Adaptive Changes in Locomotor Activity Following Botulinum Toxin Injection in Ankle Extensor Muscles of Cats

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Abstract


The present study investigated the adaptations made in motor behavior following a temporary reduction in ankle extensor activity in the walking cat. Temporary muscle weakness was induced by injecting botulinum toxin into the lateral gastrocnemius (LG), plantaris (PL), and soleus (SOL) muscles, or SOL alone. The medial gastrocnemius (MG) muscle was not injected. Adaptations in the level of muscle activity were recorded using chronically implanted electromyographic (EMG) electrodes. Serial recordings were made prior to botulinum toxin injections and for several days following the injections. Kinematic analysis of ankle joint movements was made from video records to assess the impact of the botulinum toxin injections on the function of the ankle joint during walking. Following injection of the LG, PL, and SOL muscles with botulinum toxin, the amplitude of the MG burst increased over a period of a few days to a week. This increase was similar to the previously reported changes produced in MG following transection of the nerves serving LG, PL, and SOL. Following the weakening of the ankle extensor muscles, there was a temporary deficit in ankle function during walking as evidenced by a marked increase in the amount of ankle flexion that occurred at stance onset. This functional deficit recovered relatively quickly and was not associated with a return of the EMG pattern to the preinjection pattern. After recovery from the initial injections, a second injection of botulinum toxin into SOL alone was performed. No functional deficits were observed in the ankle movements during walking following this second injection. However, weakening SOL produced increases in the burst amplitudes of the MG, LG, and PL muscles over a period of a few days. This suggests that normal movements at the ankle during walking can be generated with more than one pattern of ankle extensor activity and that there is flexibility in how the necessary torque is produced. A final procedure, transection of the nerves serving LG, PL, and SOL, failed to produce any functional deficits in ankle movements. The implication is that adaptations to the neural control of ankle extensor activity that were induced by the initial procedure persisted after the recovery of the injected muscles and were sufficient to compensate for the subsequent challenges.

INTRODUCTION

The ability to produce purposeful movements requires an accurate estimate of the biomechanical properties of the moving structures. Over the life span of an animal, the properties of these structures change during the natural course of development and aging and may be altered as a result of use, injury, or disease. Thus, if movement accuracy is to be maintained over a long period of time, changes in the mechanical properties of the moving structures must be detected by the CNS to produce the appropriate adaptive modification of motor output. There are now numerous examples of adaptive changes in motor output and/or behavior in response to either modifying the mechanical properties of a motor system (Carrier et al. 1997; Optican et al. 1985; Pearson et al. 1999), or altering the physical properties of the environment in which the movement is performed (Du Lac et al. 1995; Knudson 1994; Krakauer et al. 1999; Thoroughman and Shadmehr 1999).

Recently, we demonstrated that the pattern of activity in the medial gastrocnemius (MG) muscle in walking cats changes in an adaptive manner after cutting the nerves to the synergist muscles: lateral gastrocnemius (LG), soleus (SOL), and plantaris (PL) (Pearson et al. 1999). The electromyographic (EMG) activity in MG progressively increased over several days, while the excessive ankle yield at ground contact progressively decreased. Over the first few days the portion of the MG EMG occurring after ground contact increased rapidly, and this increase was associated with enhancement of ankle extension during mid- to late stance. The portion of the MG EMG occurring prior to ground contact increased more slowly. The increase in the early component was related to the decrease in ankle yield that occurred over a period of about 1 wk (see Fig. 6B in Pearson et al. 1999). These adaptive changes were found to be use dependent, implying that the altered afferent feedback associated with the deficit initiated the adaptive increase in the magnitude of MG activity.

In our previous study (Pearson et al. 1999) the cutting of the nerves to the synergist muscles permanently eliminated these muscles from the locomotor system. In addition, this procedure also transected the sensory afferents of the denervated muscles, thus raising the possibility that the use-dependent increases in MG activity might depend on trophic modification of the terminal processes of the cut afferents. For example, modification of these terminals may facilitate use-dependent changes in terminals of uncut afferents from MG. Thus one of the purposes of the current investigation was to determine whether weakening synergist muscles without damage to their afferents could produce similar adaptive increases in MG activity. The method we chose was to inject botulinum toxin into the bellies of the other ankle extensor muscles. Botulinum toxin blocks
transmission at the neuromuscular junction by impairing the release of acetylcholine (Jankovic 1994). An attractive feature of using botulinum toxin is that the neuromuscular blockade is not permanent. Recovery of neuromuscular transmission returns over a period of a few weeks to a few months. This feature allowed us to address the question of whether any modification of MG activity resulting from toxin injection was fully reversible. This does not necessarily have to occur since it is conceivable that on recovery of neuromuscular transmission the relative contribution of each muscle in controlling ankle extension is altered, but the net effect of all four muscles remains normal. Furthermore, it is possible that some of the adaptive modifications of MG activity are irreversible, and compensatory change must occur in the activity of synergists for the production of normal movements. Preliminary results of this investigation have appeared in abstract form (Misiaszek and Pearson 1999).

