Preferential suppression of transmission and candidate neurons mediating reflex actions from muscle group II afferents during fictive motor activity

by

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Abstract

This thesis examined two aspects of information processing by the feline spinal cord during centrally-evoked motor activity: 1) the modification of transmission from different sensory afferents and 2) the neuronal elements of reflex pathways from group II muscle afferents during fictive motor behaviours (i.e motoneuron activity under neuromuscular blockade). Fictive locomotion was evoked by electrical stimulation in the midbrain and fictive scratch was triggered by stimulation of the skin covering the ears following curare application to cervical dorsal roots in decerebrate *in vivo* feline preparations.

Both monosynaptic and longer latency components of muscle and cutaneous afferent-evoked field potentials were reduced in amplitude during fictive locomotion and scratch, but field potentials evoked by muscle group II afferents were suppressed more than those evoked by cutaneous and group I muscle afferents recorded at the same spinal locations. The novel finding, that field potentials evoked at the same spinal locations by muscle and cutaneous afferents are suprressed differently, suggests that there is a preferential and non-uniform control of transmission from muscle and cutaneous fibres during motor activity.

Extracellular recordings from neurons within the lumbar spinal segments showed that suppression of group II afferent input during fictive motor activity results in a powerful reduction of the activation of neurons with input from muscle group II afferents in 93% of the examined neurons after short trains of stimuli were delivered to peripheral nerves. However, more neurons remained recruitable by group II intensity stimulation if train duration was sufficiently long with only 33% showing a reduction in sensory-evoked firing. The majority of the neurons that remained responsive to muscle group II afferent input during fictive locomotion had axonal projections to supralumbar, or supraspinal areas and showed spontaneous, often rhythmic, firing activity.
Overall, the studies presented in this thesis provide insights into the mechanisms by which the mammalian spinal cord processes sensory information and on how sensory input is able to control motor activity in spite of suppressive control provided by the nervous system.

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I express my sincere appreciation to my supervisor, Dave McCrea, all my committee members, namely, Susan Shefchyk, Brent Fedirchuk, Tony Szturm and Brian Schmidt, and our collaborator, John Riddell, for their time, efforts and patience while working with me.

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I am proud to have received my training at the Spinal Cord Research Centre of Winnipeg, and I am thankful to Larry Jordan and David Bashor for introducing me to the opportunity of studying here.

Dedication

I would like to dedicate this thesis and all the good things that may ever follow this work to my Grandmother, Ĕglerne Csörgits Ilona and to my parents Ĕgler Mària and Stecina István.

Ezt a diplomamunkát es mindazt a jòt ami ezt a munkát majd talán egyszer követni fogja az idők során szeretněm a Nagymamám, Ĕglerne Csörgits Ilona, ês a szüleim, Ĕgler Mària ês Stecina István, javára ajánlani.

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General Introduction

As indicated in the title of the thesis, reflex pathways from sensory afferents will be under examination. In the Introduction, an overview of the receptor origin, the activation and the central projections of muscle group II and cutaneous afferents will be provided (part 1 and 2). There will be a discussion on the reflex actions of muscle group II afferents (part 3) and a brief summary of the known mechanisms involved in the modulation of sensory transmission from muscle group II afferents (part 4). The last part of the introduction (part 5) will summarize the hypothesis, the goals and the rationale of the studies described in the two papers that follow the Introduction.

1. Group II afferents

1.1. Fibre types and receptor origin of muscle afferents

Myelinated nerve fibres with the largest diameter innervating mammalian skeletal muscles are afferents originating from primary endings of spindle organs (McIntyre 1974). According to the terminology developed by Lloyd (1943), afferents with diameters of 12-16 μm and conduction velocity of 60-100 m/s are group I fibres. Afferents originating from primary muscle spindles as well as from Golgi tendon organs belong to the group I fibre category. Fibres with diameters ranging from 4-12 μm and conduction velocity of 24-72 m/s are group II fibres (Lloyd 1943). As illustrated in figure 1, group II afferents in feline muscle nerves may originate from several sources such as Pacinian corpuscles (Hunt and McIntyre 1960a), intramuscular pressure receptors (Paintal 1960), periostial pressure receptors (MacLennan 1972), free nerve endings (Hunt 1960), tap and tension receptors (Barker 1962), and even from joints (Hunt 1954); but the majority has been shown to arise from secondary endings of spindle organs (Jack 1978). In the ankle flexor tibialis anterior (TA) and peroneus longus (PerL) muscles, 46% of the large afferents are classified as low threshold group II fibres (Jack 1978). In the hip extensor and knee flexor muscle semitendinosus, and the ankle extensor soleus and medial gastrocnemius muscles, only 20-35% of the large afferents were found to be in the group II range (Jack 1978). Thus, the ratio of group I and group II afferents cannot be generalized for all muscles.

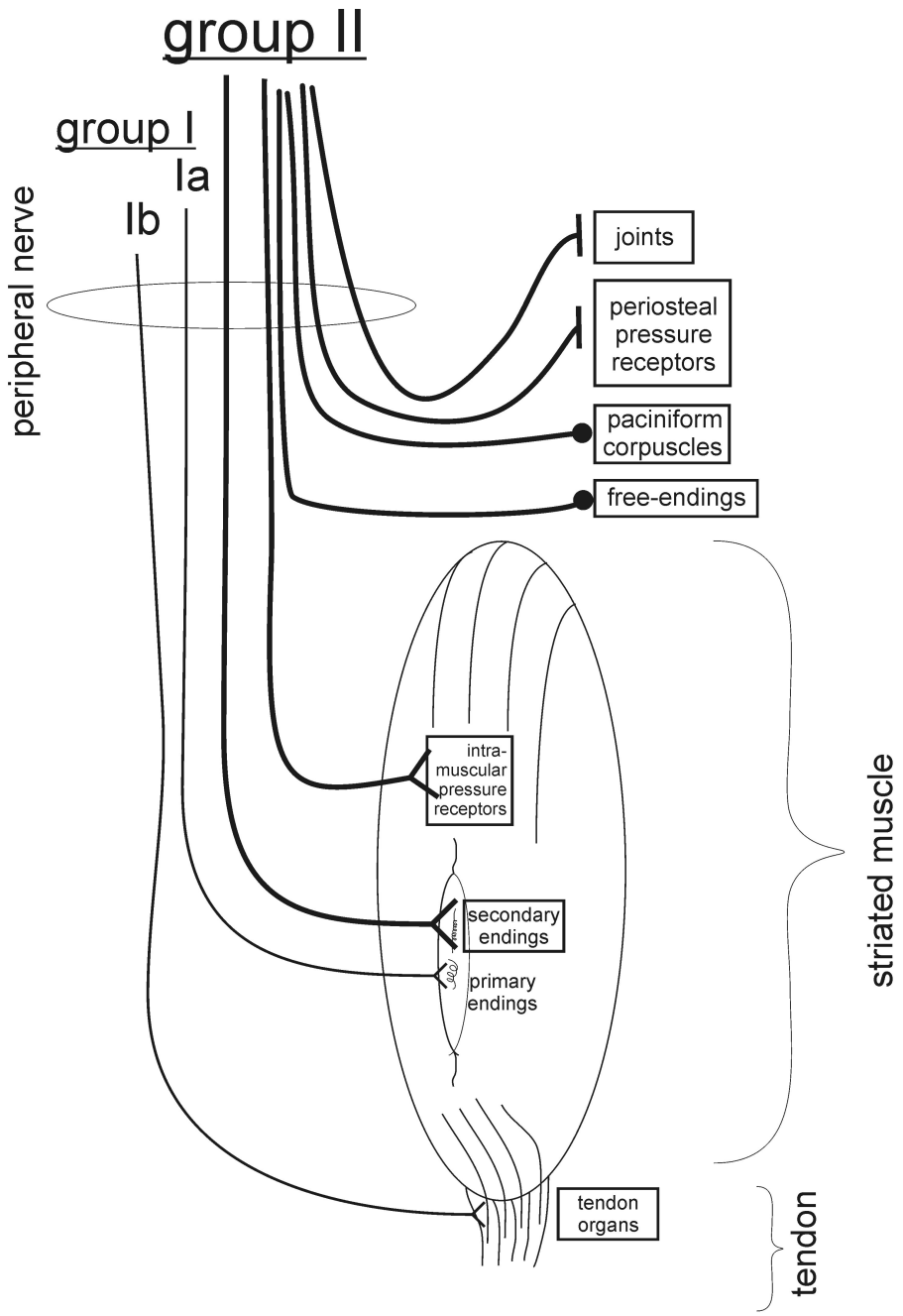

Figure 1. Sources of group II category afferent fibres running in mammalian peripheral nerves

The schematic representation illustrates the receptor origin of the group I and group II afferent fibres running in mammalian peripheral nerves. The boxes highlight the receptors giving rise to afferents in the group II category from intra- and extra-muscular sources.

1.2. Activation of muscle afferents

Primary endings of spindle organs (i.e. group Ia fibres) are activated naturally by increases in muscle fibre length with the afferent discharge proportional to the rate of muscle length change because the Ia fibres have a high dynamic sensitivity (Jack 1978). Group Ib afferents originating from Golgi tendon organs are activated by the load (force) on the tendons. Thus, together group Ia and Ib afferents signal mainly the dynamic length change and loading of a muscle. Group II muscle afferents also respond to length changes but with a low dynamic sensitivity, i.e. they are static length sensors (Matthews 1981). Even when stretched passively, secondary endings stretch more than primary endings (Poppele and Quick 1985). During treadmill locomotion in cats, the peak rate and the depth of modulation of group II afferent firing can exceed that of Ia fibres (Prochazka and Gorassini 1998). Secondary spindle afferent firing is also strongly influenced by gamma-motoneurone activity. When gamma-motoneurones are active, they can "drive" both secondary and primary endings of muscle spindles, often with the input from the secondary endings leaving the muscle spindle first (Gladden and Matsuzaki 2002). Thus when there is increased activity in gamma-motoneurones, muscle group II afferent input can have strong synaptic actions as it is coupled with group I input. During fictive locomotion, a complex drive to gamma motoneurones can result in continued activity of group II afferents throughout both phases of the step cycle, even with peaks of activity at times when the muscles are at shortened length (Bessou et al. 1990, Taylor et al. 2000).

In order to selectively activate spindle secondary endings, vibration can be combined with increasing muscle length changes (Matthews 1969, Jack 1978). The vibratory stimuli activate the majority of the primary endings and in this background of steady Ia firing, the fibres responding to additional muscle length changes are going to be those originating from secondary spindle endings. When applying electrical stimulation instead of natural activation, a repetitive stimulation pattern blocks most of the group I fibres. Then a pulse with a brief rise time and a slow decay can be superimposed on the repetitive stimulation pattern and it is used to activate group II muscle afferents (e.g. Jack and Roberts 1978). In general, most of the studies describing

group II muscle afferent-evoked reflexes utilized electrical stimulation with an intensity that is sufficient to recruit group II fibres (reviewed in McCrea and Perreault 1998). Stimulation at twice the threshold (T) used for the activation of the most excitable (i.e. largest diameter) fibres recruits almost all of the group I afferents and a few, low threshold, group II afferents (Jack 1978). Figure 2 is taken and modified from Jack (1978, Fig. 4a), to illustrate the relationship between the conduction velocity and the excitability of fibres when using electrical nerve stimulation. Excitability is the inverse of the threshold for activation. At around 0.5 on the excitability scale (the same as twice threshold, 2T) fibres with conduction velocity slower than the most excitable group I afferents also become activated. When the stimulus intensity is raised to five times threshold (5T), all group II category fibres are recruited (black box). Because one of the goals of the thesis was to compare the modulation of different types of sensory input (i.e. group I and group II input) during fictive motor activity, there were no attempts made to selectively activate only the group II fibres during the experiments. Electrical stimulation at 5T was used to activate group II fibres, but group I afferents were also activated concomitantly. The reason for focussing on reflex pathways from flexor group II afferents is because extensor group II afferent reflex actions seem to be negligible (Guertin et al. 1995) and functionally less important when compared to flexor group II afferent reflex actions during fictive locomotion. As the thesis examined muscle group II afferents from the sartorius (Sart), quadriceps (Q), tibialis anterior (TA) and extensor digitorum longus (EDL) muscles, a brief description on the anatomy of these muscles is provided below. All of the anatomical descriptions were adapted from Text-Atlas of Cat Anatomy by J. Crouch (1969). The Sart muscle consists of a lateral (anterior) and a medial (posterior) portion (Sherrington 1910, Eccles and Lundberg 1958). The lateral branch inserts into the patella and acts as a hip flexor and knee extensor. The smaller, medial part inserts on the tibia and flexes both the hip and the knee. During locomotion, the activity of lSart spans both flexion and extension, while the mSart shows activity only during the flexion phase (Hoffer et al. 1987, Pratt and Loeb 1991). The Quad muscle is composed of four muscles inserting on the patella and the lateral shaft of the tibia with origins on the shaft and greater trochanter of the femur. There are 3 knee

extensor muscles, Vastus medialis, lateralis and intermedialis, and the fourth component, the rectus femoris muscle is bifunctional (knee extensor and hip flexor).

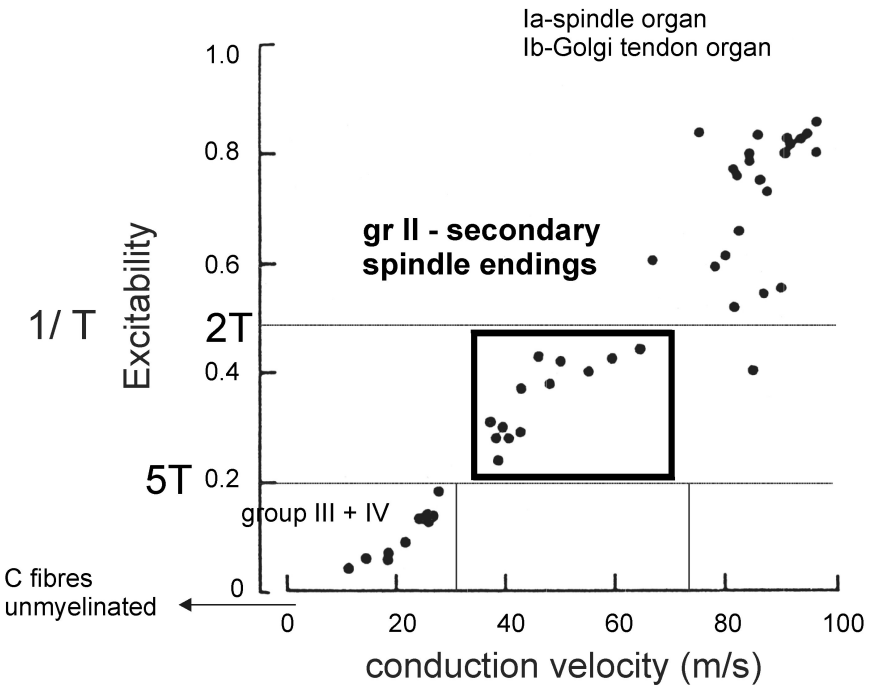

Figure 2. Electrical activation of afferents in the group II range occurs at stimulation strengths of 2 to 5 times the threshold (T)

For afferent fibres of the soleus muscle, the relationship between the excitability and the conduction velocity is illustrated following the stimulation of the muscle nerve with rectangular pulses. The data points plotted were adapted from Jack 1978 (see Figure 4). Electrical stimulation of peripheral nerves activates the fastest conducting, largest diameter fibres before the smaller, slower conducting fibres. The threshold of activation of the most excitable fibres is represented by the value of 1 on the excitability (the reciprocal of threshold) scale. Note that at 2 to 5T, muscle fibres conducting in the range of 30-70 m/s are activated (black box) that are mainly group II afferents. These results can be generalized to all other feline muscle afferents.

