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RESEARCH ARTICLE

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Recruitment of gastrocnemius muscles during the swing phase of stepping following partial denervation of knee flexor muscles in the cat

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Abstract In walking cats, the biarticular medial and lateral gastrocnemius (MG–LG) muscles act to produce extension and flexion torques at the ankle and knee, respectively, and they usually display only one burst of activity beginning just before ground contact and ending near the end of the stance phase. Currently, the MG–LG muscles are considered to function primarily to control extension movements around the ankle joint during the stance phase. However, their flexion action at the knee means that they have the capacity to regulate rotations at the knee, but this role has not yet been clearly defined. Following partial denervation of the other muscles that normally act to flex the knee during swing, we observed that the MG–LG muscles, but not the Soleus muscle (a pure ankle extensor), often generated strong bursts of activity during early swing. These bursts were enhanced following mechanical stimulation of the paw, and they were especially prominent when the leg trailed over an object. They were absent when the leg led over an object. During treadmill walking the swing-related bursts in MG and LG had little influence on ankle flexion at the beginning of swing, but they were associated with slowing of ankle flexion when the leg trailed over an object. We hypothesized that the recruitment of these bursts functions to partially compensate for the reduction in knee torque resulting from the denervation of other knee flexors. Consistent with this hypothesis was our finding that the magnitude of the swing-related activity in the MG–LG muscles was linearly correlated to the extent of the knee flexion and to the peak angular velocity of knee flexion, and that the timing of the bursts was similar to that in the denervated muscles prior to denervation. Our findings suggest that an excitatory pathway exists from the flexor half-center of the central pattern-generating network to MG–LG motoneurons, and that this pathway is strongly regulated by central and/or peripheral signals.

Keywords Walking · Nerve injury · Muscle synergies · Motor patterns · Spinal cord

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Introduction

The patterns of activity of many muscles in the hind legs of walking cats have been well characterized (Engberg and Lundberg 1969; Abraham and Loeb 1985; Rossignol 1996; Yakovenko et al. 2002). The bursts of activity in individual muscles can generally be assigned to a flexor or an extensor synergy, since most muscles exhibit clear bursts of activity associated with either the flexion (F phase) or one or more of the extension phases (E1, E2, and E3) of the step cycle (Engberg and Lundberg 1969). Of special interest is that the burst activity in some muscles can be either recruited to or removed from a synergy when the locomotor task changes. For example, an additional burst is recruited into the extensor synergy in the iliopsoas muscle (a hip flexor) when animals walk down steep slopes (Smith et al. 1998), and in the semitendinosus muscle (knee flexor, hip extensor) when animals increase their walking speed (Smith et al. 1993). Additionally, during fictive locomotion, the bursts of activity in the motoneurons innervating the biarticular muscles acting at the knee and hip (semitendinosus and rectus femoris) can be

completely switched from one synergy to another by tonic sensory input (Perret and Cabelguen 1980). These observations clearly demonstrate that the mechanisms establishing membership of flexor and extensor synergies are highly flexible, and they have suggested plausible schemes for the neuronal basis of this flexibility (Perret 1983; Smith et al. 1998).

In a preliminary study aimed at examining functional deficits produced by partially denervating muscles that flex the knee in walking cats, we unexpectedly observed that the medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles (ankle extensors, knee flexors) often produced bursts of activity during late stance and early swing in addition to the bursts that are normally associated with the stance phase. This observation, together with the fact that the MG and LG muscles cross the knee joint, indicated that under some circumstances these muscles might contribute to the production of flexion movements at the knee during the swing phase. Furthermore, it also suggested that a pathway exists from the system generating flexor bursts during early swing onto the motoneurons innervating the MG and LG muscles. The only reported occurrence of bursts of activity in LG during the flexion (F) phase of swing is during the stumbling-corrective reaction produced by mechanical stimulation of the dorsum of the paw (Forssberg 1979; Wand et al. 1980; Buford and Smith 1993). One function of the swing-associated bursts in LG is to assist paw clearance of the perturbing stimulus by stopping or reversing flexion movements at the ankle (Wand et al. 1980; Buford and Smith 1993). Another function may be to act synergistically with other muscles, such as semitendinosus, to enhance knee flexion. Recently, the pathways underlying the stumbling-corrective reaction have been examined during fictive locomotion in decerebrate cats (Quevedo et al. 2005a, b).

The primary objectives of the present investigation were to establish some of the conditions that lead to robust burst activity in the LG and MG muscles after partial denervation of knee flexors, and to assess whether these bursts contribute to producing knee flexion during early swing. We focused on two situations that we knew would normally result in enhanced knee flexion movements at the knee: (1) following a repeated sequence of mechanical stimuli to the paw (McVea et al. 2004) and (2) when the animals step over an object (Widajewicz et al. 1994). The rationale for this strategy was that if the bursts in MG and LG during the F phase were substituting functionally for the loss of other knee flexors, then the magnitude of the bursts should be significantly increased in these two situations. Furthermore, we expected to find a strong correlation between the magnitude of the F-related activity in MG and LG and the magnitude and speed of knee flexion. Thus, our main experimental approach was to examine quantitatively the relationships between the kinematics of knee movement and the intensity of activity in the MG and LG muscles during the F phase of swing in the two experimental situations mentioned above.

Materials and methods

Electromyographic (EMG) and video recordings were made from the muscles of a hind leg in three adult cats (2.5–3.5 kg) while walking on a treadmill and stepping over an object on a stationary walkway. All experimental procedures were approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

Training

Each animal was first trained daily for 1–2 weeks to walk consistently on a treadmill at a speed of about 0.5 m/s and across a stationary walkway. In the latter situation, a block (height 6.5 cm, width 3.5 cm) was sometimes placed in the center of the walkway to force the animal to flex their hind legs more than normal to avoid contacting the block.

Surgical procedures

Following the initial training, bipolar EMG electrodes (Cooner wire AS632) were implanted into the following muscles of the right hind leg under general anesthetic (isoflurane) and aseptic conditions: the hip flexor iliopsoas (IP) and the ankle extensors soleus (SOL), LG, and MG in all three animals, the knee flexor semitendinosus (ST) in two animals, and the ankle flexor tibialis anterior (TA) in one of the animals with ST implanted. The leads from the EMG electrodes were led under the skin to a multi-pin socket fixed with screws and dental acrylic to the animal's skull. While anesthetized, adhesive reflective markers (diameter 0.5 cm) were placed over the iliac crest, the hip, knee and ankle joints, and on the end of the paw and the fifth digit of the right hind leg. A drop of Krazy glue was placed on the adhesive surface of these markers to help prevent detachment. If lost, these markers were replaced prior to each recording session. A second surgical procedure was performed 10, 35, and 43 days after implantation of the EMG electrodes in the three animals, respectively, to partially denervate the knee flexor muscles. The timing of this second procedure varied because the animals were used to collect data for unrelated projects that took different times to complete. Again, under general anesthesia (isoflurane) and aseptic conditions, two of the three major branches of the hamstring nerve were transected in the right hind leg. These branches contain motor axons innervating two of the main muscles flexing the knee (semitendinosus, ST and posterior biceps, PB) and a muscle extending the hip (anterior biceps, AB). The AB nerve was included because it normally provides some innervation to ST and PB (English and Weeks 1987). Prior to cutting the nerves, EMG and video data were recorded when the animals walked on the treadmill

and across the walkway. These recordings were used to provide baseline information on the EMG patterns for the current investigation.

