

**Organization of CPG control of motoneuron pools during rhythmic fictive
behaviours**

By

Myriam Lafreniere-Roula

A thesis submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

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ABSTRACT

Central pattern generators are network of cells which can produce rhythmic motor output without the presence of rhythmic sensory input or cortical input. Despite the development of theoretical models, experimental evidence to corroborate a particular organization for the locomotor CPG remains sparse. In this thesis, we will describe a set of experiments during fictive locomotion and scratch in the decerebrate and immobilized cat where we examined subthreshold (postsynaptic potentials) and suprathreshold (action potentials) events in motoneurons to infer characteristics of the last-order interneurons immediately projecting to motor pools. Fictive locomotion was elicited by electrical stimulation of the mesencephalic locomotor region and fictive scratch was elicited by application of curare on the cervical roots followed by mechanical stimulation of the pinna of the ear. During these fictive motor outputs, we performed intracellular recordings of motoneurons to examine their firing as well as their synaptic input. Using similar techniques, we have shown in previous work that the examination of spontaneous failures (deletions) during rhythmic motor activity could provide useful information to constrain theoretical models. Specifically, we provided further evidence to support the separation of the rhythm generation and the pattern formation functions of the CPG by showing that the activity of motor pools can be severely disrupted while the timing of this activity remains consistent with the ongoing rhythm. In the experiments described here, we have combined the examination of deletions to that of motoneuron activity to infer features of the organization of last-order interneurons. We will show in the first paper that deletions, which are accompanied by decreases in the locomotor drive to motoneurons also show

corresponding changes in the short-latency postsynaptic potentials which are transmitted from the mesencephalic locomotor region. Specifically, deletions of agonists were accompanied by decreases in the amplitude of the excitatory component of the postsynaptic potential while deletions of antagonists were accompanied by decreases in the inhibitory component. This is consistent with the involvement of the last-order neurons mediating CPG drive to motor pools in the transmission of MLR-PSPs. In the second paper we will show that there is a lack of synchronized firing during either fictive locomotion or scratch even in pairs of closely located homonymous motoneurons. Furthermore, this lack of synchrony is not limited to action potentials but also extends to subthreshold, underlying excitatory postsynaptic potentials. This suggests that the CPG drive to motor pools is limited to the transmission of an envelope of excitation (locomotor and scratch drive) and does not include spike-triggering events that would lead to short-term firing synchrony.

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To Elia, you have supported me in so many ways and now, finally, we can enjoy the success together. Love.

And to my parents for all their love and support, here it is. This one's for you.

LIST OF ABBREVIATIONS

cME	caudal microelectrode
CPG	central pattern generator
EDL	extensor digitorum longus
Em	resting membrane potential
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
PBSt	posterior biceps combined with semitendinosus
Plong	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)

rME rostral microelectrode

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I. GENERAL INTRODUCTION

A Central Pattern Generator (CPG) is a network of neurons capable of generating a specific pattern of motor activity. CPGs have been shown to be involved in the generation of many rhythmic behaviours essential for survival including feeding, breathing, and walking. It is reasonable to surmise that general organizational principles of CPGs for a task such as walking would be conserved across species given its importance for survival. There is evidence that spinal circuits capable of generating rhythmic alternation of flexion and extension are present in several species suggesting it is reasonable to expect they also exist in humans. Considerable progress has been made in the determination of the locomotor CPG in invertebrates. In mammals, however, despite the work of several groups of researchers, the exact structure of the locomotor CPG remains unknown. The goal of this work is to improve our understanding the structure of the CPG.

In the general introduction we will present the concepts most relevant to this work including the central generation of locomotion and scratch patterns as well as the activity of motoneurons during these fictive behaviours. This will be followed by two manuscripts describing the work. Finally, a general discussion will conclude.

Central Pattern Generators

A central pattern generator (CPG) is a network of cells capable of independently generating a specific pattern of rhythmic motor activity. The concept of the CPG arose

following demonstrations of the possibility of generating rhythmic movements of purely central origin. In the early 1900's, the dominant explanation of movement was the reflex theory put forth by Sherrington (1906) according to which movement is the result of the activation, by sensory stimuli, of a chain of reflexes. Graham Brown (1914) proposed a mechanism by which spinal neurons could, independently of sensory input, generate locomotion. This suggestion followed his earlier reports on a series of experiments in spinal cats where rhythmic muscle contractions were recorded following the interruption of sensory input by either transection of dorsal roots or peripheral nerves (Graham Brown 1911). The possibility of generating rhythmic movements of purely central origin was also demonstrated in other organisms (e.g., swimming in fish by von Holst (1935), flying in the locust by Wilson (1961)). Since these early studies, CPGs have been implicated, in a wide variety of behaviours including feeding and respiration. In this section, following a brief introduction of CPGs in general, we will primarily review literature directly concerning the locomotor and scratch CPGs in the cat. Selected studies of locomotion and scratch in other animals will be presented when relevant to this work.

Rhythmic activity, although it can be generated by cells which have bursting properties in certain types of networks, most likely emerges from a combination of cellular and synaptic properties and patterns of neuronal connectivity in the mammalian locomotor CPG (i.e., Getting's "building blocks") (Getting 1989). Little is known of the cells comprising the adult mammalian CPG for locomotion although information is starting to emerge in rodents through the combination of molecular, genetic and classical electrophysiological techniques (For review see Kiehn 2006). Beyond the contribution of

theoretical models of CPG structure, however, the pattern of neuronal connectivity within the CPG remains unclear. The goal of this thesis is to further our understanding of CPG structure.

Although they are capable of doing so, CPGs do not operate in isolation under normal circumstances. Instead, there seems to be a complex relationship between sensory inputs and centrally generated patterns. For example, muscle afferent feedback is important in the regulation of the transition from stance to swing in the decerebrate cat (Hiebert et al. 1996, Duysens and Pearson 1980) and also contributes to the recruitment of extensor motoneuron pools (Hiebert and Pearson, 1999). Moreover, sensory stimulation can profoundly affect ongoing locomotion (reviewed in McCrea 2001), giving rise to complex reflexive actions such as the stumbling corrective reaction which arises from the need to clear an obstacle (Forssberg 1979, Quevedo et al. 2005a,b). In addition, some sensory-evoked actions are modified during centrally generated movements. For example, the inhibition of extensor motoneurons by stimulation of the superficial peroneal nerve is not observed during fictive locomotion (Quevedo et al. 2005b). This close integration of centrally generated rhythmic movements and sensory information is essential for achieving behavioural goals. Although we recognize the importance of afferent feedback in regulating motor behaviours, we have chosen to study preparations where it is absent in an effort to focus on the specific aspect of CPG organization.

Before proceeding further, one should note that, although the definition of the CPG stated at the beginning of this section may seem to imply that it is a dedicated neural circuitry

for the purpose of generating a unique motor task, there is evidence that the neural elements comprising the CPG can take part in the generation of more than one such task. For example, it has been shown, in invertebrates, that neuromodulators can configure neuronal circuits to perform particular patterns, thus affording the overlap of neuronal circuitry for the generation of various tasks (discussed in Marder et al. 2005). Recently, evidence from work on the CPG for the generation of scratch patterns in the turtle has shown that individual interneurons can be active during more than one type of fictive scratch (Berkowitz A. 2005) supporting the idea that neuronal populations may be involved in the generation of more than a single rhythmic motor pattern. This will be of particular interest with respect to some of the findings to be presented herein.

Locomotor CPG in the cat

Architecture of the CPG

The study of the locomotor CPG in the cat initiated at the beginning of the past century following the demonstration by Sherrington that spinalized cats could make rhythmic walking-like movements (Sherrington, 1913). Following early investigations into the stepping mechanism (reviewed in Lundberg, 1969), Graham-Brown (1914) suggested that locomotion in the spinalized cat could be attributed to the existence of a spinal circuit sufficient to generate the alternating limb movements required for locomotion and he described the half-centre model, the first and to this day classical, locomotor CPG model. His proposal, modified by Lundberg and his colleagues (Jankowska et al. 1967a,b)

consisted in the suggestion that the CPG may be composed of two half-centers, one controlling all flexors of a hindlimb and the other controlling all extensors. The combination of reciprocal inhibition between the half-centres and of a fatigue process in the neurons comprising the half-centres would generate alternation of flexor and extensor activity. In such a way, this organization can account for the rhythmic alternation of flexor and extensor activities with each half-center controlling all agonist pools across the limb.

Another early model where agonists within the limb are controlled as a unit is the ring model proposed by Gurfinkel and Shik (1973; reviewed in Shik and Orlovski, 1976). This model is composed of neurons connected in a ring configuration where flexor-related neurons alternate with extensor projecting neurons. Neurons within the ring project to the various motoneuron pools either directly or through interneurons. The ring configuration accounts for the successive activation of flexor pools and extensor pools. The ring itself is activated by descending influences.

The activity of certain motor pools during various forms of walking is, however, more complex than simple alternation. For example, motor pools innervating muscles crossing more than one joint can be active during both phases of the step cycle. In order to account for more complex patterns of activity Grillner suggested that each group of close synergists at a joint be controlled by a unit burst generator (UBG) (Grillner 1981). This differs from the half-centre model where each half-centre controls all the agonist motor pools within the limb. Tight coupling between the UBGs of a single limb could account

for the synchronized activity of agonist pools within each limb during forward locomotion. In turn, this coupling could be reconfigured to allow for more complex patterns of muscle activity during other forms of walking.

Underlying both the half-centre and UBG models is the assumption that neurons responsible for generating rhythmicity are also providing the excitatory drive to motor pools. More recently, Jordan proposed a modular hypothesis (1991) whereby groups of neurons could be involved in different aspects of locomotor production. It has also been suggested by a number of investigators (e.g., Burke et al. 2001, Koshland and Smith, 1989, Kriellaars et al. 1994) that the CPG should contain separate networks specifically for rhythm generation and for motoneurons excitation since each feature can be altered independently of the other by using different types of sensory stimuli. In addition, the model proposed by Burke and colleagues (2001) included a third layer of last-order interneurons interposed between the pattern formation layer and the motor pools. This layer filters the locomotor drive and modulates transmission through reflex pathways.

Recently, further evidence has been given to support the separation of rhythm generation and pattern formation (Lafreniere-Roula and McCrea 2005) and this development has led to the full development and implementation of a large-scale computational model of the CPG for a single limb (Rybak et al. 2006a and Rybak et al. 2006b). The Rybak-McCrea model is centered around the separation of the generation of rhythmicity from that of excitation. Experimental data described during my Master's thesis on the occurrence of deletions in nerve activity during fictive locomotion in the cat provided empirical

justification for this separation. Deletions are spontaneously occurring failures of excitation (decreased ENG activity) which generally occur in all agonist motor pools within the limb during fictive locomotion (Lafreniere-Roula and McCrea, 2005). During deletions, the depolarization which is normally present in motoneurons during their active phase is reduced or even abolished thus preventing firing. This decrease of activity in agonists is accompanied with corresponding sustained activity of antagonists such that motor activity remains alternating. We observed that, following a deletion, rhythmic activity often resumes at a time consistent with the pre-existing rhythmic activity. This suggests that, even though the excitation to motor pools was severely disrupted, the timing of this excitation was maintained.

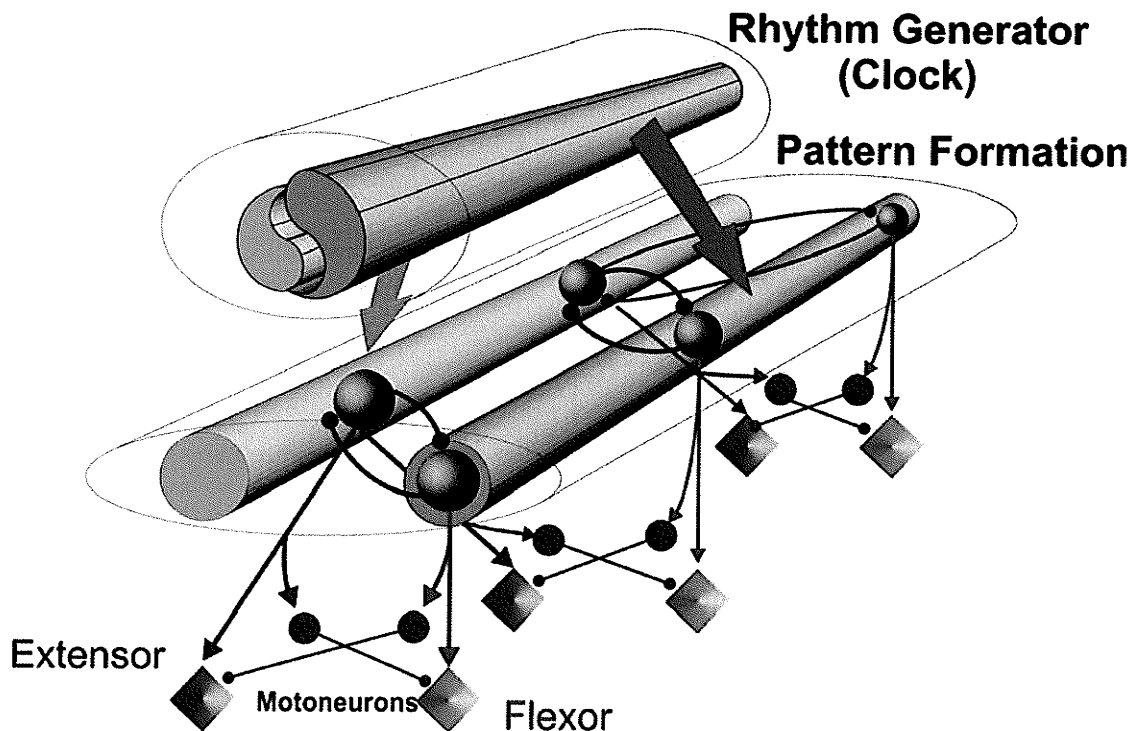


Figure 1. Two-layer model of the CPG.

A schematic version of the Rybak-McCrea model is provided in Figure 1. The Rybak-McCrea model is organized in two layers, one responsible for rhythm generation (RG layer, clock) and the other one for pattern formation or the excitation of motoneuron pools (PF layer). In this model, there is a single rhythm generator (cylinder within the blue shaded area) shared by all pools. The PF layer (within the yellow shaded area), on the other hand, contains several pattern formation modules. A single pattern formation module is represented in the diagram of Figure 1 by a pair of reciprocally inhibiting green circles (one on the extensor side and another on the flexor side). Motor pools (represented by diamonds) receive locomotor drive from a single or from multiple pattern formation modules. The particular configuration of pattern formation modules projections to motor pools is not meant to illustrate any particular configuration but rather simply to convey the potential complexity of those projections. The current implementation of the model only includes a single flexor pool and a single extensor pool (Rybak et al. 2006a,b). The advantage of this two-layer organization is that it can accommodate the features of spontaneous deletions which we have described above, namely that excitation to motoneurons can be reduced and even completely abolished while evidence remains that rhythmicity is still present. Effects of sensory stimulation on the locomotor cycle have also been incorporated in the model (Rybak et al. 2006b) but are not illustrated in this schematic.

Localization of the CPG

In parallel with attempts at understanding of the theoretical architecture of the CPG, researchers have had some success identifying regions of the spinal cord active during locomotion and characterizing the activity of identified neuronal populations during locomotion. This type of information is a prerequisite in order to localize and identify putative CPG neurons. Mapping of field potentials elicited by MLR stimulation sufficient to evoke fictive locomotion in the adult cat revealed short latency field potentials mostly in laminae VII and, to a lesser extent, in lamina VIII (Noga et al, 1995). The largest lamina-VII fields were in the L5-L6 region while the largest lamina-VIII fields were observed more rostrally in L4-L5 (Noga et al, 1995). The modulation of those field potentials in phase with the locomotor cycle suggests that the interneurons responsible are part of the locomotor CPG. Comparison of c-fos and ChAT labelling in the lumbar spinal cord of cats who had been subjected to locomotor training and cats who had not, confirmed that most active cells (c-fos stained) were in the L5-L7 segments (Dai et al 2005) and that cholinergic interneurons are activated and participate in the production of fictive locomotion (Huang et al 2000). Locomotor tasks in rats also induced increased expression of c-fos in lamina VII compared to control animals (Ahn et al 2005, Jasmin et al. 1994).

Another class of approaches to identifying the spinal segments necessary and sufficient for the production of locomotion is their isolation by either transverse sectioning or by bath partitioning of in vitro preparations combined with locally applied rhythmogenic drugs. The transverse sectioning approach in cats (Grillner and Zangger 1979) and

newborn rodents (e.g., Kjaerulff and Kiehn, 1996, Cowley and Schmidt 1997, Kremer and Lev-Tov 1997, Bonnot and Morin 1998) has led to the conclusion that the lumbar enlargement has rhythmogenic capabilities (specifically its more rostral lumbar segments). On the other hand, partitioning experiments where sections of the spinal cord were selectively exposed to rhythmogenic drugs have led to a somewhat different conclusion that rhythmogenic capabilities are limited to more rostral segments (lower thoracic and upper lumbar) and that the lower lumbar segments do not have rhythmogenic capabilities (Cazalets et al. 1995, Bertrand and Cazalets 2002). These differences may be explained by different receptor distributions in the spinal cord and by differences in the concentrations used in different protocols.

In addition to identifying regions of activity in the spinal cord during locomotion, the activity, during locomotion, of both identified and unidentified interneurons was investigated. Studies of unidentified spinal neurons during various forms of locomotion have demonstrated neurons with firing either limited to a specific phase of the step cycle of active at the transition between phases (for example, see Feldman and Orlovsky 1972, Edgerton et al. 1976). The activity of identified interneuron populations mediating a variety of reflex pathways has also been studied during fictive locomotion (Reviewed in Rossignol et al. 2006). Again, varying patterns of activity have been observed from maximal firing in the phase during which afferent stimulation is received (for example in Renshaw cells) to tonic inhibition (for example non-reciprocal group I interneurons). Despite the localization of appropriately active spinal neurons, however, CPG neurons have not been identified per se. Defining criteria for considering a particular neuron to be

part of the CPG is difficult. Rhythmic activity during locomotion is one such criteria. Using the framework of their model (Rybak et al. 2006), however, Rybak and colleagues have been able to outline a series of criteria that would allow the identification of putative CPG interneurons. This will be discussed in greater details in the General Discussion.

While it is necessary to localize neuronal populations active during locomotion, this approach does not, on its own, lead to an understanding of the role of those cells within the CPG. In order to achieve this, such findings need to be combined with a theoretical CPG architecture which can be used to make verifiable hypotheses regarding the behaviour of these putative CPG neurons in various situations. Studying failures (deletions) during locomotor activity allows us to anchor a theoretical CPG model in empirical physiological evidence making the combined approach that much more likely to be successful.

Scratch CPG in the cat

Locomotor-like movements are not the only ones capable of being generated by a CPG. Sherrington's early investigations included the study of the scratch reflex. The scratch reflex is the automatic response of a cat to a noxious stimulus in its outer ear and consists in an initial postural phase where the ipsilateral hindlimb is positioned closed to the ear followed by rapid rhythmic movements of the hindlimb aiming to dislodge the noxious stimulus. The description of the pattern of muscle activity in intact cats (e.g. Deliagina et

al. 1975, Kuhta and Smith, 1990) has shown that the rhythmic alternation of flexor and extensor pools is very similar to that occurring during locomotion with some exceptions. A fictive scratch reflex can be elicited in decerebrate, paralyzed cats by first applying curare to the dorsal roots at the C1-C2 level followed by stimulation of the pinna of the ear (Deliagina et al. 1975) and the pattern of nerve activity during fictive scratch is similar to that during real scratch (Deliagina et al. 1981). C-fos labeling of activated cells following the induction of prolonged fictive scratch demonstrated activation of cells, mostly on the scratching side, in the lumbar ventral horn (Barajon et al. 1992) but it seems the distribution was more lateral compared to what was observed during locomotion (Dai et al. 2005)

The structure of the CPG for scratch in the cat has been given less attention than that of the locomotor CPG. The activity of identified interneurons (namely the Ia interneurons) (Deliagina and Orlovsky, 1980) and of motoneurons (Berkinblit et al., 1980) has been studied and these and other data were used to elaborate a model for the generation of both the postural and the rhythmic phase of fictive scratch (Shadmehr, 1989).

The extent to which the neuronal circuits comprising the locomotor and scratch CPGs are shared in the cat is unknown at this point. As stated before, the pattern of activity in the scratching limb is similar to that during locomotion in the overall pattern of alternation of flexor and extensor activities. Among the differences, however, is the much shorter cycle period during scratch than locomotion (at least 4-5 times shorter than MLR-evoked fictive locomotion) and the changes occurring in the phase of activity of certain motor

pools (e.g., Plong activity is extensor-like during fictive scratch (Deliagina et al 1981, Lafreniere-Roula and McCrea, 2005,) but flexor-like during fictive locomotion. Differences in the activity of the bifunctional pool PBST have also been observed (Degtyarenko et al. 1998a) although this pool also displays varying patterns of activity during fictive locomotion (Chakrabarty et al. 2003) Importantly, locomotion involves coordinated alternating in all four limbs of the cat while scratch involves rhythmic activity in a single limb while the others are engaged in maintaining appropriate posture. For our purposes, however, we will limit our analysis to a single limb and can thus consider the patterns of activity during scratch and locomotion as quite similar. The occurrence of strict alternation between flexors and extensors during both fictive locomotion and scratch (in the scratching limb) suggests the possibility that both are produced by shared CPG networks. Orlovsky's group made this suggestion based on the observation that there was a continuum between the pattern of activity observed during fictive locomotion and scratch and that the activity of some spinal neurons was similar in both behaviours (Berkinblit et al. 1978). On the other hand, differences in the regions of spinal activity detected with c-fos following (Dai et al. 2005, Barajon et al. 1992) as well as in the modulation of certain afferent pathways (Degtyarenko 1998b) across both fictive locomotor and scratch suggest that they may be only partially overlapping.

