −10 to 7, \(\delta_1 = −0.31\)) and swing (CrI\textsubscript{95%} = −16 to 12, \(\delta_1 = −0.50\)) duration (Fig. 5). The root mean square values of the EMG (RMS\textsubscript{EMG}), an indicator of the signal’s amplitude, were on average higher during perturbed locomotion, but only when perturbations were exclusively mediolateral and administered at regular intervals of 2 s (CrI\textsubscript{95%} = 0.2–1.3, \(\delta_1 = 1.28\)). When perturbations were administered in both the mediolateral and the anteroposterior direction at random time intervals, the effect on the RMS\textsubscript{EMG} was negligible (CrI\textsubscript{95%} = −0.3 to 0.8, \(\delta_1 = 0.32\)) (Fig. 6C).

To uncover the modular structure of muscle activations, we decomposed EMG activity into muscle synergies via non-negative matrix factorization (Bizzi et al., 2008; Lee & Seung, 1999). Muscle synergies are represented by a set of time-invariant muscle weights (describing the relative contribution of each muscle to a specific synergy) and a set of time-dependent activation patterns. Three synergies were sufficient to reconstruct the original EMG signals of both unperturbed and perturbed walking (Fig. 6D). The obtained muscle weights and activation patterns described three main phases of the gait cycle: (1) the weight acceptance, with the major contribution of knee extensors; (2) the propulsion, mostly involving the ankle extensors; and (3) the swing, characterized by the contribution of hip abductors and knee and ankle flexors.

During perturbed locomotion, compared to unperturbed walking, the timing of synergistic activation patterns did not undergo any noteworthy shift, as measured by the centre of activity (weight acceptance: CrI\textsubscript{95%} = −7 to 25, \(\delta_1 = 0.46\); propulsion: CrI\textsubscript{95%} = −6 to 14, \(\delta_1 = 0.33\); swing: CrI\textsubscript{95%} = −2 to 6, \(\delta_1 = 0.48\)) (Fig. 6D). However, all activation patterns were longer, as shown by the increased full width at half-maximum (weight acceptance: CrI\textsubscript{95%} = 10–21, \(\delta_1 = 2.48\); propulsion: CrI\textsubscript{95%} = 7–27, \(\delta_1 = 1.21\); swing: CrI\textsubscript{95%} = 9–17, \(\delta_1 = 2.87\)) (Fig. 6D).

To further investigate the reasons for this widening, we expanded the analysis towards non-linear metrics that could give more information about, for example, an increased variability or regularity of the activation patterns that might have caused the longer activation profiles. The global (Hurst exponent, CrI\textsubscript{95%} = 0.052–0.163, \(\delta_1 = 1.71\)) but not the local (Higuchi’s fractal dimension, CrI\textsubscript{95%} = −0.004 to 0.004, \(\delta_1 = −0.07\)) complexity of activation patterns was affected by perturbations in wild-type mice (Fig. 6E). The local complexity can be seen as a measure of ‘roughness’ (or noise content) in the signal within each gait cycle, whereas the global complexity is a measure of how accurate each cycle’s activation motifs are, when compared with the others in the same trial (Fig. 3D). Activation patterns were less accurate and complex (i.e. higher Hurst exponent) when the animals were challenged by perturbations. An outcome that might explain the widening found with the analysis of the full width at half-maximum. These first observations in wild-type animals could show that perturbations to locomotion in intact animals elicited: (a) higher variability of the hip angle at touchdown and lower flexion of the knee joint in the early swing; (b) similar amplitude of EMG signals; and (c) wider, less accurate and globally less complex activation patterns.