Acquisition of EMG and video data

While the animals were walking on the treadmill, EMG data were recorded on an eight-channel Vetter 4000A PCM recorder, and the stepping movements of the right hind leg were captured at the same time by video recording at 60 frames/s. One channel of the Vetter recorder was reserved for a signal for later use in synchronizing the EMGs with video data using the Peak Motus 8.2 motion analysis system (ViconPeak, Centennial, Co). The synchronizing signals were delivered at a rate of about one every 10 s when the animals were walking on the treadmill, and close to the times the right leg stepped over the object on the walkway.

During some sequences of walking on the treadmill, we examined the aftereffects of mechanically stimulating the paw of the right leg on the patterns of activity in the ankle extensor muscles. This was done by manually positioning a horizontal bar attached to a long stick in front of the paw so that the paw contacted the bar approximately mid-way through the swing phase. Multiple stimuli applied to the paw in a naïve animal eventually led to a persistent increase in knee flexion following the termination of the stimuli. Once conditioned, a single stimulus led to an increase in flexion at the knee that lasted for many hours in intact animals (McVea et al. 2004), and from five to ten cycles following a single stimulus in animals with the knee flexors partially denervated.

Data analysis

After the EMG and video recordings were made, the two sets of data were synchronized off-line using the Peak Motus system. Files of synchronized EMG and video data were made for selected sequences of treadmill walking and for all the trials where the animals stepped over the object on the treadmill. The positions of the markers on the right leg were digitized and stored in files in the Peak system. Because the knee marker shifted significantly relative to the knee joint position, we calculated the knee joint position by triangulation from the hip and ankle joint positions and measurements of the thigh and shank lengths. This calculation was done using software in the Peak system. The Peak system also calculated joint angles and joint angular velocities, and stored these calculated parameters within the file for each trial.

To display the EMG and kinematic data in the Peak files, we used custom-written software (Matlab, Mathworks, Natwick, MA) and plotted the data in formats of our choosing. Custom-written software was also used to determine the relationships between the magnitude of EMG activity and kinematic parameters. The magnitude of EMG activity in the LG and MG muscles was determined by calculating the mean of the full-wave rectified and filtered EMG (50 Hz low pass first-order one-way Butterworth filter) over a defined time interval. In the treadmill walking cats, this interval was from the beginning to the end of bursts in the LG and MG muscles associated with flexion (Fig. 3). When the animals stepped over the block on the walkway, the interval was the first half of the flexion-associated LG and MG bursts (Fig. 6).

Results

Treadmill walking after partial denervation of knee flexors

As expected, the most obvious effect of partially denervating the knee flexor muscles was a marked reduction in the extent of knee flexion during the flexion (F) phase of the swing (Fig. 1). However, an unexpected observation was that strong bursts of activity often occurred in the LG muscle in all the three animals beginning just before the onset of swing and terminating in the latter half of the F phase. Bursts of activity also occurred at the same time in the MG muscle of all three animals, but in two animals the bursts in MG were relatively weak compared to the stance-related EMG activity. Figure 1c (filled sections) shows an example of these flexion-associated bursts in LG and MG in the animal in which both muscles were strongly activated (see Fig. 8 for an example from another animal in which the magnitude of the flexion-associated LG bursts was much larger than in the case of the MG muscle). We refer to these bursts as MG_F and LG_F bursts. These bursts were never accompanied by bursts of activity in SOL.

Because the main focus of this study was on the occurrence and associated movements at the knee and ankle joints, we did not make a detailed comparison of joint movements before and after the partial denervation of the knee flexors. Nevertheless, during the treadmill walking, any changes in the movements at the hip and ankle appeared to be relatively small (compare the hip and ankle movements in Fig. 1a, b). The absence of an obvious influence on hip extension must be reconciled with the fact that the denervated muscles, especially AB, act to extend the hip. A possibility is that hip extension following denervation may rely more on the mechanical effects produced by propulsive forces of the other legs. The duration of the swing phase was also not noticeably influenced by the denervation in two animals, but it did increase in the third (Fig. 1) due to a prolongation of the duration of the first extension phase (defined by extension at the knee and ankle).

We were unable to establish any specific condition that led to the occurrence of the MG_F and LG_F bursts when the animals were walking on the treadmill (and