The TA and EDL muscles are synergist ankle flexors. The TA muscle originates from the shaft of the tibia, fibula and from the intervening interosseous ligament and inserts into the lateral surface of the first metatarsals. Thus, TA mainly acts across the ankle joint. The EDL muscle originates from the femur and inserts through 4 tendons on the base of the distal phalanges acting therefore not only across the ankle joint, but also at the knee and the phalanges. The activity of the EDL muscle seems to be more important than activity in TA for adduction (Nichols 1994). During overground locomotion, activity recorded from the EDL muscle starts and ends later than activity recorded from the TA muscle within a step cycle (Abraham and Loeb 1985, Trank et al. 1996). Thus EDL activity overlaps more with the extension phase than TA activity during real as well as fictive (McCrea et al. 1998) locomotion. The EDL muscle also displays larger length change during locomotion than that of TA (Goslow et al. 1977). During stance-to-swing transitions, the EDL muscle lengthens more than TA due to reduction in the joint angles at the metatarsophalangeal and interphalangeal joints (Kuhtz -Buschbeck et al. 1994).

1.3. Central projections of group II afferents

Because the staining of the smaller diameter group II afferents is more difficult than of group I and even cutaneous fibres, there are relatively few studies describing the morphology of group II afferent projections. Figure 3 illustrates the three main areas of group II terminal arborisation in the lumbar enlargement based on data reported by Brown (1981) and Hongo (1992). The spinal locations where group Ia (white), Ib (gray) and group II (black) muscle afferent terminals have been identified and illustrates the average relationship and overlap between group I and group II afferents. These main areas of muscle group II afferent terminations are in the dorsal horn (laminae IV-V), in the intermediate zone (lamina VI and dorsal VII) and in the ventral horn (laminae VII & IX). In the dorsal horn, group II afferents can arborize at more dorsal locations (as high as lamina III) and there is no overlap with group I afferents (Brown 1981, Hongo 1992). In the intermediate regions, there is some overlap with group Ia afferent terminals, but in general, the group II terminal arborisation is more lateral to group Ia and Ib collaterals from the same muscle. In the ventral horn, some terminals are dorsal to the motor nuclei

while others occur within the motor nuclei so there is some overlap between group Ia and group II terminals (Brown 1981). Arborisation of group II fibres, at least of the lateral gastrocnemius-soleus muscle, is more variable than that of group I afferents from the same muscle (Brown 1981).

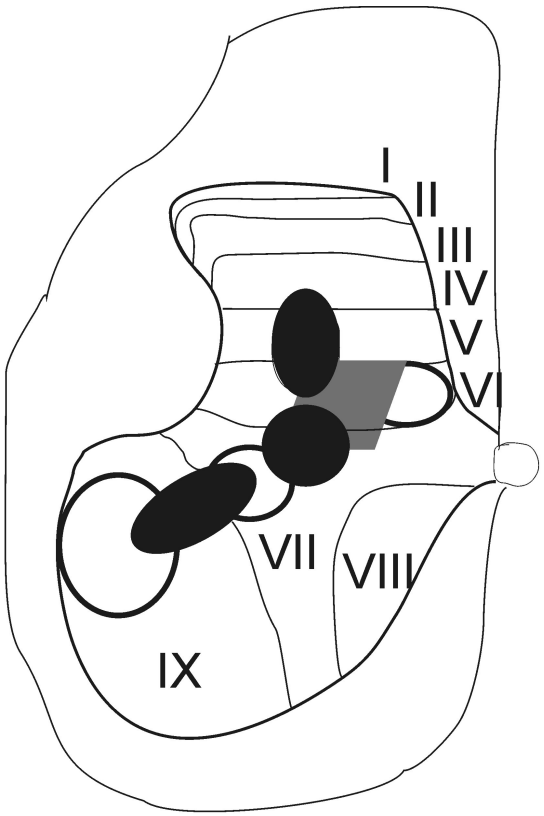

Figure 3. Terminals of group II muscle afferents

The schematic drawing of the average areas of terminations given off by group Ia, Ib and group II muscle afferents from the ankle extensor gastrocnemius muscle based on data from Brown (1981, see Fig. 3). Note, that the most overlap between group I and group II afferents occurs in the intermediate zone (laminae VI and VII) and in the ventral horn (laminae IX) but there is no (or very small) overlap in the dorsal laminae (IV-V).

Tracings of identified muscle group II afferents from plantaris, a cat ankle extensor muscle shown that fibres can have terminations only in the dorsal horn, or in the in the dorsal horn as well as in the intermediate zone and even within the motor

nuclei. Intra-axonal staining of group II muscle afferents showed bifurcation of almost all fibres in the dorsal funiculus into an ascending and a descending branch (Brown 1981, Mannen et al. 1981). Two or more collaterals are given off the same node and some collaterals bifurcate into a secondary collateral in the dorsal funiculus. Nearly all collaterals entering the gray matter at the level of the homonymous motor nucleus terminate in laminae III to VII, with the majority of the terminals in lamina V (Mannen et al. 1981).

The distribution pattern of group II afferent terminals depends on what spinal segments they enter the cord relative to the distribution of the motoneurones innervating the same muscle from which the fibres originate. In the segments where the motor columns innervating the muscle of origin are located, group II terminations were seen in both dorsal and ventral areas (Hongo 1992). Rostral and caudal to the segments where motor columns of the muscle of origin are located, group II collaterals terminate mainly in the dorsal horn (Hongo 1992). The distribution of group II afferent boutons from medial gastrocnemius, plantaris and flexor digitorum brevis muscles in relation to the homonynous motor nuclei were shown to extend rostrally and caudally outside of the corresponding motor nuclei with terminals mainly in lamina IV and V (Ishizuka et al. 1984). The terminals entering lamina IX gradually decrease in number and eventually disappear outside of the corresponding motor columns (Ishizuka et al. 1984). This suggests that the larger distribution a motor column has, the larger is the rostro-caudal extent of the ventral termination of group II afferents originating from the same muscle associated with the motor column.

One of the earliest reports on the projections of muscle group II afferents from the *gastrocnemius* muscle of the cat utilized electrical stimulation to trace the impulses of these nerves in the spinal cord (McIntyre and Lloyd 1948). This study found that group II afferents had a reduced conduction velocity past their entry points in the dorsal roots. They also found projections from muscle group II afferents of ankle extensors in the upper thoracic segments and concluded that *gastrocnemius* group II afferents pass the group I fibres in the lower thoracic cord and continue upward, as they probably contribute to the tract of Goll, i.e. fasiculus gracilis (McIntyre and Lloyd 1948).

Although, Fern and colleagues (1988) could not verify the existence of ascending projections of group II afferents as far as the cervical segments.

Coombs and colleagues (1956) have identified field potentials evoked by biceps-semitendinosus (BSt), quadriceps and gastrocnemius group II afferents in the 6th and 7th lumbar segments of the feline cord. Extracellular field potential recordings reflect the flow of current in the vicinity of the recording microelectrode. This current is generated by several events such as action potentials in nerve fibres, postsynaptic potentials in neurones within the gray matter of the cord, and by the depolarization of afferent terminals (for review see Willis 1980). The field potentials evoked by the activation of muscle group II afferents have the same onset latency but a shorter duration than the postsynaptic potentials evoked in the neurones around the recording electrode. Although, Coombs and colleagues (1956) reported some of the latencies of field potentials, they did not attempt to discriminate between the monosynaptic and the later components. Detailed measurements of onset latencies from group II field potentials evoked by gastrocnemius-soleus afferents in a series of investigations established that the onset latency of monosynaptic, muscle group II-evoked field potentials ranges from 0.6 to 1 ms in the dorsal horn, and from 1.2 to 2.5 ms in the intermediate zone and the ventral horn as measured from the group II afferent volley (Fu et al. 1974, Fu and Schomburg 1974). Responses evoked by the fastest conducting group II afferent fibres overlap with those evoked by the slowest conducting group I afferents (see page 1 for discussion of conduction velocity). The latency of monosynaptic group II field potentials in the ventral horn is much greater than in the dorsal horn because these afferents become greatly reduced in their diameter after entering the dorsal horn (see above McIntyre and Lloyd 1948).

Describing monosynaptic connections from secondary muscle spindles to motoneurones represented a break-through concept at the time when the Ia afferent fibres were thought to be the only source of monosynaptic excitatory potentials to spinal motoneurones. Although, group II evoked EPSPs have been fairly extensively described in ankle extensor motorneurones (in triceps surae), there is a lack of experimental data for other hindlimb motoneurone pools. It is also important to bring into perspective that

unitary group II evoked monosynaptic EPSPs in motoneurones not only occur less frequently than EPSPs evoked by group I afferents, but also they produce only 10-15% of the size of group I evoked EPSPs (Lundberg et al. 1977). The group II-evoked EPSPs elicited in the motoneurones are also smaller in amplitude than the EPSPs described in spinal interneurone populations (see McCrea 1986).

1.4 Field potentials

Field potentials - in the context of my thesis - refer to extracellular recordings from intraspinal sites and are the result of the net movement of charge (i.e ions) in the vicinity of the recording electrode following the stimulation of sensory nerves. Figure 4A is a schematic of an intraspinally positioned microelectrode used to record field potentials evoked by the stimulation of peripheral nerves. In the experiments conducted during the course of this thesis work, the microelectrode was positioned near spinal neurones that received afferent input from more than one sensory nerve. The schematic of figure 4B illustrates a recording electrode positioned in the vicinity of neurones that receive input from three different afferents (black lines: muscle input from nerves 1 and 2 and gray line: cutaneous input from nerve 3) and panel C illustrates recordings collected from one of the experiments used for the thesis in the caudal portion of the 5th lumbar segment (cL5) of the spinal cord 1.8 mm deep from the surface (top trace). At this location, field potentials were evoked by the stimulation of two muscle nerves (TA and EDL) and a cutaneous nerve (SP). The bottom trace in panel C is a simultaneous recording from the surface of the spinal cord (the cord dorsum potential).

What are the components contributing to field potential recordings? Without peripheral nerve stimulation, the baseline is (of course) flat. Following the stimulation of muscles nerves (stimulus artefacts labelled SA) that have terminals close to the electrode, a positive deflection is evoked by action potentials moving through the stimulated nerves, closer and closer to the afferent terminals. When the action potential reaches the afferent terminals, the depolarzation accompanying it is called the terminal potential. Depolarization of the afferent terminals is followed by excitatory neurotransmitter release which in turn evokes depolarization in the postsynaptic cells.

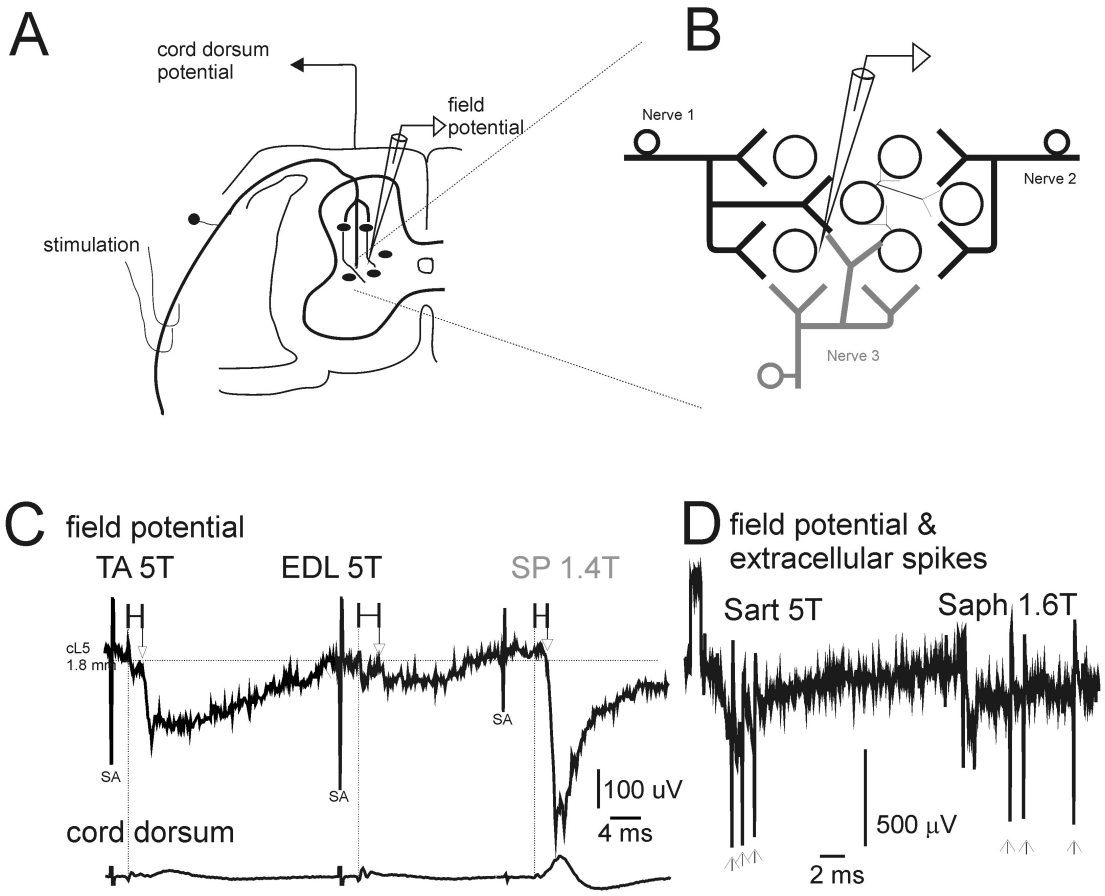

Figure 4. Recording field potentials from spinal neurones

A. Illustration of the experimental set-up for recording field potentials evoked by afferent stimulation intraspinally**.**

B. Schematic representation of a recording electrode used to collect field potential recordings from spinal locations where multiple sensory nerves have terminals. Nerve 1 and 2 (black) represent connections from different muscles afferents and nerve 3 (gray) show connections from a cutaneous afferent exciting spinal neurons in the vicinity of the recording electrode.

C. Averaged field potential recordings (n=12) evoked by the stimulation of the tibialis anterior (TA) and extensor digitorum longus (EDL) muscle nerves at 5T and by the superficial peroneal (SP) cutaneous nerves in the caudal L5 segments, 1.8 mm deep. Stimulus artefacts (SA) are followed by the onset of a large negative deflection in the recorded potentials with onsets marked by downward arrows.

D. Field potential recordings with action potentials (upward arrows) evoked in a neuron with input from the sartorius (Sart) muscle and the saphenous (Saph) cutaneous nerve. The cord dorsum recording is not shown for clarity.