Feedback from muscle spindles regulates locomotion

Next, we set out to investigate the aftermath of genetic removal of one class of proprioceptors: the muscle spindles. We used Egr3\textsuperscript{−/−} mice (Tourtellotte & Milbrandt, 1998), a model in which muscle spindles, but not GTOs, regress after birth (see Supporting information, Movie S2). In those mutant animals, the number of steps per minute was higher than in wild-type (CrI\textsubscript{95%} = 54–240, \(\delta_1 = 1.93\)) and the swing phase was somewhat shorter (CrI\textsubscript{95%} = −33 to 10, \(\delta_1 = −1.14\)), while stance times were similar to those found in wild-type mice (CrI\textsubscript{95%} = −15 to 13, \(\delta_1 = −0.10\)) (Fig. 5). Moreover, in the mutant group kinematics was generally not affected by the presence of external perturbations (Fig. 7B), similar to gait temporal parameters (post hoc; cadence: HPD = −89 to 6; stance duration: HPD = −9 to 11; swing duration: HPD = −8 to 11). Yet, the variability of the hip joint angle at touchdown was higher than in wild-type (CrI\textsubscript{95%} = 0.00–0.28, \(\delta_1 = 0.99\)) and not affected by perturbations (post hoc; HPD = −0.112 to 0.051) (Fig. 7A). The lack of feedback from muscle spindles in Egr3\textsuperscript{−/−} animals did not have an effect on the RMS\textsubscript{EMG} (CrI\textsubscript{95%} = −0.2 to 0.2, \(\delta_1 = 0.06\), similar to the presence of perturbations (CrI\textsubscript{95%} = −0.1 to 0.2, \(\delta_1 = 0.22\)) (Fig. 7C). Yet, mutants showed increased duration of the weight acceptance (CrI\textsubscript{95%} = 6–24, \(\delta_1 = 2.59\)) and swing (CrI\textsubscript{95%} = 1–12, \(\delta_1 = 1.06\)) synergistic activation patterns compared to their wild-type littermates and the main activity shifted later in time in the propulsion (CrI\textsubscript{95%} = 0–25,
Figure 4. Hindlimb joint angles and muscle activities during unperturbed and perturbed locomotion.

A, treadmill speed and mediolateral position, joint angles and rectified raw muscle activities of a representative wild-type mouse, without time-normalisation of the gait cycles. Vertical lines represent the touchdown of each gait cycle. Muscle abbreviations are reported in the legend to Fig. 3.

B, the same as in (A), but for a representative Egr3−/− mouse.

C, the same as in (A), but for a representative PγCre::AvliDTR mouse after DTX injection.

D, the same as in (A), but for a representative wild-type mouse after surgical lesion of the DCML pathway in the spinal cord. [Colour figure can be viewed at wileyonlinelibrary.com]
\[ \delta_t = 0.87 \] and swing activation patterns (CrI95\% = 4–10, \[ \delta_t = 2.07 \]) (Fig. 7D). When exposed to perturbations though, mutants did not tune the timing (\textit{post hoc}; weight acceptance: HPD = −10 to 35; propulsion: HPD = −4 to 21; swing: HPD = −3 to 2), nor the duration (\textit{post hoc}; weight acceptance: HPD = −2 to 15; propulsion: HPD = −13 to 1; swing: HPD = −10 to 1) of activation patterns (Fig. 7D). As in wild-type animals, the roughness (i.e. the local complexity) of activation patterns was not interestingly affected by the lack of feedback from spindles (CrI95\% = −0.007 to 0.000, \[ \delta_t = −0.92 \]) or perturbations (CrI95\% = −0.004 to 0.003, \[ \delta_t = 0.12 \]) (Fig. 7E). However, in contrast to that found in the wild-type, activation patterns in normal locomotion were as accurate (i.e. same global complexity) as those of perturbed locomotion (\textit{post hoc}; HPD = −0.099 to 0.004) (Fig. 7E). In short, this second part of the experiment revealed that the systemic lack of muscle spindles produced: (a) increased variability of kinematics; (b) the inability to modulate the hind-limb kinematics or EMG amplitude in the presence of perturbations; and (c) wider, less accurate (i.e. globally less complex) activation patterns compared to wild-type, with
Figure 6. Gait performance parameters of wild-type mice during unperturbed and perturbed locomotion

