Long-Lasting, Context-Dependent Modification of Stepping in the Cat After Repeated Stumbling-Corrective Responses

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Submitted 30 August 2006; accepted in final form 7 November 2006

INTRODUCTION

As animals move through the environment, they encounter uneven terrain, obstacles, and other impediments to their progress. The unique requirements of each of these challenges highlight the need for flexibility in the neuromuscular control of locomotion. Two broad categories of modifications are used to this end (Pearson 2000). The first is a step-to-step adjustment of motor activity to accommodate the immediate features of the environment. The second is long-term changes in the pattern of motor activity during walking to accommodate a persistent change in the environment. In this study we report a form of long-term modification of stepping in normal cats: a persistent enhancement of the swing movement of the hind leg of cats to avoid an obstacle that repeatedly perturbed the swing movement. This adaptive response was discovered in the course of an investigation on sensory regulation of the swing movement. This adaptive response was discovered in the course of an investigation on sensory regulation of the swing movement. We unexpectedly observed that repeated mechanical stimulation of the paw led to an increase in knee flexion and step height (here we will refer to this behavior as persistent hyperflexion) even in the absence of further stimuli (not reported in McVea et al. 2005). The hyperflexion of the leg in response to mechanical stimulation of the paw is often referred to as the “stumbling-corrective response,” and previous investigations in cats documented the neural pathways and changes in leg kinematics and muscle activity underlying this response (Forssberg 1979; Quevedo et al. 2005; Wand et al. 1980). None of these studies, however, examined the modifications after multiple steps obstructed during swing in normal walking cats, although brief aftereffects (lasting for one to ten steps) after repeated stumbling-corrective responses were reported in infants (Pang et al. 2003), decerebrate ferrets (Lou and Bloedel 1988a), and spinalized cats (Edgerton et al. 2004). In contrast, the aftereffects we describe in this study lasted for much longer periods, >24 h in some cases.

The discovery of the conditioned persistent hyperflexion during treadmill walking raised the question of whether this modification of stepping is context dependent. Context-dependent modification of locomotor behavior was previously reported in cats walking on inclined walkways (Carlson-Kuhta et al. 1998; Smith et al. 1998a) and humans walking on compliant and slippery surfaces (MacLellan and Patla 2006; Marigold and Patla 2002). To investigate this issue we conditioned the hyperflexion while animals walked on a treadmill and then examined leg movements when animals walked in a different environmental context. In addition, we tested whether the persistent hyperflexion could be evoked in decerebrate walking animals and after denervation of the main flexor muscles of the knee, posterior biceps, and semitendinosus. Our main findings were that the persistent hyperflexion is context dependent and that it cannot be produced in either decerebrate walking animals or after partial denervation of the main knee flexor muscles.

A brief report of some of the findings in this study was previously published in abstract form (McVea et al. 2004).

METHODS

The Health Sciences Animal Welfare Committee at the University of Alberta reviewed and approved all of the procedures involved in this study.

Surgical procedures

The main objective of this study was to investigate the long-term aftereffects after a series of stumbling-corrective responses during walking in the cat. To do this, six adult cats were trained to walk on a custom-built treadmill at speeds ranging from 0.3 to 1.0 m/s. Once
cats were walking confidently and reliably, they were anesthetized (Isoflurane) and bipolar recording electrodes (Cooner wire AS632) were implanted unilaterally in the hind leg under aseptic conditions. The muscles implanted varied from animal to animal, but generally were the ankle extensors soleus (SOL), lateral gastrocnemius (LG), medial gastrocnemius (MG), the knee extensor vastus lateralis (VL), the hip flexor iliopsoas (IP), and semitendinosus (ST, knee flexor/hip extensor) muscle. Sartorius (Sart), a hip and knee flexor, was implanted in two animals. For this project, only data from the IP, ST, and Sart muscles were analyzed. Data recorded from other muscles were used in unrelated projects. A multipin socket attached to the animal’s skull using dental acrylic allowed access to the electrodes.