An elaborate model of a rhythm generator capable of generating the scratch reflex with this particular sequence of tonic and rhythmic activity has been proposed (Shadmehr, 1989). This model can generate the scratch reflex from a tonic stimulus such as the

nociceptive stimulus which initiates the scratch reflex in the intact cat. The model focuses on the generation of appropriate rhythmic activity and makes no distinction between different agonist motor pools (See Fig. 2 of Shadmehr 1989).

Preparations to study spinal CPGs

Fictive locomotion

Following the contributions of Graham Brown (1911, 1914) and Sherrington (1906, 1910, 1913), a number of reduced cat preparations were developed in Russia in the 1960's. In particular, Drs. Shik and Orlovsky developed preparations in which cats produced co-ordinated locomotor movements of all four limbs while on a treadmill (Shik *et al.*, 1966a,b). The most commonly employed preparation, the decerebrate preparation, consisted of a removal of the cortex followed by a transection of the brainstem which kept most of it intact (precollicular-postmamillary transection). Electrical stimulation of the midbrain in the decerebrate preparation generated appropriately co-ordinated locomotion that persisted until either the electrical stimulation was turned off or the treadmill stopped (Shik *et al.*, 1966b). This "locomotor region" was subsequently located to a particular area of the reticular formation of on the edge of the pons (Shik *et al.* 1967) which later came to be known as the "mesencephalic locomotor region" or MLR (Shik and Orlovskii 1976). It is now known that the MLR corresponds to the cuneiform nucleus which in turn projects to the reticular formation (Steeves & Jordan, 1984; Jordan, 1991). The generation of locomotor activity in this preparation is not dependent on the presence

of rhythmic sensory feedback as was demonstrated by the experiments of Grillner and Zangger (1975, 1984) who observed rhythmic activity in hindlimb nerves in a limb which had been completely deafferented by transection of all related dorsal roots. Perret and Cabelguen (1976) confirmed those results in decorticate cats. Moreover, transection of ventral roots to remove ventral afferents did not prevent the generation of fictive locomotion by MLR stimulation in mesencephalic paralysed cats (Jordan *et al.*, 1979).

In addition to electrical stimulation of the MLR and of other regions of the neuraxis (See for examples Mori *et al.*, 2001, Kinoshita & Yamaguchi, 2001, Mori *et al.*, 1999), pharmacological stimulation can evoke fictive locomotion. It was also in the mid 1960's, this time in Sweden and in particular in Lundberg's group, that the foundations were laid for the study of the pharmacological stimulation of the locomotor CPG. Lundberg and his colleagues showed that intravenous administration of L-DOPA (dopamine and norepinephrine precursor) to spinalized cats was capable of eliciting a locomotor pattern (Anden *et al.* 1966a, Anden *et al.* 1966b, Grillner *et al.*, 1967; Jankowska *et al.*, 1967a; Jankowska *et al.*, 1967b). This capability was later found to be potentiated by adding nialamide to the mix (Grillner and Zangger, 1979). Nialamide works to prevent degradation of norepinephrine

The results during fictive locomotion presented in this thesis were exclusively obtained using MLR stimulation in the decerebrate, precollicular-postmamillary preparation. A major advantage to the use of preparations where fictive locomotion is electrically evoked is that it is possible to cease electrical stimulation and to return to a resting state.

This is much more difficult, if not impossible to truly assess with pharmacological manipulations in a live animal since intravenous administration guarantees that the drug will be distributed and remain for substantial periods of time, longer than the duration of the experiment. Therefore, any changes induced by the drugs can be assumed to be present from administration (allowing time for the drug to take action) and until the end of the experiment. Another difference of fictive locomotion induced by drugs such as DOPA compared to MLR-evoked fictive locomotion is that the step cycle of the locomotion it generates is much longer than that observed during locomotion in intact cats and during MLR-evoked fictive locomotion (Grillner and Zangger, 1979). From this point on, fictive locomotion will refer to MLR-evoked fictive locomotion in precollicular-postmamillary decerebrate and paralyzed cats.

Fictive scratch

The scratch reflex was also studied by Sherrington who elicited it by stimulating the skin in the region of the pinna of the ear and stimulating the cervical spinal cord electrically (Sherrington, 1910). It was later described that curare as well as other irritants applied on the cervical roots were able to potentiate electrical stimulation in animals where that alone did not work (Deliagina *et al.*, 1975). Furthermore, chemical sensitization of the cervical cord was found to be sufficient to render the mechanical stimulation of the pinna successful in eliciting scratch without the need for electrical stimulation (Domer & Feldberg, 1960). The combination of these developments led to the way in which fictive scratch was elicited for this thesis which is mechanical stimulation of the pinna following

curare application on the cervical spinal cord. This is what will be referred to as fictive scratch in the remaining.

Motoneuron activity during fictive locomotion and scratch

Sherrington (1906) recognized motoneurons as the “final common pathway” relaying the information from spinal and supraspinal motor circuits to the effectors of movement, the muscles. The CPG provides rhythmic excitation and inhibition to motor pools which results in observable motor activity. One main objective of this thesis is to understand the nature of this excitation. Motoneurons, however, are more than passive relays. It is now understood that motoneurons derive their firing patterns not only from their synaptic inputs but also from their intrinsic properties. This section will briefly review sources of motoneuron excitation, factors governing motoneuron integration and the translation of motoneuron activity into motor unit firing of those inputs and more recently described state-dependent properties modifying this relationship.

Motoneuron excitation

Motoneurons receive a wide variety of inputs from sensory afferents as well as descending systems. During fictive locomotion and fictive scratch, however rhythmic sensory inflow is prevented by immobilization of the animal. Furthermore, many of the descending inputs are absent due to decerebration. What remains are descending influences from the brainstem which we will review briefly.

Supraspinal projections to motoneurons include monosynaptic projections from the vestibulospinal and reticulospinal tracts (largely uncrossed) and from the corticospinal and rubrospinal tracts (mostly crossed) (Reviewed in Burke and Rudomin 1977). The vast majority of supraspinal input to motoneurons, however, is via interneuronal pathways (See Baldissera et al. 1981 for review). Of particular interest is the role of reticulospinal projections in motor control ever since Lloyd (1941) showed that activation of bulbospinal pathways elicited discharges in spinal neurons and facilitated hindlimb motoneurons. Reticulospinal projections can be classified in 2 main pathways: the medial reticulospinal tract (MRST) originates from the pontine region of the reticular formation (nuclei pontis oralis and pontis caudalis) and projects mostly ipsilaterally via the ventromedial funiculus and the lateral reticulospinal pathway (LRST) originating from the medullary portion of the reticular formation (nuclei gigantocellularis and ventralis) and projecting also mostly ipsilaterally but via the ventrolateral funiculus (Reviewed in Peterson 1979). The MRST directly excites axial musculature and limb flexors and extensors while the LRST has both excitatory and inhibitory connections to the neck motor pools and excitatory connections to back motor pools.

Reticulospinal projections involved in the generation of locomotion

Reticulospinal projections convey signals involved in the initiation and generation of locomotion. Electrical stimulation of 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) have all been demonstrated to evoke locomotor-like movements in the hindlimb of the decerebrate cat and it is thought that the signals originating in the SLR, MLR, and P-BLR are propagated to the spinal cord via reticulospinal projections. Specifically, it is thought that the MLR initiates locomotion through an indirect pathway to the spinal cord proceeding via the medial pontomedullary reticular formation (MRF) (Steeves and Jordan 1984, Garcia-Rill and Skinner 1987a,b reviewed in Jordan, 1991). The MRF in turn projects to the spinal cord within the ventrolateral funiculus (VLF) (Steeves & Jordan, 1980). Cells that are rhythmically active during spontaneous locomotion have been identified in both the MLR and the MRF, supporting the involvement of those regions in the generation of rhythmic behaviour (reviewed in Jordan, 1991).

MLR stimulation to elicit fictive locomotion evokes short-latency postsynaptic potentials in lumbar motoneurons (Shefchyk and Jordan 1985, Degtyarenko et al. 1998a, Noga et al, 2003). These PSPs are modulated in amplitude and sign during the step cycle (Shefchyk and Jordan 1985, Noga et al. 2003) and they are abolished when locomotor activity ceases, either due to cessation of the MLR stimulation or to cooling along the transmission pathway of MLR excitation (Noga et al. 2003). MLR pathways have also been shown to converge with FRA pathways (Shefchyk and Jordan, 1985, Leblond, Menard and Gossard, 2000). Stimulation of the medial longitudinal fasciculus (MLF) also produces both mono- and disynaptic excitation of hindlimb motoneurons (For example, Grillner and Lund, 1968) that are modulated during locomotion (Floeter et al.

1993, Degtyarenko et al. 1998a) but the pattern of modulation may be specific to particular motor pools (Degtyarenko et al. 1998a).

Common drive to motoneurons

The concept of a common drive to motoneurons emerged following early studies of motor unit synchronization (e.g. Milner-Brown et al. 1975, De Luca et al. 1982). The idea behind the common drive is that members of a motoneuron pool are controlled as a group by some common source during contraction based on the observation that the changes in firing rates of homonymous motor units follow each other across different units. The individual firing patterns of motor units are therefore derived not by individual commands to each but rather by the differences in which each responds to the common drive. The source of the common drive could be either peripheral or central and De Luca and Erim (1994) argued that further experiments were required to determine its true source.

CPG common drive: Locomotor and scratch drive potentials as common drive

The locomotor drive potential provides a common drive during fictive locomotion. Rhythmic depolarizations and hyperpolarizations occur in motoneurons during fictive locomotion and this is termed the locomotor drive potential (LDP) (Jordan 1983). The LDP is therefore a central common drive to synergistic motoneurons during fictive locomotion. Descriptions of intracellular activity of motoneurons during fictive

locomotion (e.g. Edgerton *et al.* 1976, Perret and Cabelguen 1980) have established that the membrane potential of individual flexor and extensor motoneurons is modulated throughout the step cycle such that it is most depolarized when the corresponding pool and its agonist pools are firing and most hyperpolarized when those are quiescent and antagonist pools are firing. Similar membrane fluctuations have been observed during fictive scratch (Berkinblit *et al.*, 1980; Berkinblit *et al.*, 1978) and by analogy have been referred to as the scratch drive potential (SDP) (Brownstone *et al.* 1994 and Perreault *et al.* 1999). The common drive provided by the LDP and the SDP is an envelope of excitation. The rhythmic depolarizations and hyperpolarizations of the LDP have similar shapes among motoneurons from a given pool and contribute to constrain the firing to the active phase. This applies to the SDP as well. This type of common drive is different from that provided by the branching of afferents onto multiple motoneurons within a given pool.

Motoneuron firing

Factors governing the relationship between motoneuron input and firing

Given a certain synaptic current, several factors may affect the translation of this excitation into firing by the motoneuron (reviewed in Binder *et al.* 1996). Motoneurons are recruited when their somatic voltage (E_m) reaches threshold (V_{th}). The somatic voltage reached given a fixed incoming synaptic current depends on the neuron's input resistance (R_{in}). Input resistance is a function of cell size, morphology, membrane composition

(which determines membrane resistivity) and active conductances. Increases in synaptic current above that required to reach threshold cause repeated firing whose rate is governed by the frequency-current ($f-I$) relationship.

In order for firing to occur, threshold voltage must be reached at the axon hillock. Several processes govern the propagation of synaptic currents to the soma and will be reviewed here after the discussion presented in the review by Binder and colleagues (1996). The current transferred from the synapse to the soma will depend on the location of that synapse, the geometry of the dendritic tree and the resistivity of the membrane interposed between that synapse and the soma. This current will decrease with increased distance from the soma and with decreased resistivity. One should note that membrane resistivity is not static, especially if one considers that a multitude of synaptic inputs are impinging the motoneuron simultaneously. Synaptic activity decreases resistivity due to the increased synaptic conductances at active synapses. Therefore, paradoxical as it may seem, concurrent synaptic activity results in smaller combined somatic potential increases than would be expected by linear summation. The difference is, however, not large. Sufficient distance between the synapses involved could account for this lack of interaction between synapses.

Whether threshold is reached will depend on the sum total of the synaptic input received by the cell. Motoneurons receive the majority of their inputs from segmental interneurons as well as inputs from a variety of peripheral afferents and descending tracts. The distribution of these inputs will affect which motoneurons in the pool will reach threshold

and be recruited. Recurrent inhibition is also a source of synaptic input to the motor pool and a hypothesis was put forth by Hultborn and colleagues (1979 , see review Hultborn et al, 2004) stating that recurrent inhibition may contribute to setting the gain of a motor pool. Although the hypothesis was met with objections it has not yet been entirely ruled out.

Once threshold is reached, voltage-gated fast sodium channels are activated causing a rapid influx of sodium in the neuron (rising phase of the action potential). Delayed voltage activation of potassium channels combined with the inactivation of sodium channels then cause the membrane potential to repolarize. The prolonged afterhyperpolarization which follows is caused by a calcium-activated potassium current.

Threshold is a dynamic property of motoneurons and has been demonstrated to be lowered during fictive locomotion in the cat (Krawitz et al, 2001). Over the past 25 years, much progress has been made in the determination of intrinsic motoneuron properties such as plateau potentials (Crone et al. 1988) and their modulation and how this affects the transformation of synaptic input into firing (reviewed in Hultborn et al. 2004). Active dendritic conductances, for example, have the power to amplify synaptic input up to five fold and thus contribute significantly to the transduction of synaptic input into motoneuron firing (Lee et al. 2003, reviewed in Heckman, Lee and Brownstone 2003). Intrinsic motoneuron properties are state-dependent. For example voltage threshold is lowered during locomotion (Krawitz et al. 2001) as is the post-spike

afterhyperpolarization (Brownstone et al. 1992, Schmidt 1994) lowering is mediated by monoamines

Methods to study motoneuron firing

The study of neural circuitry for the control of movement in humans has benefited from the use of cross-correlation and coherence analyses which are now being used to study the CPG circuitry for locomotion in the cat (Hamm and McCurdy 1995, Hamm et al. 2001, Nielsen et al. 2005). Cross-correlation is a technique to analyze the relationship between two spike trains, whereby the times of occurrence of action potentials in one spike train are compared to those in the other train in order to identify synchronized events. The results of cross-correlation is also referred to as the peristimulus time histogram (PSTH), pre and post correlogram and other variations of those names. In all cases, it is simply a histogram of the time delays between spikes in two trains, with the pairs of spikes considered being determined by the time window chosen. The presence of a significant peak of short-term synchrony between the spike trains is thought to indicate underlying common input between the two neurons in question. Characteristics of the histogram peak are thought to reflect the amplitude and shape of the common synaptic input (Reviewed in Kirkwood, 1979). The significance of the correlation can be evaluated using indices of synchrony. Two such indices that are widely used in the field (e.g., Turker and Powers, 2001) are the E index and the CIS index. Both indices offer a measurement of the spike count in the peak which exceeds what would be expected to occur by chance. The E index (Datta and Stephens, 1990) is expressed as $E = [A - (\mu \times$

$B_p)/R$ where A is the number of 4 added spikes in the peak, μ is the average number of spikes per bin in the histogram, B_p is the number of bins in the correlogram peak and R is the number of reference events used to compile the histogram. The CIS index (Nordstrom et al., 1992) by comparison, is normalized to the duration of the spike train used for the PSTH and is thus expressed as $CIS = [A - (\mu \times B_p)]/T$ where T is the duration of the spike train used to compile the histogram.

In addition to describing the relationship between the timing of action potentials in two spike trains, one can describe the relationship between frequency domain measures of those spike trains. One of the most commonly used measure, coherence can be used to infer the frequency content of the common input and it can reflect the nature of the common input between the cells (See Rosenberg et al. 1998 for details).

Coherence and correlation analysis have been used during fictive locomotion in the cat to elucidate certain aspects of CPG organization. The work of Hamm and colleagues in particular has focused on frequency domain analysis of composite recordings of nerve activity. Using ENG recordings and coherence analysis, they reported correlation between many motor pools suggesting that several pools, even some with actions at different joints, receive common drive from the CPG during fictive locomotion and scratching (Reviewed in Hamm et al. 2001). More recently, studies by Nielsen and colleagues (2005) have confirmed correlations between the activity of motor pools acting at a single joint. They have not, however, witnessed as widespread correlations as Hamm and colleagues, and no correlation between muscles acting at different joints. This is at

least in part attributable to the fact that both groups used different preparations for eliciting fictive locomotion, namely brainstem electrical stimulation in the case of Hamm and colleagues and DOPA in the case of Nielsen and colleagues. Both groups have acknowledge that the presences of the MLR could provide potentially synchronizing input that would increase the correlations observed but Hamm did report that such widespread correlations were also observed during spontaneous locomotion. Definite conclusions thus remain to be drawn.

Thesis Overview

The goal of this thesis is to contribute to the understanding of the structure of the CPG in general and specifically of the last-order interneurons and their projections to motor pools. Our approach is to examine both subthreshold (postsynaptic potentials) and suprathreshold (action potentials) events in motoneurons under different conditions of fictive movement including fictive locomotion and scratch as well as during deletions.

The results will be presented in two parts, each taking the form of a manuscript. Paper #1 will address the hypothesis that locomotor modulated short-latency MLR-PSPs are affected by deletions of ENG activity. This will be accomplished by a comparison of MLR-PSPs during locomotion and during deletions. Paper #2 will address the extent to which the common drive provided by the CPG during fictive locomotion and scratch contributes to the synchronization of motoneuron firing within a pool and in synergists.

II. Paper 1: Effects of deletions on the locomotor modulation of MLR-PSPs during fictive locomotion

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Abstract

Electrical stimulation of the mesencephalic locomotor region (MLR) elicits fictive locomotion in the decerebrate cat and also gives rise to stimulus-locked, locomotor-modulated postsynaptic potentials (PSPs) in lumbar motoneurons. We examined MLR-PSPs during spontaneous deletions of activity occurring during fictive locomotion. Deletions of ENG activity were accompanied by changes in the amplitude of the locomotor drive potential as well as changes in MLR-PSP amplitude. Deletions in agonist motoneuron activity were accompanied by a decreased depolarized phase of the LDP and by a decrease in the excitatory component of the MLR-PSPs. Conversely, deletions in antagonist activity were characterized by a decreased hyperpolarized phase of the LDP and decreased inhibitory component of the MLR-PSPs. These results give further support to the hypothesis that MLR-PSPs are mediated through lumbar interneurons involved in the rhythmic depolarization and hyperpolarization of motoneurons during locomotion. Furthermore, our results show that MLR stimulation during fictive scratch also produces phase-modulated PSPs in lumbar motoneurons. In turn, this suggests that the CPGs for locomotion and scratch behaviours may share common elements.

Introduction

Electrical stimulation of the brainstem in the mesencephalic locomotor (MLR) region of the decerebrate and immobilized cat elicits fictive locomotion consisting in rhythmic nerve activity resembling that occurring during normal over ground locomotion. This is

accompanied by rhythmic depolarizations and hyperpolarizations of the motoneurons in the corresponding motor pools that were termed locomotor drive potentials (LDP) (Jordan 1983). In addition, stimulation in the MLR region gives rise to short-latency, stimulus-locked post-synaptic potentials in lumbar motoneurons (Shefchyk and Jordan, 1985). The latencies of the earliest components of these effects are consistent with a disynaptic segmental link although oligosynaptic effects are also present (Degtyarenko et al. 1998a, Noga et al. 2003). In a large number of cases, the sign and amplitude of these MLR-PSPs are modulated throughout the locomotor cycle such that excitatory MLR-PSPs are observed during the depolarized phase of the LDP while inhibitory MLR-PSPs are observed during the hyperpolarized phase (Shefchyk and Jordan, 1985, Degtyarenko et al. 1998a, Noga et al. 2003). This suggests that the spinal interneurons mediating MLR-PSPs are also involved in the production of the LDP and thus, of the locomotor output. Additional support for this idea was obtained from experiments where cooling was used to reversibly prevent activity and transmission along the pathways involved in MLR-evoked locomotion (Noga et al. 2003). In these experiments, cooling was found to result in the disappearance of MLR-PSPs in addition to the cessation of fictive locomotor activity (Noga et al., 2003). These disruptions, however, were reversible and both locomotion and MLR-PSPs resumed when cooling was stopped.