A, stick diagrams of one representative animal (left) and Poincaré maps (right) of the hip angle $\theta_{\text{hip}}$ at touchdown ($n$) and ($n + 1$) for all animals, with descriptor ellipse (see Methods). B, average joint angles (thick lines) and SD (shaded bands) for all animals. Vertical shaded areas denote differences detected by statistical parametric mapping. C, average electromyographic activity (thick lines) of the eight recorded muscles and individual trials (thin lines) from all five mice. RMS is normalized to unperturbed walking. Muscle abbreviations are reported in the legend to Fig. 3. D, muscle weights and activation patterns of the three bootstrapped muscle synergies. Weights are presented on a normalized y-axis base. For simplification of the graphs, each point represents 10 nearest neighbours of the 1000 bootstrapped trials. For the activation patterns, the x-axis full scale represents the averaged step cycle (stance and swing normalized to the same amount of points) and the y-axis is the normalized amplitude. Full width at half-maximum (FWHM) and centre of activity (CoA) of activation patterns are reported schematically (right). E, boxplots describing the Higuchi's fractal dimension (local complexity HFD or 'roughness') and Hurst exponent (global complexity H or 'accuracy') of the bootstrapped activation patterns for two perturbation protocols: random (Fig. 2) and one mediolateral displacement every 2 s (ramp). Raw data points (each point represents 10 nearest neighbours of the 1000 bootstrapped trials) and their density estimates are presented to the right of each boxplot. [Colour figure can be viewed at wileyonlinelibrary.com]
Figure 7. Gait performance parameters of Egr3−/− mice during unperturbed and perturbed locomotion
A, the descriptor ellipses in the Poincaré maps are overlaid to those of wild-type (Fig. 6). B–D, control is wild-type. The RMS in (B) is normalized to unperturbed walking of Egr3−/−. For all other details of A–E, see the legend to Fig. 6. [Colour figure can be viewed at wileyonlinelibrary.com]
Acute ablation of muscle spindles and GTOs severely disrupts locomotion

The genetic removal of muscle spindles from birth can nevertheless raise the question of whether the animals could adapt to the partial lack of sensory feedback during the first weeks of life. Moreover, the force-sensitive GTOs are known to be important players in the control of locomotion (Donelan et al., 2009; Grillner & El Manira, 2020), but the Egr3<sup>−/−</sup> model only targets muscle spindles. Lastly, the Egr3 transcription factor is widely expressed in the nervous system and its mutation might disrupt the development and function of other sensory and motor circuit elements. To tackle these three potential issues, we acutely and selectively ablated muscle spindles and GTOs in adult mice by systemic injection of diphtheria toxin in PV<sup>Cre::Avil IDTR</sup> mice (see Supporting information, Movie S3), as previously performed by Takeoka & Arber (2019).

Before injection, these mice behaved largely similar to wild-type animals (Fig. 10). After injection however, they underwent major degradation of sensory neurons resulting in severely disrupted kinematics, largely independently on the presence of perturbations (Figs 5 and 11A and B; see also Supporting information, Movie S3). Specifically, the step-to-step variability of the hip joint angle at touchdown increased dramatically as compared to pre-injection recordings (CrI<sub>95%</sub> = 0.09–0.25, δ<sub>t</sub> = 1.78) and the ankle joint was less flexed throughout almost all of the stance phase (Fig. 11B). Moreover, there was a large effect of sensory ablation on the cadence (CrI<sub>95%</sub> = −193 to −48, δ<sub>t</sub> = −1.45) and on the stance (CrI<sub>95%</sub> = 46–103, δ<sub>t</sub> = 2.77) and swing duration (CrI<sub>95%</sub> = 7–40, δ<sub>t</sub> = 1.30) (Fig. 5). The RMS<sub>EMG</sub> was on average higher after injection (CrI<sub>95%</sub> = 0.0–0.70, δ<sub>t</sub> = 1.01) and unaffected by perturbations (CrI<sub>95%</sub> = −0.3 to 0.4, δ<sub>t</sub> = 0.11) (Fig. 11C). Although the number and function of muscle synergies (Fig. 11D) were not influenced by acute sensory ablation, the timing of synergistic activation patterns was indeed. When comparing pre- and post-injection recordings, we detected a main effect on the timing of activation, which was shifted to the right (CrI<sub>95%</sub> = 38–52, δ<sub>t</sub> = 5.65) and of shorter duration (CrI<sub>95%</sub> = −14 to 2, δ<sub>t</sub> = −0.81) in the propulsion synergy (Fig. 11D). All the remaining main effects were small and interactions had large effect size in