In two animals (cats 1 and 3) a second surgical procedure was performed 35 and 43 days later, respectively. After anesthetizing with Isoflurane, the nerves innervating ST, posterior biceps (PB), and anterior biceps (AB) of the right hind limb were cut just distal of their divergence from the sciatic nerve to denervate the main flexor muscles of the knee. The nerve innervating AB (hip extensor) was included because it includes motor axons supplying PB and ST (English and Weeks 1987).

Stumbling-corrective responses

A key element of this study was initiating repeated stumbling-corrective responses in walking cats. To do this, we used a handheld wooden rod, about 1 m long, with a 4-cm-long screw fixed perpendicularly in one end to form a hook. The screw was wrapped in gauze and medical tape to pad it. This hook was positioned manually so it made contact with the dorsum of the foot of the right hind leg early in the swing cycle while cats were walking on a treadmill (Fig. 1). We did not actively move the stimulating hook backward during each trial so the contact force (which was not measured) was established entirely by the collision of the moving paw with the stationary hook. During the conditioning procedure the stimuli were delivered in blocks of ≥20 consecutive trials, i.e., once every step for 20 steps. We will refer to each stimulus as a perturbation. During the first three or four perturbed steps, the cats occasionally moved forward or backward in the treadmill to avoid the wooden rod. However, because the perturbations were relatively innocuous, cats quickly became familiar with this process and walked comfortably during repeated perturbations. Occasionally, the animal moved quickly forward or backward within the treadmill, making it difficult to perturb every step, resulting in a different number of perturbed steps. The exact number of perturbed steps for each cat is shown on the abscissa of Fig. 2. We generally did not position the hook at a consistent level because steps became progressively higher through a sequence of perturbations so it was necessary to raise the hook to continue to elicit flexion responses. As much as possible, we restricted contact of the hook to the dorsum of the paw. After a sequence of 20 consecutive trials, the perturbing rod was removed and the animal walked for between 30 s and 1 min with no perturbations. After the unperturbed sequence, another set of perturbations was begun.

Investigation of context specificity

In five animals, we observed walking in contexts different from the treadmill to ascertain whether an increase in step height resulted from increased flexion in all walking conditions. In these experiments, the steps of cats were repeatedly perturbed while walking in a treadmill until they were exhibiting persistent hyperflexion. We then compared the expression of the persistent hyperflexion in the treadmill and in a different context (either a level walkway or a series of offset pegs). Usually, this comparison was made immediately. In other cases, when the persistent hyperflexion was still evident a number of days after the perturbed steps, the comparison was made days later.

In three animals, we delivered a sequence of perturbations at a specific speed (0.5 or 0.6 m/s) and then observed walking at different speeds, to determine whether the persistent hyperflexion was specific to one speed.

Stumbling-corrective responses in decerebrate walking cats

The general procedure for the decerebrate walking preparation was described extensively in previous publications from our laboratory (Donelan and Pearson 2004b; Hiebert and Pearson 1999). Five animals were anesthetized with Isoflurane and a tracheal cannula was inserted for continued administration of the anesthetic. A cannula, inserted into one of the carotid arteries, was used to monitor blood pressure. Drugs and fluids were delivered by a second cannula inserted into one jugular vein. Bipolar recording electrodes (Cooner wire AS632) were then sewn into the lateral gastrocnemius (LG), medial gastrocnemius (MG), the hip flexor iliopsoas (IP), and semitendinosus (ST, knee flexor/hip extensor) muscles of one hind leg. Data from recordings from LG and MG were used for unrelated projects. In one decerebrate cat, the ankle extensors of the contralateral (unexamined) leg had been denervated many months earlier and the cat had fully recovered. A multiterminal connector positioned above the animal’s back allowed recordings of muscle activity throughout the experiment. To support the animal’s hindquarters, a thick wire was threaded through holes drilled in both iliac crests. This wire was later clamped to an external frame to support the hips while the animal was walking on a treadmill. Reflective markers were placed above the iliac crest, the hip joint, knee and ankle joints, and on the paw and toe of the left leg. These markers were used to...
determine the kinematics of leg movements using the Peak Motus 8.5 motion analysis system (ViconPeak).