Spontaneous disruptions of locomotor output can occur during fictive locomotion without any experimental intervention (Duysens 1977, Grillner and Zangger 1979, Jordan 1991, Lafreniere-Roula and McCrea 2005). These disruptions, which we termed deletions (Lafreniere-Roula and McCrea, 2005), consists of the absence of the expected rhythmic

ENG burst in one or more agonist during otherwise normal rhythmic motor output. Activity in antagonists during deletions is usually sustained and sometimes modulated although the periods of quiescence normally present in antagonist pools are absent. These events, called deletions, consist in the absence or great reduction of the ENG burst amplitude for short periods of time, followed by a return to normal fictive locomotor activity. A previous study from our laboratory (Lafreniere-Roula and McCrea, 2005) revealed that the absence of ENG activity during deletions usually occurs across a number of synergist motor pools and that it is accompanied by similarly widely distributed sustained activity in antagonist motor pools. We also reported that deletions in ENG activity can be accompanied by changes in the amplitude of the LDP and that despite sometimes large effects on LDP amplitude, deletions can occur without any significant alteration of the cycle period of the locomotor activity (Lafreniere-Roula and McCrea 2005). The disruption caused by deletions is temporary and normal fictive locomotion reappears spontaneously as well.

The disappearance of MLR-PSPs during spinal cooling which disrupts fictive locomotion (Noga et al., 2003) has led us to postulate that MLR PSPs will also be affected during spontaneous deletions. Moreover, the relationship between MLR-PSP sign and the phase of the locomotor cycle during normal locomotion suggests that the effects of deletions on MLR-PSPs will be related to the motor pools affected (i.e. agonists or antagonists). In order to test these hypotheses, paired intracellular motoneuron recordings were performed during fictive locomotion and MLR-PSPs were compared during normal locomotion and during deletions. To further investigate the link between the production

of motor output and MLR-PSPs, MLR stimulation was combined with stimulation to elicit the fictive scratch reflex in order to see whether time-locked PSPs could be observed in this behaviour as well.

Methods

Decerebrate cat preparation

Surgical and experimental protocols complied with guidelines of the Canadian Council for Animal Care and the University of Manitoba. Detailed methods were described in Lafreniere-Roula and McCrea (2005) Briefly, 9 adult cats were anaesthetised with a mixture of halothane (1-2%), nitrous oxide (70%), and oxygen (30%) using a face mask. Atropine (0.05 mg/kg s.c.), saline (10cc s.c.) and dexamethasone (2mg/kg i.v.) were administered. The left femoral and the right jugular veins were cannulated for drug administration. Blood pressure was monitored using a transducer in the right carotid. A buffer solution (5% glucose, 0.84% bicarbonate solution) was infused throughout the experiment to maintain blood pH and to replace lost fluids. CO₂ levels and respiratory rhythm were monitored via a sensor inserted into the tracheotomy tube.

The left hindlimb was subjected to extensive nerve dissection (SmAB, PBSt, LGS, MG, TA, EDL, Plong, FDHL, Tib, and SP) for recording and stimulation using bipolar silver ball electrodes in a mineral oil pool. Left hip flexor nerves (Sart, Quad) were recorded

and stimulated using a multi-compartment cuff electrode. Adductor tendons of both hips were cut and the right hindlimb was completely denervated.

Following laminectomy (L4 to L7) the cat was transferred to a stereotaxic frame. A craniotomy was performed and followed by a mechanical decerebration involving removal of the cerebral cortex followed by a transection of the brainstem at the post-collicular, pre-mamillary level. Anesthesia was discontinued following decerebration and the animal was paralysed (Pancuronium bromide, 0.1 mg/kg per hour) and ventilated. Bilateral openings in the chest wall were made to minimize mechanical movement due to respiration during intracellular recordings. Animal body temperature was kept normal using a heating pad underneath the belly and feedback-controlled heating lamps.

Stimulation and recording

A two-hour delay was observed between the end of decerebration and the start of brainstem stimulation. The MLR was stimulated using monopolar with square current pulses (0.5 ms, 50-500 μ A, 10-20 Hz). MLR stimulation was either unilateral or bilateral. Stimulation parameters were optimized based on the locomotor output produced but kept constant within each run of data captured. The upper range of current intensity was seldom used. It was sometimes useful to swing the forelimbs in order to initiate rhythmic activity. In addition to fictive locomotion, fictive scratch was elicited by touching the pinna of the ear or the lateral aspect of the face of the cat following the application of a curare solution (0.01-0.1%) on the C1 dorsal roots.

Filtered (30 Hz - 3 kHz) and rectified ENGs were digitized (500 Hz sampling rate) and recorded along with intracellular recordings (5-10 kHz sampling rate). Capture and analysis were done using in-house software (Spinal Cord Research Center) running on a Pentium PC under Linux Redhat operating system. Intracellular recordings of motoneurons were performed using sharp glass electrodes (1.6-1.9 μm) connected to an Axoclamp 2A amplifier (Axon Instruments Inc, California, USA) and filled with 1.5 M sodium citrate solution. Simultaneous paired recordings from antidromically identified lumbar motoneurons were made possible by the use of two, independently moving, motorized microdrives. Cord dorsum potentials were recorded using bipolar silver ball electrodes.

Data analysis

For each recording analyzed, an effort was made to identify a control period where locomotor activity was not elicited by the MLR stimulation in order to determine that the MLR-PSPs examined were locomotor specific. Total and segmental latencies of MLR-PSPs were measured using the recording of the MLR current pulse and of the onset of CDP. Total latency was measured from the stimulus artifact from the MLR pulse and segmental latency was measured from the peak of the first wave of the CDP. ENG onsets were used to perform cycle-based averages of AC-coupled intracellular recordings of MLR-PSPs (10 bins per step cycle) were performed during normal fictive locomotion to examine the modulation of PSP amplitude during the locomotor cycle. Trace amplitude was measured at the peak of the earliest excitatory and inhibitory components. Traces

were included in a given bin if the first 10 ms following the MLR stimulus occurred entirely within that particular portion of the step cycle. Deletions of nerve activity were identified by visual inspection. Deletions were selected for analysis if they consisted of a sudden reduction of ENG amplitude and were followed by a return to normal locomotion. Traces occurring during deletions were grouped according to whether they occurred within the extensor or flexor phase if sufficient rhythmic activity remained to establish the active and inactive phases. Otherwise, traces were averaged throughout the deletion or examined individually. Amplitude measurements of MLR-PSPs were performed on trace averages pooled in two groups, one for each phase of the step cycle. Significance of modulation was established by a t-test between trace amplitude measurements on the individual traces during the active phase and during the inactive phase. Unless otherwise specified, all values are reported as the mean \pm standard deviation.

Results

Data was collected from 9 adult female cats (2.7 ± 0.3 kg). Results during fictive locomotion are based on recordings in 21 motoneurons (11 paired recordings). Details regarding motoneuron species are provided in Table 1. On average, the resting membrane potential prior to activity was -57 mV (SD=13 mV). In addition, MLR stimulation during fictive scratch was performed in 2 of the animals and three additional motoneuron pairs were recorded during this type of stimulation.

- Table 1 here -

Patterns and latencies of MLR-PSPs observed

We examined MLR-PSPs in 19 motoneurons during stimulation of either or both sides of the MLR (iMLR: n=5 cells, cMLR: n=9 cells, bilateral MLR: n=4 cells). One cell was examined twice, once with iMLR stimulation and another time with cMLR stimulation and the response to MLR stimulation was different in both cases (data not shown). Thus, there were a total of 18 observations. The patterns of MLR-PSPs was purely excitatory in 61% of cases (11 of 18 observations), purely inhibitory in 33% of cases (6 of 18 observations) and mixed in the remaining case.

The average latency recorded at the lumbar cord dorsum (CDP) from the artifact of the electrical shock delivered in the midbrain was 3.2 ms (n=9 measurements, one per pair, SD=0.2ms). The segmental latency of the initial MLR-EPSPs from this CDP recording was 1.8 ms (n=12, SD=0.6 ms) and that of initial MLR-IPSPs was 2.0 ms (n=7, SD=0.6 ms). The average segmental latencies for a second distinct component of MLR-PSPs were 6.4 ms (n=6, SD=3.6 ms) and 10.5 ms (n=1) for MLR-EPSPs and MLR-IPSPs respectively. These latencies are within the range of previously reported values (Noga et al., 2003)

Modulation of MLR-PSPs during the step cycle

We observed single and double distinct components of MLR-PSPs almost equally often (9 of 18 for single, 8 of 18 for double). In one case there were even three components observed. In almost two thirds of observations (11 of 18), there was at least one MLR-EPSPs present during the active phase of fictive locomotion that was either decreased in amplitude during the inactive phase (8 of 11) or were replaced by an MLR-IPSP (3 of 11). There was a single observation of MLR-IPSP during the active phase which became larger during the inactive phase. In the remaining 6 observations, there was either no modulation or only slight modulation of MLR-PSP amplitude, likely attributable to the effects of rhythmic fluctuations in membrane potential (LDP) during the step cycle

Effect of agonist deletions on MLR-PSPs

- Figure 1 here -

In all cases, we were able to make comparisons between periods of normal locomotion and deletions. Observations during deletions were focused on the early components of MLR-PSPs as the longer latency components are more difficult to interpret. In general terms, deletions of agonist activity resulted in a reduction in the size of MLR-EPSPs. Figure 1 shows an example of the effects of extensor deletions on an MLR-PSPs recorded in an extensor motoneuron during normal fictive locomotion. Figure 1A shows the ENG and intracellular recording of MG248 during normal fictive locomotion where the

rhythmic activity of flexors (TA, EDL) and extensors (LGS, MG) was regular and alternating. Figure B shows an expanded view of a single step cycle (top panel) and the normalized average cycle (bottom panel). Note that individual MLR-PSPs alternate between EPSPs during the depolarized phase and IPSPs during the hyperpolarized phase (vertical traces above top panel). The reversal from excitatory MLR-PSPs to inhibitory MLR-PSPs during locomotion is further illustrated by the cycle-triggered trace averages shown in panel C. MLR-EPSPs during extension (top 2 traces) were replaced by inhibitory MLR-PSPs during flexion. Figure 1D shows the graph of trace amplitudes measured at two points corresponding to the peak of the MLR-EPSP during the active phase (top graph) and the peak of the MLR-IPSP during the inactive phase (bottom graph). These points are indicated in panel C by the vertical dashed lines labeled c and d respectively. At both time points, the amplitude of the MLR-PSP in the MG₂₄₈ motoneuron was positive during the depolarized phase (extension) and negative during the hyperpolarized phase (flexion). Figure 1E shows an example of a deletion of extensor activity (dashed box) that occurred during constant MLR stimulation and while recording from the motoneuron. Flexor activity remained rhythmic and only small depolarizations were observed in conjunction with flexor quiescence (red arrows). Based on this rhythmic activity, traces occurring during the deletion were averaged in two groups, namely the poorly developed extensor phase and the flexor phase. Figure 1F shows the comparison of those averages with similar averages obtained during normal locomotion such as that illustrated in panel A. During the extensor phase, MLR-EPSPs were reduced to approximately half the normal amplitude (compare the red trace to the black one). During the flexor phase, no changes were observed. Thus, during the extensor deletion,

the decreased depolarization of the MG motoneuron was accompanied by a decrease in the amplitude of MLR-EPSPs.

Of 6 MLR-PSPs recorded in which excitation during the active phase was replaced by inhibition during the inactive phase, 4 were examined during deletions of agonist activity. All four displayed reduced or absent excitatory components. In 3 cases, only IPSPs were observed during the failed active phase of the deletion. In the fourth case, small EPSPs were still present. Two modulated MLR-PSPs were examined during deletions of antagonist activity and are discussed below.

Effect of antagonist deletions on MLR-PSPs

- Figure 2 here -

Deletions of activity in antagonists resulted in a decrease of the inhibitory component of MLR-PSPs. Two extensor motoneurons displaying reversing MLR-PSPs during normal fictive locomotion were examined during deletions of flexor activity and results are shown in Figure 2. Panel A illustrates the period of fictive locomotion during which two deletions of flexor activity (TA, EDL) occurred (dashed boxes) while extensor activity was sustained. During the deletion, action potentials were abolished in the one cell that was firing prior (MG₂₃₄, action potentials truncated in the leftmost cycle of activity illustrated in panel A) but the membrane potential of both extensor motoneurons (MG₂₃₄ and Plant₂₃₃) remained depolarized during the active phase. Slight rhythmic decreases in

membrane potential were observed in the intracellular recordings (red arrows) which were accompanied by slight drops in the tonic extensor nerve activity (LGS, MG, red arrows). Panels B to D illustrate the MLR-PSPs in the Plant₂₃₃ motoneuron. MLR-PSPs were excitatory during extension and inhibitory during flexion as shown in the raw individual traces (panel B, top) and in the cycle-based average (panel C). During deletions of flexor activity, the inhibitory component of the MLR-PSP disappeared leaving only the excitatory component. This is shown in panel D where the averages of the traces during the two deletions shown in Fig. 2A (black traces) were overlaid with the average during the most depolarized phase of the LDP (blue trace) and the average during the most hyperpolarized phase (green trace). Note how the MLR-PSP during deletions (black traces) are similar to those during normal extension (blue trace) and lack the inhibitory component present during fully developed flexion (green trace). Similar results are illustrated in panels E to G for the second motoneuron in the pair, MG₂₃₄. Figure 2E shows a single step cycle during normal locomotion where no firing occurred and MLR-PSPs could be best observed. Note the reduction of the excitatory MLR-PSP at the transition between the depolarized and hyperpolarized phases of the LDP. This is observed more clearly in the cycle-based averages (panel F) from which traces where action potentials occurred were excluded. The overlay of averaged MLR-PSP during deletions (black traces) shown in panel G shows that in this cell also, the inhibitory component present during fully developed flexor activity (green trace) was absent during flexor deletions.

All MLR-PSPs observed which showed reversal during normal locomotion also showed decreased excitatory components during deletion of agonist activity and decreased inhibitory components during deletions of antagonist activity. Moreover, as reported previously (Noga et al., 2003), a number of MLR-EPSPs were examined that, while not reversing, were modulated in amplitude throughout the step cycle. In general, those MLR-PSPs were also decreased in amplitude during deletions of agonist activity. Some examples will be presented in the following figures.

MLR-PSPs in simultaneously recorded pairs of motoneurons

- Figure 3 here -

The combination of excitatory and inhibitory components and whether those are modulated during locomotion varies between motoneurons. We examined MLR-PSPs in 5 pairs of simultaneously recorded extensor motoneurons (4 pairs of MG motoneurons and 1 pair of plant motoneurons) and observed differences in the patterns of MLR-PSPs even in closely located homonymous motoneurons. Figure 3 illustrates two examples of our recordings. Figure 3A and 3B presents the locomotor modulation of MLR-PSPs in two pairs of MG motoneurons. In one case, one member of the pair had an MLR-IPSP which was larger during extension (top overlay, MG₈₉, blue trace) than flexion (green trace) but remained inhibitory in both phases. In contrast, the other MG motoneuron recorded simultaneously, and thus under the same conditions of MLR stimulation, had slightly excitatory MLR-PSP during extension (bottom overlay, MG₉₀, blue trace) which

became inhibitory during flexion (green trace). Thus, although both MLR-PSPs were modulated during the step cycle, they were quite different since excitation was completely absent in one case. The second pair recorded is shown in panel B. In this case, both MG motoneurons (MG₂₄₉ and MG₂₄₈) displayed excitatory MLR-PSPs during extension (panel B, blue traces) although they had different patterns (EI for MG₂₄₇ and EE for MG₂₄₈). In both cases, the excitatory component was decreased during flexion and particularly so in MG₂₄₈. Both pairs were also examined during deletions of extensor activity. Pair #1 was examined during an extensor deletion which was accompanied by tonic flexion and the resulting averages (black) are overlaid with averages during each phase of the normal locomotor cycle (blue for extension, green for flexion) in panel C. In MG₈₉, the MLR-IPSP present during fully developed extension (blue trace) was reduced in amplitude during the extensor deletion (black trace). In MG₉₀, the slight MLR-EPSP normally present during extension (blue trace) was abolished and replaced by an MLR-IPSP during the deletion of extension. Similarly in pair # 2, a deletion of extensor activity gave rise to a reduction of excitation during the extensor phase (panel D). In pair #2, rhythmic flexor activity was maintained during the extensor deletion and it was thus possible to average traces separately in both phases (dashed black for rhythmic flexion and solid black for poorly developed extension). Averaged MLR-PSPs were similar in both phases and showed a larger inhibitory component than had been observed during normal extension (panel B).

Similar to reversing MLR-PSPs, MLR-EPSPs that were modulated in amplitude throughout the step cycle without reversing (i.e., larger EPSPs in the depolarized phase and smaller EPSPs in the hyperpolarized phase) also showed a decrease in amplitude

during deletions of agonist activity (i.e. a decreased excitatory component). Conversely, during deletions of antagonists, they showed increased amplitude. Such changes occurred in the majority of observations (75%). The remaining modulated PSPs showed slight or no changes during deletions.

MLR-PSPs during fictive scratch

- Figure 4 here -

Six additional motoneurons were examined during a combination of MLR stimulation and fictive scratch. MLR stimulation during scratch gave rise to CDPs which were comparable to those obtained during fictive locomotion as well as cycle-modulated MLR-PSPs. The combination of MLR and scratch stimuli gave rise to a variety of intermediate patterns of nerve activity where the alternation of flexor and extensor activities remained but the cycle duration and the patterns of activity of specific motoneuron pools were variable.

Figure 4 shows two examples of the resulting activity. Figure 4A illustrates how MLR stimulation (10 Hz) initiated during a period of fictive scratch led to a delayed switch to locomotor-like activity. Following the start of MLR stimulation scratch activity persisted for approximately 1 second. CDPs in response to MLR stimulation appeared immediately when MLR stimulation was initiated (data not shown) although the nerve and motoneuron activity remained consistent with scratch for a short time. Following a transition period, proper fictive locomotion emerged with an appropriately longer cycle

period. Plong nerve activity transitioned from extensor-like during scratch to flexor-like during locomotion. In another instance where MLR and scratch stimulation were combined, the changes in cycle parameters were gradual and occurred while rhythmic alternation persisted (data not shown). In that case, the onset of Plong activity was delayed starting immediately after the start of MLR stimulation and the delay progressively increased over the first 10 cycles following the start of MLR stimulation until Plong active became completely in phase with flexor activity. The cycle period also gradually increased following the start of MLR activity and reached values consistent for fictive locomotion.

MLR-PSPs were present in lumbar motoneurons during combined MLR and scratch stimulation. Figure 4C shows a pair of motoneurons which were recorded both during fictive locomotion (Fig. 4B, left panel) and fictive scratch (Fig. 4B, right panel). During both fictive locomotion and while MLR stimulation was performed simultaneously to stimulation eliciting fictive scratch, CDPs of similar latency and amplitude were recorded (top traces of panel C, blue for extension and green for flexion). During locomotion, the MG₂₅₉ motoneuron was firing during extension (middle left overlay, blue trace) and an MLR-EPSP remained during flexion (green trace). These are shown, for comparison, with MLR-PSPs recorded in the same cell during MLR-stimulation but prior to the development of full locomotor activity (black trace). Clearly, the MLR-PSPs are much larger during locomotion than during MLR stimulation which does not elicit motor output. The middle right panel shows the MLR-PSPs recorded in the same cell but during fictive scratch. They are much smaller than those during fictive locomotion, more on par

with the amplitudes recorded in the control condition without motor output (black trace). Nevertheless, they are clearly modulated during the scratch cycle, with mostly excitatory components during extension (blue trace) and some reversal to inhibition during flexion (green trace). The bottom panels illustrate the results obtained for the other motoneuron of this pair, PBST₂₅₈. During locomotion, modulated MLR-EPSPs were present. During fictive scratch, smaller MLR-PSPs were present and were larger than those observed during the control condition without locomotor output. These PSPs also seemed modulated, especially the later component which was larger during extension (blue trace) than during flexion (green trace).

Discussion

In this study, MLR-evoked postsynaptic potentials were compared during normal fictive locomotion and during spontaneous deletions of activity in the decerebrate, paralyzed cat. Consistent with previous findings (Shefchyk and Jordan 1985, Degtyarenko et al. 1998a, Noga et al. 2003), we observed MLR-PSPs that were modulated in amplitude during the step cycle and where excitation during the active (depolarized) phase was replaced by inhibition during the inactive phase. The main finding was that the amplitude of these locomotor-modulated MLR-PSPs was reduced during spontaneous deletions of ENG activity. Specifically, decreases in the amplitude of the depolarized phase of the LDP which led to deletions were accompanied by a decrease or a disappearance of the amplitude of the excitatory component of all phase reversing MLR-PSPs (Figure 1). These spontaneous changes in MLR-evoked excitation occurred against a background of constant MLR stimulation. Similarly, deletions of antagonist activity that led to a decreased hyperpolarized phase of the LDP were associated with a reduction or disappearance of the inhibitory component of the MLR-PSP. Similar changes were also observed in the majority of those MLR-PSPs that were modulated in amplitude during the step cycle but whose sign did not change during the inactive phase of the LDP. These findings are in agreement with previous work showing that reversible cooling, which completely abolished locomotion, also abolished MLR-PSPs (Noga et al. 2003). The present study extends those observations to spontaneous reductions in locomotor output by showing that MLR-PSPs are affected for the brief duration of the deletion. This observation is further evidence for convergence of the generation of excitation to the

motor pool during rhythmic motor output and the transmission of phase-reversing MLR-PSPs.

