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RESEARCH ARTICLE

Stumbling corrective reaction elicited by mechanical and electrical stimulation of the saphenous nerve in walking mice

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ABSTRACT

The ability to walk around in a natural environment requires the capacity to cope with unexpected obstacles that may disrupt locomotion. One such mechanism is called the stumbling corrective reaction (SCR) that enables animals to step over obstacles that would otherwise disturb the progression of swing movement. Here we use *in vivo* motion analysis and physiological recording techniques to describe the SCR in mice. We show that SCR can be elicited consistently in mice during locomotion by inserting an obstacle along the path of leg movement during swing phase. Furthermore, we show that the same behavior can be elicited if the saphenous nerve, a cutaneous nerve that would detect contact of the leg with an object, is stimulated electrically. This suggests that cutaneous afferent feedback is sufficient to elicit SCR. We further show that the SCR is phase dependent, occurring only with stimulation during swing phase, but not during early stance. During SCR elicited by either method, the foot is lifted higher to clear the object by flexing the knee, via the semitendinosus muscle, and ankle joint, by tibialis anterior contraction. The tibialis anterior also exhibits a brief extension before flexion onset. Our data provide a detailed description of SCR in mice and will be crucial for future research that aims to identify the interneurons of the premotor network controlling SCR and its neuronal mechanisms by combining motion analysis, electrophysiology and mouse genetics.

KEY WORDS: Spinal circuitry, Electromyogram, Kinematics, Mice, Motor behavior

INTRODUCTION

Animals have the ability to adapt stepping movements to perturbations that would otherwise disrupt locomotion. For example, an unexpected obstacle intercepting the normal path of the foot without visual perception requires the central nervous system (CNS) to respond with a 'stumbling corrective reaction/response' (SCR) (Forssberg et al., 1975, 1977; Prochazka et al., 1978; Forssberg, 1979). Although SCR has been described in detail using the cat as the animal model (Quevedo et al., 2005a,b; McVea and Pearson, 2007) as well as in humans (Potocanac et al., 2016), information regarding the spinal circuits mediating this reflex remain obscure. This is at least in part due to a lack of SCR studies in genetically tractable animal models, such as the mouse. In this article, we provide a comprehensive analysis of the SCR in mice *in vivo*, elicited by either mechanically perturbing the leg during swing phase or by electrical stimulation of the saphenous nerve during stepping on a treadmill. This work will serve as a basis for future research to understand the premotor interneuronal network that controls the SCR.

The typical locomotor behavior of terrestrial mammals is achieved by rhythmic and coordinated movement of two (bipeds) or four legs (quadrupeds) with multiple joints (Grillner, 1981). The rhythmic stepping movement of the legs is divided into stance and swing phases. Throughout the stance phase, the foot is on the ground, carries the body weight and provides propulsion. As the body moves forward, the foot moves backward relative to the body, beginning from an anterior extreme position (AEP) towards the posterior extreme position (PEP) (Cruse et al., 1998). Once the foot reaches the PEP at the end of stance phase, it lifts off the ground and moves forward (swing phase) to reach the AEP to begin the next stance phase. Whenever an object collides with the leg during swing phase, cutaneous mechanoreceptors are activated, eliciting a flexor response that moves the foot higher to clear the obstacle (Wand et al., 1980). However, if this same perturbation occurs during a stance phase, no flexor response is triggered but an enhanced extensor activation is observed (Forssberg et al., 1977; Forssberg, 1979). This phenomenon shows an example of reflex reversal: that is when identical stimuli cause opposite effects depending on the context of the stimulation (Duysens et al., 1990; Pearson and Collins, 1993).

Traditionally, insights into the neural control of SCR have been provided by kinematic and electromyographic (EMG) analyses of the step cycle, with a particular focus on the cat hindlimb (Doperalski et al., 2011). By using the cat as an animal model, it has been shown that stimulation of cutaneous afferents is sufficient to elicit SCR (Wand et al., 1980; Buford and Smith, 1993; Quevedo et al., 2005a). There is further evidence that the network controlling the SCR is located within the spinal cord, as SCR can be elicited in spinalized cats (Forssberg et al., 1977). Electrical stimulation of the superficial peroneal nerve, activating cutaneous afferent fibers that otherwise would signal obstacle touch on the dorsum of the paw, have been shown to elicit a motor response closely resembling the SCR. These experiments have been done during fictive locomotion in the absence of any physical movement, and consequently movement related (phasic) sensory feedback (Quevedo et al., 2005a). Because of the lack of any physical movement in these experiments, Quevedo et al. (2005a) named these reactions 'fictive' SCR. Furthermore, the same authors performed intracellular recordings from different motor neurons during fictive SCR to infer that di-, oligo- and polysynaptic pathways mediate SCR (Quevedo et al., 2005b). In addition, SCR with similar characteristics has been characterized in humans (Schillings et al., 1996). As in cats, electrical stimulation of the saphenous nerve elicits SCR in a phase-dependent manner in humans (Van Wezel

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et al., 1997; Zehr et al., 1997). We have gained considerable insights into the neuronal mechanisms that control SCR from experiments on humans and cats, but a clear understanding of the spinal interneuronal network is lacking.

In recent years, mice have become a preferred animal model for studying locomotion because of the possibilities of genetic manipulation of the neural circuits, and capability for measuring its effect on locomotor behavior in either *in vivo* or *in vitro* experiments (Goulding, 2009; Grillner and Jessell, 2009; Kiehn, 2016). By combining genetics and behavioral observations, a recent study identified a group of interneurons, distinguished by selective retinoid-related orphan receptor (ROR) alpha expression that were important for corrective reflex movements during walking on a narrow beam (Bourane et al., 2015b). Furthermore, many more interneurons have been identified based on gene expression patterns that are interconnected between sensory afferents and motor neurons (Alvarez et al., 2005; Zhang et al., 2008; Zagoraiou et al., 2009; Bui et al., 2013; Bourane et al., 2015a; Hilde et al., 2016; Koch et al., 2017). Nevertheless, it is not known whether any of the previously identified interneurons are part of the neuronal network underlying the SCR. This is mainly because methods to elicit SCR in freely behaving mice have not been available. A reliable method to trigger SCR in mice would allow experiments in combination with mouse genetics to identify the neuronal circuit underlying the SCR.