After these surgical procedures, the animal was transferred to a frame mounted above a treadmill. The wire through the iliac crests was clamped to an external frame and the head was placed in a stereotaxic holder. The brain stem was subsequently transected rostral to the superior colliculus and mammillary bodies and the animal was removed from the anesthetic. All animals began to walk spontaneously about 30 min later.

During regular walking sequences, a hook similar to that described above for use in intact cats was used to perturb the animal’s leg during the swing phase of walking.

Data acquisition and analysis

Data were collected daily from intact animals for 10–14 days and over the course of 1–3 h from decerebrate animals. Both video and EMG signals were recorded through the entire course of each experiment; video was recorded using a Peak Motus system, and EMG was recorded with an eight-channel Vetter 4000A PCM recorder (band-pass filtered from 30 to 30,000 Hz). The Peak Motus system placed a signal periodically on both the video and the EMG recordings, which was used for off-line synchronization. The Peak Motus system was also used to track reflective markers (0.5-cm diameter) placed over iliac crest, the hip, the ankle, the paw, and on the fifth digit of the toe. The position of the knee was calculated using triangulation from the hip and ankle locations and measurement of thigh and shank lengths. These data were used to calculate relevant kinematic data, such as joint position and angle and toe height above the supporting surface.

Custom programs written in Matlab (The MathWorks) were used to analyze the synchronized EMG and kinematic data. Kinematic data were first low-pass filtered (fourth-order Butterworth, zero-lag) and then analyzed to determine maximum step height, angle flexions, and other parameters of interest. EMG data were rectified, filtered (first-order Butterworth, zero-lag), and analyzed.

Statistical tests

Comparison of multiple means was done using the Kruskal–Wallis test, a nonparametric alternative to the ANOVA test. Comparison of two means was done using Student’s *t*-test, with allowances for unequal variances.

RESULTS

This study describes a long-lasting modification of the swing phase in walking cats. This modification was produced by repetitively evoking stumbling-corrective responses in one hind leg using a handheld device while animals walked on a treadmill (Fig. 1). The main observation was that the flexion of the knee progressively increased during the perturbation sequence and that the increase in knee flexion was long-lasting and context dependent (throughout we will refer to this behavior as persistent hyperflexion). An easily quantified measure of this hyperflexion is the maximum height of the toe during the swing phase of walking and we use this as a measure throughout this study. Of six cats studied, five showed a long-lasting increase in step height after such a sequence of perturbed steps. We noted two relevant differences between the cat that exhibited only temporary increases in step height and the remaining

FIG. 2. Bar graphs showing mean maximum step height after sequences of continually perturbed steps and extended delay periods (24 h, cats 1 and 2; 20 min, cats 3 and 4) for 4 cats. Data are taken from steps immediately after conditions described on abscissa. Asterisks denote significant difference from control step height (*P < 0.05, Kruskal–Wallis test). Error bars denote 1 SD.
cats. The first was that this cat was much more timid and nervous in our laboratory and the second was that this cat walked with a very crouched gait. For the remainder of the analysis and discussion, we will refer only to the five cats that did exhibit persistent hyperflexion.