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**Figure 8. Acute ablation of proprioceptive afferents with diphtheria toxin (DTX)**

Dorsal root ganglia (left) and spinal cord (right) sections taken at the fourth lumbar spinal segment (L4) in wild-type (WT) and mutant (PV<sup>Cre::Avil IDTR</sup>) mice. Runt-related transcription factor 3 (RUNX3) is selectively expressed in intact proprioceptive sensory neurons located in dorsal root ganglia. Vesicular glutamate transporter 1 (VGLUT1) is expressed in central sensory terminals. After DTX injection in PV<sup>Cre::Avil IDTR</sup> mice, the proprioceptive neuron terminals are eliminated at all spinal levels, leaving the fewer in number corticospinal and non-proprioceptive sensory terminals unaffected. [Colour figure can be viewed at wileyonlinelibrary.com]
all synergies for both duration and main peak activity, a sign of cross-over interaction or opposite effects in the two groups. Similar to that observed in Egr3−/− mice, the acute disruption of feedback from proprioceptors produced less accurate and complex (CrI95% = 0.065–0.156, δt = −2.08) activation patterns (i.e. higher Hurst exponent), once more making unperturbed and perturbed locomotion numerically indistinguishable (post hoc; HPD = −0.035 to 0.062) (Fig. 11E). In addition, we found that the sensory ablation was followed by a large increase in the roughness of activation patterns (CrI95% = 0.06–0.079, δt = 6.81) (Fig. 11E). In summary, the acute ablation of both muscle

Figure 9. Sham experiments for diphtheria toxin (DTX) injections and dorsal column-medial lemniscus pathway lesion (DCMLL)

A, for the sham experiments, three animals underwent both DTX injection and DCMLL surgery (lesion excluded). Protein kinase C gamma (PKCG) is expressed in the axons of the corticospinal tract (CST). Here, both rostral (first thoracic spinal segment T1) and caudal (fourth lumbar spinal segment L4) sections with respect to the sham surgery site show intact CST. Runt-related transcription factor 3 (RUNX3) is selectively expressed in intact proprioceptive sensory neurons located in dorsal root ganglia. After DTX injection in sham mice that do not express human DTX receptors, the proprioceptive neuron terminals are left intact.

B, stance (left) and swing (right) phase duration for each step recorded in every animal used for the sham experiments. Individual step values, pictured as dots and their distributions are shown next to the relevant boxplot. C, the 95% credible intervals and their probability distributions (shaded areas) describe the effects and interaction of the investigated conditions and perturbations administered during locomotion on the stance duration (left), swing duration (middle) and cadence (right). Effect size in the style of Hedges (i.e. considering all the variance sources in the model) are shown on the graphs and called δt. D, the same as in (C), but for the two Poincaré map descriptors (SD1 and SD2). E, the same as in (C), but for the root mean square of the electromyographic signals (RMSEMG). [Colour figure can be viewed at wileyonlinelibrary.com]
Figure 10. Largely similar behaviour of wild-type and PVCre::AviliDTR mice, before diphtheria toxin injection

A, the 95% credible intervals and their probability distributions (shaded areas) describe the effects and interaction of the investigated groups and perturbations administered during locomotion on the stance duration (left), swing duration (middle) and cadence (right). Effect size in the style of Hedges (i.e. considering all the variance sources in the model) are shown on the graphs and called $\delta_t$. B, Poincaré maps (left) of the hip angle $\theta_{\text{hip}}$ at touchdown.
spindles and GTOs induced: (a) increased kinematic variability and EMG amplitude and the inability to modulate them in the presence of perturbations and (b) rougher, less accurate and globally less complex activation patterns, with substantial reorganization of timing.