We cannot rule out a passive component to the modulation of MLR-PSPs. In fact, some of the MLR-PSPs we observed were only passively modulated. The effects of passive modulation due to membrane potential fluctuations are likely to be small, however, especially for EPSPs. Furthermore, the presence of actively modulated net MLR-PSPs suggests that the locomotor modulation of MLR-PSPs components is obscuring any passive modulation. We may therefore be underestimating the magnitude of the locomotor modulation if we take into account that the membrane fluctuations likely cause some small magnitude passive effects as well.

Phasic conductance changes occurring during fictive locomotion could affect the amplitude of MLR-PSPs. Modeling studies have shown, however, that conductance changes may only have limited influence on PSP amplitude. For example, for EPSPs occurring at distances consistent with the synapses for Ia afferents onto motoneurons, doubling of the conductance resulted in only small changes in EPSP amplitude (McCrea, Shefchyk and Carlen 1990).

As outlined in the General Introduction (Fig.1), our observations on cycle timing maintenance during spontaneous deletions (Lafreniere-Roula & McCrea 2005) led to the development of a novel scheme for the organization of the locomotor CPG. In this scheme, the rhythmic depolarization of motoneurons during locomotion is produced by

rhythmic activity in excitatory neurons within the Pattern Formation layer of the CPG. According to our computational model of the CPG developed from this scheme (Rybak et al. 2006) deletions of motoneuron activity occur with reductions in the excitability of PF layer neurons that consequently reduce the size of the depolarizing component of the LDP. Another postulate of our scheme is that descending systems that initiate locomotion activate PF (and other) layer interneurons. Consequently, MLR-evoked activation of these PF interneurons should produce short latency (i.e. disynaptic as measured from the lumbar CDP) MLR-EPSPs in motoneurons during the active locomotor phase. The present observations showing the reduction of amplitude of the phase-modulated MLR-evoked PSPs during deletions are thus consistent with our hypothetical CPG organization.

In the present study, only cells with MLR-PSPs were chosen for analysis. While no attempt to was made to assess the prevalence of MLR-PSPs in motoneurons, it is clear from this and previous studies (Degtyarenko et al. 1998a, Noga et al. 2003) that MLR-PSPs are absent from some motoneurons despite the fact that those cells have well developed LDPs (see however, Shefchyk & Jordan 1985). The absence of fully modulated reversing MLR-PSPs would appear to be inconsistent with the mediation of MLR-PSPs through PF layer neurons.

There are several possible explanations for an absence of MLR-PSPs in the presence of well developed LDPs that could accommodate our hypothesis about PF-layer mediation of MLR-PSPs. First, MLR-PSP characteristics may be related to the exact location of

brainstem stimulation. In this study, no attempt was made to standardize the location of stimulation within the brainstem across experiments. Electrode placement in the brainstem was dictated by the presence and robustness of locomotor behavior. CDP recordings were used only to assess the effect of stimulation and to obtain a reference for the measurement of segmental latencies of synaptic potentials. Unlike a previous study concerning MLR effects in the spinal cord (Noga et al. 1995), the MLR site was not optimized to maximize the excitation observed from the CDP. Given that several regions of the brainstem can elicit fictive locomotion (e.g., pontobulbar locomotor region, ventral tegmental field of the caudal pons, reviewed in Jordan 1991) it is possible that locomotion was elicited in some of the present experiments by indirect activation of the MLR or by activation of downstream structures such as the medial reticular formation. The activation of other descending systems in conjunction with MLR stimulation could have produced synaptic effects in motoneurons that obscured or overwhelmed more direct MLR (i.e. midbrain-reticulospinal) effects. Similarly, the recruitment of strong, non-phase dependent PSPs in motoneurons could obscure detection of phase-dependent modulation of MLR-evoked PSPs.

Second, there appears to be heterogeneity within the MLR itself. For example, stimulation of more dorsal portions of the MLR preferentially elicits fictive locomotion in the forelimbs (Amemiya and Yamaguchi 1984). It is therefore possible that, in some cases, MLR effects in lumbar motor pools were mediated indirectly via propriospinal projections from the rostral spinal cord (forelimb CPG).

A third possible explanation for the lack of MLR-PSPs may be a high excitability of the spinal interneurons mediating MLR-effects. According to our hypothesis that PF-layer neurons mediate MLR-PSPs and according to arguments raised in previous studies (Burke et al 2001, Noga et al. 2003), interneurons mediating MLR-effects should be rhythmically active during the cycle phase when motoneurons are depolarized. High interneuron excitability and rapid firing rates (e.g. via the activation of state-dependent intrinsic properties) could reduce the ability of the MLR stimulation to reliably recruit stimulus-locked action potentials in the interneurons. Thus, if interneuron firing is controlled and dominated by CPG network activity or intrinsic repetitive firing, stimulus-locked MLR-evoked spiking may fail and few MLR effects would be seen in the motoneurons. Averaging would further obscure detection of the few MLR-PSPs that were synchronized to the stimulus delivery and there would be no or only small stimulus-locked responses recorded in motoneurons.

Another and perhaps more interesting explanation, is the possibility that there are alternate descending pathways mediating MLR excitation to motoneurons with only some of those pathways being directly involved in the production of motor output. Thus MLR-PSPs in motoneurons could consist of locomotor-related phase modulated components as well other effects unrelated to locomotion. Evidence to support this hypothesis comes from our observation that differences in MLR-PSPs observed in different cells are not only preparation dependent (i.e. brainstem electrode placement). Our finding that MLR effects recorded in pairs of closely located homonymous motoneurons under similar conditions may be different (see Figure 3) is an important observation in this regard. A

shortcoming of the present data set, however, is that the membrane potential of cells within pairs is sometimes quite different (several millivolts). Differences between MLR-PSPs in homonymous motoneuron pairs should be further examined. For example, those differences may be related to specific physiological differences between the impaled motoneurons such as the muscle fibre type (eg. slow vs fast fatigable) innervated by homonymous motoneuron pool members remains unknown. Finding the basis for differences between MLR-PSPs in homonymous motoneuron pairs would be worthy of further exploration in future studies

Lastly, we have shown that MLR stimulation during the scratch reflex can produce a variety of intermediate rhythmic motor output. Moreover, we have preliminary evidence that modulated MLR-PSPs can also be observed during the generation of fictive scratch. Although they are of smaller amplitude than those recorded during fictive locomotion in the same cells (Fig.4) they may be different than those observed during MLR stimulation in the absence of any motor output. Our observation of a CDP in response to MLR stimulation during is at odds with previous reports (Degtyarenko et al. 1998b) in which MLR stimulation following the initiation of fictive scratch ceased to evoke MLR-PSPs and cord dorsum potentials during scratch. While we have no explanation for these discrepancies, our findings suggest that at least in some circumstances, MLR stimulation evokes qualitatively similar effects during fictive locomotion and scratch. In turn this is further support for the suggestion (Gelfand et al. 1988, Perreault et al 1999; Gosgnach 2003, Lafreniere-Roula & McCrea 2005) that the locomotor and scratch CPG networks share common features and neurons.

In summary, our results show that MLR-PSPS with a clear phase dependent modulation during locomotion are reduced during spontaneous deletions. In these cases, the disappearance of MLR-evoked activity in lumbar neurons that project to agonist motoneurons during deletions might be used as a part of the electrophysiological identification of PF-layer excitatory interneurons (discussed in Rybak et al 2006). The data presented is consistent with our hypothesis that last-order pattern formation interneuron mediate both locomotor drive and short-latency MLR-PSPs. We cannot rule out that last-order interneurons outside of the pattern formation layer may also be receiving convergent input from the locomotor CPG as well as be in the short-latency MLR pathways to motoneurons.

Figure legends

Figure 1. Deletions of agonist result in decreased MLR-EPSPs

A. ENG and intracellular recording of an MG motoneuron (MG_{248}) during fictive locomotion. Note the alternation of flexor (TA and EDL) activities with that of extensors (LGS, MG). MG_{248} was not recruited for firing during this episode but displayed well developed LDPs (about 8 mV). The lack of action potentials allowed the examination of MLR-PSPs. *B.* Top panel: Expanded view of a single cycle of activity in MG_{248} with individual MLR-triggered traces on top (vertical traces) showing the time-locked responses to MLR stimulation which were excitatory during the depolarized phase of the LDP and inhibitory during the hyperpolarized phase. Bottom panel: Normalized average of activity in MG_{248} during normal locomotion showing well developed LDP (average cycle=776 ms, SD=37ms). *C.* Average CDP recording and cycle-based average of intracellular traces triggered on the MLR stimuli. The top trace shows the average CDP at the onset of each current pulse delivered to the MLR, traces of short duration (typically 30 ms) were captured and averaged according to the portion of the step cycle in which they occurred. In this example, extension (MG) occupied 20% of the step cycle and the top 2 bins are therefore averages of traces occurring during the extensor phase while the bottom 8 traces are averages during flexion. The average of the CPD trace is also shown. The filled triangle at the bottom indicates the time of MLR stimulation. Vertical lines indicate (a) the onset of the CDP, (b) the onset of the MLR-EPSP (central latency from CDP recording = 1.3ms), (c) the peak of the MLR-EPSP during extension and (d) the peak of the MLR-IPSP during flexion. *D.* Graphs of average peak amplitude of MLR-

PSPs. Measurements of MLR-PSP amplitude were taken at the peak of the excitatory response measured at point c shown in panel C(top graph) and at the peak of the inhibitory response measured at point d shown in panel C (bottom graph) and averaged throughout the normalized step cycle. showing the cycling between EPSPs during the depolarized phase of the LDP and IPSPs during the hyperpolarized phase (compare with panel B, bottom graph). *E.* ENG and intracellular recordings during a deletion of extensor activity (dashed box). During the deletions, LDPs in the extensor motoneuron were reduced but present (red arrows) and flexor activity remained rhythmic with intervening quiescent periods (red arrows) in between activity bursts. *F.* The average of the MLR-PSPs occurring during deletions (red traces) were compared to those occurring in the control period of normal locomotion (black traces). The remaining rhythmic activity (decreased LDP, flexor rhythmic activity) was used to separate the cycle into the active (extensor deletion) and inactive (flexor phase) phases. During the failed extensor phases (smaller LDPs indicated by red arrows in MG₂₄₈ in panel E), the amplitude of the excitatory MLR-PSP was decreased to about half of its value during normal extension (compare red trace to black trace in top overlay labeled extensor phase). During the flexor phase, which remained rhythmic during the deletion, there were no apparent changes in the response to MLR stimulation during the flexor phase (bottom overlay).

Figure 2. Deletions of antagonist activity result in decreased MLR-IPSPs

A. ENG and intracellular recordings in a pair of extensor motoneurons (MG_{234} and $Plant_{233}$) during fictive locomotion in which two deletions of flexor activity occurred (dashed boxes). Action potentials are clipped on the MG_{234} trace. Following normal rhythmic locomotor activity, deletions of flexor activity occurred that lasted a few (2-3 seconds) each. Tonic extensor ENG activity remained during the deletions and was slightly modulated as observed either by slight decreases in ENG activity (red arrows on LGS) or by slight drops in the tonic depolarization of the motoneurons (red arrows over the MG_{234} and $Plant_{233}$ recordings). *B-G* MLR-PSPs in both cells during a period of normal fictive locomotion lasting 6 seconds and the deletions illustrated in panel A. Both cells had well developed LDPs and displayed a reversal of excitatory MLR-PSPs to inhibitory MLR-PSPs throughout the cell cycle (panels B and E for raw traces, extension occupying 69% of the step cycle). The reversal from excitation to inhibition is also apparent on the cycle-based averages presented in panels C and F. Filled triangle indicates the time of MLR stimulation as in figure 1. Vertical lines indicate the onsets of (a) the CDP and (b) the MLR-EPSP (central latency of 1.7 ms for both motoneurons). The comparison of MLR-PSPs averaged during the active phase (extension – blue traces) and the inactive phase (flexion – green traces) of normal locomotion and during the deletions of flexion (black traces) is shown in panels D and G for $Plant_{233}$ and MG_{234} respectively. In both cases, the inhibitory component that was present during normal flexion (green) was absent during deletions (black traces, one for each of the deletions shown in panel A). In fact, during the deletions of flexion, extensor activity was sustained

and the MLR-PSPs during this sustained extensor activity (black) were similar to those during normal extension (blue).

Figure 3. MLR-PSPs can be variable even in homonymous motoneurons

Comparison of MLR-PSPs in two pairs (Pair #1: *A-C*, Pair #2: *B-D*) of homonymous MG motoneurons during (*A-B*) normal locomotion and (*C-D*) extensor deletions. *A*. MLR-PSPs were dissimilar in Pair #1 where MG₈₉ displayed MLR-IPSPs throughout the locomotor cycle (passively modulated, IPSPs larger during the depolarized phase) while MG₉₀ showed modulated PSPs (MLR-IPSPs during flexion and a small EPSP or no PSP during extension). In Pair #2, one motoneuron MG₂₄₇ had similar MLR-PSPs during both phases of the step cycle while MG₂₄₈ had locomotor modulated and reversing MLR-PSPs. *C*. In both cells, MLR-PSPs were inhibitory during extensor deletions (black traces) and were very similar to the MLR-IPSPs that had been observed during the flexor phase of normal locomotion (blue traces). *D*. During deletions where pair #2 was recorded, some rhythmic flexor activity was maintained which allowed the averaging of traces occurring during deletions to be grouped by phase of activity. MLR-PSPs were however similar in both phases (compare dashed and solid black traces) and in both cells. In MG₂₄₇ the inhibitory component was increased during the extensor deletion (the average during normal locomotion were overlaid in muted colors for comparison). In MG₂₄₈ the inhibitory component was also increased and the excitatory component decreased.

Figure 4. MLR-PSPs can be evoked during scratch

A. ENG and intracellular recordings of a pair of PBST motoneurons during fictive scratch alone (indicated by the white box) and during combined fictive scratch and MLR stimulation (shaded box). Patterns of nerve activity were typical of fictive scratch even for a short time (about 1 sec) following the initiation MLR stimulation. A transition period during which there was tonic extensor activity (MG) was followed by a locomotor-like rhythm. Note the striking difference in the pattern of activity of Plong during fictive scratch (extensor-like) and locomotion (flexor-like). Both PBST motoneurons had extensor-like activity and were tonically depolarized during the transition period. *B.* ENG recordings during fictive locomotion (left panel) and fictive scratch (right panel) evoked a few seconds apart while recording from another pair of motoneurons. *C.* Comparison of CDPs (top overlays) and MLR-PSPs evoked during the fictive activity shown in panel B. Fictive locomotion averages (left side) and combined MLR and scratch stimulation averages (right side) show that comparable CDP recordings were obtained in both cases. Averaged MLR-PSPs are compared to an average control MLR-PSP recorded during MLR stimulation that did not evoke nerve activity (black traces). Both cells had clear excitation during the extensor phase in locomotion and this excitation was reduced during the flexor phase. In both phases, MLR-PSPs were clearly distinct from the non-locomotor control PSP. During the combined stimulation of the MLR and of the pinna of the ear for scratch, MLR-PSPs were more subdued, and in the MG motoneuron, comparable to the non-locomotor control. Nonetheless, they were clearly modulated throughout the step cycle with the excitatory component larger during

extension and the inhibitory component larger during flexion. In the PBST cell, MLR-PSPs during scratch were mostly inhibitory during in both phases.

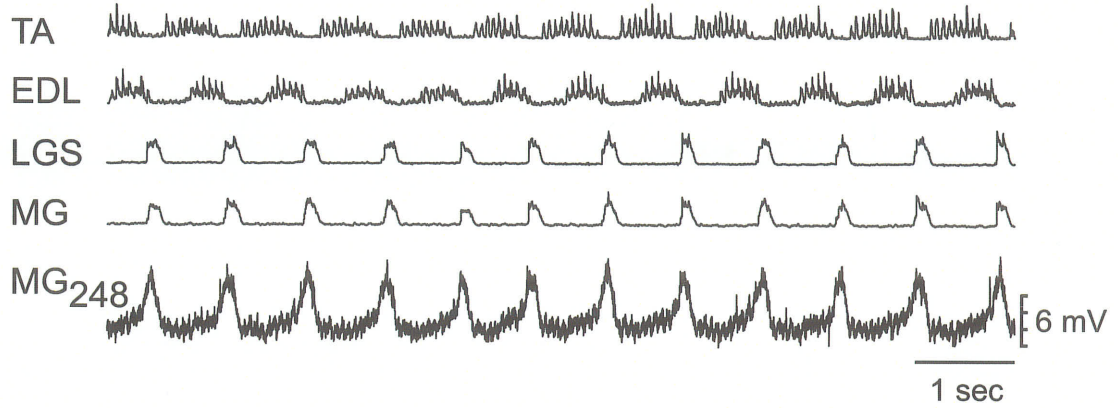
Tables

	pair		Em	rest	Em	rest
	number	cME	cME	rME	rME	
Homonymous	38	mg90	-67	mg89	-44	
	39	mg90	-41	mg91	-49	
	40	mg92	-63	mg93	-67	
	60	plant140	-58	plant139	-84	
	109	mg247	-49	mg248	-72	
Synergists	69	lgs154	-59	fdh1155	-69	
	105	plant233	-81	mg234	-60	
	113	mg248	-73	ext252	-50	
	119	pbst258	-52	mg259	-44	
Antagonists	18	mg41	-53	pbst42	-51	
	81	mg182	-38	edl183	-42	

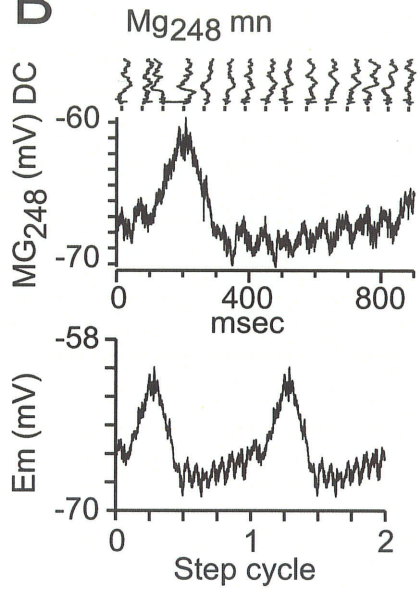
Table 1. Motoneuron resting potentials in the caudal microelectrode (cME) and the rostral microelectrode (rME).