In this study, we set out to investigate SCR in mice. Leg movements during normal stepping and SCR were measured, in mice stepping on a treadmill, by using kinematic methods along with recordings of EMG muscle activity pattern from multiple muscles (Akay et al., 2014). The SCRs were elicited either by inserting a rod along the pathway or electrically stimulating a cutaneous nerve, the saphenous nerve (SPN), during the swing phase. Both stimulations were sufficient to modify the swing movement, consistent with the SCR. Furthermore, the EMG activation patterns of muscles during SCR in mice were similar to the pattern previously described in cats (Forssberg, 1979; Wand et al., 1980; McVea and Pearson, 2007), where increased flexor muscle activation lifted the leg over the rod. In addition, when the electrical stimulation of the saphenous nerve was delivered during stance phase, activation of the flexor muscle was absent, congruent with the cat experiments (Forssberg, 1979). Our data will set the stage for future research that will combine kinematic measurements and EMG recordings with mouse genetic manipulation to gain insights into neuronal control mechanisms of SCR.

MATERIALS AND METHODS

Animals

Experiments were carried out on nine C57Bl6/J wild-type adult mice of either sex, with ages ranging from 74 to 141 days (Table 1).

Table 1. Number of control cycles and stumbling corrective responses involved in the data analysis in this study

Mechanical SCR			Electrical SCR		
Mouse ID	Control cycles	SCR	Mouse ID	Control cycles	SCR
M1 (m)	24	8	M5 (m)	21	7
M2 (m)	48	16	M6 (m)	54	18
M3 (f)	90	30	M7 (f)	39	13
M4 (f)	30	10	M8 (f)	66	22
			M9 (f)	87	29
Total	192	64	Total	267	89

m, male; f, female; ID, identity of individual mouse.

None of the mice was trained prior to the experiments. All procedures were in accordance with the Canadian Council on Animal Care and were approved by the University Committee on Laboratory Animals at Dalhousie University.

Electrode implantation surgeries

Each mouse underwent electrode implantation surgery as previously described (Akay et al., 2014). Briefly, mice were anesthetized with isoflurane, ophthalmic eye ointment was applied to the eyes, and the skin of the mouse was sterilized with three-part skin scrub using hibitane, alcohol and povidone-iodine. A set of six bipolar EMG electrodes were implanted in all animals (Pearson et al., 2005; Akay et al., 2006) and five animals received an additional nerve stimulation cuff electrode implant (Akay, 2014). Small skin incisions were made to the neck region and to the right hindleg to expose the target muscles and the saphenous nerve (SPN). Electrodes were drawn subcutaneously from the neck incision to the leg incisions, and the head piece connector was stitched to the skin around the neck incision. The EMG recording electrodes were implanted into hip flexor (iliopsoas, Ip) and extensor (anterior biceps femoris, BF), knee flexor (semitendinosus, St) and extensor (vastus lateralis, VL), and ankle flexor (tibialis anterior, TA) and extensor (gastrocnemius, Gs). The cuff electrode was implanted around the saphenous nerve, a nerve carrying cutaneous afferent fibers from the anterior part of the distal hindleg to the spinal cord. The leg incisions were then closed and anesthetic was discontinued. Analgesics (0.03 mg kg^{-1} buprenorphine and 5 mg kg^{-1} ketoprofen) were injected subcutaneously 1 h before surgery to avoid pain. Additional injections were performed at 12 h intervals for 48 h. Mice were housed separately, placed in a warmed cage with a fresh mass of hydrogel for the first 3 days, and then returned to their regular mouse rack. Any handling of the mouse was avoided until mice were fully recovered, and the first recording session started at least 10 days after electrode implantation surgery.

Behavioral recording sessions

Following full recovery from electrode implantation surgery, the behavioral recordings were performed as previously described (Pearson et al., 2005; Akay et al., 2006). Under brief anesthesia with isoflurane, custom-made cone-shaped reflective markers (1–2 mm diameter) were attached to the skin at the level of the anterior tip of the iliac crest, hip, knee, ankle, the metatarsal phalangeal joint (MTP), and the tip of the fourth digit (toe). The anesthesia was discontinued, and the mouse was placed on a mouse treadmill (model MA 102; custom built in the workshop of the Zoological Institute, University of Cologne, Germany). The electrodes were connected to an amplifier (model 102; custom built in the workshop of the Zoological Institute) and to a stimulus insulation unit (Iso-flex). We waited at least 5 min to begin the recording session to allow the mice to fully recover from anesthesia. The mice started stepping on the treadmill when it was turned on. The speed of the treadmill was set to 0.3 m s^{-1}. In the four mice that did not receive the nerve cuff electrode, a custom-made metallic hook was placed in the path of the moving foot during the swing phase to elicit a stumbling corrective reaction. For the other five mice, with the nerve cuff electrode implanted, the saphenous nerve was electrically stimulated with five brief impulses (0.2 ms duration, 500 Hz frequency). Only one recording session was performed with each mouse to avoid a learning effect. Therefore, mice in which SCRs were elicited by mechanical stimulation were different from the mice in which SCRs were elicited by electrical nerve stimulation (Table 1). The strength of the stimulation was set to be 1.2 times the

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current that was necessary to elicit the slightest response in the tibialis anterior muscle during resting, which varied between 96 and 1200 µA from animal to animal. The stepping mouse was filmed from the sagittal plane with a high-speed video camera (IL3, Fastec Imaging) at 250 frames s^{-1}, and video files were stored on a computer for later motion analysis. The EMG data were stored separately on the computer using a digitizer (Power 1401, Cambridge Electronic Design, UK) combined with Spike2 software (version 8, Cambridge Electronic Design).

Data analysis

The kinematic parameters of stepping were obtained from the video files using Vicon Motus (Version 9.2, Vicon Motus Inc.) or custom-made software written by Dr Nicolas Stifani with ImageJ (KinemaJ) and R (KinemaR) (Bui et al., 2016; Fiander et al., 2017). The coordinates and the angular joint movements were then imported into the Spike2 files containing the EMG data in a way that the kinematic and EMG data were synchronized with a custom-written Spike2 script. The data analysis was performed using this final Spike2 file containing the merged EMG and kinematic data. All plots were done using Excel 2016 software, and statistical analyses with the data analysis package for Excel: statistiXL (version 1.8). Comparisons of swing durations and amplitudes during control steps and SCR were performed with a Mann–Whitney test using statistiXL.