Stumbling-corrective responses elicit long-lasting increases in step height

Normally, after contact with an object during walking, the basic pattern of leg movements is modified for only the perturbed step, a response described as the stumbling-corrective response (Forssberg 1979; Wand et al. 1980) and shown in Fig. 1B. In work related to an earlier study in our laboratory (McVea et al. 2005), we made the observation that after sequences of repeated stumbling-corrective reactions, much longer-lasting modifications resulted. On further investigation, we observed that the maximum step height dramatically increased after a series of stumbling-corrective responses [hereafter referred to as perturbations or perturbed steps (defined in METHODS), as shown in Fig. 1C. An analysis of the results from four cats is presented in Fig. 2. Note that these data describe swing movements in the absence of perturbations. These data show that swing movements changed rapidly after a small number of perturbed steps. For example, all cats increased their step height by at least one third after only 20 perturbed steps. Larger numbers of perturbed steps produced a further increase in step height and this increase in step height persisted for ≥24 h in two animals (cats 1 and 2) and ≥20 min in the remaining animals when returned to the treadmill without further perturbations. Figure 3 shows that this increase in step height resulted primarily from an increase in flexion at the knee after perturbed steps. Changes in hip and ankle excursion were not significant except in one animal (cat 2) in which ankle flexion increased. Small changes in the timing and extent of ankle flexion were also seen in cat 3, as shown in Fig. 3A but the excursion did not change significantly. Figure 4 shows that another feature of the modification in response to repeated perturbations was a change in the response to a single stimulus. Even after the persistent hyperflexion had waned (Fig. 4A), a perturbation during swing led to a series of higher steps, which was not evident in the control case. (Although the first step in the bottom panel of Fig. 4A is higher than the control steps in the top panel, this is only because the cat was taking long steps while moving forward through the treadmill. Figure 2 shows this cat did not have persistent hyperflexion lasting for >24 h.) In one interesting case, a perturbation of the contralateral leg (which never developed persistent hyperflexion) led to a sudden increase in step height in the ipsilateral leg (Fig. 4B). This was not observed in other animals.

EMG recordings from hip and knee flexor muscles were consistent with these observations on movements at the knee and hip; activity in ST, an important knee flexor, increased concurrently with step height (Fig. 5, A and B), whereas activity in IP, a hip flexor, did not (Fig. 5A).

Expression of persistent hyperflexion is context dependent

We next examined whether the persistent hyperflexion was expressed in all environments or restricted to the environment in which the perturbations were encountered. We tested this by first perturbing the steps of cats as they walked on the treadmill until the persistent hyperflexion was evident (between 80 and 120 perturbed steps). We then compared the maximum step height during unperturbed steps in the original treadmill and in a new environment (either a series of offset pegs or a level walkway). In some cases, this comparison was done within a few minutes after the development of persistent hyperflexion, whereas in other cases, it was done some days later. Figure 6A illustrates the basic phenomenon, showing increased step height after perturbations on the treadmill, much lower step height on a walkway, and a return of increased step height after return to the treadmill. Figure 6B presents averaged maximum step height from four cats, showing a significant difference between the maximum steps height in the different contexts.
Note in the data from cat 2 that increased step height on the treadmill persisted for at least 3 days after perturbed steps. Four of the five cats that displayed a persistent hyperflexion on the treadmill showed a context-dependent expression of the increase in step height. In one cat (cat 4), step height increased on return to the treadmill.

Consistent with this clear evidence that the expression of the persistent hyperflexion was restricted to certain contexts, we occasionally observed that particular contextual information was sufficient for initiating this expression. Figure 7 shows evidence that visual cues related to the animal’s environment are one source of contextual information. Data in the top panel show that step height increased considerably when the rod used for perturbing steps was observed by the cat. Although this phenomenon was not thoroughly examined, similar but smaller effects were seen in a second cat (not shown). In the bottom panel, data from a sequence of steps in which cat 1 increased step height over the course of four steps after the treadmill was engaged are shown. There was no stimulus applied to the paw in either the top or bottom panel of Fig. 7. We also obtained evidence in one cat that the speed of walking was a cue that triggered expression of the increased maximum step height. Data from this cat are shown in Fig. 7B. Note that the step height at 0.5 m/s, which is the speed at which the animal initially experienced the perturbed steps, was the highest over the range of speeds tested. In contrast, the step height of the contralateral toe changed very little across these different speeds, supporting our conclusion that the peak in step height at 0.5 m/s is a context-dependent adaptation to the prior perturbed steps. We believe the change in step height was related directly to the speed of the treadmill and not to other cues, such as verbal announcements of treadmill speed, be-

**FIG. 4.** Stumbling-corrective response is enhanced even in the absence of persistent hyperflexion. *A:* representative steps from cat 4 show the response to a single perturbation after a sequence of continually perturbed steps (in this case, 20 min later, after persistence had waned (bottom), led to an increase in step height for a number of steps, which is not present in the normal stumbling-corrective response (top). Perturbed step is denoted by an arrow. *B:* in one animal, evoking a stumbling-corrective response in the opposite leg led to an increase in step height in the leg that had experienced multiple perturbations. Black line shows the step height of the ipsilateral (persistent hyperflexion) limb; gray line shows the step height of the contralateral limb. Perturbed step of contralateral leg is denoted by an arrow.

