FAILURE MODES OF CENTRALLY GENERATED PATTERNS: CLUES TO THE ORGANIZATION OF THE SPINAL CENTRAL PATTERN GENERATOR

BY

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A Thesis Submitted to
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in Partial Fulfilment of the Requirements for the Degree of

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ABSTRACT

In adult decerebrate cats, the rhythmic alternating activity of hindlimb flexor and extensor motoneurons during fictive locomotion and fictive scratch can be interrupted by deletions, the brief absence of activity in some motoneuron pools. During these deletions the activity of agonist motoneurons operating throughout the limb disappears or is greatly attenuated while the activity of antagonists continues. Intracellular recordings show that the depolarizing portion of locomotor or scratch drive potential of motoneurons is reduced during deletions of agonists while the magnitude of the hyperpolarized phase is reduced during deletions of antagonists. In many cases, the timing of the rhythmic bursts following a deletion is preserved, suggesting that the period of the fictive locomotor or scratch cycle has not been changed by the deletion. This in turn suggests that the central circuitry generating the timing of these rhythmic motor behaviours may be functionally distinct from that distributing the rhythmic depolarization and hyperpolarization of hindlimb motoneurons.

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LIST OF ABBREVIATIONS

CPG	central pattern generator
EDL	extensor digitorum longus
ENG	electroneurogram
FDHL	flexor digitorum hallucis longus combined with flexor digitorum longus
GS	lateral gastrocnemius, medial gastrocnemius and soleus
LDP	locomotor drive potential
LG	lateral gastrocnemius
LGS	lateral gastrocnemius and soleus
MG	medial gastrocnemius
MLR	mesencephalic locomotor region
mn	motoneuron
PBSt	posterior biceps combined with semitendinosus
PerL	peroneus longus
Plant	plantaris
Quad	quadriceps (includes vastus lateralis, vastus medialis, vastus intermedius and rectus femoris)
Sart	sartorius
SD	standard deviation
SDP	scratch drive potential
SmAB	semimembranosus combined with anterior biceps
TA	tibialis anterior
Tib	tibialis (includes muscle and cutaneous branches innervating the plantar surface of the paw)

INTRODUCTION

Motor behaviours such as locomotion are obviously important for the survival of animals. It is, therefore, not surprising that several general principles of the organization of locomotor systems are conserved across species in which locomotion varies from swimming in fish to walking in quadrupeds. One striking feature of locomotion is the rhythmic alternating activity of two groups of muscles acting at opposite sides of 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).

Overwhelming evidence (summarised below) indicates that in all vertebrates studied, there are neuronal circuits in the spinal cord that can produce the basic alternation of muscle groups for locomotion. In mammals, this spinal circuitry appears sophisticated enough to also produce different locomotor gaits and coordinated activity between the limbs. These spinal organizations of neurons are called central pattern generators (CPGs). Unfortunately, the mammalian CPG cannot currently be described as a clearly defined set of interconnections between nerve cells. Despite the work of several groups of researchers the description of the structure of the CPG remains abstract. While general features of the activity of motoneuron pools have been described during locomotion, we presently have little knowledge about any of the interneurons that may make up the CPG.

The robust alternation of flexor and extensor activity is observed not only in real locomotion but also during fictive locomotion. Fictive locomotion is the rhythmic activity of motoneurons that closely resembles activity recorded in the corresponding muscles during real locomotion. It occurs in a reduced, decerebrate, preparation in which neuromuscular blockade prevents muscle contraction. In adult mammals such as the cat, fictive locomotion can be produced by a variety of procedures including intravenous administration of drugs or continuous electrical stimulation of the brainstem.

One advantage of fictive motor behaviours is that they are generated in the absence of either rhythmic sensory feedback or cortical control. They thus reflect the operation of intrinsic (spinal) pattern generators more so than intact preparations. A

second advantage of fictive behaviours for the study of motoneuron activity is that these behaviours can be produced even in the presence of neuromuscular blockade. This allows stable and reliable recordings from motoneurons and interneurons during CPG operation.

During fictive locomotion or scratch, the normally robust and alternating activity of flexor and extensor motoneurons can sometimes fail, reappearing after a second or two. This thesis explores a particular type of failures namely spontaneous deletions of ENG activity occurring during the operation of the locomotor circuitry in an attempt to gain a conceptual insight into the internal organization of the adult mammalian CPG. All observations to be described were made during fictive locomotion evoked by electrical stimulation of the midbrain or during fictive scratch evoked by topical application of curare to the cervical spinal cord in decerebrate cats.

This thesis will characterize the occurrence of deletions, a particular type of failure consisting of a missing ENG burst in otherwise rhythmic activity. This term was introduced by Stein and Grossman (1980) who first described this phenomenon during the turtle scratch reflex. One goal of describing deletions during cat fictive behaviours is to infer principles governing CPG organization. The Introduction will review the characteristics of normal and fictive locomotion as well as the evidence for the existence of spinal central pattern generators (CPGs) capable of generating locomotor-like activity. We will also present the characteristics of normal and fictive scratch as well as of the paw shake reflex. Effects of afferent input on rhythmic patterns will be briefly introduced. Preparations which have been used to study CPGs will be discussed and an overview of some proposed models for the CPG will be given. Finally, reports of failures of rhythmic activity in the literature will be presented.

Activity patterns during real locomotion

The step cycle of the cat (reviewed in Rossignol, 1996) consists of two phases, stance and swing. For simplicity, we will consider only the movement of and activity in structures from a single hindlimb. During swing, the limb is lifted from the ground by flexion of the knee and ankle and travels from its posterior position to an

anterior position. Kinematically, extension begins with extension of the ankle and knee while the hip is still flexed. As the foot contacts the ground, the ankle and knee are passively stretched by loading of the limb. The final part of the extensor phase consists in extension of all three joints and propulsion of the body forward.

The pattern of muscle activation that underlies these complex kinematic events is, however, much simpler. Electromyogram (EMG) recordings show that most limb muscles can be classified as belonging to one of two groups, with each group being active once per step cycle. These groups are the pure extensors and flexors. These groups display strictly alternating activity during locomotion. At the hip, knee and ankle, EMG recordings show that there is simultaneous activity in all of the pure extensors during stance followed by simultaneous activity in all of the pure flexors during swing in cats engaged in unrestrained over ground locomotion.

The pure flexor and extensor muscles, activated during swing and stance respectively, correspond well to the classification of flexors and extensors made by Sherrington (1910). Using decerebrated cats, he noted which muscles contracted during the flexion reflex and the crossed extension reflex. This gave rise to the modern definition of hindlimb muscles as flexors (active during flexion reflexes) and extensors (Sherrington, 1910) which in some cases contradicts the terminology arising from strictly anatomical studies. For example, extensor digitorum longus is primarily an ankle flexor and "plantar flexion" is produced by ankle extensor muscles. From his studies, Sherrington had two other profound insights. The first was that there was a reciprocal organization of excitation and inhibition of motoneurons during the flexion reflex. During flexion, extensors were inhibited and during crossed extension flexors were inhibited. The second was that this organization could allow for the production of stepping movements by alternation of these reflexes.

Bifunctional muscles, that is, muscles that span two joints, display more complex patterns of activity during real locomotion. They may be active during the transition between phases or even burst more than once per step cycle (eg. Engberg & Lundberg, 1969). The question arose as to whether muscles that display more than one burst of activity per step are innervated by two separate groups of motoneurons or

whether individual motoneurons can be active twice during a step cycle. This issue was addressed by studies of intracellular recordings from hamstrings motoneurons during fictive locomotion which showed that single motoneurons were depolarized twice during each step cycle (Perret & Cabelguen, 1980). Modulation of the relative amplitudes of depolarization during flexion and extension could, as discussed in Perret (1983), give rise to activity in bifunctional muscles that is purely extensor- or flexor-like, present in both phases, or occurs only at the transition between the two phases. The nervous system could therefore produce complex muscle activity by controlling the depolarization of motoneurons innervating a bifunctional muscle.

Similarities and differences in the activity patterns during various forms of walking

Cats readily and smoothly switch between a number of locomotor tasks such as forward and backward walking, walking up and down inclined surfaces and walking in a crouched position and all at different speeds. In a series of careful observations, Dr. Judy Smith and her colleagues at UCLA have described the kinematics, muscle activation patterns, and limb forces during these different locomotor behaviours. They have shown that, whereas patterns of activity of flexor and extensor muscles remain essentially the same under several conditions of locomotion, activity of bifunctional muscles is much more variable.

Studies of backward locomotion (Buford & Smith, 1990; Buford *et al.*, 1990) have shown that, although backward walking requires different posture and hindlimb kinematics (Buford & Smith, 1990; Buford *et al.*, 1990), the basic flexor and extensor synergies remain, with flexors active during swing and extensors active during stance (Buford & Smith, 1990). Contrary to earlier suggestions (e.g. Grillner, 1981), mixed synergies (co-contractions) are not observed during backward walking. The proportion of stance and swing phases is similar in both forward and backward walking although the stride length is shorter in backward walking. However, the main difference between forward and backward walking is that the horizontal displacement of the hindlimb is done mainly through hip extension combined with knee flexion during backward walking

but through the combination of hip flexion and knee extension during forward walking. This is accomplished by a change in the timing and amplitude of recruitment of bifunctional muscles. Semitendinosus (St) (hip extensor, knee flexor) has a single burst of activity during flexion in backward walking, but two bursts of activity during forward walking (Buford & Smith, 1990).

The pattern of activity of St also varies in other types of walking. During upslope walking, it is active beginning at the transition between swing and stance and until mid-stance (Carlson-Kuhta *et al.*, 1998). In comparison, during level forward walking it is active once at the end of stance and into the transition to swing and a second time at the end of swing and into the transition to stance. Other variations in ST activation have been observed in crouched walking (Trank *et al.*, 1996) and downslope walking (Smith & Carlson-Kuhta, 1995; Smith *et al.*, 1998). These changes in the pattern of activity during different locomotor tasks is true of other bifunctional muscles such as the lateral sartorius (hip flexor, knee extensor) which has two bursts of activity per step cycle during locomotion in unrestrained cats (Engberg & Lundberg, 1969; Pratt & Loeb, 1991).

Cats can also display gaits of varying speed such as the walk, trot and gallop. In general, the various gaits involve different patterns of interlimb coordination but the basic flexor and extensor synergies within a limb are the same even in normal and chronic spinal cats (Barbeau & Rossignol, 1987).

Therefore, while the pattern of activity of bifunctional muscles is complex and subject to vary from one mode of locomotion to another, patterns and synergies of pure flexors and extensors are very stable across behaviours. Moreover, intralimb synergies are also maintained across different gaits. All this is consistent with common neural mechanisms being involved in the generation of intralimb synergies for a variety of gaits and movements. In this thesis we will limit our study to the patterns of activity of pure flexors and extensors in order to gain insight into the nature of the centrally generated pattern underlying various motor tasks.

Patterns of motoneuron activity

It is understandably difficult to examine motoneurons during real behaviours since a mechanically stable preparation is required for intracellular recordings. It is however possible to record from their axons in dissected ventral root filaments. This was performed by Zajac and Young (1976 and 1980) in decerebrate cats during treadmill locomotion induced by brainstem stimulation. Moreover, a technique was developed by Loeb and colleagues to perform long-term ventral root filament recordings during treadmill walking in intact cats (Hoffer *et al.* 1981, Hoffer *et al.* 1987). Both groups found that motoneurons innervating flexor and extensor muscles fire a single burst that lasts throughout the corresponding ENG burst (Zajac and Young, 1980; Hoffer *et al.* 1981; Hoffer *et al.* 1987). This indicates that the ENG activity is not the result of the sequential firing of subsets of motoneurons within the pool. Rather, each active motoneuron in the pool fires throughout the burst suggesting that they receive a similar time course of synaptic drive during locomotion.

The intracellular activity of motoneurons has been described during fictive behaviours (e.g. Edgerton *et al.* 1976, Perret and Cabelguen 1980 and others). During fictive behaviours, the membrane potential of individual flexor and extensor motoneurons varies cyclically in phase with the efferent activity in the corresponding nerves. Motoneurons are depolarized during the burst of activity in the corresponding nerve and hyperpolarized during activity in antagonist nerves. This has been shown in the spinal cat during pharmacologically evoked fictive locomotion (Edgerton *et al.* 1976), in the thalamic paralysed cat during spontaneous locomotor activity (Perret and Cabelguen, 1980), and in the decerebrate cat during MLR-evoked fictive locomotion (see Jordan 1983). Motoneurons to bifunctional muscles have a more complex pattern of membrane potential fluctuations, in keeping with the more complex activity pattern observed in these muscles. The ST muscle can display two activations per cycle (Perret & Cabelguen, 1976; Perret & Cabelguen 1980) and it was shown that ST motoneurons can display double depolarizations for each cycle during spontaneous fictive activity in the thalamic paralysed cat (Perret & Cabelguen, 1980). This suggests that the complexity of the St

muscle activation pattern is due to a complex drive received by individual motoneurons and not to the combination of simple activation patterns distributed to different subsets of motoneurons. Muscles innervated by a combination of subpools of motoneurons, each active only once per cycle are called heterogeneous muscles. It was thought that heterogeneous muscles do not exist in the cat (Perret, 1983). However, there is some evidence in the freely moving cat that PerL may be composed of two parts (anterior and posterior) with task-related antagonistic actions (Hensbergen & Kernell, 1992). Similarly Sart, which displays 2 bursts of EMG activity during locomotion (Engberg and Lundberg, 1969; Pratt and Loeb, 1991).

Preparations for studying the mammalian CPG

Fictive locomotion in the mesencephalic cat

In the mid 1960's Moscow investigators, and in particular Drs. Shik and Orlovsky, developed reduced cat preparations in which cats were capable of co-ordinated locomotor movements of all four limbs on a treadmill (Shik *et al.*, 1966b). Under general anaesthesia, the cortices were removed and a blunt transection of the brainstem-thalamus was made at a particular level. This preparation is a particular form of the decerebrate preparation. Shortly after anaesthesia was discontinued, it was possible to obtain locomotor movements that kept up with the speed of the treadmill surface. Spontaneous locomotion occurred if the transection was rostral enough to leave the region around the subthalamic nuclei intact. However, the most commonly employed preparation was one in which the plane of transection was rostral to the superior colliculli and caudal to the mamillary bodies. This precollicular-postmamillary transection preserves the brainstem, including the cuneiform nuclei. Electrical stimulation in the midbrain elicited co-ordinated four limb locomotion that persisted until either the stimulation was turned off or the treadmill stopped (Shik *et al.*, 1966b). Orlovsky and colleagues also performed a more precise localization of the "locomotor region" to a particular area of the reticular formation on the edge of the pons (Shik *et al.* 1967). The locomotor region later became referred to as "mesencephalic locomotor region" or MLR (Shik and Orlovsky 1976) and

it is now known that it corresponds to the cuneiform nucleus and that this nucleus projects to the reticular formation (Steeves & Jordan, 1984; Jordan, 1991).

Afferent information is not required for MLR stimulation to produce rhythmic discharges in the motoneurons. Using mesencephalic cats with MLR stimulation and walking on a treadmill, Grillner and Zangger (1975, 1984) showed that rhythmic activity in hindlimb nerves can occur after transection of all dorsal roots carrying afferents from that hindlimb. This was also shown in decorticate curarized cats (Perret & Cabelguen, 1976). Moreover, it was shown that removal of ventral afferents by transection of ventral roots in mesencephalic paralysed cats did not prevent the generation of fictive locomotion by MLR stimulation (Jordan *et al.*, 1979).

Treadmill walking in spinal cats

Another important type of reduced preparation which gained acceptance in the 1970's is that of the spinalized cat. It was shown in the early 1970's that kittens spinalized shortly after birth can display locomotor-like activity when held over a treadmill with the limbs touching the moving band (Grillner 1973, Forssberg *et al.* 1980). The locomotor pattern can be improved by regular treadmill training where the hindlimbs are placed on the belt while the forelimbs rest on a stationary platform (reviewed briefly in Rossignol *et al.* 2002). These kittens eventually are be able to walk and gallop on the treadmill. Even cats spinalized as adults can regain locomotor function provided that they are extensively trained with a treadmill (Barbeau and Rossignol, 1987). The chronic spinal cat preparation again demonstrates the ability of spinal circuits to generate the locomotor pattern and highlights the ability of phasic sensory feedback to promote locomotor activity.

The concept of the central pattern generator

Experiments showing the possibility of eliciting rhythmic activity in decerebrate cats in the absence of rhythmic afferent information as well as the possibility of eliciting rhythmic activity in spinal cats, made the concept of central pattern generators very popular. A CPG is an ensemble of neurons working together to generate

a specific pattern of neural activity associated with the execution of one or several behaviours, in our case locomotor behaviours.

The CPG is thought to be located in the spinal cord after the evidence provided by several investigators. Brown in the 1910's did a series of experiments which addressed this issue. In the spinal cat with transected dorsal roots, he recorded alternating rhythmic contractions in ankle muscles following acute transection of the spinal cord (Graham Brown, 1911). In a different preparation, where most of the lumbosacral afferent input was abolished by transection of peripheral nerves in a spinal animal, he elicited rhythmic activity by stimulating the cut end of branches to the saphenous nerve (Graham Brown, 1911). In these experiments, the muscles investigated were not deafferented.

Sherrington, using decerebrate cats instead of spinal ones, deafferented them in the same manner, namely by peripheral nerve transection to the exclusion of the nerves to the muscles of interest, in this case vastocrureus (vastus intermedius). Simultaneously stimulating both peroneal nerves, he recorded clear spontaneous alternating contractions of the vastocrureus muscles lasting for as long as the bilateral stimulation was maintained (Sherrington, 1913). He noted that these rhythmic reactions lead to clear stepping of the animal and that withdrawing either stimuli lead to the end of the rhythm. Grillner and Zangger (1979) used spinal animals where all dorsal roots were transected and elicited rhythmic alternating movements by continuous stimulation of the cut dorsal roots. These movements were elicited even after neuromuscular blockade with curare. Together, these experiments provide strong evidence for the existence of a CPG located within the spinal cord.

The work of Lundberg and colleagues in the mid 1960's (Anden *et al.*, 1966a; Anden *et al.*, 1966b; Anden *et al.*, 1966c; Grillner *et al.*, 1967; Jankowska *et al.*, 1967a; Jankowska *et al.*, 1967b) initiated research into the pharmacological induction of locomotion by showing that intravenous administration of the dopamine and norepinephrine precursor L-DOPA to spinalized cats was capable of inducing a locomotor pattern. This could be enhanced by co-administration of nialamide (Grillner & Zangger, 1979) a monoamine oxidase inhibitor thereby preventing the degradation of norepinephrine. Moreover, norepinephrine agonists acting at the α_2 receptor site such as

clonidine can also elicit locomotor like activity (Grillner & Zangger, 1979). Pharmacological induction of locomotion has two major disadvantages. First, locomotor activity generated pharmacologically displays several important differences with patterns of normal animals. The step cycle is often much longer (up to 4 seconds) and the activity of antagonists can overlap at the transition between flexion and extension (Grillner and Zangger 1979). Second, drugs injected intravenously or applied topically to the spinal cord are not easily removed and neither are their effects. This results in an imprecise distinction between control (i.e. no motoneuron activity) and locomotor (i.e. alternating activity) states.

Locomotor patterns can also be elicited in cats pretreated with nialamide and DOPA using electrical stimulation of the dorsal roots or the dorsal columns (Grillner and Zangger, 1979). Moreover, in a variety of suprapinal sites (reviewed in Mori *et al.*, 2001) as well as in the lateral funiculus at the level of the cervical spinal cord (Kinoshita & Yamaguchi, 2001) and in the white matter of the cerebellum (Mori *et al.*, 1999) electrical stimulation alone is sufficient to induce locomotor-like movements in decerebrate cats. Cerebellar stimulation in the hook bundle of Russell in the midline of the cerebellar white matter produces locomotor-like movements in forelimbs and hindlimbs of the decerebrate cat on a moving treadmill (Mori *et al.*, 1999). Electrical stimulation of the lateral funiculus at the cervical level evokes locomotor-like activity in the forelimbs of the decerebrate cat similar to patterns of stepping in the immobilized animal (Yamaguchi, 1986). Electrical stimulation of at least 4 regions in the brain of the decerebrate cat can evoke locomotor-like movements in the hindlimbs. These regions are the subthalamic locomotor region (SLR), the MLR (Shik *et al.*, 1966b), the pontobulbar locomotor region (P-BLR) (Mori *et al.*, 1977) and the ventral tegmental field of the caudal pons (VTF). It is thought that the signals originating in the SLR, MLR, and P-BLR are conveyed to the spinal cord via reticulospinal projections. Indeed, it was shown that the MLR, which corresponds to the caudal portion of the cuneiform nucleus, projects to the reticular formation and not directly to the spinal cord (Steeves & Jordan, 1984). Electrical stimulation in the pontomedullary locomotor strip (PLS) (Mori *et al.*, 1977), elicits hindlimb locomotor-like activity resembling the activity elicited via MLR stimulation.