RESULTS

Kinematics and muscle activity pattern before gait perturbation

During the stance phase, while the foot was on the ground and the leg supporting the body weight, the foot moved in a caudal direction from the AEP towards the PEP (Fig. 1Ai). At the end of the stance phase, when the leg was extended, the foot reached the PEP and the swing movement began. During the swing, the foot was lifted off the surface and moved forwards with a smooth trajectory to be placed back on the treadmill belt at the AEP (Fig. 1Aii). As the foot advanced its path from the PEP to AEP, it crossed the level of the hip

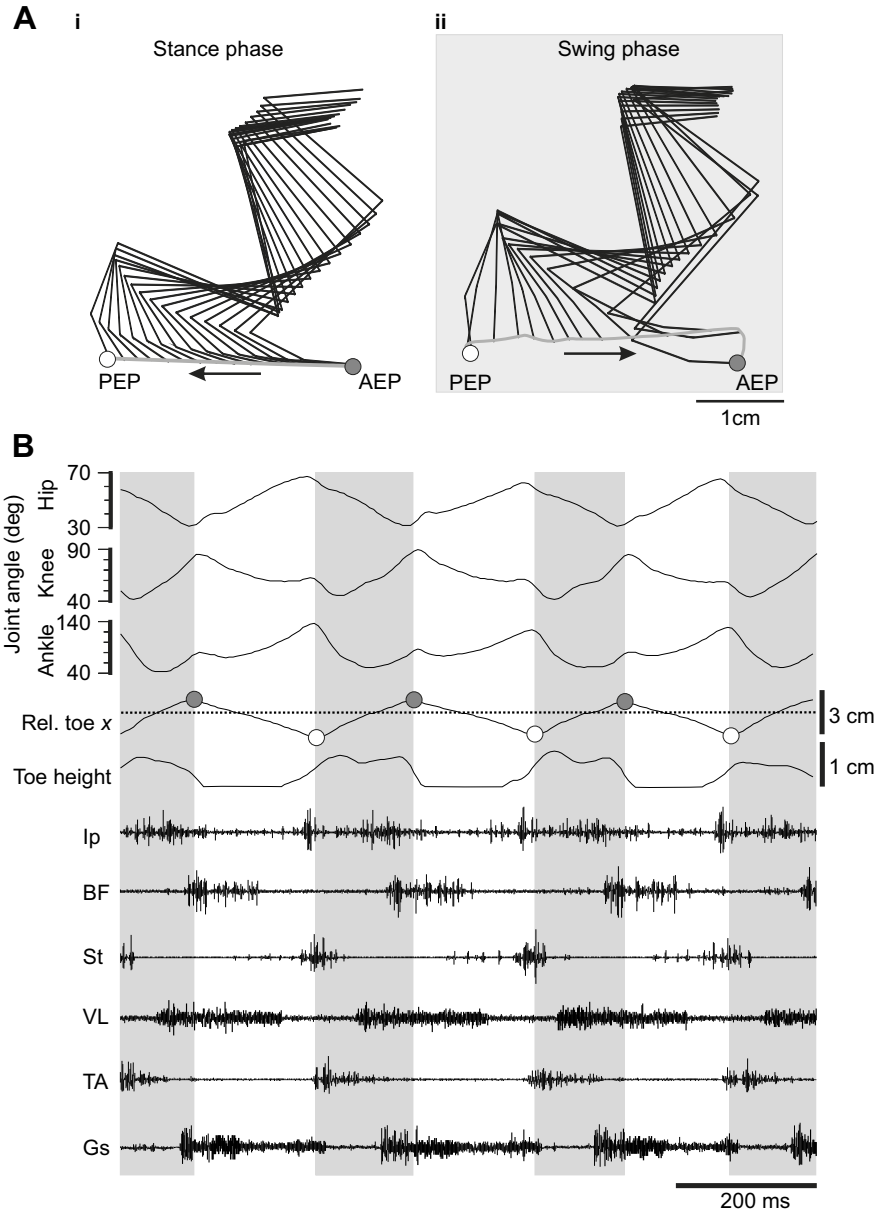

Fig. 1. Kinematics and EMG pattern of stepping mice. (A) Stick reconstruction of one stance (i) and one swing phase (ii). Toe trajectory is indicated by the grey line and the direction of the foot movement is indicated by the black arrows. PEP, posterior extreme position (white circle); AEP, anterior extreme position (grey circle). The slightly elevated position of the AEP and PEP is because the most distal marker is attached to the tip of the fourth digit which is not the most distal portion of the foot. At the beginning and the end of the swing phase, the last marker position slightly elevates before the most distal part of the foot is lifted off or placed on the ground, leading to a slightly elevated position of the AEP and PEP. (B) Joint angles, toe position on the horizontal axis relative to hip (rel. toe *x*; dotted horizontal line indicates hip position), toe height, and raw EMG data from flexor and extensor muscles during an undisturbed stepping sequence that includes four swing phases (shaded background) and four stance phases (white background). Ip, iliopsoas (hip flexor); BF, anterior head of biceps femoris (hip extensor); St, semitendinosus (knee flexor); VL, vastus lateralis (knee extensor); TA, tibialis anterior (ankle flexor); Gs, gastrocnemius (ankle extensor). As in A, PEP and AEP are indicated by white and grey circles, respectively.

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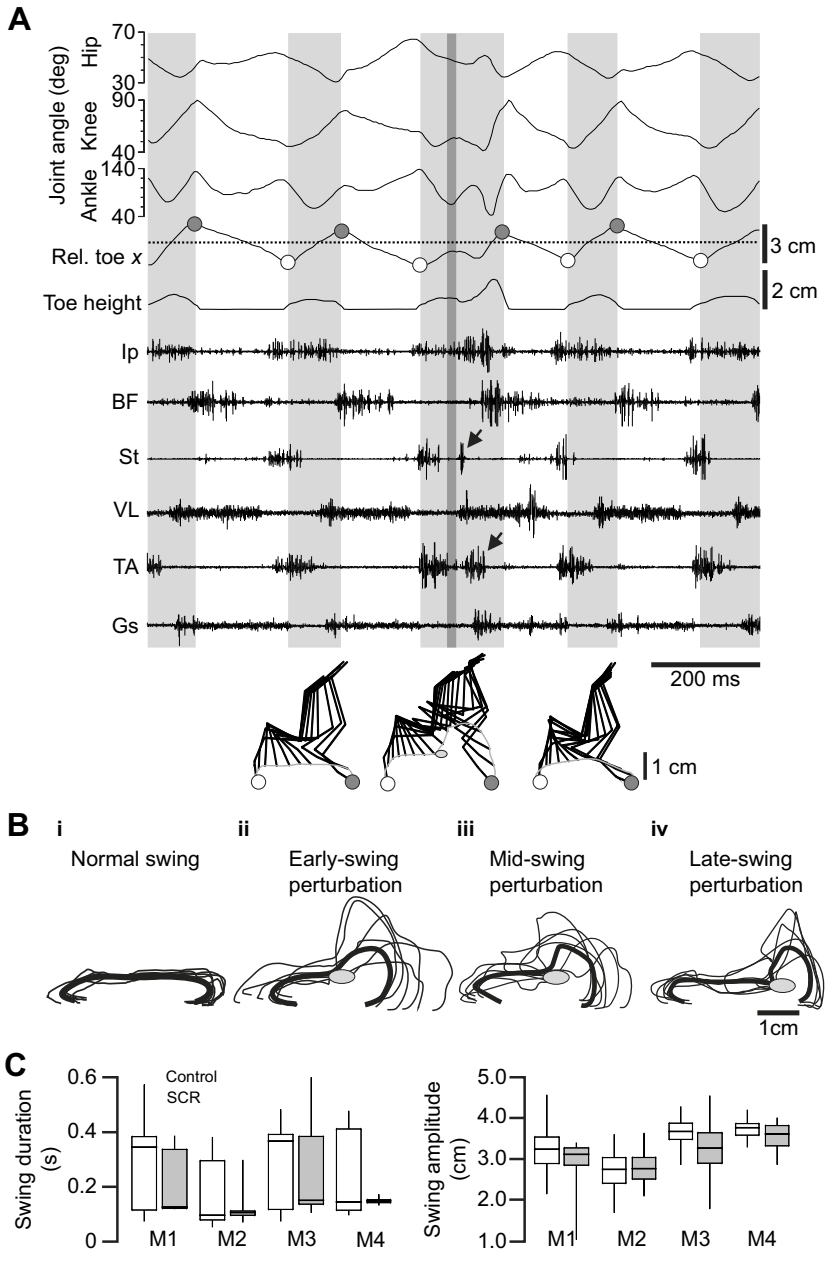

