Deletions of Rhythmic Motoneuron Activity During Fictive Locomotion and Scratch Provide Clues to the Organization of the Mammalian Central Pattern Generator

Myriam Lafreniere-Roula and David A. McCrea
Spinal Cord Research Centre and Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada

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Lafreniere-Roula, Myriam and David A. McCrea. Deletions of rhythmic motoneuron activity during fictive locomotion and scratch provide clues to the organization of the mammalian central pattern generator. J Neurophysiol 94: 1120–1132, 2005; doi:10.1152/jn.00216.2005. We examined the features of spontaneous deletions of bursts of motoneuron activity that can occur within otherwise rhythmic alternating flexor and extensor activity during fictive locomotion and scratch in adult decerebrate cats. Deletions of activity were observed both in hindlimb flexor and extensor motoneuron pools during brain stem–stimulation-evoked fictive locomotion but only in extensors during fictive scratch. Paired intracellular motoneuron recordings showed that deletions reduced the depolarization of homonymous motoneurons in qualitatively similar ways. Differences occurred in the extent to which activity in synergist motoneuron pools operating at other joints within the limb was reduced during deletions. The timing of the rhythmic activity that followed a deletion was often at an integer multiple of the preexisting locomotor or scratch cycle period. This maintenance of cycle period was also seen during deletions in which there was a complete failure of motoneuron depolarization. The activity of antagonist motoneurons was usually sustained during deletions with some rhythmic modulation at intervals of the preexisting cycle period. We discuss an organization of the central pattern generator for locomotion and scratch that functions as a single rhythm generator with separate and multiple pattern formation modules for controlling the hyper- and depolarization of subsets of motoneurons within the limb.

INTRODUCTION

One feature of locomotion conserved across species is the rhythmic alternating activity of muscles with opposing actions at a joint (e.g., agonist/antagonist pairs in mammals) or on opposite sides of the body (e.g., corresponding left and right segments in fish). This basic locomotor pattern is produced by neuronal networks in the spinal cord and can be elicited in animals in which supraspinal influences are removed by transection of the spinal cord (Graham Brown 1911) and in the fictive locomotion preparation in which rhythmic sensory afferent feedback is removed by neuromuscular blockade (see Orlovsky et al. 1999). These observations support the concept of spinal central pattern generators (CPGs) for the production of rhythmic alternating movements such as locomotion and the scratch reflex (reviewed in Grillner 1981; Orlovsky et al. 1999; Rossignol 1996). Although progress has been made in understanding the organization of pattern-generating circuitry in lower vertebrates (e.g., Grillner and Wallen 2002) we presently have little knowledge about the neuronal circuitry that constitutes the mammalian CPG for locomotion or scratch.

The half-center model of the locomotor CPG (Graham Brown 1911), as developed by Lundberg and colleagues (see Lundberg 1981), often forms the basis for discussion of the mammalian CPG. In this model, populations of interneurons in the flexor and extensor half-centers excite flexor and extensor muscles throughout the limb. Simultaneous activity of flexors and extensors is prevented by mutual inhibitory interconnections between the half-centers. A high level of excitability within the half-center generates activity that is eventually curtailed by a fatigue process. Slowing of firing in the active half-center (fatigue) releases the opposing half-center from inhibition and allows it to create activity in antagonist motoneurons. The process repeats and the system oscillates. According to this model, a failure of the fatigue process would result in the system’s being locked in its current state of flexion or extension and a sustained inhibition of the antagonist half-center. An important feature of the classic half-center model is that the interneurons constituting the half-centers are responsible for both the generation of timing as well as the excitatory drive to agonist motoneuron pools. Others have suggested that the CPG organization should include a separation of the networks for rhythm generation and motoneuron excitation (e.g., Burke et al. 2001; Koshland and Smith 1989; Kriellaars et al. 1994).

The fictive locomotion preparation using electrical stimulation of the mesencephalic locomotor region (MLR) in adult decerebrate cats after neuromuscular blockade offers several advantages for studying mammalian CPG operation. These include the use of a mature nervous system, the generation of motor patterns in the absence of both rhythmic sensory feedback and cortical control as well as the lack of systemic or bath application of neurotransmitter agents. Similar advantages apply to the fictive scratch preparation. In response to a noxious stimulus around the ear, head, or neck, the feline scratch reflex consists of an initial flexion phase which positions the ipsilateral hindlimb near the head followed by several seconds of rapidly repeated contact between the foot and the head (e.g., Kuhta and Smith 1990). A similar sequence of motoneuron activation can be evoked in decerebrate and paralyzed cats by mechanical stimulation of the ear after topical application of

Address for reprint requests and other correspondence: D. A. McCrea, Spinal Cord Research Center, University of Manitoba, 730 William Avenue, Winnipeg, Manitoba R3E3J7, Canada (E-mail: dave@scrc.umanitoba.ca).

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curare or strychnine to the cervical spinal cord (e.g., Deliagina et al. 1981).

We consider the spinal CPG to be a fundamentally a bipartite organization that produces alternating and out of phase activation of flexor and extensor muscles throughout the hindlimb. Additional networks shape the activity of individual motoneuron pools, including those innervating bifunctional muscles. The activity in proximal and distal motoneuron pools occurs within 20–30 ms of each other (e.g., Quevedo et al. 2000). During both fictive locomotion and scratch, many hindlimb muscles can be classified as belonging to one of two groups, the flexors and extenders, with each group active once per activity cycle and in strict alternation. Other muscles, such as those which have actions across two joints (the bifunctionals) may show activity twice per step cycle or change their activity under different conditions or gaits (reviewed in Rossignol 1996; Stein and Smith 1997) and receive a more complex pattern of excitation and inhibition during locomotion than flexors and extenders (Perret and Cabelguen 1980). These complex patterns of bifunctional motoneuron activity and other observations have led some to question the half-center organization of the CPG and to propose alternate schemes for rhythmic alternating activity during locomotion (e.g., Grillner and Zangger 1979; Orlovsky et al. 1999; Stein and Smith 1997; but see Lundberg 1981).