**Pivotal role of supraspinal integration in the control of challenging locomotion**

The neurophysiological changes genetically induced in *Egr3*−/− and *PvCrCre::AvilDTR* animals were affecting both the spinal, as well as the ascending proprioceptive information conduction, thus hindering any reasoning on the specific contribution of supraspinal proprioceptive integration to locomotion. To overcome this last hurdle in our experimental design, we surgically lesioned the dorsal column in wild-type mice to disrupt proprioceptive information projecting to the brain through the DCML and partly the dorsal spinocerebellar pathways (see Supporting information, Movie S4). After lesion, the animals did not show notable changes in either the kinematics (hip angle variability at touchdown: CrI95% = −0.04 to 0.11, δt = 0.37), gait temporal parameters (cadence: CrI95% = −43 to 49, δt = 0.01; stance duration: CrI95% = 3–24, δt = 1.47; swing duration: CrI95% = −18 to 3, δt = −0.66) (Fig. 5) or EMG amplitude (CrI95% = −0.3 to 0.2, δt = −0.12) compared to pre-lesion recordings (Fig. 12A, B and C; see also Supporting information, Movie S4). The number and function of synergies was not affected by the lesion either (Fig. 12D). Yet, the timing of synergistic activation patterns underwent interesting modifications. First, in lesioned animals there was a negligible effect of perturbations on both the timing (*post hoc*; weight acceptance: HPD = −18 to 10; propulsion: HPD = −9 to 7; swing: HPD = −5 to 0) and the duration (*post hoc*; weight acceptance: HPD = 0–9; propulsion: HPD = −17 to 0; swing: HPD = −7 to 2) of the main activity, in contrast to that occurring before the lesion. Moreover, the activation patterns during unperturbed and perturbed locomotion were similarly accurate (*post hoc*; HPD = −0.032 to 0.050) and the values resembled those observed for unperturbed walking in intact animals (*post hoc*; HPD = −0.016 to 0.072) (Fig. 12E). Surprisingly, three out of five animals would not manage sudden accelerations of the treadmill belt, interrupting locomotion immediately after the stimulus was administered. This outcome did not correlate with the extent of the lesion, which was checked for accuracy after each surgery through immunohistochemistry as illustrated in Fig. 1. In summary, with this last part, we could show that inhibiting the flow of proprioceptive information to the brain in wild-type animals caused minimal changes to unperturbed locomotion but almost completely hindered the ability to interact with perturbations, leaving the animals unable to tune the motor output and produce robust, uninterrupted locomotion.

**Discussion**

We designed a simple experiment to assess whether the murine brain integrates proprioceptive information from the hindlimb through the DCML pathway during locomotion. Using a mix of mouse genetics, *in vivo* electrophysiology and a spinal lesion model, we showed that supraspinal integration of signals from proprioceptors is crucial for locomotion in the presence of perturbations. This implies that spinal proprioceptive circuits alone are not sufficient to guarantee effective responses to external perturbations during locomotion.

**The systemic removal of proprioceptors undermines locomotor robustness**

As previously reported (Mayer & Akay, 2018; Santuz et al., 2019), wild-type mice are exceptional at coping with external perturbations during locomotion. Our results showed that the neural strategies adopted to safely overcome sudden mediolateral displacements and accelerations produced lower joint flexion and increased kinematic variability. Despite similar amplitudes of the EMG signals, the synergistic activation patterns were tuned in response to perturbations. Specifically, as usually found in both mice (Santuz et al., 2019) and humans (Cappellini et al., 2016; Martino et al., 2014; Santuz et al., 2018), time-dependent activation motifs (i.e. activation patterns) were wider relative to the gait cycle in the presence of perturbations, a modulation that makes locomotion robust. In addition, activation patterns were substantially less accurate and complex in perturbed than in normal walking, similar to that previously found in mice (Santuz & Akay, 2020) and humans (Santuz, Brüll et al., 2020) locomoting in a similar experimental environment. As expected, most of these observations
Figure 11. Gait performance parameters of PVCre::AvilDTR mice during unperturbed and perturbed locomotion

A, the descriptor ellipses in the Poincaré maps are overlaid to those of pre-DTX injection recordings. B–D, control is pre-injection. The RMS in (B) is normalized to unperturbed walking recorded pre-injection. For all other details of A–E, see the legend to Fig. 6. [Colour figure can be viewed at wileyonlinelibrary.com]
Figure 12. Gait performance parameters of wild-type mice during unperturbed and perturbed locomotion after DCML pathway lesion. A, the descriptor ellipses in the Poincaré maps are overlaid to those of wild-type, pre-lesion (Fig. 6). B–D, control is pre-lesion. The RMS in (B) is normalized to unperturbed walking recorded pre-lesion (Fig. 6). For all other details of A–E, see the legend to Fig. 6. [Colour figure can be viewed at wileyonlinelibrary.com]
changed when feedback from proprioceptors was either genetically inhibited from birth or acutely removed in adult mice.