Fig. 2. Stumbling corrective reaction elicited by mechanically perturbing leg movement during swing phase. Kinematics and EMG pattern during a stepping sequence that includes two swing phases (shaded background) before and two swing phases after mechanically evoked stumbling corrective reaction (SCR). (A) Hip, knee and ankle joint angles, rel. toe *x* and *y* coordinates (toe height) synchronized with raw EMG activity of flexor (Ip, St, TA) and extensor (BF, VL, Gs) muscles. Mechanical perturbation of swing phase is represented by the darker grey inside the third swing phase. Arrows point to the activity of knee and ankle flexor muscle initiated by the perturbation. Stick diagram reconstruction of a swing phase before SCR, an SCR, and the swing phase after SCR are illustrated below. (B) Average toe trajectories during control swing phase (i), SCR elicited during early- (ii), mid- (iii) and late-swing (iv). Thin lines indicate toe trajectories from individual trials from one animal and the bold line is the average trajectory. (C) Box and whisker diagrams illustrating average swing duration (left) and average swing amplitude (right) from control unperturbed swing phases (white bars, 24<n<90 swing phases) and SCRs elicited by mechanical perturbation (grey bars, 8<n<30 SCRs) from four mice. None of these comparisons was statistically significant with the Mann–Whitney test. M1 to M4 represent the four individual mice used in this study.

joint at the horizontal axis (Fig. 1B, rel. toe *x*) and landed on average 14±2 mm in front of the hip joint at the AEP. The step cycle was defined as one swing phase with the following stance phase.

The coordinated movement of three leg joints (hip, knee and ankle) during a step cycle was controlled by patterned contraction of multiple flexor and extensor muscles. Angular movement of the hip, knee and ankle joints, synchronized with EMG activity of six muscles throughout four swing phases (shaded background) and three stance phases (white background) are illustrated in Fig. 1B. The kinematic and EMG pattern was very similar to the pattern observed previously (Akay et al., 2014).

Mice respond with a 'stumbling corrective reaction' if swing phase is perturbed with an obstacle

When the hindleg encountered an obstacle (the rod in our experiments) during the swing phase, the foot was lifted higher to clear the obstacle and placed on the AEP without disrupting the ongoing stepping, a response previously described as SCR (Forssberg, 1979) (Fig. 2; Movie 1). In these experiments, mice stepped on the treadmill at a constant speed of 0.3 m s^{-1} and in a random sequence, a rod was manually placed briefly along the path of the leg movement during swing phase. When the leg touched the rod during swing phase, the foot was lifted higher to clear the obstacle, and the swing continued until the foot touched the ground at the AEP (Fig. 2A and B). The lifting of the foot over the obstacle was achieved by an extra burst of activity in the knee and ankle flexor muscles (Fig. 2A, arrows). The SCR could be elicited regardless of whether the swing was perturbed during early-, mid- or late-swing (Fig. 2Bii–iv). Of the perturbed swing phases, neither the swing amplitude (distance between PEP and AEP) (P≥0.674, Mann–Whitney test) nor the duration of the swing phase (P≥0.170, Mann–Whitney test) changed compared with the unperturbed swing phases (Fig. 2C). These data indicated that SCR can be elicited in mice, as previously described in cats (Forssberg et al., 1977;