The present study stems from our observations of brief spontaneous failures in the normally robust alternating flexor and extensor activity during fictive locomotion and scratch. Such failures in CPG operation have been noted before. For example, a missing burst in knee extensor activity was accompanied by sustained knee flexor activity during otherwise rhythmic locomotion in a chronic-spinal decerebrate cat (Grillner and Zangger 1979; their Fig. 4). A concurrent failure to depolarize the agonist and to hyperpolarize the antagonist motoneuron pools was reported during MLR-evoked fictive locomotion in the cat (Jordan 1991; Turkin and Hamm 2004; Turkin et al. 2003). The most extensively described failures of motoneuron activation during rhythmic activity are, however, the missing bursts of activity during the turtle scratch reflex (Stein 2005; Stein and Grossman 1980). These deletions of activity bursts have also been studied using intracellular recordings from motoneurons (Robertson and Stein 1988) and extracellular recordings of activity of premotor interneurons (Stein and Daniels-McQueen 2002). The “hip-extensor deletion,” for example, consists of a spontaneous period of silence in hip-extensor motoneuron activity during which hip flexor motoneuron activities display amplitude-modulated bursts with no intervening quiescence (Stein et al. 1995, 1998; termed B-phase deletion by Stein and Grossman 1980; termed HR-KF deletion by Robertson and Stein 1988). We will use the term “deletion” to refer to the spontaneous events to be described here as an analogy to the hip-extensor deletion reported during the turtle scratch reflex (Stein et al. 1995, 1998). In our examples of burst deletions, however, the quiescent period between bursts of the antagonist sometimes remained.

The present study describes changes in peripheral nerve (electroneurographic [ENG]) activity and intracellularly recorded events in hindlimb motoneurons during spontaneous deletions occurring in fictive locomotion and scratch in adult decerebrate cats. The results will show that most deletions of activity affect proximal and distal motoneuron groups simultaneously and that the ongoing cycle period is often maintained through a deletion. These results form the basis of a new computational model of the organization of the locomotor CPG (Rybak et al. 2004). Preliminary results have been presented in abstract form (Lafreniere-Roula et al. 2001, 2004).

METHODS

Surgical and experimental protocols were in compliance with guidelines of the Canadian Council for Animal Care and the University of Manitoba. Anesthesia was induced in purpose-bred adult cats (2.4–3.2 kg) using halothane (1–2%) in a mixture of, nitrous oxide (70%), and oxygen (30%). Administration of the anesthetic mixture was maintained by a tracheotomy tube. The level of anesthesia was monitored by confirming the absence of pedal withdrawal reflexes periodically and by monitoring arterial blood pressure and muscle tone. Atropine (0.05 mg/kg subcutaneous [sc]), saline (10 ml sc), and dexamethasone (2 mg/kg intravenous) were given at the beginning of the surgery. Cervical nerves were inserted in the left femoral and the right jugular veins for drug administration. A buffer solution (5% glucose, 0.84% bicarbonate solution; 5 ml/h) was continuously infused through the jugular vein for blood pH maintenance. Blood pressure was monitored from the right carotid artery using a transducer. The CO2 levels and respiratory rhythm were monitored by a sensor inserted into the tracheotomy tube. The bladder was catheterized through the urethra.

Several nerves were dissected in the left hindlimb and mounted on conventional hook electrodes for recording and stimulation: semimembranosus and anterior biceps, SmAB; posterior biceps and semitendinosus, PBS; lateral gastrocnemius and soleus, LGS; medial gastrocnemius, MG; the combination of LGS and MG, GS; tibialis anterior, TA; Plant, plantaris; extensor digitorum longus, EDL; peroneous longus, PerL; flexor digitorum and hallucis longus, FDHL; the mixed tibial nerve innervating plantar foot structures, Tib. Ventrally located cuff electrodes were used to record from hip flexor (sartorius, Sart) and knee extensor (quadriceps, Quad, sometimes including the bifunctional, rectus femoris) nerves. The adductor tendons of both hips were cut and the right hindlimb was denervated by cutting the sciatic nerve, nerves to SmAB and PBS, as well as the obturator and femoral nerves.

A laminectomy was performed to expose the L4 to L7 segments of the spinal cord and the cat was transferred to a stereotactic frame. The dorsal aspect of the cervical spinal cord was exposed at C1 for topical application of curare to elicit fictive scratch as described below. A mineral oil pool was made for mounting the dissected left hindlimb nerves. The temperature of the animal was maintained by a heating pad and radiant heat lamps. After mechanical removal of the cortex, a blunt transection of the brain stem was performed at the postcollicular, premammillary level. All tissue rostral to the transection was removed. After this decerebration, anesthesia was discontinued and the animal was paralyzed (pancuronium bromide, 0.1 mg/kg per hour) and ventilated. Bilateral openings in the chest wall were used to minimize respiratory movements.

Fictive locomotion was induced by unilateral or bilateral stimulation of the brain stem with 0.5 ms duration current pulses (50–500 mA, 10–20 Hz). Fictive scratch was induced in the ipsilateral (left) hindlimb by applying a small piece of cotton soaked in a 0.01 or 0.1% solution of curare on the left C1 dorsal root entry zone followed by mechanical stimulation of the left pinna or left side of the face.

Hindlimb ENG recordings were filtered (30 Hz to 3 kHz), rectified and integrated before digitization at 500 Hz. Intracellular recordings (digitized at 5 or 10 kHz) were made from antidromically-identified lumbar motoneurons using 1.5 M sodium citrate filled glass electrodes (tip size 1.6–1.9 mm) and an Axoclamp 2A amplifier (Axon Instruments, Union City, CA). Paired intracellular recordings were achieved using independent positioning devices and microdrives. All signals
were captured and analyzed using software developed within the Spinal Cord Research Center and running on a Pentium PC under the Linux operating system. Runs of fictive locomotion or fictive scratch were typically captured in 1- to 2-min segments.