The major disruption of locomotor patterns in Egr3−/− (Tourtellotte & Milbrandt, 1998) and PVCre::AvilDTR (Takeoka & Arber, 2019) mice is well known. Egr3−/− lack muscle spindles from birth and typically show gait ataxia (Tourtellotte & Milbrandt, 1998), especially to be seen in the timing of the ankle joint flexors during the swing phase (Akay et al., 2014). By contrast, PVCre::AvilDTR mice are born intact and only after acute ablation of feedback from both muscle spindles and GTOs they undergo a swift and irreversible degradation of locomotor capabilities (Takeoka & Arber, 19), which is by far worse than that observed in Egr3−/− mutants. Here, we built up on previous research further characterising kinematics and muscle activation patterns during locomotion with and without perturbations. We found that both mouse lines showed kinematics and muscle activity patterns that were numerically undistinguishable when comparing unperturbed and perturbed locomotion, showing the lack of ability to adapt to external perturbations. Moreover, the vast majority of analysed metrics had values that resembled those found in the wild-type during perturbed locomotion. When compared to controls (i.e. wild-type for Egr3−/− and pre-ablation recordings for PVCre::AvilDTR), the outcomes were all similar: increased variability of kinematics and wider, less accurate activation patterns, independently on whether perturbations were administered or not. This indicates an inherently disrupted control of locomotion because of the systemic lack of feedback from proprioceptors, independently on the external challenges.

Although Egr3−/− and PVCre::AvilDTR mice shared many of the outcomes, a few peculiarities were found in the latter that were not visible in the former. The dramatic effects on the timing of propulsion in PVCre::AvilDTR, which were not evident in Egr3−/− mice, might be explained by the absence of feedback from GTOs. It has been previously shown that mice lacking muscle spindles but not GTOs exhibit increased ankle flexion and longer ankle flexor activity during the swing phase, with little to no effects on the stance phase (Akay et al., 2014). During stance, GTOs in the ankle extensors signal the level of loading in the limb and dictate the transition to the swing phase, in which they become mostly silent (Duyssens & Pearson, 1980; Pearson, 2008; Prochazka et al., 1989). Taken together, these two observations might explain the reason for finding most of the time-related disruption in the propulsion synergy in PVCre::AvilDTR rather than in Egr3−/− mice. Moreover, experiments in the cat during unperturbed walking (Donelan et al., 2009) showed that force feedback is responsible for around one third of the total muscle activity in the flexors of the ankle, muscles that are mostly contributing to the propulsion synergy. This share increases with the task demand (Donelan et al., 2009) and/or the length at which muscle fascicles work (Donelan & Pearson, 2004) and could thus additionally explain the effect of concurrent spindle and GTO removal on the timing of the propulsion synergy. The sensory ablation in PVCre::AvilDTR mice was also followed by a large increase in the roughness of activation patterns. However, rather than the lack of GTOs, this might be a result of the known effect of speed on the Higuchi’s fractal dimension (Santuz, Ekizos et al., 2020): after injection, the animals could not cope with pre-injection speeds because of the remarkable loss of locomotor robustness and had to be tested at around half those speeds (0.15 instead of 0.25 and 0.30 m s−1), also resulting in longer cycle times and lower cadence.

**Ascending proprioceptive pathways in the dorsal column carry crucial information for locomotion in challenging conditions**

Surgical lesion of dorsal column in wild-type mice allowed us to interrupt a major part of the proprioceptive information flowing from the hindlimb to the brain, leaving all spinal circuits intact. The murine DCML pathway is composed, caudal to the sixth thoracic segment, of two tracts in the dorsal column of the white matter (Niu et al., 2013): the gracile fasciculus (in the dorsal region) and the corticospinal tract (in the ventral region). Given its extremely dorsal location (Watson & Harrison, 2012), the dorsal column can be easily exposed during surgery. The lesion disrupts the ascending information to the brainstem from proprioceptors and exteroceptive touch sensors, mostly from hindlimb proprioceptors when surgery is carried out on the lower thoracic spinal segments (Niu et al., 2013). This surgery, however, can partially or fully damage the corticospinal tract (mainly connecting the motor cortex to the spinal cord) as its projection is just ventral to the ascending projections in the dorsal column (Lieu et al., 2013). Moreover, the dorsal spinocerebellar tract is left mostly intact because this pathway projects, rostral to the sixth thoracic spinal segment, through the lateral funiculus to the cerebellum (Mann, 1973; English, 1985).