When excitatory post synaptic potentials are evoked, there is a movement of positively charged particles into these neurones, thus generating a local negativity in the vicinity of the recording electrode. This is seen as a downward deflection in the trace. In Fig 4C, the monosynaptic field potential components are seen at the large, early onset downward deflections following TA and SP nerve stimulation. The onset of the excitatory postsynaptic potentials can be matched up with the onset of the field potentials, and depending on the number of postsynaptic cells depolarized and firing, the shape, the size and the duration of the field potentials can greatly vary. The onset of the field potentials (marked by downward arrows) evoked by the TA, EDL and SP nerves and measured from the arrival of the afferent volley recorded with the cord dorsum electrode (vertical dotted lines) were at latencies of 2.0, 2.2 and 1.5 ms, respectively. While the latency of group I muscle afferent evoked field potentials is very similar at all depth within the gray matter in lumbar segments, the latency of field potentials evoked by muscle group II afferents varies greatly with the depths where they are evoked. The majority of the recordings that will be described in this thesis, however, come from less than 2 mm from the surface of the spinal cord and constitute a homogenous pool of data in this respect. Field potentials evoked monosynaptically (i.e. afferents making direct contacts with first order neurones) by group II muscle afferents originating from pretibial flexor muscles and by cutaneous afferents in the mid-lumbar dorsal horn have been shown to range from 1.5 to 2.9 ms and from 1.3 - 1.7 ms respectively (Edgley and Jankowska 1987a). Examining the changes in monosynaptic field potentials evoked by afferents provides insights about mechanisms that modulate synaptic transmission at the presynaptic level. When measuring the peak amplitude of the monosynaptic field potentials within 0.8 ms from the onset and thus before second order neurones could be activated, amplitude changes will reflect modulation in the degree of depolarization of first-order neurones contacted by these afferents. In turn this gives an indication of the amount of transmitter released from the afferent terminals. Accordingly, postsynaptic levels of ionic movements and the recorded field potentials will be different in size. This presynaptic interpretation of changes in the monosynaptic components of field potentials is supported by a large body of experimental evidence (e.g. Rossignol et al. 1998,

Rudomin 1999). It is also important to acknowledge that changes in the monosynaptic components of field potentials evoked by primary afferents could also reflect changes in the sensitivity and operation of the receptors of postsynaptic neurones that bind the excitatory neurotransmitters released by the afferents or changes in the driving force for ion movements. One other factor could be the size postsynaptic potentials arriving from different sources at the time of afferent input from afferents being examined (see discussion in Perreault et al. 1999).

The experiments described in the thesis have utilized field potentials evoked by activation of peripheral nerves and compared the amplitude of different types of field potentials (i.e. evoked by muscle group I, group II or cutaneous afferents) at the same spinal locations in order to examine the possibility of a differential modulation of synaptic transmission during different motor tasks. The underlying assumptions used for the interpretation of the data were that 1) mainly excitatory postsynaptic potentials and action potentials evoked by afferent input contribute to the field potentials recorded; 2) changes in the monosynaptic field potentials reflect modifications occurring in the presynaptic milieu leading to altered neurotransmitter release and that 3) changes in later components of field potentials (i.e. peak amplitude measured at more than 0.8 ms after the onsets) reflect postsynaptic modulation of transmission in reflex pathways mediating actions of muscle or cutaneous afferents. Although, inhibitory postsynaptic potentials also evoke movement of charged particles and under some conditions can greatly contribute to extracellular field potentials (for references see Leung 1990), they are typically slower, later and of lower amplitude than excitatory postsynaptic potentials and therefore contribute to later components and to a lesser extent than excitatory PSPs. However, there are no known monosynaptic inhibitory PSPs evoked from sensory afferents in the mammalian spinal cord. Thus contributions of IPSPs to the field potentials under study here would be longer latency (i. e. at least disynaptic) and would produce depolarizing fields (unlike the exclusively hyperpolarizing components reported here).

By manipulating the position of the microelectrode, we were able to recorded both postsynaptic field potentials and the action potentials of interneurones with

monosynaptic sensory input. Panel 4D illustrates the spiking of a neurone that was activated by the stimulation of both a muscle (Sart) and a cutaneous nerve (SP). The arowheads indicate the action potentials fired by this single unit, and note that they fall onto a field potential evoked by the same afferents (i.e. wider negative deflection from baseline). In the second part of the thesis, similar recordings to this will be described that were used to gain further insights on the modulation of synaptic transmission in reflex pathways from muscle and cutaneous afferents during different motor tasks. In the case of these extracellular recordings of neuronal activity, the latency of the action potentials and the number of responses evoked per stimulus was used as a measure of synaptic efficacy, and more discussion is provided on the interpretation of the data in later sections of this thesis (e.g. see Paper 2).

Until the late 1980s, investigations regarding the central projections of muscle group II afferents had focussed mainly on areas in the lower lumbar and sacral segments. Then Edgley and Jankowska (1987a) demonstrated that in the mid-lumbar (L3 - L5) spinal segments, several hindlimb group II muscle afferents evoke relatively large field potentials at two main locations: in the dorsal horn (laminae IV-V) and in the intermediate zone (laminae VII - VIII). In the dorsal horn, field potentials evoked by the stimulation of muscle afferents at 5T did not have a group I component, but field potentials located in the intermediate zone had both group I and group II components. At both dorsal and intermediate locations, monosynaptic connections exist between group II fibres and spinal neurones. The receptor origin of these mid-lumbar field potentials was investigated by using selective activation of group II afferents following the fatigue of extrafusal and group I fibres (Harrison et al. 1988). These studies were critical for establishing that fibres originating from secondary spindle endings give rise to excitation in these mid-lumbar regions but some contribution to this excitation evoked by afferents arising from other receptors than secondary spindle endings, although probably minor, cannot be excluded. It is also important to point out that although the studies by Harrison and colleagues (1988) confirmed that muscle group II afferents of the Quad and Sart muscles originate from secondary spindle ending, they did not study afferents from other hindlimb muscles and neither have other studies addressed this

issue up to date.

Throughout the lower lumbar segments (L6 and L7), group II afferents from the hip extensor, pre-tibial flexor and some ankle extensor muscles also evoke field potentials (Riddell and Hadian 2000a). These potentials were located in laminae IV-V, lateral to the intermediate zone group I field potentials and sometimes even present in lamina VII with latencies suggesting monosynaptic connections.

As mentioned above, field potential recordings are thought to represent-in part-the activity of populations of neurones with input from muscle group II afferents and they can be used as a preliminary step in identifying the location of such neurones within different segments of the spinal cord. In turn, the activity of these neurones can be recorded by an intracellularly placed microelectrode in order to decipher their input and output patterns and the role they play in reflex pathways originating from muscle group II afferents. In the following section, populations of interneurones that receive input from group II muscle afferents will be described in more detail.

1.5. Spinal neuronal relays of group II muscle afferents

Investigations mostly done by Elzbieta Jankowska and her colleagues have revealed several populations of spinal neurones with monosynaptic input from group II muscle afferents in the cat lumbar spinal cord (see review Jankowska 1992 and Jankowska and Hammar 2002). Most of the current information known about populations of spinal neurones with input from muscle group II afferents originated from studies on deeply anaesthetized animals. The act of recording intracellularly from spinal neurones that are non-motoneurones can be difficult because of the small size of the cells and the experimental conditions must be very stable to get records with appropriate quality. Hence the anaesthetized preparation is far more advantageous than any other condition.

One population is located in the mid-lumbar segments (L3-L5) that receives group II input mainly from hip flexor, knee extensor and pretibial flexor muscles (Edgley and Jankowska 1987b). Cells with a soma in the dorsal horn (laminae IV or V) are likely functionally different from those located in the deeper laminae. Dorsal mid-lumbar interneurones receive only group II afferent input while neurones in the

intermediate and ventral horn get both group I and group II input. Some group II interneurones in the intermediate laminae have direct projections to lumbar motoneurones several segments away while dorsal interneurones usually do not contact motoneurones directly (Edgley and Jankowska 1987b). Another population of interneurones with monosynaptic group II afferent input is located in the sacral segments of the feline spinal cord (L7-S2). The sacral group II interneurones receive input mainly from the knee flexor posterior biceps and semitendinosus and the ankle extensor triceps surae muscles (Jankowska & Riddell 1993). These sacral interneurones are located in the dorsal horn within the lateral half of laminae IV and V (Jankowska & Riddell 1993). The majority of these interneurones receive excitation from cutaneous afferents from the skin over the hindlimbs and the perineal region. They project to other group II interneurones in the mid-lumbar segments. The third population of group II activated interneurones is located in the lateral parts of laminae IV-VII of lower lumbar segments (L6-L7) (Lundberg et al. 1987a, Riddell and Hadian 2000b). There is no clear dorsal-intermediate division of these cells as for those found in the mid-lumbar region. The source of group II muscle afferent excitation to L6-L7 interneurones comes from a wide range of muscle nerves including mainly knee and ankle flexors (Lundberg et al. 1987a). Most of these interneurones receive convergent input from cutaneous and group I afferents as well as different group II afferents. There are a few last order interneurones within this lower lumbar group of cells, but most of them project to other interneurones (Riddell and Hadian 2000b). Figure 5 illustrates the schematic relationship of the three main groups of group II-activated interneurones. The mid-lumbar population is subdivided into the dorsal (dark circle) and intermediate (white circle) pools. The arrows point from the source to the target populations. The lower-lumbar and sacral intermediate group II interneurones are shown to send projections to more rostral areas, eventually reaching the mid-lumbar intermediate interneurone pools. For each population of group II-activated neurones, the afferents evoking the most prominent excitation are listed above the cord. Several of the experiments described in the thesis examine the modulation of transmission from muscle afferents to spinal neurones and compare it to the modulation of cutaneous afferent input reaching the same

neurones. So far, the receptor origin, the activation and the spinal projections of muscle afferents have been discussed. A similar discussion on cutaneous afferents is to follow.

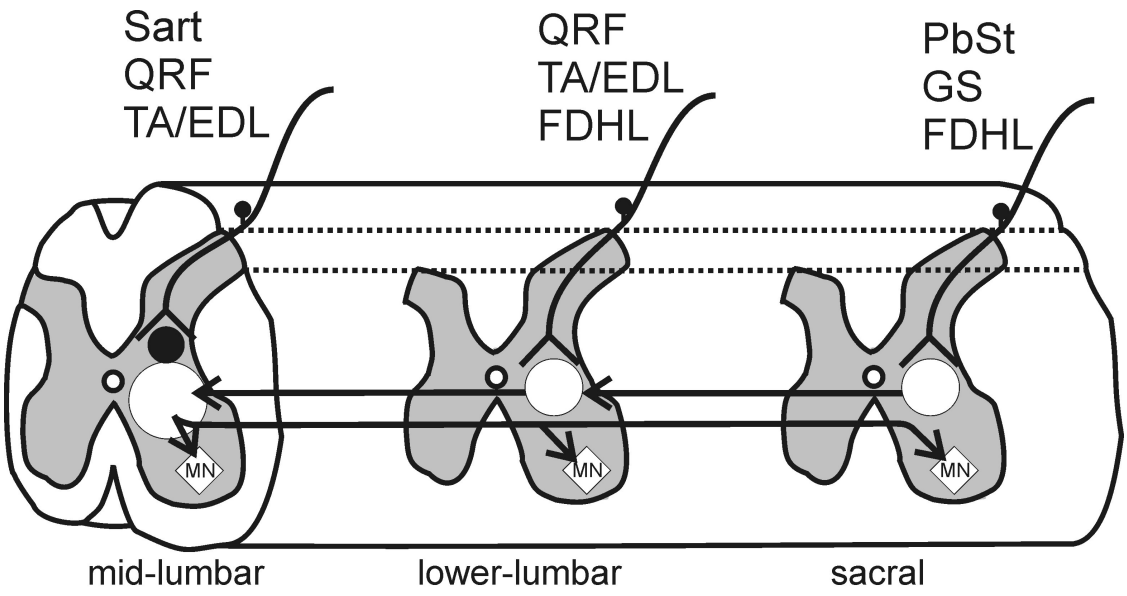

Figure 5. Populations of spinal interneurons with input from muscle group II afferents
Schematic representation of spinal interneuron populations with monosynaptic input in 3 main areas of the feline spinal cord: in the mid-lumbar, the lower-lumbar and the sacral segments. In the mid-lumbar segment there is a dorsal (black circle) and an intermediate (white circle) subpopulation of neurons.The intermediate group II neurons project to motoneurons in the same or even more caudal segments. Note the axonal projections of sacral dorsal interneurons to more rostrally located group II interneurons. The major afferent input in each of the main areas is listed above the spinal cord.

2. Cutaneous afferents

2.1. Fibre types, receptors and activation of cutaneous afferents

The nomenclature created by Lloyd (1943) has been the most accepted standard for characterizing cutaneous afferents. The low-threshold, large diameter cutaneous afferents (group II category, or A-alpha fibres) originate from several different types of mechanoreceptors such as hair follicles, Merkel bodies and Pacinian corpuscles (Brown et al. 1980). The high-threshold, small diameter fibres (group III and IV category) originate from mechanoreceptors and mainly from free nerve endings involved in pain and temperature sensation (Brown 1981).

The studies that will be described in the thesis examine cutaneous nerves that innervate the skin over the thigh, the lower-leg and the paw of the cat, namely the saphenous (Saph), the sural (Sur) and the superficial peroneal (SP) nerves. The saphenous nerve supplies the skin of the medial aspects of the lower thigh and the leg. Myelinated Saph fibres innervate all types of mechanoreceptors (Heaney et al. 1984). The Sur nerve innervates the lateral and posterior aspect of the thigh and the lower-leg, including the tarsus, metatarsus and the calcaneus. The majority of these fibres innervate hair receptors (Hunt and McIntyre 1960b) and about 70% of the axons are myelinated (Langford 1983). The superficial peroneal nerve, distally, innervates the dorsum of the foot and the toes. Low-threshold afferents from SP are likely to arise from hair follicles as air-puff stimulation aimed at the dorsum of the foot evokes the same kind of stumbling corrective reaction as the electrical stimulation of the SP nerve (Forssberg 1979).

Specialized nerve endings in the skin have preferential sensitivity for certain types of stimuli. The hair follicles respond to light touch and vibratory stimuli and they adapt quickly. The Merkel bodies respond to touch and pressure and are slowly adapting. Pacinian corpuscles also respond to fast vibrations and touch and have a very fast adaptation mechanism. The free nerve endings respond to cooling, warming, painful pressures, chemical irritants and are slowly adapting. In the studies presented in the thesis, the cutaneous fibres were activated only by electrical stimulation instead of the natural activation of the receptors.

Electrical stimulation is commonly used to study the central reflex pathways from cutaneous afferents. Typically, two peaks of evoked potentials can be observed following the stimulation of the sural nerve (Boyd and Kalu 1979). These two peaks reflect the activation of the large and the small diameter fibres. Electrical stimulation at 2-2.4T is sufficient to recruit all the large, low-threshold cutaneous fibres in the sural nerve while the small, high-threshold myelinated nerves have a mean threshold of 8.7T (Boyd and Kalu 1979). In the experiments presented in the thesis, mainly 1.2 to 2T and occasionally 5T stimulation of cutaneous nerves was used with electrical pulses (0.1 - 0.2 ms long, 1-2 shocks) in order to activate only low-threshold fibres.