Data were obtained from ten experiments in which there were episodes of good rhythmic ENG activity during fictive locomotion or scratch. These episodes were then examined for missing bursts of ENG activity and cycle periods calculated before, during, and after the missing ENG bursts. Deletions were included in the analysis only if there was a sudden reduction in ENG amplitude (less than half the amplitude of neighboring bursts). Only deletions that were flanked by well-developed rhythm were considered for analysis. This led to the elimination of periods of activity immediately at the beginning or end of fictive locomotion or scratch.

Statistical analysis of cycle period during deletions

We sought to determine whether the rhythmic activity after a deletion appeared at an integer multiple of the control step-cycle period. Such an interval would suggest that the rhythm had not changed during the deletion and that a motoneuron activity in an integer number of cycles was missing. The interval was measured between burst onsets in the ENG record or between onsets of the intracellular depolarization in motoneurons occurring before and after the deletion. The possibility of changes in cycle period during deletions was assessed using two methods.

In the first, a hypothesis was made that the deleted activity spanned an integer number of cycles (such as 1 or 2). The hypothesized number of cycles \( x \) present in the deletion was based on the presence of at least one of the following criteria during the deletion: 1) Distinguishable bursts in the ENG of interest or in other ENGs (i.e., average normal cycle according to the formula). 2) Modulation of activity in antagonist ENGs. 3) Rhythmic bursting in other ENGs. 4) Intracellular recordings displaying rhythmic depolarizations. A running average \((Y_2\) with sample size \( n_2 = 5 \) and variance \( s_2 \) of \( x \)-cycle intervals was calculated during the period just before the deletion to reduce the effects of any fluctuations in cycle period that might occur during fictive locomotion and scratch. The single observation \((Y_1)\) was then compared with the mean \((Y_2)\) according to the procedure outlined in Sokal and Rohlf (1969). Briefly, the \( t \) statistic was calculated using the formula

\[
t = \frac{Y_1 - Y_2 - (\mu_1 - \mu_2)}{s_2 \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}
\]

where the hypothesized difference is \( \mu_1 - \mu_2 = 0 \).

The second method compared each cycle period \( y \) (including the deletion \( y_{del} \)) to the average cycle period in all normal (undeleted) cycles \( (T) \). This difference was expressed as phase with respect to the average normal cycle according to the formula \( \Phi = 2\pi[y - T/k] \). During deletions this difference was expressed as \( \Phi_{del} = 2\pi(y_{del} - T/k) \), where \( k \) is an integer chosen such that \( -\pi < \Phi_{del} \). The comparison of \( \Phi_{del} \) to \( \Phi \) values was made by calculating the \( t \) statistic using the above formula. In 43 of 49 cases, we were able to base our hypothesis of the number of cycles missing during the deletion using at least one of the criteria listed above. In the remaining cases \( k \) was chosen to satisfy \( -\pi < \Phi_{del} \). A significance level of \( P < 0.05 \) was used. Values are reported as means \( \pm \) SD.

RESULTS

This study describes spontaneous disturbances in the rhythmic alternating activity of flexor and extensor motoneurons during episodes of fictive locomotion and scratch displaying otherwise stable rhythmic activity elicited without any peripheral nerve stimulation. Records from ten experiments (three during fictive locomotion, five during fictive scratch, and two during both behaviors) were examined and in eight there was at least one example of a deletion. Specifically, five of the seven episodes of fictive locomotion examined contained between two and four deletions of rhythmic activity and ten of the 17 episodes of fictive scratch examined (in five cats) contained one to four deletions each. Although no further attempt was made to estimate their incidence, it appears that spontaneous disturbances in rhythmic activity occur in most decerebrate preparations. Cycle duration was measured from the onset of consecutive ENG bursts in a nerve and averages were calculated from cycles in which the rhythmic activity was not perturbed by deletions. During the five episodes of fictive locomotion examined, the average cycle duration was 729 ms (SD 95). In four cases, the flexion phase was of longer duration than the extension phase with a flexion:extension ratio of 1.2 (SD 0.3). In the remaining episode, extensor bursts were longer than flexor bursts (flexion:extension ratio of 0.63).

Fictive scratch usually begins with a tonic flexion phase before the rhythmic and rapidly alternating flexor and extensor motoneuron activity (Deliagina et al. 1981). In nine episodes of fictive scratch examined, 2–3 s of tonic flexor activity preceded the period of phasic flexor and extensor ENG alternation, which lasted 9 s (SD 1). In one instance, there were several episodes of fictive scratch in immediate succession without intervening rest or tonic flexor activity for a total duration of 50 s. The average cycle duration (measured as the period between the onset of successive extensor bursts) during fictive scratch was 217 ms (SD 141). During fictive scratch, the flexor (F) bursts were always longer than extensor (E) bursts with an average F:E ratio of 3.9 (SD 0.8).

Deletions of either extensor or flexor activity are observed during fictive locomotion

The rectified and integrated ENG records illustrated in Fig. 1A are a 9-s portion of a 21-s run of MLR-evoked fictive locomotion. During this run, two spontaneous deletions were observed. Flexor burst number 5 is followed by the delayed appearance of the sixth flexor burst in both distal (EDL, an ankle flexor) and proximal (Sart, a hip flexor) flexor nerves. During this absence of flexor activity (i.e., a flexor deletion) the activity of extensors acting at the hip (SmAB) and ankle (LGS, MG, Plant) is sustained. There is a slight modulation of the sustained SmAB activity in the period between the fifth and sixth burst of Sart activity (indicated by *). After the sixth flexor burst there is another prolongation of extensor activity (i.e., a second flexor deletion).

In the records in Fig. 1B from another fictive locomotion preparation, the interval between the second and third extensor bursts is much longer than that between either the first and second or the third and fourth bursts. This is an example of a deletion of extensor nerve activity affecting ankle (GS, Plant, FDHL, Tib) and hip (SmAB) motoneurons during fictive locomotion. During this extensor deletion, there is tonic activity in flexors (PerL, TA, EDL).