In unperturbed locomotion, the lesion did not produce notable changes in the kinematics, EMG amplitude or activation pattern timings compared to data collected before surgery in the same animals. This is in agreement with previous findings after dorsal column lesion in the cat (English, 1980, 1985). Yet, a striking fact emerged when administering external perturbations: the animals behaved almost identically as in the unperturbed state, leading to similarities in the vast majority of the analysed parameters, from kinematics to the complexity of muscle activation patterns, as summarised in Fig. 13.
In other words, lesioned animals were unable to discern the perturbed state from the unperturbed and this can be confirmed by recalling the observations made in proprioception-deficient mutants. Both $Egr3^{-/-}$ and $PV_{Cre::AvilIDTR}$ mice showed some adaptation to the partial or total lack of proprioceptive information, which was visible in the constant, perturbation independent: (a) an increase in the variability of kinematics; (b) an increase in the relative duration of activation patterns; and (c) a decrease in the accuracy of activation patterns. Similar adaptations were found in spinal cats, where the albeit limited compensation capabilities of spinal circuits (Grillner & Zangger, 1979) produced modifications in both kinematics and muscle activation patterns in response to perturbations (Zhong et al., 2012). By contrast, lesioned wild-type mice did not substantially modify any of the aforementioned parameters, indicating a major absence of adaptation that even led, in three out of five animals, to abrupt stops on the treadmill right after a sudden acceleration of the belt. It follows that the system failed to maintain function and thus robustness (defined above as the ability to maintain the system’s function despite perturbations or errors of execution). Hence, our results reveal a key principle in supraspinal somatosensory processing: proprioceptive information from the DCML pathway is absolutely crucial to guarantee robust locomotion in challenging settings. Most probably, even though not supported by our data, the first crucial supraspinal elaboration of proprioceptive information already occurs in the brainstem. Direct ascending proprioceptive afferents convey in the cuneate and gracile nuclei of the dorsal column. In the cat, it has been shown that intra-cuneate networks are responsible for the potentiation of proprioceptive feedback from the forelimb and that cuneate cells project to the reticular formation and the mesencephalic locomotor region (Leiras et al., 2010), with both known to have key roles in the initiation, control and termination of locomotion (Caggiano et al., 2018; Cregg et al., 2020; Grillner & El Manira, 2020; Leiras et al., 2022). To further elucidate the specific roles of the aforementioned structures in the regulation of proprioceptive information during locomotion, additional experiments are certainly needed.

**Limitations and future directions**

Lesions of the DCML pathway were carried out at the ninth thoracic segment. Thus, we cannot exclude that some dorsal spinocerebellar tract fibres were damaged because it has been shown in the cat that the dorsal spinocerebellar tract neurons disappear completely from the dorsal column only at the sixth thoracic segment (English, 1985). However, the dorsal spinocerebellar tract also carries proprioceptive information from the hindlimb, and thus our main goal of hindering ascending proprioceptive pathways would not be affected by a partial lesion of it. Moreover, both the DCML pathway and the dorsal spinocerebellar tract are known to additionally carry information from exteroceptors. Specifically, the DCML carries information from $A_{β}$ low-threshold rapidly-adaptive mechanoreceptors (Niu et al., 2013), whereas the dorsal spinocerebellar tract carries a mixture of touch and pressure signals (Hantman & Jessell, 2010; Lundberg, 1964). This should not substantially modify our conclusions because, although cutaneous feedback is