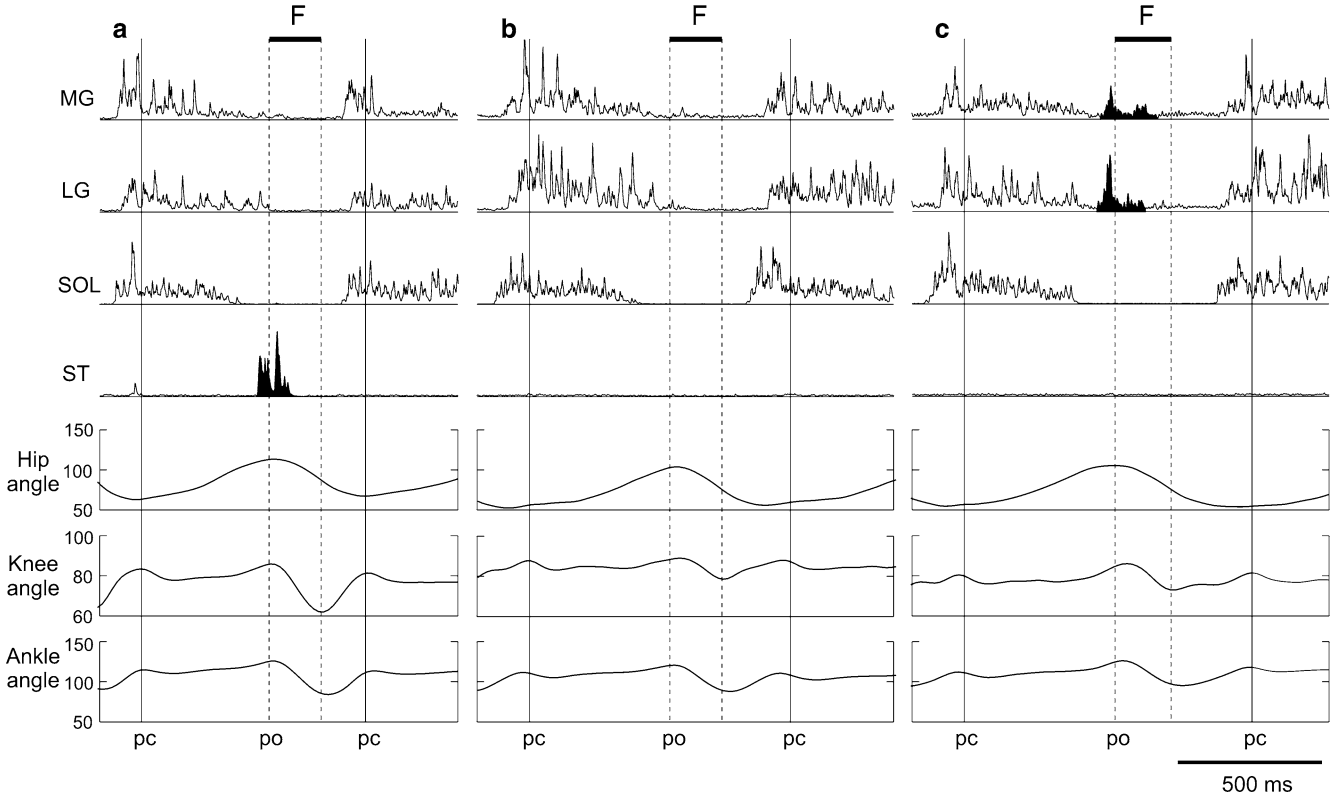

Fig. 1 Electromyographic (EMG) activity in MG and LG muscles during the early swing in a treadmill walking cat following partial denervation of the knee flexors. *Top four traces*—rectified and filtered EMGs from the MG, LG, SOL, and ST muscles. *Bottom three traces*—plots of the corresponding movements at the hip, knee, and ankle joints. Treadmill speed 0.5 m/s. *pc* paw contact, *po* paw off. a A typical step cycle recorded before partial denervation of the knee flexors showing burst activity in ST and the absence of activity in the extensor muscles during the flexion (*F*) phase. b, c Step cycles following partial denervation of the knee flexors, one in which little activity occurred in MG and LG during the F phase (b) and another in which distinct bursts of activity where generated in MG and LG (*filled regions*) near the time of onset of the F phase (c). Note the absence of bursting in SOL during the F phase, the abolition of ST activity after denervation, the reduction in amplitude of knee flexion following denervation, and the increase in knee flexion associated with the MG_F and LG_F bursts. All recordings were from the same animal

across the walkway without the object being present), but one possibility we did exclude was that dragging of the paw on the treadmill belt caused the bursts due to activation of cutaneous receptors on the dorsum of the paw. It was certainly the case that the paw often dragged along the treadmill belt during early swing, especially in the first few days following partial denervation of the knee flexors. However, we were unable to correlate the occurrence of the MG_F and LG_F bursts to paw drag, and on most occasions these bursts occurred without any noticeable paw drag. Most importantly, the onset of the bursts preceded the onset of the swing phase by 60 to 80 ms, and the ventral (plantar) side of the paw was always firmly in contact with the supporting surface and moving backwards relative to the hip at the time of onset of MG_F and LG_F bursts. In addition, it was clear from the analysis of the leg kinematics that the MG_F and LG_F bursts began before the onset of flexion at the hip, knee, and ankle joints, that is, prior to the time of onset of any forward movement of the paw, and there were no indications that the leg was held in an extended position prior to the initiation of flexion to cause movement of the treadmill belt across the stationary paw.

A noteworthy observation was that the timing of the onset of the MG_F and LG_F bursts relative to the extensor-related activity in the ankle extensors corresponded closely to the time of onset activity of the main knee flexors prior to denervation. This can be seen by comparing the timing of the onset of ST burst relative to the movements at the hip, knee, and ankle in Fig. 1a (before denervation) to the timing of the onset of the MG_F and LG_F bursts in Fig. 1c (after denervation), and it is shown clearly in averages of the LG_F bursts after denervation and the ST bursts before denervation (Fig. 2). Note in Fig. 2 that the time of the onset of the LG_F bursts relative to bursts in other muscles (panels B and D) corresponds quite closely to the time of the onset of ST bursts relative to the same muscles (panels A and C). The main implication of the close correspondence of the normal ST bursts and the MG_F and LG_F bursts is that the MG_F and LG_F bursts may be recruited in an attempt to substitute for the loss of knee flexors.

Another important point to note in Fig. 1 is that the occurrence of the MG_F and LG_F bursts was associated with a larger flexion movement at the knee (compare knee angle in Fig. 1b, c) without a large influence on

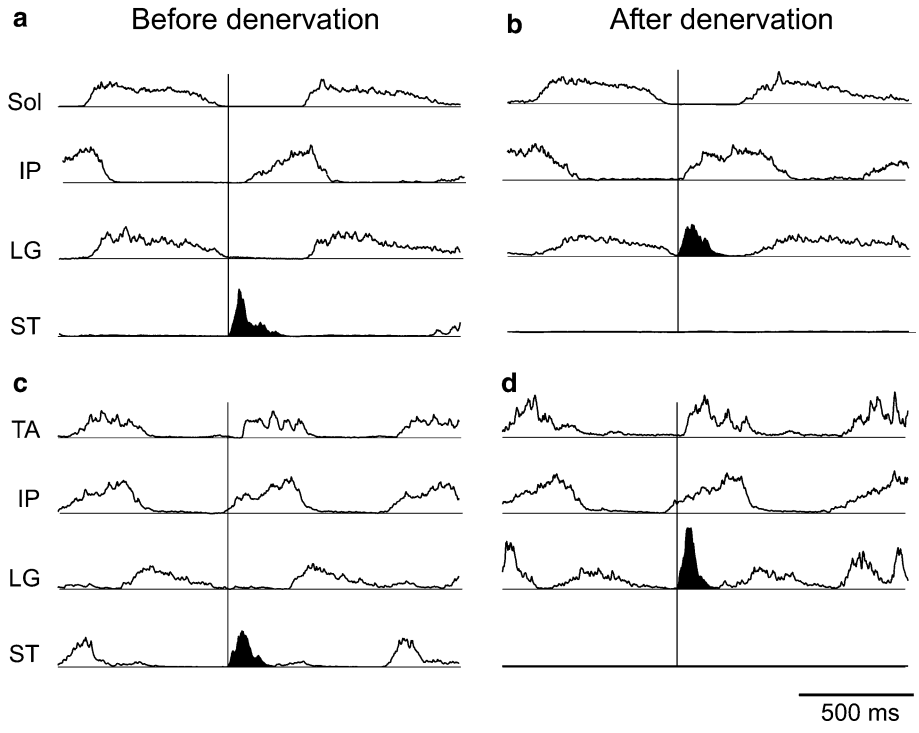

Fig. 2 The timing and duration of the LG_F bursts (*filled in LG traces*) after partial denervation of the knee flexors (**b**, **d**) are similar to the timing and duration of the ST bursts (*filled in ST traces*) before denervation (**a** , **c**) relative to bursts in other muscles. **a**, **b** Same animal as that yielding data from Fig. 1. **c**, **d** Same animal as that yielding data for Fig. 5. Each trace is an average of ten steps referenced (*vertical lines*) to either the onset of the ST (**a**, **c**) or LG_F (**b**, **d**) bursts. Treadmill speed 0.5 m/s

movement at the ankle joint (see also Fig. 5 for absence of large effects on the ankle in another animal). This observation is consistent with the notion that the MG_F and LG_F bursts are recruited to substitute for the loss of other muscles flexing the knee. To assess whether the MG_F and LG_F bursts could be contributing to knee flexion, we examined the relationships between the magnitude of the bursts and the extent and velocity of knee flexion movements. We chose to do this analysis for the step cycles following a mechanical stimulus to the dorsum of paw because the MG_F and LG_F bursts were consistently evoked on every step for five to ten steps following the stimulus, and because there was a progressive decline in the amplitude of the MG_F and LG_F bursts. Figure 3 shows an example of the EMGs in MG, LG, and SOL during eight cycles following the stimulus, as well as the hip and knee angles, knee angular velocity, and toe height during the sequence. These data clearly show that the progressive decline in the magnitude of the MG_F and LG_F bursts was associated with declines in the extent of knee flexion, the magnitude of knee angular velocity, and the amplitude of toe height. The plots in Fig. 4 show the correlation between these kinematic parameters and the amplitude of the MG_F and LG_F bursts. Figure 3 also shows that the SOL muscle was never activated (asterisks) at the same time as the MG_F and LG_F bursts, despite the very large initial amplitudes of the MG_F and LG_F bursts.