METHODS

Experiments were performed on three adult cats (2 female, 1 male) weighing between 2 and 3.5 kg. The experimental procedures were approved by the Health Sciences Animal Policy and Welfare Committee at the University of Alberta.

Experimental procedure

The animals were trained to walk on a motor-driven treadmill at speeds ranging from 0.4 to 1 m/s. Once the animals were sufficiently trained, EMG recording electrodes were implanted into the MG, LG, SOL, and PL muscles of the right hind leg. Two pairs of electrodes were implanted into MG to provide a safeguard in the event of failure of one set of electrodes. In addition, the similarity between the EMG profiles recorded from the two sets of electrodes in MG provided assurance that the changes in the EMG activity were not due to movement of the electrodes. The EMG electrodes were comprised of a multi-stranded stainless steel wire (Cooner Wire Company, AS632) insulated except for a 3- to 4-mm length positioned in the muscle. To implant the wires into the muscles, the ends of the wires were secured to a 21-gauge needle that was then passed through the belly of the muscle. The two wires of an electrode pairing were then knotted and secured to the muscle with a silk suture. The electrodes were fed subcutaneously to the head of the animal where they were soldered to a plug adapter, which was then secured with dental acrylic to screws embedded into the skull. A cable connection to the amplifiers was inserted into the plug during recording sessions. This cable was supported above the animal by a retractable tether so that the cat was free to move about the treadmill. Two to 3 days following the implantation of the electrodes, EMGs were recorded while the animal walked on the treadmill. For cat 1 we permitted the animal to recover for 2 days following the injection prior to recording the first session. We initiated recordings in cat 2 24 h after the injection. In cat 3, the first recordings were made about 6 h after the injections, once the animal had sufficiently recovered from the anesthetic. Recordings were made over the next 5–6 wk (Table 1) while the activity in the injected muscles recovered. Subsequently, a second botulinum injection was administered to only the SOL muscle. Injection of SOL alone was selected for this second series as the recovery of the MG muscle activity toward control values appeared to be closely related to the recovery of function in SOL activity in the first series (Fig. 4). Moreover, the injection of the botulinum toxin appeared to be most effective in blocking the SOL muscle activity. For each cat, the dose used for this second injection was 40 units. Initial EMG and video recordings were made 5–6 h after the injection and continued over the next 4–6 wk (Table 1). Following recovery from this second injection, LG, SOL, and PL were exposed, but not injected with the toxin. This sham operation was performed on two animals, and recordings were made for the next 7 days. In one animal (cat 1), we performed a tenotomy of SOL, and recordings were made for the next 14 days. The results of the procedure in this first cat were unremarkable. Therefore this procedure was not repeated in the other cats. Finally, in all animals the nerves to LG, SOL, and PL were transected repeating the procedure performed in our previous paper (Pearson et al. 1999). Data were recorded within 6 h of the transection and repeated over the next 3 wk.

Data analysis

The EMG signals were amplified (×500–20,000) and filtered (30–10,000 Hz, Grass P511 preamplifier, Astro-Med, West Warwick, RI) prior to storage onto magnetic tape (VHS, Vetter 4000A PCM recording unit). The data were later full-wave rectified, filtered (low-pass 20 Hz), digitized (sampling at 700 Hz) using an Axotape data acquisition system.

| Table 1. Dosage and recovery durations of botulinum injections for each cat |
|--------------------------|--------------------------|--------------------------|
|                         | Cat 1                    | Cat 2                    | Cat 3                    |
| Injection into LG, SOL, and PL |
| Doses                    |                         |                          |                          |
| LG                       | 50 units                 | 90 units                 | 80 units                 |
| PL                       | 30 units                 | 70 units                 | 60 units                 |
| SOL                      | 20 units                 | 30 units                 | 40 units                 |
| Days recovery            | 41                       | 35                       | 40                       |
| Injection into SOL alone |
| Dose                     | 40 units                 | 40 units                 | 40 units                 |
| Days recovery            | 41                       | 27                       | 31                       |
| Sham                     |                          |                          |                          |
| Days recovery            | Yes                      | Yes                      | No                       |
|                         |                          |                          |                          |
| Section of nerves to LG, SOL, and PL |
| Days recovery            | 21                       | 19                       | 20                       |
| LG, lateral gastrocnemius; SOL, soleus; PL, plantaris; N/A, not applicable. “Units” refers to mouse LD50 units of botulinum toxinn, reconstituted in saline (10 units/0.1 ml).
system (Axon Instruments), and stored to computer disk. The timing and magnitude of the EMGs were measured using custom software.