2.2. Central projections of cutaneous afferents

The terminations of Saph, Sur and SP afferents in the dorsal horn of the lumbar spinal segments will be reviewed here without a discussion of their more wide-spread projections. Afferents from the Saph nerve enter to the spinal cord at levels ranging from the 4th to the 7th lumbar segments but predominantly in the 5th and 6th (Koerber and Brown 1982). Distribution of the Saph fibres was found to be somatotopically organized, with proximal parts of the nerve projecting to more rostral areas (Heaney et al. 1984). The majority of the Saph terminals have been found in laminae III and IV (Koerber and Brown 1982) but Brown and Fuchs (1975) have shown terminals from this nerve in laminae V and VI as well. Projections of the Sur nerve have been found to enter through dorsal roots ranging from the 6th lumbar to the 1st sacral segments, but most frequently in the 7th lumbar segment while SP afferents enter in the caudal parts of the 6th lumbar and in the rostral half of the 7th lumbar segment (Light and Durkovic 1984).

The terminations of Saph, Sur and SP afferents overlap with muscle group II input from various hindlimb nerves mainly in laminae IV-VI in the lumbar segments. At the majority of the sites selected for recording in the experiments described in the thesis, field potentials were evoked by both muscle group II and cutaneous afferents. Both low-threshold cutaneous and group II muscle afferents are proprioceptors and they convey important sensory information about external perturbations and muscle position. The reflex actions evoked by these afferents during motor activity are powerful.

3. Reflex actions of muscle group II afferents

3.1. Reflexes evoked in quiescent preparations

Early discussions on the function of group II afferents arose from work on flexion reflex systems. In anaesthetised, low spinal animals, the activation of group II afferents produces essentially the same response as does the activation of group III muscle, high threshold cutaneous and joint afferents (Eccles & Lundberg 1959), namely the flexion reflex. This response consists of excitation of ipsilateral flexor muscles and inhibition of ipsilateral extensor muscles along with a crossed extension reflex (excitation of contralateral extensors and inhibition of contralateral flexors). Consequently, group II afferent fibres were considered as being a part of the flexor reflex afferent (FRA) system (Eccles & Lundberg 1959, for review see McCrea 1992).

Reflex actions of group II afferents after low-pontine lesions in the cat change to evoke excitation of extensor and inhibition of flexor motoneurones and suggest that an alternate reflex pathway controlled partly by descending systems exists for group II afferents (Holmqvist and Lundberg 1961). Eccles and Lundberg (1959) were well aware of the proprioceptive activity of group II afferents and discussed the potentially destabilizing effects of group II fibres if their activation resulted in flexion reflexes during stepping. They argued for the need to prevent the group II muscle afferents from evoking flexion reflexes during movements such as stepping. Forty years after Lundberg's insights, Perreault and colleagues (1999) showed that in the decerebrate cat preparation (with intact spinal cord) group II flexion reflexes are depressed with the onset of fictive locomotion. A detailed discussion of non-FRA type reflexes evoked by muscle group II afferents under different conditions will be given in section 3.2 of the introduction. Another important characteristic of muscle group II reflex actions is that unlike group Ia afferents, they can influence motoneurone activity bilaterally, therefore possibly controlling reflexive left and right hindlimb coordination (Arya et al. 1991, Bajwa et al. 1992). Actions of muscle group II afferents on contralateral motoneurones seem to be exaggerated after trassection of the spinal cord (Arya et al. 1991), suggesting that these reflex pathways are under strong descending control. Evidence for gr II afferents to influence bilateral motor activity during locomotor-like behaviour has been

reported (e.g. Perreault et al. 1995) but are scanty. Spike-triggered averaging was used by Kirkwood & Sears (1975) to link synaptic potentials in soleus and intercostal motoneurones to group II muscle afferent firing. Monosynaptic connections from triceps surae group II afferents to other hindlimb motoneurones were shown to be similarly organized as the Ia afferent connectivity between synergistic muscles (Stauffer et al. 1976). It should be emphasized that the group II monosynaptic input to motoneurones is very small and infrequent. In this author's opinion, direct group II actions have little effect on lumbar motoneurone excitability and for the purposes of the present discussion, can be ignored. Earlier suggestions that the activation of muscle group II afferents also contributes to the stretch reflex have been re-interpreted (Matthews 1989) with the conclusion that activation of group II afferents contributes to the amplitude-increase of the short (but still polysynaptic) but mainly the long latency components of the stretch reflex. Most recently, the view that interneuronally mediated actions of both primary and secondary endings take part in reflexly regulating human voluntary movements, at least in the hand, has also been verified (Lourenco et al. 2006, also see Matthews 2006).

Muscle group II afferents seem to be particularly important for the generation of the medium and longer latency component of the stretch reflex in humans. Evidence for muscle group II afferents contributing to the late component of the stretch reflex comes from several different studies in which selective activation of muscle group II afferents was applied by vibration of group I fibres (Bove et al. 2003), or by nerve cooling and ischemia (Grey et al. 2001). The functional role of the large, late component of the stretch reflex in lower limb muscles is thought to be the generation of torque that is strong enough to anchor the toes to the platform and provide a good base for the responses in other ankle extensors to stabilize posture and movements should a perturbation happen during locomotion (Schiepatti and Nardone 1999). In humans with Charcot-Marie-Tooth polyneuropathy, there is a nearly complete loss of Ia afferent-evoked (i.e. short latency) component of the stretch reflex. The medium latency, group II -evoked reflexes are unaffected and are likely to be the reason why these patients do not have larger body sways during stance than healthy humans (Nardone et al. 2000,

reviewed in Schiepatti and Nardone 1999). Other types of neuropathies that effect both small and large diameter muscle afferents often result in poor balance and loss of postural control. In patients with diabetic neuropathy, there was a clear relationship between the impairment of medium size myelinated fibres (i.e. increase of the latency in the medium-latency component of several ankle muscles, decreased conduction velocity of group II muscle afferents) and the impairment of postural control as measured by the increase of body sway area (Nardone and Schieppati 2004). Thus muscle group II fibres may have a major role in the segmental control of stance.

There is also increasing evidence that reflex actions evoked by muscle group II afferents are a significant component of spasticity (discussed in Eriksson et al. 1996). Antispastic medications, such as L-DOPA and tizanidine suppress reflex actions of muscle group II afferents in cats (Bras et al. 1988, 1989, Schomburg and Steffens 1988). These same agents were also found to suppress group II activation of motoneurones (Jankowska et al. 1998). The late component of the stretch reflex is decreased after the oral administration of tizanidine in humans, while the short latency component is not affected (Corna et al. 1995).

In humans, one population of segmental interneurones that have been found to evoke excitation of proximal leg muscles (of the quadriceps) after stimulation in the cortex has been shown to receive sensory input from group II afferents of the common peroneal (CP) nerve (Marchand-Pauvert et al. 1999). Based on these observations, it has been hypothesized by Marchand-Pauvert and colleagues (1999), that the human neuronal correlates receiving convergent input from descending cortical pathways and segmental afferents are neurones that correspond in function to the mid-lumbar group II interneurones described in the feline spinal cord by Edgley and Jankowska (1987b). Several observations suggest that muscle group II afferents contribute more to the spastic reflexes than muscle group I fibres (Eriksson et al. 1996 and see review by Pierrot-Deseiligny and Burke 2005). For example, monoamine agonists reducing spasticity suppress the facilitation of peroneal group II afferent evoked excitation of quadriceps motoneurones in spastic patients more than the excitation produced by group I afferents (Remy-Neris et al. 2003). In non-medicated patients with Parkinson's

disease, the muscle group II afferent-evoked facilitation of the quadriceps H-reflex was greatly increased in the rigid compared to the non-rigid limb (Moreau et al. 2002). It is hypothesized that the rigidity of the lower limbs in patients with Parkinson's disease also mainly results from the increased excitability of muscle group II afferent reflex pathways.

3.2. Re-organization of reflexes during motor activity

Like the group Ia and Ib afferents, group II and cutaneous afferents are also positioned to be an important part of the step-by-step regulation of motoneurone output during locomotion. Unlike muscle group I afferents, however, our knowledge about the reflex function of muscle spindle secondaries during locomotion remains rudimentary. During fictive locomotion in cats with intact spinal cords, reflexes from muscle group II afferents become separated from those of the FRA system. The suppression of flexion reflexes during fictive locomotion does not mean that there are no reflex actions evoked, rather, the activation of flexor muscle group II afferents has powerful actions on the step cycle. Stimulation of flexor group II afferents originating from the Sart, TA and PbSt muscles during flexion can curtail ongoing flexor activity and phase advance the transition to extension (Perreault et al. 1995). Additional studies have shown that reflex actions of flexor group II afferents originating from EDL, PerL, and iliopsoas muscles enhance ongoing flexor activity (Stecina et al. 2005). Thus, flexor group II afferents can be differentiated into at least two classes based on how they perturb the step cycle. This is also supported by similar observations obtained in decerebrate cats during treadmill stepping (Hiebert et al. 1996, Lam and Pearson 2001, 2002).

Segmental afferent-evoked reflex actions are powerful during many behavioural states. Therefore, segmental sensory input must be gated appropriately to prevent unwanted reflex effects. In the first part of the thesis, gating of transmission from muscle and cutaneous afferent input during fictive locomotion and scratch is examined. There are presynaptic mechanisms that seem to be involved in the gating processes evoked by the operation of the central pattern generator. In the following section, an overview of the presynaptic control mechanisms that modulate transmission in group II and cutaneous reflex pathways will be provided.

4. Presynaptic modulation of transmission from muscle group II afferents

The spinal cord is the first site in the mammalian nervous system where sensory information from proprioceptors is processed and integrated with commands originating from higher neuronal centres. Receptors sensing changes in muscles length and tension, as well as sensory organs located in the skin covering the limbs provide sensory input from the periphery that alters centrally generated motor commands. In general, the mechanisms that modify transmission in sensory pathways are controlled by three major sources: descending projections of supraspinal centres, segmental sensory afferents and the central spinal circuitry. Reduction of transmission from segmental afferents can occur at both presynaptic and postsynaptic levels and most likely, a combination of several types of mechanisms is used to achieve appropriate information flow. The term presynaptic inhibition refers to physiological control mechanisms that act to modify the amount of transmitter released from nerve terminals.

Descending systems that control transmission from group II afferents at presynaptic sites in the feline spinal cord include the corticospinal tracts (Carpenter et al. 1963), the locus coeruleus and the raphe nucleus (Noga et al. 1992), as well as the cuneiform nucleus (Noga et al. 1995, Riddell et al. 1992). Both monoaminergic and non-monoaminergic descending pathways seem to be involved in the depression of muscle group II input as the suppression of group II field potentials is only partially countered by noradrenergic antagonists (Skoog and Noga 1995). Local application of monoamines onto spinal neurones excited by muscle group II afferent input revealed that both serotonine (5-HT) and noradrenalin (NA) can modify the efficacy of transmission from muscle group II afferents (Jankowska et al. 2000). These studies have also led to the realization that 5-HT and NA modulate transmission differently, based on the type of the afferent input and on the functional type of interneurones that receive the input. In the mid-lumbar areas, NA suppresses activation of intermediate zone group II interneurones and of a sub-population of dorsal horn interneurones while 5-HT application has the reverse actions (Jankowska et al. 2000). These observations suggest that muscle group II afferent transmission is strongly controlled by descending monoaminergic projections but it is yet to be examined how much these monoaminergic systems contribute to gating

of muscle group II afferent input during motor activity.

Sensory-evoked presynaptic control of transmission from group II afferents has also an important function in regulating segmental input but so far, it has been studied mainly in the absence of motor activity. Stimulation of cutaneous and group II muscle afferents strongly depolarizes group II fibres but stimulation of group I muscle afferents does not evoke significant depolarization of group II terminals in the mid-lumbar and sacral segments of anaesthetized cats (Riddell et al. 1995). Also, group II afferent-evoked field potentials are depressed by the conditioning stimulation of the same afferents that evoke depolarization in group II fibres. Interestingly, group I afferent evoked-field potentials in the intermediate mid-lumbar segments are reduced to only about two-thirds of their control levels following the stimulation of selected muscle afferents while the group II afferent-evoked field potentials at the same locations are often completely obliterated by the same sensory stimuli (Riddell et al. 1995). Thus, transmission from group II muscle afferents seems to be affected by sensory-evoked presynaptic inhibition more than the input from group I muscle afferents (Riddell et al. 1995). Similarly, the presynaptic reduction of cutaneous afferent-evoked field potentials at the same location where group II afferents also evoked field potentials is much weaker than that of group II afferents (Jankowska et al. 2002). These observations taken together suggest that there is a preferential sensory-evoked suppression of muscle group II afferent input.

During locomotor activity, three main indicators for presynaptic modulation of sensory transmission have been examined in decerebrate cats: membrane potential oscillations, antidromic afferent firing, and modulation of afferent excitability (for review see Rossignol et al. 1998). All of these indicators are based on events that are generated at the level of afferent terminals in the spinal cord and are conducted back into primary afferent axons. The cyclic oscillations of afferent membrane potentials can be recorded at the dorsal rootlets (Baev 1980, Baev and Kostyuk 1981, Dubuc et al. 1985), or from single, identified afferents (Gossard et al. 1989, 1991) or recorded as changes in excitability (Baev 1980, Duenas and Rudomin 1988). Antidromic firing of afferents (often coupled to locomotor activity) can be recorded from dorsal rootlets (e.g. Dubuc et

al. 1985, or see in Rossignol et al. 1998), or in single muscle (Gossard et al. 1989) and cutaneous (Gossard et al. 1991) afferents. The amplitude of the depolarization of primary afferents (PAD) is modulated often in a phase dependent manner and it can be recorded in individually identified cutaneous (Gossard et al. 1990) and muscle (Menard et al. 1999) afferents, or recorded as dorsal root potentials (e.g. Gossard and Rossignol 1990). Recording presynaptic modulation of group II sensory transmission is more difficult than that of group I or cutaneous fibres. As the group II afferents are considerably smaller in diameter and more difficult to isolate, antidromic firing or membrane potential changes have not (yet) been recorded from them during any type of motor activity.

In the dorsal horn within the mid- and lower-lumbar spinal cord, field potentials evoked by muscle group II fibres are reduced to 80% of their control amplitude during fictive locomotion. This reduction is comparable to the suppression of group I and cutaneous afferent-evoked potentials seen in the dorsal laminae during fictive locomotion. However, in the intermediate regions there is a much greater reduction of group II-evoked field potentials than that of group I and cutaneous-evoked fields. Based on these observations, Perreault and colleagues (1999) hypothesized that there is a preferential suppression of muscle group II afferent input based on the location (i.e. in the intermediate regions) in the lumbar segments. The results presented by Perreault and colleagues (1999) are from a post-hoc analysis of pooled data and they provide only indirect evidence for the preferential suppression of muscle group II input. In the studies described in the first part of the thesis, the hypothesis put forth by Perreualt and colleagues (1999) was examined by using a different experimental design. In the experiments described in this thesis, recordings of muscle group II and cutaneous afferent-evoked field potentials at the same spinal locations during fictive locomotion and/or fictive scratch were collected. Therefore, the modulation of several different types (i.e. cutaneous, group I and group II) of field potentials could be compared during the same bout of motor activity in order to provide direct evidence for the preferential suppression of group II input. Additionally, the thesis also examined how the activity of individual neurones is influenced by the centrally-evoked mechanisms that alter

transmission from group II afferents. The term ‘centrally-evoked’ refers to the idea that the spinal cord and the components of the central nervous system responsible for generating rhythmic motor activity are also responsible for the modulation of transmission from these afferents. This term differentiates the type of presynaptic control to be presented in this thesis from the more commonly studied "sensory-evoked" presynaptic inhibition. Sensory-evoked control of afferent transmission is typically evoked by conditioning stimulation of (usually another) a peripheral nerve and it has been reviewed extensively (e.g. Willis 1999; Rudomin Schmidt 1999).