The small bursts of activity in the Quad and PBSt ENGs during the deletion in Fig. 1B deserve comment. As dissected in this experiment the Quad nerve contained branches to both the knee extensor (vasti) and the bifunctional rectus femoris muscle. Note that the onset of Quad activity during undeleted
cycles (e.g., 4–6) precedes that of pure hip (SmAB) and ankle (GS, Plant) extensor as well as the activity in the nerves supplying the FDHL muscles and plantar foot structures (Tib). Similarly, the activity of PBSt (bifunctional: hip extensor–knee flexor actions) motoneurons is unlike that of flexors, being active only at the onset of ankle flexor activity. Thus the locomotor drives to both Quad and PBSt motoneurons were different from those to pure extensor and flexor motoneurons in this experiment. The residual activity in these ENGs during the extensor deletions may thus be a consequence of the specialized excitatory drive to bifunctional motoneuron pools during locomotion.

Sustained activity in antagonists accompanying agonist inactivity (e.g., Fig. 1) was the most common type of deletion observed in the present study. During deletions examined in seven episodes of fictive locomotion from five preparations, twelve deletions occurred in extensor and six in flexor motoneuron pools. In 14 of these 18 deletions, activity in motor pools operating at different joints was similarly affected and activity in antagonists was sustained.

Only deletions of extensor activity are observed during fictive scratch

Deletions observed during fictive scratch had features similar to those observed during fictive locomotion. Figure 2A shows a typical example where five successive and regular bursts of extensor activity are followed by a period of silence in an ankle extensor (MG) ENG before the regular rhythm returns. During this complete deletion of MG ENG activity, the hip extensor (SmAB) burst is substantially reduced (a partial

![FIG. 2. Deletions of extensor activity during fictive scratch. A: dotted box indicates where, after the 5 rhythmic bursts illustrated, the MG nerve became inactive (a complete deletion). During this period the PBSt and SmAB bursts were reduced in amplitude (partial deletions). Ankle flexor activity (TA) was modulated but did not return to baseline. Intervals on the horizontal bar were calculated from the immediately preceding 5 cycle periods (197 ms, SD 2). Interval spanning the deletion was not significantly different from control 2-cycle intervals ($P > 0.40$). B: another example of a deletion of activity during fictive scratch in the hip extensor (SmAB) as well as the bifunctional PBSt and the ankle flexor (PerL) nerves. This deletion is accompanied by sustained activity in another ankle flexor (TA). Scratch cycle period indicated on the horizontal bar was 204 ms (SD 14). Rhythmic bursts after the deletion reappear at an interval corresponding to 3 control step-cycle periods (3 vertical tick marks). Interval spanning this deletion was not significantly different from 3-cycle intervals taken from the control period ($P = 0.65$).]
deletion). Note that PBSt is active with extensor motoneurons during scratch in this preparation and its burst is also reduced during the deletion. Ankle flexor activity (TA) persists (but is modulated) during this deletion. In Fig. 2A, the PBSt bursts immediately before and after the deletion (5 and 6) were of similar amplitude to that of the other bursts (1–4, 7–9). In MG, however, the fifth and sixth bursts are smaller than any other.

Figure 2B is from another fictive scratch preparation. Again, the regular and rhythmic alternation of flexor and extensor bursts is interrupted by a period of silence in the hip extensor (SmAB) associated with prolonged ankle flexor (TA) activity between extensor bursts 7 and 8. This extensor deletion also affects PBSt activity and is preceded and followed by cycles of regular rhythmic activity.

Overall during 11 episodes of fictive scratch, there were 31 deletions affecting extensor activity but none affecting flexor activity. The majority (29/31) involved multiple motor pools. In eight of 31 cases, the antagonists showed sustained activity without intervening quiescent periods. In the other cases, the sustained activity in antagonists was reduced during the deletion (e.g., the TA record in Fig. 2A).

**PerL behavior during deletions changes as its activity changes in locomotion and scratch**

During fictive locomotion, the activity of the ankle flexor PerL is in phase with other ankle flexors (compare TA, EDL, and PerL activities in Fig. 1B); during fictive scratch, however, it becomes more like that of limb extensors (compare PerL and SmAB activities in Fig. 2B). The extensor-like activity of PerL during scratch has been noted previously (Deliagina et al. 1981) and was seen in all fictive scratch preparations examined in the present series in which PerL activity was recorded. Figure 3 shows the switch from flexor to extensor-phase activity of PerL that occurs with the change in motor task from fictive locomotion to fictive scratch. In Fig. 3A, fictive locomotion was obtained by using electrical stimulation of the MLR; some minutes later in the same preparation, fictive scratch was evoked by manual stimulation of the ipsilateral pinna. Figure 3A shows fictive locomotion (left) and fictive scratch (right) activities plotted on different timescales to facilitate comparison. During fictive locomotion PerL activity is in phase with Sart and out of phase with MG and SmAB. During fictive scratch, PerL activity overlaps with extensor activity (MG) and continues into the flexion phase.

The changes in PerL activity in locomotion and scratch are further illustrated in Fig. 3B where averaged and cycle normalized records of MG (dashed line), PerL (solid line), and PBSt (dotted line) ENGs from the runs in Fig. 3A are superimposed. The activity of PerL changes from being out of phase with the extensor MG during fictive locomotion to being slightly delayed from the onset, but overlapping with MG activity during fictive scratch. During fictive scratch, the activity profile of PerL (solid line) is strikingly similar to that of the bifunctional motor pool PBSt (Fig. 3B, dotted line). The activities of both begin during the extensor phase and continue into the early portion of the flexor phase. In this preparation, PBSt was active only at the onset of the flexor phase during fictive locomotion. The activity of the ankle flexor (TA) remained out of phase with ankle and hip extensor activity during fictive scratch (Fig. 3A).