Paper 1 - Figure 1

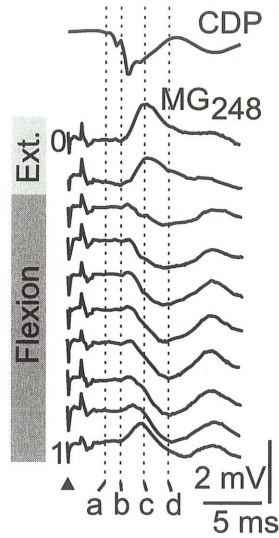
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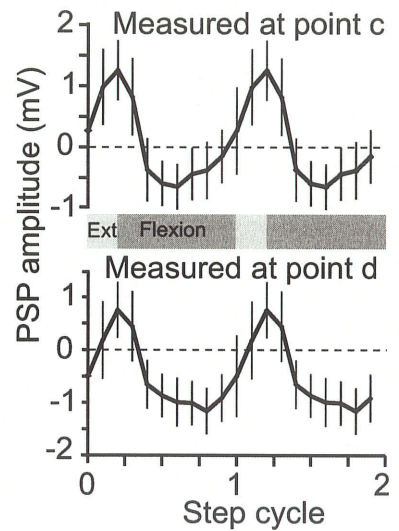
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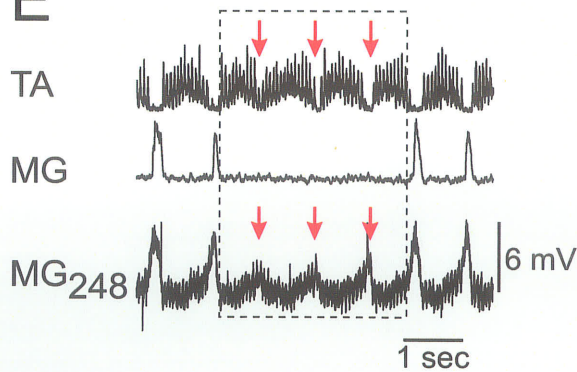
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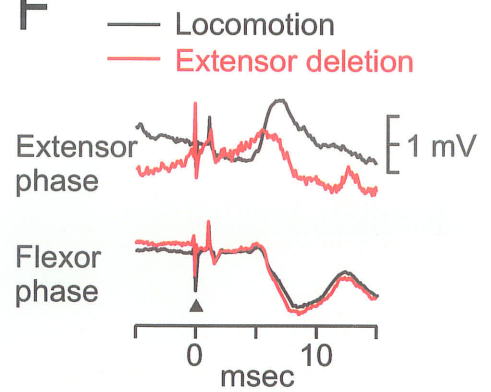
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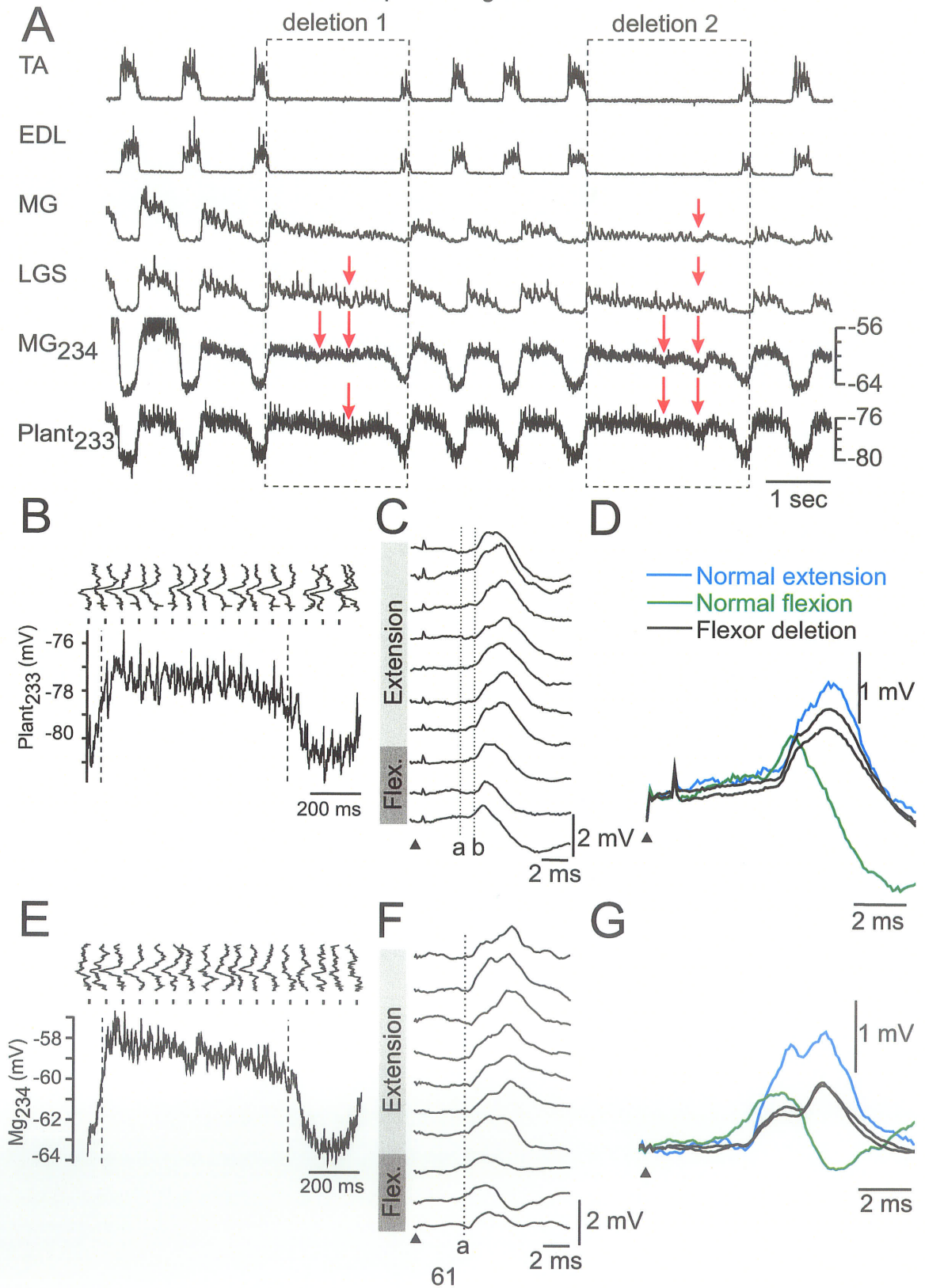
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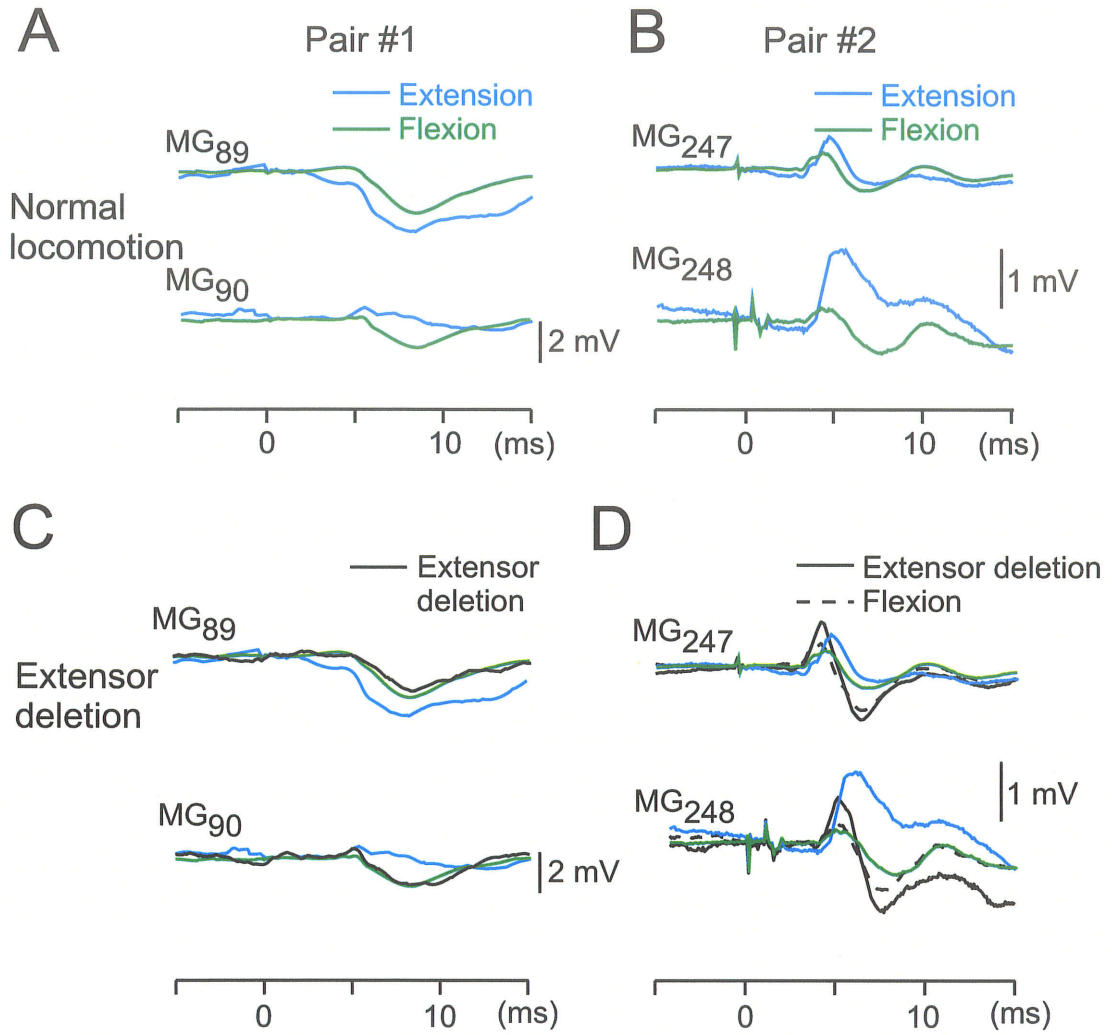
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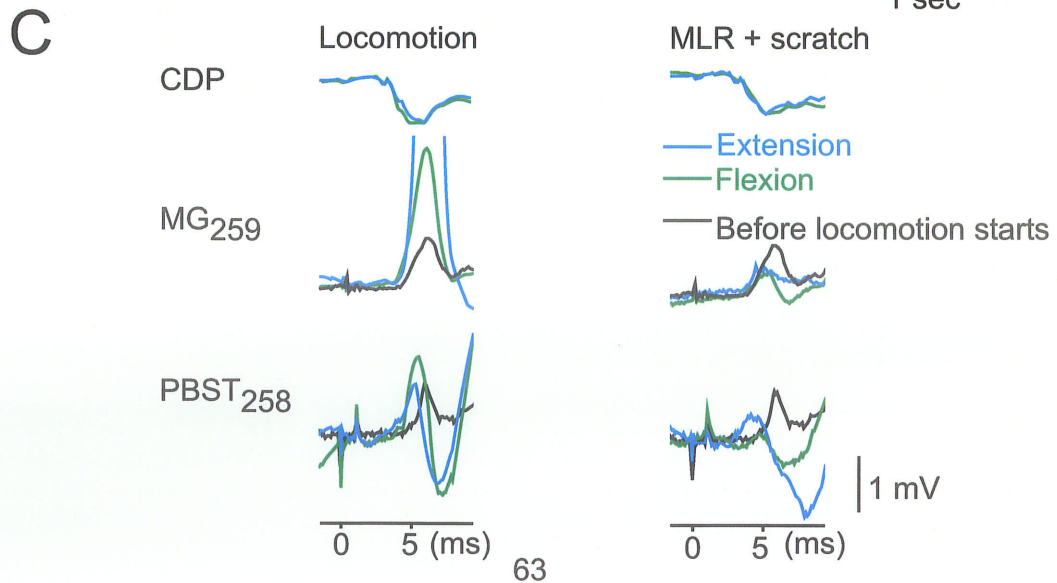
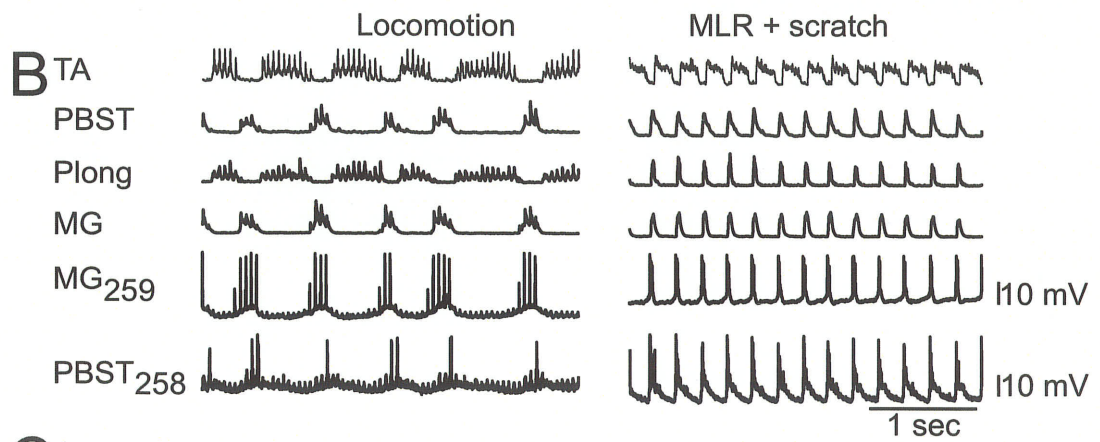
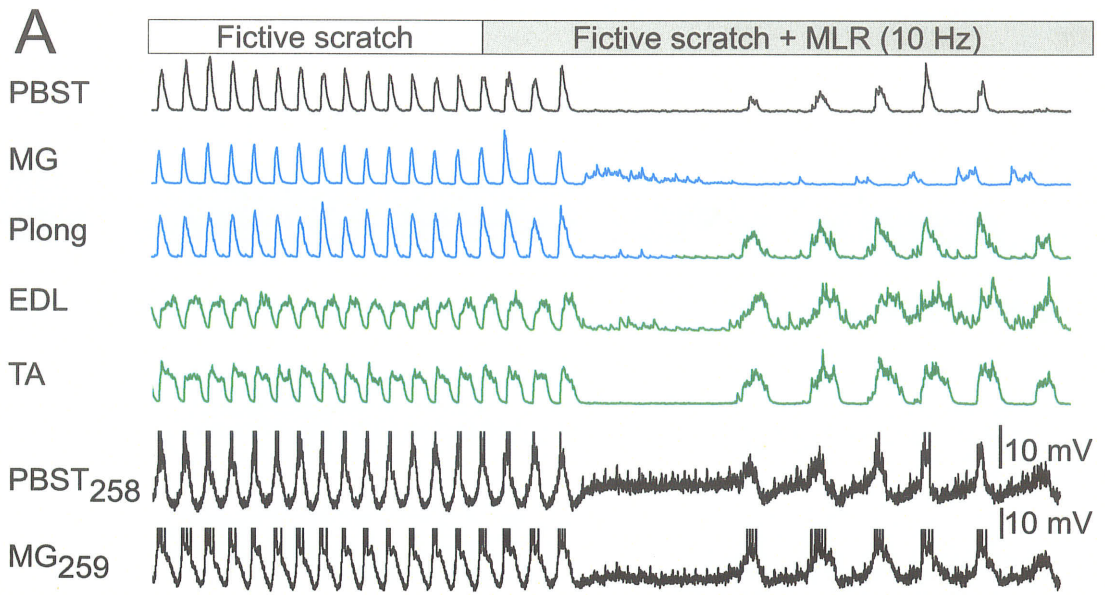
Paper 1 - Figure 2



Paper 1 - Figure 3



Paper 1 - Figure 4



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III. Paper 2: Lack of firing synchrony in homonymous motoneurons during fictive locomotion and scratch in the cat

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Abbreviated title: Firing synchrony during locomotion and scratch

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Abstract

Previous studies have suggested the presence of correlated common synaptic input to synergist motoneurons during fictive locomotion in the cat. The present study addresses whether this common drive also gives rise to a significant correlation between the timing of action potential occurrence in homonymous motoneurons. Intracellular recordings from pairs of motoneurons were used to detect short-term firing synchronization and the existence of synchronized EPSPs in homonymous motoneurons during fictive locomotion evoked by electrical stimulation of the brainstem. Fictive scratch was also studied since it is evoked by a combination of chemical and mechanical stimulation and therefore in the absence of potentially synchronizing electrical input. Cross-correlation and spike-triggered averaging failed to reveal significant synchronization of motoneuron firing in pairs either during fictive locomotion or scratch. Despite the possibly synchronizing effect of continuous electrical stimulation of the brainstem, the degree of synchronized firing between motoneurons during fictive locomotion remained low. This was also observed during fictive scratch which is not dependent on rhythmic electrical stimulation. Additional analysis that was limited to the initial spike in each cycle was performed to minimize the effects of preceding action potentials on the membrane trajectory. This also failed to reveal significant synchronized firing during either fictive locomotion or scratch. These results suggest that the common drive provided by the CPG to motoneurons is limited to an envelope of excitation but does not include the tight determination of the firing times of individual motoneurons. The synchrony observed in other preparations may be a reflexion of the differences in experimental preparation and analysis.

Introduction

Motor unit synchrony has been studied in a variety of motor tasks in human and is viewed as a possible strategy for the control of force during voluntary contraction. The study of correlation during human walking and cat fictive locomotion has been used to determine the extent of the common drive to the various motor pools. During human walking, analysis of motor unit firing suggests some level of synchrony especially in synergist muscles acting at a particular joint (Hansen et al. 2001). Generally, the firing synchrony observed in humans is attributed to the effects of the corticospinal tract (Hansen et al., 2005). Locomotion, however, can in many species, be generated in the absence of cortical control by the activation of a spinal central pattern generator (CPG). The structure of the mammalian CPG remains unclear and how closely the CPG controls the exact timing of motoneuron firing and could thus contribute to synchronizing motoneuron firing is also unknown.

Fictive behaviours such as fictive locomotion and scratch represent ideal preparations to study the operation of the CPG in isolation. Synchronization of firing during fictive locomotion in the cat has been studied both in the time (correlation) and frequency (coherence) domains. Correlation analysis examines the temporal relationship between spike trains while coherence analysis compares the frequency components of two signals. Frequency domain analysis of ENG or combinations of one ENG and one intracellular recording in the decerebrate cat during electrically evoked fictive locomotion has suggested that there is correlated firing between synergist motoneurons (Hamm, Trank

and Turkin 1999). This has been supported, for motoneurons of synergistic pools acting at one particular joint, by correlation analysis during chemically evoked fictive locomotion in the spinal cat (Nielsen et al. 2005). In the work of Nielsen and colleagues (2005), coherence analysis was performed in spinalized cats in pairs of ENG recordings during fictive locomotion induced by nialamide and DOPA. Significant coherence at frequencies below 50 Hz was observed, both in pairs of synergists and antagonists, but characteristic features indicating the presence of short-term synchronization were only present in pairs of close synergists. Furthermore, correlation of synaptic activity using pairs of intracellular motoneuron recordings in the presence of blockers of fast sodium channel to prevent firing, did not detect significant coupling other than what is attributable to the slow depolarizations and hyperpolarizations that motoneurons undergo during locomotion (locomotor drive potentials, LDPs) (Nielsen et al., 2005). Hence, there seems to be no evidence for short term synchrony of synaptic inputs to homonymous motoneurons. To our knowledge, however, no direct analysis of firing in pairs of single units during spinally generated behaviours in the cat has been reported to date.

In order to test the hypothesis that motoneurons receive branching inputs from the CPG capable of producing short-term synchrony during fictive behaviours, we performed correlation analysis of firing in simultaneous intracellular recordings of extensor motoneurons pairs during fictive locomotion evoked by electrical stimulation of the brainstem and during fictive scratch. Bursts of extensor activity in the scratching limb are very brief (about 50 ms) and their onsets are near simultaneous across synergists. This should favor the detection of synchronized firing. In addition to the analysis of spike

trains, we performed spike-triggered average analysis in order to detect possible subthreshold EPSPs synchronized to firing activity in the other cell. Lastly, in addition to examining full spike trains, we also analyzed specifically the initial action potential of each cycle in order to circumvent the activation of voltage-gated conductances and their potential effects on the timing of motoneuron action potentials.

Consistent with previous reports of low correlation of synaptic activity in homonymous motoneurons (Nielsen et al. 2005), our results will show that there is relatively little short-term synchrony of action potentials in homonymous pairs during both fictive locomotion and scratch. Preliminary results have been presented in abstract form (Lafreniere-Roula and McCrea, 2002, Lafreniere-Roula and McCrea, 2003).

Methods

Animal surgery

All surgical and experimental protocols were in compliance with guidelines of the University of Manitoba and the Canadian Council for Animal Care. Surgical methods have been described in detail elsewhere (Lafreniere-Roula and McCrea 2005). Data was collected from 7 purpose-bred adult cats who were anesthetised using a mixture of halothane (1-2%), nitrous oxide (70%), and oxygen (30%) delivered initially via a face mask and later via a tracheotomy tube. The left femoral and the right jugular veins were cannulated for drug administration as well as for continuous infusion of a buffer solution

(5% glucose, 0.84% bicarbonate solution; 5 ml/hr). Blood pressure was monitored using a transducer connected to the right carotid circulation and CO₂ levels and respiratory rhythm were monitored as well.

Nerves of the left hindlimb were dissected and mounted for recording and stimulation using bipolar silver ball electrodes in a mineral oil pool. These included: semimembranosus and anterior biceps, SmAB; posterior biceps and semitendinosus, PBSt; lateral gastrocnemius and soleus, LGS; medial gastrocnemius, MG; the combination of LGS and MG, GS; tibialis anterior, TA; extensor digitorum longus, EDL; peroneus longus, Plong; flexor digitorum and hallucis longus, FDHL; the mixed tibial nerve innervating plantar foot structures, Tib. Ventrally located cuff electrodes were used to record from hip flexor (sartorius, Sart) and knee extensor (quadriceps, Quad) nerves. Adductor tendons were cut and the right hindlimb was denervated.

Following a laminectomy exposing segments L4 to L7 of the spinal cord, the cat was transferred to a stereotactic frame where body temperature was maintained using a combination of a heating pad and radiant heat lamps. Removal of the cerebral cortex was followed by a transection of the midbrain at the precollicular-postmamillary level and discontinuation of the anesthetic as well as administration of a neuromuscular blocker (pancuronium bromide, 0.1 mg/kg per hour) and artificial ventilation. Bilateral openings in the chest wall were used to minimize respiratory movements. The dorsal aspect of the cervical spinal cord was exposed at C1 for topical application of curare in order to elicit fictive scratch as described below.

Stimulation

Fictive locomotion was induced using a combination of unilateral or bilateral stimulation of the mesencephalic locomotor region of the brainstem with 0.5 ms duration current pulses (50-500 μ A, 10-20 Hz). Fictive scratch was evoked by mechanical stimulation of the pinna of the left ear or of the lateral aspect of the left side of the face after application of a cotton pledget soaked in a curare solution (0.01 or 0.1% solution) on the left C1 dorsal roots.

Hindlimb ENG recordings were filtered (30 Hz - 3 kHz), rectified and integrated before digitization at 500 Hz. Intracellular recordings (digitized at 5 or 10 kHz) of antidromically-identified motoneurons were performed using sharp glass electrodes (1.5 M sodium citrate, tip size between 1.6-1.9 μ m) and an Axoclamp 2A amplifier (Axon Instruments Inc, California, USA). Two independent positioning devices and microdrives were used to achieve simultaneous paired intracellular recordings. Signals were captured and analyzed using in-house software (Spinal Cord Research Center) running on a Pentium PC under the Linux operating system.