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**FIG. 5.** Increase in step height is accompanied by increases in semitendinosus (ST) activity. *A:* data from cat 1 showing the same sequence of steps as in Fig. 7A (bottom). Step height is shown above unrectified EMG traces from iliopsoas (IP) and ST muscles. Note the increase in ST activity accompanies the increase in step height, whereas IP is unchanged. *B:* mean ST activity from cats 1 and 5 shows increased magnitude and duration of activity. Gray traces: ST activity during control steps. Black traces: ST activity after the development of persistent hyperflexion. Bursts are aligned on the initiation of ST activity.
cause of the partial increase at speeds close to 0.5 m/s. Although we tested other cats for this phenomenon, we observed a connection between walking speed and persistent hyperflexion in only one cat.

Main knee flexor muscles are essential for long-term retention of persistent hyperflexion

As Fig. 3 shows, knee flexion increased significantly in cats after a sequence of perturbed steps and this increase was attributed at least in part to an increase in ST activity (Fig. 5). As part of a concurrent project in our laboratory concerning the recovery of flexion movements after the denervation of the PB and ST muscles (Tachibana et al. 2006) we were able to examine the ability of cats to adapt to sequences of perturbations after the denervation of the PB and ST muscles. The important observation from these experiments was that, although short-term increases in stepping height were evident after a sequence of perturbed steps, these increases did not persist. This can be seen by comparing the increases in step height for cat 1 when intact (Fig. 2) and after the denervation of PB and ST (Fig. 8B). Note that the increase in step height persisted for 24 h in the former case, while disappearing after 5 min in the latter case. Similar differences were evident for cat 3. Despite the absence of PB and ST activity, the transient increase in step height was associated with increased knee flexion as well as increased ankle flexion (Fig. 8A). We could not clarify which muscles were responsible for the increased knee flexion in the absence of PB and ST, but some possibilities include gracilis, posterior Sartorius, and gastrocnemius (Tachibana et al. 2006).

FIG. 6. Increase in step height was restricted to the context in which perturbed steps were experienced. A: representative examples from cat 4 show step height after a sequence of perturbed steps on the treadmill (gray line), upon transfer to walkway (dotted black line), and upon return to treadmill (solid black line). B: mean step height for cats 1, 2, 4, and 5 after a sequence of perturbed steps, in the walkway and upon return to the treadmill. Note that the increase in step height persisted for 3 days in cat 2. Dashed lines indicate mean unperturbed step height on treadmill. Asterisks denote significant difference from step height on walkway ($P < 0.05$, 1-tailed $t$-test). Error bars represent 1 SD.

FIG. 7. Sensory cues can evoke expression of persistent hyperflexion. A, top: step height from a sequence of steps from cat 2. Step height increases when the handheld obstacle enters the treadmill, although no cutaneous stimulation was experienced. Bottom: gradual increase in step height in cat 1 after the treadmill motion begins. Again, no cutaneous stimulation was experienced. B: step height in cat 1 was maximum at the treadmill speed at which perturbations had been experienced (0.5 m/s). All data are from steps after a sequence of perturbations had led to an increase in step height. Black bars: perturbed leg; gray bars: opposite leg. Asterisks denote significant difference from step height at 0.5 m/s. Error bars represent 1 SD. Note that opposite leg shows little change in step height at different speeds.