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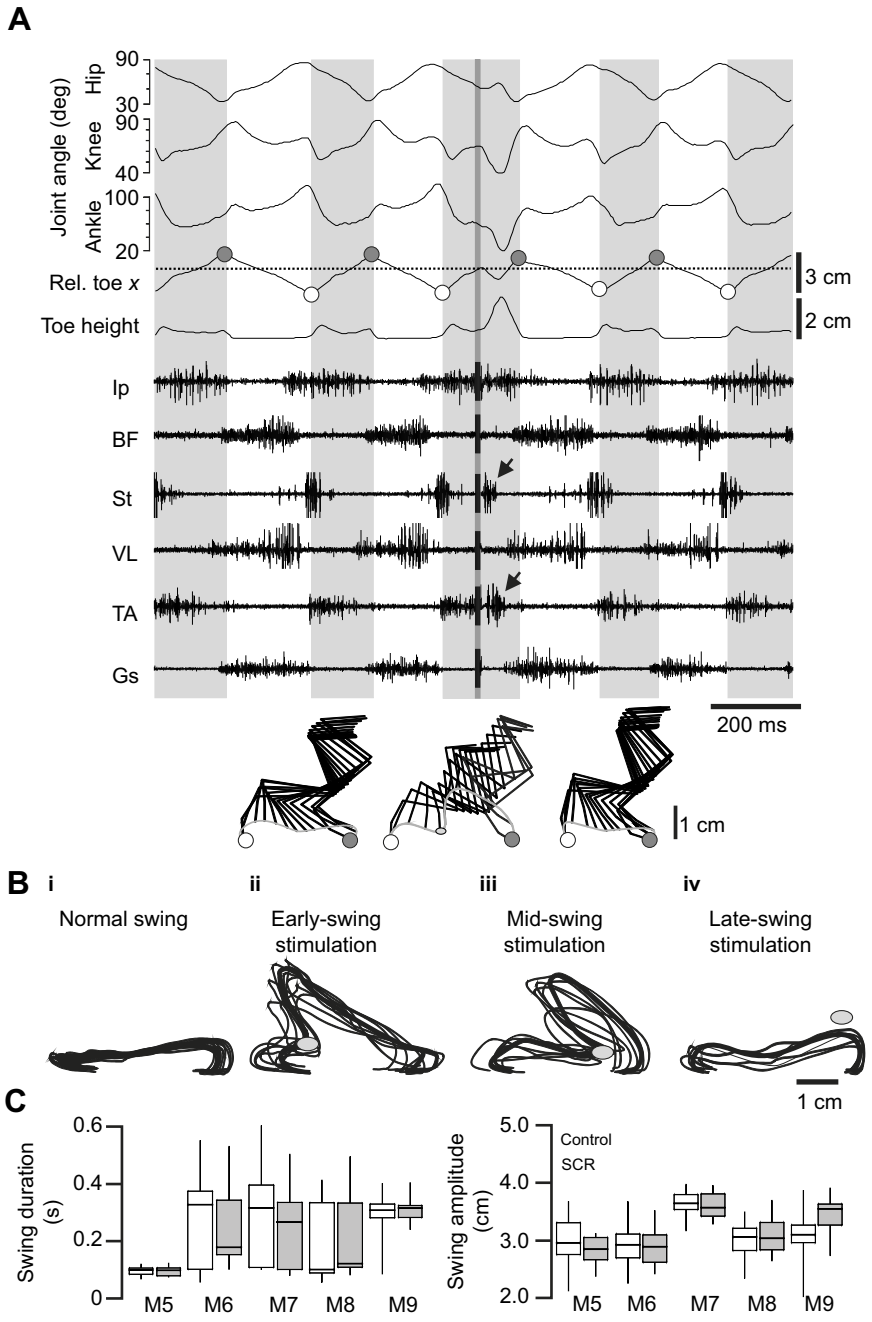

Fig. 3. Stumbling corrective reaction elicited by electrical stimulation of the saphenous nerve during swing phase. Kinematics and EMG pattern during a stepping sequence including two swing phases (shaded background) before and two swing phases after electrically evoked SCR. (A) Hip, knee and ankle joint angles, rel. toe *x* and *y* coordinates (toe height) synchronized with raw EMG activity of flexor (Ip, St, TA) and extensor (BF, VL, Gs) muscles. Electrical stimulation of the saphenous nerve during swing phase is indicated by the darker grey inside the third swing phase. Arrows point to the activity of knee and ankle flexor muscle initiated by the stimulation. Stick diagram reconstruction of a swing phase before SCR, an SCR, and a swing phase after SCR are illustrated below. (B) Average toe trajectories during control swing phase (i), SCR elicited during early- (ii), mid- (iii) and late-swing (iv). Thin lines indicate toe trajectories from individual trials from one animal and the bold line is the average trajectory. (C) Box and whisker diagrams illustrating average swing duration (left) and average swing amplitude (right) from control unperturbed swing phases (white bars, 21<*n*<87 swing phases) and SCRs elicited by electrical stimulation (grey bars, 7<*n*<29 SCRs) from four mice. None of these comparisons was statistically significant with the Mann–Whitney test. M5 to M9 represent the five individual mice used in this study.

Forssberg, 1979) and humans (Schillings et al., 1996; Van Wezel et al., 1997; Zehr et al., 1997).

The high stepping frequency (4.14±0.91 Hz) and short swing duration (0.113±0.017 s) made mechanical perturbation of the swing phase without additionally disturbing the following stance phase very challenging. Therefore, a large number of trials (188 trials) were performed and analysed in which the rod only touched the foot a single time during swing phase (64 trials). This presented a major limitation to the feasibility of this method as a tool for further projects. To overcome this limitation, one could imagine that an automated system could be developed to carry out the mechanical perturbation of the swing movement in a much more precise way. Alternatively, the SCR could be elicited by electrical stimulation of peripheral nerves, activating sensory afferents selectively that would signal obstacle touch. This has been done in the past in cats and humans by stimulating the superficial peroneal nerve (Van Wezel et al., 1997; Zehr et al., 1997; Quevedo et al., 2005a). However, implanting cuff electrodes around the superficial peroneal nerve was not feasible, due to the small size of mice. Therefore, we hypothesized that the saphenous nerve, which also innervates the skin on the dorsal site of the foot (Zimmermann et al., 2009; Dezhdar et al., 2015) might be similarly effective in mice.

Saphenous nerve stimulation elicits SCR in mice during stepping

To overcome the limitations of mechanical perturbation, we sought to elicit the SCR with an alternative method. Previously in cats, it was shown that SCR can be evoked either by electrically stimulating the superficial peroneal nerve in intact animals (Buford and Smith, 1993) or during fictive locomotion (Quevedo et al., 2005a,b). We

thought that a similar approach in mice would have the potential of overcoming the limitations of mechanical stimulation.

When SPN was electrically stimulated during the swing phase, angular joint movements, toe trajectory and EMG pattern of muscles reacted in a similar fashion to the SCR evoked by mechanical stimulation (Fig. 3A,B; Movie 2). Furthermore, during electrical SCR, the lifting of the foot to clear the virtual obstacle was achieved by activation of flexor muscles moving knee (St) and ankle (TA) joints (Fig. 3A, arrows). Only when the stimulation occurred later in swing phase did the leg tend to terminate the swing and proceed to the next stance phase instead of clearing the virtual obstacle (Fig. 3Biv). When the saphenous nerve was electrically stimulated, as in the mechanical stimulation, we could not detect any changes either in the swing amplitude ($P \geq 0.629$, Mann–Whitney test) or the swing duration ($P \geq 0.580$, Mann–Whitney test) (Fig. 3C) compared with unperturbed swing phases. Our data provide evidence that electrical stimulation of the SPN during ongoing swing phase consistently elicits a response that strongly resembles the SCR elicited by mechanical stimulation.

Angular movements of the hip, knee and ankle joints, as well as the toe trajectory during SCR, showed striking similarities regardless of being elicited by mechanical or electrical SPN stimulation (Fig. 4). When mechanical or electrical stimulation occurred within the first third of the swing phase (early-swing) while the knee joint was performing flexion movement, the knee joint continued the flexion movement for a brief period before the extension of the knee began (Fig. 4Aii and Bii, white circles). Stimulation occurring during the second third of the swing phase (mid-swing), after the knee started extension movement, caused the knee joint to switch to flexion movement which was followed by an extension (Fig. 4Aiii and Biii, white circles). The ankle joint reacted consistently with a short extension during early- and mid-swing mechanical SCR, followed by a brief flexion and finally switched to extension (Fig. 4Aii and iii and Fig. 4Bii and iii, black circles). The hip joint reaction was generally less consistent during mechanical and electrical SCR. That is, brief extension followed by flexion was observed only when stimulation occurred at mid-swing in two out of five mice during mechanical SCR, and three out of five mice for electrical SCR (Fig. 4Aiii and Biii, grey circles). No consistent joint reaction could be detected if the SCR was electrically elicited late-swing.