Data from our previous paper (Pearson et al. 1999) suggested that there were two distinct components of the MG EMG trace and that each might be regulated by different mechanisms following the transection of the LG, SOL, and PL nerves. Consequently, in the present study we quantified the MG EMG over these same two periods (Fig. 4). The first period was the initial 80–100 ms of the burst, which varied in duration from cat to cat depending on the time of EMG onset to the initiation of stance phase (time of ground contact). The second period was a window of equal duration (dependent on the duration of the first interval) centered on the peak of the EMG activity that followed ground contact. We refer to these two periods as the initial and late components, respectively. We wished to determine changes in the profile of the MG EMG in the days following the injection of LG, SOL, and PL with botulinum toxin. Consequently, all averages were normalized to the values obtained on the day of the injections. This recording session occurred prior to the injections and is referred to as day 0. Typically, average EMG amplitudes were obtained from measuring 50–60 individual steps while the animal was walking regularly at one position of the treadmill.

The amplitudes of the EMGs from LG, SOL, and PL were also quantified over the days subsequent to injection to determine the extent and duration of the neuromuscular block of these muscles. For this analysis, the MG EMG was used to determine the timing of the measurement window as the reduced EMG levels in the affected muscles made onset times difficult to determine (see Fig. 1). For these muscles, we measured the average EMG amplitude over the first 400 ms of the burst.

Analysis of the EMG data were similar following each of the additional procedures (reinjection of SOL alone, sham and nerve transection). The amplitudes of the measured EMGs were always normalized to the values obtained during the recording session immediately preceding the procedure. This allowed us to assess the effects of the procedure per se, rather than comparing the results to a control value obtained prior to a number of intervening procedures. However, this also meant that the control value used for normalization included some residual influences from the preceding procedure. Consequently, we are restricted to a largely qualitative analysis of the influence of these later procedures. Nevertheless, the changes in the MG EMG are robust, suggesting any residual influences of the preceding procedures are minor in comparison.

The kinematics of stepping of the right hind leg were determined from digitized images from the video recordings. For each recording session, reflective markers were placed on the iliac crest, the hip, knee, and ankle joints, and the paw to allow measurement of the joint angles. To ensure the markers were placed consistently between each session, the position of each marker was outlined on the shaved skin with indelible ink. A video capture card (Miro DC20) digitized the images at 30 frames/s. Custom software was used to calculate the joint angles from these images. Our primary interest was the angle of the ankle joint. The ankle kinematics were determined by averaging 10–15 steps. The video data were obtained from the same sequence of walking used for the EMG analysis. We used data obtained during the preferred walking speed of the individual animal for analysis. Two cats provided consistent walking at 0.6 m/s, the other cat preferred stepping at 0.5 m/s.

**RESULTS**

**Effectiveness and time course of action of botulinum toxin injection**

EMG recordings from LG, SOL, and PL demonstrated that injecting botulinum toxin into these muscles led to neuromuscular blockade (Fig. 1). Blockade began within a few hours of injection and was maximal in 1–2 days (Figs. 2, 3, and 5). The amount of blockade varied depending on the muscle. In SOL, blockade was almost complete in all but one case (Fig. 2), whereas in LG and PL blockade was never complete (Fig. 3; for cat 3, the LG electrodes were damaged during the injection surgery; as a result, LG was not recorded from this animal). In LG and PL the maximum suppression of EMG activity varied between 53 and 92%. We are uncertain as to why botulinum toxin injection was less effective in LG and PL. One possibility is simply that these muscles are larger than SOL, thus reducing the probability that the injection sites were close to neuromuscular junctions. Another is that botulinum toxin may preferen-

**FIG. 1.** Sample electromyography (EMG) traces from one animal that received botulinum toxin injection into LG, SOL, and PL. **A:** a sequence of 4 steps recorded just prior to the injections of the toxin. **B:** a similar sequence of 4 steps recorded 2 days following the injections. **C:** average EMG traces for each muscle before (thick line) and 2 days after (thin line) the injections. Each trace is the average of 50 steps. The time window of analysis (see METHODS) is indicated by the vertical lines and the bar below the SOL traces. MG, medial gastrocnemius; LG, lateral gastrocnemius; SOL, soleus; PL, plantaris.
tially block transmission in slow motor units as evidenced by the earlier onset and more rapid progression of atrophy in “slow” muscle fibers following botulinum toxin injection (Duchen 1970; Rosales et al. 1996).

One of the objectives of this investigation was to determine whether weakening of the synergists of MG produced modifications of MG bursts similar to those occurring following transection of the nerves innervating synergists. Since the latter primarily occur over a period of 1 wk, it was important to first estimate the time course of recovery from the neuromuscular blockade. This is not a straightforward matter of simply monitoring the reduction in the magnitude of EMG bursts in an injected muscle because a reduction in the number of functional motor units may be overshadowed to some extent by compensatory increases in the level of activity of motor units that are not blocked. For example, in the SOL muscles in which near complete block was achieved (5 of 6 times SOL was injected), the magnitude of burst activity began to progressively increase a few days after injection, and it returned to a value close to control over a period of about 4 wk (Fig. 2). This period probably underestimates the actual time course of recovery from neuromuscular blockade. The progressive increase in SOL EMG may be due in part to a progressive enhancement of motor unit activation and not entirely due to recovery from neuromuscular blockade. In any event, it was clear that the duration of action of botulinum toxin in SOL, and probably in LG and PL as well, was sufficient to allow us to examine the influence on burst activity in the noninjected MG muscle over the first week following muscle injections.