**Other forms of deletions**

Pooled results from fictive locomotion and scratch indicated that most deletions affected synergists throughout the limb. Thus deletions of ankle motoneuron firing were accompanied by deletions of hip motoneuron firing in 43 of 49 cases. In the other cases, activity was absent in some motor pools but only reduced in others. Figure 4A shows an example of differences in the deletion of activity in distal and proximal motor pools. During this episode of fictive locomotion, the hip extensor (SmAB) burst is almost completely absent (third burst in A1; second and third bursts in A2), whereas ankle extensor ENG activity (LGS, MG) is unaffected.

Figure 4B shows nearly 2 s of an 8-s-long episode of fictive scratch during which a long-lasting deletion of extensor bursts
can be seen. Numbers above the traces correspond to extensor-like activity bursts in PerL. The fifth extensor burst is missing in all extensor ENGs recorded (SmAB, LGS, MG). During cycles 7–12, there is no activity in the LGS nerve and only small-amplitude bursts are seen in MG. Bursts in the SmAB ENG reappear and recover to previous amplitudes by the twelfth cycle when ankle extensor nerve activity continues to be deleted. Unlike the example in Fig. 2B where the extensor-like activity of PBSt and PerL fails during extensor deletions, PBSt and PerL ENGs are only reduced in Fig. 4B, being most affected during the fifth and sixth bursts. This figure and Fig. 2A show that, although deletions typically affect motoneurons operating at both proximal and distal muscles, there are variations in the completeness of the deletion as seen in the ENG as well as the number of cycles in which reduced activity occurs.

As illustrated in Figs. 1 and 2, the deletion of activity in some motor pools may be accompanied by sustained activity in the antagonist pools. On the other hand, during the deletions in Fig. 4, A–C, such reciprocity between the failure to excite agonists and the failure to silence antagonists is not seen. In Fig. 4A flexor activity continues to be rhythmic during reductions in SmAB activity. In Fig. 4B, TA activity remains rhythmic during the period in which ankle extensor (MG and LGS) activity is absent and hip extensor (SmAB) activity reduced during fictive scratch. In Fig. 4C the rhythmic bursting of hip (Sart) and ankle flexors (TA, PerL) and the bifunctional PBSt motoneuron pools are unaltered during the period where hip (SmAB) and ankle (MG, LGS) extensor activity is absent. The intracellular traces from the PerL and MG motoneurons in Fig. 4B and the EDL motoneuron in Fig. 4C are discussed in the following text.

Timing of rhythmic activity can be maintained during deletions

During the deletions illustrated in Fig. 4, A and B, the step-cycle period appears to be more or less consistent throughout the period in which motoneuron activity failed and rhythmic activity continues in antagonists. Such a maintenance of cycle period during failures to recruit motoneuron pools (i.e., ENG deletions) was seen frequently in the present series including deletions in which there was a tonic activity in antagonists while agonist activity failed (Figs. 1 and 2). Vertical tick marks representing the mean step cycle calculated from the five cycles preceding the deletion are plotted in Figs. 1 and 2. In those examples during fictive locomotion (Fig. 1) and scratch (Fig. 2), the reemergence of deleted ENG activity occurs at an integer multiple of the preexisting cycle period. In Fig. 1A, the sixth flexor burst occurs at an interval close to three step-cycle periods and the seventh burst at an interval of two step-cycle periods. Furthermore, between flexor bursts 5 and 6 the tonic activity in the extensor SmAB is decreased slightly (stars) at intervals of the preexisting step-cycle period. In Fig. 1B, the interval between extensor bursts 2 and 3 is three times the preexisting step-cycle period. Similarly cycle period appears to be maintained during deletions observed in fictive scratch in Fig. 2 with extensor activity returning at very near the interval of one missing in Fig. 2A. In Fig. 2B, PBSt, PerL, and SMAB bursts occur after what would appear to be two missing cycles of the preexisting cycle period.

Figure 5, A and B, shows rectified and integrated ENGs and cycle-period measurements during fictive scratch and locomotion, respectively. In Fig. 5A, an extensor deletion occurs at the end of the period of rhythmic activity in which the average interburst interval during normal cycles was 198 ms (SD 9, n = 29 cycles). The interval between extensor bursts when the...
deletion occurred (open symbol) was 404 ms or nearly twice the average value. In Fig. 5B the average interburst interval between successive Quad ENG bursts during normal cycles (filled symbols) was 798 ms (SD 113, n = 21 cycles). During the first extensor deletions in which GS activity is absent and Quad activity is reduced (open symbols), there was no change in cycle period. The second deletion is discussed in the following text.

The maintenance of cycle-period timing during a deletion is illustrated in the recordings of Fig. 6 in which, during the 7 s of rhythmic flexor and extensor motoneuron alternation during fictive scratch (Fig. 6A), a deletion of extensor activity occurred in three of the 37 cycles. Continuous ENG and intracellular records were broken into 37 segments of 500-ms duration. These traces start 250 ms before and end 250 ms after the onset of PerL activity and are overlaid in Fig. 6B. Traces without deletions are plotted with dotted lines and those during the three extensor deletions with solid lines in Fig. 6B. Portions of the records with deletions are plotted in Fig. 6C. There is an absence of activity in LGS and SmAB accompanied by a substantial reduction of activity in PerL during two of those deletions. In those two cases, the activity is sustained in TA. In the third deletion, the activity is reduced in extensor (LGS, SmAB) and PerL ENGs and the activity in the TA ENG is similar to that during control cycles. The overlaid dotted and solid ENG traces show that SmAB and LGS ENG bursts after deletions occur within a few milliseconds of those occurring without deletions.

Further insights into deletions from the intracellular motoneuron recordings

During the episode of fictive scratch illustrated in Fig. 6A, intracellular recordings were made from two LGS motoneurons (bottom traces, Fig. 6, B and C). During normal cycles (dotted), both motoneurons were rhythmically depolarized with a peak-to-peak scratch drive potential (SDP) of about 10 mV and fired two to five action potentials per cycle. Segments of the three traces in which deletions occurred are replotted in Fig. 6C. In both motoneurons, the depolarizing SDP was absent during the two complete deletions of LGS activity (solid
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lines) and reduced in the partial deletion (dotted line, Fig. 6C). As judged from the intracellular records in Fig. 6B, cycle-period timing was maintained during both the incomplete and complete deletions of extensor activity.