A surprising observation was that ankle extension at the end of stance was not noticeably influenced by the occurrence of the MG_F and LG_F bursts (compare Fig. 1b, c). We examined this more closely by averaging the ankle movements following the onset of the MG_F and LG_F bursts in one animal in which we had implanted the TA muscle (Fig. 5). This was a different animal from that yielding the data in Figs. 1 and 3, and one in which the LG_F bursts were relatively large. These averages clearly showed that the augmented LG_F bursts following paw stimulation had a negligible effect on ankle extension. Corresponding to the enhanced activity in LG was an earlier onset of activity in TA, and an increase in the period of coactivation of the LG and TA muscles. It is likely that this increase in coactivation of LG and TA (and possibly other ankle flexor muscles, such as extensor digitorum longus) is the reason for the small influence of the augmented LG_F bursts on ankle extension. The most noticeable effect on the ankle movements in the steps following a mechanical stimulus of the paw was an increase in the magnitude of ankle flexion during the F phase (Fig. 5). Correspondingly there was a marked increase in the magnitude of the TA bursts following the termination of the LG_F bursts (Fig. 5) that very likely was largely responsible for the enhanced ankle flexion.

Recruitment of MG and LG activity during swing when stepping over an object

To further investigate the conditions leading to the production of the MG_F and LG_F bursts after partial denervation of the knee flexors, we examined the activity in the ankle extensors when the animals stepped over an object (Fig. 6). There was a clear difference in the activity in MG and LG depending on whether the leg was trailing or leading relative to the contralateral leg.

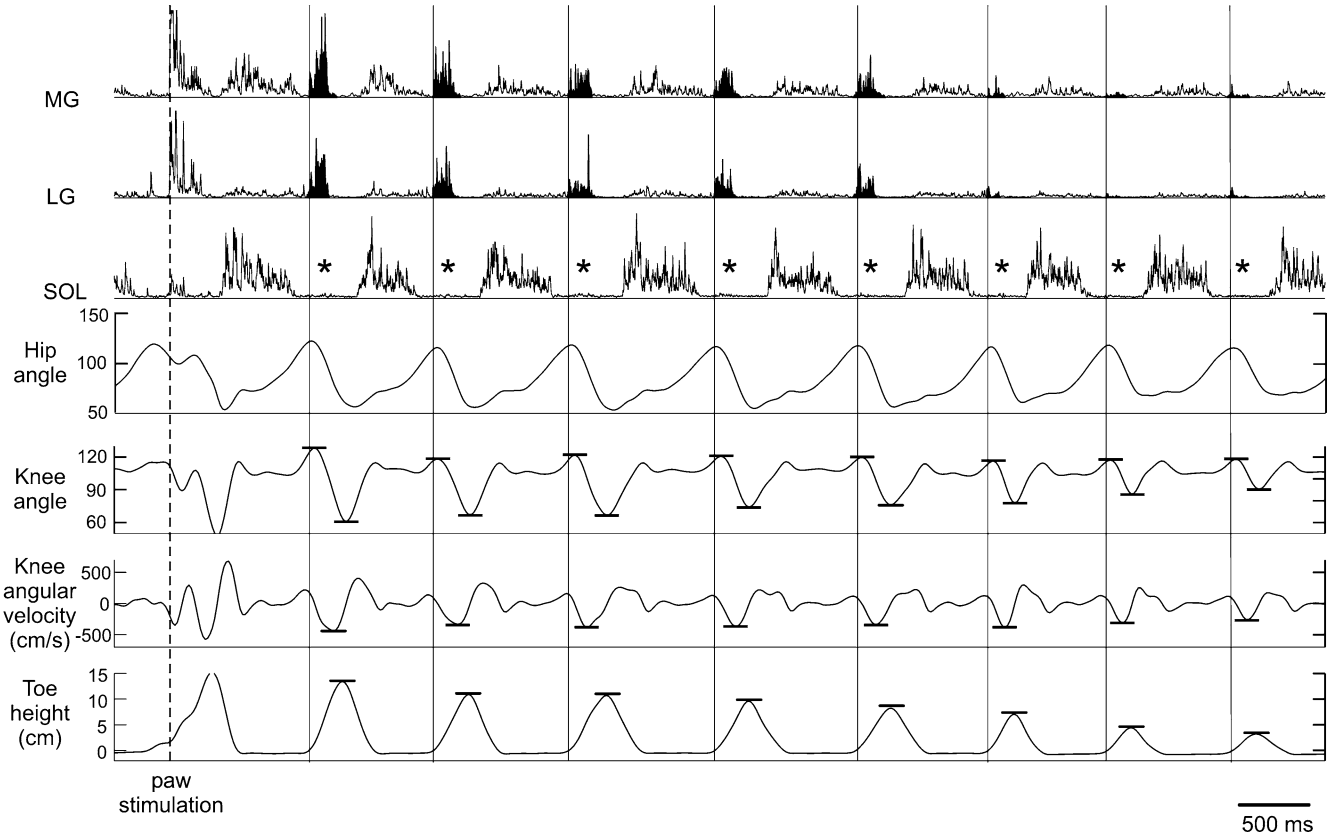

Fig. 3 The magnitude and velocity of knee flexion and toe height above the treadmill during the swing phase are related to the magnitude of the MG_F and LG_F bursts. *Top three records*—rectified and filtered EMGs from the MG, LG, and SOL muscles during a sequence of steps following a mechanical stimulus to the paw (*vertical dashed line*); *bottom four records*—plots of hip angle, knee angle, knee angular velocity, and toe height. *Vertical lines* indicate the times of onset of the MG_F bursts. Treadmill speed 0.5 m/s. Note that the progressive decline in the magnitude of the MG_F and LG_F bursts following the stimulus was associated with progressive declines in the magnitude of flexion at the knee (*distance between short horizontal lines*), the maximum knee angular velocity during flexion (*short horizontal lines*), and the peak toe height during the swing phase. The SOL muscle was not active during the MG_F and LG_F bursts (*asterisks*). By the end of this stepping sequence the EMGs and kinematics had returned to values close to those prior to the stimulus

When trailing, large MG_F and LG_F bursts were generated commencing before the onset of swing (Fig. 6a), whereas there was usually no activation of the MG and LG muscles during the F phase when the leg led (Fig. 6b). Similar to our observations in treadmill walking animals, no activity occurred in SOL during the MG_F and LG_F bursts when the leg trailed (asterisk in Fig. 6a). In addition, the extent of knee flexion and maximum knee angular velocity were correlated with the magnitude of the MG_F and LG_F bursts (Fig. 7).