Changes in MG bursts following weakening of synergists

When LG, SOL, and PL were injected with botulinum toxin, the magnitude of the bursts of activity in MG increased significantly (Fig. 4). We quantified changes in the initial and late components of the MG bursts (see Fig. 4A) since previously we found a difference in the time course of changes in these two components (Pearson et al. 1999). Figure 4, B–D, illustrates that the magnitude of the late component increased rapidly over the first few days following the injection and then continued to increase more gradually over a period of a week or more until reaching a peak increase ranging from 150 to 400%.

FIG. 2. Neuromuscular blockade of SOL following botulinum toxin (BOTOX) injection. Each panel displays the average SOL burst amplitude recorded for each day following injection of toxin. The left column of panels shows the data from the initial injection paradigm (injections into SOL, LG, and PL) for each cat. The right column of panels shows the data from the 2nd injection series of SOL alone. Burst amplitudes have been normalized to the day 0 preinjection burst amplitudes (100%, depicted by the broken line extending across each panel). Each data point is the average of ≥50 steps. Error bars have been excluded for clarity.
Following this large increase, there was a slow decline in magnitude over a period of 3–4 wk toward control values. The pattern of the increase in the late component of the MG bursts over the first week differed in one obvious aspect from that observed in our previous study using nerve transections to denervate LG, SOL, and PL (see Fig. 5 in Pearson et al. 1999). In that study the late component increased rapidly within 2 days of the nerve transection, but thereafter increased only slightly or decreased, whereas in this study the initial increase in the late component was more gradual and continued over several days. One possibility for this difference is that the neuromuscular blocking action of botulinum toxin requires a number of days to be fully effective. Consistent with this view is that when SOL muscle alone was reinnervated, the late component of the MG bursts increased to maximum values within 2–3 days, corresponding to the time of maximum suppression of activity in SOL (Fig. 5A). Another possible explanation is that injection of the muscles does not eliminate the afferent feedback from those muscles, in contrast to the immediate loss of afferent feedback with a nerve transection. Thus proprioceptive signals from LG, PL, and SOL would continue to be delivered to the spinal cord. It is difficult to speculate what impact this might have on the adaptive processes responsible for the functional recovery as the afferent signals from these muscles would themselves be altered by the changing state of the parent muscle.

A notable feature of the change in the magnitude of the late component of the MG bursts was that the recovery toward normal values was closely associated with an increase in the magnitude of the SOL EMG. This was seen when LG, PL, and SOL were injected (Fig. 4) and when only SOL was reinnervated (Fig. 5). Note that this association also occurred in the one unusual animal (cat 2 in Fig. 4) in which activity in SOL was not completely blocked and increased well above normal after 10 days.

The initial component of the MG EMG also increased following the injection of botulinum toxin into synergist muscles (Fig. 4, C). Unlike the increase in the late component, however, the increase in the initial component was relatively modest (ranging from 60 to 100%), and it did not show a large increase on the day following injection (cats 2 and 3 in Fig. 4). Another difference was that the initial component, although decreasing slightly following a peak increase, did not return to a value close to control within 4–5 wk.

The initial and late components of the MG bursts were influenced differently following reinnervation of SOL alone. In the example shown in Fig. 5 (all 3 cats yielded similar data), activity in SOL was almost completely suppressed within 2 days of toxin injection, and its activity returned slowly over a period of about 3 wk. Corresponding to the decreased activity in SOL was an increase in the activity of MG, LG, and PL (Fig. 5A). A closer analysis of the MG bursts revealed that the increase was primarily in the late component (Fig. 5B). On day 4 for instance, the late component had increased by over 60%, whereas the initial component had increased by <10%.

Changes in ankle kinematics following botulinum toxin injections

Not unexpectedly, injection of botulinum toxin into LG, SOL, and PL resulted in deficits in movements at the ankle during the stance phase (Fig. 6, A–C). At stance onset in the normal walking cat, there is a flexion of the ankle joint as the weight of the animal loads the leg. This ankle flexion at early stance was markedly increased following the weakening of LG, SOL, and PL with botulinum toxin. Figure 6A shows plots of the ankle angle during the stance phase for one cat before, 2 days after, and 2 wk after the injections. Each trace begins 66 ms prior to ground contact. These plots show an exaggerated yield (flexion) at the ankle 2 days after the injections compared with normal, and a virtually complete recovery by 2 wk.