MLR-evoked fictive locomotion in the decerebrate cat is obtained by stimulating the brainstem, either unilaterally or bilaterally, with a continuous stimulus train (Shik *et al.*, 1966b). As mentioned, it is thought that the MLR initiates locomotion through an indirect pathway to the spinal cord proceeding via the medial pontomedullary reticular formation (MRF) (reviewed in Jordan, 1991). The MRF then projects to the spinal cord within the ventrolateral funiculus (VLF) (Steeves & Jordan, 1980). Cells in both the MLR and the MRF have been shown to be rhythmically active during spontaneous locomotion which supports their involvement in the generation of rhythmic behaviour (reviewed in Jordan, 1991). The fictive activity evoked displays characteristic rhythmic alternation of flexors and extensors. Thus, one of the more striking features of locomotor movement is present in MLR-evoked fictive locomotion. The cycle period of MLR-evoked activity can be controlled to some extent by strength of stimulation of the MLR (Shik *et al.*, 1966b). Because locomotor activity subsides once electrical simulation is discontinued, the MLR preparation permits an easy comparison of motoneuron membrane potentials in control and locomotor states.

Patterns of activity during real scratch

Scratching is an example of a cyclical hindlimb motor behaviour that is not locomotor in nature. The scratching behaviour is an automatic response of the cat to a noxious stimulus in its outer ear and consists in dislodging the noxious stimulus from the ear by a series of rapid rhythmic movements of the ipsilateral hindlimb to scratch the pinna of the ear. In the intact adult cat, it consists of three phases, the approach, the cyclic phase and the return phase (Kuhta & Smith, 1990). More specifically, the approach is mainly a postural phase where the cat adopts one of a few possible preparatory postures to perform scratch. The animal may sit, lie down, or stand. During this phase, the head is positioned to orient the ear towards the ipsilateral hindlimb which will perform the actual scratching. There is an initial phase of tonic flexion followed by strict and rapid alternation of flexors and extensors. In intact cats, the extensor and flexor phases are of almost the same duration and the cyclic phase lasts on average 13 cycles with a range of 1 to 60 cycles (Kuhta & Smith, 1990). During most cycles the paw contacts the head

except sometimes for a few cycles at the end of a response. During the return period the head and the hindlimb come back to their original positions (Sherrington, 1906). Scratch can also be elicited in deafferented animals (Sherrington, 1910) but in this case, the paw cycles in the air without actually touching the ear (Jankowska, 1959). Muscle activity remains very similar in the deafferented preparation with alternating activities of flexors and extensors (Deliagina *et al.* 1975).

The robust rhythmic alternation of flexors and extensors which is common to both fictive locomotion and scratch suggests the possibility that the same CPG be involved in the production of both. This was proposed by Orlovsky's group (Berkinblit *et al.* 1978) who observed a continuum between the temporal pattern of fictive locomotion and that of fictive scratch as well as some similarities in the activity of spinal interneurons during both behaviours. The circuit responsible for the alternation of flexors and extensors could be common to both behaviours and supplemented or modified during each specific task to account for the particularities in each.

Preparations to evoke fictive scratch

There are various means of evoking fictive scratch. These include mechanical stimulation of the skin in the region of the pinna of the ear and electrical stimulation of the cervical spinal cord at the C1-C2 level, both of which were used by Sherrington (Sherrington, 1910). In cases where electrical stimulation of the spinal cord does not succeed, it is possible to elicit fictive scratch in decerebrate, curarized cats (deafferented or not) by first applying curare to the cervical spinal cord and then stimulating electrically (Deliagina *et al.*, 1975). Curare can also be replaced by strychnine (Deliagina *et al.* 1981). Moreover, in decerebrate, mesencephalic, paralysed cats, chemical stimulation of the cervical spinal cord with curare can also sensitize it such that subsequent mechanical stimulation of the ear by the experimenter is sufficient to elicit fictive scratch (Domer & Feldberg, 1960). From this point on, we will use the term fictive scratch to refer specifically to this method of evoking fictive scratch by mechanical stimulation of the pinna following curare application on the cervical spinal cord. Regardless of the method used, however, the evoked activity employs strict

alternation of flexors and extensors. Efferent activity recorded in decerebrate, curarized cats reveals that there is an initial period of tonic activity in flexor nerves followed by rhythmic alternation of flexor and extensor activities (Deliagina *et al.*, 1981). Intracellular recordings during fictive scratch show that the membrane potential of motoneurons fluctuates rhythmically (Berkinblit *et al.*, 1980; Berkinblit *et al.*, 1978). These fluctuations are referred to here as the scratch drive potential (SDP) (Brownstone *et al.* 1994 and Perreault *et al.* 1999) by analogy to the locomotor drive potential (LDP) present in motoneurons during fictive locomotion.

Effects of afferent input on motor patterns

In intact animals, sensory input from cutaneous and muscle afferents contributes to the modulation of the motor pattern produced by the CPG in two ways. First, it allows the animal to deal with unusual locomotor situations such as obstacles, holes, inclines, steps and so on. Second, muscle afferent feedback appears also important for the step-by-step generation of actual locomotor activity. In the decerebrate cat, muscle afferent feedback has been shown to be involved in the regulation of the transition from stance to swing (Hiebert *et al.*, 1996; Duysens and Pearson, 1980) and to contribute to the recruitment of extensor motoneuron pools (Hiebert & Pearson, 1999). Afferent feedback is therefore crucial in regulating the precise motor pattern in all motor behaviours.

Models of central pattern generators

As discussed previously, the central nervous system is capable of producing rhythmic motor output in the absence of cortical input and sensory feedback and even in the isolated lumbo-sacral spinal cord. This has given rise to the hypothesis of a central pattern generator (CPG), a neuronal circuit in the spinal cord capable of producing this rhythmic activity. There is good evidence for the existence of a CPG in cats and primates (reviewed in Duysens & Van de Crommert, 1998) but the organization of the CPG remains unknown. A number of possible models have been proposed for the CPG

(reviewed in Grillner, 1981) but there is little experimental data that would favour one over the other. Brown's half-center hypothesis (Graham Brown, 1914; modified by Jankowska *et al.* 1967) suggests that the CPG may be composed of two half-centers, one controlling all flexors of a hindlimb and the other controlling all extensors. These two half-centers reciprocally inhibit each other and a fatigue process generates alternation of flexor and extensor activity. On the other hand, Grillner suggested that each group of close synergists at a joint is controlled by a unit burst generator (UBG) (Grillner, 1981). One purpose of this thesis is to gain insight into the organization of the CPG through analysis of fictive patterns centrally generated with neither supraspinal control nor afferent feedback.

Failures observed in centrally generated patterns

As was described in the previous sections on fictive locomotion and fictive scratch, centrally generated patterns display the robust alternation between flexors and extensors. However, centrally generated patterns sometimes exhibit various kinds of failures. Using the chronic spinal decerebrate cat preparation, Grillner and Zangger (1979) showed an example of a missing burst in extensor (Q) activity accompanied by sustained flexor (St) activity (Figure 4 of Grillner & Zangger, 1979). They also described two patterns of activity which they suggested indicate that there is variability among the activity of synergist muscles at different joints. These patterns were 1) tonic flexor activity at a joint accompanied by rhythmic flexor activity at another joint, and 2) a high level of rhythmic extensor activity at one joint accompanied by none at another. In this thesis, deletions are defined as missing bursts of ENG activity. The example of the missing Quad burst mentioned previously (Figure 4 of Grillner and Zangger (1979) is an example of something we would call a deletion. Deletions have also been observed during MLR-evoked fictive locomotion in the decerebrate cat (Jordan, 1991) where a deletion was shown in a TA ENG was accompanied by a reduced hyperpolarization in an intracellularly recorded MG motoneuron.

Failures have also been observed during the paw-shake response in the cat (Smith *et al.*, 1985). The paw-shake is a mixed synergy characterized by alternating

flexion and extension of the ankle and by activity in the knee extensor VL overlapping with both ankle extension and flexion. This behaviour can be elicited by wrapping adhesive tape on the cat's hindpaw at the level of the plantar pad. It can also be evoked in the spinal cat with similar cycle characteristics (Sabin and Smith 1984) as well as in the spinal deafferented cat where proprioception is eliminated but some cutaneous innervation remains intact (Koshland and Smith 1989). In some of the spinal non-deafferented animals in which they studied the paw-shake response, Smith and colleagues observed missing VL bursts while the TA and LG nerves remained rhythmically active (Figure 3 of Smith *et al.*, 1985).

In addition to the deletions observed in cat preparations, there is substantial work on the occurrence of deletions in a particular muscle during the turtle scratch reflex. It was shown that during turtle scratch there is usually alternation between the activities of a hip retractor/knee flexor muscle (flexor cruris pars flexor tibialis internus denoted HR-KF) and a knee extensor muscle (triceps femoris pars iliotibialis denoted IT-KE) (Stein and Grossman 1980). During deletions, there is a missing burst of activity in HR-KF while IT-KE remains active (Stein and Grossman 1980). The synaptic input to motoneurons during these deletions was studied (Robertson & Stein, 1988) as well as the activity of pre-motor interneurons (Stein & Daniels-McQueen, 2002). In this thesis, the concept of deletion is expanded to all muscles in the cat hindlimb during fictive locomotion and fictive scratch.

Objectives

In this thesis, we present an analysis of deletions occurring in centrally generated fictive motor patterns in the cat. We studied two fictive behaviours in the decerebrate cat, namely fictive locomotion evoked by electrical stimulation of the brainstem and fictive scratch evoked by topical application of curare to the cervical spinal cord and mechanical manipulation of the ipsilateral pinna. The results will address the following five questions:

1. What is the pattern of occurrence of deletions during fictive locomotion and fictive scratch?
2. Does a deletion of activity of motoneurons operating at one joint occur while

rhythmic motoneuron activity persists at other joints?

3. Does a deletion in one step affect the timing of the next step cycle?
4. Are antagonist pools affected when a deletion occurs?
5. What are the intracellular correlates of these deletions?

To address the first four questions we compiled a list of deletions and looked at the pattern of ENGs with deletions of activity. To investigate the intracellular correlates of deletions, we recorded from lumbar motoneurons during fictive behaviours and compared the membrane potential in cycles with and without deletions.

METHODS

Surgery

Surgical and experimental protocols were in compliance with guidelines set by the Canadian Council for Animal Care and the University of Manitoba. Adult cats were fasted overnight and brought to a surgical plane of anesthesia in a closed chamber using a mixture of halothane (1-2%), nitrous oxide (70%), and oxygen (30%). During the surgery, the anesthesia was administered first through a mask and then directly via 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 s.c.), saline (10cc s.c.) and dexamethasone (2mg/kg i.v.) were given at the beginning of the surgery. Canulae were inserted in the left femoral and the right jugular veins for drug administration. The right carotid artery was canulated and connected to a transducer for blood pressure monitoring and to a peristaltic pump for infusion of a buffer solution (5% glucose, 0.84% bicarbonate solution) blood pH maintenance. The CO_2 levels and respiratory rhythm were monitored via a sensor inserted into the tracheotomy tube. The bladder was catheterized through the urethra.

Several nerves were dissected on left hindlimb for recording and stimulation: SmAB, PBSt, LGS, MG, TA, EDL, PerL, FDHL, Tib, and SP. A multi-compartment cuff electrode was put ventrally to record from hip flexor nerves. The adductor tendons of both hips were cut and the right hindlimb was denervated by cutting the sciatic nerve as well as its muscular branch which supplies SmAB and PBSt.

A laminectomy was performed to expose L4 to L7 segments of the spinal cord and the cat was transferred to a stereotaxic frame. A craniotomy was then performed followed by a mechanical decerebration involving first a removal of cerebral cortex followed by a transection of the brainstem at the post-collicular, pre-mamillary level. Following the decerebration, anesthesia was discontinued and the animal was paralysed (Pavulon, 0.1 mg/kg per hour) and ventilated. The cervical spinal cord was exposed (C1) to allow access to the roots for application of curare for scratch described below. Bilateral openings in the chest wall were made to minimize mechanical movement due to

respiration during intracellular recordings. Hindlimb nerves which were not in a cuff electrodes were recorded from using bipolar silver ball electrodes. Mineral oil was used to cover and protect the exposed nerves and spinal cord The animal was warmed with an electrical heating pad wrapped around the body and by radiant heat lamps.

Stimulation

Fictive locomotion was induced by unilateral or bilateral stimulation of the brainstem with 0.5 ms current pulses (50-500 μA, 10-20 Hz). Mechanical stimulation of the forelimb was sometimes used to initiate the rhythm. The forelimbs were either swung rhythmically, squeezed, maintained in a flexed position, or one paw was flexed while the other was extended. Fictive scratch was induced by application of curare (0.01-0.1%) on the left C1 roots followed by mechanical stimulation of the pinna of the left ear or of the lateral aspect of the left side of the face.

Recording

Hindlimb nerve activity was recorded with bipolar silver ball electrodes except for ventrally located nerves where cuff electrodes were used. Intracellular recordings of motoneurons in the lumbar spinal cord were made using glass electrodes (tip size between 1.6-1.9 μm) filled with a 1.5 M sodium citrate solution connected to an Axoclamp 2A amplifier (Axon Instruments Inc, California, USA). Electrodes were moved within the spinal cord using motorized microdrives. Simultaneous recordings of pairs of motoneurons were made using two electrodes moved independently from each other with individual microdrives. Motoneurons were identified by stimulating individual nerve branches and determining which branch could elicit antidromic activation of that motoneuron. Motoneuron recordings where the baseline membrane potential remained stable over the course of the period of rhythmic activity were accepted for analysis.

ENGs were filtered (30 Hz - 3 kHz), rectified and integrated. The sampling rate for digitization was 500 Hz for ENGs and 5-10 kHz for intracellular recordings. All signals were captured and analyzed using software developed within the Spinal Cord

Research Center and running on a Pentium PC under Linux Redhat operating system. Data was typically captured in 2 minute segments.

Data analysis

Data was taken from experiments with 10 cats. Periods of good rhythmic activity during which there was at least one intracellular recording were selected for analysis. Factors taken into account when selecting the data included the regularity of the rhythmic pattern as well as the number of ENGs available where bursts were clearly distinguishable from background nerve activity.

Once suitable periods of activity were selected, they were broken into cycles using the ENG corresponding to the species of motoneuron recorded intracellularly or the ENG of an agonist. Periods of activity were examined and missing bursts noted. For each cycle in which a burst was missing, the amplitude of the rectified and integrated ENGs during that cycle was recorded in a database (Microsoft Works). Each level was recorded as either normal, small excitation (on-phase activity is less than it should be), no excitation (no on-phase activity at all), small inhibition (some ENG activity remaining during the off-phase), no inhibition (as much activity during the off-phase as there was during the on-phase), or not available (ENG was not recorded during that particular run). The amplitude of the locomotor or scratch drive potential was then calculated for each cycle by subtracting the minimum level (hyperpolarizing phase) during a cycle from the maximum level (depolarizing phase). When cycles contained spikes, the maximum level of the drive potential was approximated as the depolarizing level at which the spike occurred. The values of the drive potential amplitudes were exported to a spreadsheet for further analysis and plotting. Unless otherwise specified, all values are reported as the mean ± standard deviation.

RESULTS

The first part of the Results section will describe nerve (ENG) activity observed during fictive locomotion and scratch. The second part will describe the activity of motoneurons during deletions using both ENGs and intracellular recordings.

In this study, we present data from 10 animals where 7 of those were used for an analysis of deletions in ENG activity (2 during fictive locomotion only, 3 during fictive scratch only, and 2 during both behaviours). The other three animals were each used to illustrate a particular example: the example of PerL activity during fictive scratch and locomotion (Figure 3), the atypical example of a deletion (Figure 7), and the example of intracellular recordings from a pair of antagonist motoneurons (Figure 10).

Periods of rhythmic activity were selected for analysis if they included at least one intracellular recording and displayed good rhythmic activity as judged by the regularity of the cycle length and the magnitude of the activity in ipsilateral hip and ankle nerves. Note that in the case of fictive scratch, the initial period of tonic flexion was not analyzed and only the period of phasic activity was considered. For the analysis of deletions, we selected 7 periods of fictive locomotion (total of 209 seconds of activity corresponding to 288 cycles) and 15 periods of fictive scratch (total of 156 seconds of activity corresponding to 743 cycles). The periods of activity used are listed in Table 1. We examined intracellular recordings from 22 motoneurons including 16 recorded as pairs. The identity of the cells examined is given in Table 1. Data from 3 additional episodes (not listed in Table 1), each within a different animal, were used to illustrate specific examples as mentioned previously (Figures 3, 7 and 10).

The activity of pure flexors and extensors is strictly alternating during fictive locomotion and scratch

Characteristics of fictive locomotion activity

The activity shown in Figure 1 is an example of the fictive locomotion analyzed in this study. MLR stimulation (paired current pulses, 0.5 ms, 50-500 μA, 27 Hz) lasting for approximately 2 minutes in total evoked a 21-second long period of

fictive locomotion of which the last 8 seconds are shown in Figure 1. The initial 13 seconds of the rhythmic activity occurred during MLR stimulation and the rhythmic activity continued for 8 seconds after MLR stimulation was stopped. This period of spontaneous activity immediately after MLR stimulation is shown in Figure 1 where ENG activity from 11 hindlimb nerves is shown. One can note that bursting was rhythmic and the activity alternated between flexor and extensor nerves. The cycle length was reasonably stable throughout the run and the magnitude of ENG bursts was comparable in size from one cycle to the next. In this example, the average cycle length was 768 ± 96 ms (standard deviation (SD), n = 26 cycles).

Cycle parameters were measured for all episodes of fictive locomotion. The cycle length was measured from the onset of consecutive flexor bursts using the TA ENG in most cases and the EDL ENG in others. Overall, the average cycle length during episodes of MLR-evoked activity was 721 ± 158 ms (n=6 episodes, 4 animals). Extensor burst lengths were measured using either the LG, MG or GS ENGs. In one episode it was necessary to use the PBSt ENG as this was the only extensor-like activity in that episode. The ratio of flexor burst length over extensor burst length was then calculated. Usually, the flexor burst was longer than the extensor burst. On average, the ratio of the flexor burst to extensor burst length was 1.6 ± 0.9 (n=6 episodes, 4 animals). This corresponds to the flexor burst occupying on average 62% of the step cycle. In a one experiment, the flexor burst was shorter than the extensor burst (ratio of flexor burst over extensor burst was 0.45).

In Figure 1, the ENGs are arranged according to their classification with flexors in the top 4 traces, extensors in the bottom 5 traces and bifunctional and combined nerves in the middle. It can be seen by looking at the extensor ENGs in Figure 1 that extensors innervating different joints (eg. Tib at the toe, GS and Plant at the ankle and SmAB at the hip) had very similar onsets, terminations and envelopes of activity. Similarly, Figure 1 shows that ankle flexors (TA, EDL, PerL) have similar onsets and terminations of activity. Their envelopes of activity are however different. Specifically, EDL activity increased as flexion proceeded whereas TA and PerL activities rose sharply at the onset of flexion and were maintained throughout the flexor phase. For all flexors shown, the activity fell sharply with the onset of extensor activity. Figure 4A, which will

be discussed in greater detail later, shows another example where the hip flexor, Sart, and ankle flexor, EDL, had a similar onsets and terminations of activity but different envelopes. Again, EDL activity in Figure 4 started at a low level and increased whereas Sart activity level was maintained throughout the flexor phase. Moreover, Figure 4B shows that the termination of activity can sometimes vary among ankle flexors. For example, the termination of activity in EDL occurs after the termination of activity in TA and PerL.