**Figure 13. Perturbation-induced effects on locomotor kinematics and muscle activation patterns**

A, the descriptor ellipses of the Poincaré maps express the variability of the hip joint angle at touchdown. Bigger ellipses correspond to higher variability and vice versa. WT, wild-type; $Egr3^{-/-}$, ablation of exclusively muscle spindles from birth; $PV_{Cre::AvilIDTR}$, acute ablation of both muscle spindles and Golgi tendon organs in the adult; WTDCML, lesion of the ascending proprioceptive pathways in the wild-type dorsal column. $H$, the Hurst exponent describes the global complexity or ‘accuracy’ of muscle activation patterns. Only in WT was a modulation numerically distinguishable and, in WTDCML, the values were not affected by perturbations and were similar to those found in unperturbed locomotion in WT. [Colour figure can be viewed at wileyonlinelibrary.com]
important for perturbed locomotion, it has been shown that it is not required for the generation of the basic locomotor activity (Bolton & Misiaszek, 2009; Rossignol et al., 2006).

Muscle synergy analysis does not commonly involve data resampling as we performed in the present study, as well as previously (Santuz et al., 2019). The unusual choice of extracting synergies from muscle activities coming from different animals was taken mainly to avoid discarding data from mice showing faulty EMG channels for disparate reasons (e.g. faulty implantation, postoperative electrode migration, recording artefacts, etc.). However, this approach has some downsides. The resampling shuffles muscle activity recorded in different gait cycles and might thus hide some of the effects occurring at time scales larger than one gait cycle. Similarly, the postprocessing mixes together muscle activities coming from different animals, potentially hiding animal-specific synergistic interactions. Nonetheless, this approach provides us with the tremendous advantage of reducing the number of animals needed to conduct the analysis at the same time as still allowing for comparisons between groups.

Stopping behaviours such as those found in wild-type animals after lesion of the DCML pathway are still poorly understood. Three conditions can lead to halting locomotion: goal completion, fear and startle (Roseberry & Kreitzer, 2017). With the results of the present study, we cannot directly confirm the origin of this behaviour and can only speculate that the animals in our experiment probably stopped because fear and/or startle, rather than to complete a goal. There is strong evidence that the arrest of locomotor activity originates from supraspinal circuits (Roseberry & Kreitzer, 2017). In mice, the optogenetic activations of glutamatergic excitatory neurons in the reticular formation (Bouvier et al., 2015) or glycinergic inhibitory neurons in the caudal part of the brainstem (Capelli et al., 2017) have been shown to effectively suppress locomotion. Those regions of the brainstem have synaptic interactions with the mesencephalic locomotor region that, if electrically or optogenetically stimulated, can arrest locomotion in the lamprey (Gräch et al., 2019) and in the mouse (van der Zouwen et al., 2021), respectively. Only further research will identify whether proprioceptive afferent input can directly or indirectly halt locomotion through any of these brainstem regions.

**Conclusions**

In conclusion, we showed that the systemic removal of proprioceptors in mice resulted in impaired locomotion proportionally to the level of ablation (i.e. of only muscle spindles or of both spindles and GTOs). This confirms that sensory information from proprioceptors is needed to cope with external perturbations and that the lack of sensory feedback has direct implications on the behaviour of the animal, negatively affecting movement and making muscle activation patterns less accurate (e.g. less complex) and adaptable. Moreover, the surgical interruption of ascending pathways carrying proprioceptive information to the brainstem through the spinal cord compromises the ability of wild-type mice to tune the motor output and cope with perturbations: a demonstration that supraspinal integration of sensory information from proprioceptors is crucial to ensure robust locomotion.

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Additional information

Data availability statement

The datasets generated and analysed during the current study and an exhaustive explanation of their structure are available at the following Zenodo repository: https://doi.org/10.5281/zenodo.4724765.

Competing interests

The authors declare that they have no competing interests.

Author contributions

AS and TA were responsible for study conceptualization. AS was responsible for data curation; formal analysis; software; visualization; and writing – original draft. AS and ODL were responsible for study investigations. TA was responsible for study supervision. AS, ODL and TA were responsible for writing – review & editing.

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Keywords

locomotion, muscle synergies, perturbations, proprioception, sensory feedback

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Statistical Summary Document
Peer Review History
Movie S1
Movie S2
Movie S3
Movie S4

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