The increased flexion of the ankle during stance onset is quantified in Fig. 6B for the same cat as in Fig. 6A (cat 2). The ankle flexion increased over several days following the injections, then decreased toward control values beginning about a week after the injection. Prior to the injection of the toxin, the average amplitude of ankle flexion at ground contact was $46.1 \pm 1.83^\circ$ (mean $\pm$ SE). This increased to a maximum amplitude of $35.5 \pm 5.58^\circ$ 4 days following injection. The amount of ankle flexion remained relatively stable for 2–3 days before decreasing toward control. Within 11 days of the injection, the exaggerated flexion of the ankle at ground contact was no longer evident. No further changes were evident thereafter. This pattern of deficit and recovery was similar for all three
cats. Figure 6C shows the average data from the three cats over a period of 35 days following the injection of the LG, SOL, and PL muscles. In all instances the hyperflexion of the ankle at ground contact was reduced to values close to normal within 2 wk of the injection.

In addition, as shown in Fig. 6A, the overall kinematic profile of the stance phase of the cat step cycle was qualitatively indistinguishable from normal 2 wk following the injection of the toxin. Two days following injection, there was an obvious reduction in the extent of extension of the ankle joint.

FIG. 4. Adaptive changes in MG burst amplitude following injection of botulinum toxin into LG, PL, and SOL. A: sample of average EMG traces from one animal demonstrating the increase in amplitude of the MG burst 3 and 7 days following injection of LG, PL, and SOL. The time windows used to obtain the average for the Initial and Late components of the MG burst are depicted. Each trace is the average of 50 steps. B–D: each panel depicts data from 1 cat and shows the average Initial MG (■), late MG (●), and SOL (▲) burst amplitude for each day following injection of LG, PL, and SOL. The burst amplitudes have been normalized to the preinjection amplitudes (100%, depicted as the broken line). Each data point is the average of ≥50 steps. Error bars have been excluded for clarity.

FIG. 5. Adaptations to ankle extensor burst amplitudes following a 2nd injection of botulinum toxin into SOL alone. A: average burst amplitude for each muscle on each day following injection of botulinum toxin into SOL. Only the late component of the MG burst is included. B: average burst amplitude for the initial and late components of the MG burst. Each data point is the average of ≥50 steps. The data have been normalized to the preinjection amplitudes (100%, depicted by the broken line). The data are from a single animal (cat 2).
at the end of the stance phase, i.e., during the propulsive portion of the step cycle (maximum extension was 134.5° prior to injection, and only 111.4° 2 days following injection). However, within 2 wk of the injection the ankle extension at the end of stance was very similar to the preinjection value (132.6°). This was a consistent observation in all cats. The restoration of the kinematic profile of the ankle movements was evident when viewing the behavior of the animal. In the first week following the toxin injections, the animal walked with a noticeable drop in the level of the hips and a marked asymmetry in the stepping pattern. However, by the second week the walking behavior of all the animals appeared completely normal.

A surprising result of the investigation was that reinjection of botulinum toxin into SOL alone (4–5 wk following the injections into SOL, LG, and PL) had no noticeable influence on the kinematics of ankle movements (Fig. 7). Neither the profile of ankle movement during stance (Fig. 7A) nor the magnitude of ankle flexion during early stance (Fig. 7B) was altered. Visual observation of the animals walking on the treadmill or in unrestrained situations also failed to indicate any behavioral deficit produced by the second injection. Despite the absence of any noticeable effects on ankle kinematics, the blockage of neuromuscular transmission in the SOL muscle must have had some mechanical influence on the synergists. This was indicated by the fact that activity in all three synergists increased when SOL activity was reduced (Fig. 5A).

Effects of transecting the LGS and PL nerves after botulinum toxin treatment

The final stage of these experiments was sectioning of the nerves serving LG, SOL, and PL. As with the preceding stages, the MG EMG burst amplitude was standardized to the value that was obtained during the recording session immediately preceding the nerve section. The first recording session following nerve section was performed 5–6 h later.

In one animal (cat 1) an additional procedure had been performed prior to the nerve transection. The additional procedure was a tenotomy of SOL. The tenotomy resulted in a progressive increase in the burst amplitudes of MG, LG, PL, and SOL in the days following, suggesting that adaptive processes similar to those induced by the botulinum toxin injections were also induced by the tenotomy. In this cat, the transection of the nerves to LG, PL, and SOL resulted in an increase in the activity of the MG muscle, as was seen in all cats (see following text), but the magnitude of change was substantially less. Presumably, this was due to the adaptive changes that had occurred in this cat following the tenotomy of SOL. Consequently, the data from this animal were excluded from the analysis of the final phase of the study. However, qualitatively the results from the nerve transection in this cat are consistent with the results described below for the two other cats, indicating that the data from this cat support the general conclusions.