In these preparations, some nerves were combined and recorded from as a group for convenience. This is the case for FDHL, Quad, and Tib. FDHL is the combination of FDL (flexor digitorum longus) and FHL (flexor hallucis longus) nerves. They are anatomical synergists which share a common tendon and plantarflex the toes. However, they have very different actions during locomotion, as was demonstrated in normal cats during unrestrained treadmill locomotion and fictive locomotion (Fleshman *et al.*, 1984). Electromyogram recordings showed that FDL activity occurs mostly during swing whereas FHL activity resembles that of gastrocnemius (O'Donovan *et al.*, 1982). In the fictive locomotion preparation, the combined activity of FDL and FHL nerves was mostly extensor-like with some activity in early flexion as can be seen in Figure 1. The Quad ENG is the combination of nerves to the uniarticular knee extensors vastus lateralis, vastus medialis and vastus intermedius as well as to the biarticular rectus femoris (knee extensor/hip flexor). This combination accounts for the fact that, during fictive locomotion, Quad is mostly active during extension (vasti) but has some activity during flexion as well (rectus femoris). Finally, Tib represents the caudal end of the tibial nerve (distal to FDL and FHL branches). It contains both cutaneous and muscular branches which innervate plantar structures of the foot. The primary activity of Tib efferents during fictive locomotion is in phase with ankle extensors.

Bifunctional muscles have more complex and varied patterns of activity as required by their function at two joints. PBSt is composed of posterior biceps and semitendinosus, both of which act at the hip and knee to cause hip extension and knee flexion. In the example of fictive locomotion shown in Figure 1, PBSt activity is brief and occurs at the onset of flexion. This pattern was also observed in the example in

Figure 3A. In other animals, PBSt activity was mostly extensor-like (See Figure 4B, 7, 10).

Characteristics of fictive scratch activity

Fictive scratch was obtained by first applying a curare solution on the left C1 dorsal roots for a few minutes. Following the removal and washing away of the solution and waiting for 10-15 minutes, the left ear of the animal was gently manipulated (scratched or pinched). This initiated an episode of fictive scratch (See Deliagina *et al.*, 1981 and Perreault *et al.*, 1999) for description). Figure 2A shows an example of fictive scratch activity. The episode began with a period of tonic flexor activity which lasted approximately 500 ms. This was followed by rhythmic alternating activity of flexor and extensor nerves which lasted for approximately 12 seconds. Importantly, in contrast to fictive locomotion, no electrical stimulation is used to evoke the rhythmic activity in fictive scratch.

The pattern of tonic flexor activity (tonic portion) followed by rhythmic alternation of flexor and extensor activities (rhythmic portion) is characteristic of fictive scratch (e.g. Deliagina *et al.* 1981) and was observed in almost all episodes of fictive scratch elicited. Fictive scratch usually shows a gradual reduction in ENG amplitude towards the end of the episode (See Figure 2). There was one instance (not illustrated) in which several episodes of fictive scratch were observed immediately one after the other for a total duration of about 50 seconds without intervening periods of tonic flexor activity to separate them. The activity nonetheless appeared to be a succession of individual episodes of fictive scratch because of the modulation of the amplitude of the ENG activity. In this case, the ENG bursts decreased in amplitude, as usually occurs towards the end of a fictive scratch episode, and then increased in amplitude again as a new episode began. This was therefore considered as several individual episodes.

A segment of the rhythmic activity shown in Figure 2A is expanded in Figure 2B. Flexors (EDL and Sart) were clearly active in phase with each other. The extensors (MG, Plant, SmAB) were also active in phase with each other. In all examples of fictive scratch analyzed, PBSt was coactive with extensors (See Figures 2, 5, 6B) as was reported by Burke and colleagues (Degtyarenko *et al.*, 1998). In the preparations

illustrated in Figures 3A and 3B (right panel), PBSt was also active with extensors but had some additional activity during the flexor phase. The strict alternation of flexor and extensor activities was observed in all episodes. Overall, the rhythmic portion of the fictive scratch activity lasted on average 7.5 ± 2.9 seconds (n=15 episodes, 5 animals). The average cycle length during phasic activity was 193 ± 20 ms (n=15 episodes, 5 animals). During fictive scratch, the extensor phase was always shorter than the flexor phase. Overall, the ratio of the flexor burst length over the extensor burst length was 3.9 ± 0.9 (n=14 episodes, 5 animals) corresponding to the flexor phase occupying on average 79% of the cycle.

Differences in ENG activity between fictive locomotion and fictive scratch

One interesting difference in ENG activity between the two behaviours is the change in the activity of PerL. In all cases of fictive locomotion (3 animals) we observed PerL active in phase with flexors (See Figures 1and 4B for examples) but in all cases of fictive scratch (4 animals) PerL was active in phase with extensors (See Figures 2B, 3B and 5B for examples). This is consistent with the pattern of activity reported by Deliagina and colleagues where, recording simultaneously from Sart and PerL during fictive scratch, they reported PerL activity starting 40-50 ms before that of Sart (Deliagina *et al.* 1981). In three cases, we were able to observe this switch from flexor-like to extensor-like activity in the same animal. One such case is illustrated in Figure 3 where both fictive locomotion and fictive scratch were elicited in the same animal within a few minutes of each other. Figure 3A shows fictive locomotion (left panel) and fictive scratch (right panel) activities plotted on different time scales to ease comparison. During fictive locomotion (left panel), PerL activity is in phase with Sart and out of phase with MG and SmAB. During fictive scratch (right panel), PerL activity overlaps with extensor activity (MG) and continues into the flexion phase. This is further illustrated in Figure 3B which shows the superposition of the averaged and normalized activity in MG (dashed line), PerL (solid line) and PBSt (grey area) during both behaviours (same examples as in Figure 3A). The activity of PerL switches from flexor-like during fictive locomotion to extensor-like during fictive scratch. In comparison, TA activity remains strictly out of phase with ankle extensors (Figure 3A, left panel).

During fictive scratch, the activity profile of PerL (solid line) is in fact very similar to that of PBSt (Figure 3B shaded profile). This is also apparent in the example shown in Figure 2B. During fictive locomotion, however, the activity of PerL continues throughout flexion while that of PBSt often stops (Figures 1 and 3A left panel). Different patterns of PBSt activity were observed during fictive locomotion. The PBSt nerve is either active during early flexion (Figure 1, 3A left panel, 4B) or in phase with extensor activity (Figure 10). During Fictive scratch, the activity of PBSt is extensor-like. For example, in Figure 2B, the onset of activity of PBSt occurs with that of pure extensors (SmAB) and the activity lasts into the early phase of flexion. This pattern is consistent with previously reported patterns for PB and St separately (Deliagina *et al.* 1981). The PB pattern they reported consisted of activity starting after that of GS and continuing into early flexion. The St pattern was a single burst in early flexion in most instances and a double burst pattern in some cases where the bursts were flanking each side of the GS activity burst. The combination of the reported PB and St activity corresponds well with the activity recorded in our preparations in the combined PBSt nerve. In summary, PerL activity always switches from flexor-like during fictive locomotion to extensor-like during fictive scratch. PBSt activity during fictive scratch is also extensor-like which may or may not represent a change from its phase of activity during fictive locomotion.

FDHL activity was also variable during fictive scratch as seen in Figure 5 where recordings of fictive scratch in 2 different animals are presented. The peak of FDHL activity occurs mostly during extension in Figure 5A although lower amplitude activity extends into the flexor phase. In Figure 5B, FDHL activity starts after the onset of flexor activity (TA), peaking towards the end of flexion and extending through extensor activity. During fictive locomotion, FDHL is often active in phase with extensor activity (Figures 1 and 4B) and can sometimes have additional activity during the flexor phase (Figure 10).

The activity of Quad was also consistently different during fictive scratch and fictive locomotion. Figure 1 shows Quad mostly active in phase with extensors during fictive locomotion. During fictive scratch in Figure 2B, Quad activity was seen mainly during flexion with a clear second burst during extensor activity. Presumably, this reflects a greater activation of the bifunctional RF motoneuron pool during scratch. It is

unlikely that knee extensors would be strongly recruited during real scratch since the main movement is at the ankle with a smaller range of motion of the knee than is required than during fictive locomotion.

The deletions observed during fictive locomotion and fictive scratch have similar features

The main goal of this study was to describe the deletions of ENG activity occurring during fictive locomotion and scratch. We defined deletions as missing ENG bursts flanked by otherwise rhythmic activity. Examples of deletions during cat fictive locomotion have been reported previously (Figure 4 of Grillner & Zangger, 1979 and Jordan, 1991). We defined deletions as "complete deletions" when the ENG burst was absent and as "partial deletions" when the ENG burst was reduced but not absent. The visual criterion used to assess partial deletions was that bursts had to be less than half the height of their neighbouring bursts. In this study, we focussed on deletions of activity in motor pools which behave the same in fictive locomotion and in fictive scratch (flexors Sart, TA, EDL and extensors SmAB, LGS, MG, Plant). We also considered one motor pool (PerL) which changes its activity in fictive locomotion and scratch.

Deletions occurred in most animals and in the majority of episodes of activity that were examined. Of 22 episodes of activity (7 animals, 7 episodes of fictive locomotion and 15 of fictive scratch) selected for the analysis of deletions, 13 contained at least one deleted cycle (5 of 7 fictive locomotion episodes and 8 of 15 fictive scratch episodes). Pooling all episodes which contained deletions, the overall frequency of deleted cycles was 16% during fictive locomotion and similar (17%) during fictive scratch. Therefore, although deletions occurred in most of the periods of fictive activity, their frequency of occurrence was low. Examples of deletions shown in Figures 4 and 5 will now be described.

In Figure 4A, 5 cycles of rhythmic alternating activity in flexors and extensors are followed by a prolonged period of inactivity in flexors (Sart and EDL) that is accompanied by sustained activity in extensors (LG, MG, Plant, SmAB). The expected bursts are missing in both distal flexor (EDL at the ankle) and proximal (Sart at the hip)

flexors. During the flexor deletions, extensor activity was either tonic (LG, MG, Quad) or sustained and slightly modulated (Plant) as was the activity in SmAB. In the absence of flexor bursts, extensor activity did not stop as would be expected if the deletions resulted from a brief cessation of the locomotor behaviour (i.e. just stopped). Rather the activity of extensor continues with obvious, albeit small, decreases in the activity of the Plant and SmAB ENGs shown in 4A.

Figure 4B shows deletions in the activity of extensor nerves innervating ankle (GS, Plant, FDHL, Tib) and hip (SmAB) muscles during fictive locomotion in a different animal. Panel B starts on the left with 2 rhythmic bursts in extensors (SmAB, GS, Plant, FDHL, Tib) followed by a period of silence in these same nerves. This period is accompanied by tonic activity in flexors (PerL, TA, EDL). Thus during both the flexor deletions shown in Figure 4A and the extensor deletions shown in Figure 4B, the activity in the antagonists persists. In almost all examples (see Figure 7 for exception), deletions occurring at agonists were accompanied by sustained activity in antagonists. Deletions during fictive locomotion occurred in both flexors and extensors. An example of a flexor deletion is shown in Figure 4A where there is a complete deletion in EDL and Sart after the 5th flexor burst accompanied by sustained activity in extensors (LG, MG, Plant). An example of a flexor deletion in another preparation is shown in Figure 11C (right panel). In this case, the TA burst in cycle number 20 was partially deleted. In the same cycle the GS burst was also reduced. This example was unusual in that there was no sustained activity in GS during the TA partial deletion. Rather, there was a reduced extensor burst and a reduced flexor burst in the same cycle. Finally, flexor deletions were also observed during rhythmic flexor activity (data not shown). In these episodes of MLR stimulation, the only extensor-like activity was recorded in Quad. During deletions of flexor-like ENG activity (TA, EDL, PerL) the Quad ENG showed modulated but sustained activity (not shown). In summary, during fictive locomotion, deletions occur in agonists throughout the limb, are usually accompanied by sustained activity in the antagonists, and occur in both flexors and extensors.

Similar features are present in the deletions occurring during fictive scratch. In Figure 5A, five consecutive rhythmic extensor bursts are followed by a period of silence in ankle extensor MG ENG. During this silent period (a complete deletion of MG

ENG activity), the hip extensor SmAB burst is greatly reduced (partial deletion). Note that PBSt was active with extensors in this preparation and that its burst is also attenuated during the deletion of hip and extensor activities. Ankle flexor activity (TA) activity persists (i.e. does not return to baseline) during this deletion. Figure 5B shows another preparation in which a deletion of extensor-like activity (SmAB, PBSt and PerL) occurs and in which flexor activity (TA) is maintained. Again, the quiescent period occurs after several cycles of rhythmic activity and is followed by cycles of rhythmic activity. In Figure 5 the deletions occurred without any gradual decrease in ENG burst amplitude in the cycles immediately preceding the deletion. In Figure 5B, the flexor-like FDHL activity is modulated but does not return to baseline during the extensor deletion. In Figure 5A, the peak of FDHL activity was in phase with extensors and not obviously altered during the extensor deletion. Overall, the examples in Figure 5 show that deletions during fictive scratch also engage synergists operating at different joints, are accompanied by sustained activity in the antagonists, and can be graded. These features were observed in all episodes of fictive scratch studied. The cycle during fictive scratch is dominated by flexion (see Fig. 2, 3A right panel, 5 and 6 for examples) with the average flexor over extensor burst ratio of 3.9 as stated earlier. During fictive scratch in these preparations, deletions occurred exclusively in extensors.

Summarizing for both fictive behaviours, deletions engaged synergists throughout the limb. Considering only motor pools with stable activity either during the flexor phase or the extensor phase but not both (flexors Sart, TA, EDL and extensors SmAB, LGS, MG, Plant), 92% (45/50) of deletions of ankle motoneuron activity were accompanied by deletions of hip motoneuron activity. Similarly, when there was a deletion of hip motoneuron activity there was usually (88%, 45/52) one of ankle motoneuron activity. Overall, deletions occurring in both distal and proximal motoneuron pools accounted for 88% (70 out of 80 deletions) of all deletions observed. This suggests that the deletion process is a parallel failure to excite distal and proximal motoneuron pools.

Exceptions to the parallel failure of excitation of distal and proximal motor pools were observed in two episodes (4 deletions out of 80). These examples are shown in Figure 6. Figure 6A shows an example during fictive locomotion, where the hip

extensor SmAB burst is almost completely absent (left panel, 3rd burst and right panel 2nd and 3rd bursts) while ankle extensor activity (LG, MG) is not affected at all. Figure 6B shows the example during fictive scratch in which there was a deletion of activity in motoneurons acting at a single joint. It shows 12 cycles from an 8 second episode of fictive scratch. Several deletions of extensor bursts can be observed in this segment. The 5th extensor burst is missing in all recorded extensors (SmAB, LG, MG). The SmAB bursts do reappear and get larger as the behaviour proceeds. By the 12th cycle, SmAB reaches normal levels of activity while bursts are still absent in LG and of very small amplitude in MG. Therefore, at the end of this episode of scratch, ankle extensor nerve activity was missing while hip extensor nerve activity remained robust. This shows that the amount of depolarization sufficient to evoke spiking in different extensors somehow changed throughout the period of activity. The significance of this observation will be explored in the Discussion.

We mentioned earlier that the activity of PerL and PBSt is different in fictive scratch compared to fictive locomotion. In the same way, their activity or lack thereof during deletions is related to the phase of their activity during rhythmic behaviours. In figure 5B, the extensor-like bursts of PBSt and PerL are missing during deletions in SmAB. In Figure 4A Quad nerve activity was mostly extensor-like and, like other extensors, it remained active during flexor deletions.

Deletions were usually accompanied by sustained activity in antagonists. We did observe, however, one unusual case which is shown in Figure 7. In this example, there are 5 consecutive deletions of extensor activity (hip extensor SmAB and ankle extensors MG and LGS), the first ones being complete and the last partial. During these particular deletions, unlike in other examples, the activity in flexor ENGs remained rhythmic without any visible difference from the activity before and after the deletions. Moreover, an intracellular recording from a flexor (EDL) motoneuron did not show any changes in its LDP when extensor activation failed.

Deletions are not necessarily accompanied by changes in the timing of the rhythmic activity

An important feature of the deletions described in this thesis is that, when the burst amplitude is reduced or bursts are absent, in many cases the timing of subsequent bursts timing is not disturbed. Thus bursts immediately following a deletion can reappear at an interval at which they would have been expected had there not been any deletion. Consider Figure 4A. The average cycle length in the 5 cycles preceding the deletion was 830 ± 86 ms (n=5 cycles) measured from consecutive onsets of EDL ENG activity. On the bar shown above the ENG traces, vertical tick marks are placed at intervals of 830 ms for the duration of the deletion of flexor activity. The bar is aligned at the left on the onset of EDL ENG activity during the 5th burst. Note that the onset of the 6th burst in EDL and Sart occurs at the 4th vertical tick mark. The onset of the 7th burst in EDL and Sart activity occurs close (76 ms) to 2 tick marks later. Furthermore, the dashed boxes, positioned where the sustained activity in SmAB and Plant decreased slightly, correspond well to the vertical tick marks. In Figure 4B the average cycle length in the 5 cycles preceding the deletion was 718 ± 66 ms (GS ENG, n=5 cycles) and the vertical tick marks on the horizontal bar are placed at intervals of 718 ms. The 3rd burst in SmAB, GS and Plant occurs not long after (107 ms) the 4th tick mark. The boxes indicating periods of decreased activity in PerL occur either at (left box) or not long after (right box) the 2nd and 3rd tick mark respectively. In summary, the data in Figure 4 suggests that the timing of rhythmic activity may be maintained although the activity is completely absent in several motor pools for integer multiples of the cycle period.

Figure 7 shows another example during fictive locomotion where the timing of rhythmic activity seems to be maintained. The distance between the tick marks corresponds to 772 ms and the activity resumes in extensors (6th burst) 77 ms after the 6th tick mark.

Further support for the hypothesis that deletions can affect motoneuron recruitment while the cycle period is maintained can be found during fictive scratch. Consider Figure 5A where there is a deletion after 5 rhythmic bursts of extensor activity. The average cycle length for the five cycles preceding the deletion was 196 ± 19 ms

(SmAB ENG, n=5 cycles) and the 6th MG and SmAB ENG bursts occur exactly at the 3rd tick mark. Similarly in Figure 5B the average cycle length was 200 ± 19 ms (SmAB ENG, n=5 cycles) and the 8th SmAB ENG burst occurred just 12 ms after the 4th tick mark. Again in Figure 7, the average cycle length before the deletions was 772 ± 50 ms (SmAB ENG, n=5 cycles) and the 7th burst in the SmAB ENG occurs shortly before (56 ms) to the 6th tick mark after 4 cycle periods without extensor activity.

The possibility that timing can be maintained during deletions is also illustrated in Figure 8 which was constructed from a 7.5-second bout of fictive scratch in which intracellular recordings were made from 2 LGS motoneurons and in which a deletion of extensor motoneuron activity occurred in 3 of the 37 fictive scratch cycles. The overlaid recordings in Figure 8 are 500 ms traces obtained as follows. Cycles were defined from the onsets of successive activity bursts in the PerL ENG. The extensor-like activity of PerL during scratch (See Figure 3) was reduced during the 3 extensor deletions but remained of sufficient amplitude to allow determination of the onset of activity during deletions. The activity in ankle flexor TA was sustained during the extensor deletions. The continuous records were then broken into 500 ms segments. Thirty-seven such segments are overlaid in Figure 8, centered on the onset of activity in PerL.. This presentation was chosen to illustrate and compare both ENGs and intracellular recordings during spontaneous deletions. Of the 37 cycles, 34 were normal (dotted lines) and 3 displayed a deletion of ENG extensor activity (solid lines) clearly visible in the ankle extensor LGS and the hip extensor SmAB ENGs. Traces were aligned to the onset of PerL activity (time zero, vertical dashed line). The timing of the burst following a deletion is consistent with the cycle lengths observed during normal activity. In 2 of the 3 extensor deletions, there was no LG or SmAB activity. During these same deletions both of the LG motoneurons impaled showed no depolarization. The third deletion consisted of small SmAB and LGS activities. It was accompanied by the depolarization of both LG motoneurons and a single action potential. Note that the timing of the post-deletion burst (i.e. the one to the right side of Figure 8) falls within a few milliseconds of the bursts occurring without deletions (dotted traces).