In all the three animals, the maximum angular velocity of knee flexion was larger when the leg trailed over the object compared to when it led (9, 25 and 37% higher when trailing). This is consistent with the notion that the MG_F and LG_F bursts contributed to knee flexion, since the MG_F and LG_F bursts only occurred when the leg trailed. The wide range of the difference in the magnitude of the knee angular velocities of trailing vs leading steps in the three animals may be partially due to differences in the magnitude of the hip angular velocity. In the animal with the smallest difference in knee angular velocity (9%) the difference in maximum hip angular velocity was largest (always higher during leading step), and vice versa for the animal with the largest difference in knee angular velocity. A mechanical consequence of a large difference in the angular velocity of hip flexion would be to provide additional assistance for knee flexion, thus reducing the difference in the knee angular velocity. In all the animals, the onset of activity in the hip flexor IP commenced earlier and was larger in amplitude when the leg was leading compared to when the leg was trailing (Fig. 6). The earlier and larger activity in IP during leading steps was associated with a faster flexion of the hip, as illustrated in the plots of hip angular velocity in Fig. 6.

The occurrence of the MG_F and LG_F bursts during trailing steps was also associated with an obvious slowing and delay in flexion of the ankle joint (Fig. 8). Functionally, this was appropriate because with the weakening of knee flexion it was necessary to keep the ankle extended during early swing on trailing steps to ensure that the toe cleared the object. On trailing

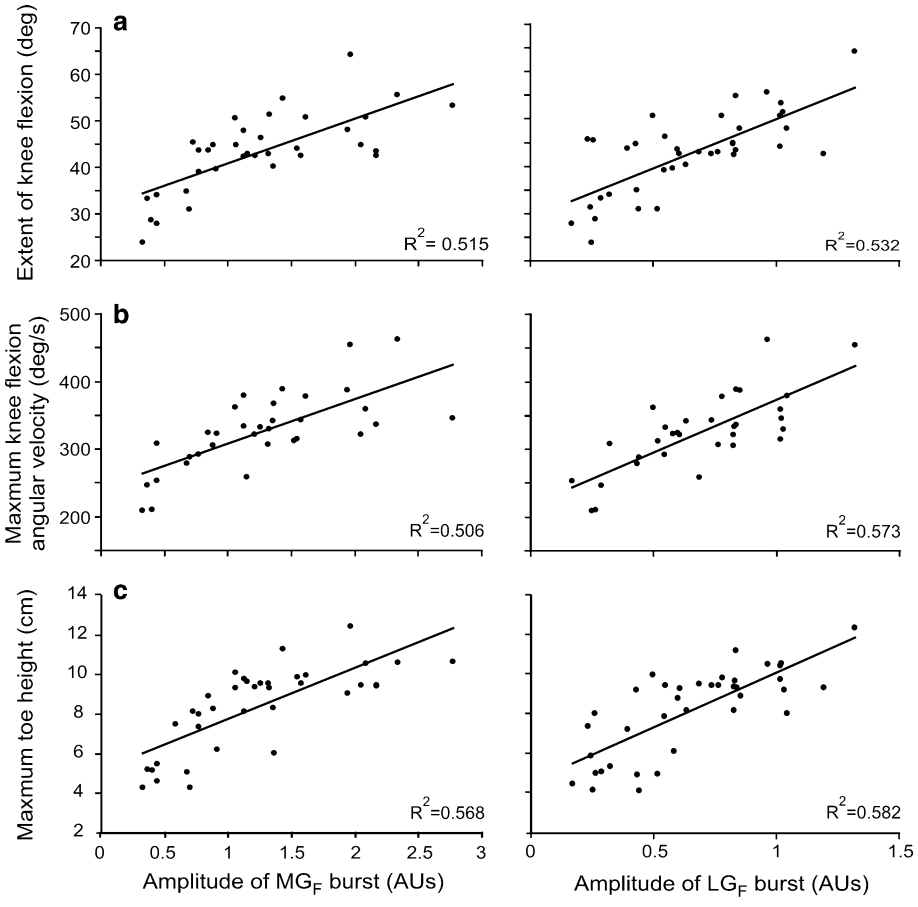

Fig. 4 Scatter plots of the relationships between the magnitude of the MG_F and LG_F bursts (*left* and *right*, respectively) and the extent of knee flexion (**a**), the maximum knee flexion angular velocity (**b**), and maximum toe height (**c**) during stepping following a mechanical stimulus to the paw. The line in each plot is the best fitting linear regression line. *AU* arbitrary units

steps, the position of the paw at the onset of swing was close to the object (Fig. 6, top left), so a weak knee flexion combined with an immediate flexion at the ankle would have increased the likelihood of the foot contacting the object. The occurrence of the MG_F and LG_F bursts likely contributed to the slowing of ankle flexion because the ankle angular velocity during early swing was inversely related to the magnitude of the MG_F and LG_F bursts (Fig. 9). The association of the MG_F and LG_F bursts with the slowing of ankle flexion during early swing was distinctly different from our observations during treadmill walking, in which the enhancement of the MG_F and LG_F bursts had little or no influence on initial part of ankle flexion (Fig. 5). A possible reason for this difference is that when the leg trailed over the object there was a substantial reduction in the magnitude of activity of the main ankle flexor TA at the time of the occurrence of the MG_F and LG_F bursts compared to the magnitude of the TA bursts when the leg led over the object (Fig. 8). This observation indicates that when the leg trails over an object, TA motoneurons are inhibited by the system generating the MG_F and LG_F bursts, thus reducing the flexor torque at the ankle joint. A reduction in TA activity was not observed when the MG_F and LG_F bursts were enhanced following the mechanical stimulation of the paw (Fig. 5).

Discussion

The main finding of this investigation was that the biarticular MG and LG muscles (ankle extensors/knee flexors) can be briefly activated near the onset of the swing phase in walking cats following partial denervation of muscles flexing the knee. This resulted in two bursts of activity during walking: one commencing just before the onset of stance and associated with the E1, E2, and E3 phases of the step cycle, and the other commencing just before swing and lasting well into the F phase. We refer to the latter bursts in MG and LG as the MG_F and LG_F bursts. The occurrence of these bursts have not been reported in previous studies on the EMG patterns during unperturbed walking, although it has been known for some time that mechanical stimulation of the dorsum paw during the swing phase can evoke strong bursts of activity in the LG muscles (Forssberg 1979; Wand et al. 1980). Because cutaneous stimulation of the paw evokes responses qualitatively similar to those we found in this study, it is important to consider the possibility that the MG_F and LG_F bursts were reflexively produced by cutaneous stimulation of the paw. The most compelling observations demonstrating that this was not the case was that these bursts began about 60–80 ms before the end of stance (Fig. 1c), and