FIG. 6. Changes in ankle kinematics following injection of botulinum toxin into LG, PL, and SOL. A: sample kinematic traces of the ankle joint during the stance phase for one cat. Each trace is the average of 10 steps. In B, the peak ankle flexion that occurs during early stance phase is averaged for 10 steps for each day following the injection of LG, PL, and SOL. The broken line represents the amount of ankle flexion that occurred prior to the injections. Error bars represent 1 SD. The data in A and B are from 1 cat (cat 2). C: data from all 3 cats have been averaged for 6 time points following the injections to show the consistency of the results across animals. Error bars represent 1 SE.

FIG. 7. Absence of change in ankle kinematics following the 2nd injection of botulinum toxin into SOL. A: sample kinematic traces of the ankle joint during the stance phase for 1 cat (cat 3). The traces represent preinjection (thickest), day 2 (middle thickness), and day 14 (thinnest) steps. Each trace is the average of 10 steps. B: the average ankle flexion at stance onset across animals for 4 time points following injection of SOL. Error bars represent 1 SE.
Figure 8A displays the kinematic profile for the ankle movements during stance phase from one cat (cat 3). Remarkably, there is little difference in the pattern of movement 5 h following nerve section compared with the presection trace. The only noticeable difference is reduced extension during terminal stance. There was no noticeable increase in the amount of ankle flexion at the onset of stance. A similar result was observed in the other cat.

The amplitude of MG EMG for cat 3 is shown in Fig. 8B. The amplitude of the late component was dramatically increased 5 h after the section and remained close to this level for the following 20 days. There was no noticeable change in the amplitude of the initial component of the MG burst during this period. Interestingly, the late component of the MG burst increased to a value close to the maximum level achieved during either of the previous two stages of the experiment. This is represented as the solid line in Fig. 8B, representing the recording from day 2 from the second injection series (SOL reinjected alone). Similar results were obtained in the other cat.

**Discussion**

The present study investigated the adaptations made in motor behavior following a temporary reduction of ankle extensor activity in the walking cat. Temporary muscle weakness was induced in LG, SOL, and PL, or SOL alone, as a result of botulinum toxin injection. The MG muscle was not injected and thus was temporarily forced to generate greater ankle extensor torque during the stance phase of locomotion. Three main findings arise from this study. First, the amplitude of the MG burst increased over a period of a few days following the injection of botulinum toxin into LG, SOL, and PL, and this increase was similar to that produced by transection of the nerves serving these muscles (Pearson et al. 1999). This indicates that the adaptations in MG burst amplitude observed in the previous study were not in response to trophic events initiated by the injury of the transected afferents. Second, a functional deficit in ankle movement produced by the first injection of botulinum toxin recovered relatively quickly, and this return to normal function was not associated with a return of the EMG pattern to that seen prior to botulinum toxin injection. Furthermore, the overall EMG pattern continued to change after the return of normal ankle movements (Figs. 4 and 6). Taken together, these observations demonstrate that there is not a unique pattern of activation of the ankle extensors for controlling the normal movements at the ankle during level over ground walking. Third, after an initial period of functional deficit following the first injections, subsequent procedures, including a final transection of the nerves serving LG, SOL, and PL, failed to produce any additional periods of functional deficit. The implication is that the adaptations to the neural control of ankle extensor activity that were induced by the initial procedure (increase in MG activity prior to ground contact and presumed increase in reflex gain contributing to the late component of the EMG burst) persisted after the recovery of the injected muscles and were sufficient to compensate for the subsequent challenges.

It is important to note that our conclusions are limited to level treadmill walking. It is possible that deficits in function of the ankle joint would be noticed in other activities. We cannot address this issue with our current data. Nevertheless, the results of the present study highlight the adaptability available within the nervous system to generate movements. Presumably, this adaptability is not restricted to level walking, but would also be invoked to reduce functional deficits that are sure to occur in other tasks.

**Technical considerations**

This study involved the recording and measurement of EMG activity over a period of ≈15 wk. A critical issue is the reliability of the chronic EMG recordings over this time period. It is possible that the large changes in the amplitude of the bursts observed following the injections was the result of changes in the positioning or physical properties of the electrodes. The most compelling evidence against this possibility was the similarity of the results obtained from the first and second injection series. Injection of LG, SOL, and PL was more invasive than the reinjection of SOL alone. Nevertheless, qualitatively similar changes to the MG burst amplitude were observed. Another compelling observation was the different time course of change observed in the two components of the MG burst (Fig. 4). If the changes in MG burst amplitude were the result of displacement of electrodes or changes in properties, then it would be expected that both components would change in parallel. A third observation was that the recordings obtained from the two pairs of electrodes implanted into MG were similar (data not shown). This indicates that there were no major shifts in the position of the electrodes. One final obser-
vation was that a sham operation performed on two of the cats failed to produce any changes in EMG profiles.