Main knee flexor muscles are essential for long-term retention of persistent hyperflexion
Forebrain regions are required to encode and express the persistent hyperflexion

As shown in Fig. 7, most cats expressed the increased step height only in the context in which they encountered perturbations and Fig. 5 shows examples of this recognition of environmental cues related to context. This implies that forebrain regions, especially visual and auditory regions of the cortex, are involved in the expression of persistent hyperflexion. We thus predicted that the persistent hyperflexion would not be evident in decerebrate walking animals. Figure 9 shows that this was the case. Although a transient adaptation was evident, it lasted for only short periods, as the progressively decreasing step height in Fig. 9A shows. Figure 9B shows averaged data from four animals, one of which had very small increases in step height, whereas two had significant but short-lived increases in step height. In one cat, mean step height decreased slightly after perturbed steps. In total, we attempted but failed to evoke long-lasting persistent hyperflexion in five decerebrate cats.

DISCUSSION

Characteristics of persistent hyperflexion

Locomotor systems can be readily modified by changes of the musculoskeletal system or the environment to maintain...
stable and efficient movement (Emken and Reinkensmeyer 2005; Jensen et al. 1998; Lam et al. 2006; Rossignol et al. 2004; Tachibana et al. 2006). In this study we describe a persistent modification of stepping in a hind leg of walking cats after a small number of mechanical stimuli to the paw, that is, a significant increase the maximum step height of the perturbed leg during swing. Figure 2 shows that this effect is persistent, lasting for $\geq 24$ h in two animals in the absence of further stimuli. In the remaining three animals, the maximum step height was elevated 24 h later but this increase was not statistically significant (not shown). However, in these three animals, an enhanced response to a single stimulus was evident even in the absence of persistent hyperflexion (Fig. 4). Figure 3 shows that an increase in knee flexion was primarily responsible for the increase in step height, although ankle flexion also increased in one animal. This increase in knee flexion is similar to previously described flexion responses when cats encounter obstacles during swing (Buford and Smith 1993). Increases in the activity of the ST muscle (Fig. 5), as well as the absence of the persistent hyperflexion in animals after the denervation of PB and ST, suggest that these primary knee flexors are an important part of the hyperflexion. We also examined the activity of IP (a hip flexor) and Sart (a hip/knee flexor) in two animals and saw no significant increase (data not shown).

One clear feature of this modification is that it does not generalize to all environments. Figure 6 shows that maximum step height returned to normal when the cat was placed in a new environment, but once again increased when returned to the environment in which it had experienced the stimuli while walking. In some cases, an increase in step height in response to a visual or auditory cue that a cat associated with perturbed steps was very striking (Fig. 7A). In addition to increasing maximum step height during all steps, cats also developed an increased response to additional perturbations while walking. Figure 4A shows that the steps after contact with an obstacle during swing remained much higher in animals that had repeatedly been perturbed while walking, even after the persistent hyperflexion had waned. In one intriguing case, the contact with an object by the opposite leg, which had a normal stepping height and flexion response, led to a sudden and marked increase in stepping height in the leg that had developed persistent hyperflexion (Fig. 4B). This suggests that the persistent hyperflexion may reflect an enhancement of the output of spinal networks by a supraspinal signal related to the environment in which the animal is walking. Contact with an obstacle by either leg would provide sensory cues that would reinforce the recognition of the environment.

The persistent hyperflexion we describe herein is superficially similar to classically conditioned behavior in that the conditioned response (leg hyperflexion) is associated with the environmental context (treadmill and its surrounding). However, it is distinctly different from previously described classically conditioned flexor responses in spinal (Durkovic 1983; Patterson et al. 1973) and intact (Bracha et al. 1999) animals, in which a conditioned stimulus is associated in time with an unconditioned stimulus that naturally provokes a flexion response. Although we do have evidence that visual and/or auditory cues can provoke a sudden increase in flexion (Fig. 7A), it is important to note that in our study there was no consistent temporal relationship between the stimulus provoking the flexion response (perturbed step) and any feature of the environment. In contrast, a short, precise interval between the conditioned and the unconditioned stimuli is a necessity for classical conditioning of the flexion response as shown by Durkovic and Damianopoulos (1986), who investigated the strengths of conditioned responses over a range of time intervals separating conditioned and unconditioned stimuli in the spinal cat. Conditioning of the flexor response in the intact cat also relies on a short, consistent time interval (Bracha et al. 1999). Furthermore, our data did not reveal any rapid extinction of the hyperflexion (increased flexion persisted during hundreds of unperturbed steps on the treadmill). Nonetheless, we cannot say for certain to what extent the persistent hyperflexion we describe overlaps with other examples of classically conditioned flexion responses. It may be that the visual and/or auditory cues of a unique environment become associated with a modified walking pattern at some level of the locomotor system.