It is clear that stimulation of brainstem areas evoking locomotion in decerebrate cats can suppress muscle group II afferent input in anaesthetized cats in the absence of locomotion. However, it is unclear whether this suppression results from the direct stimulation of the mesencephalic locomotor region and in turn, the activation of descending systems involved in the generation of locomotor-like activity. Therefore, fictive scratch behaviour was evoked in some of the experiments described in the thesis to further examine the factors involved in the modulation of afferent transmission from muscle group II afferents in the absence of brainstem stimulation.

Scratching is a site-specific motor response to tactile stimulation applied on the skin of mammals so the limbs rhythmically rub against the stimulated skin surface to remove the source of irritation. activity pattern of hindlimb muscle nerves during fictive and real scratch is very similar. The scratch reflex can be divided into 3 phases: the approach, the cyclic, and the return part (Kuhta and Smith 1990). In the approach phase, the ipsilateral hindlimb on the stimulated side is lifted to the head in order to reach the ears with the paw through the tonic activation of hip and ankle flexors and knee extensors. The cyclic phase consists of alternating flexor and extensor motoneurone activity at a cycle period of about 120-250 ms. In the return phase, the hindpaw is re-positioned to touch the ground. In cats, fictive scratch can also be evoked by applying curare or bicucculine on the dorsal roots of the first or second cervical roots followed by the mechanical stimulation of the skin covering the ear and/or the face on the ipsilateral side (Orlovsky and Deliagina 1999). Direct electrical stimulation of the brainstem is not required for evoking fictive scratch, therefore it is a good model to assess whether

suppression of field potentials evoked by muscle group II afferents occurs without electrical activation stimulation in brainstem. Although, it is acknowledged that the central circuitry producing scratch could also cause activation of some brainstem areas and activity in bulbo-spinal descending pathways (see Arshavsky et al.1978a, 1978b, 1978c).

5. How can strong reflex actions from group II afferents occur during fictive behaviours when transmission from these afferents is reduced?

Several characteristics of muscle group II and cutaneous afferents have been discussed in the previous parts of the Introduction. Both muscle group II and cutaneous afferents are important for proprioception, the sensing of the position of body parts, relative to other neighbouring areas of the body. Their main role, i.e. as static length sensors, makes them one of the key players in helping to relate muscle length across different areas of our body and with the numerous sites in the spinal cord where their afferent terminals converge with other types of muscle sensory and cutaneous input they are likely to be especially important for proprioception. However, as of yet, there is not a clear understanding of how their inputs are combined with tactile or vestibular or other sensory input that also plays a role in proprioceptive control. What is known, is that muscle group II afferents can evoke a variety of strong reflexes under various behavioural states. As described above, there is also a strong modulation of the efficacy of transmission from muscle group II afferents evoked by several sensory afferents, descending pathways, and from the central spinal circuitry generating patterned motor activity. **The first aim of the thesis will be to examine whether there is a centrally-evoked preferential suppression of muscle group II input at the same spinal locations in lumbar segments during fictive locomotion and during fictive scratch**. To this end, extracellular recordings of field potentials evoked by muscle and cutaneous afferents will be collected prior to and during fictive motor activity and the amplitude modulations of the field potentials will be compared. By evoking two different fictive motor behaviours (locomotion and scratch) the relative contribution of the midbrain

stimulation to the suppression of these sensory inputs will also be assessed.

The fact that transmission from muscle group II afferents is reduced during fictive locomotion (Perreault et al. 1999) but strong reflex actions can be triggered by these sensory fibres (Perreault et al. 1999, Stecina et al. 2005) seems paradoxical. One could assume that there should be specific locations in the spinal cord where muscle afferent input is less suppressed during motor activity in order to allow some reflex actions. This assumption does not seem to hold true: at every location examined to date (in Perreault et al. 1999) there was a significant reduction of muscle afferent input. However, if the reduction of group II afferent transmission was not uniform but selective, according to the muscle of origin, then group II reflexes evoked during locomotion from one muscle could be more powerful than those from another. More recent experiments in our laboratory examined the modulation of transmission from group I afferents and found that although the overall depression of group I input from different muscles is the same on average, field potentials from two different muscles at the same location were often reduced to different levels (Gosgnach 2003). Such selective presynaptic control of transmission from group II afferents originating from different muscles at certain spinal locations would allow for differential modulation of transmission from group II fibres of specific muscles and also could explain the differences seen in their reflex actions. **The second aim will be to test the hypothesis that the suppression of group II afferent input from different muscles at the same spinal location is not uniform.** This will be addressed by recording field potentials following the stimulation of group II muscle afferents in two or more peripheral nerves terminating in the same regions within the lumbar segments. Paper 1 will present the results in regard to the first and the second aims.

Another mechanism that could play a role in regulating and differentiating the reflex actions of group II afferents during fictive locomotion is the control of spinal interneuronal excitability. Although there are several interneurone pools that have been associated with muscle group II input, our knowledge about the activity of candidate interneurones mediating group II reflex actions during fictive locomotion is limited. A study by Edgley and colleagues (1988) provided evidence for the activation of some

group II activated mid-lumbar interneurones by the mesencephalic locomotor region. Based on their observations, these interneurones were hypothesized to be active during fictive locomotion. Shefchyk and colleagues (1990) reported that some mid-lumbar group II interneurones projecting to motoneurones show phasic activity during fictive locomotion. Interestingly, the short-latency afferent input to the phasicaly active interneurones was suppressed during locomotion. This suppression was originally thought to be the result of postsynaptic control of mid-lumbar interneurones, however, the strong presynaptic modulation of transmission from group II afferents in this region (Perreault et al. 1999) could also partly account for it. Because the sensory-evoked activity of these neurones was inhibited during locomotion, they were considered as unlikely candidates for mediating group II reflex actions during fictive locomotion. Interestingly, some sacral group II activated interneurones were also found to have reduced spontaneous activity during micturition in decerebrate cats (Buss and Shefchyk 2003).

The second part of the thesis will examine the activity of group II activated spinal neurones that may mediate group II reflex actions during fictive locomotion. To this end, extracellular neuronal activity of cells in the lumbar segments that are activated by muscle group II afferent input will be collected. These neurones will be described electrophysiologically during fictive locomotion. Evidence for these neurones being involved in muscle group II afferent-evoked reflexes will be obtained by recording their activity during perturbation of the locomotor rhythm following electrical stimulation of peripheral muscle nerves. Paper 2 will present the results from these studies. Lastly, in the General Discussion, the interpretation of the results from both parts of the thesis and the future potential applications of the results will be discussed.

Paper 1

Preferential Suppression of Group II and Cutaneous Afferent Transmission During Fictive Locomotion and Scratch in the Cat

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Summary

A comparison was made in decerebrate cats of the effects of fictive locomotion and fictive scratch on field potentials evoked by different types of afferents terminating at the same locations in the lumbar spinal cord. Both monosynaptic and longer latency components of field potentials evoked by electrical stimulation of peripheral muscle nerves were reduced during fictive locomotion following midbrain stimulation and a similar, but less pronounced, depression of the same field potentials was evident during fictive scratch evoked by curare application to dorsal roots and mechanical stimulation of the skin. At the same locations, field potentials evoked by cutaneous and by group I muscle afferents were reduced less (to 67 ± 5% and 85 ± 7% of control, respectively) than those evoked by muscle group II afferents (to 51 ± 3% of control on average) during fictive locomotion. Moreover, field potentials evoked by group II afferents of different muscles at the same locations were found to be suppressed non-uniformly during fictive motor activity in 10/15 cases. Although, some field potentials were rhythmically modulated during fictive motor activity, the phase in which the maximal depression occurred was variable. The rhythmic modulation was generally less pronounced than the tonic depression during both fictive locomotion and scratch. During the approach phase of fictive scratch, the suppression of the field potentials evoked by muscle group II afferents was 10% less on average than during rhythmic scratching. Together the results suggest that rhythmic motor activity with and without the direct activation of the mesencephalic locomotor region can trigger a tonic suppression of transmission from myelinated cutaneous and hindlimb muscle afferents. This suppression seems to exert a preferential control of reflexes evoked by muscle group II afferents and it is presynaptic in part.

Introduction

The central nervous system has powerful actions on synaptic transmission from propriospinal input during motor activity. One line of evidence for changes in the efficacy of synaptic transmission from muscle and cutaneous afferents in the feline spinal cord comes from observations on rhythmic membrane potential oscillations of individual group I afferents during locomotion (for references see review: Rossignol 1996). These oscillations are thought to result from the depolarization of primary afferents (PAD). Recordings from identified single muscle group I and cutaneous afferent fibres revealed tonic (Duenas and Rudomin 1988) as well as phasic (Gossard et al. 1989) depolarization of primary afferents during fictive locomotion and during fictive scratch (Cote and Gossard 2003). As PAD represents changes in excitability, it has been used as an indicator of reduced transmission in spinal pathways.

There is less evidence for motor-activity related changes in afferent transmission occurring in reflex pathways mediating actions of group II muscle afferents. To measure the PAD of muscle group II afferents is not easy as they are smaller in diameter and are more difficult to identify than group I fibres. Assessing the monosynaptic component of composite extracellular excitatory postsynaptic potentials (i.e. field potentials) evoked by muscle group II afferents during fictive motor activity offers a good opportunity to examine changes in sensory evoked transmission at presynaptic sites. Monosynaptic field potentials evoked by group II muscle afferents have been shown previously to be reduced to about 80% of control levels during fictive locomotion in the dorsal horn of the feline mid and lower lumbar spinal cord during fictive locomotion (Perreault et al. 1999). In the dorsal horn, there is also a comparable reduction of group I and cutaneous afferent-evoked field potentials as well as (Perreault et al. 1999). In the intermediate zone, however, the reduction of the field potentials evoked by muscle group II afferents has been found to be greater (on average to 49% of control levels) than the reduction in the dorsal horn (Perreault et al. 1999). Based on the differences found between the reduction of field potentials evoked by different types of muscle afferents, the hypothesis, that sensory input from muscle group II afferents is preferentially suppressed during fictive locomotion has been proposed (Perreault et al. 1999).

The studies reported in this paper further examined the modulation of synaptic transmission in reflex pathways from group II muscle afferents during fictive motor activity. The aims of this study were to determine 1) whether a preferential reduction of transmission from group II muscle afferents exists on fibres terminating at the same locations in the spinal cord during fictive locomotion, 2) whether there is a non-uniform suppression of group II input from different muscles at the same spinal locations, and 3) whether a similar reduction of field potentials also occurs during rhythmic motor activity evoked without the direct, electrical stimulation of the brainstem, i.e. during fictive scratch. The new approach taken in these experiments was that two or three different field potentials at the same spinal locations (i.e. evoked by different muscle afferents and/or cutaneous afferents) were recorded during fictive motor activity in the lumbar (L4-L7) segments. Fictive locomotion was evoked by the stimulation of the mesencephalic locomotor region (MLR) and fictive scratch was evoked by mechanical stimulation of the skin covering the ear and the face following topical application of curare onto cervical dorsal roots in decerebrate cats. Comparisons of the changes in amplitude of the different types of field potentials evoked by electrical stimulation of peripheral nerves are reported in the results. Evidence will be presented to support the hypothesis that a preferential and a non-uniform suppression of muscle group II input during fictive locomotion and scratch exists. The putative functional role of this preferential motor-activity related control of muscle group II afferent input in differentiating reflex actions will also be discussed. Preliminary data have been presented earlier (Riddell et al. 2001, Stecina et al. 2002).

Methods

Animal preparation and surgery

Data presented in this paper were collected from 17 cats (2.1 - 3.9 kg). Canadian Council on Animal Care guidelines were followed and all procedures were approved by the animal care committee of the University of Manitoba. Halothene-nitrous oxide anaesthesia was applied from the start and during tracheotomy; cannulation of the carotid artery and of the jugular and femoral veins. At the start of the surgery, subcutaneous injection of atropine (0.05 mg kg^{-1}) and saline (1 mL) was given along with intravenous

injection of dexamethasone (2 mg kg $^{-1}$). A buffer solution of 5% glucose and 0.5% $NaHCO_3$ was infused intravenously (5 mL h^{-1}) throughout the experiments.

The following nerves in the left hindlimb were dissected and placed on bipolar hook electrodes for recording or stimulation: posterior biceps and semitendinosus, semimembrinosus and anterior biceps (SmAB), sural (Sur), medial gastrocnemius, lateral gastrocnemius and soleus (GS), plantaris, flexor digitorum and hallucis longus, peroneus longus, tibialis anterior (TA), extensor digitorum longus (EDL), and superficial peroneal (SP). The saphenus (Saph), sartorius (Sart), the vasti and the rectus femoris nerves together as quadriceps (Quad) were placed in cuff electrodes. The remaining ventral and dorsal nerve branches of the left and right hindlimbs were cut and the tendons around the hip were sectioned bilaterally.

A dorsal laminectomy was performed to expose the spinal cord from the 2nd to the 7th lumbar vertebrae, at the 12th and 13th thoracic vertebrae and at the first and second cervical levels bilaterally. Following surgical preparations, the animal was transferred to a rigid frame. The head was positioned in a stereotaxic holder and the hips were fixated. The skin of the left hindlimb was used to create a pool filled with mineral oil to suspend the nerves on silver hook electrodes. Precollicular-postmammillary decerebration was performed and the brain tissue rostral to the transsection was removed. Anaesthesia was discontinued and the animal was paralysed with pancuronium bromide (1.2 mg initially then 0.4 mg hr^{-1}). During the experiment, the CO_2 level was maintained between 3 and 5% by artificial ventilation, the blood pressure was kept above 80 mmHg by dextran injections when needed. Core body temperature was kept stable near 38 ^{0}C by infrared lamps and a heating pad positioned under the trunk of the animal. In 2 cats 4-aminopyridine (4-AP, initial dose 100 μg kg $^{-1}$) was given intravenously to improve locomotor activity. At the termination of the experiments, the animals were euthanised by a lethal injection of Euthanyl.

Stimulation and recordings

Fictive locomotion was evoked by electrical stimulation (30-400 μA, 0.5 - 1 ms pulses, 10-30 Hz) of the mesencephalic locomotor region (MLR). Fictive scratch was evoked by applying curarae (0.01 - 1%) on the dorsal roots of the first cervical segment

and mechanically stimulating the pinna or the skin of the face and neck area. Bipolar ball electrodes were used to stimulate selected hindlimb muscle and cutaneous nerves. Peripheral nerves were stimulated at intensities expressed in multiples of threshold (T) for the most excitable fibres as measured at the cord dorsum. Muscle afferents were electrically stimulated by 1-2 shocks (0.1 ms duration) usually at 5 times threshold in order to maximally recruit all group II fibres. In order to recruit only low-threshold cutaneous fibres, cutaneous nerves were stimulated at ≤2 times threshold in most cases and occasionally at 5T.