The focus of this study was the adaptation in the ankle extensor muscle MG, following botulinum toxin injection of three other ankle extensor muscles LG, PL, and SOL. Previously it has been reported that flexor hallucis longus (FHL) is capable of producing a plantarflexion torque roughly equivalent to SOL (Lawrence et al. 1993). Moreover, it has been demonstrated that the pattern of activity in FHL is similar to that of the other ankle extensors during treadmill walking (Abraham and Loeb 1985). Therefore it is possible that some of the functional recovery is the result of adaptations in the use of FHL. However, the extensor torque produced by FHL is minor compared with MG (Lawrence et al. 1993), and the adaptations in MG activity in the present study were substantial, suggesting that the adaptations in MG activity were important to the recovery of function. Nevertheless, it is reasonable to speculate that a portion of the functional recovery was due to adaptations in FHL activity. If so, the adaptations in FHL activity likely paralleled the adaptations observed in MG. Indeed, the activity of LG and PL changed in parallel with MG when SOL alone was reinjected (Fig. 5).

Functional adaptation of MG EMG amplitude

Following injection of LG, SOL, and PL with botulinum toxin the MG burst amplitude progressively increased over a period of several days (Fig. 4). At least three factors may have contributed to this increase. First, the botulinum toxin required 2–3 days for maximum effect. Thus over this time period there was a progressive increase in the flexion of the ankle joint at ground contact (Fig. 6). This increase in ankle flexion caused a greater stretch of the ankle extensors, including MG. This in turn would lead to greater activation of the stretch reflex and a resultant increase in the amplitude of the late component of the MG burst. However, not all of the increase in the MG burst amplitude can be explained by an increased stretch of the muscles because the MG burst amplitude continued to rise after the maximum ankle flexion occurred.

A second factor that could contribute to increasing the MG burst amplitude is an increase in the gain of the stretch reflex. Evidence that the gain of the stretch reflex in MG can change in this manner is offered by a previous study (Pearson and Misiaszek 2000; Pearson et al. 1999). Following section of the LG, SOL, and PL nerves, the amplitude of the late component of the MG burst progressively increased before reaching a plateau (Pearson et al. 1999). This increase in the late component of the MG burst occurred even though the excessive ankle flexion (an immediate result of the nerve section) was progressively decreasing. In addition, the increase in the late component of the MG burst is associated with an increase in the slope of the relationship between the magnitude of the late component of the MG burst and the amplitude of ankle flexion that occurs in early stance (Pearson and Misiaszek 2000). This indicates that the EMG produced in MG subsequent to a given amount of muscle stretch increased following the adaptation in burst amplitude, suggestive of an increase in the gain of the stretch reflex.

A third factor that could contribute to the increase in the MG burst amplitude is an increase in central drive (Gritsenko et al. 2001). Previous reports have shown that both afferent feedback and central dive contribute to the amplitude of ankle extensor activity during the stance phase (Stein et al. 2000). A change in central drive most likely explains the changes observed in the initial component of the MG burst in the present study (Fig. 4). This component of the MG burst occurs prior to ground contact and is presumably largely generated from central circuitry.

Recovery of function in muscles poisoned with botulinum toxin

One intriguing observation from this study was the finding that the burst amplitudes of many of the muscles injected with botulinum toxin overshot the preinjection control values during recovery (Figs. 2 and 3). For reasons given earlier in the DISCUSSION, this is unlikely to be due to alteration in the position and properties of the electrodes. A more likely explanation is that the overshoot in the burst amplitude of the injected muscles was the result of a similar adaptive process that led to the increase in MG burst amplitude. That is, the functional deficit that leads to the adaptive changes in the MG bursts could lead to similar increases in the EMG amplitudes of the injected muscles. However, the expression of this adaptive increase is masked until the neuromuscular transmission is restored. From the results of the present study, we cannot speculate on the afferent source that might lead to such adaptations in the poisoned muscles. If we assume that the adaptations in MG activity are initiated by homonymous afferent feedback (see Pearson et al. 1999), it is possible that this source leads to heteronymous adaptation of LG, PL, and SOL activity. Alternatively, poisoning of LG, PL, and SOL does not block transmission in afferents serving these muscles. Thus homonymous afferent feedback, signaling increased length of the muscles, or perhaps reduced force could also initiate the adaptive process in these muscles.

Another possibility is that the overshoot in the EMG burst amplitude in the injected muscles might be a secondary effect of remodeling of the muscle fibers by neuromuscular blockade (Anguat-Petit et al. 1990; Brown et al. 1980; Duchen 1970; Holland and Brown 1981; Spencer and McNeer 1987; Yee and Pestronk 1987). In particular, the muscle fibers display morphological changes associated with denervation atrophy. This includes atrophy of all fiber types, abnormalities in the sarcoplasmic reticulum including migration of the mitochondria, as well as vascular changes, including capillary withdrawal. The implication is that the functional characteristics of the muscle fibers are altered, which could lead to altered contractile strength and subsequent changes in the length-tension characteristics of the injected muscles. Functionally, this could mean that larger amplitude bursts might be required to produce the same contractile force. The output from the nervous system to these muscles must then adapt to reflect the progressively altering state of the muscle.