On first sight our results also appear to be related to avoidance conditioning of legs in spinal rats (Grau et al. 1998) and headless cockroaches (Horridge 1962). In these animals, electrical stimulation of a leg when it is lowered below a preset level results in a maintained elevation of the leg to avoid the stimulus. This phenomenon has not been reported in cats. Nevertheless, the fact that we were unable to produce long-lasting hyperflexion responses in decerebrate animals (Fig. 9) suggests that the stimulus pattern we used in this study is unable to produce a conditioned avoidance response similar to those found in spinal rats and headless cockroaches. The conditioned hyperflexion we found in the cat clearly requires normal functioning of supraspinal regions for its expression, whereas the avoidance response can be conditioned in the absence of these structures in rats and cockroaches. We thus conclude that the neuronal basis for the conditioned hyperflexion in intact walking cats is different from that for the conditioned avoidance responses in other animals.

It is possible that the animals we studied may have hyperflexed the leg using an avoidance strategy requiring planning and cognitive processes more complex than those of associative conditioning. However, we think this is unlikely for a number of reasons. First of all, it is unlikely that all five animals would choose the same strategy (hyperflexion) to avoid the obstacle. Other strategies that could have been used include refusing to walk in the treadmill, walking near the extreme front or back of the treadmill, or walking erratically from side to side to avoid the obstacle. We did not see any consistent use of such strategies in any animals. Second, we occasionally observed instances where the hyperflexion became expressed gradually in response to cues associated with perturbed steps, such as the movement of the treadmill or the appearance of the perturbing device (Figs. 4B and 7A). In these cases, step height increased and decreased over three or four steps, which is inconsistent with a cognitively planned strategy to begin stepping high to avoid the perturbations. Finally, one cat expressed the increased step height at only one speed, with step heights at close speeds being somewhat elevated (Fig. 7B). Again, this graded response is inconsistent with a planned strategy to increase step height.
Site of modification

The persistence and the context-dependent nature of the locomotor adaptation we describe in this study motivated us to try to gain insight into the neural structures that are involved. Locomotor output is the product of complex interactions of many areas of the nervous system, including spinal pattern generators (Dietz 2003; Rossignol and Dubuc 1994), muscle afferents (Dietz and Duysens 2000; Pearson 2004), and the forebrain (Drew et al. 1996, 2002). One theme that has emerged from the study of locomotor adaptation is that many regions of the nervous system are involved in the process of locomotor adaptation (Bouyer et al. 2001; Carrier et al. 1997; Chen and Wolpaw 2005; Whelan and Pearson 1997; Wolpaw and Tennissen 2001).

Nonetheless, a number of studies showed that spinal circuits alone are able to learn new locomotor behavior. Clear demonstrations of this are that spinalized cats can recover stepping after training (Barbeau and Rossignol 1987), both spinalized cats (Edgerton et al. 2004) and decerebrate ferrets (Lou and Bloedel 1988a) increase flexion to avoid obstacles in the environment, and spinalized rats decrease stance duration to accommodate a force field applied to the leg (Timoszyk et al. 2002). However, we do not believe that spinal circuits are the site of long-lasting changes during the development of persistent hyperflexion because one clear aspect of this adaptation is the dependency on context (Fig. 6), which implicates descending signals from cortical regions of the brain.