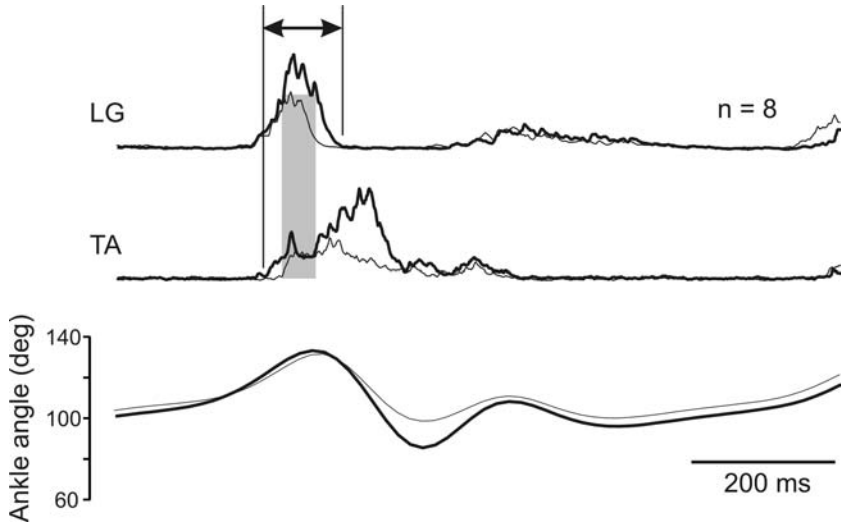

Fig. 5 Enhancement of the LG_F bursts by mechanical stimulation of the paw during treadmill walking has little effect on late ankle extension and early ankle flexion. Superimposed averages ($n = 8$) of rectified and filtered EMG records in TA and LG (*top two sets*) and ankle movement (*bottom set*) before (*thin traces*) and after (*thick traces*) a mechanical stimulus to the paw. Note the increase in the duration of the coactivation of the LG and TA muscles following the stimulus. The periods of coactivation before and after the stimulus are indicated by the *width of shaded area* and the *horizontal arrowed line*, respectively. Averages were made relative to the onset of the LG_F burst. See text for more details

at the time of onset of the bursts the ventral (plantar) surface of the paw was firmly in contact with the supporting surface and moving backwards relative to the hip. Furthermore, the MG_F and LG_F bursts usually occurred in the absence of any noticeable paw drag that might have activated cutaneous receptors to evoke a stumbling-corrective response. Thus, we conclude that the MG_F and LG_F bursts are produced by a central network linked to the system generating motor output for the flexion component of the swing phase.

The first issue we wish to consider is the function of the MG_F and LG_F bursts. Because the occurrence of the MG_F and LG_F bursts was a consequence of partial denervation of other muscles producing flexion torques at the knee, the most obvious possibility is that the MG_F and LG_F bursts are recruited to compensate for the loss of function of the denervated knee flexors. This possibility is strongly supported by the following observations. First, the timing of the MG_F and LG_F bursts corresponded very closely to the timing of bursts of activity in ST that normally commenced just before the onset of swing and terminated mid-way during the F phase (Figs. 1a, 2). Second, conditions that normally led to an increase in the magnitude of activity in ST prior to denervation, such as enhanced leg flexion following mechanical stimulation of the paw and when the leg trails over an object (unpublished observations and Drew et al. 2002), also led to an increase in the magnitude of the MG_F and LG_F bursts (Figs. 3, 8). It is also relevant that ST activity increases more when the leg trails than when it leads in normal animals (unpublished observations and Drew et al. 2002), which parallels our finding that the MG_F and LG_F bursts only occurred when the leg was trailing (Fig. 6a). The third observation supporting the notion that the MG_F and LG_F bursts function to assist in knee flexion was that the pure ankle extensor muscle SOL was never found to be activated with the MG_F and LG_F bursts (Figs. 1, 3, 6). This suggests that the MG and LG muscles participate in a specialized function that is not shared with SOL. The obvious possibility for this specialized function is producing a flexion torque at the knee, since the action of all three muscles at the ankle (extension) is similar.

On the other hand, it is conceivable that the MG_F and LG_F bursts could function to control movements at the ankle joint without the participation of SOL during early swing. Indeed, our finding that the velocity of ankle flexion was inversely related to the magnitude of the MG_F and LG_F bursts when the leg trailed over an object (Fig. 9) indicates that the MG_F and LG_F bursts are involved in controlling ankle flexion in this situation. The slowing of ankle flexion is functionally appropriate because on trailing steps a rapid ankle flexion at the beginning of swing would increase the likelihood of the paw contacting the object because the swing begins from a paw position close to the object. The slowing of ankle flexion may also be assisted by a reduction in the magnitude of the TA bursts relative to their magnitude during leading steps (Fig. 8). On the other hand, the influence of the MG_F and LG_F bursts on extension movements at the ankle when the animals were walking on the treadmill was not nearly so obvious (Figs. 1, 5). This was initially surprising because the small inertia of the paw would seem to enable contractions of the MG and LG muscles to have a strong influence on ankle movements. However, recordings from the antagonist TA showed this muscle to be active at the same time as the MG_F and LG_F bursts and the extent of the overlap