Data analysis

Action potentials were identified using a combination of automatic threshold detection and visual verification. Peristimulus time histograms (PSTH) were computed using the in-house Analysis software (± 20 ms window, bin width of 1ms). The firing train with the

least firing was defined as the trigger train while the other was defined as the response. For each action potential in the trigger train, the response train was examined over the ± 20 ms window and action potentials from the response train within that window were binned in 1ms bins. The PSTH was plotted as the probability of firing in the response train for each bin. The probability of firing is defined as the probability that an action potential will be observed at a given time lag from the trigger. For example, if $p=0.05$ it indicates that 5% of the trigger action potentials were accompanied by an action potential in the response train at the given time lag. Spike triggered averaging was also used to detect possibly synchronized subthreshold events (EPSPs).

Significance of the peak of the PSTH was assessed using the k ratio after the method described in Sears and Stagg (1976). Briefly, k is the ratio of the counts in the peak over the mean counts in the control region. The peak was defined as the single bin with the highest probability of firing and the control as all the other bins.

Two commonly used indices of synchrony were also calculated, namely the E index and the CIS index. Both offer a normalized measurement of the spike count in the peak which is in excess of what would be expected by chance. The E index (Datta and Stephens, 1990) is expressed as $E = [A - (\mu \times B_p)] / R$ where A is the number of added spikes in the peak, μ is the average number of spikes per bin in the histogram, B_p is the number of bins in the correlogram peak and R is the number of reference events used to compile the histogram. The CIS index (Nordstrom et al., 1992), by comparison, is normalized to the duration of the spike train used for the PSTH and is thus expressed as $CIS = [A - (\mu \times$

$B_p)/T$ where T is the duration of the spike train used to compile the histogram. Peak width was defined as 1 bin.

RESULTS

The data presented were obtained from 7 adult cats (2.7 kg SD = 0.2). Results are based on 20 pairs of simultaneous intracellular recordings (39 different motoneurons) collected during either MLR-evoked fictive locomotion (10 pairs) or fictive scratch (10 pairs). When both motoneurons of the pair fired, cross-correlation analysis was performed (4 pairs during locomotion, 6 pairs during scratch). In the remaining pairs, only one motoneuron was firing and spike-triggered averaging was used to detect synchronized subthreshold events.

Correlation during MLR-evoked fictive locomotion

- Figure 1 here -

Correlation analysis performed during fictive locomotion failed to reveal significant peaks of short-term synchrony. Figure 1 shows two examples of MG motoneuron pairs examined for synchronized firing. Figure 1A shows an example of a period of fictive locomotion during which two MG motoneurons were rhythmically firing. Spike-triggered averaging based on the MLR-stimuli (Fig. 1B) shows that both motoneurons received synchronized excitation from the MLR at a latency consistent with what has been

demonstrated for MLR-EPSPs during locomotion (Shefchyk and Jordan, 1985, Degtyarenko et al., 1998, Noga et al., 2003). A peristimulus time histogram was computed using the spike trains of the two motoneurons illustrated in Figure 1A over a period of 12 seconds and is shown in Figure 1C. Correlated firing caused by branching inputs would appear as a peak around time zero. In this case, there was clearly no peak at time zero since several other bins at various time delays had the same probability of firing as the center bin. The firing pooled in a 2 ms time window around the center comprised only 7% of the total firing observed during the period analyzed. The k-test confirmed that the peak was not significantly larger and the E and CIS indices indicated low probability of additional firing in the center bin compared to the rest of the histogram (see values in Table 1). PSTH analysis was performed on an additional 3 homonymous pairs of extensor motoneurons during fictive locomotion with similar results (Table 1 for values).

- Table 1 here -

Pairs of homonymous motoneurons where a single cell fired were examined using spike-triggered averaging (6 pairs). An example of a pair of MG motoneurons examined is shown in Figure 1, panels D and E. Figure 1D shows an excerpt of the locomotor period used. Figure 1E shows overlaid traces triggered at the MLR stimulus. In this case, as in the pair illustrated in Figure 1A-C, both cells responded to the MLR stimuli by either action potentials or MLR-EPSPs. In the cell which was recruited for firing these responses were larger while in the other motoneuron (MG93) MLR-EPSPs were smaller

in amplitude. Thus, overall, despite the presence of MLR-PSPs the correlation of firing was not very strong in the pairs examined during fictive locomotion.

Correlation during fictive scratch

In order to investigate firing synchrony in the absence of potential synchronizing inputs, correlation analysis was performed using 6 homonymous pairs recorded during fictive scratch (details in Table 1). This analysis also failed to reveal significant synchronization of firing.

- Figure 2 here -

Figure 2 shows an example of cross-correlation analysis in a pair of MG motoneurons during fictive scratch. Figure 2A shows ENG and intracellular recordings during the entire bout of scratch. Each bout of fictive scratch starts with a period of tonic flexor activity (e.g. see TA) which corresponds to a postural phase serving to position the hindlimb appropriately for scratching. The tonic flexor phase is normally followed by fast, rhythmic alternation of flexor and extensor activity, in this example ongoing for 5-7 seconds. Note the similar onset and termination of ENG activity recorded in the extensor motoneurons pools (SmAB, LG, MG) during each scratch cycle. In this example of fictive scratch the average duration of extensor activity recorded in the ENG was 186.4 ms (SD = 8.7 ms). The activity of two MG motoneurons was also recorded during this bout of scratch. Figures 2B and 2C show these recordings at greater magnification. Figure 2B shows a short sequence of 6 cycles of activity. The action potentials were clipped for

better viewing of the scratch drive potential (SDP). Note the similarity of onset and termination times of depolarization in these 2 motoneurons. The shape of the SDP is also similar in both cells. As Figure 2C shows, however, the timing of individual action potentials in the 2 neurons was not synchronized. This lack of synchronization is reflected in the PSTH illustrated in Fig. 2D and computed from the firing times of the motoneurons during the phasic part of the fictive scratch reflex (Fig. 2A, dashed box). In this example 110 spikes in MG 12 were used to generate the histogram. If spikes in the 2 motoneurons were evoked on the rising phase or peak of a commonly occurring scratch CPG-generated EPSP, one would expect a strong narrow peak in the PSTH around time zero. There was no such peak of short-term synchrony observed. In this example, the summed firing in the 2 bins surrounding time zero (i.e. a 2ms epoch) represents only about 8% of the firing. Thus only a small proportion of the firing was tightly correlated in the two motoneurons.

- Figure 3 here -

Figure 3 shows another example of cross-correlation analysis in pair of pbst motoneurons. The data in Fig. 3A was computed using 49 trigger spikes from a single bout of fictive scratch and like the example in Fig. 2, show little evidence for strongly correlated spiking activity in the two motoneurons. The firing within a 2 ms time window around zero comprised 7% of the total firing. Similarly, the firing which occurred in the 2 ms window around the peak (located at the -2 ms bin) also comprise a similar percentage of the total firing, namely 8%. Figure 3B shows the PSTH constructed from the data in Fig. 3A and 3 additional bouts of fictive scratch evoked within 2 minutes of each other

(total number of trigger = 121) while recording from the same pair of PBSt motoneurons. Again, there is no evidence for a significant peak of short-term synchrony observed. The inclusion of a much larger number of spikes did not change the qualitative appearance of the histogram.

- Figure 4 here -

Figure 4 shows PSTHs obtained from another 4 pairs of homonymous motoneurons during fictive scratch. Again, in all cases, k-test analysis did not reveal significant peaks of short-term synchrony. The PSTH in Fig 4B does seem to have a peak at 4 ms post-trigger. This peak, however, occurs against a background of little spiking activity (data not shown). This peak was not significant using the k-test and the strength of synchrony in this pair as calculated by the E and CIS index was well within the boundaries of what was observed in the other pairs (See Table 1). This is at least partly due to the fact that the E and CIS are normalized measures which take into account either the number of triggers (E) or the length of the interval (CIS).

Similar analysis as presented in Figures 2 and 3 was performed in an additional 4 pairs of synergist motoneurons combining extensors (plant-mg, unidentified ext-mg) or bifunctional motoneurons active during extension (pbst-smab, pbst-tib). The differences in the trajectories of the SDP between motoneurons in such pairs gave rise to time-shifts of the PSTH peak. No significant peak of short-term synchrony was observed in those pairs (data not shown).

Overall, PSTHs computed over a 40 ms window to match the active phase of extensors during fictive scratch showed action potentials distributed over that interval. There was no peak of short-term synchrony detected. Some of the histograms showed a tendency for increased firing around zero time lag but that can simply be attributed to the shape of the scratch drive potential. In extensors during scratch, the SDP is fairly narrow and this the time period over which threshold can be reached reduced thus forcing action potentials to occur over a narrow time period. Moreover, the peak heights remained low, ranging in firing probability between 0.05 to 0.23 This is consistent with the fact that the percentage of action potentials occurring in the 2 ms window of each trigger was often less than 10%.

Analysis of initial spikes during fictive scratch

- Figure 5 here -

It is well known that firing is not entirely determined by synaptic input. Intrinsic properties of motoneurons such as active conductances can have strong effects on the initiation of firing as well as the inter-spike interval and thus intrinsic differences between cells could affect synchrony between spike trains. In order to minimize the effects of such confounding factors we performed spike-triggered averaging using only the first (initial) spike within each cycle of fictive scratch in 5 of the 6 homonymous pairs listed in Table 1. Figure 5 shows an example of this analysis on the pair of MG

motoneurons which was presented in Figure 2. Figure 5A shows an overlay of portions from the intracellular recordings showing the closeness of the SDP profiles in the 2 MG motoneurons (spikes clipped for better viewing of the SDP and scales adjusted to normalize SDP amplitude) As shown before in Figure 2D, the distribution of spiking intervals in the 2 cells is broad and approximates the duration of firing during each scratch cycle. This is again the case as shown in Figure 5B (top panel) where the trigger spike train was chosen to be MG13. The bottom panel of Figure 5B shows the PSTH obtained from using only the first-occurring spikes in the each scratch cycle (indicated by arrows in panel A). The histogram of the first-spike analysis shows that the delay between initial spikes varies within a 10 ms window around the zero lag point. Figure 5C shows the superposition of traces triggered using the first spike in MG13 (top traces). Although some action potentials in the second motoneuron (MG12) occur near the trigger spikes in MG13 (red traces), most of first spike firing in the second cell (MG12) occurs several milliseconds later. In this example in 34% of the cycles examined (11 of 32 cycles) there was an action potential synchronized to the trigger spike. In the remaining cycles however, there was none.

Figure 6A shows the initial spike analysis for the same pair of PBSt motoneurons as presented in Figure 3. Figure 6A shows overlaid intracellular recordings from the two motoneurons and, as in Fig.5, illustrates the strong similarities in the shapes of SDPs within a given pool. As illustrated in previous figures, the correlation histogram from all spikes (upper histogram in panel B) in this homonymous motoneuron pair does not show a strong, short latency synchronization. The first spike histogram shows that the first

spike in the second (blue) motoneuron is consistently delayed from that in the other cell (6B, lower histogram). The traces in Fig. 6C are the intracellular records from PBST₁₀ triggered from the spike in PBST₁₁. This spike-triggered analysis was used to detect any subthreshold depolarizing events (EPSPs) that were nearly synchronous with activity in one cell and that might have led to spiking in the second cell. The traces are grouped according to the presence of such short-latency, sub-threshold, depolarizations occurring in the individual sweeps. In this example, an initial EPSP was detected within a 2 ms window in 26% of cycles (action potential in 3 of 38 cycles shown in the top grouping and EPSP in 7 of 38 cycles shown in the middle grouping). Note, however, that these EPSPs are of variable latency and small amplitude. Only in 3 sweeps (top group) did it appear that a short latency EPSP might have given rise to an action potential. In the majority of cases, the action potentials in the second motoneuron occurred either well after the peak of the EPSP (middle group) or without a preceding EPSP locked to the occurrence of the action potential in the other motoneuron.

Overall, using first-spike analysis we detected synchronized events (EPSP or action potential, top or middle grouping in Figure 6) in as little as 5% of cycles or as much as 34% of cycles. Thus, including subthreshold events in our analysis as well as looking specifically at initial spikes increased the maximum probability of synchronized events observed but the numbers of synchronized events remained relatively low.

Discussion

General approach

Last-order interneurons provide common drive to motoneuron pools in the form of the LDP and SDP and each last-order interneuron likely projects to several motoneurons. The goal of this study was to determine whether the common drive provided to homonymous motoneurons includes, in addition to an envelope of excitation, spike triggering events. If spike-triggering events were distributed by the CPG to homonymous motoneurons, then this should be reflected in the firing of motoneurons and there should be some evidence for short-term synchronization of firing in homonymous pairs. Parallel approaches were used to detect synchronized excitatory activity during centrally generated rhythms. These included an evaluation of synchrony during fictive locomotion and during the highly stereotyped fast rhythmic activity of fictive scratch. Separate analyses were also performed that examined only the initial action potential within a burst. Finally, we extended the initial spike analysis to include detection of subthreshold excitation (EPSPs) by using spike-triggered averaging.

The lack of synchronized firing in the pairs we examined suggests that the common drive provided to homonymous motoneurons by last-order interneurons is limited to an envelope of excitation (LDP/SDP) and does not include spike-triggering events. Moreover, the fact that several action potentials we observed were not preceded by

EPSPs (e.g. see Paper 2, figure 5, panel C top and middle groupings) suggests that those action potentials may have been the result of the activation of repetitive firing properties as opposed to synaptic events.

The rapidly alternating hindlimb movements used by the cat to scratch the head and neck and to generate the basic alternating pattern of rhythmic flexion and extension during locomotion are produced through a spinally-located network of neurons, the central pattern generators (CPGs) for scratch and locomotion. During both real and fictive behaviours, the onsets and terminations of hindlimb flexor and extensor motoneuron population activities are tightly regulated suggested that they are controlled by common inputs from the CPG. The failure to find short-term synchrony in homonymous or close synergist pairs (MG and Plant for example) during scratch, suggests that spike occurrence in individual motoneurons is not determined by spike-triggering events during fictive scratch and locomotion.

The presence of either time-locked inhibition or shunting inhibition could be obscuring synchronized spike-triggering events in motoneurons and cannot be ruled out. There is no evidence, however, that shunting inhibition similar to that observed in turtle motoneurons during the scratch reflex (Alaburda et al. 2005) is present in cat motoneurons.

Results and limitations

The rhythmic alternating depolarizations and hyperpolarizations observed in motoneurons during locomotion and scratch (LDP and SDP, respectively) constrain the envelope of action potential firing within a motoneuron pool. Our cross-correlation analysis between the time of occurrence of action potentials in pairs of homonymous or close synergist motoneurons revealed only a broad peak of low amplitude of variable width. We suggest that this broad correlogram peak reflects the presence of the common envelope of excitation imposed by the LDP or SDP. In extensor motoneurons this peak is about 40 ms wide during fictive scratch (see Fig. 2D) and 600 ms wide in fictive locomotion (not shown). Cross correlation failed to detect any significant peaks reflecting short-term synchrony in the 10 pairs of homonymous (see Table 1) or the 4 pairs of agonist motoneurons examined. Thus the “k” statistic (see Methods) was not significant for any pair of motoneurons and the “E” and “CIS” values were uniformly low.

A 2 ms interval (2 bins) was chosen for the analysis of correlogram significance and for illustration purposes of subthreshold depolarizations (eg, Fig. 1E). This short interval was based on 2 assumptions. The first was that if the CPG was distributing spike-synchronizing EPSPs to the motoneuron pool, then these EPSPs would arrive almost simultaneously in the pair of impaled motoneurons. This seems a reasonable assumption since the distances between the impaled pairs of motoneurons were typically within 5 mm of each other (measured at the dorsal surface of the spinal cord). Even with a slow intraspinal conduction velocity of 10 m/s a distance of 5 mm would result in a delayed

arrival of an EPSP at one motoneuron of only 0.5 ms and would be easily accommodated in the bin width chosen.

The second assumption is that any action potential-synchronizing EPSPs would have a reasonably short time to peak so that their effect on increasing the probability of action potential initiation would be present for only a short time. Although no direct measurements of the characteristics of EPSPs that might give rise to synchronization were made in the present study, Fig. 1E shows that they are probably rapidly rising events with time courses typical of other synaptic inputs to motoneurons. For example, monosynaptic EPSPs recorded in motoneurons typically have rise times on the order of 1 ms, (e.g sensory Ia EPSPs, see Hochman & McCrea 1994a; MLR-PSPs see Fig 1C of Paper 1). Thus, if common spike-triggering events were distributed by the CPG to closely located motoneurons, the resulting EPSPs should have similar latencies in the 2 motoneurons and give rise to action potentials within one ms of each other. Therefore, the use of a 2 ms window around the time of occurrence of the trigger action potential to detect synchrony in occurrences of action potentials should have provided a generous time window to see synchronization. It is worth noting that Sears and Stagg (1976) found large peaks in the correlograms obtained from pairs of intercostal motoneurons during breathing using 1.0 ms bins and concluded that there was considerable synchronization of the firing of these motoneurons

It is well known from intracellular current injection studies in motoneurons that locomotor-specific changes in the afterhyperpolarization (Brownstone et al. 1992) and

the voltage threshold (Krawitz et al. 2001) of motoneurons can alter the occurrence of subsequent spikes in a train. These factors, along with accommodation and other intrinsic motoneuron properties (Hultborn et al. 2004) create an F/I relationship in motoneurons that is significantly altered during fictive locomotion. It is not yet clear to what extent these properties may be activated during fictive scratch. Preliminary data indicates, however, that motoneuron voltage threshold is also lowered during fictive scratch in the cat (Power et al. 2006). These intrinsic motoneuron processes could have diminished the amount of detectable synchrony. For example, it has been shown that persistent inward currents generated in motoneuron dendrites provide large excitatory drive which could potentially overshadow putative common inputs from the CPG (reviewed in Binder 2002). Theoretical studies have also shown that spike synchrony can be decreased by lowering the spike threshold using a steady depolarizing current (Svirakis and Hounsgaard 2003). Active dendritic conductances have also been shown to decrease motor-unit synchronization in a modeling study (Taylor and Enoka 2004a,b).

Because cellular properties might have played a role in reducing the amount of synchrony that was observed in the present study, two procedures were used to minimize the influence of intrinsic membrane properties in obscuring CPG-derived synaptic synchronization.

The first was to look for synchronization using only the first spike in a train during a locomotor or scratch burst. In this way the influence of preceding spikes on membrane trajectory to threshold was avoided. Limiting the analysis to only the first-occurring spike

during a scratch activity cycle, however, similarly failed to reveal strong synchrony between firing in pairs of homonymous motoneurons. Even when using only the initial action potential of each cycle as the trigger, spikes in one motoneuron were infrequently associated with excitatory events in another cell (usually in less than one third of cycles of activity examined). This argues against the hypothesis that the CPG distributes EPSPs that synchronize action potential firing within the motor pool.

Perhaps the strongest evidence against the common CPG-derived EPSP hypothesis is the observations using spike triggered averaging. In these cases, the lack of firing in the motoneuron (i.e. the SDP was below threshold) allowed visualizing the presence of EPSPs occurring around the time of spike initiation in the trigger cell (Fig. 1E). Again, few action potentials in the trigger cell were associated with EPSPs in the second motoneuron.

Relationship to previous findings

Coherence analysis performed on ENGs and combinations of ENGs and intracellular recordings during MLR-evoked fictive locomotion has suggested not only that there may be common input to close synergists but also to agonists acting at different joints across the limb (reviewed in Hamm, Trank and Turkin 1999). However, coherence was not observed by another group when looking at activity in chemically evoked locomotion (DOPA-nialamide) (Nielsen et al. 2005). The main reason put forth to explain this difference was the presence, in the MLR-evoked locomotion of correlating input from the

MLR (Nielsen et al. 2005). However, although they acknowledged the possible contribution of MLR-locked firing to the coherence observed, Hamm and colleagues (2001) reported that they observed similar and even increased amounts of coherence in spontaneous locomotion occurring following the cessation of MLR stimulation (Hamm and McCurdy 1995). They suggested that their observation could indicate flexibility in the pattern of activation of motoneurons from the CPG that would depend on the particular mechanism of evoking locomotion.

Besides the possibility of preparation-dependent particularities in the CPG inputs to motoneurons resulting from differences in the way in which locomotion was evoked, a direct comparison of the results from Hamm's group and our own is difficult. They used composite recordings of motoneuron activity (i.e. ENG recordings) for the majority of their analyses. Thus they performed a coherence (i.e. frequency domain) analysis either between ENG recordings of firing activity from the entire motor pool or between one motoneuron and the its own motor pool. Our results are consistent, however, with previous findings using intracellular paired recordings during chemically evoked fictive locomotion. In their work, Nielsen and colleagues (2005) examined synaptic activity in homonymous pairs in which motoneuron spiking was prevented the intracellular diffusion of a fast sodium channel blocker (QX314) in the microelectrode. In agreement with our results on the lack of firing synchrony, Nielsen and colleagues (2005) failed to find significant evidence for correlated synaptic inputs in any of the pairs they examined.

Why is there a discrepancy with human data on motor synchrony?