Recovery of ankle function after botulinum toxin injection

One of the most remarkable findings in this study is the lack of change in the kinematics of the ankle movements during stance phase once the initial functional deficit produced by the first toxin injections has ameliorated. No substantial changes in ankle function were observed 1) over most of the period of recovery following the first injections, 2) following reinjection
of only SOL, or 3) following section of the nerves to LG, SOL, and PL.

The magnitude of flexion at the ankle joint during stance onset was transiently increased after the initial injection of botulinum toxin into LG, SOL, and PL. The functional recovery of this portion of the ankle movement is likely related to the increased amplitude of the initial component of the MG burst (Pearson and Misiaszek 2000; Pearson et al. 1999). This early improvement in function is unlikely the result of changes to muscle properties, such as from hypertrophy. Other studies using muscle ablation or tenotomy to induce compensatory hypertrophy in synergist muscles have shown that hypertrophy does occur within days, even hours of the surgery (Armstrong et al. 1979; Goldberg et al. 1975). However, this early hypertrophy is largely due to edema and inflammation, resulting in a larger wet weight of the muscle, but no change in the dry weight (Armstrong et al. 1979). Degens et al. (1995) report that the larger wet weight of the muscle, but no change in the dry weight (Armstrong et al. 1979). Degens et al. (1995) report that true hypertrophy of PL was first observed 10 days after denervation of LG, MG, and SOL. Moreover, it has been reported that the tension produced by a chronically loaded muscle does not change during the first 4 days of these hypertrophic events, indicating that the early hypertrophy following tenotomy of synergists is not likely due to changes in contractile elements of the muscle (Goldberg et al. 1975). Indeed, our own observations have shown that 7 days after denervation of the LG, SOL, and PL, the peak tension developed in the chronically loaded MG is not different from control (previously unreported).

Following the re-injection of SOL alone, or section of the nerves to LG, SOL, and PL, none of the cats in the present study showed an increase in ankle flexion at stance onset, and there was no increase in the amplitude of the initial component of the MG burst. This suggests that the amplitude of the initial component of the MG burst was rescaled following the initial functional deficit produced by the first botulinum injection and that this new setting was retained for the duration of the experiment. Indeed, there was only a slight decrease in the initial component during the time the late component was returning toward normal (Fig. 4). Thus we conclude that the activity of the MG muscle prior to ground contact was sufficient to set the appropriate stiffness of the ankle joint at all times after recovery from the initial deficit. The overall stiffness at the ankle joint is determined by the in-series elastic elements of the muscle and tendon. If the muscle component is sufficiently high, then the stiffness at the ankle is approximated by the tendinous component (Griffiths 1991). Thus the increase in the muscle component that would be produced as a result of the increase in the initial component of the MG burst might be sufficient to establish a high enough muscle stiffness in MG that the overall stiffness was determined primarily by the tendon, and this did not change throughout the experiment.

The late component of the ankle extensor activity occurs following ground contact. Therefore afferent feedback associated with ground contact could contribute to the generation of the burst amplitude. The force generated as a result of the late component of the MG burst contributes to the support during stance phase as well as propulsive forces at the end of stance. The results from the present study suggest that the burst amplitude of the ankle extensor muscles following ground contact is regulated to achieve an accurate net force production. Moreover, the force production appears to be shared among all available ankle extensors and is adaptive to the changing capabilities of those muscles. This suggests that the amplitudes of the ankle extensor bursts are accurately controlled for the production of a required net force by all ankle extensors.

A simple means of accomplishing this would be to utilize a force-feedback reflex pathway for each muscle. Thus loading the muscle during stance phase would result in an increase in the burst amplitude for that muscle. The results of the present study support such a system. With the weakening of the injected muscles, the late component of the MG burst increases in amplitude as this muscle bears more load. As the injected muscles begin to recover and contribute some force to ankle extension, the load borne by the MG muscle is lessened, and the amplitude of the late component decreases. Such a mechanism would be able to account for 1) the decrease in the late component of the MG burst with the recovery of the injected muscles, 2) the stable ankle kinematics at late stance during the period of recovery as the injected muscles regain activity and contractile strength, and 3) the parallel pattern of increased activity in MG, LG, and PL following re-injection of SOL alone. The implication is that the force produced by individual muscles is sensed and the net force production of all muscles is distributed. Thus an important finding from the present study is the maintenance of stable ankle kinematics following recovery of the initial deficit, despite marked variation in the burst amplitudes of the various ankle extensor muscles. This functional stability suggests that the generation of force by ankle extensor muscles is accurately controlled for each individual muscle to ensure the adequate and appropriate net extensor torque during the stance phase of locomotion.

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REFERENCES


GAIT ADAPTATIONS FOLLOWING TOXIN INJECTIONS