Our data suggest that SCR can be evoked when mechanical or electrical perturbation occurs within the first- and second-third of the swing phase. The movement of joints during SCR evoked by mechanical stimulation and electrical stimulation are very similar. The only difference we could detect was, when the electrical stimulation occurred at the end of swing phase, SCR was not elicited but the swing was terminated. In contrast, mechanical stimulation at the end of swing phase still consistently initiated SCR. Nevertheless, the SCR elicited by electrical stimulation provides advantages over mechanical stimulation as it can be applied with more accuracy and hence elicit cleaner responses.

Distal flexor muscles are activated during stumbling corrective reaction

During SCR elicited by mechanical perturbation of the swing movement, the flexor muscles St and TA were activated with short latency regardless of whether the perturbation occurred early-, mid- or late-swing (Fig. 5Aii–iv, black arrows). In contrast to the flexor muscles, knee extensors exhibited a clear response only when the perturbation was delivered late in swing phase (Fig. 5Aiv, grey arrowhead). Therefore, the activation pattern of flexor and extensor muscles during SCR described in this mouse model are very similar to the pattern described during SCR in cats (Wand et al., 1980).

During electrical SCR elicited at early- and mid-swing, St and TA muscles were activated in a similar manner as in the mechanical SCR (Fig. 5Bii,iii, black arrows). In contrast to the mechanical SCR, when the electrical stimulation occurred during late-swing, no change in activity in either St or TA could be detected (Fig. 5B); in accordance with this observation, late-swing electrical stimulation did not elicit SCR as described above (Figs 3Biv and 4Biv). When electrical stimulation occurred during stance, we observed a slight increase in extensor muscle activity (Fig. 5Bv, grey arrows) and no SCR. We conclude that cutaneous afferent stimulation during swing phase elicits SCR that includes activation of the flexor muscles. In contrast, if these same afferents are stimulated during stance phase, it activates extensor muscles, a phenomenon previously described as an example of reflex reversal (Forssberg et al., 1975).

DISCUSSION

The primary goal of our study was to gain insights into the stumbling corrective reaction in mice. We have shown that if the swing phase is perturbed by inserting an obstacle in the pathway of the foot, moving from the posterior extreme position to the anterior extreme position elicits an SCR. The kinematics of leg movement during SCR and the activation pattern of multiple flexors and extensor muscles closely resemble the pattern previously described in cats. The knee joint flexed as a response to the obstacle, whereas the ankle joint initially extended and then flexed, hence the combination of these movements lifted the foot higher to clear the obstacle. Accordingly, we detected activation of flexor muscle as a response to the obstacle contact during swing phase. Furthermore, electrical stimulation of the saphenous nerve, activating cutaneous afferent neurons that would normally signal the object contact with the leg, elicits SCR. Both SCRs, regardless of whether they were elicited by mechanical or electrical stimulation, were similar in kinematic parameters and muscle activation patterns. Moreover, we have shown that when electrical stimulation of the saphenous nerve occurs during ongoing stance phase, it did not generate any kinematic changes of flexor muscle activation. The flexor muscle activation during stance depended on its natural activity onset, also in accordance with previous observations in cats (Forssberg et al., 1975, 1977; Forssberg, 1979). Our data provide a detailed description of the SCR in mice and will be crucial for future attempts to address neuronal control mechanisms of SCR by combining this method with mouse genetics.

Stumbling corrective reaction in mice

When animals, including humans, encounter an obstacle during walking, they can modify their step in a way that the walking sequence is not disrupted (Rossignol, 2011). When the movement of the foot during swing is disrupted by an obstacle, the foot is elevated higher to clear the obstacle, a response previously called the stumbling corrective reaction (Forssberg et al., 1977). We have shown that mice also react with an SCR when an obstacle is introduced during swing phase without affecting the fluency of the locomotion (Fig. 2).

In previous cat experiments, it has been shown that the elevation of the foot is mainly achieved through the activation of the flexor muscles of distal leg joints leading to increased flexion (Wand et al., 1980). Our data suggest that SCR characterized in cats also occurs in mice with striking similarities (Fig. 2). That is, when the foot encounters an obstacle during swing movement, regardless of early-, mid- or late-swing, the knee joint simply flexes, and the ankle joint

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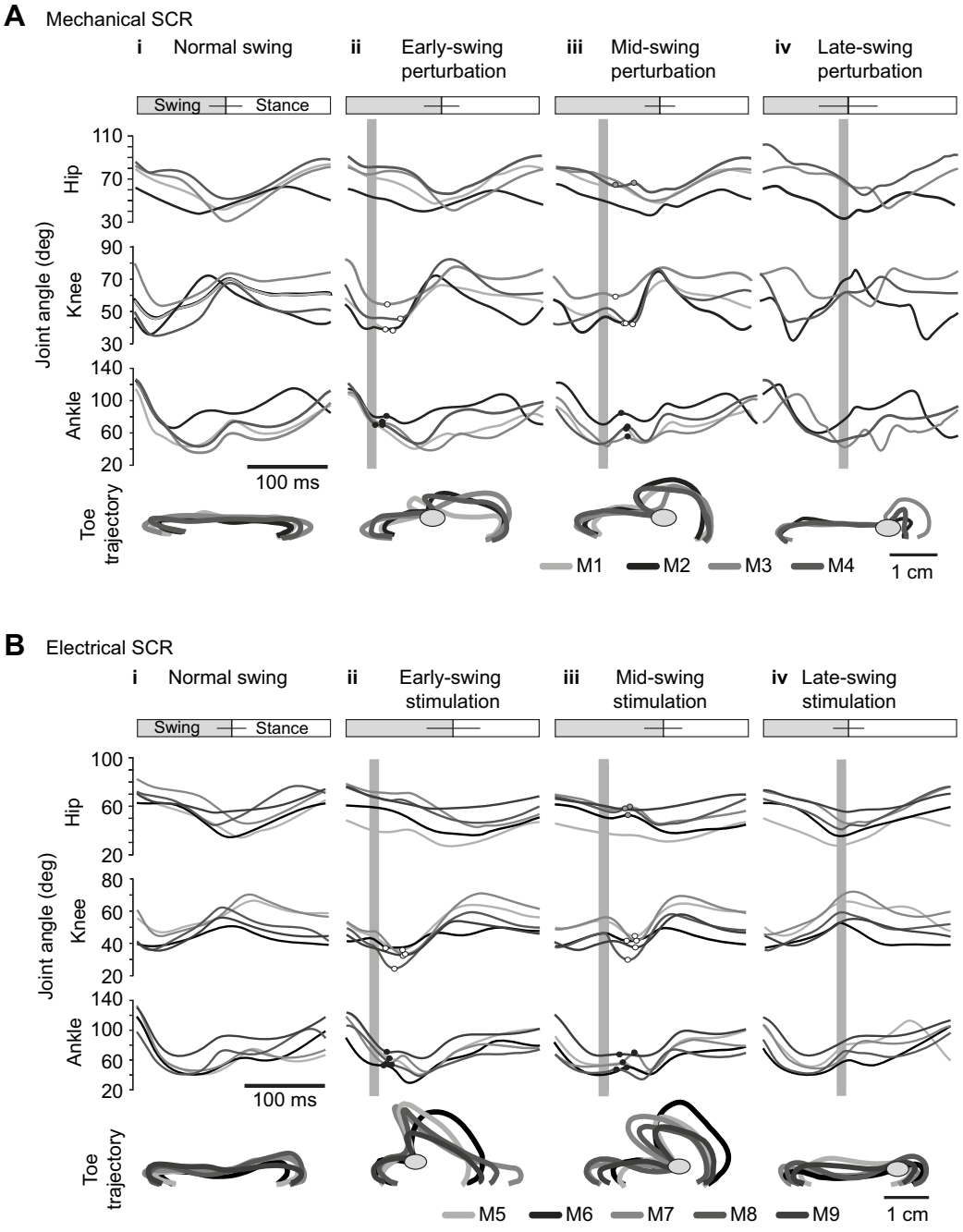