Motor unit synchrony has been extensively studied during voluntary contractions in humans especially during skilled movements such as fine finger movements. One source of interest in motor unit synchrony in humans stems from its potential effects on contraction force (e.g. Semmler et al. 2000). However, motor-unit coupling can also be used as a non-invasive method to study the organization of inputs to motor pools in humans. This has been done during walking in healthy human subjects (Hansen et al. 2001, Halliday et al. 2003) as well as in patients with partial spinal cord lesions (Hansen et al. 2005). The reduction or absence of evidence for common drive observed in spinal cord injured patients compared with non-injured subjects (Hansen et al. 2005) suggests a supraspinal origin of the synchrony observed in humans. Thus, our findings are in agreement with human data in that there doesn't seem to be a spinal source of synchronizing input neither during cat fictive behaviours nor human walking.

A simulation study by Binder and Powers (2001) suggests that the sensitivity of the cross-correlation techniques is in fact quite limited and that a large amount of common input would be required for synchronization to emerge. In this study, Binder and Powers (2001) injected current in lumbar motoneurons of anesthetized cats. The injected currents were based on physiologically observed synaptic currents and were augmented with varying amounts of common input. The results of this study demonstrated that under their conditions, a large amount of common input was required under such condition for synchronized firing to occur (Binder and Powers, 2001). The common input used in that

study, however, was a noise waveform and not synaptic potentials. Furthermore, current injections were made serially in motoneurons and not simultaneously as in the present study. Thus, the extent to which their conclusion on the weakness of the cross-correlation approach apply to the present study remains unclear.

Similar conclusions about the lack of strong correlated firing with similar inputs to different motoneurons were reached in a theoretical study by Taylor and Enoka (2004b). They used a 6 cylindrical compartment model to show that only when over 60% of the total excitatory input was common to 2 cells was there a significant effect on correlated firing (Taylor and Enoka 2004b). Accordingly, even if the CPG were to distribute large common EPSPs to motoneurons within a pool, there might not be a large degree of synchronization. Again, how these data relate to real motoneurons with real synaptic currents is unclear. It is clear, however, that synchronization among motoneurons can occur during respiration in which there is rhythmic sensory input to the spinal cord (eg. Sears and Stagg 1976). We suggest that our failure to frequently detect large common EPSPs in homonymous motoneuron pairs is evidence against a similar CPG-derived synchronization occurring during fictive locomotion and scratch.

In conclusion, our results suggest that the CPG control of motoneuron excitation appears not to extend to the tight synchronization of action potentials in members of a motor pool. Consequently it appears that CPG generated motoneuron activities are created by an envelope of depolarization upon which the intrinsic membrane properties initiate action potentials in individual motoneurons.

Figure Legends

Figure 1. Correlation analysis during fictive locomotion

A. ENGs and intracellular recordings of a pair of firing MG motoneurons during MLR-evoked fictive locomotion. *B.* Spike-triggered averages of intracellular recordings triggered on the onset of the MLR current pulse (indicated by the filled triangle at time zero). Both motoneurons had time-locked responses to MLR stimulation. *C.* Peristimulus-time histogram (PSTH) computed from the spike trains of the cells shown in *A* and *B*. The x-axis represents the time from trigger, that is the delay between the trigger spike and the spikes in the other motoneuron. The probability of an action potential occurring in each particular bin (or at each particular time delay) can be read on the y-axis. The probability of detecting a spike at time zero lag was not higher than at other time lags. This probability was calculated as the ratio of the count per bin to total number of spikes in the response train. There was no peak indicative of short term synchrony. Rather, firing was distributed over the range of delays (-20 ms to + 20 ms) examined. *D.* ENG recordings and intracellular recordings for another pair of MG motoneurons during fictive locomotion. In this case, only one motoneuron fired and thus, spike-triggered averaging was used instead of crosscorrelation analysis. *E.* Overlay of intracellular traces synchronized to the MLR stimuli which occurred during the extensor phase only. The non-firing motoneuron (Mg92, top panel) had time-locked EPSPs with virtually each MLR pulse. The firing motoneuron (mg93, bottom panel) had either an action potential or an EPSP (solid traces) in the majority of cases (56%). The latency of the excitatory events was similar in both cells. (Time scale indicates time from MLR stimulus).

Figure 2. Cross-correlation analysis of firing in a pair of MG motoneurons during fictive scratch. *A.* ENG and intracellular activity during a bout of fictive scratch illustrating the initial postural phase marked by tonic flexor activity (for example TA) followed by the fast rhythmic alternation of flexion and extension. *B.* Expanded view of the intracellular recordings over a few cycles to show the similarity between the LDP in the two cells (spikes clipped). *C.* Overlay of a single cycle of activity for both intracellular recordings showing that, despite similarities in the onset and profile of scratch drive potential (SDP) in both cells, there are differences in the exact timing of the individual action potentials. *D.* PSTH computed during the rhythmic phase (dashed box) of the fictive scratch bout shown in panel A. Time lags between spikes in both trains are distributed over the range corresponding to the duration of the active phase of those motoneurons.

Figure 3. Increasing the number of spikes analyzed did not result in qualitative changes in the histogram. PSTH for a pair of PBST motoneurons where several episodes of fictive scratch activity were available for analysis. *A.* PSTH computed from a single epoch of fictive scratch. The peak at -2 ms was not significant. *B.* PSTH compiled from the pooled data from 4 epochs of fictive scratch during which activity was recorded in the same pair of motoneurons. Again, there was no significant peak indicative of short-term synchrony. There were no qualitative differences that arise from the histogram being compiled from a larger number of spikes.

Figure 4. PSTH of homonymous pairs during fictive scratch

Histograms computed in other pairs of homonymous motoneurons. In all new cases of MG pairs (A,C,D) and PBST (B), no significant short-term synchrony was observed (See table for coefficient values). Intracellular profiles are shown in C and D to indicate the similarities between the members of the pairs.

Figure 5. Analysis of the initial spike within each cycle of activity A. Overlay of simultaneous recordings of two MG motoneurons during fictive scratch. The scale for the motoneuron MG13 (red) is on the left while that for Mg12 (blue) is on the right. Note that the scales are the same. Action potentials were clipped to better display the voltage trajectory of the scratch drive potential (SDP). The recordings are displayed on the same scales although the absolute voltage values are different. The overall shape of the SDP was similar in both motoneurons although the initial spikes (indicated by the arrow) were not synchronous in all cycles displayed. B. PSTH obtained from the same pair as shown in panel A. The top histogram was obtained by including all action potentials and the bottom histogram included only the initial action potential of each scratch cycle. When all spikes are included, there is no peak of short-term synchrony, just a general tendency for action potentials to cluster in accordance with the shape of the SDP. In the histogram of initial spikes, there is no short-term peak. In fact, the initial spikes are distributed within the first 6 ms following the trigger initial spike. C. Overlay of all the initial spikes included in the bottom histogram in panel B. Top trace shows the initial spike in MG13 which was chosen as the trigger. The middle trace shows an overlay of all the cycles in Mg12 where there was an initial spike within 2 ms of the initial spike in Mg13. This occurred in 11 of 32 cycles examined. The bottom trace shows an overlay of the other 21 cycles where the initial spikes in Mg12 occurred at a variety of times following, or even preceding in one case, the initial spike in Mg13.

Figure 6. Inclusion of subthreshold excitatory events does not increase the number of synchronous events detected

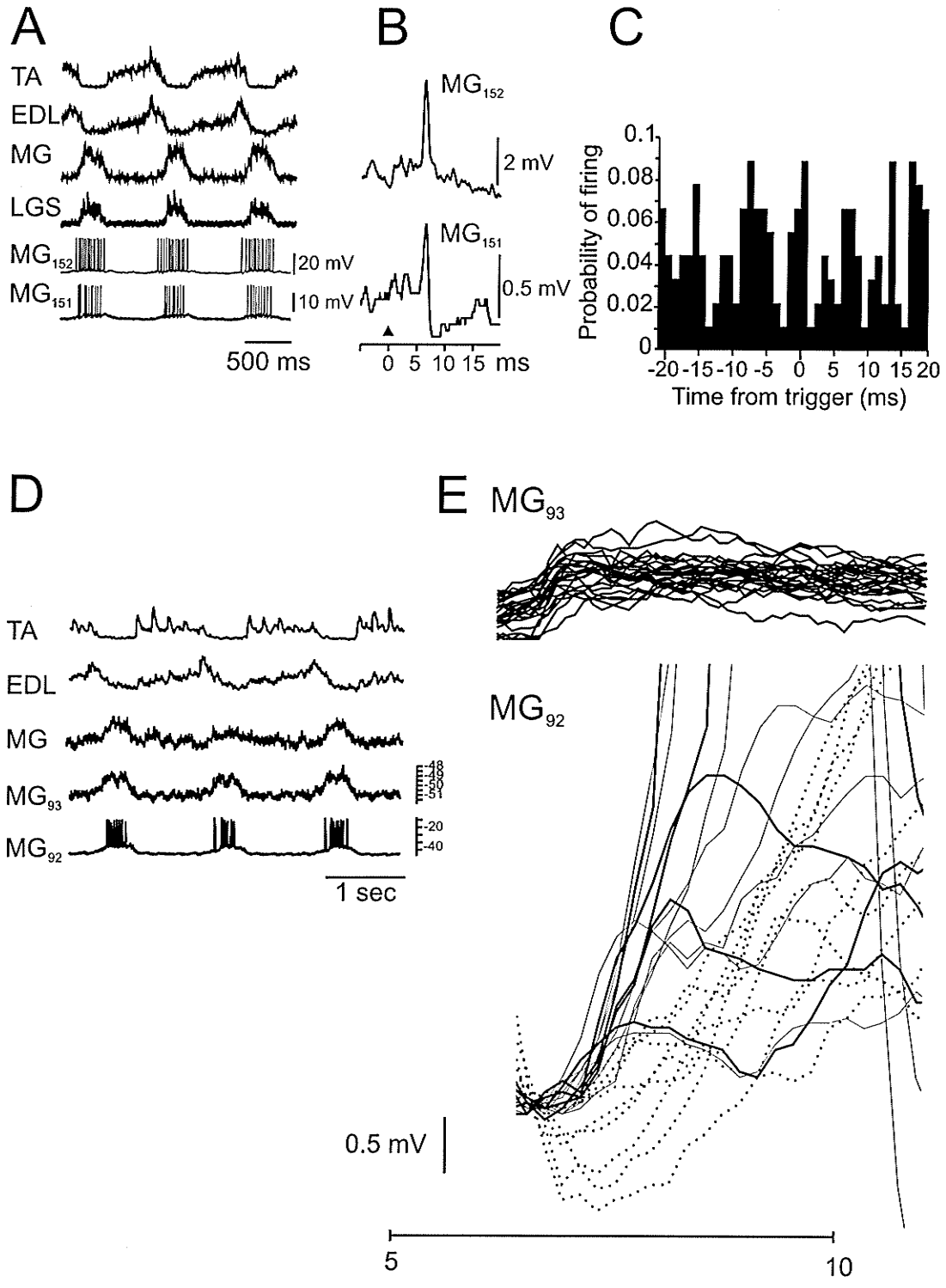
A. Overlay of simultaneous recordings of two PBST motoneurons during fictive scratch.
B. Histograms including all action potentials (top) and only the initial action potentials of each cycle.
C. Spike triggered average where traces were grouped according to whether a synchronous EPSP leading to an action potential was detected (top group), a synchronous action potential was detected (middle group) or no synchronous event was detected (bottom group). Only in less than a third of cases was a synchronized EPSP detected within a 2 ms time window from the trigger spike.

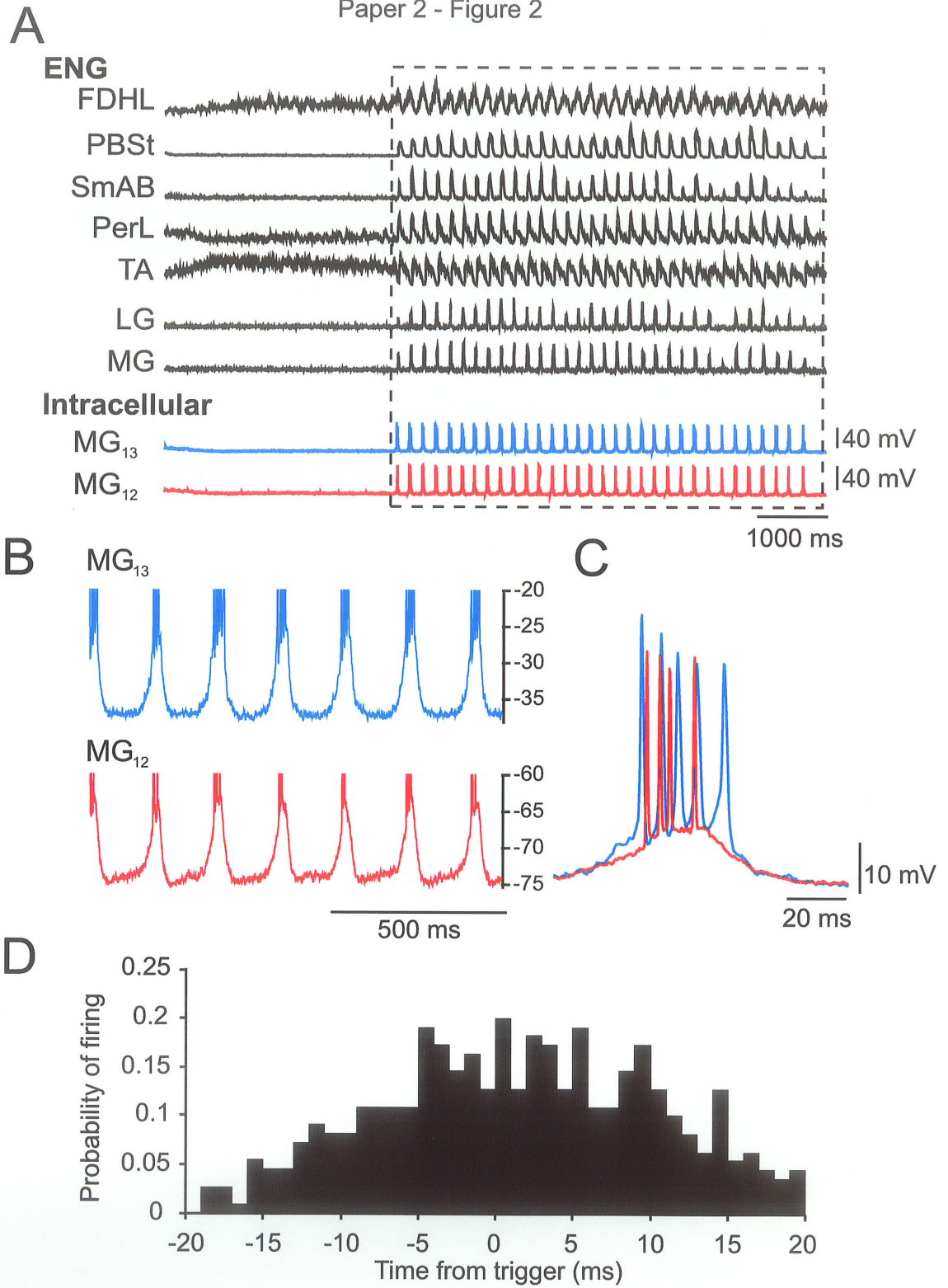
Tables

	pair #	ID	type	k		significance	E	CIS
locomotion	1	58	plant - plant	1.8	± 2.2	not sig	0.03	0.21
	2	59	plant - plant	2.5	± 2.0	not sig	0.02	0.66
	3	60	plant - plant	3.3	± 3.0	not sig	0.06	0.30
	4	67	mg - mg	2.7	± 2.3	not sig	0.08	0.57
scratch	5	6	mg - mg	3.3	± 5	not sig	0.23	4.20
	6	52	mg - mg	2.9	± 3.4	not sig	0.12	0.83
	7	54	mg - mg	2.3	± 2.2	not sig	0.04	0.21
	8	50	smab - smab	2.5	± 6.1	not sig	0.05	0.07
	9	5	pbst - pbst	3.0	± 2.0	not sig	0.21	1.37
	10	16	pbst - pbst	2.6	± 5.5	not sig	0.05	0.04

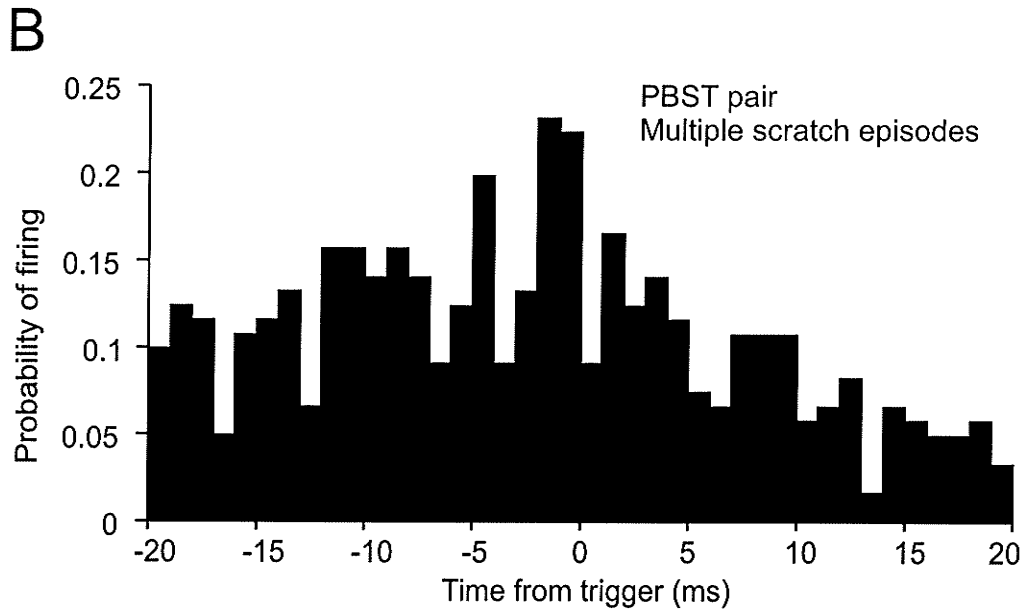
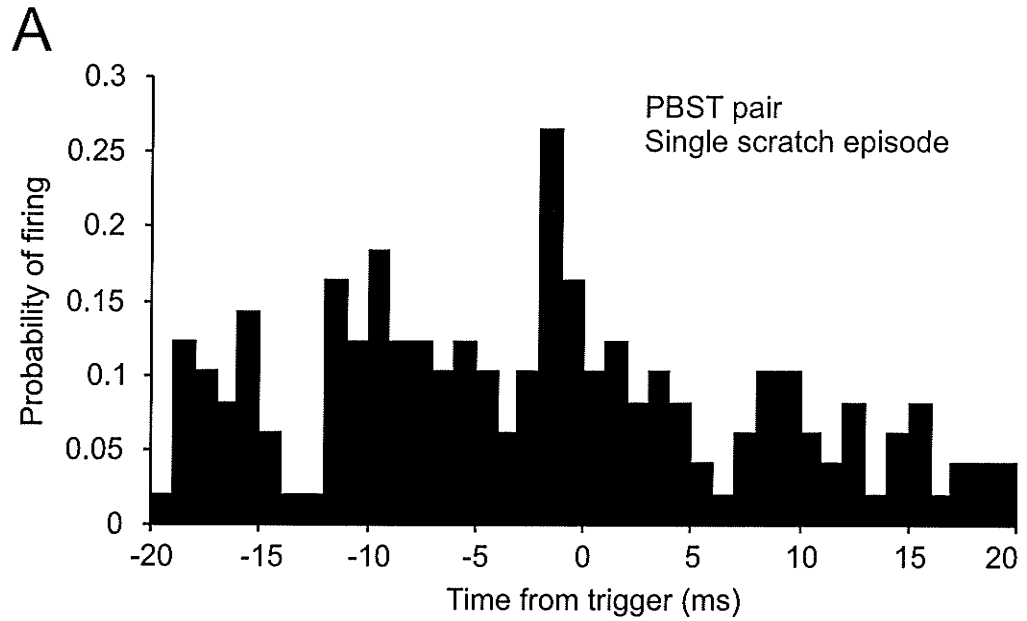
Table 1. Firing synchrony results