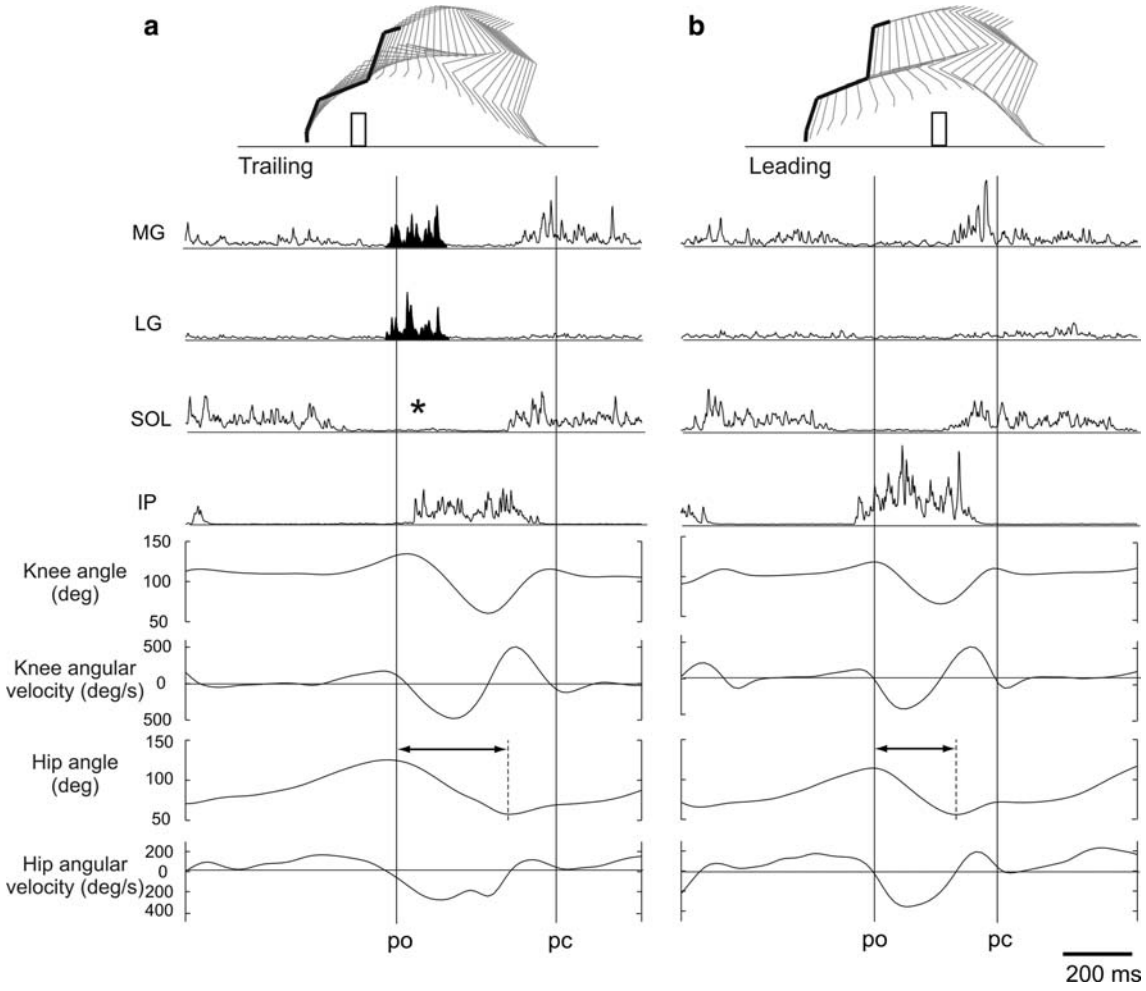

Fig. 6 Generation of MG_F and LG_F bursts when the leg trails over an object (*filled sections* in **a**) but not when it leads (**b**). *Top diagrams*—superimposed stick figures of the hind leg (60/s), *top four traces*—rectified and filtered EMGs from MG, LG, SOL, and IP muscles, *bottom four traces*—corresponding records of knee angle, knee angular velocity, hip angle, and hip angular velocity. No burst activity occurred in SOL at the same time as MG_F and LG_F bursts (*asterisk* in **a**), and the extent of knee flexion was larger when the leg trailed over the object. Note also that the IP bursts had a smaller magnitude and later onset time when the leg trailed over the object. The smaller and delayed IP bursts during trailing steps were associated with a longer and slower flexion movement at the hip (*horizontal arrowed lines*)

increased when the MG_F and LG_F bursts were enhanced (Fig. 5). This activity in the TA muscle, and perhaps other ankle flexors (such as extensor digitorum longus), would act to resist extensor torques produced by the MG_F and LG_F bursts. This resistance to extension at the ankle would enable a greater force to be generated in the MG and LG muscles due to a reduction in the velocity of shortening and hence produce a greater flexion torque at the knee. One caveat to these proposals for the role of the TA muscle to regulate ankle movements produced by the MG_F and LG_F bursts is that our observations on TA burst activity were made in a single animal. To fully explain the neuromuscular mechanisms underlying the modification of ankle movements in the different behavioral tasks requires a more extensive examination of activity of TA and other muscles producing torques at the ankle. One muscle that may be especially interesting to examine would be flexor digitorus longus (FDL). This muscle produces an extensor torque at the ankle in addition to its main function of plantar flexing the toes (Lawrence and Nichols 1999). Of special relevance is that FDL is briefly activated close to the onset of the swing phase (O'Donovan et al. 1982). The timing and duration of the bursts in FDL closely resemble the MG_F and LG_F bursts we observed. Therefore, it is conceivable that the interneuronal system generating the FDL bursts is recruited to produce the MG_F and LG_F bursts. If this is true, then significant changes may also occur in FDL bursts following denervation of the knee flexors.

Another issue we wish to discuss is the mechanism that enables the generation of the MG_F and LG_F bursts following partial denervation of the knee flexors. The first point to consider is whether these bursts are produced by an immediate reorganization of the pattern-generating network by removal or changes of sensory signals (due to loss of afferent signals from the denervated muscles or modifications in the movement of the

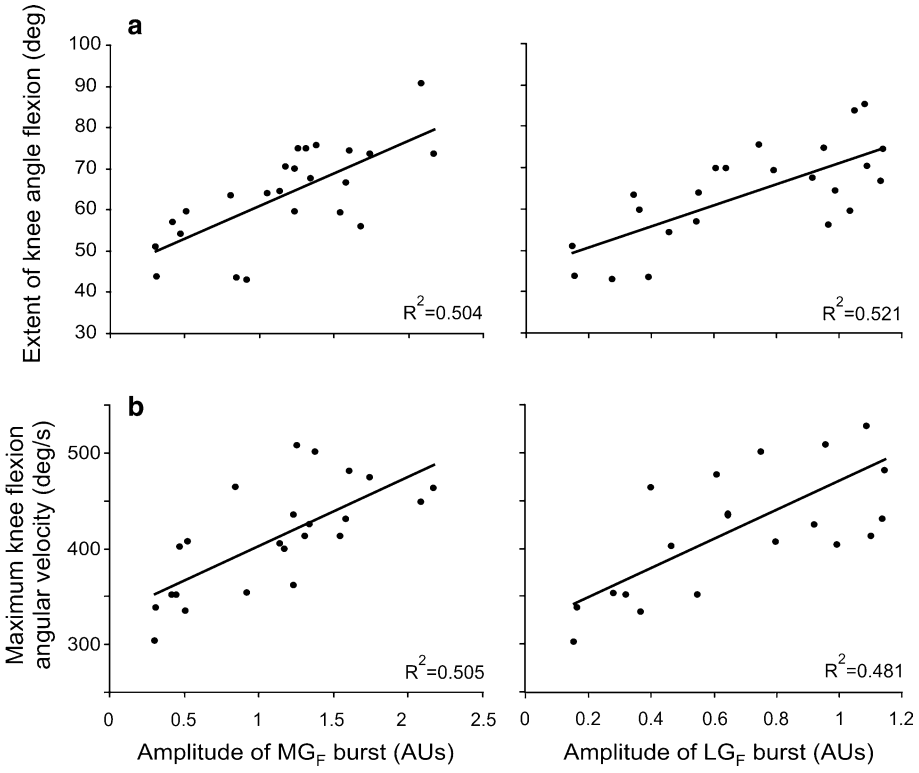

Fig. 7 *Scatter plots* of the relationships between the magnitude of the MG_F and LG_F bursts (*left* and *right*, respectively) and the extent of knee flexion (**a**) and the maximum knee flexion angular velocity (**b**) When the hind leg trailed over an object. Each dot is a single trial, and the line in each plot is the best fitting linear regression line. *AU* arbitrary units