The locomotor drive potential of motoneurons is reduced during deletions

Deletions of ENG activity are the absence of firing in the corresponding motoneuron pool. Does this absence arise from a small decrease in membrane depolarization to just subthreshold levels or from a complete failure to depolarize during rhythmic behaviour? This can only be answered by examining intracellular recordings of motoneurons during deletions.

Figure 8 shows intracellular recordings made simultaneously from 2 LG motoneurons (bottom 2 traces). During normal cycles (dotted), both motoneurons were rhythmically depolarized (scratch drive potential (SDP) approximately 10 mV) and fired 2 to 5 action potentials per cycle. During deletions however, the SDP and number of action potentials were reduced. Note that in both motoneurons, the depolarizing SDP is absent during 2 deletions and reduced in the third where it produces only a single action potential in each motoneuron.

The three deleted cycles of Figure 8 are plotted again in Figure 9. Of these three cycles, two show a complete deletion of the ankle and hip extensor bursts (solid and dashed lines) while the third shows a partial deletion (dotted line). During the two complete deletions, the ENG activity in LG and SmAB nerves is completely abolished and the activity in PerL is greatly reduced. In these cases, the SDP was totally abolished in both motoneurons. In contrast, during the partial deletion, the activity is reduced in LG and SmAB and unaltered in PerL. In this case, the SDP of the motoneurons was reduced compared to normal cycles (Figure 7, dotted traces) but not completely abolished. Motoneurons fired a single action potential in this cycle. In all 3 cases, the behaviour of both motoneurons was similar (i.e. no SDP in complete deletions in either motoneuron and smaller SDP in both motoneurons during partial deletions). The fact that the effects are similar in this sample of two motoneurons within the pool and in two other pairs (see Figures 11 and 12 for the other two examples) suggests that the changes that lead to deletions of ENG activity affect the entire motor pool in a qualitatively homogeneous way. In this example, the underlying event during two deletions was a complete failure to depolarize and in the other a partial failure

Figures 11 and 12 show measurements of the drive potentials in pairs of motoneurons recorded during periods of fictive locomotion (Figure 11) and scratch (Figure 12) where deletions occurred. In both figures, the ENG corresponding to the intracellular recordings was first examined to determine which cycles had a deletion (The EDL ENG (not shown) was used for Figure 11A and the TA ENG (shown in Figure 11C) was used for Figure 11B). In the two pairs where the intracellular recordings were from different motor pools (Figure 12B and 12D) the MG ENG was used to assess deletions since, in both cases, both intracellular recordings were from motoneurons active during extension. LDP amplitudes were measured as the difference between the highest and lowest voltages during each cycle. Within each period of activity, LDP measurements were normalized to the average LDP amplitude without deletions and then plotted as a percentage of the average against the step cycle number. In cases where a motoneuron was firing during the active phase, the highest voltage value for LDP measurements (i.e. 100%) was the voltage just subthreshold for firing. Examples of how these 100% values were determined are shown in Figure 11C for cycles 1 and 19 as the height of the shaded rectangles. The 100% value is plotted as a solid line in Figures 11 and 12 where the dotted horizontal lines indicate one standard deviation above and below the mean.

Figure 11A shows the measured LDP amplitudes in an EDL motoneuron during an episode of fictive locomotion (nerve recordings not shown). The average LDP (i.e. the 100% value) during control cycles (i.e. without deletion) in that episode was 4.6 mV. The LDP was clearly smaller during the 3 deletions (filled circles) than during cycles which did not have deletions (open circles). Figure 11B shows LDP measurements in a pair of simultaneously recorded TA motoneurons. Filled diamonds correspond to cycles in which there was a GS ENG deletion and will be discussed later. The filled triangle indicates a cycle where there was a partial deletion of both the TA ENG and of the GS ENG (Figure 11C, right panel, cycle 20). During that deletion (filled triangle) both TA LDPs were reduced compared to normal cycles (open circles). Figure 11C shows the ENGs and intracellular recordings corresponding to the data in panel B. Cycle numbers are shown above the traces. Cycles were defined from the termination of GS bursts and their boundaries are indicated by the dashed vertical lines. The right panel of Figure 11C shows the period (cycle 20) in which there was a TA deletion (filled triangle)

and the decreased depolarization of both motoneurons (bottom 2 traces). In addition to a deletion of TA activity, cycle 20 also had a deletion of GS activity that will be discussed below. Note that this example is unusual in that there is both a deletion of flexor and extensor ENG activity and also in that the cycle during which this deletion occurs appears to be shorter than the other cycles. More commonly, deletions in one group of ENGs are accompanied by sustained activity in the other group (e.g. 11C right panel). Overall, the average LDP during all deletions observed in fictive locomotion was reduced to 34 ± 14 % of the average value during control cycles (n=4 motoneurons, 3 episodes, 2 animals).

Figure 12 shows further examples of SDP changes during deletions in fictive scratch. In panel A, a pair of PBSt motoneurons was recorded from during two episodes of fictive scratch (trial 1 and trial 2). In both trials, SDP amplitudes were reduced in both motoneurons during deletions in the PBSt nerve. Panel E also shows another PBSt motoneuron in which the SDP was reduced during cycles where deletions in the homonymous ENG occurred. In Figure 12B, during MG deletions, the SDP of the MG motoneuron was reduced but not that of the PerL motoneuron recorded at the same time. Although the PerL ENG was active with extensors, it remained rhythmically active during MG deletions (not shown) and the amplitude of the PerL motoneuron SDP was maintained (see Discussion). Panels C and D show more complex relationships between changes in SDP amplitude and deletions in ENG activity. Panel C shows measurements from a pair of LG motoneurons during a period of fictive scratch where there were 4 deletions (Same pair as in Figure 8 but different episode of fictive scratch). The SDP was clearly reduced in the first and last deletions. In the other two deletions (cyles 28 and 29), the SDP was either decreased (cycle 29 in bottom graph), in the lower end of the normal range (cycles 28 and 29, top graph) or normal (cycle 28 in bottom graph). This is possible since these deletions were partial deletions. Figure 12D shows another pair of extensor motoneurons. As observed in Figure 12C, the SDP was decreased during complete deletions (cycles 1, 25, 59-63) and within the normal range during partial deletions (cycles 2 and 26). In summary, Figure 12 shows that in fictive scratch, as in fictive locomotion, deletions are often accompanied by a reduction in the SDP. Overall, the average SDP during fictive scratch was reduced to 30 ± 25 % of the average value during

control cycles (13 motoneurons, 8 episodes, 3 animals). Figure 12 also shows that SDP measurements from individual motoneurons are not necessarily sensitive predictors of changes in the ENG because ENG activity reflects the recruitment of the motoneuron pool as a whole.

Antagonist pools show sustained activity during deletions and their motoneurons show a reduced hyperpolarization

It was shown in Figures 4, 5 and 8 that, during deletions, antagonist ENGs often display sustained activity without the phasic quiescent periods that normally occur. Figure 11B and C show recordings of a pair of TA motoneurons where their LDPs could be examined during both deletions of an agonist ENG (TA, triangle) and of an antagonist ENG (GS, diamonds). As mentioned in the previous section, the depolarized phase of the LDP was reduced during deletions the TA ENG (Figure 11C, right panel, cycle 20, filled dot). During deletions in the antagonist (cycles 2,3,4) the TA motoneurons maintained their depolarization but failed to hyperpolarize. There was a reduction of the hyperpolarized phase during all reduced or absent extensor (GS, Plant) bursts (cycles 2, 3, 20). The potential of the cell during the third cycle fluctuated very little because there was no hyperpolarizing phase at all. In summary, the decreased LDP values plotted during deletions of an antagonist ENG are due to a smaller hyperpolarized phase.

The reciprocal effect of failure to activate agonists accompanied by a failure to inactivate antagonists is illustrated in Figure 10 in a pair of antagonist motoneurons recorded during fictive scratch. The SDP of the flexor-like PBSt motoneuron has a complex shape. The membrane potential begins to depolarize in the middle of extension, peaks at the end of extension, decreases at the beginning of flexion during which it maintains its level to finally decrease to the most hyperpolarized level by early extension. Therefore, during extension, this motoneuron is in the hyperpolarized phase of the SDP. This example shows a missing burst of activity (burst number 2) in extensors (SmAB, MG, FDHL). As expected, the extensor (SmAB) motoneuron fails to completely depolarize during the deletion. Note the accompanying failure to hyperpolarize the PBSt motoneuron during this deletion (cycle 2).

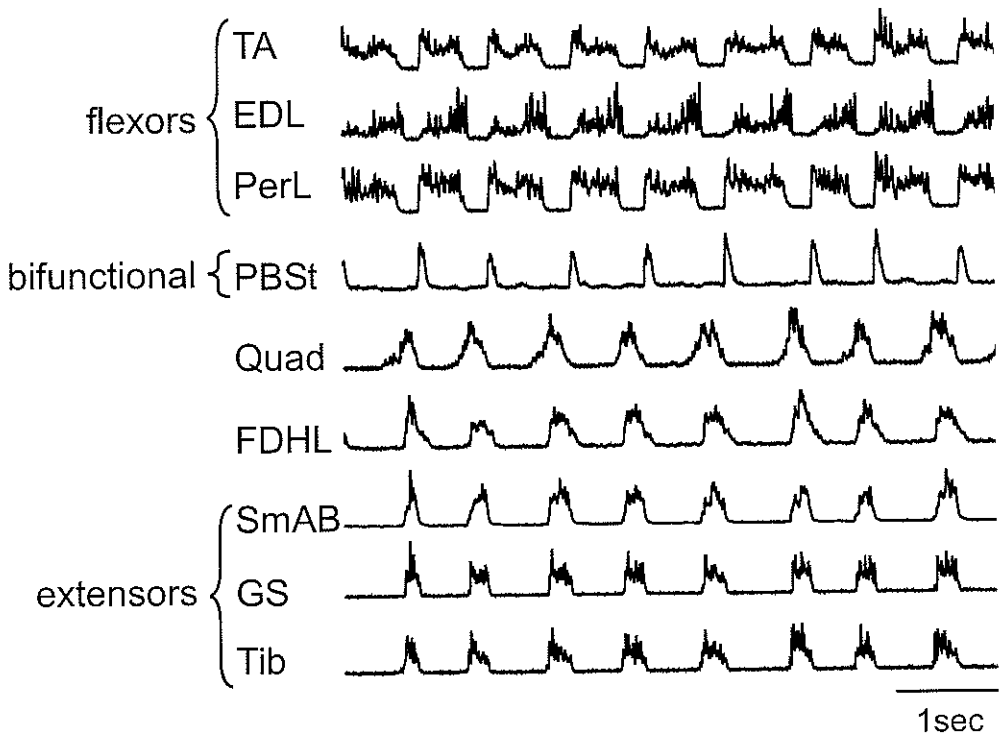

Figure 1. MLR-evoked fictive locomotion
This represents 8 seconds of a 21 second period of stable rhythmic activity extracted from a 2 minute run of MLR stimulation. This particular segment of 8 seconds occurred immediately after the MLR stimulation was stopped. The rhythm is robust and the alternation of flexor and extensor activity is clear. The recordings are grouped according to the extensor-like or flexor-like characteristics of their activity. Pbst is a bifunctional muscle with actions at two joints. The Quad nerve contains both knee extensor branches (vasti) as well as a bifunctional knee extensor/hip flexor branch (RF). It is therefore active during both phases of the step cycle. FDHL and Tib nerves also contain several branches each but their combined activity is mostly in phase with extensor activity.

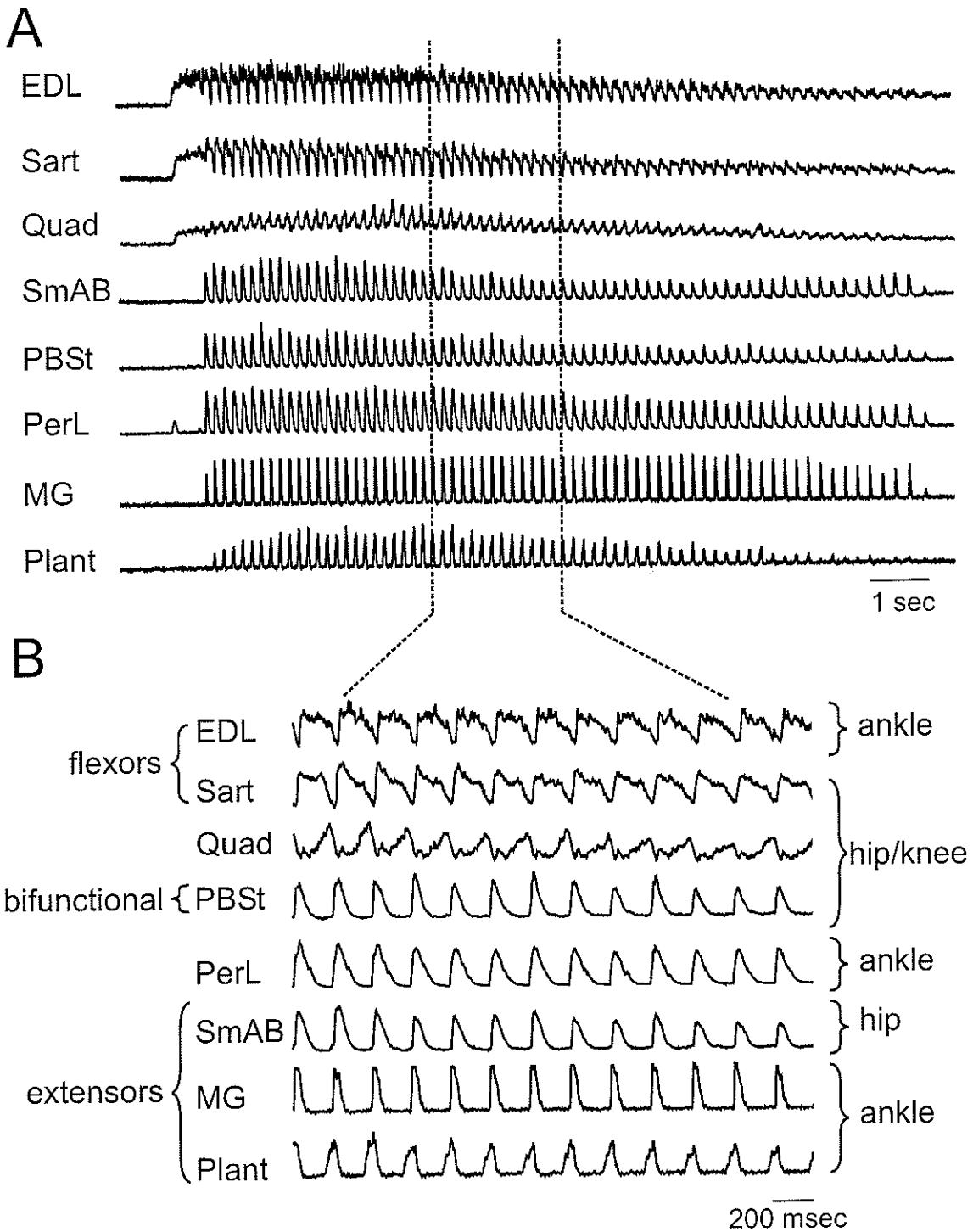

Figure 2. Fictive scratch. This is an example of fictive scratch activity. (A) One epoch of fictive scratch lasting about 14 seconds. An initial period of postural tonic flexion lasting about 500 ms is followed by alternation of rhythmic flexion and extension. (B) Magnified view of a section of the rhythmic activity shown in panel A. The alternation of flexors and extensors is very robust. Note also that the cycle length is shorter in fictive scratch than in fictive locomotion (Figure 1) and that the extensor bursts are very short in this behaviour.

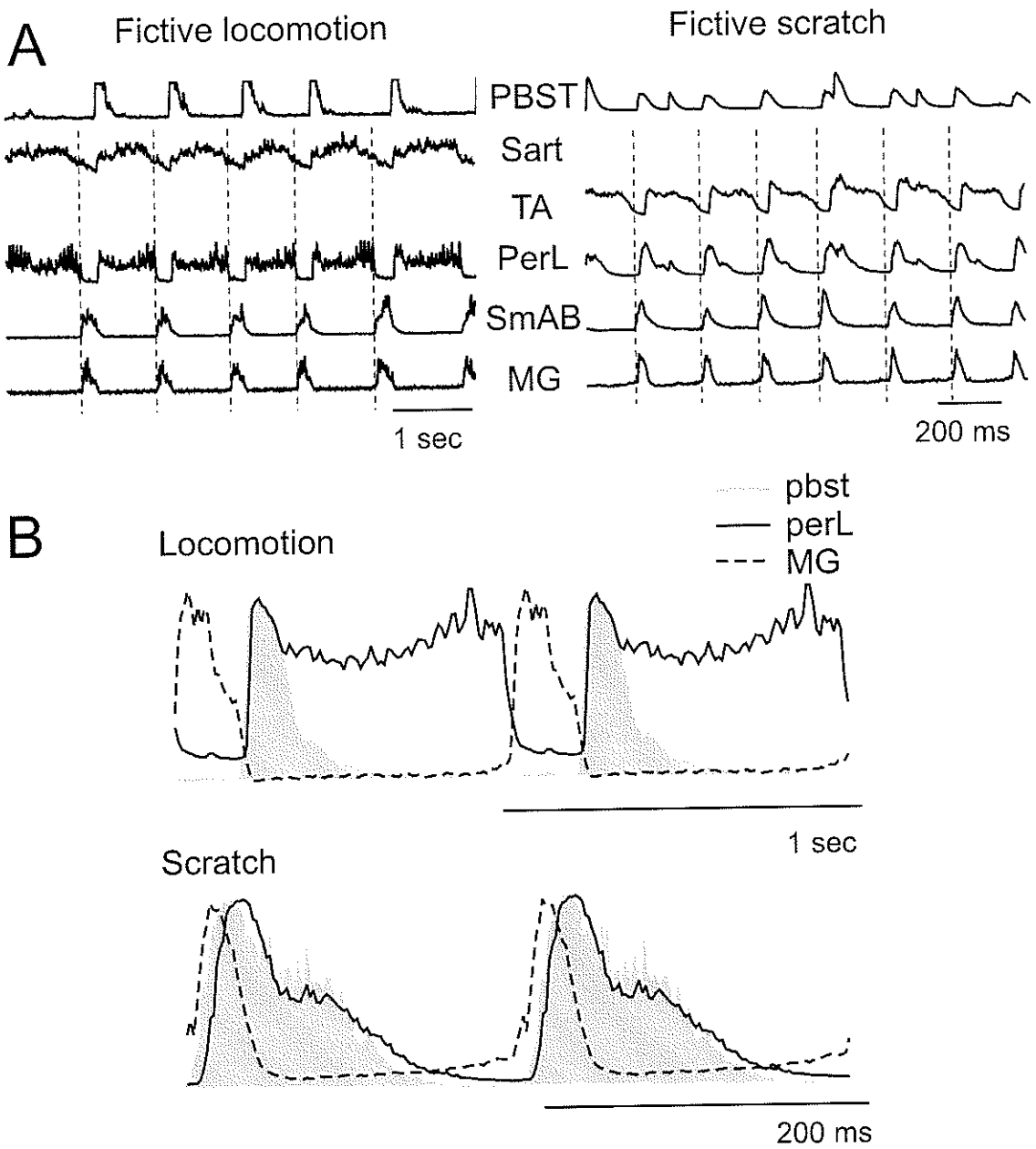

Figure 3. PerL nerve activity changes in different behaviours. (A) Nerve recordings during fictive locomotion and scratch in the same animal. The dashed lines indicate the onset of extensor activity. During fictive locomotion, PerL is active in phase with flexors such as Sart. During fictive scratch, PerL activity is more extensor-like although slightly delayed from the onset of pure extensor activity (MG). Moreover, PerL nerve activity continues during flexion. (B) Overlaid averaged nerve activity of PerL (solid line) and MG (dashed line) from the example shown in (A). Average activity in Pbst, PerL, and MG ENGs during 10 cycles of fictive locomotion and 11 of fictive scratch. PerL nerve activity is clearly out of phase with MG nerve activity during fictive locomotion and in-phase with MG nerve activity during fictive scratch. PerL activity is clearly out of phase with MG activity during fictive locomotion and in-phase with MG activity during fictive scratch. PerL activity is in fact very similar to the activity in Pbst.