The bottom trace in Fig. 5A illustrates the scratch drive potential (action potentials truncated) in an extensor (LGS) motoneuron that fired action potentials during every scratch cycle except the one in which SmAB, PBSt, and MG ENG activities failed. The horizontal box shows the average excursion of the SDP (5 mV, SD 1). During the extensor deletion, there is a substantial reduction in the depolarization of this LGS motoneuron.

Figure 5B shows intracellular recordings from two TA motoneurons (bottom two traces) during a period of fictive locomotion with deletions of extensor activity. The horizontal lines on the intracellular records show the average locomotor-drive potential (LDP) excursion during normal cycles (34 mV, SD 1, for TA motoneuron [mn] 1; 23 mV, SD 4 for TA mn 2). During the deletion of extensor activity on the left, the membrane potential of both cells remains depolarized throughout. In the deletion on the right, extensor (GS and Quad) activities are partially reduced; in the next cycle, there is a reduction in amplitude of the TA ENG. During the reduced TA burst, the depolarization of the TA motoneuron 1 is unaffected, whereas that of the second one is reduced slightly (see DISCUSSION). The hyperpolarization of both TA motoneurons is reduced during the partial deletion of GS activity.

The paired intracellular motoneuron recordings in Figs. 5A and 6 illustrate several features of deletions. The first is that motoneurons in a pool are affected in qualitatively similar ways. The second is that deletions can occur in which there is a complete failure to depolarize motoneurons (Fig. 6). The third is that membrane potential excursions after a deletion can be similar in amplitude to those occurring before the deletion without a gradual recovery in LDP amplitude. The fourth is that the timing of the onset of the subsequent depolarization can be unaffected by the deletion. Thus in Fig. 6, despite the complete failure to depolarize both LGS motoneurons during two deletions, the scratch pattern–generating circuitry maintains the interburst interval.

Apparent changes in cycle period during deletions were also encountered in the present series that were not statistically significant. In the second deletion illustrated in Fig. 5B, the cycle period spanning the deleted burst is 1,162 ms, which is not an integer multiple of the control cycle period of 780 ms. The lack of a statistically significant difference in cycle period during the deletion (see legend), may have resulted from the variability of the control cycle period. As indicated by the vertical tick marks in Fig. 4C, the interburst interval changes somewhat throughout the deletion with a small shift in the occurrence of extensor bursts when extensor activity resumes, although this was not significant.

Figure 8 presents P values obtained from the comparison of deletions to control cycles for all 49 deletions reported. These values were obtained by comparing cycle intervals during deletions to the cycles immediately before the deletion. In 18 of 49 cases, the deletion was significantly different from the sample of control intervals (P < 0.05). For the remaining cases (63%) there was no evidence that the cycle timing had changed during the deletion. Twenty-four values in Fig. 8 are >0.2. The comparison using the few cycles before a deletion for the control was an attempt to minimize effects of cycle period fluctuations occurring over longer time intervals during the run. Almost identical results (not shown) were obtained with a comparison of the deleted interval with all cycles in the run using the oscillator phase analysis (see METHODS). In this comparison, 17/49 resulted in P < 0.05 with 24 observations, P > 0.2. Thus the most common deletion observed was one in which the onset of burst activity was maintained during the deletion with the reappearance of activity occurring at an integer multiple of the normal step-cycle period.

Maintenance of reciprocity during deletions

During the deletions illustrated in Figs. 1 and 2, the failure to activate agonists is associated with a failure to inactivate antagonists (i.e., sustained activity). Figure 9 shows a similar
example of reciprocal effects in antagonist motoneurons using both ENGs and intracellular recordings. In Fig. 9A, there is a prolongation of the flexor burst between extensor bursts 5 and 6. During this event the Sart motoneuron, which had only a moderate cycle by cycle fluctuations in ENG burst amplitude. Simultaneous recordings from pairs of homonymous motoneurons suggest that deletions occur in a qualitatively homogeneous way throughout the motor pool (Figs. 5B and 6). They also show that deletions were either accompanied by a reduction or the complete absence of the expected motoneuron depolarization (e.g., Figs. 6 and 7). One complication in assessing changes in the amplitude of SDPs and LDPs is that they may include a voltage-dependent component that can saturation in amplitude (Brownstone et al. 1994). Consequently, small reductions in premotoneuronal depolarizing drive could have little influence on LDP amplitude in some motoneurons if the membrane potential remained in the voltage-dependent region. This could, for example, explain the lack of change in the level of depolarization in the TA motoneurons during the partial TA deletion illustrated in Fig. 5B. On the other hand, even small decreases in premotoneuronal drive could result in substantial loss of motoneuron recruitment in those motoneurons depolarized to levels just threshold for voltage-dependent excitation.

Deletions during fictive locomotion and scratch

Deletions during fictive locomotion and scratch were similar in that they could be complete or partial and they often affected the

DISCUSSION

During fictive locomotion and scratch, the rhythmic activation of flexor and extensor motoneurons can be interrupted by spontaneous deletions of activity. Intracellular recording revealed deletions in which the depolarization that produces motoneuron firing during locomotion and scratch was reduced and others in which this depolarization was missing (e.g., Fig. 6). In terms of the locomotor rhythm, deletions occurred in which the cycle period after the deletion was altered and others occurred in which cycle period timing was maintained. The maintenance of cycle period timing during some deletions suggests a separation of the functions of rhythm generation and the distribution of excitation to motoneurons in the organization of locomotor and scratch CPGs.
activity of synergists throughout the limb. Deletions of extensor activity were observed in both behaviors. Whether the absence of flexor deletions during scratch is related to the more robust activity of flexors during this behavior remains unknown. Deletions with and without alterations in cycle period were found in both scratch and locomotion. The common characteristics of deletions suggest that there may be a similar organization of the networks responsible for fictive locomotion and scratch.