Paper 2 - Figure 1

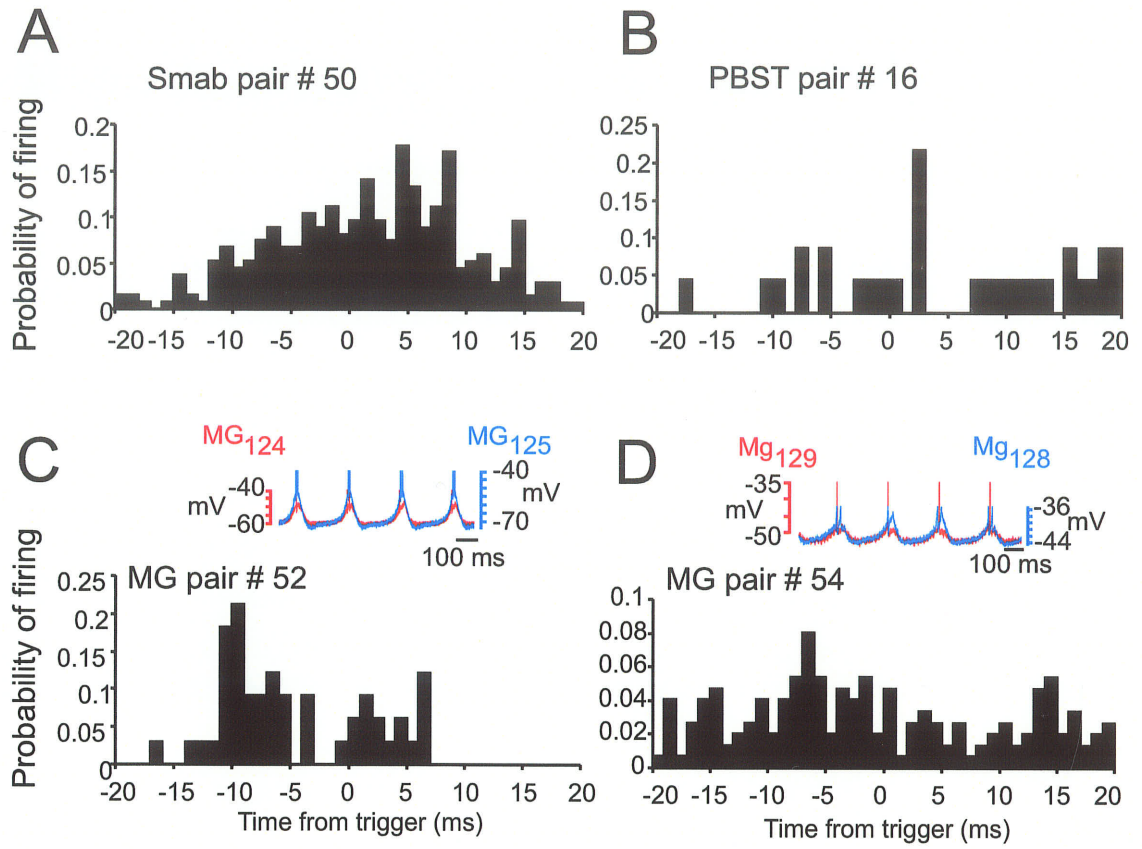




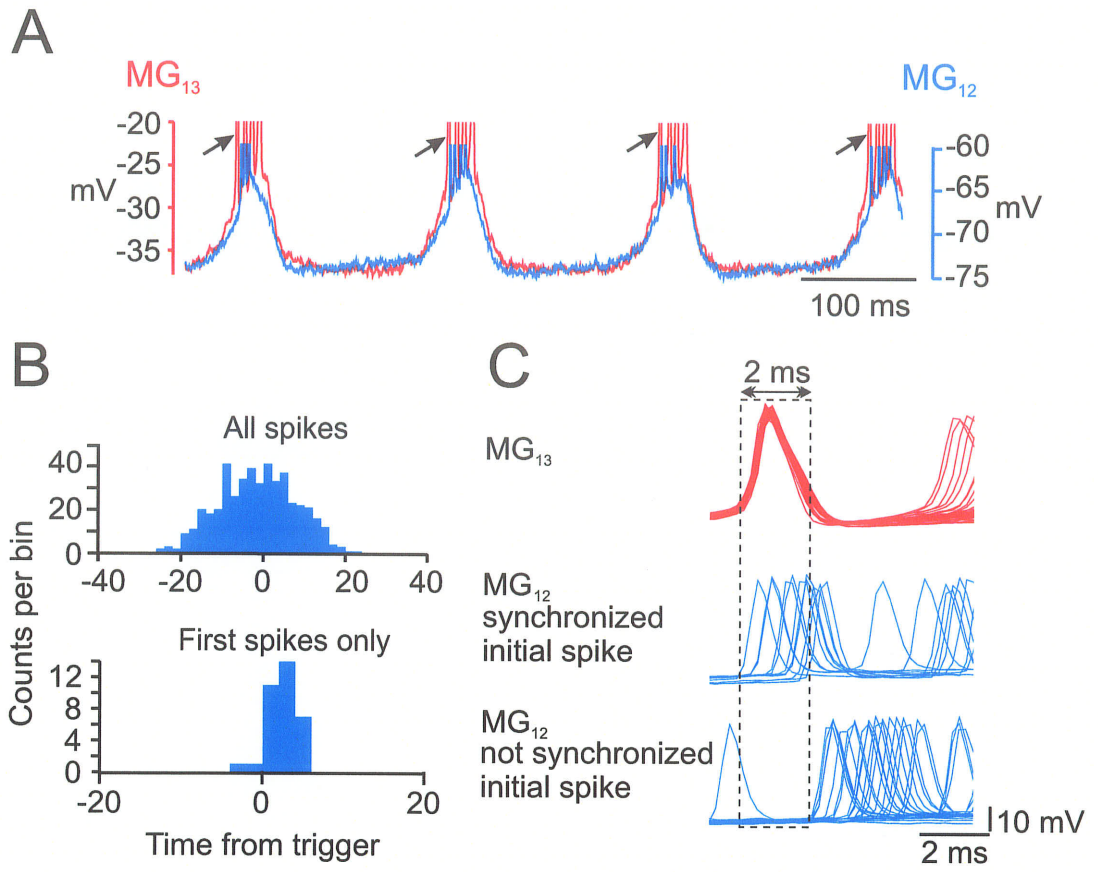
Paper 2 - Figure 3



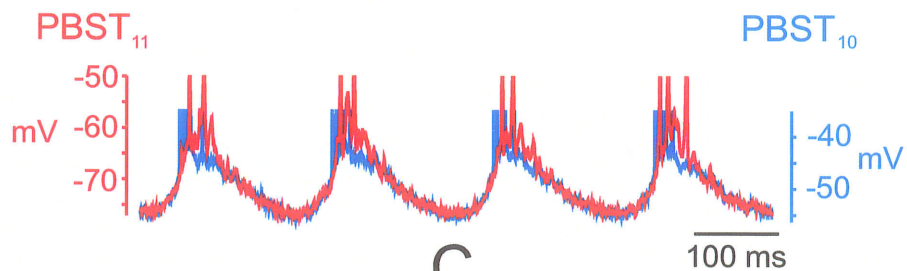
Paper 2 - Figure 4



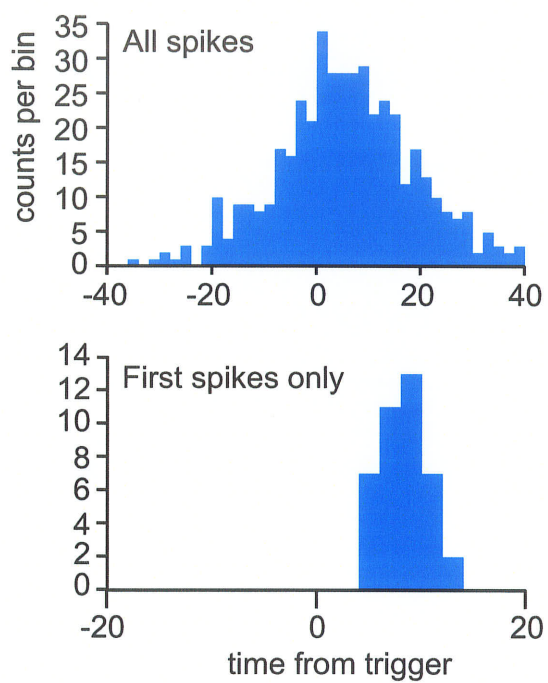
Paper 2 - Figure 5



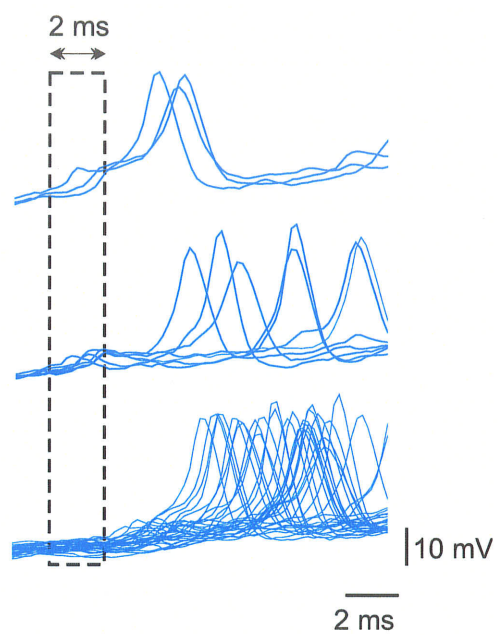
A



B



C



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IV. GENERAL DISCUSSION

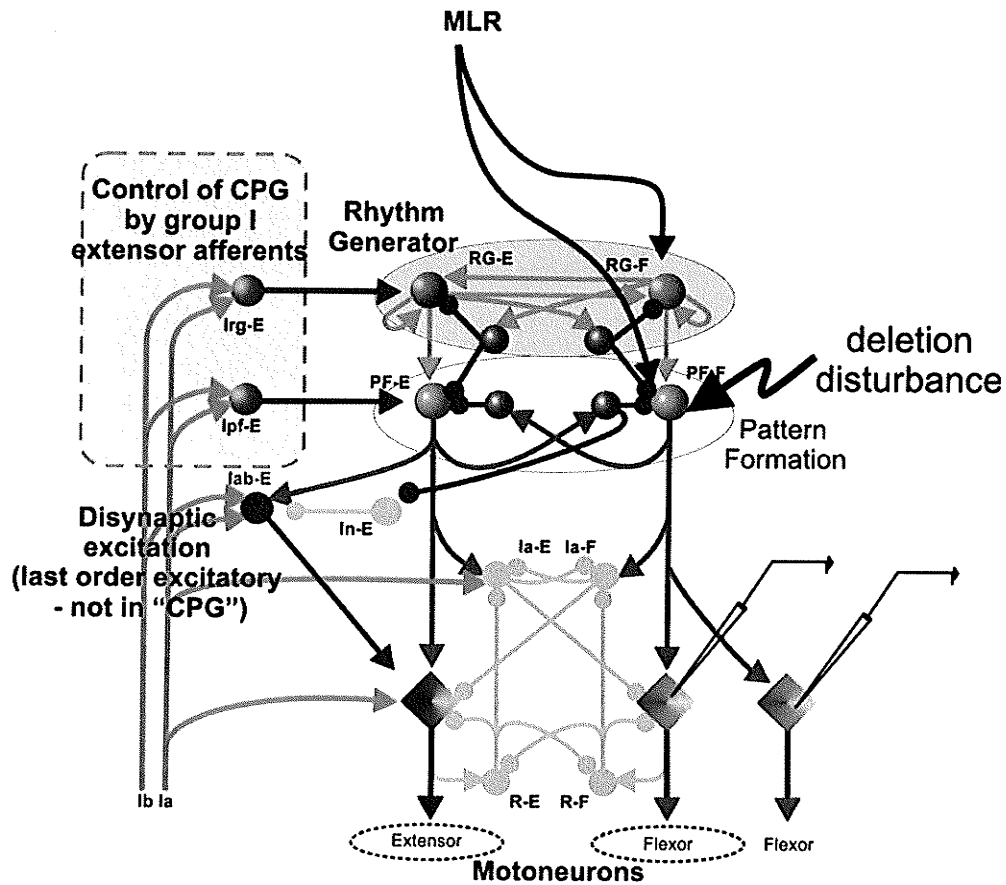


Figure 1. Detailed schematic of the Rybak-McCrea model (Rybak et al. 2006a,b)

Implications of findings for the structure of the locomotor CPG

The general goal of this thesis was to provide information regarding the possible organization of the locomotor CPG. Although a number of theoretical CPG models have been introduced over the years (reviewed in the Introduction) we have chosen, as a framework for the discussion of the results, the model developed by Rybak and colleagues (2006a,b) and shown in Figure 1 above (a more schematic version was shown in Figure 1 of the General Introduction). One of the reasons is that the original data on the

occurrence of deletions during fictive locomotion and scratch (Lafreniere-Roula and McCrea, 2005) was key in the development of this model.

The detailed representation of the Rybak-McCrea model shown in Figure 1 of the current section illustrates the internal organization of the rhythm generation layer (within the light blue shaded ellipse) and of the pattern formation layer (within the light yellow shaded ellipse) as they were implemented by Rybak and colleagues (2006a,b). These will not be discussed here in details here. The Renshaw and Ia systems were also implemented within the model and are shown in grey. MLR drive is provided to the RG neurons as well as the PF neurons. In the model, deletions of ENG activity where the timing of rhythmic activity was maintained were implemented by altering the drive of PF-layer model neurons (labeled deletion disturbance in Figure 1). Although sensory effects were not examined in the experiments described in this thesis, the model specifically implemented known effects of stimulation of group I afferents on CPG generated rhythm by stimulus trains.

The data we presented in the thesis supports the separation of rhythm generation and pattern formation. We cannot rule out the existence of last-order interneurons mediating CPG excitation and that are not within the pattern formation layer. Burke and colleagues (2001) have proposed a layer of last-order interneurons interposed between the pattern formation layer and the motor pools. In fact, previous work in the laboratory (Angel et al. 2005) has shown that the interneurons mediating group I di-synaptic excitation of extensor motoneurons are facilitated by MLR stimulation and therefore may be such an

example. The reason why these interneurons are thought to be outside of the pattern formation layer is that some fictive locomotor preparations do not display group I disynaptic excitation while showing good locomotion. The activation of the group I disynaptic pathway is therefore not necessary for the production of appropriate locomotor output.

We have shown that closely located homonymous motoneurons may vary in the details of their activity. The variability described in motoneurons from the same motor pool is evidenced by the following three observations. First, MLR-PSPs can vary even in pairs of closely located homonymous motoneurons (Paper 1). Second, the correlation of firing between homonymous motoneurons was low (Paper 2). Moreover, the synchronization of subthreshold excitatory events was also low (Paper 2). The lack of synchronized firing in the pairs we examined suggests that the common drive provided to homonymous motoneurons by last-order interneurons is limited to an envelope of excitation (LDP/SDP) and does not include spike-triggering events. Moreover, the fact that several action potentials we observed were not preceded by EPSPs (e.g. see Paper 2, figure 5, panel C top and middle groupings) suggests that those action potentials may have been the result of the activation of repetitive firing properties as opposed to synaptic events.

We have also shown that, in a majority of cases (but not all), changes in MLR-PSPs during deletions can be predicted by the effects of these deletions on the locomotor drive potential. This confirms the suggestion that MLR-PSPs are related to the production of locomotor output. It is also consistent with the hypothesis that PF layer neurons also

mediate MLR-PSPs since failure of excitatory drive to motor pools (deletions) are accompanied with decreases in the excitatory components of the MLR-PSPs.

Although this confirms the relationship of MLR-PSPs to the production of locomotion, the fact that MLR-PSPs are not always modulated with the locomotor cycle, suggest that multiple pathways must be available for the transmission of MLR-excitation and that only some of those are directly involved with the production of the rhythmic excitatory/inhibitory drives to motoneurons. Moreover, the absence of MLR-PSPs in some motoneurons that are nonetheless displaying well-developed LDPs, although possibly related to differences in brainstem stimulation (discussed in Paper 1 – Discussion), may also suggest the existence of PF neurons either not directly activated by the MLR or not sensitive to those inputs. Therefore, because of this apparent heterogeneity, it seems that the analysis of MLR-PSPs we have done so far is not sufficient in identifying a unique population of putative pattern formation neurons, making it difficult to proceed to identification and localization without further studies.

Cross-correlation analysis on intracellular recordings of spike trains has failed to reveal short-term synchrony of firing between individual motoneurons. Although this is in agreement with studies showing a lack of correlation of synaptic inputs to motoneurons during fictive locomotion (Nielsen et al. 2005), it seems at odds with findings of short-term synchrony in motor unit firing in humans during various tasks, including walking. There are, however, several key differences between the two paradigms which can explain this difference. Let us consider specifically the question of synchrony during

walking since it directly relates to this thesis. Firstly, there is strong evidence that the synchrony during walking in humans originates from the corticospinal tract and not to be caused by spinal circuitry (Hansen et al. 2005) since it has been shown that humans with incomplete spinal lesions display little or no synchrony in the discharge of the tibialis anterior muscle during treadmill walking compared to healthy subjects (Hansen et al. 2005). This is not to diminish the importance of spinally generated synchrony in other systems such as the respiratory system for example (Sears and Stagg 1976). It supports the finding that synchronized motor unit activity during walking does not arise from the activity of spinal circuits.

Implications of findings for the structure of the scratch CPG

The particular characteristics of the pattern of activity during fictive scratch compared to fictive locomotion suggest differences between the two CPGs. First, the fact that activity is strikingly different between the scratching limb and the other limbs (used for posture) raises the issue of interlimb coordination which is beyond the scope of this thesis. Second, within the scratching limb, the rhythmic activity is preceded by a period of tonic flexion phase followed by a fast rhythmic alternation of flexion and extension. Third, there are differences in the patterns of activity of certain motor pools during fictive scratch and locomotion.

Full consideration as to how the Rybak-McCrea model could be applied to the generation of scratch has not been given yet. In our study of deletions occurring during fictive

locomotion and scratch we have found essentially no differences between the characteristics of deletions during both activities. Namely, in both cases, we observed some deletions where, although the magnitude of excitation to the motor pools (as displayed by ENG and LDP amplitudes) was strikingly different, there was no evidence for alteration of the locomotor or scratch rhythm (as judged both by examination of rhythmic activity in other motor pools and by statistical means. For examples, see Figure 5B (leftmost box) and Figure 6 of Lafreniere-Roula and McCrea, 2005). Thus, it seems reasonable to postulate that the scratch CPG also has a two-layer architecture.

Our results showing the lack of synchrony during fictive scratch suggest that, although fictive scratch is a behaviour where we would have expected tighter control of motoneuron firing due to the very stereotyped pattern of activity involved, there is still limited evidence for a role of the CPG in generating the precise timing of action potentials.

Are the CPGs for locomotion and scratch shared?

Differences in the patterns of activity during fictive locomotion and scratch suggest differences between the scratch and locomotor CPG. The ability to produce patterns of motoneuron activity that are intermediate between those observed during scratch and locomotion suggests, however, that they may even converge under some circumstances. In both cases, the lack of synchrony suggests a similar role for the CPG and that is the generation of the LDP/SDP but not including the distribution of spike-triggering events.

In addition to similarities in the organization of the scratch and locomotor CPG networks, it seems that state dependent changes in motoneuron properties that have previously been identified during fictive locomotion such as voltage-threshold lowering for example (Krawitz et al. 2001) are also now being observed during fictive scratch as well (Power et al. 2006). This is further evidence that at least some of the same processes are engaged during both behaviours. It is clearly a good strategy for the nervous system to generate similar but different motor tasks by reconfiguration of the spinal neural circuitry. Moreover, it has been shown in other organisms, that this can occur and a striking related example is the involvement of individual interneurons in more than one patterns of fictive scratch in the turtle (Berkowitz et al. 2005, 2006).

What is the role of the CPG in determining motoneuron firing patterns?

From our combined results, it seems that the role of the CPG is in providing the boundaries within which motoneuron firing may occur, that is the locomotor of scratch drive potential. Clearly, the CPG provides the envelope of excitation which determines the phase of excitation of various motor pools and constrains firing in a particular motor pool to a limited portion of the step cycle. Motoneurons may also be in a state of excitability which renders it impossible for the MLR to drive them on each stimulus. This excitability may have in turn been caused by the activation of state dependent intrinsic properties.

During movement in humans, the corticospinal tract plays an important role in generating the motor unit synchrony observed although in some specific systems such as the innervation of intercostal muscles. In addition to corticospinal inputs, sensory inputs may potentially be better candidates for providing strong synchronizing drive to the motor pool. For example, perturbations of the step cycle elicited by sensory input may need to act on the motor pool so rapidly that a mechanism for overriding CPG evoked excitation may be needed. Sensory afferents, which have been shown to fire synchronously (Hamm et al. 1985) may have this capacity.

Future directions

One of the main advantages of using deletions to study the CPG is also one of its main limitations: the fact that deletions are spontaneous events that occur relatively rarely. It would clearly be desirable to observe changes in MLR-PSPs in controlled situations. One such situation would be during sensory perturbations of the step cycle which can be reproducibly produced by graded electrical stimulation of particular afferents. Moreover, sensory perturbations can affect the rhythm of motor activity independently of its amplitude which would provide a way to test a hypothesis put forth by Jordan and colleagues (Noga et al. 2003) that some of the longer latency MLR-PSPs are mediated by rhythm-generating neurons. Finally, because some of the interneurons mediating those sensory effects are identified, it may be possible to examine their activity during deletions and to establish whether they are or not part of the circuitry mediating MLR-PSPs.

Although analysis of synchronous firing has led to tremendous insights on motor control in humans, it seems unlikely that it will be a useful tool in the future for the specific purpose of unraveling the details of CPG organization. Theoretical studies have shown that large amounts of common input are necessary for synchrony to be detectable and it seems unlikely that the CPG would be providing this type of input to motor pools. This is especially apparent when one considers that the patterns of motor activity capable of being generated by the CPG for different types of walking are variable (different patterns of motor activity for forward and backward walking for example) and that motoneuron recruitment is, at least in part, determined by intrinsic motoneuron properties.

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