Comparison of durations of the persistent hyperflexion and other forms of adaptation

One unusual aspect of the persistent hyperflexion we described was its duration. Figure 6 shows that the adaptation persisted for up to 3 days in one animal, in the absence of any further stimuli. Aftereffects were described previously for other forms of locomotor adaptation but they persist for much less time. For example, when stepping in an artificial force field, humans quickly adapt to overcome an initial change in step height. After the removal of the force field, there is a brief aftereffect that decays over the course of three to five steps (Emken and Reinkensmeyer 2005). Similarly, humans adapted to walking in a robotic device that alters the torques necessary to flex the joints of the leg display an aftereffect that lasts on the order of tens of steps when the device is shut off (Lam et al. 2006). An important difference between these forms of adaptation and the persistent hyperflexion we describe herein is that the former involve the modification of the dynamic properties of the leg; that is, they change the movement that results from a given amount of muscle activity. This form of adaptation has been widely studied in the context of reaching movement and has been hypothesized to involve modifications of an internal model of limb dynamics (Kawato 1999; Lackner and DiZio 2005; Ostry and Feldman 2003; Wolpert and Ghahramani 2000). These modifications could be based on a difference between expected and actual movements, caused by the unusual limb dynamics (Wolpert and Ghahramani 2000). Adaptations to walking on rotating disks (Gordon et al. 1995) and split-belt treadmills (Jensen et al. 1998), which also display short-lived aftereffects, would similarly involve a discrepancy between the actual and the expected movement. This process would be equally active once the normal limb dynamics are restored (during which expected and actual movements would again be different), leading to a rapidly declining aftereffect.

Figure 10 highlights an important difference between this form of adaptation and the one we describe here. We did not change the dynamics of the limb—rather, we introduced a change to the environment. When this change was removed, there was no positive signal that the environment has returned to normal, as there is when an artificial force field is removed or split-belt treadmills are returned to the same speed. We hypothesize the lack of a positive signal that the environmental change has ceased is a crucial feature in the persistence of this hyperflexion.

Functional relevance of persistent hyperflexion

The increased flexion during walking that we describe here is an example of a learned modification of the locomotor pattern that minimizes the disruptive effects of a particular environment (context). It is therefore related to a number of previous studies that showed that the basic pattern of walking is altered in functionally relevant ways in certain contexts with particular environmental challenges. For example, when cats walk up (Carlson-Kuhta et al. 1998) or down (Smith et al. 1998b) inclines, there are changes to the flexor and extensor synergies that are normally evident during level walking. A second example is that when adult humans walk on a compliant surface, there are changes in muscle activity, step height, and center of mass height when compared with walking on a solid surface (MacLellan and Patla 2006). It is possible that the

FIG. 10. Conceptual model showing 2 potential forms of locomotor adaptation. In A, discrepancies between the goal and the actual movement (reported by afferent feedback) lead to changes in the internal model of limb dynamics to eliminate or reduce the discrepancy. In B, recognition of a particular context results in a goal specific to that context (in this case, stepping high to avoid obstacle). This form of adaptation would be longer lasting because there is no discrepancy between expected and actual movements when the obstacle is removed, and less generalized because it is expressed only when the appropriate context is recognized.

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particular patterns of locomotor activity that are needed to negotiate these situations are learned and are then expressed as needed within the correct context. An example can be seen in adult humans walking onto a moving platform that can slide forward. Subjects in this situation develop an adaptive response to minimize the resulting sway (Reynolds and Bronstein 2003) and this adaptation shows only limited expression in other contexts (Reynolds and Bronstein 2004). These modifications maintain stability and efficiency in the particular environment where they are expressed. They are unlikely to be induced by immediate feedback from the environment because they involve major changes in the timing of muscle activity (such as the appearance of flexor bursts during the stance phase of walking; Smith et al. 1998b) and they persist in the absence of the perturbing stimuli (Reynolds and Bronstein 2004). The persistent hyperflexion that we describe in this study, as well as these examples, may reflect a crucial ability of the locomotor system to rapidly learn the particular challenges of different environments and to enact long-term, context-dependent modifications of walking output to accommodate these challenges.

ACKNOWLEDGMENTS

We thank Drs. Atsumichi Tachibana and J. Maxwell Donelan for help collecting the data and offering comments and advice. We thank R. Gramlich for expert technical help.

GRANTS

This work was supported by Canadian Institutes of Health Research and the Alberta Heritage Foundation for Medical Research.

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