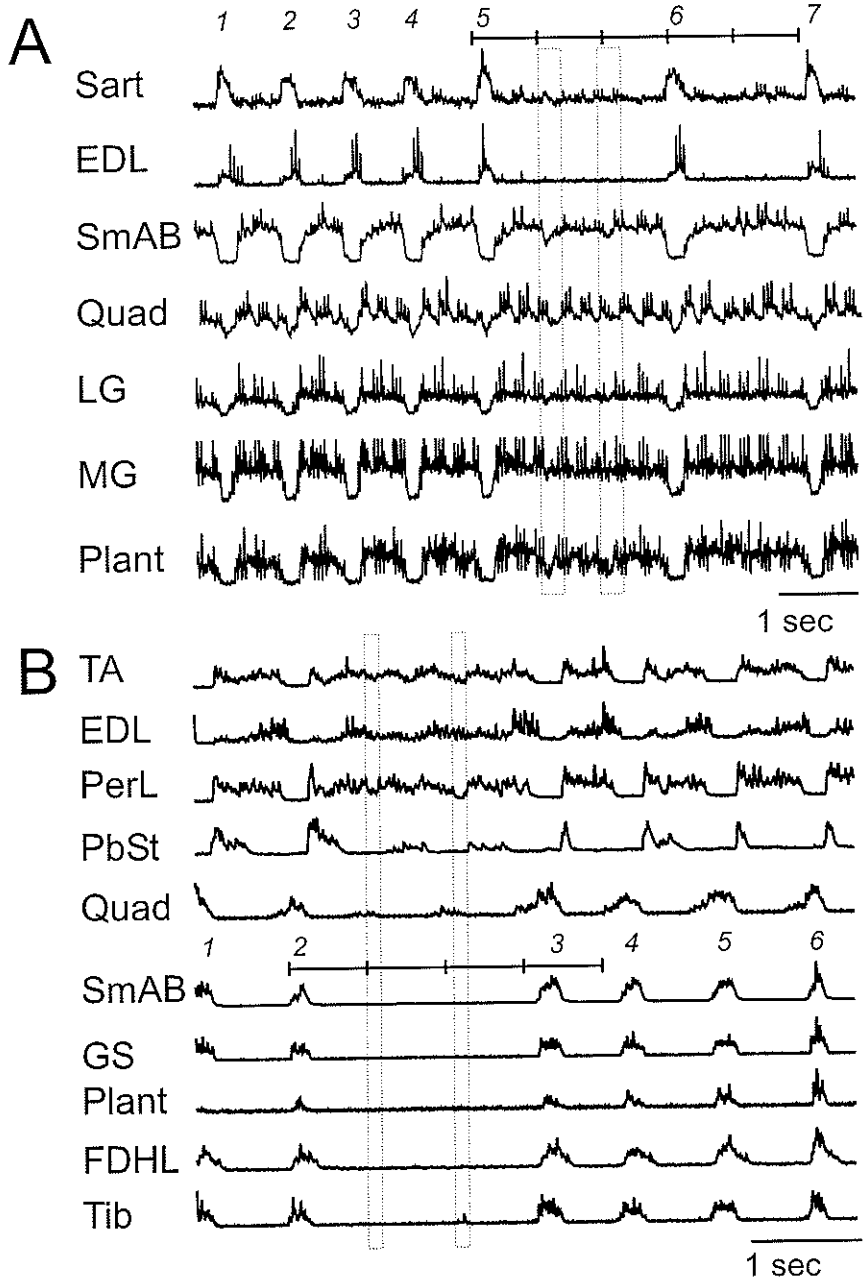

Figure 4. Examples of deletions during fictive locomotion Deletions can occur in either flexor or extensor nerve activity. (A) Two consecutive deletions of flexor activity following 5 rhythmic flexor bursts. Dotted boxes indicate the location of reduced activity in Plant and PBSt. The bursts are clearly missing in ankle (EDL) and hip (Sart) flexors. This is accompanied by sustained tonic activity in all extensors (LG, MG, Plant) as well as in PBSt and Quad. The tick marks on the horizontal bar are separated by a distance equivalent to the average cycle length in the 5 cycles preceding the deletions (830 ms). (B) Deletion of two consecutive ankle extensor bursts (GS, Plant, Fdhl, and Tib) accompanied by sustained activity in ankle flexors (PerL, TA, EDL). The hip extensor SmAB is also deleted while the bifunctional PBSt (hip extensor/knee flexor) shows ENG bursts of decreased magnitude. The dotted boxes are positioned just before the subtle extensor-like activity in Quad between the second and third extensor bursts. The distance between the tick marks on the horizontal bar was calculated as for panel A and is 718 ms. Note that in both panels the rhythmic bursts reappear very close to a tick mark (see Results and Discussion).

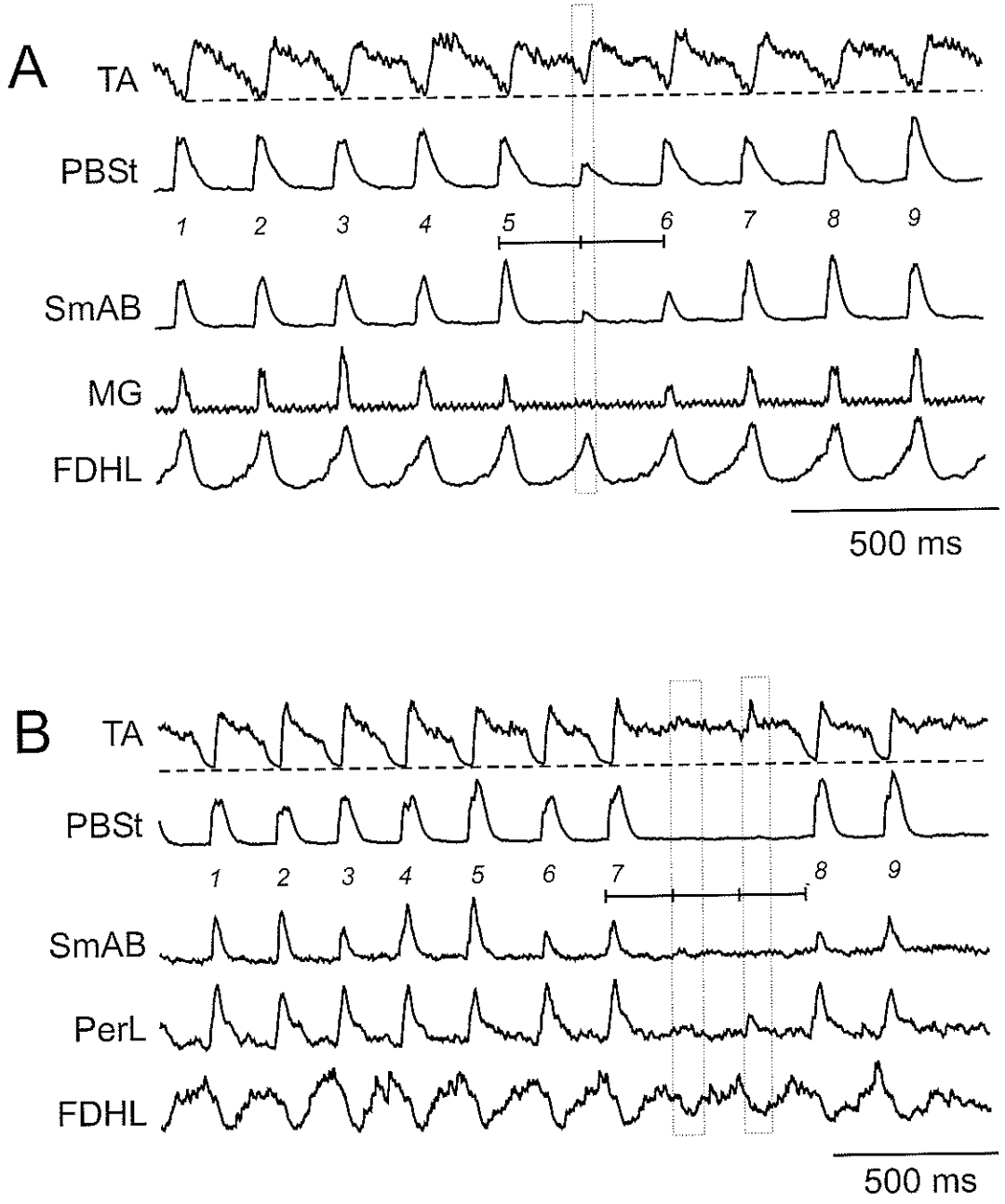

Figure 5. Deletion examples during fictive scratch. (A) Deletion of one extensor burst (MG) following 5 rhythmic bursts. This is accompanied by a partial return to baseline of the ankle flexor activity (TA) as well as by a reduction of the burst in the hip extensor (SMAB) and in PBST. The dotted box indicates the location of reduced activity in the TA ENG and the separation between the vertical tick marks on the horizontal was calculated as for Figure 4 (196 ms). (B) In another example from the same run as (A), the deletion of the extensor-like activity of PerL is accompanied by a complete absence of quiescence in the ankle flexor (TA). There is also a concurrent deletion in the hip extensor SMAB and in PBST. The dotted boxes indicate the reduction in the FDHL activity and the tick marks on the horizontal bar are 200 ms apart. Note that as was the case in Figure 4, in both panels, the rhythmic bursts reappear very close to a tick mark (see Results and Discussion).

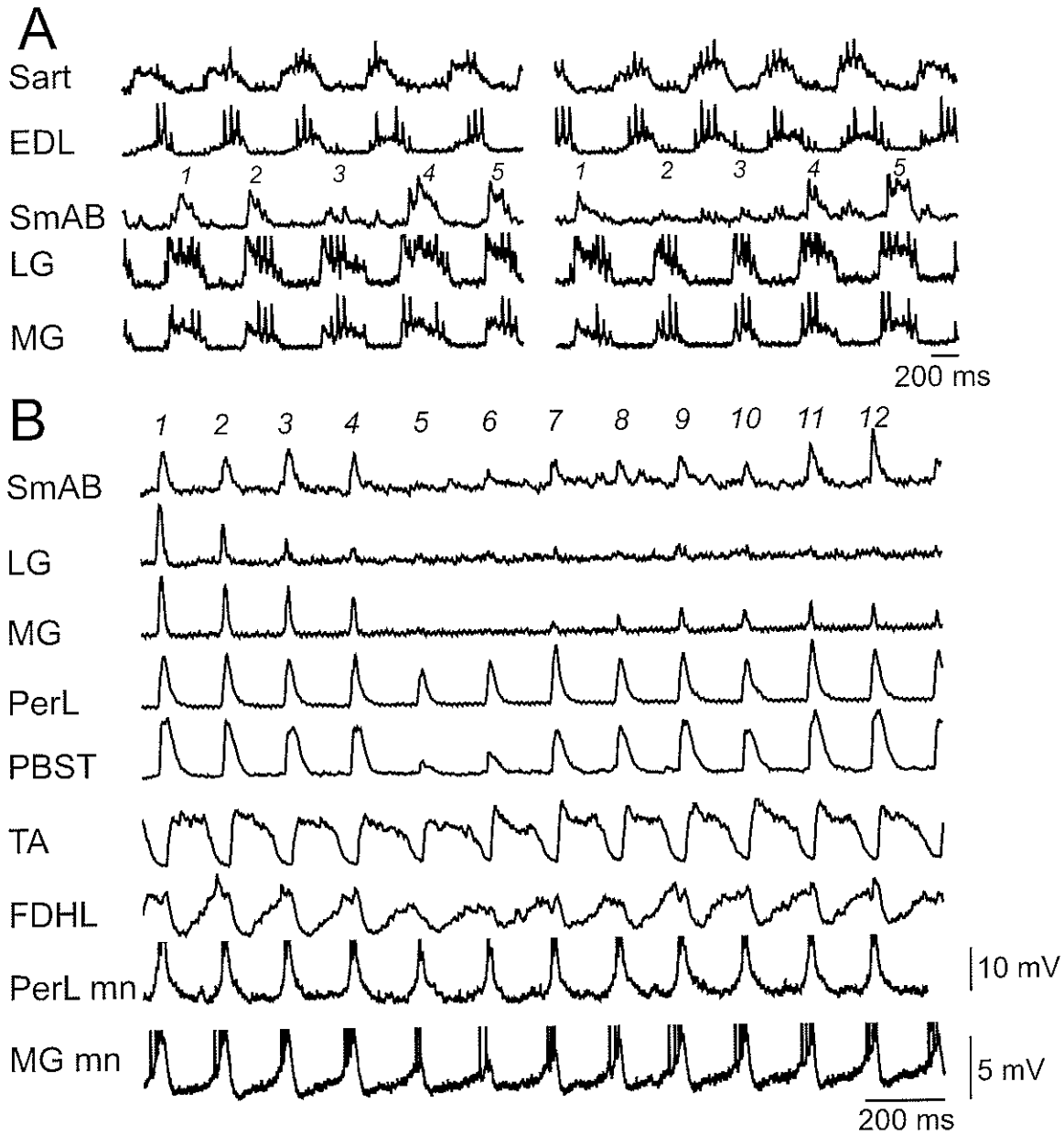

Figure 6. Uncoupling of synergists (A) ENG recordings during fictive locomotion showing deletions in the hip extensor nerve SmAB while other extensors (LG and MG) remain active. (B) Nerve and intracellular recordings during the last 2.6 seconds in an episode of fictive scratch. Action potentials were clipped in both intracellular recordings. Action potential height was approximately 25 mV for the MG motoneuron and 19 mV for the extensor motoneuron. Resting membrane potential was -78 mV for MG motoneuron and -39 mV for PerL motoneuron. Extensor activity decreases in LG during bursts 3 and 4 and completely stops in all extensors in bursts 5 and 6. Thereafter, MG activity slowly increases although never back to a full-size burst, LG activity remains absent and SMAB activity completely recovers by burst 12. During these fluctuations in the MG burst size, the SDP in an MG motoneuron remains remarkably consistent. In the other extensor motoneuron, the LDP is decreases during bursts 5 and 6 but are also relatively consistent in all other bursts.

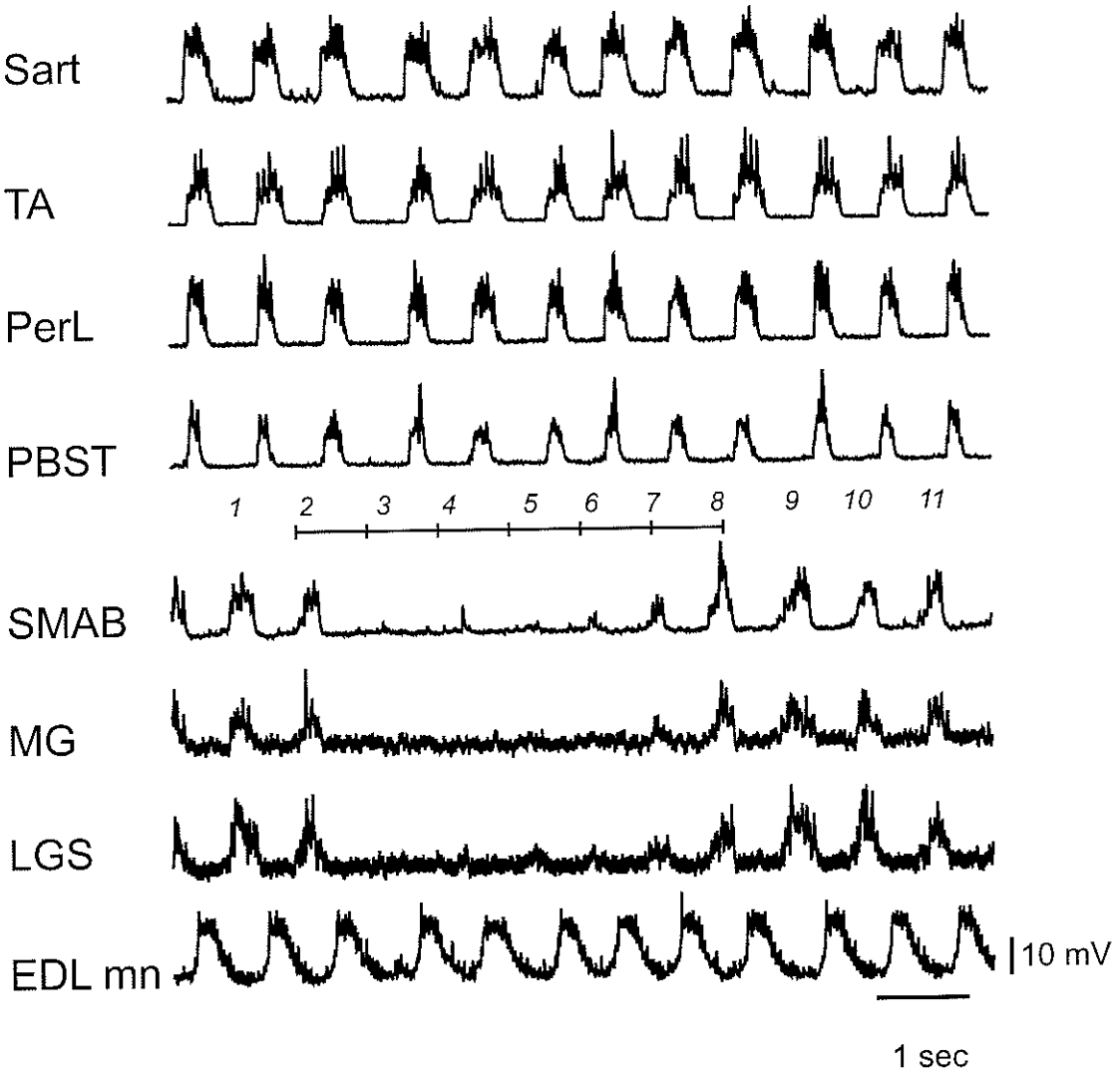

Figure 7. The flexor and extensor modules can be dissimilar. This shows an atypical example of deletions occurring during fictive locomotion. Five consecutive deletions occured during rhythmic activity. The tick marks on the horizontal bar are separated by 772 ms (average cycle length in the 5 cycles preceding the deletions. Bursts are deleted in extensors at all joints (ankle extensors MG and LGS, hip extensor SmAB). This is not accompanied by the usual tonic activtiy in flexors however. Instead, it is accompanied by perfectly rhythmic activity in flexors throughout the limb (ankle flexor PerL and TA, hip flexor Sart). Moreover, an intracellular recording from a flexor (EDL) motoneuron did not display any change in the LDP during deletions compared to normal cycles. Note that rhythmic activity resumes in SmAB (7^{th} burst) shortly after the 6^{th} tick mark.