Except for PerL and PBSt, the phase of activity of motoneuron pools did not change from fictive locomotion to fictive scratch. Figure 3 shows the switch in the phase of PerL activity from flexor-like in fictive locomotion to more extensor-like during fictive scratch in the same preparation. During locomotion, PerL motoneuron activity is similar to that of its close synergist TA and in phase with hip flexor (Sart) activity. During fictive scratch, PerL activity begins 40–50 ms before that of Sart (Deligianna et al. 1981) and overlaps with extensor motoneuron activity. In Fig. 3B, the averaged profiles of PerL and PBSt activity are almost identical during scratch. During deletions of extensor activity during fictive locomotion, PerL activity was sustained like that of other flexors (Fig. 1B). During fictive scratch, however, PerL activity was reduced along with that of the extensor motoneuron pools (complete deletion in Fig. 2B, partial deletions in Figs. 6 and 7). Thus the pattern of PerL activity during deletions followed the changes in PerL activity during fictive locomotion and scratch.

**Deletions are missing bursts of activity**

Deletions are reductions or absences of one or more expected rhythmic bursts of activity in multiple agonist motoneuron pools. They can occur spontaneously and during rhythmic fictive locomotion and scratch. In contrast to a failure in motoneuron activation that might result from a local reduction in excitability at the level of the motoneurons, the deletions of activity in multiple agonist motoneuron pools are presumably related to a failure in operation in some common spinal structures (e.g., the CPG). However, this definition does not address the question of whether the phase of the locomotor rhythm is changed during the deletion. In other words, this definition does not address whether the deletion is accompanied by a resetting of the locomotor (scratching) rhythm. To our knowledge this issue has not been explicitly addressed in previous studies. If a deletion does not affect the phase of the locomotor rhythm, then an integer number of cycle periods should be missing during the deletion. One problem with determining whether this has occurred is that there are spontaneous variations of the cycle period during fictive locomotion (Burke et al. 2001; Yakovenko et al. 2005) and scratch (e.g., Berkinblit et al. 1978). Because deletions are rare events, a single measurement of the cycle period surrounding a deletion is compared with the average control cycle period, which can fluctuate. Given the small number of events for comparison, statistical results must be treated cautiously. We suggest, however, that the present classification (Fig. 8) clearly identified a number of deletions during which a change of the phase of oscillations occurred (was statistically significant: $P < 0.05$; 18/49) and deletions that occurred without rhythm resetting (in which a shift of the phase was not found by statistical analysis; e.g., $P > 0.20$; 24/49). The results in Fig. 7 show that these two types of deletions can occur in the same run. In that example, the longer deletion was without change in the cycle period. Figure 8 suggests that deletions in which cycle period timing was not altered during the deletion were common.

Complete deletions in which there was no evidence for resetting are shown in Figs. 1A, 2B, 5A, and 6. The paired intracellular extensor motoneuron recordings during fictive scratch in Fig. 6 show an unchanged scratch cycle period despite the complete failure of motoneuron depolarization during some deletions. The maintenance of cycle timing (and thus phase of the locomotor rhythm) has also been suggested during deletions seen in fictive locomotion elicited by dorsal root stimulation in a chronic spinal cat after decerebration (Fig. 4 in Grillner and Zangger 1979). Taken together, the evidence argues strongly for a CPG organization in which the cycle period timing can be maintained despite failures to activate agonists (missing bursts) and inactivate (continuous bursts) antagonist motoneurons. Accordingly, the deletions examined here are more likely to be failures in motoneuron excitation than abrupt, temporary changes in step-cycle period.

**Deletions provide clues to CPG organization**

Figure 10 presents an organization of the mammalian CPG operating within one limb that can accommodate the features of the spontaneous deletions reported here. For the purposes of this discussion we consider the scratch and locomotor networks to be similar. As outlined in the Introduction, in the classic half-center model of the CPG, the same network is responsible for both the generation of cycle timing and the excitation of motoneurons. Deletions in which cycle-period timing was maintained are an important observation in this regard.

A maintenance of cycle period during deletions suggests that the failure to recruit motoneurons is not inextricably linked to the generation of the locomotor or scratch rhythm. In Fig. 10, the CPG is shown as organized into two layers, one for rhythm generation (RG) and the other for pattern formation (PF). A separation of the tasks of rhythm generation and motoneuron
excitation was previously proposed to account for the observation that variations in ENG amplitude during entrainment of the locomotor rhythm were not related to cycle frequency (Kriellaars et al. 1994). Burke and colleagues (2001) also suggested that cutaneous afferents can access separately the rhythm-generating and the pattern-formation components of the CPG. Kosland and Smith (1989) proposed a circuit controlling paw-shake where pattern formation and rhythmo-genesis were separated. The two-layer organization in Fig. 10 is in keeping with these observations and offers an explanation for the deletions observed here.

First consider a spontaneous alteration of excitability within the PF networks that does not affect the RG network. Such a disturbance would alter motoneuron activity but would not produce rhythm resetting or phase shift. Depending on interactions within the PF networks, this type of deletion would be accompanied by a complete suppression of activity or a reduction (partial deletion) in ENG amplitude. On removal of the deletion-producing disturbance, motoneuron activity would reappear at the time that would have been expected had the deletion not occurred. According to our analyses, this type of deletion occurred frequently in these preparations. Now consider the result of a disturbance that affects the excitability of only the RG network. A disturbance at the RG level may directly affect rhythm generation for an arbitrary time period. Thus the rhythm after this disturbance could have a phase shift relative to the predeletion rhythm.

We suggest that deletions can be classified into two types. One is produced by a perturbation occurring at the pattern-formation level without affecting the rhythm generator (see Fig. 10) and is associated with the maintenance of the phase of the locomotor (or scratching) rhythm. The other type results from a perturbation that affects the RG network. This would cause a deletion accompanied by a shift of the phase (resetting) in the locomotor (or scratching) rhythm (see Fig. 10).