Glass micropipettes (1-2 μm tip diameter) filled with sodium-citrate were used as recording electrodes. Maximal cord dorsum potentials evoked by group II afferents from specific muscle nerves were mapped prior to selecting a location for intraspinal electrode positioning. Fictive motor output was monitored by using rectified, integrated and digitized electroneurograms (ENG) collected at 5 kHz, with low and high pass filtering (30 Hz-3 kHz). Data capture and analysis was done with software developed by the Winnipeg Spinal Cord Research Centre using Pentium PCs running Linux.

Recordings of field potentials prior to the onsets of fictive motor activity were used as control periods but data collection was continued throughout and after the cessation of motor activity. During the follow-up data analysis 10-40 individual traces were averaged during control, locomotor/scratch and post locomotor/scratch (i.e. recovery) periods to measure field potential peak amplitude and area. Peak amplitude and area measurements were taken at selected time points following the onset of the field potentials. Time points selected for measurements were the same for records collected in control, locomotion/scratch and recovery periods of the same field potentials. The onset latencies of the field potentials were measured from the arrival of the group I or cutaneous afferent volleys as identified by the cord dorsum electrode recordings that were evoked by the first stimulus if more than one shocks were used. Field potentials evoked at onset latencies ≤ 1 ms by group I, ≤2 ms by group II (see Edgley and Jankowska 1987a, Riddell et al. 1995) and ≤1.5 ms by cutaneous afferents (see Edgley and Jankowska 1987b) were considered to be compatible with monosynaptic coupling between the afferents and the neurones located in the vicinity of the recording electrode.

Those measurements of peak amplitude that were taken at ≤0.8 ms from the onsets were considered to reflect changes of the monosynaptic components of the field potentials (see Riddell et al. 1995, Jankowska et al 2002).

Based on the standard deviation and the number of averages used, an F-test was performed to assess whether the control, locomotor/scratch and recovery conditions had the same standard deviations (using 95% confidence level). Student's t-test was used to compare the mean peak amplitude and area measurements between the control and the locomotion/scratch as well as the control and the recovery periods (two-tailed, 95% confidence level). Then paired comparisons were done between the modulation of the field potentials evoked by cutaneous and muscle group II afferents at the same locations as well as group I and group II muscle afferents at the same locations by using paired t-tests (95% confidence level). Differences in the suppression of the field potentials evoked by the same afferents during both locomotion and scratch in the same preparations were compared by paired t-tests (95% confidence level). The standard error of the difference between two means (± SE) is reported with the mean values. Area measurements were used to asses field potential changes in a subset of the data and the average results were the same as obtained by peak amplitude measurements. In the Results section of this paper, only data based on amplitude measurements are reported.

Results

Field potentials evoked by hindlimb muscle and cutaneous afferents at the same locations in lumbar spinal segments of decerebrate cats were recorded during fictive locomotion and/or scratch. The reduction of group II-evoked field potentials at the same spinal location was larger than the reduction of group I and cutaneous-evoked potentials during fictive locomotion in 23/27 cases and during fictive scratch in 10/13 cases. This preferential suppression of group II input seemed to be partly mediated by mechanisms acting at the presynaptic terminals as not only the later but also the monosynaptic components of the examined field potentials were affected. Comparisons of field potential reduction during two different behaviours in the same animals revealed that direct electrical stimulation of the MLR is not necessary for evoking suppression of

muscle and cutaneous afferent field potentials. Additionally, there was a non-uniform suppression of field potentials evoked by muscle group II afferents of different muscles found at two-thirds of the locations examined during both fictive locomotion and scratch.

Preferential suppression of field potentials evoked by muscle group II afferents during fictive locomotion

Recordings of field potentials evoked by electrical stimulation of muscle and cutaneous afferents at the same locations throughout the lumbar spinal cord were collected during fictive locomotion as shown in figure 1. Field potentials evoked at the same location by different afferents are shown in figure 1A from the mid L6 segment, 1.45 mm deep. The trace in A1 shows that using 1.5 times the strength required to activate the fibres with the lowest threshold (T), only the EDL nerve stimulation evoked a field potential. Stimulation at 2T strength (thin trace in panel A2) increased the amplitude of the EDL-evoked field potential from 84 to 127 μV and gave rise to a small (14 μV) field potential evoked by the stimulation of the TA nerve. Increasing the stimulus strength to 5T (thick trace in panel A2) did not change the EDL-evoked field potential but it greatly increased (to 138 μV) the TA-evoked field. Thus the EDL-evoked field potential was mainly due to the activation of group I afferents while the TA-evoked field potential resulted from the recruitment of muscle group II fibres. Field potential recordings were collected continuously at a repetitive 4 Hz rate throughout the runs prior to (i.e. control), during and after fictive locomotion. The hip flexor Sart, the hip extensor SmAB and the ankle extensor GS nerves illustrated in panel B show alternating flexor and extensor motoneurone activity following MLR-stimulation. Along with the stimulation of the TA and the EDL nerves at 5T, the SP nerve was also stimulated at 1.3T. As show in figure 1C, group I afferents of the EDL nerve, group II afferents of the TA nerve and low-threshold cutaneous afferents of the SP nerve evoked field potentials at this location with onset latencies of 2.0, 0.7 and 1.3 ms respectively. Averaged recordings during the control period (dotted) and during fictive locomotion (solid trace) are superimposed in panel C. The onset latencies are marked by the open arrows at the crossings of the baseline (horizontal dotted line) and they were measured from the arrival

of afferent volleys recorded on the surface of the spinal cord (dotted vertical lines). The amplitude of the field potentials before, during and after locomotor activity was measured at 2.0, 1.1 and 5.4 ms following the onsets (filled arrows). The TA-evoked field potential was 41 ± 12%, while the SP-evoked field potential was 75 ± 12% of its control value with a significant reduction (t-test, $p \leq 0.01$). The EDL-evoked field potential was not reduced significantly ($p=0.2$). After locomotion ceased, within 2 minutes, the field potential amplitudes returned to levels that were not significantly different from those during control (data not shown).

-Figure 1 here-

Similar field potential recordings to that shown in figure 1 were collected from 19 different sites ranging from the 4th to the 7th lumbar segments. At 7/19 locations two different muscle nerves evoked field potentials along with a cutaneous nerve and at 1/19 locations two different cutaneous nerves evoked field potentials along with a muscle nerve. There were no systematic attempts made to record field potentials evoked by the same group II and cutaneous afferent fibres in the dorsal and in the intermediate or deeper regions within the same tracks. The depth of the recordings ranged from 1.2 to 2 mm below the surface of the spinal cord, thus, are likely to be from laminae IV-V of the dorsal horn (see Edgley and Jankowska 1987a). However, at 4 locations field potentials were also evoked by group I afferents of one or more muscle nerves, that is characteristic of recordings found in the intermediate zone. It is important to point to out, that the location of the recordings taken in the studies reported here cannot be directly compared to the dorsal and intermediate classification of group II field potentials established by previous studies (e.g. Edgley and Jankowska 1987a). One of the reasons for this is that in the studies described here, not all muscle nerves evoking field potentials at a given locations were explored, as field potentials evoked by hip and ankle flexor muscle afferents were of main interest. Also, in the studies described here, the TA and the EDL nerves were always separately activated, while in the previous studies they were always combined. In total, 26 muscle group II afferent-evoked, 20 cutaneous afferent-evoked and 8 muscle group I afferent-evoked field potential recordings were collected during control, locomotor and post locomotor conditions. Field potentials evoked by group II

muscle afferents in all cases, those evoked by cutaneous afferents in 16/20 cases and those evoked by group I afferents in 5/8 cases were reduced significantly during locomotion when compared to control.

-Figure 2 here-

Figure 2 illustrates the amplitude of each field potential evoked by muscle group II and cutaneous afferents during control periods expressed as millivolts (mV) and the amplitude of the same field potential expressed as percent of control levels during fictive locomotion. There was no apparent relation found between the size the and the degree of suppression of either the cutaneous or the group II afferent-evoked field potentials.

The onset latency of the 26 group II field potentials ranged from 1.3 to 3.9 ms. There were 12/26 potentials with latencies equal or less than 2 ms, suggesting monosynaptic activation from group II afferents (see Edgley and Jankowska 1987a, Riddell et al. 1995). The amplitude of 6/12 monosynaptic group II-evoked field potentials was measured at less than 0.8 ms from the onset, while in the other cases, the measurements were taken within 2.0 to 3.1 ms from the onset. During fictive locomotion, the average amplitude of those monosynaptic field potentials evoked by muscle group II afferents that were measured at a window $\leq$ 0.8 ms from the onset (filled triangles figure 2A) was 53 ± 5% of control levels (n=6), and that of longer latency (i.e. non-monosynaptic) field potentials was 52 ± 5% of control levels (n=14) without any significant difference.

The onset latency of cutaneous-evoked field potentials ranged from 0.8 to 3.4 ms and 8 were compatible with monosynaptic activation ($\leq$ 1.5 ms, see Edgley and Jankowska 1987b). At 5/8 locations the amplitude measurements of cutaneous field potentials were taken within 0.8 ms from the onset. For field potentials evoked by cutaneous afferents, the average amplitude of those monosynaptic field potentials evoked by muscle group II afferents that were measured at a window $\leq$ 0.8 ms from the onset (filled triangles figure 2B) was 82 ± 5% of control values (n=3), and was not significantly different from that of longer latency field potentials which were suppressed to 64 ± 7% of control (n=12). Thus, field potentials evoked by both muscle group II and cutaneous afferent with onset latencies compatible with monosynaptic coupling (triangles) showed

reduction to similar levels than those with longer onset latencies (squares).

There were no differences found in the average suppression of muscle group II or cutaneous afferent-evoked field potentials based on depth (data not shown) within the data set collected in this study, likely because all of the recordings have been recorded at depth ≤ 2 mm from the surface of the spinal cord. Interestingly, field potentials evoked by the SP cutaneous nerve showed a difference in the level of reduction based on the rostro-caudal location of the recordings. The amplitude of the SP-evoked potentials in the L4 segments during locomotion was 96 ± 2% of control on average (n=4) that is significantly different ($p \leq 0.01$) from the size of those in the L5-L7 segments (66 ± 6% of control (n=9). When the SP field potentials were ranked in rostro-caudal order and a trendline was calculated, there was a weak linear relationship ($r^2=0.5$) suggesting that there is less reduction in the efficacy of synaptic transmission in reflex pathways mediating actions of the SP nerve in the L4 segment compared to that in more caudal levels.

Comparing the reduction in field potentials evoked by group II muscle and cutaneous afferents at the same locations in the spinal cord, it was found that during fictive locomotion in 23/27 cases the reduction of the muscle group II afferent-evoked field potentials was larger than that of cutaneous afferent-evoked field potentials. Cutaneous afferent-evoked field potentials did not change significantly during fictive locomotion (n=4) or increased (n=1) in amplitude while the field potentials evoked by group II afferents were reduced. In the remaining 4 cases, cutaneous afferent-evoked field potentials were reduced more, although to a small extent, than those evoked by muscle group II afferents. Monosynaptic components of group II and cutaneous afferent-evoked field potentials evoked at the same locations were compared in 3/27 cases, in all of which there was a larger reduction of the field potentials evoked by muscle group II afferents than that of field potentials evoked by cutaneous afferents.

Panel A of figure 3 illustrates the summary of paired comparisons made between the reduction of different types of field potentials evoked by group II (open bars) and by cutaneous (filled bars) afferents during fictive locomotion. The nerves stimulated and evoking the field potentials and the number of comparisons are shown on the horizontal

axis. The height of the bars represents the averaged amplitude of the field potentials during fictive locomotion expressed as percent of control values (with SE indicated). Paired t-tests were used to compare field potential amplitudes between those evoked by muscle group II and cutaneous afferents. There was a significant difference (asterisks) between the mean suppression of group II muscle afferent-evoked field potentials of TA and SP ($p \leq 0.02$), of QRF and SP ($p \leq 0.01$), and of Sart and SP ($p \leq 0.03$). There was no significant difference between the suppression of EDL and SP ($p \leq 0.51$) or EDL and Sural ($p \leq 0.56$) afferent-evoked field potentials. Although, the comparison of TA and Sural, of QRF and Sural, and of GS and Sural, is based on recordings from only one location, it also shows that field potentials evoked by cutaneous afferents are less suppressed than those evoked by group II fibres. Overall, during fictive locomotion, all the field potentials evoked by muscle group II afferents taken together were reduced to $51 \pm 3\%$ of control, while those evoked by cutaneous afferents were $67 \pm 5\%$ of control with a significant difference between them ($p \leq 0.01$).

-Figure 3 here-

Differences between averaged field potential amplitudes in the flexion and extension phases were often less than the tonic suppression during both phases taken together as illustrated in figure 3B. The circles represent averaged values expressed as percent of control levels measured when taking together data collected in both phases of locomotion for field potentials evoked by cutaneous (gray symbols) and by group II muscle (open symbols) afferents. The vertical lines passing through each circle represents the difference in the amplitude of field potential averages obtained during flexion and extension. The horizontal lines at the end of each vertical line mark values obtained during the extension phase while the other end of the lines shows the values obtained during the flexion phase. Amplitudes (expressed as percent of control values) of field potentials evoked by group II afferents were ranked in an increasing order the values shown for field potentials evoked by cutaneous afferents are shown with a corresponding group II afferent field taken at the same location. Note that when 2 muscle group II-evoked potentials were recorded with one cutaneous field potential at the same location, only one of the muscle group II afferent-evoked field potential is plotted with the

cutaneous afferent-evoked field for the sake of clarity of the illustration. In some cases, the suppression was larger in flexion, while in others it was larger in extension. The largest difference of field potential amplitudes measured as percent of control during flexion and during extension was 46% for group II and 23% for cutaneous afferent-evoked potentials. There was only a small difference between the average values during flexion and extension (54 ± 3% and 51 ± 4% of control, respectively) and in both phases taken together (51 ± 3% of control) for group II afferent-evoked field potentials as well as for cutaneous field potentials (69 ±5% of control for flexion, 65 ± 6% of control for extension and 67 ±5 %, of control for both phases together). There seemed to be no relation between the source of the field potentials and the phase in which it was most suppressed (data not shown).

Preferential suppression of field potentials evoked by muscle group II afferents during fictive scratch

Changes in field potentials evoked by different afferents at the same spinal locations were also examined during fictive scratch. Figure 4A illustrates recordings from hip (Sart) and ankle (GS) nerves following the application of curare on the first cervical dorsal roots and mechanical stimulation of the skin covering the ear and face on the ipsilateral side. After applying mechanical stimulation, there is an approach phase characterized by tonic flexor (Sart) activity. Then alternating flexor and extensor motoneurone activity starts that is referred to as cyclic scratch. Stimulation of the TA and the EDL muscle nerves (2 stimuli at 200 Hz, strengths of 5T) and of the SP nerve at 1.2T with single shocks was repeated at a 4 Hz rate and it evoked field potentials in the mid L5 segment, 1.5 mm deep. Averaged field potentials (n=10) from the control (i.e. prior to scratch), the approach and the fictive scratch periods are overlaid in panel B. The onset latency of the field potentials evoked by the stimulation of the TA, the EDL and the SP nerves was 2.3, 2.0 and 2.0 ms respectively (open arrows). The maximal field potential amplitudes were measured at more than 4 ms after the onsets (filled arrows). The amplitude of the control recordings (thin black trace) evoked by the TA, the EDL and the SP nerves was 187, 149, and 246 μV, respectively. During cyclic scratch, the field

potentials evoked by the TA and the EDL muscle nerves were reduced to 59 ± 15% and 72 ± 19% of control levels, while the SP-evoked field potential increased to 105 ± 6% of control (thick black trace). Note in figure 4B, that changes in the field potential amplitude were evident not only during cyclic scratch but even during the approach phase as shown by the gray trace. During the approach phase, the amplitude of the TA and the EDL-evoked potentials was 87% and 89% of control, respectively, while that of the field potential evoked by the SP nerve was increased to 105% of control.