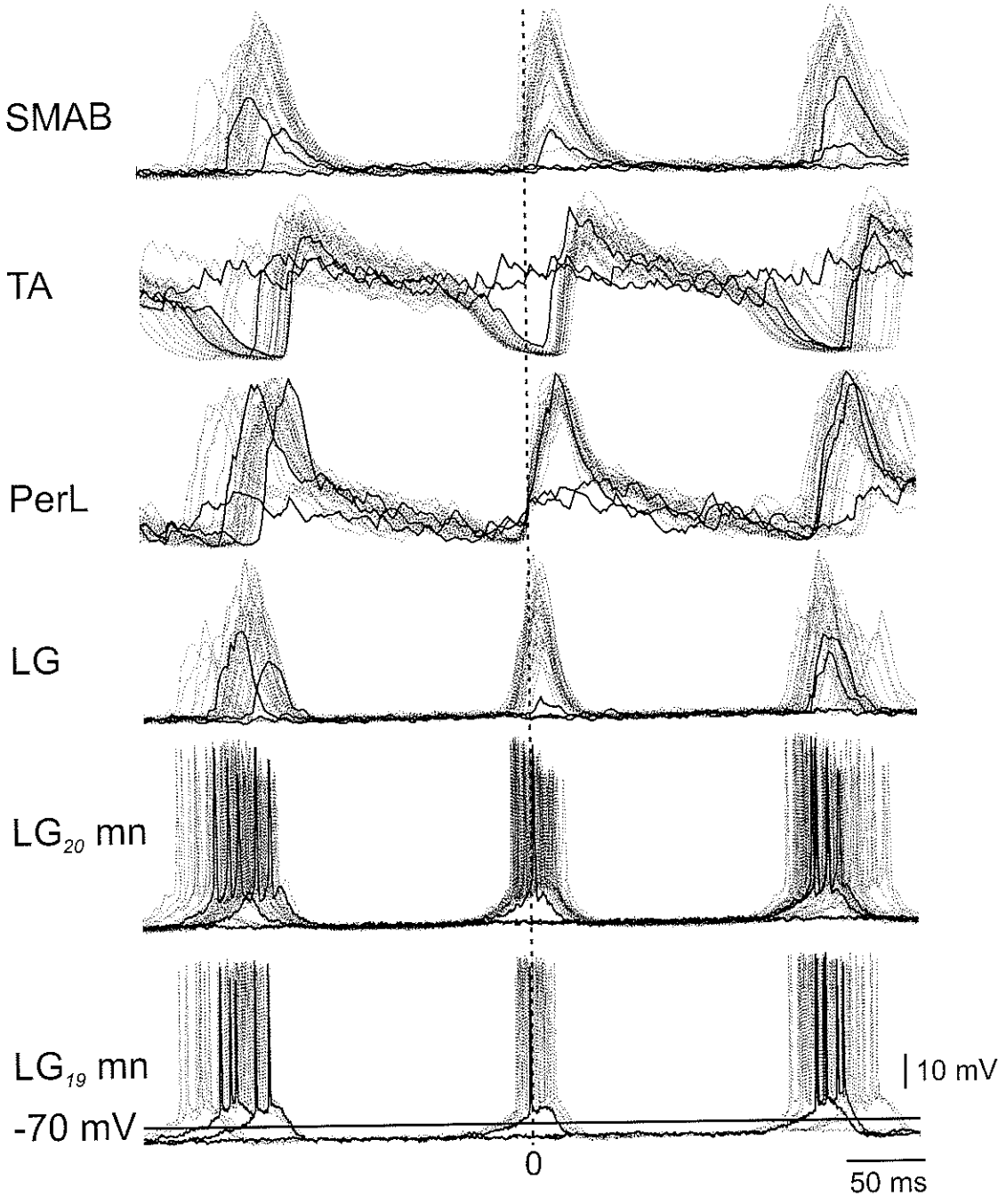

Figure 8. The timing of activity is maintained during deletions. Overlay of all the cycles of rhythmic activity in a single bout of fictive scratch. Each cycle is shown with its left and right neighbours. Normal cycles (dotted lines) are overlayed with deleted cycles (solid lines). The top 4 traces represent ENGs whereas the bottom 2 represent intracellular recordings from a pair of LG motoneurons. Deletions occured in extensors LG and SmAB while the activity was maintained (2 traces) or only partially reduced (1 trace) in the flexor nerve TA The SDP in both motoneurons is abolished or reduced during the deletion in the homonymous nerve.

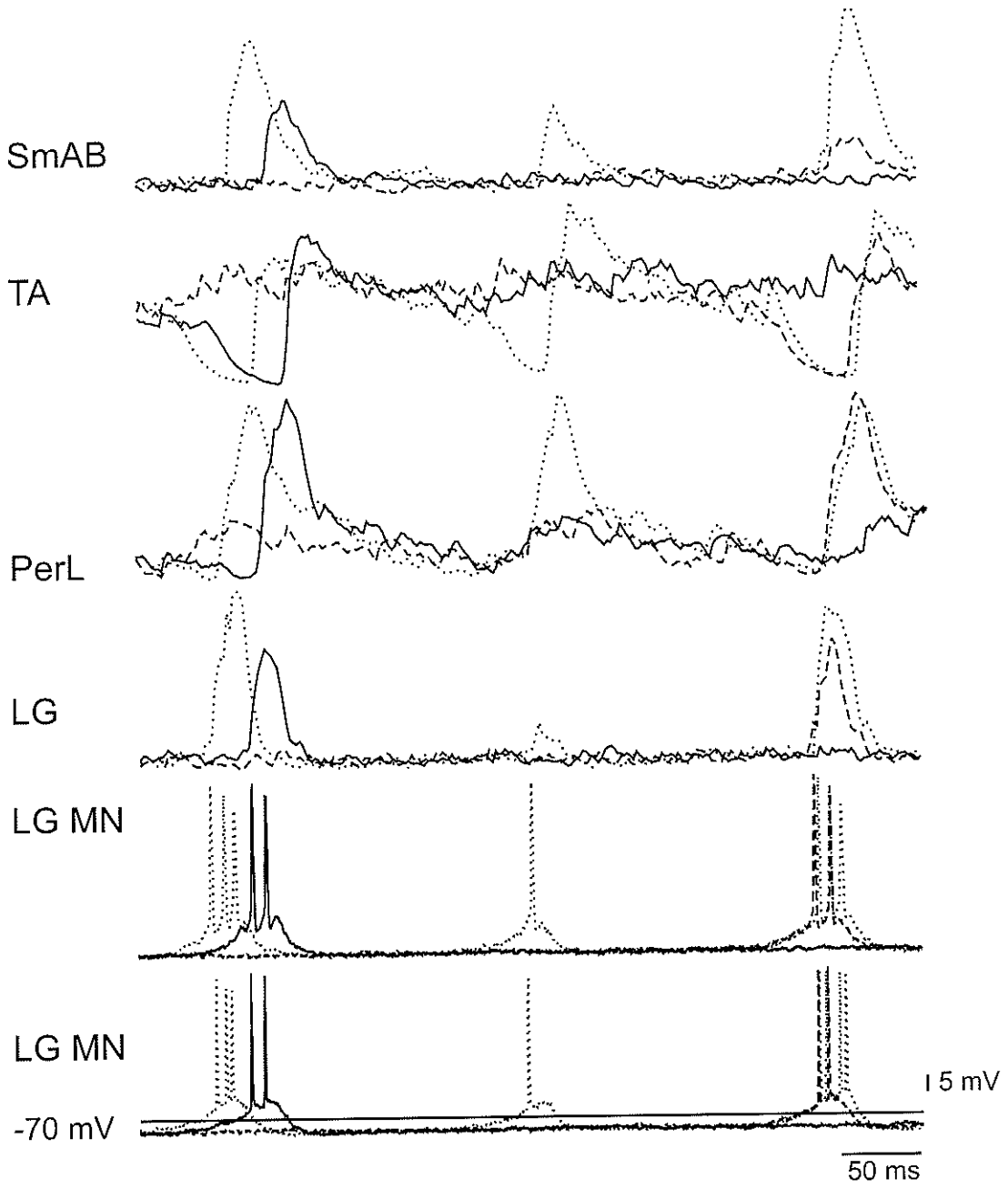

Figure 9. Deletions can be complete or partial. The 3 deleted cycles were extracted from the example of deletions during fictive scratch in Figure 8. Corresponding traces are identified by their line pattern. Two cycles showed a complete deletion (dashed and solid lines) and the third showed a partial deletion (dotted line). During complete deletions in LG, the TA nerve remained tonically active and did not display the expected silent period. At the same time, the LDP of LG motoneurons was completely abolished and no depolarization was observed. During the partial deletion of LG, the activity in the TA nerve did decrease but it was not abolished as is expected in the off-phase. Moreover, the depolarized phase of the LDP of the LG motoneurons was reduced during those cycles. Threshold was reached briefly, allowing a single spike.

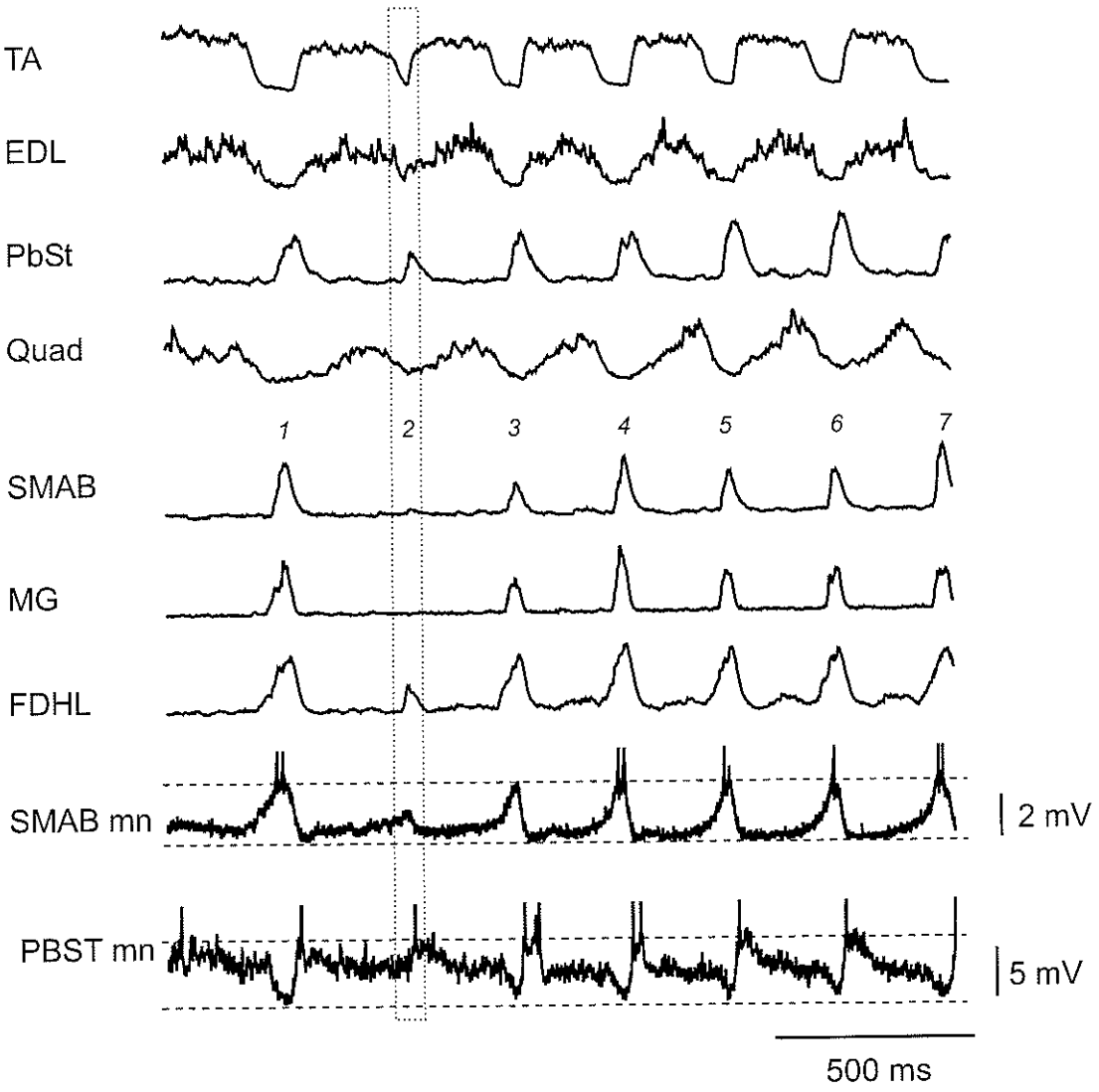

Figure 10. Antagonist motoneurons show reciprocal changes in the LDP during deletions. ENG recordings (top traces) and intracellular recordings (bottom two traces) of a pair of motoneurons active in opposite phases of the step cycle during fictive scratch. During the deletion of extensor activity (burst 2), the depolarizing phase of the LDP of the SmAB motoneuron is reduced as is the hyperpolarizing phase of the LDP in the flexor-linked motoneuron. The dashed horizontal lines show the depolarization and hyperpolarization observed in the normal cycles.

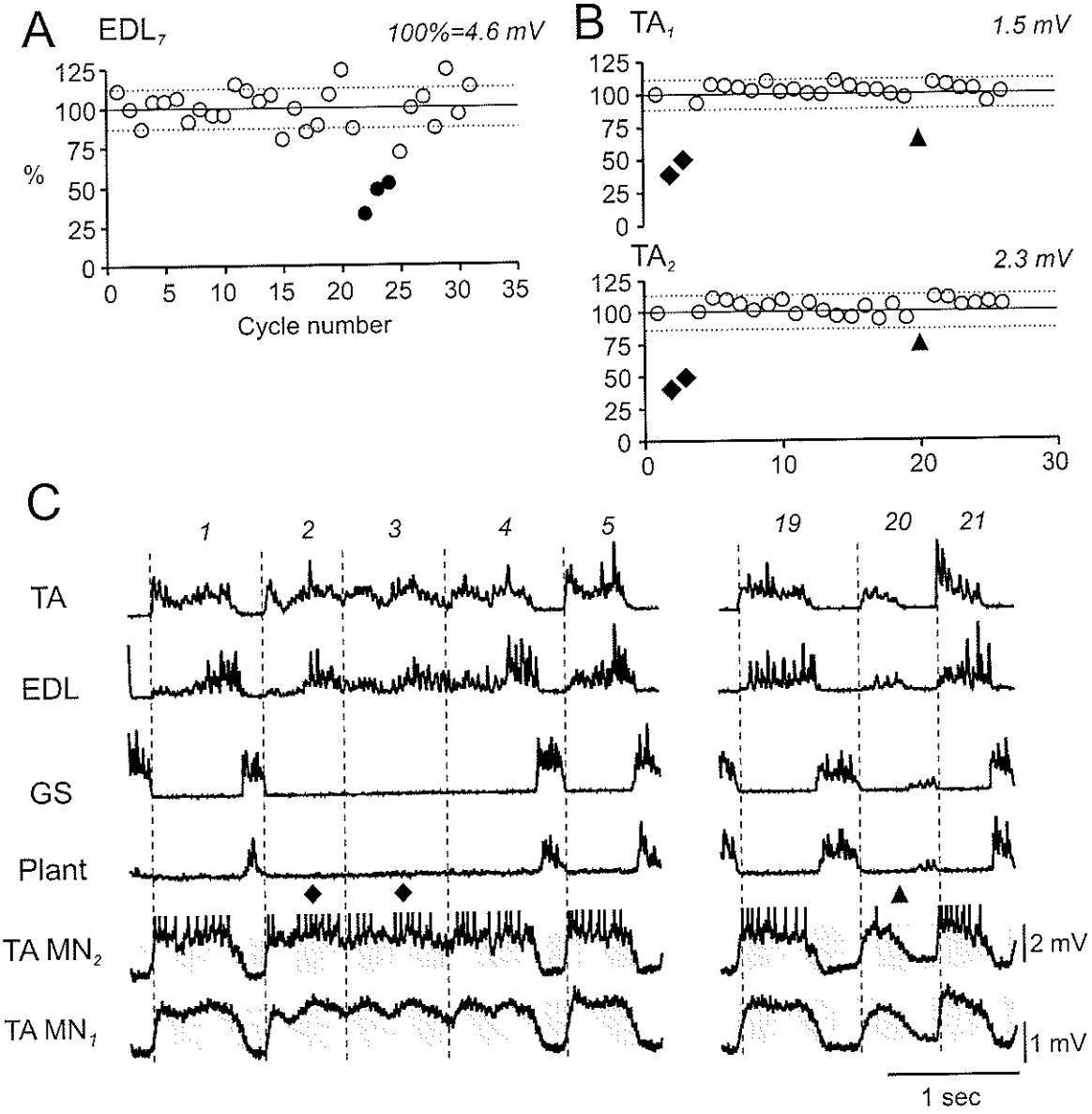

Figure 11. LDP measurements during episodes of fictive locomotion. (A-B) LDP measurements for each cycle, expressed as a percentage of the mean LDP during control cycles (average of open circles) measured for a given cell within a particular episode of fictive locomotion. (A) LDP measurements in an EDL motoneuron during an episode of fictive locomotion. Open circles denote normal cycles and filled circles denote cycles where the EDL ENG was deleted. In this example, the average LDP during control cycles was 4.6 mV. The LDP was reduced when the EDL ENG was deleted. (B) LDP measurements in a pair of TA motoneurons. Open circles denote normal cycles, filled diamonds denote cycles where the GS ENG was deleted and filled triangles denote cycles where both the TA and GS ENGs were deleted.. The LDP was reduced during the TA deletion and during one but not the other two MG deletions. (C) ENGs recorded during the same episode as in panel B with deleted cycles indicated with the same symbols. Cycle numbers are shown above traces. Shaded boxes overlaid on the intracellular recordings show the expected maximum and minimum of the LDP based on normal cycles.

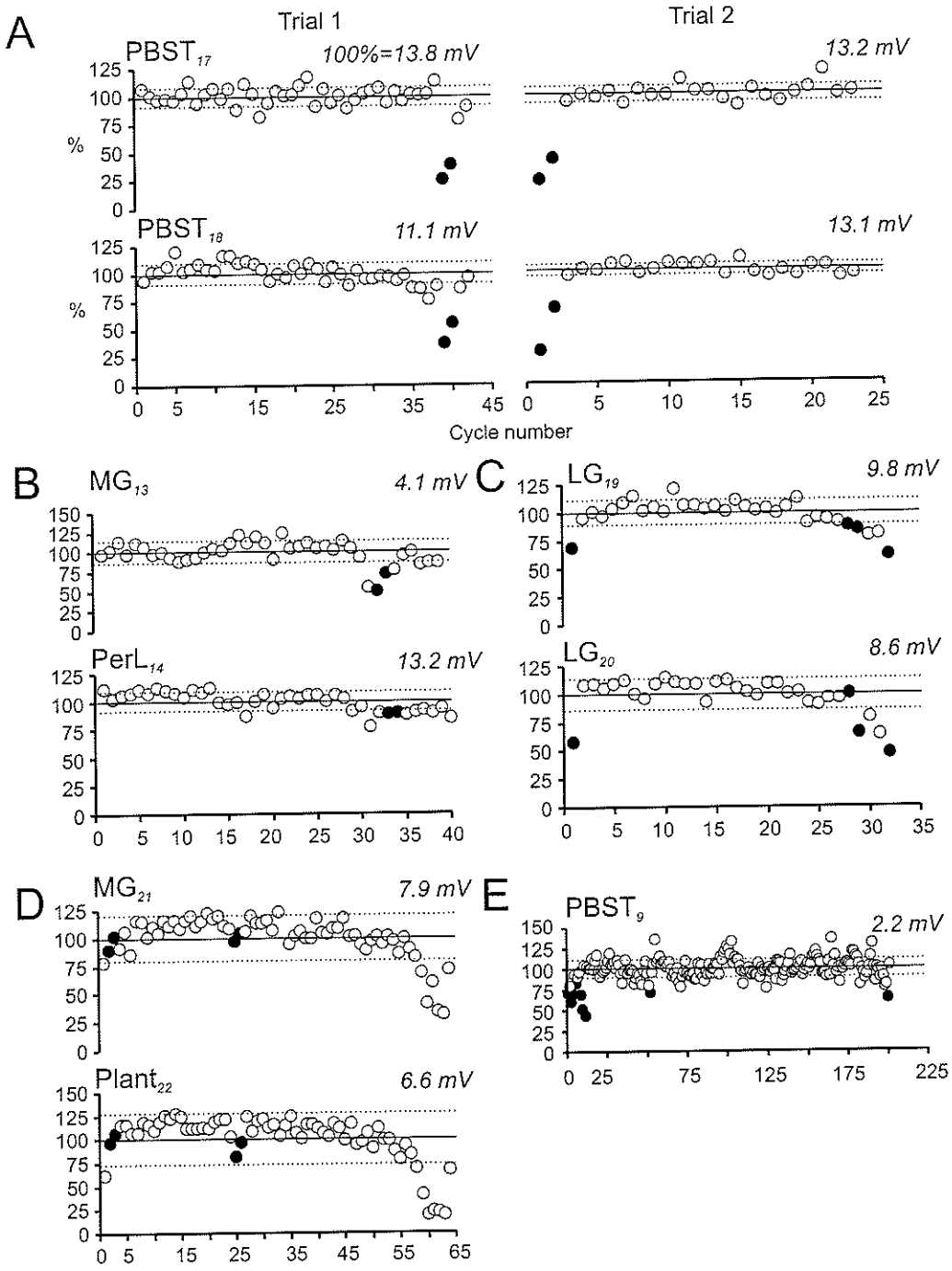

Figure 12. SDP measurements during episodes of fictive scratch. (A-E) The SDP was measured during episodes of fictive scratch in 9 motoneurons including (A-D) 4 paired recordings and (E) 1 single recording. The measurements were made as described in Figure 11. ENGs homonymous to the intracellular recordings were used to determine deletions. (A) SDP values during two separate episodes of fictive scratch where the same two PBST motoneurons were recorded from. (B, D) In two example where cells were from different motor pools (MG and PerL in panel B, MG and Plant in panel D), the MG ENG was used to determine deletions since the activity of both cells within each pair was in-phase with extensors.