Fig. 4. Angular changes of leg joint during stumbling corrective reaction. Angular changes in leg joints during SCR initiated by mechanical stimulation (A) or electrical SPN stimulation (B). (A) Average angular movement of the hip, knee and ankle joints during unperturbed stepping (i) and during SCR elicited early- (ii), mid- (iii) and late-swing (iv) by mechanical perturbation. Lines are averages of each animal. Swing phase is indicated by the shaded bar and stance phase by the white bar above the traces (horizontal lines at transition indicate standard deviation). Grey vertical lines indicate mechanical perturbation. Below each graph are average toe trajectories from each animal during mechanical SCR (light grey elliptic indicates foot contact with the obstacle). M1 to M4 represent the four individual mice used in this study. (B) Same as in A, but the perturbation is electrical stimulation of the SPN. Below each graph are average toe trajectories from each animal during electrical SCR (light grey elliptic indicates foot position when the SPN was stimulated). M5 to M9 represent the five individual mice used in this study.

initially extends but then flexes (Fig. 4A). In accordance with this, we recorded that flexor muscles of the knee and ankle joints are activated during this movement with no consistent change in activity in the hip flexor muscle (Fig. 5A).

What is the reason for the short latency initial ankle extension prior to ankle flexion? Two explanations for this kinematic behavior during SCR could be explored. First, it is likely we simply have not recorded the extensor muscle that would underlie the early ankle extension. Multiple extensor muscles extend the ankle joint, and we only recorded from one head of the gastrocnemius muscle (lateral gastrocnemius). It is conceivable that other ankle extensors, with no EMG electrodes implanted, such as the medial gastrocnemius, soleus or the plantaris muscles might have had increased activity assisting the movement. Second, the initial extension of the ankle joint occurs passively due to ongoing flexion of the hip joint that would push the foot towards the obstacle causing the ankle joint to

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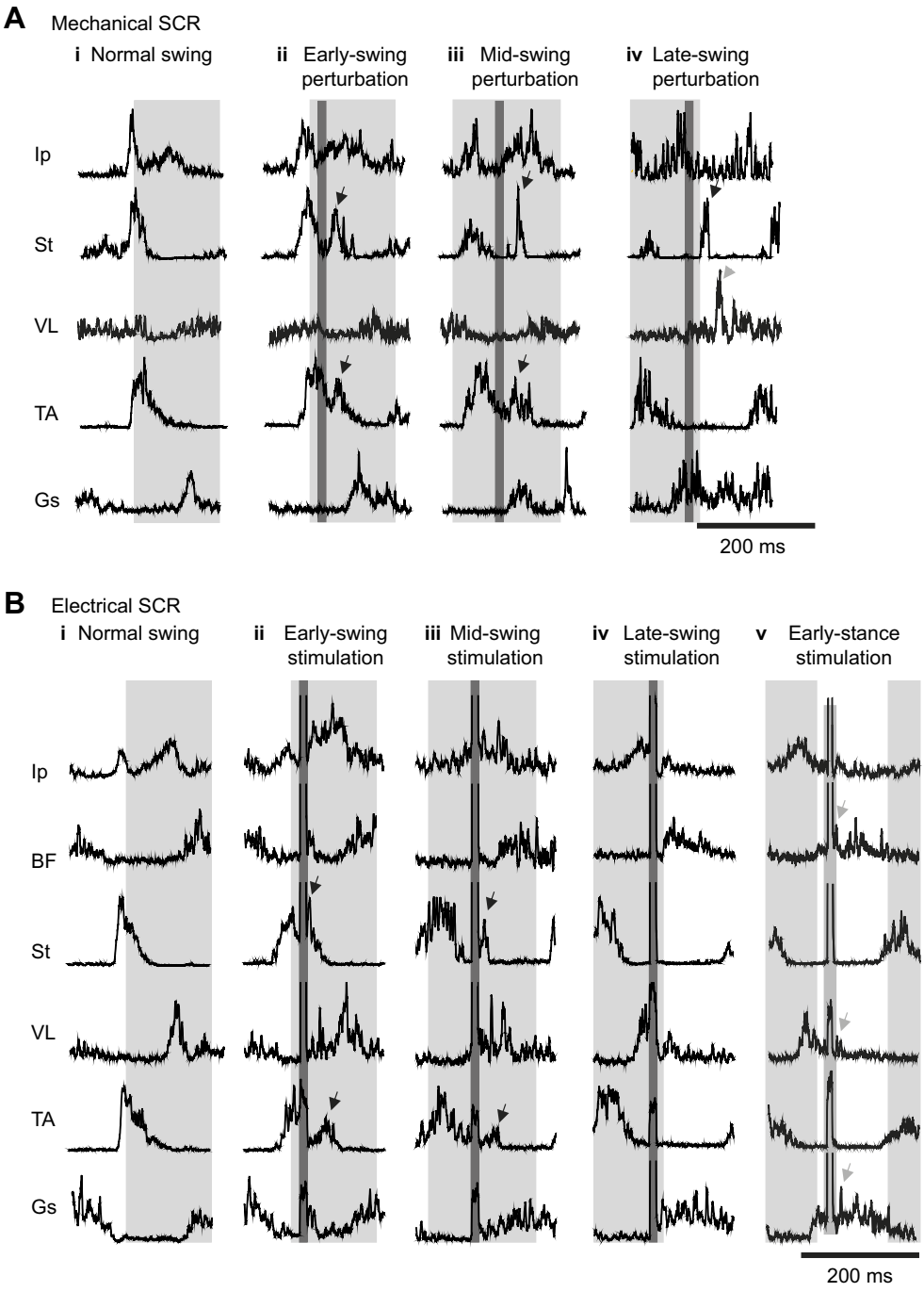