-Figure 4 here-

Fourteen muscle group II afferent-evoked field potentials were assessed during the approach as well as the cyclic scratch periods which are shown in panel 4C. Overall, field potentials evoked by muscle group II afferent input during the approach (white squares) and the scratch periods (black squares) showed a significant difference ($p \leq 0.03$) with means of 68 ± 7% and 57 ± 5% of control levels during approach and scratch, respectively. In 7/14 cases, the difference between the suppression of muscle group II field potential was more than 10%, with more reduction during cyclic scratch than during the approach phase, in 3 cases the suppression during the approach phase was within 5% of that measured during cyclic scratch, in 3 other cases the suppression was larger during the approach phase than during cyclic scratch. Interestingly, in 1 case there was an increase in amplitude during the approach phase but a suppression during cyclic scratch. Changes in field potentials evoked by cutaneous afferents did not show a significant difference during the approach and the cyclic scratch periods on average (triangles in panel 4C). They were reduced to 91 ± 7% and 90 ± 7% of control values during approach and scratch, respectively.

At 3/9 locations during fictive scratch, two different muscle group II afferents evoked field potentials along with one cutaneous field and at 1/9 location two different cutaneous nerves evoked field potentials along with a group II-evoked field potential. Overall, there were 13 comparisons made between field potentials evoked by muscle group II (n=12) and cutaneous (n=10) afferents during fictive scratch. Preferential suppression of muscle group II afferent evoked field potentials was evident in 10/13 cases. Muscle group II-evoked field potentials were reduced while cutaneous afferent

field potentials were not significantly changed or increased in 2 and in 6 cases, respectively. More suppression of cutaneous than that of muscle group II field potentials was evident in 3 cases with 2%, 3% and 20% difference between the percent of control levels of the group II and the cutaneous afferents during fictive locomotion. Monosynaptic components of muscle group II and cutaneous field potentials were compared in 2/13 cases, and both showed greater suppression of muscle group II than of cutaneous afferent-evoked field potentials.

Figure 5A illustrates the grouped comparisons made between the amplitude of muscle group II and cutaneous afferent-evoked field potentials at the same locations during fictive scratch. The reduction of field potentials evoked by Sart group II fibres was significantly larger than the modulation of the field potentials evoked by the SP nerve ($p \leq 0.03$) although it is based on measurements taken only at 2 locations. Note that field potentials evoked by the stimulation of the SP nerve increased to 114 ± 13% on average. There was no significant difference between the suppression of field potentials evoked by TA and SP ($p \leq 0.26$) and by QRF and SP ($p \leq 0.09$) afferents. The comparison of EDL and SP, and of QRF and Sural afferent-evoked potentials show more reduction of group II than of cutaneous-evoked potentials, but each of these observations comes from data collected only at one location. Overall, together all of the field potentials evoked by muscle group II afferents were 69 ± 3 % of control (solid line) and those evoked by cutaneous afferents were 87 ± 7% of control during fictive scratch (dashed line) with a significant difference ($p \leq 0.03$) between them.

-Figure 5 here-

Similar to the observations during fictive locomotion, differences between averaged field potential amplitudes in the flexion and extension phases were often less than the tonic suppression during both phases taken together. In 4/12 and 4/10 field potentials evoked by cutaneous and muscle group II afferents respectively, the difference between the averages obtained during flexion and extension was greater than 10% (see Fig. 5B, same format as Fig. 3B). In some cases the suppression was larger in flexion while in others it was larger in extension with only a small (2%) difference in the average values of group II and cutaneous-evoked potentials taken during flexion,

extension, and together in both phases.

Comparisons of changes in field potentials evoked by muscle group I and group II afferents at the same spinal locations

During fictive locomotion, at locations where both group I and group II muscle afferents evoked field potentials (n=8, data not illustrated), comparing field potential changes revealed that overall the reduction of the field potentials evoked by muscle group I afferents was to 85 ± 7% of control. This was significantly less ($p \leq 0.01$) than that of field potentials evoked by muscle group II afferents (to 59 ± 7% of control). Homonymous field potentials (i.e. evoked by the group I and group II afferents of the same nerve) were compared in 6/8 cases. The remaining 2 comparisons were made between heteronymous group I and group II field potentials (i.e. EDL group I vs. TA group II and QRF group I vs. TA group II). In both homonymous and heteronymous comparisons, the suppression of the field potentials evoked by the muscle group II afferents was less than that of those evoked by group II fibres. Changes in the monosynaptic components of field potentials evoked by muscle group I and group II afferents were compared in 4/8 cases and showed reduction to 88 ± 11% and 51 ± 11% of control, respectively. Thus similar changes were seen by the comparison of monosynaptic field potentials to those evoked and/or measured at latencies that were longer than values representing monosynaptic coupling.

There were 6 comparisons made between the reduction of field potentials evoked by group I and group II afferents (all evoked by QRF afferents) during fictive scratch (data not shown). All of these 6 comparisons were made between the monosynaptic components of field potentials. Overall, field potentials evoked by muscle group I and group II afferents at the same regions of the spinal cord were reduced to 73 ± 5% and 51 ± 11% of control levels during fictive scratch without a significant difference (paired t-test, $p \leq 0.07$). Although, in 4/6 cases, the reduction of the group II afferent fields was greater than that of group I fields and in one other case the reduction of the group I field potential was not significant while the group II field was significantly reduced. Only in one case was the reduction of the group I field larger (by 2%) than the reduction of the

group II field.

Comparison of field potential changes during fictive locomotion and scratch

At 6 locations within the L4 and L7 lumbar segments, 16 different field potentials (9 muscle group II and 7 cutaneous-evoked) were recorded during both fictive locomotion and fictive scratch evoked in the same preparation. The bouts of locomotor and scratch activity were evoked 3-18 minutes apart from each other. The comparison of changes in field potential amplitude evoked by different group II muscle (TA, QRF, Sart, EDL) and cutaneous (SP and Sural) afferents during locomotion (open bars) and scratch (filled bars) are shown in figure 6. For all field potentials evoked by group II muscle afferents taken together (n=9), the average amplitudes were 58 ± 5% and 72 ± 4% of control levels during fictive locomotion (solid line) and scratch (dashed line), respectively, with a significant difference between them ($p \leq 0.01$). There was a significant difference in the reduction of the field potentials evoked by the TA muscle nerve with 27% less reduction during scratch than during locomotion ($p \leq 0.02$). Although, the suppression of QRF, Sart and EDL-evoked field potentials was less during scratch than during locomotion, they were without a significant difference ($p \leq 0.09$, 0.84, 0.13 respectively).

-Figure 6 here-

All cutaneous afferent-evoked field potentials taken together (n=7) were reduced to 81 ± 5% and to 98 ± 6% of control values during fictive locomotion (solid line) and scratch (dashed line), respectively. This 17% difference, however, was not statistically significant ($p \leq 0.06$) because of the large variability of the levels measured during fictive scratch (i.e. some reduced by 30% while others increased by 26%). Interestingly, in 3/6 occasions some field potentials evoked by cutaneous afferents increased in amplitude during scratch but decreased during locomotion.

Non-uniform suppression of group II afferent input from different hindlimb muscles

Field potentials were evoked by more than one type of muscle afferent at several

locations and this allowed for assessing the differential suppression of various types of muscle group II input. Figure 7 illustrates the amplitude of each muscle group II afferent field potential recorded during locomotion (filled symbols) and scratch (open symbols) along with a field potential at the same location that was evoked by group II afferents of another muscle nerve. The muscle afferents evoking the field potentials are indicated along the horizontal axis. Overall, during fictive locomotion, there was more than 10% difference between the suppression of two different muscle group II afferent-evoked field potentials at 6/9 locations. Of these 9 comparisons, 6 was made between field potentials evoked by TA and EDL afferents, with 23 ± 7% difference between them on average. During fictive scratch, overall, there was more than 10% difference between the suppression of two different types of muscle group II inputs at 4/6 locations. Monosynaptic field potentials evoked by 2 types of group II afferents were compared at 2 and at 1 locations during fictive locomotion and scratch, respectively, and they were different by 40, 14 and 20%.

-Figure 7 here-

Discussion

The present approach of simultaneously recording field potentials evoked by the electrical stimulation of two or three peripheral nerves permitted a direct comparison of the relative change in the effectiveness of synaptic transmission from different afferents at the same locations in lumbar spinal segments. The principle finding is that during both fictive locomotion and scratch, field potentials evoked by muscle group II afferents are reduced more than those evoked by cutaneous afferents at the same locations. On average, field potentials evoked by group II muscle afferents were reduced to 51 ± 3% of control while those evoked by cutaneous afferents at the same locations were reduced to 67 ± 5% of control levels during fictive locomotion (see Fig. 3A). For the first time, the reduction of field potentials evoked by muscle group II afferents during fictive scratch is reported in this paper (see Fig 4 and 5), that was similar to the modulation occurring during fictive locomotion, although the mean depression of field potentials evoked by group II muscle (to 69 ± 3% of control) as well as cutaneous (to 87 ± 7% of control) afferents was smaller during scratch than during locomotion.

These results further support the idea proposed by Perreault and colleagues (1999) that during centrally generated rhythmic movements, there is a preferential depression of transmission from group II muscle afferents to spinal neuronees. The average group II field potential depression found here (to 51 ± 3% of control) during fictive locomotion was somewhat more than that reported previously in the dorsal horn (to 80% of control) and more comparable to that recorded in the intermediate zone (to 49% of control) by Perreault and colleagues (1999). This could be due to some of the differences between the field potentials under examination in the two studies. Previously, field potentials evoked by Quad, Sart and PbSt muscle afferents were examined mainly from the mid-lumbar segments, while in the present study, field potentials evoked by TA and EDL muscle afferents were in focus (12 and 7 respectively from a total of 26). These field potentials were also located in more caudal segments than those investigated before. Also, in order to make reliable comparisons between the modulation of field potentials evoked by different afferents, we recorded only from regions in which distinct group II and cutaneous fields could be recorded, which was not the purpose of the previous study.

A major finding of Perreault et al. (1999) was that dorsally-located group II fields were suppressed less during fictive locomotion than were fields evoked by the same afferents in the intermediate spinal laminae. Thus, that study showed a clear location-dependent preferential suppression of transmission from hindlimb group II afferents. The present study extended the analysis of unequal suppression of sensory input to fields evoked from different nerves but recorded in the same location. The preferential depression of group II over cutaneous fields is also evidenced by the finding that no group II fields in the present and only one in the earlier study (in Perreault et al. 1999) were unaffected by locomotion, while several cutaneous field potentials remained close to control values or in a few cases became larger during locomotion. The present study also extend the observations on the selective suppression of field potentials evoked by group II over those evoked by cutaneous muscle afferents during fictive scratch.

The suppression of field potentials evoked by muscle group I and group II afferents was reported to be comparable in the intermediate regions of the mid-lumbar segments (Perreault et al. 1999). In this study, the mean suppression of muscle group I

afferents was found to be significantly less during fictive locomotion than that of those evoked by muscle group II afferents. This discrepancy from the previous report could also be likely due to the differences of the afferents under examination or the location of recordings. Previously, group I afferents of the QRF, the GS and the FDHL muscle nerves were stimulated while in this study, more than half of the recorded field potentials were evoked by the stimulation of the Sart, the TA and the EDL muscle nerves. Also, in the previous study, only homonymous field potentials were compared while in this study, heteronymous group I and group II field potentials were also included in the comparisons. During fictive scratch, more suppression of field potentials evoked by group II than suppression of those evoked by group I afferents were found in the majority of the examined cases, however, there a statistically significant difference could not be established. More detailed investigations are needed to estimate the relative difference between the supersession of group I and group II muscle afferent input during fictive scratch.

Similarities in the suppression of muscle group II afferent transmission during fictive locomotion and scratch

The finding that muscle group II afferent input is more suppressed during fictive locomotion than input from cutaneous afferents at the same spinal locations is not so surprising in the light of the previous hypothesis proposed by Perreult ad colleagues (1999). However, it is more of a surprise that such a preferential suppression of muscle group II afferent input also occurs during fictive scratch (Fig. 5A). Although, locomotion and fictive scratch require similar activity patterns from most of the hindlimb muscles, they are different functional tasks. One could imagine, that during scratching, when the animal is using the hindlimb to release some irritation, all input from all sensory afferents is attenuated strongly while engaged in this intense activity. However, this did not seem to be the case, as the input from the muscle group II afferents was more reduced than that from cutaneous afferents.

Based on our data, maximal reduction of muscle group II afferent input during fictive locomotion and scratch occurs during rhythmic motoneurone activity. Neither

during MLR stimulation without any phasic activity on the nerves (Perreault et al. 1999) nor during tonic flexor activity in the approach phase (Fig. 4B) is there a full reduction evoked of the field potentials generated by muscle group II afferents. A comparison of field potential amplitudes during the flexion and the extension phases of fictive locomotion and scratch showed that some muscle group II as well as cutaneous field potentials were phasically modulated with the locomotor or the scratch cycles (see Fig. 3B and 5B). In agreement with the results of Perreault et al. (1999), however, the phase in which the maximal depression occurred was overall inconsistent and there was mainly a tonic depression with little difference in the amplitudes between the two phases. Smaller phasic than tonic suppression of all types of field potentials has been suggested before (Perreult et al. 1999) and now it has been extended to fictive scratch. This constitutes another similarity between the two behaviours.

Another common feature of locomotor and scratch-related control of synaptic transmission from peripheral afferents is the occasional increase seen in field potentials evoked by cutaneous afferents (1 during fictive locomotion and 3 during scratch) and in a few cases variable patterns, such as suppression followed by increase. Such increases in the efficacy of synaptic transmission have been reported previously during micturition from urethral afferents (Angel et al. 1994) and during fictive locomotion from group I muscle afferents (Gosgnach et al. 2000). Increased field potential amplitudes could reflect the increased excitability of cutaneous afferents and the variations of excitability at different locations of the examined afferents could reflect the functional role of a particular reflex pathway similarly to what has been suggested in regard to the feline micturition circuitry (Buss and Shefchyk 1999). The excitability profile of the urethral afferents has also shown to be variable and it has been suggested that urethral afferents linked with excitatory bladder reflexes could be expected to show increased excitability during voiding while other urethral afferents that facilitate sphincter reflexes would have a more variable excitability profile during voiding (Buss and Shefchyk 1999). Analogous to this idea, it could be hypothesized, that field potentials increasing in amplitude during fictive locomotion could reflect neuronal pathways that are linked with facilitatory actions of ongoing locomotor activity, while at locations where field potentials were

suppressed, neuronal pathways linked to other actions that are primarily inhibitory to motoneuronees engaged in rhythmic firing during the phase in which the peripheral nerve was stimulated. More detailed studies on the location and the subgroups of cutaneous afferents that show variable and increased excitability changes could help to define whether reflex pathways with different functional tasks do show such differences in their excitability profile. An increases in the efficacy of synaptic transmission during motor activity such as micturition (Angel et al. 1994) and fictive locomotion (Gosgnach et al. 2000) could also be compatible with the removal of tonic inhibition of synaptic transmission operating in the quiescent state.