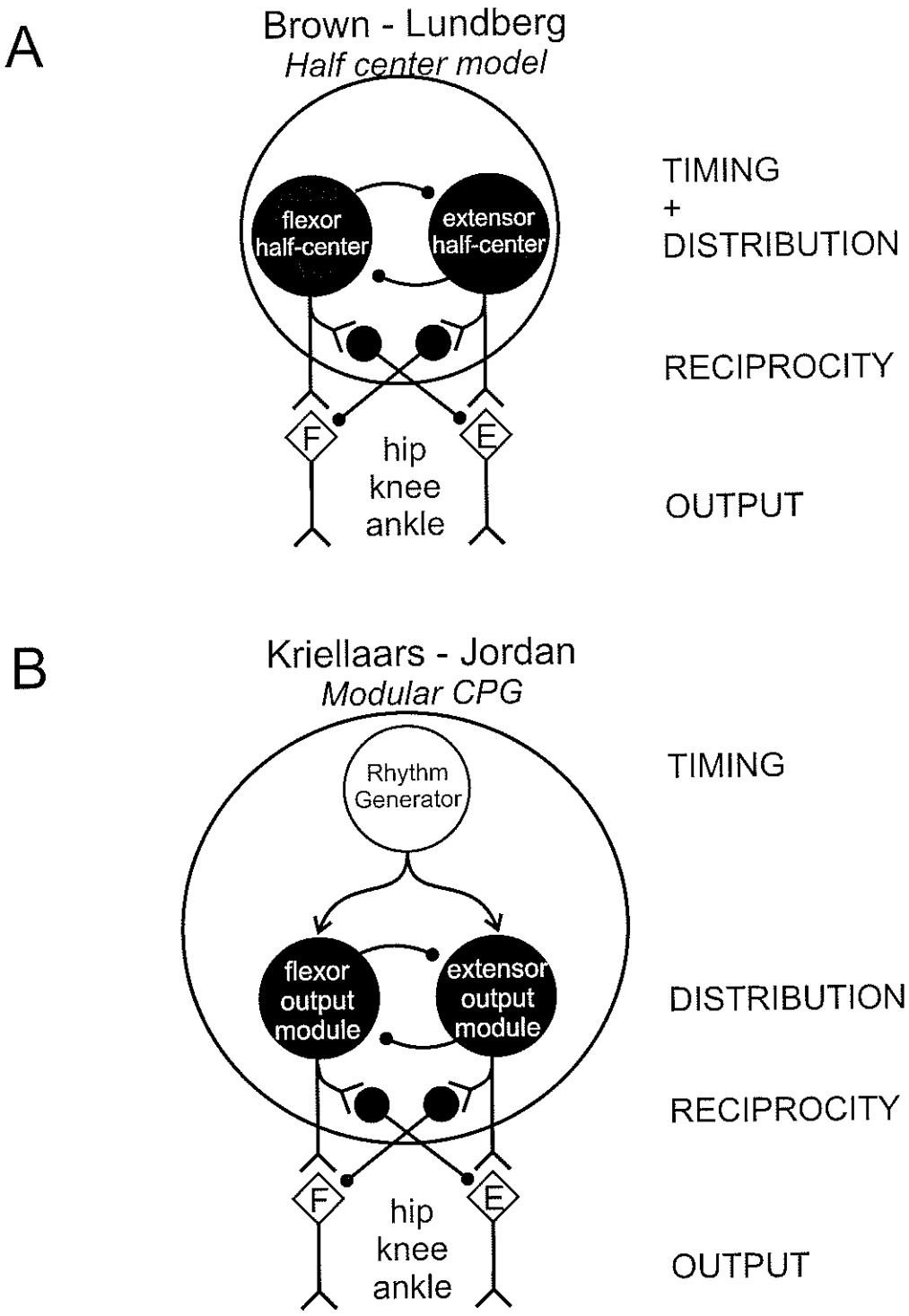

Figure 13. Models of CPG organization (A) The half-center model of Brown (1914) and Lundberg (reviewed 1980) consists of two modules which reciprocally inhibit each other. Each half-center is responsible for excitation of all agonist motoneuron pools in the hindlimb. (B) The modular CPG proposed by Kriellaars and Jordan separates the generation of timing from the distribution of excitation to motoneuron pools. For more details, see Discussion.

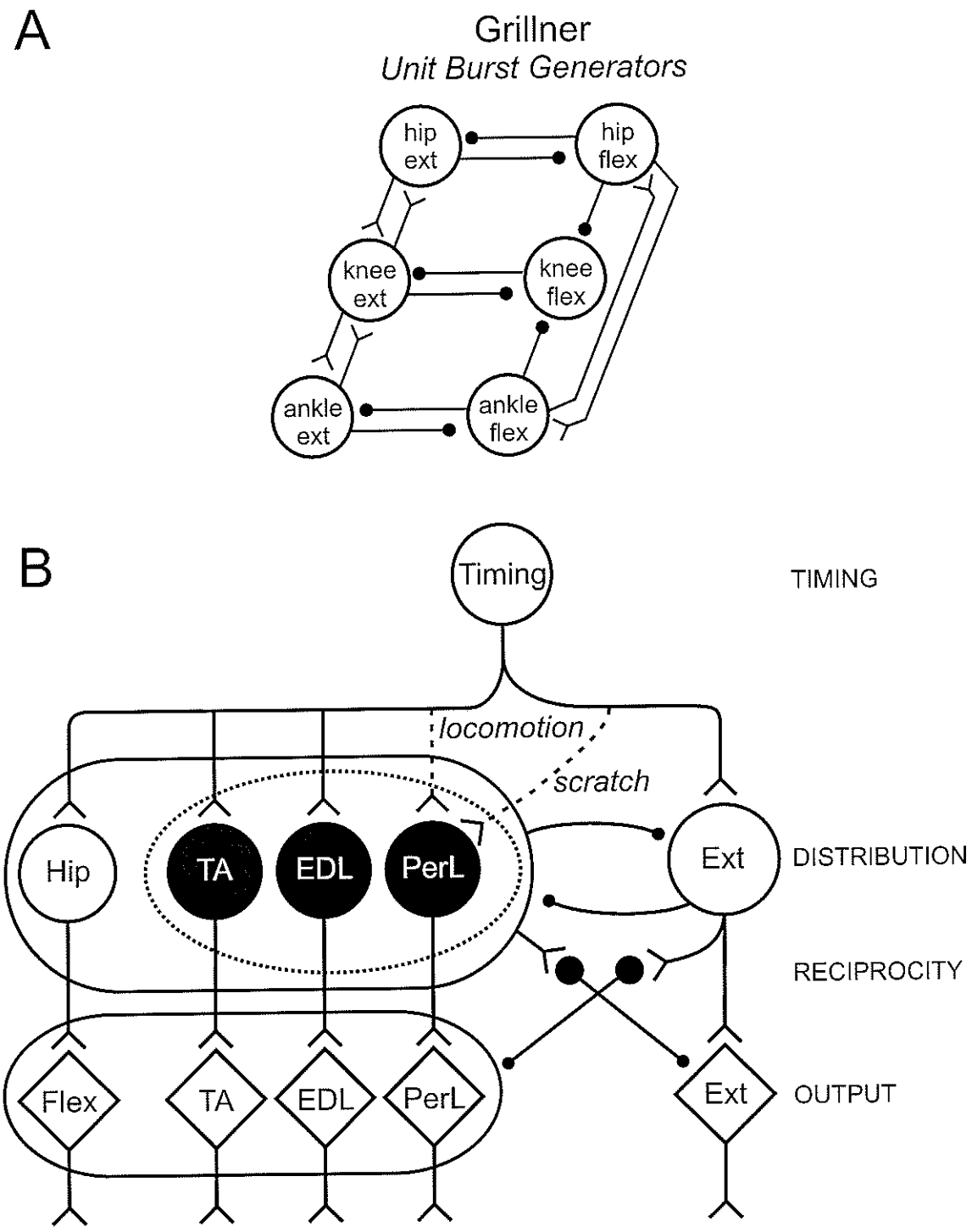

Figure 14. Organizations of the limb controller. (A) Grillner's Unit Burst Generator (UBG) model (1981) contains a module for each group of synergists at each joint. Coupling between the UBGs varies depending on the task. (B) Schematic drawing of our current understanding of the conceptual components of a CPG model which can account for the findings described in this study and elsewhere. For details see Discussion.

	Animal	Motoneurons			
Fictive locomotion					
	dt	ta	*(1)*	ta	*(2)*
	dt	ta	*(3)*	plong	*(4)*
	ee			sart	*(5)*
	ek			edl	*(6)*
	ek			edl	*(6)*
	el			edl	*(7)*
	el			lg	*(8)*
Fictive scratch					
	dx			pbst	*(9)*
	dx			pbst	*(9)*
	ek			unknown flexor	*(10)*
	el			edl	*(7)*
	el			edl	*(7)*
	em	pbst	*(11)*	tib	*(12)*
	em	mg	*(13)*	perl	*(14)*
	em	mg	*(15)*	fdhl	*(16)*
	em	pbst	*(17)*	pbst	*(18)*
	em	pbst	*(17)*	pbst	*(18)*
	em	pbst	*(17)*	pbst	*(18)*
	em	pbst	*(17)*	pbst	*(18)*
	em	lg	*(19)*	lg	*(20)*
	em	lg	*(19)*	lg	*(20)*
	en	mg	*(21)*	plant	*(22)*

Table 1. Motoneurons recorded from intracellularly and analyzed for deletions
Intracellular recordings were obtained in from 22 motoneurons including 8 pairs. A unique cell number is shown italics.

DISCUSSION

This study described the spontaneously occurring deletions during fictive locomotion and fictive scratch as well as their intracellular correlates. While such errors would represent unacceptable failures during normal behaviours, their occurrence in fictive behaviours can provide insights into the organization of the CPG operating on its own. Fictive behaviours allow us a unique opportunity to study the spinal CPG since they are produced in the absence of either cortical input or phasic afferent feedback. Moreover, while there are some differences between fictive and real behaviours such as spontaneous variations in the cycle length (e.g. Burke *et al.* 2001), the rhythmic alternation of activity in pure flexors and extensors observed during fictive locomotion and scratch is a strong feature of several variations of real walking (see Introduction) as well as of real scratch (Carlson-Kuhta and Smith, 1990). An exception to the flexor/extensor synergies is the behaviour of the bifunctional muscle St which changes in different forms of normal walking (Buford and Smith 1990). There are also behaviours such as the paw shake reflex which display mixed synergies (Smith *et al.*, 1985). Overall, however, both fictive and real locomotion and scratch display similar strict alternation of pure flexor and extensor activities. We studied deletions during fictive behaviours in 7 animals. While this a relatively small number, our findings are consistent with previous observations made informally in the laboratory in a larger group of animals.

We used both fictive locomotion and scratch to study the nature of the failures which can occur in centrally generated patterns. Apart from the fact that fictive locomotion requires ongoing electrical stimulation of the brainstem whereas fictive scratch does not, a key difference between the two behaviours is how the activity proceeds. Fictive scratch occurs in brief episodes, consisting of a period of tonic flexion followed by alternation of flexion and extension that spontaneously subsides. Fictive locomotion by contrast, occurs at slower rates and consists of rhythmic alternation which can persist for as long as the stimulus is maintained. Beyond these differences, however, fictive locomotion and scratch have the common feature of strict alternation of activity in flexors and extensors. The present study was limited to the behaviour of pure flexors and extensors which are active in strict alternation during fictive locomotion and scratch. We

never observed co-contraction of pure flexors and extensors during either fictive locomotion or scratch.

This study focussed on a particular type of failure which we call a deletion after the term used to discuss the missing bursts of hip extensor activity that occur during fictive rostral scratch in the turtle (Stein and Grossman, 1980; Robertson & Stein, 1988), a behaviour where knee extensor activity is strictly alternating with knee flexor activity. Stein and Grossman (1980) observed that in some cycles during a scratch response, there was an absence of knee flexor activity following the termination of knee extensor activity. This is what they termed a deletion. Robertson and Stein (1988) later described intracellular recordings during deletions of knee flexor activity. Their recording from a motoneuron belonging to the knee flexor motor pool showed a complete absence of depolarization during deletions of knee flexor activity (Figure 2B of Robertson and Stein, 1988). Furthermore, a recording from a hip protractor motoneuron (a motor pool usually active strictly out of phase with the knee flexor) showed a decreased hyperpolarization during deletions of knee flexor activity (Figure 2A of Robertson and Stein, 1988). In the present study, a deletion was defined as one or several missing bursts in any ENG during otherwise rhythmic activity, in either fictive locomotion or scratch. For example, the PBSt ENG remains rhythmically active during deletions in Figures 4B and 5A. In Figure 5B, it is the FDHL ENG which remains rhythmically active during deletions. In Figure 4A, the evidence for maintained rhythm during the deletion of flexor activity between flexor bursts 5 and 6 comes from the rhythmic decreased activity in the SmAB ENG (indicated by the dotted boxes). The period between flexor bursts 6 and 7 could also be considered as a "long step" but we prefer to classify it as a deletion because of the immediately preceding deletion. In general terms a deletion is different from a longer step in that some evidence of rhythmicity must be present during the deletion.

Deletions were defined as either complete (ENG burst entirely absent) or partial (ENG burst reduced to less than half the height of their neighbouring bursts. There are two potential problems with this. The first is whether the height of the integrated and rectified ENG burst represents the amount of activity in the nerve as accurately as a measure of the area under the curve. Given that, for a given motoneuron pool, ENG bursts are usually consistent with each other in length and shape, changes in height

should be a good estimate of changes in activity. The second issue is that, even during normal rhythmic activity, there can sometimes be a tendency for burst size to change slowly over several cycles. In this study, only decreases in ENG amplitude that were sudden were considered as deletions. Slow decreases in ENG amplitude over a few cycles were not classified as deletions.

Summary of results

The main findings of the study are as follows. Deletions occurred in otherwise rhythmic fictive locomotion (Figures 4, 7, 11) and scratch (Figures 5, 6-8, 12). The frequency of occurrence was similar in both behaviours and overall low (16% in fictive locomotion and 17% in fictive scratch). Although infrequent, they occurred in most animals (9/10) and most episodes (16/25) of activity showed at least one deletion. Deletions of extensor activity were observed in both behaviours (Figure 4B for fictive locomotion and Figure 5 for fictive scratch) but deletions of flexor activity occurred only during fictive locomotion (Figure 4A). Deletions consisted of a failure to activate synergist motor pools innervating both distal and proximal muscles. Simultaneous recordings from pairs of homonymous motoneurons during deletions showed that these changes occur in a qualitatively homogeneous way throughout the motor pool (Figures 11B and 12A). Deletions were almost always accompanied by a failure to inactivate antagonists in both fictive locomotion (Figures 4,8,10,11) and scratch (Figures 5,7). Despite these failures of motoneuron recruitment, subsequent ENG bursts often occurred very close to integer multiples of the cycle length preceding the deletion. This confirms the original observation of Grillner and Zangger (1979, Figure 4). Intracellular recordings from motoneurons showed that deletions of agonists were accompanied by a reduced depolarization of the motoneuron (Figures 9, 10, 11, 12). Deletions of agonists were accompanied by a reduced hyperpolarization of antagonists (Figures 10, 11). Thus the reciprocal depolarization and hyperpolarization of antagonist motoneurons seen during normal fictive behaviours was usually maintained during deletions.

Implication of findings for CPG organization

In order to discuss our findings in relation to models of CPG organization, we will first introduce a terminology to describe some of the processes that a CPG would need to control in order to produce the rhythmic behaviours we observe. There are several aspects of rhythmic behaviours that should be accounted for by a model of the CPG. For activity in a single limb these would include the following:

1) *Cycle period* : determines the frequency of the behaviour, its cadence

2) Cycle phase: proportion of flexor and extensor activity within a cycle

3) ENG amplitude: level of recruitment of the motoneuron pool

4) Distribution of drive to motor pools throughout the limb: preferential excitation of motor pools

5) Reciprocity: pattern of strict alternation between flexor and extensor activities

6) Pattern sculpting: production of varying patterns of activity of motor pools within a group of synergists

7) Variable patterns: production of different patterns of activity of a given motoneuron pool in different behaviours

Several models have been proposed for the CPG (see Introduction and below). Figure 13A shows the half-center model (Brown, 1914; Lundberg, 1981). It consists in a flexor and an extensor half-center, each of which excites the respective muscles of the entire hindlimb. The half-centers inhibit each other as well as the antagonist motoneuron pools. Brown initially thought that the motoneurons constituted the half-centers but the modern interpretation (reviewed in Lundberg, 1981) is that they are comprised of interneurons which then excite the motoneuron pools. The three features of the half-center model that allow it to produce rhythmic activity (Grillner, 1975) are that 1) the half-centers inhibit each other 2) each half-center is excitable enough such that it will fire when not inhibited and 3) a fatigue process causes cessation of firing of one of the half-centers and leads to oscillation of the system. In this model, the rhythm is essentially

dependent on the fatigue process. Failure of the fatigue process results in the system becoming locked in a state of either flexion or extension. The return to oscillation can occur when the half-center which is currently active fatigues or when the other half-center becomes active and inhibits the tonically active half-center. An important feature of this model in the context of this discussion is that the interneurons comprising the half-centers are responsible for both the generation of timing as well as the drive to the motoneuron pools. Applying the terminology presented in the list above to the half-center model shown in Figure 13A, one can say that the interneurons comprising the half-centers are responsible for timing generation involving the cycle period (number 1), cycle phase (number 2) as well as for generating of the ENG activity (number 3) and the reciprocity (number 5).

1) Cycle period

A key question concerning the organization of the CPG is whether the same interneurons are responsible for both the generation of rhythm and of the drive to the motoneuron pools. If the same interneurons populations are involved in both processes, as is assumed by the half-center model, then it would be reasonable to expect that changes in the excitation of the motoneuron pools would be accompanied by changes in the timing of the activity as well. One key observation from our data is that, despite a spontaneous deletion of an ENG burst, the timing of the subsequent burst of rhythmic activity can be maintained. Examples showing that bursts following deletions can occur at times consistent with the observed frequency of rhythmic activity prior to the deletion were presented in Figures 4, 5 and 7. This supports the functional separation of timing and pattern generation and agrees with an earlier observation made by Grillner and Zangger during treadmill locomotion of an absence of Quad activity accompanied by tonic St activity (Grillner & Zangger, 1979) Figure 4). The importance of the observation made in the present study is that this maintained periodicity occurred in the absence of any rhythmic afferent feedback. Such observations cannot be easily accommodated by the half-center model described by Lundberg (Figure 13A).

Previous work has suggested that afferent feedback pathways can access the CPG at various levels and thus modify timing and amplitude of rhythmic activity independently of each other (Lennard and Hermanson, 1985; Kriellaars, 1992; Whelan and Pearson 1997; Perreault *et al.,* 1995; Guertin *et al.* 1995; Burke *et al.,* 2001; Stecina *et al.*, personal communication). Lennard and Hermanson (1985), reviewing the organization of the central locomotor network in several animals, suggested a model where a central intralimb pattern generator (CIPG), responsible for organizing the phase relationships (*cycle phase, #2*) between muscles within a limb, would be interposed between the central timing network (CTN) and the premotor and motor nuclei. The CTN can be directly influenced by proprioceptive afferents while cutaneous afferents exert their influence on the CIPG (Lennard and Hermanson 1985, Figure 3). This suggests a separation between the control of the *cycle period* (this section) from that of the *cycle phase* (discussed in the following section). The only comment by Lennard and Hermanson (1985) related to the generation of ENG amplitude was to say that their model does not exclude proprioceptive inputs on premotor and motor nuclei. A separation between the *cycle period* and the *cycle phase* was also suggested by Perret and Cabelguen (1983) who pointed out that "there is no close relation between the frequency and the internal organization of the cycle" and that the frequency generator was therefore probably functionally separated from the output network. In their discussion, Perret and Cabelguen therefore include the cycle phase generation as part of the functionality built into the output network. As will be discussed, we have a different definition of the output module as being responsible only for the recruitment of the motor pool (*ENG amplitude, #3).*

Kriellaars (1992), in his thesis concerning entrainment of the locomotor rhythm in mesencephalic cats, suggested that the generation of timing and pattern were independent based on the observation that entrainment can modify the amplitude and frequency of the locomotor rhythm independently of each other. His proposed variation of the half center model is schematized in Figure 13b. According to the terminology introduced above, the Kriellaars-Jordan model has a timing module called the rhythm generator (RG) (Kriellaars 1992) (responsible for *cycle period, #1*). This feeds into the reciprocity module (RM) which combines the distribution of CPG excitation (*drive, #4*)

with CPG inhibition (*reciprocity, # 5*). Afferent input accesses the rhythm generator directly thus modifying the timing of locomotion without altering its amplitude.