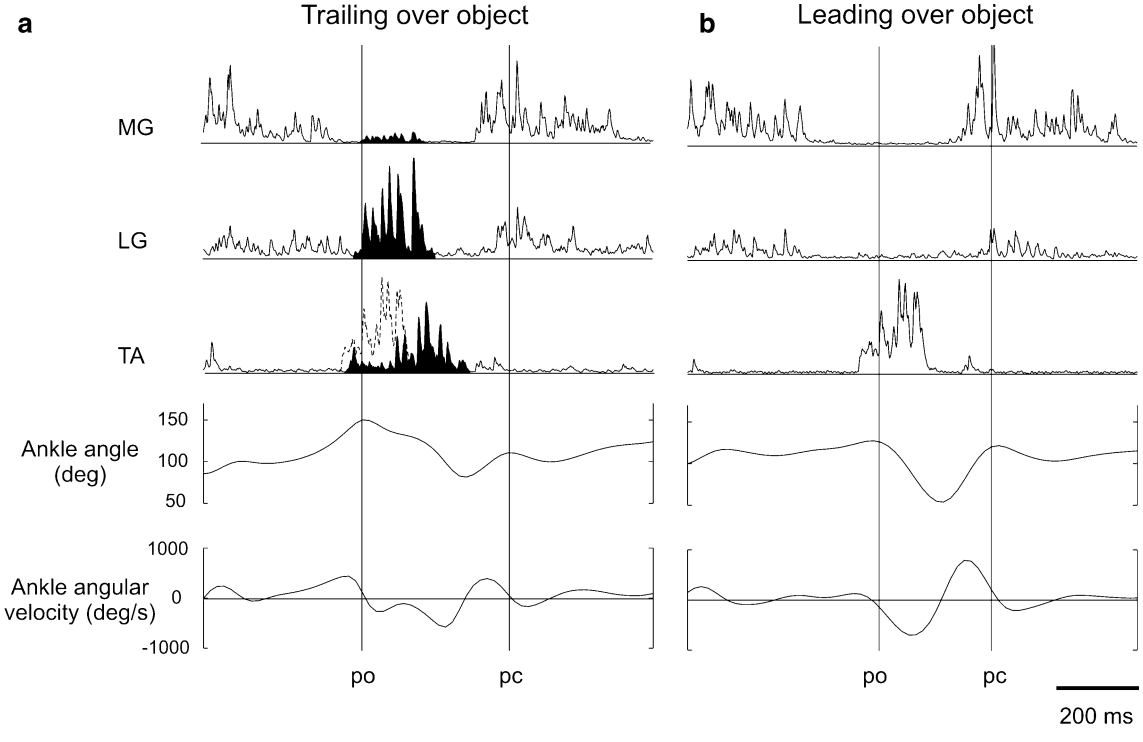

Fig. 8 The occurrence of the MG_F and LG_F bursts when a leg trails over an object is associated with a slowing of ankle flexion. *Top three trace*— rectified and filtered EMGs from MG, LG, and TA; *bottom two traces*—corresponding records of ankle angle and ankle angular velocity. Note that the TA burst was smaller and longer when the leg trailed (**a**) than when the leg led over the object (**b**). The *dotted line* in the TA record in (**a**) shows the TA burst during the leading step for comparison with the TA burst (*filled region*) during a trailing step

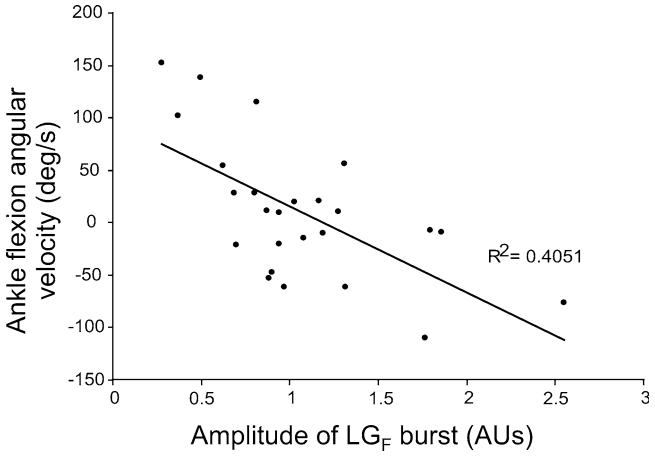

Fig. 9 Ankle flexion when the leg trails over an object is slowed in proportion to the magnitude of the MG_F and LG_F bursts. *Scatter plot* showing the inverse relationship between the ankle flexion angular velocity and the amplitude of the LG_F bursts. Each *dot* is a single trial, and the *line* in the plot is the best fitting linear regression line. *AU* arbitrary units

leg, respectively), or whether the generation of the MG_F and LG_F bursts develops over time as a result of some sort of learning process. One indication that learning was not a major factor was that MG_F and LG_F bursts were observed on the first recording session 5–6 h following the denervation surgery. During the short recovery period the animals were confined to a small-animal transport cage that prevented locomotion. However, all the animals did walk on the treadmill or walkway for short periods (a few seconds each time) while being prepared for each recording session, so there remains the possibility that the animals quickly learned to generate the MG_F and LG_F bursts during these short periods of walking prior to the first recording session. If this occurred, it would be in contrast to the much slower adaptations in the activity of the MG muscle following denervation or weakening of the LG, SOL, and plantaris muscles (Misiaszek and Pearson 1999; Pearson et al. 1999). At present, however, we favor the notion that the MG_F and LG_F bursts are produced by an immediate reorganization of the spinal pattern-generating network.

The reorganization of the spinal pattern-generating network appears to require input from supraspinal regions because the MG_F and LG_F bursts have never been reported in walking decerebrate or spinal cats, or during fictive locomotion in these preparations. Nor do they occur in decerebrate walking cats in which the hamstring nerve has been cut (unpublished observations). A likely source of these supraspinal signals is the motor cortex. The activity of many neurons in the hind leg region of the motor cortex is modulated during walking, with maximal activity occurring during the F phase of the step cycle (Widajewicz et al. 1994; Drew et al. 2002). Moreover, the activity in these modulated neurons increases significantly when the contralateral hind leg steps over an object. Thus, it would be of some interest to know whether the activity of these neurons also increases following partial denervation of the contralateral knee flexors. If they do, then the next question is what could be the cause of the increased activity? This question also applies to any other descending pathway showing modified activity after partial denervation of knee flexors.

Another fact of potential relevance to understanding the mechanism for the generation of the MG_F and LG_F bursts is that activation of the LG and MG muscles occurs without activity in SOL during paw shaking (Smith et al. 1980). Thus, we know that a spinal interneuronal network exists that can selectively activate LG and MG motoneurons, so it is reasonable to suppose that this same network is recruited to produce the MG_F and LG_F bursts following partial denervation of the knee flexors. If, in addition, supraspinal inputs are involved in generating the MG_F and LG_F bursts, as we suspect (see above), then we predict that some descending pathways must be directed onto the interneuronal system selectively activating the MG and LG motoneurons. It is also likely that this interneuronal system receives input from, or may be part of, the central pattern generating network that produces the basic locomotor rhythm, since the MG_F and LG_F bursts can be consistently linked with the onset of flexor activity during walking (Fig. 3).

The findings of the present investigation add to the growing body of data that has revealed considerable flexibility in the functioning of the interneuronal network controlling burst generation in hind leg motoneurons. It is now quite clear that bursts can be generated in most sets of motoneurons in either the flexion phase, one or more of the extension phases, or both flexion and extension phases of the step cycle, depending on the situation. The expression of additional bursts and/or the switching of bursting activity from one phase to another, has been reported during task-modification during normal walking (Smith et al. 1993, 1998; Carlson-Kuhta et al. 1998), in response to removal or addition of sensory signals (Perret and Cabelguen 1980; Perret 1983; Bouyer and Rossignol 2003), and as a result of weakening of a set of muscles (this study). In no instance have the underlying mechanisms been determined. One proposal is that the basic rhythm generating network consists of mutually inhibiting half-centers (flexor and extensor) and that each half-center is connected to many different groups of motoneurons via pathways that can be open or closed by sensory and/or supraspinal signals (Perret 1983). Another is that the central pattern generating network consists of a set of unit burst generators each of which drives a set of motoneurons and that the coupling between these unit burst generators can be modified by sensory and/or supraspinal signals (Grillner 1981; Smith et al. 1998). Establishing whether either of these proposals is correct is a challenging task. At the very least it requires a much greater understanding of the functional organization of interneurons in the spinal cord, as well as knowledge of the activity patterns of descending interneurons in

situations in which burst patterns are modified. Encouragingly, the feasibility of gaining this information has been demonstrated in recent studies on activity patterns of an identified class of interneurons in the lumbar cord (Angel et al. 2005) and recordings from different regions of the brain stem and cortex in behaving cats (Widajewicz et al. 1994; Matsuyama and Drew 2000; Lavoie and Drew 2002).

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