Excitability changes in the RG network would result in spontaneous changes in the cycle period and, depending on the interactions between the RG and PF networks, might not result in deletions of motoneuron activity. During fictive locomotion, the durations of both the flexion and extension phases are subject to variation as the cycle period changes spontaneously (Yakovenko et al. 2005). Those analyses suggest that the organization of rhythm generation networks in the CPG is symmetrical to flexors and extensors. In the present study no attempt was made to quantify spontaneous changes in the cycle period when no deletions occurred. Because of the possibility of spontaneous changes in the control cycle period, one analysis (Fig. 8) used the cycles immediately before the deletion to determine the control cycle durations. Because the proportions of nonresetting and resetting deletions were the same as those obtained when comparing the deleted cycle to all the cycles in a run, cycle-period fluctuations were unlikely a major factor in the present analysis.

As shown in Fig. 7, deletions in which the cycle period was maintained and deletions in which the cycle period was altered can occur in the same run. Turkin and Hamm (2003) reported a high incidence of deletions during fictive scratch in which changes in cycle-period timing occurred. Both rhythm resetting and nonresetting deletions are easily accounted for in Fig. 10 by the separation of RG and PF networks and the possibility of independent changes in the excitability of these networks.

Unit burst generators

The theoretical advantages of a mammalian CPG organization for locomotion and scratch consisting of multiple oscillators that, under some circumstances, can display independent
rhythmicity have been noted (Grillner 1981; Stein and Smith 1997). In this organization, oscillators, commonly referred to as unit burst generators (see Grillner 1981), would operate on a limited number of motoneuron pools. Using the L-DOPA preparation, Grillner and Zangger (1979) reported a case in which there was rhythmic activity in some synergist motoneuron pools while others were silent. Stein and Daniels-McQueen (2004) reported hip-extensor deletions in which knee-flexor alternation continued. During paw-shake in the cat there can be missing bursts in knee extensor activity, whereas the rhythmic activity persists in TA and LGS nerves (Smith et al. 1985). We do not consider these observations to be evidence for independent rhythm generation in subsets of motoneurons. According to our scheme of CPG organization, such deletions can occur with a failure in one of the PF modules. In our opinion, the best evidence for independent rhythm generation would be distinct cycle periods recorded intracellularly in two motoneuron pools. Continuous phase differences in the onsets of activity bursts in two motoneuron pools (unsynchronized oscillations) would also be evidence for independent rhythm generators. A lack of synchronization can sometimes be observed in the coupling of rhythms between the two hindlimbs during fictive locomotion (personal observations). In support of the unique rhythm generator within the limb is the fact that in the present material and during less rigorous examinations of records from our laboratory over several years, we have not encountered a single example in which there were different periods of rhythmic activity in ipsilateral motoneuron pools during brain stem–evoked fictive locomotion. (Bursting of bifunctional motoneurons in both the flexor and extensor phases is not considered as evidence for different rhythms within the limb.) Nor have we encountered tonic activity in some pools that was accompanied by rhythmic activity in other agonists.

Burke and colleagues (Fig. 10 in Burke et al. 2001) described the absence of extensor ENG bursts while flexors continued to burst rhythmically as an example of independent cycling of flexor bursting. Their intracellular recordings from an LGS motoneuron, however, show continued small cyclic depolarizations and small bursts of activity in the LGS ENG. Our interpretation is that this is a deletion in extensor activity and not an example of independent flexor rhythm generation. We posit that, in the rhythmic flexor bursting in Fig. 4C, there is a rhythmic but subthreshold depolarization of extensors. Although there was no intracellular recording from an extensor motoneuron during that episode, the ENG traces show hints of appropriately timed extensor motoneuron recruitment. In our opinion, all available records suggest that the oscillator circuitry responsible for cycle period within a limb functions as a single-rhythm generator in which extensor and flexor activities alternate during fictive locomotion and scratch. The present results cannot preclude the possibility that it can operate in other modes under other conditions.

The organization depicted in Fig. 10 consists of a single rhythm–generation module along with several pattern-formation modules. It could be described as a Central Rhythm Generator controlling several Unit Pattern Generator modules each responsible for excitation and inhibition of subsets of motoneurons. This organization has elements of the circuit proposed by Koshland and Smith (1989) for paw-shake in the cat. We believe that this architecture can provide the advantages of a unit burst–generator organization in generating varied patterns of motor pool activities. The single rhythm–generation module in Fig. 10 is not in conflict with an extensive distribution of rhythmicogenic capabilities throughout the spinal cord (e.g., Cowley and Schmidt 1997; Kjaerulf and Kiehn 1996). An organization that included strong propriospinal connections to ensure that rhythm generation was synchronized into a single functional entity would be consistent with the organization depicted in Fig. 10. We do not suggest that there is only one or even a spatially constrained “clock” underlying rhythm generation during locomotion in all limbs. Independent locomotor rhythms in the left and right limbs are seen during split-belt treadmill walking in spinal cats (e.g., Forsberg et al. 1980). Intrinsic rhythmic capabilities have been found in spinal neurons isolated from the larger network (e.g., Hochman et al. 1994a) and in motoneurons in isolated spinal cord preparations (Hochman et al. 1994b). The existence of such properties does not answer the question of whether such neurons ever produce independent rhythms in motoneurons when they are embedded into the intact motor network. We believe that at present there is no need to include multiple RG networks in Fig. 10. We are in agreement with Stein and Daniels-McQueen (2004) that the patterns of deletions reported in the present study are in conflict with a CPG organization that groups all agonists together into a single half-center. In the organization presented in Fig. 10, this conflict is resolved by the presence of multiple PF modules.

To summarize, the present data are evidence for an organization of the locomotor CPG in which there is a separation of rhythm generation and the distribution of excitation to motoneurons (i.e., the pattern-formation network). Such a separation forms the basis of a new half-center model and its implementation in a large-scale computational model that can replicate all of the deletions reported in the present study (Rybak et al. 2004).

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REFERENCES