Fig. 5. Activity pattern of hindleg muscle during stumbling corrective reaction. Muscle activation pattern during SCR elicited by mechanical perturbation (A) or by electrical stimulation of the SPN (B). (A) Rectified and averaged flexor and extensor EMG activities triggered around the mechanical perturbation of the swing phase indicated by dark grey are within the swing phase (shaded background). Averages are from one representative animal. (B) Same as in A, but SPN was electrically stimulated (dark grey area) instead of mechanical perturbation. Averages are from one representative animal. Ip, iliopsoas (hip flexor); BF, anterior head of biceps femoris (hip extensor); St, semitendinosus (knee flexor); VL, vastus lateralis (knee extensor); TA, tibialis anterior (ankle flexor); Gs, gastrocnemius (ankle extensor). Black arrows indicate flexor activation and grey arrowhead indicates activation of vastus lateralis. Grey arrows indicate slight extensor activation.

extend. Once the foot is cleared, the ankle would then start flexion due to flexor muscle activity. We do not think the latter is true, as we observed a similar early extension of the ankle joint during nerve stimulation where there is no actual obstacle to cause a passive ankle extension. Therefore, we believe that the early ankle extension is an active part of the SCR that is presumably caused by another ankle extensor muscle not recorded in our experiments.

Cutaneous afferent signalling is sufficient to elicit SCR

Mechanical stimulation leaves open the question of whether a functional SCR can be elicited by only cutaneous afferent signals, as suggested from experiments in cats (Forssberg, 1979). Alternatively, proprioceptive feedback that signals changes in the natural angular joint movement due to obstacle contact (McVea and Pearson, 2007) could also contribute to the initiation of the SCR. To differentiate between these two possibilities, we recorded the SCR initiated by cutaneous afferent activation by electrical stimulation of the saphenous nerve that would mimic obstacle contact. Here, as there is no actual physical object preventing leg movement, the proprioceptive component of the sensory signalling was eliminated. Therefore, we could conclude that a response can be initiated by cutaneous afferent signalling only. Indeed, electrical stimulation of the SPN, activating only cutaneous afferents, is sufficient to generate an SCR very similar to the SCR initiated by contact of the foot with an obstacle during swing phase (Figs 3, 4B and 5B). These data provide evidence that cutaneous afferent signals are sufficient to initiate SCR.

The only major difference between mechanically and electrically evoked SCRs was found at the end of swing phase. When mechanical stimulation occurred at the end of swing phase, kinematic changes were triggered to overcome the obstacle. In contrast, electrical stimulation of the SPN at the end of swing did not elicit SCR. Our current data cannot elucidate this discrepancy. However, one possible explanation is that proprioceptive feedback, although not necessary to elicit SCR in early- or mid-swing phase, might have a more important role at the end of swing phase. Future research using mutant mice lines that have modified proprioceptive sensory feedback (Akay et al., 2014; Woo et al., 2015) should shed light on this question.

Cutaneous feedback signalling during stance phase activates extensor muscles but not flexor muscles

In cat experiments, it has been shown that when the stimulus occurs during stance phase (Forssberg et al., 1975; Quevedo et al., 2005a), the cutaneous stimulation does not activate flexor muscles as observed in SCR, but activates extensor muscles. This observation was interpreted as an example for phase-dependent (swing phase versus stance phase) reflex reversal, because one particular stimulation causes a reversed output depending on which phase of the gait it occurs (Forssberg, 1979). In our experiments, when the SPN was stimulated during the stance phase, it increased activity in the extensor muscles with mild or no response in the flexor muscles. This is a reversed response from the one observed when SPN was stimulated during the swing phase. Therefore, we conclude that corresponding to the cat literature, the effect of cutaneous afferent stimulation is dependent on the timing of the stimulation. The motor output generated by spinal circuitry reverses depending on the phase of the gait in which the stimulation occurs.

Why is the SCR only observed when cutaneous stimulation occurs during swing phase? Furthermore, why is the duration of swing phase not changed when it is interrupted with an SCR? We believe that both questions could be addressed by a previously proposed two-level network configuration consisting of a rhythm-generating circuit and a pattern-generating circuit (Rybak et al., 2006; McCrea and Rybak, 2008; Rybak et al., 2015). Accordingly, the phase dependency of the afferent influence would be achieved by a gating mechanism by the rhythm-generating circuit selectively allowing the cutaneous afferent input to affect the circuitry only during the flexor phase. Moreover, the spinal circuitry underlying the muscle activation pattern during the SCR would be at the level of the pattern formation circuit bypassing the rhythm-generating circuit, which explains why swing durations did not change with SCR (Figs 2C and 3C). Therefore, the explanation to these two questions is plausible, but our future research will provide clarification to the questions.

In conclusion, our data provide a first detailed description of the stumbling corrective reaction in mice. Using the mouse as an animal model opens up new avenues of exploration to understand the role of specific classes of interneurons in the control of SCR. In this paper, we provide a comprehensive analysis of the SCR in mice, showing that electrical stimulation of the SPN is sufficient to elicit SCR in freely behaving mice, and that was strikingly similar to the SCR elicited by mechanical stimulation. These data will be crucial for future research aiming to identify the interneuron circuit and its function that control the SCR, and how the nervous system controls motion by using SCR as a tool when combining *in vivo* experiments with mouse genetics.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: W.P.M., T.A.; Methodology: W.P.M., T.A.; Validation: T.A.; Formal analysis: W.P.M., T.A.; Investigation: W.P.M., T.A.; Resources: T.A.; Writing - original draft: W.P.M., T.A.; Writing - review & editing: T.A.; Visualization: W.P.M.; Supervision: T.A.; Funding acquisition: T.A.

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Supplementary information

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