Data showing the separate modulation of amplitude and timing by afferent feedback does not, however, imply the CPG itself should comprise separate modules for the generation of timing and pattern. Rather, it addresses the nature of the interneuronal pathways mediating afferent influence on the central pattern and their relation to the CPG. While such data can reveal how the CPG is reconfigured to integrate afferent input into the generation of motor behaviours, it does not truly address the issue of the intrinsic organization of the CPG.

Burke and colleagues (2001) also suggested a CPG organization in which there was a pattern forming network separate from a rhythm generator network (#1 and #6). This conclusion was partly based on the fact that the facilitation of SP EPSPs in FDL motoneurons occurs regardless of whether FDL is active in early flexion or in extension. This suggests that the circuits mediating afferent influences on the motor pools are not part of the timing generator but rather driven by it. It does not directly address the organization of the CPG in its native form.

2) Cycle phase

The relative balance of flexor and extensor activities changes in fictive locomotion and scratch and can also vary for the same behaviour in different preparations. Deletions of both flexor and extensor activities were observed during fictive locomotion. As described earlier, flexion was usually but not always longer than extension in the fictive locomotion preparations studied here (see Fig. 1, 3A left panel, 4B for examples) with an average ratio of flexor to extensor burst of 1.6±0.9 (6 episodes). During fictive scratch, only deletions of extensor activity were observed. Moreover, during all episodes of fictive scratch, flexor activity occupied a much greater portion of the cycle than extensor activity with an average ratio of flexion to extension of 3.9 ±0.9. This suggests that there may be an association between the balance of flexor and extensor activities and the nerves which are more prone to exhibiting a deletion.

When flexor activity dominates the cycle (ratio much greater than 1) as is the case during fictive scratch, extensors may be more prone to deletions. Since a systematic analysis was not performed, one should be cautious about over-interpretation and more data would be required to substantiate this hypothesis. It remains, however, that during a behaviour where flexion occupies the major part of the cycle, deletions of flexor activity were not observed.

3) ENG amplitude

It was seen (Figures 8 and 9 in particular) that, during deletions, there may be either a complete or partial failure to depolarize motoneurons in the pool. The cases where there was no depolarization at all (Figure 9, solid and dashed traces) suggest that there was a failure of the premotor interneurons to excite the motor pools. In the case where the depolarization was decreased but not absent (Figure 9, dotted trace) it is possible that the premotor interneurons themselves remained active but at a reduced level. This level of depolarization in turn might have failed to activate voltage-gated conductances in the motoneurons during that particular cycle causing a loss of excitation of the pool.

4) Reciprocity

The model introduced by Kriellaars and Jordan (Kriellaars, 1992) and consisting of a rhythm generator and of a reciprocity module (see earlier description and Figure 13B) was used primarily to emphasize the reciprocal organization of flexor and extensor activation and reciprocal inhibition (Jordan, 1991; Kriellaars, 1992) that occurs during fictive locomotion. Jordan (1991) presents an example of a deletion during MLR-evoked fictive locomotion and emphasized the strong link between excitation of agonist pools and inhibition of antagonist pools. In that example, failure to fully excite a flexor motor pool (deletion in TA ENG) is accompanied by a reduced inhibition of an extensor (decreased hyperpolarization of an MG motoneuron). The discussion stressed that a failure to excite agonists (a deletion) should be accompanied by a concomitant failure to

inhibit antagonists. Our study fully supports this idea by showing that, failure to excite a group of synergists (deletions) is usually accompanied by failure to inhibit antagonists (see Figures 4 and 5 for examples). Therefore, the reciprocity observed during normal fictive behaviours is usually maintained during deletions. The tight reciprocity observed between agonist and antagonist nerves is also present at the motoneuronal level. During deletions, there is a decreased depolarization in agonist motoneurons and a failure to hyperpolarize in antagonist motoneurons. Note that the maintenance of reciprocity is also a feature of deletions of hip extensor activity in the turtle (Stein and Grossman 1980).

Although deletions were usually accompanied by lack of quiescence in antagonist pools, there was an exception to the tight reciprocity between flexor and extensor activities (Figure 7). In this case, the pure flexors (Sart, TA, PerL) remain rhythmically active while the extensor bursts are clearly absent. The intracellular recording from an EDL motoneuron indicates that it remained rhythmically depolarized to a similar degree with and without the deletion. Deletions in extensor motor pools accompanied by undisturbed rhythmic activity in flexor could occur if the deletion in extensor activity was the result of decreased (i.e. subthreshold) depolarization of extensor motoneurons. It is not possible to determine whether this was the case since there was no intracellular recording from an extensor motoneuron during that episode. If there was indeed a subthreshold depolarization of extensor motoneurons, although the depolarizing drive (output stage) to extensors would be insufficient to cause firing, the correspondingly reduced hyperpolarizing drive to flexors may be adequate to inhibit them rhythmically. Moreover, a reduced output stage drive may have been sufficient to rhythmically activate the flexor motor pools if they were more excitable than the extensor motor pools. Arguing for the maintained reciprocity is the fact that the amplitude of the activity in flexors did not decrease during the complete extensor deletions. In a case illustrated by Burke and colleagues (Figure 10 Burke *et al.* 2001) there is an absence of extensor ENG bursts while flexors continue to burst rhythmically. Burke and colleagues (2001) suggest that this indicates independent cycling of flexor bursting. Their intracellular recording from an LGS motoneuron, however, showed continued cyclic depolarizations. This indicates that the extensors were indeed receiving a cyclic drive but that this drive was insufficient to recruit motoneurons of the extensor pools. Moreover,

there is a small level of activity in the LGS ENG lending support to the idea that the extensor pools were receiving a drive from the CPG but that this drive was subthreshold. We therefore argue that this does not represent an example of independent flexor cycling. Figure 7 of the present study shows an absence of extensor bursts for several seconds while flexor ENGs continue to burst rhythmically. A simultaneous intracellular recording of an EDL motoneuron shows rhythmic depolarizations and hyperpolarizations, unaffected during the deletions of extensor ENG activity. Without an intracellular recording from an extensor motoneuron we do not know if this represents independent cycling.

The maintenance of reciprocity we observed during deletions contrasts with a report by Grillner & Zangger (1979) of a case where a flexor nerve (St) was tonically active while an extensor nerve (Q) remained rhythmically active. One caution for the interpretation of this observation is that, although St has been discussed in the past litterature as a flexor, from our current understanding, it functions as a bifunctional muscle which crosses two joints and has antagonistic actions at each. Its activity varies and can occur during either flexion (Figure 4) or extension (Figure 6). It can also be depolarized during both phases with recruitment at the transition between the two phases (Figure 10). Preliminary data from this laboratory (Chakrabarty *et al.*, 2003) shows that PBSt motoneurons active in phase with flexors can maintain their rhythmicity during an extensor deletion in which there is tonic activity in the pure flexors. This suggests that PBSt motoneurons receive a more complex drive than flexors such as TA, EDL, Sart. The patterns observed by Grillner are then consistent with our current understanding of St as a bifunctional muscle with a complex drive.

The examples described above (Figure 10 of Burke *et al.* 2001 and Figure 7 of this study) show that the excitability of the flexor and extensor motor pools can be different from each other. The excitability of a motoneuron pool is determined by two factors. The first factor is the threshold at which the cell will fire. The threshold has been shown to vary in different behaviours, specifically it has been shown to decrease during fictive locomotion compared to rest (Krawitz *et al.*, 2001). The second factor is the responsiveness of the cell to depolarizing current. This responsiveness is governed by the intrinsic properties of the cell, as well as voltage-activated conductances which alter the

response of the cell to the depolarizing drive it receives. The voltage-gated conductances can be activated or inactivated by neuromodulatory inputs to the motoneuron. This provides a second way to alter the excitability of the cell. Therefore, excitability is a dynamic property of the cell and it is conceivable that excitability changes as a behaviour proceeds as well as from one behaviour to another. A decreased drive to motoneurons may decrease the LDP without resulting in cessation of firing if the motoneuron pool is highly excitable. Such an event would be virtually undetectable by looking at ENGs alone. Conversely, a very small change in the drive to motoneurons decreasing the LDP might be sufficient to inhibit firing altogether which would result in completely absent ENG bursts. This highlights the importance of knowing the intracellular activity of motoneurons to determine the nature of deletions.

5) Distribution of drive to motor pools throughout the limb

In our study of deletions in the mesencephalic cat during MLR-evoked fictive locomotion and scratch, we generally observed that, during deletions, motor pools acting at all joints were affected (See Figures 4B and 5A and B for examples). This is in contrast to an anomalous pattern mentioned by Grillner and Zangger (1979) of silence in extensors at one joint accompanied by rhythmic activity in extensors at another joint. It is possible that in their unillustrated example, the deletions were complete in one extensor motoneuron pool and partial in another, thus masking the fact that they did occur. In the present study where recordings were obtained simultaneously from a large number of nerves, the vast majority of deletions observed affected all synergists (Figure 4,5). One example during fictive scratch (Figure 6) showed robust activity at the hip but deletions of the ankle extensor activity. Indeed, there was no rhythmic activity in one ankle extensor (LG), reduced rhythmic activity in another (MG), and robust rhythmic activity in a hip extensor (SmAB).This example was atypical (2 deletions out of over 80 deletions we looked at). The fact that this example was only found at the end of an episode fictive scratch could suggest that there might be additional influences on the CPG during this behaviour.

The models presented in Figure 13A and 13B both suggest that the drive is distributed to all agonist muscles of the hindlimb, i.e. there is a single half-center pair for

the entire hindlimb. Observations of variability between the activity of different groups of synergists led to the suggestion that the limb generator be divided into unit burst generators (UBGs), each of which would control a group of agonists acting at a particular joint (Grillner & Zangger, 1979). They reported two motor patterns where subsets of synergists had different activities. The first pattern (mentioned above) consisted of rhythmic activity of extensors at a joint accompanied by silence of the extensors at another joint. The second pattern was tonic activity in a flexor at one joint accompanied by rhythmic activity in a flexor at another joint. Although mentioned (Grillner & Zangger, 1979), neither pattern, to our knowledge, has been illustrated. As noted by (Lundberg, 1981), these observations, if true, would be critical evidence against a simple half-center model. The UBG model (Grillner, 1981) is shown schematically in Figure 14A. In such a model, muscles at each joint would be controlled independently by a pair of unit burst generators (UBGs), one for extensors and the other for flexors. These would have bursting capability but would not be entirely independent from the rest of the limb due to the coupling between them. This coupling was suggested to vary with the task, such that different muscle synergies be generated for walking forward or backward for example. However this suggestion was refuted (see the discussion below on the work of Smith and colleagues).

A model with UBGs would predict that deletions can be seen in isolated groups of muscles at a joint. However, the vast majority of our data on deletions shows them to occur simultaneously in all synergists. This suggests that either the CPG responsible for alternation of flexor and extensor activity is common for the limb, such as the example in Figure 13B, or that individual UBGs for each joint must be very tightly coupled during these behaviours. Tight coupling would make this arrangement indistinguishable from a single limb generator during deletions. We cannot, with the current data, differentiate between a common drive to all joints and tightly coupled yet independent UBGs.

The extensive data from Smith and colleagues on muscle activity during various forms of walking in intact cats shows that basic flexor/extensor synergies are maintained in various forms of walking but the activity of bifunctional muscles varies to accommodate the specific requirements of these different behaviours. This suggests that

the organization of the CPG must be flexible enough to allow motor pool activity to vary from one behaviour to the next. They suggested (Carlson-Kuhta *et al.*, 1998) that both Grillner's UBG model and the half-center model could account for the changing activity of St. The UBG model could reconfigure its connections in different tasks and this could result in varying St activity. On the other hand, as proposed by Perret & Cabelguen (1980), the St pool could receive input from both the flexor and the extensor half-center, the combined strength of which would determine the activity of the St muscle in a particular task. In both cases, there is a need to reconfigure the CPG for varying tasks. This reconfiguration could be controlled by supraspinal inputs and integrate various aspects of motor behaviours such as posture and motivational intent. Therefore the results of Smith and colleagues do not preclude a half-center based organization of the CPG provided that enough flexibility in the control of bifunctional muscles, specifically St, is embedded in the organization.

6) Pattern sculpting

Several lines of evidence suggest that the drive to various ankle flexors (TA, EDL and PerL) varies despite the fact that they are all active during ankle flexion in fictive locomotion. First, the burst shape of TA and EDL can be quite different from each other as can be seen in Figure 1. EDL activity is low at the beginning of the phase and increases throughout the burst whereas TA activity is relatively stable throughout the flexor phase. Therefore, it seems that these two motoneuron pools are is recruited differently. In other fictive locomotion preparations the envelope of TA and EDL activities are more or less superimposable. During various forms of real walking in cats (crouched, upslope, downslope), differences between the activity patterns of TA and EDL can also be observed (Trank & Smith, 1996; Smith *et al.* 1998; Carlson-Kuhta *et al.* 1998). The differences between the recruitment pattern of various ankle flexor pools are represented schematically in Figure 14B which shows our proposed organization for the limb CPG. This figure shows separate modules for each motor pool within the flexor module. Another feature of the organization of Figure 14B is that timing is distributed separately to hip and ankle flexors and then separately again to various ankle flexors.

There is evidence for a difference in the latencies of onset of hip and ankle flexor motoneuron depolarization (Chakrabarty *et al.*, 2001; Quevedo *et al.*, 2000).

7) Variable patterns

In addition to differences in the onset time and in the pattern of recruitment of the pool among flexors, the burst shape and timing of PerL activity is strikingly different between fictive locomotion and scratch. PerL activity is flexor-like, resembles TA activity and is strictly out-of-phase with ankle extensors during fictive locomotion. During fictive scratch, PerL burst is mainly in-phase with ankle extensors and out-of-phase with TA although it is slightly delayed from extensor onset and also shows a low-level of activity during flexion. This switch of PerL activity from being flexor-like to more extensor-like suggests that depolarization is distributed differentially to PerL in fictive locomotion and fictive scratch. This is why timing is depicted as coming from the flexor side during fictive locomotion and from the extensor side during fictive scratch in Figure 14B.

It is interesting to note that the behaviour of PerL during deletions was consistent with its pattern of activity. During fictive locomotion, it was tonically active with the other flexors during extensor deletions (Figure 4B). During fictive scratch, where PerL activity was more extensor-like, it was deleted during extensor deletions (Figure 5B). Note, however, that during fictive scratch, PerL can also remain rhythmically active despite deletions of extensor activity (not shown, discussed in the Results section related to Figure 12B).

By contrast with PerL, TA remained out of phase with extensors in both behaviours (Figure 3B) and thus showed tonic activity during extensor deletions in both cases. The need for a difference between the behaviours of these two motor pools can be understood by considering the actions of these three muscles at the ankle of the cat (Young *et al.*, 1993). TA and EDL are the main flexors of the ankle and only aid in abduction and eversion. By contrast, PerL is primarily an abductor and also evertor of the ankle. Its ankle flexor action is modest and only expressed when the ankle is flexed compared to the neutral position. It is reasonable to suggest that during locomotion, the

ankle flexor action of PerL justifies its recruitment during flexion. During scratch, however, as the ankle extends to bring the paw to the ear, eversion is required for the digits to contact with the skin during extension (Carlson-Kuhta *et al.*, 1990).

Concluding remarks on a plausible model for the CPG

Some features of Figure 14B have already been discussed. To summarize, Figure 14B shows a schematic drawing of a possible organization of the CPG responsible for alternation of flexors and extensors during fictive locomotion and scratch. The first feature of this organization is that the distribution of timing can be separated from the generation of the drive to motoneurons. This is necessary to account for our observation that timing of rhythmic activity is maintained during deletions. We have shown that the drive potential may be abolished or reduced during deletions. If the LDP is only reduced, it could be argued that timing and drive are not separated since both are maintained in such cases. This is true, but ignores the fact that the LDP is indeed completely abolished in some cases, leaving no intracellular indications of a drive to the cell. These examples suggest that the maintenance of timing in the absence of drive is possible and common during fictive locomotion.

Future directions: CPG control of bifunctional muscles may be different from that of flexors and extensors

This study focussed on pure flexors and extensors and ignored bifunctional muscles. However, bifunctional muscles have been shown to be unique in their behaviour during real and fictive locomotion (summarized in Smith 1986). Their behaviour during deletions could yield particular insights into CPG organization. Preliminary data (Chakrabarty *et al.* 2003) suggests that bifunctional motor pools behave differently from pure flexor or extensor motor pools during deletions. This is particularly relevant in comparing the present study with work on deletions in other preparations. In addition to reports of deletions in cats walking on a treadmill (Grillner & Zangger, 1979) or during

fictive locomotion (Grillner & Zangger, 1979; Jordan, 1991) deletions have been reported during the paw-shake response in the cat (Koshland & Smith, 1989) and during the turtle scratch reflex (Robertson & Stein, 1988), both of which are mixed synergy behaviours. The paw-shake response is characterized by very rapid oscillations of the paw, to an irritating stimulus, such as water or adhesive tape, on the paw of the cat. Studies of the muscle activity during paw-shake in normal cats (Smith *et al.*, 1985) showed that, in addition to the reciprocal activity of ankle extensors and flexors, there is a mixed synergy which appears midway during the behaviour and which is characterized by co-contraction of the knee extensor VL and the ankle flexor TA. The turtle scratch reflex is also characterized by a mixed synergy where agonists at one joint fire at a different time than agonists at another joint. Stein and Daniels-McQueen (2002) argue against a half-center model on the basis that knee extensor motoneurons are not quiescent during hip extensor deletions. In the mixed synergy of turtle scratch, the knee extensor in question is actually active at the end of the hip flexor phase. It therefore does not behave quite like an extensor but rather like a bifunctional muscle active at the transition between phases. In that sense, the fact that it can be uncoupled from extensors is not surprising if we consider the preliminary data (Chakrabarty *et al.* 2003) mentioned previously which suggests that bifunctional muscles have a more complex drive than flexors and extensors. The same reasoning can be applied to the paw shake response where the knee extensor (VL) is active with the ankle flexor TA. The study of mixed synergies is thus quite different from our study which focussed on strictly alternating muscles. In fact, it is probable that mixed synergies can be better compared to the behaviour of bifunctional muscles. Further study of both mixed synergies and bifunctional muscles is likely to provide unique insights into the organization and flexibility of the CPG.

To conclude, this thesis analysed the occurrence of spontaneous burst deletions during fictive behaviours. It was shown that deletions occur spontaneously although infrequently during both fictive locomotion and scratch and that they involve entire groups of synergists. Deletions were shown to have intracellular correlates in motoneurons in the form of changes to the locomotor drive potential. The reciprocal nature of the activity of flexors and extensors was maintained during deletions where a

failure to depolarize agonists was accompanied by a failure to hyperpolarize antagonists. A major contribution of this thesis was to provide evidence for the fact that timing can be maintained during failures in the drive to motoneuron pools. A second contribution was the demonstration that the knowledge of intracellular events in motoneurons is necessary to fully understand the process behind deletions of ENG activity. Further work, some of which has already been initiated in this laboratory, should consider the behaviour of bifunctional motoneuron pools during deletions as a means of uncovering how these motor pools can be activated in varying ways during various behaviours. The study of deletions in mixed synergies is also likely to provide insights into CPG organization. Understanding the organization of the CPG will be essential for the identification and characterization of the interneurons comprising it.

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