Although, the number of observations was small, this study has also provided evidence for the non-uniform suppression of muscle group II afferent-evoked field potentials during both fictive locomotion and scratch (Fig. 6). More experiments are needed to determine the absolute differences that exist between two muscle nerves at specific locations, but based on the data provided here, there are similar differences seen during locomotion and scratch. For example, in both behaviours at two-thirds of the locations there was a difference greater than 10% between the suppression of two fields.

Sources of suppression and the relative contribution of the MLR to the suppression of group II afferent input

By comparing the modulation of field potentials at the same locations in the same preparations during both fictive locomotion and scratch, the relative contribution of the electrically-activated MLR could be assessed. Based on the results obtained in this study (see Fig. 6), it can be concluded that no direct stimulation of the MLR is required to evoke reduction in field potentials evoked by either muscle or cutaneous afferents. However, there seems to be about 14-17% more reduction developing when applying electrical stimulation of the MLR (i.e. during fictive locomotion).

Electrical stimulation of brainstem regions around the functionally identified MLR can produce a depression of group II field potentials in anaesthetised preparations in the absence of locomotor activity (e.g. Noga et al. 1995). However, in decerebrate preparations, the maximal depression of synaptic transmission from both group Ia

(Gosgnach et al. 2000) and group II afferents (present results and Perreault et al. 1999) have been reported to occur not with the onset of MLR stimulation but with the development of rhythmic motor output. The increase in depression as rhythmic activity develops has been used as an argument that some of the field potential depression reported during MLR-evoked fictive locomotion is related to the operation of the central locomotor circuitry and not to the stimulation of the midbrain. This argument now has now been further supported from observations during fictive scratch (see Fig. 4). Comparisons between the suppression of field potentials evoked during episodes of spontaneous and MLR-stimulation evoked locomotion would be another way to further investigate the role of rhythmic motorneuronee activity in the suppression of afferent transmission from muscle and cutaneous afferents.

However, it is important to acknowledge that indirect activation of several areas in the brainstem and some descending systems cannot be excluded when interpreting the data collected during scratch. Stimulation used to evoke fictive scratching and the developing motoneuronal activity is thought to activate supraspinal centres and descending tracts. For example, during both actual and fictive scratch, neurones of the lateral reticular nucleus (Arshavsky et al. 1978a) and vestibulospinal tract neuronees (Arshavsky et al. 1978b) display rhythmic activity. Rubrospinal neuronees projecting to the lumbosacral spinal cord also fire in a patterned manner during scratch (Arshavsky et al. 1978c). Therefore, the suppression evoked during fictive scratch could be related to the indirect activation of supraspinal and descending centres and this possibility needs further investigations.

Another alternative preparation suitable for examining the relative role of the MLR in field potential suppression could have been the spinalized cat preparation with DOPA administered systemically in order to evoke fictive locomotion. However, as there are several known differences between the operation of the reflex pathways from muscle group II afferents during fictive locomotion in decerebrate and DOPA-treated cats, this preparation should be used with caution. Most importantly, the reflex actions of muscle group II afferents seem to be different during fictive locomotion evoked in the two different preparations. In the DOPA-treated cats, electrical stimulation of muscle group II

afferents throughout the hindlimb evoke FRA-like reflexes (Schomburg et al. 1998) while in the decerebrate cat their FRA-type actions seem to be suppressed and they evoke differential actions such as either promoting flexion or promoting extensor motoneurone activity, depending on the source of the afferent (Stecina et al. 2005). Also, in terms of cutaneous afferents, they evoke flexion reflexes in DOPA-treated cats while they have more specialized reflex actions (i.e. stumbling corrective reflex evoked by SP stimulation) in decerebrate cats during fictive locomotion (Quevedo et al. 2005). On the other hand, reflex actions evoked by several muscle group II afferents have been shown to be similar during fictive locomotion and scratch (Stecina et al. 2005).

Presynaptic and postsynaptic mechanisms evoking suppression of muscle group II and cutaneous input during rhythmic motor activity

The modulation of the non-monosynaptic field potentials was examined in the majority of the data and these results suggest that there are postsynaptic modulatory mechanisms that control afferent transmission in reflex pathways from muscle and cutaneous afferents at the same location. However, based on the comparisons made between monosynaptic group II and cutaneous field potentials with a window less than 0.8 ms from the onset (23/27 and 10/13 comparisons during fictive locomotion and scratch) preferential reduction was apparent in these monosynaptic components of group II muscle and cutaneous afferent-evoked field potentials. Although, these observations were less in number, they still suggest that not only selective postsynaptic but also selective presynaptic inhibitory mechanisms could control synaptic transmission from muscle group II afferents during fictive motor activity.

Assuming that a substantial portion of the field potential depression is due to presynaptic reduction in transmitter release, these observations suggest that field potential depression during fictive locomotion and scratch does not result primarily from the rhythmic activity of interneurones with contacts on primary afferent terminals. A similar conclusion for a tonic control of sensory transmission during fictive locomotion has been suggested based on the depression of monosynaptic group Ia EPSPs in hindlimb motoneuronees (Gosgnach et al. 2000). Thus, the centrally-generated control of

transmission from primary afferents appears to involve tonic inhibitory mechanisms. These might include effects mediated by $GABA_B$ receptors or in the case of group II afferents, a descending monaminergic control from supraspinal noradrenergic or serotonergic systems which are known to have powerful depressive actions on group II field potentials (Noga et al. 1995) and also can be preferential at specific locations in anaesthetised preparations (Jankowska et al. 2000).

Functional relevance of the preferential suppression of group II and cutaneous input

Field potentials reflect changes in extracellular current flow with negative potentials indicating the shift of positive charges from the extracellular space into surrounding neurones (i.e. depolarization). Thus the decrease in sensory-evoked field potentials during fictive locomotion and scratch is a reflection of a decreased depolarization of the neurones in the vicinity of the electrode evoked by sensory afferent stimulation (e.g. Riddell et al. 1995, Perreault et al. 1999; Gosgnach et al. 2000). The idea that the reflex actions of certain sensory afferents, and in particular the proprioceptive input from group II muscle spindle afferents, should be controlled during movement was raised by Eccles and Lundberg (1959). They recognized that since these afferents evoke flexion reflexes in low-spinal preparations, there should be some mechanism to suppress group II-evoked flexion reflexes during fictive locomotion. Evidence that group II-evoked flexion reflexes are preferentially suppressed during fictive locomotion was obtained in an earlier study using the same preparation employed here (Perreault et al. 1999). Arguments presented in that study and strengthened by several observations reported here suggest that a centrally-generated presynaptic inhibition of transmission from group II afferent terminals can be significant component of reflex suppression during locomotion. As shown by Gorassini and Prochazka (1998) intense activity in group II muscle spindle afferents is to be expected during even unperturbed overground locomotion in the intact animal. In contrast, cutaneous input from several skin areas would be expected to be modest under many conditions (Loeb 1981). Accordingly, the finding of a larger centrally programmed suppression of group II

input over input from cutaneous afferents during fictive locomotion makes functional sense.

It is more difficult to elaborate on the possible functional role of the unequal suppression of group II input from two muscle nerves recorded at the same spinal location. Unlike the broad distribution of input from most cutaneous nerves to several segments in the lumbar cord, monosynaptic group II field potentials from individual nerves have a more limited distribution. This precluded comparing group II field potential depression from a wide variety of nerves and no attempt was made to systematically compare the depression of particular pairs of group II inputs.

A recent development towards understanding the physiological role of group II afferent input during locomotion has been the recognition that there are a variety of reflexes evoked from flexor group II afferents in fictive locomotion preparations. In general terms, stimulation of some flexor nerves produces an enhancement of flexor activity while stimulation of others terminates flexion and initiates extension (Perreault et al. 1995; Stecina et al. 2005). However, the reflex effects evoked from particular nerves are reversed in some preparations and can sometimes switch for a single stimulus trial (Stecina et al. 2005). Variations in flexor nerve-evoked reflexes have also been reported during treadmill locomotion in decerebrate cats (Lam and Pearson 2002). The existence of a variety of group II-evoked reflexes (i.e. parallel reflex pathways) suggests the need for mechanisms to select between alternate reflexes evoked by particular nerves. Accordingly, the uneven suppression of muscle group II input from two close synergists, such as TA and EDL (e.g. Fig. 6) might help to control the opposing reflex actions of these two nerves (Stecina et al. 2005) in more intact preparations. It is noteworthy, that during voluntary movements in man, there can also be a selective presynaptic regulation of transmission from particular group Ia afferents expected to be activated during the movement (Hultborn et al. 1987), although yet no such evidence exists in regard for group II muscle afferents.

The present results provide insight into how the central pattern generator circuitry can regulate sensory input during locomotion and scratch by causing a reduction in transmission from sensory afferents. These processes appear to be quite specialised and

provide the substrate for fine control of sensory input from different fibre types, from the same fibre types in different nerves and from the same fibres terminating in different spinal locations. Transmission from group II muscle spindle afferents appears to be subject to the strongest control during locomotion and scratch in the decerebrate preparations studied to date. It is important to stress, however, that group II afferents can evoke a powerful reflex control over the circuitry producing locomotion and scratch in these preparations (Perreault et al 1995; Stecina et al. 2005). One important remaining issue is, therefore, an understanding of which neurones remain recruitable by group II afferents during fictive locomotion and might be involved in mediating group II actions on the locomotor pattern generating circuitry. This is addressed in the following section of my thesis (paper 2).

Figure Legends

Figure 1. The fictive-locomotion related suppression of field potentials evoked by muscle and cutaneous afferents at the same spinal location is different

Extracellular field potentials evoked by the electrical stimulation of muscle (TA and EDL) and cutaneous (SP) afferents at the same spinal locations, 1.45 mm deep, in the L6 segment.

A. Averaged (n=16) field potentials by electrical stimulation of the TA and the EDL muscle nerves at 1.5 times the threshold (T) of the activation of the most excitable fibres in the respective nerves (**A1**) and 2T and 5T (**A2**) prior to fictive locomotor activity (i.e. control period). The dotted line represents the baseline level prior to the onset of the field potentials.

B. Rectified and integrated ENG recordings from the hip flexor Sart, the hip extensor SmAB and the ankle extensor GS nerves during control and following MLR stimulation. Note the alternating flexor and extensor activity during this 3 s period of fictive locomotion.

C. Averaged field potentials evoked before (dotted trace, n=17) and during fictive locomotion (solid trace, n=15). The bottom trace illustrates the averaged cord dorsum potential. The onset of the field potentials evoked by the stimulation of the TA, the EDL and the SP nerves was 2.7, 0.7 and 1.3 ms (open arrows). The peak amplitudes of each field potential were measured at points marked by the filled arrows. The horizontal dotted line represents the baseline recording levels prior to the onset of the field potentials and the vertical dotted lines represent the afferent volleys.

Figure 2. Amplitude of muscle group II and cutaneous afferent-evoked field potentials

A-B, Field potential amplitude during locomotion expressed as percent of control levels measured during the control period and the peak amplitude of averaged field potentials during control periods are shown for all field potentials evoked by muscle group II (**A**) and cutaneous (**B**) afferents. The triangles represent monosynaptic muscle group II (in **A**)

and cutaneous (in **B**) field potentials with onset latencies ≤ 2.0 and ≤1.5 ms, respectively. The filled symbols in each panels represent monosynaptic field potentials with amplitudes measured at a time points ≤0.8 ms from the onsets.

Figure 3. Reduction of field potentials evoked by group II muscle afferents is greater thanthe reduction of those evoked by cutaneous afferents

A. Grouped amplitudes of field potentials evoked by muscle group II (open bars) and cutaneous (filled bars) afferents during fictive locomotion. The vertical axis represent the averaged amplitude of field potentials as percent of control levels. The afferents evoking the field potential and the number of locations from which recordings were collected are shown below the X axis. Group II-evoked field potentials were reduced to 51% of control (solid line) and cutaneous afferents to 67% of control (dashed line) on average during fictive locomotion. Asterisks mark significant differences between comparisons ($p \leq 0.05$).

B. Average amplitude expressed as percent of control for all field potentials recorded during fictive locomotion taken together from both the flexion and the extension phases of the step cycles are shown for those evoked by group II (open circles) and by cutaneous (gray circles) afferents. The vertical lines represent the phasic modulation of field potential amplitude. The end of each vertical line with a horizontal line (-) shows the average obtained during the extension phase and the other end of the line shows the average from the flexion phase, separately. Data were ranked in an increasing order of muscle group II-evoked field potential amplitude.

Figure 4. Muscle group II afferent-evoked field potentials are more suppressed during cyclic scratch than during the approach phase

A. Illustration of every 2nd extracellular field potentials (top traces) recorded prior to and during fictive scratch activity following the stimulation of the TA, the EDL muscle nerves at 5T and the SP nerve at 2T in the mid L5 segments, 1.4 mm deep. Recordings from hip (Sart) and ankle (GS) muscle nerves following the topical application of curare

and the mechanical simulation of the skin covering the ear and the face. Note that the approach phase characterized by tonic flexion developed before alternating flexion and extension set in (i.e. cyclic scratch).

B. Overlay of the averaged field potentials evoked by the electrical stimulation of the TA, the EDL and the SP nerves recorded during the control (thin black trace), during the approach (gray trace) and during the fictive cyclic scratch (thick black trace). The open arrows indicate the arrival of the earliest afferent volleys recorded on the cord dorsum (bottom trace). The amplitude of the field potentials was measured at points indicated by the filled arrows.

C. Averaged peak amplitude of all field potentials evoked by muscle group II (squares) and by cutaneous (triangle) afferents during the approach (open symbols) and the cyclic scratch activity (filled symbols) periods.

Figure 5. Field potentials evoked by muscle group II afferents are more suppressed than those evoked by cutaneous afferents also during fictive scratch

A. Comparison of muscle group II and cutaneous afferent-evoked field potential amplitudes expressed as percent control at the same spinal locations during fictive scratch.

B. Averaged field potential recordings during flexion and extension phases taken together (circles) and separately (vertical lines). Same format as in Figure 2.

Figure 6. Field potentials evoked by muscle group II and cutaneous afferents are reduced more during fictive locomotion than during scratch

Average amplitude of field potentials evoked by muscle group II and cutaneous afferents during fictive locomotion (white bars) and during fictive scratch (filled bars) grouped. The types of field potentials and the number of different locations are indicated on the horizontal axis. The solid and dashed lines represent the grouped averages during fictive locomotion and scratch, respectively.

Figure 7. Non-uniform suppression of field potentials evoked by different muscle group II afferents at the same spinal locations

Comparison between the suppression of field potentials evoked by muscle group II afferents originating from two different nerves at the same spinal location during fictive locomotion (filled symbols) and during fictive scratch (open symbols). Data were ranked in increasing order of the % control difference between the amplitude of the field potentials (horizontal axis). Note that the difference is greater than 10% in two-third of the observations.

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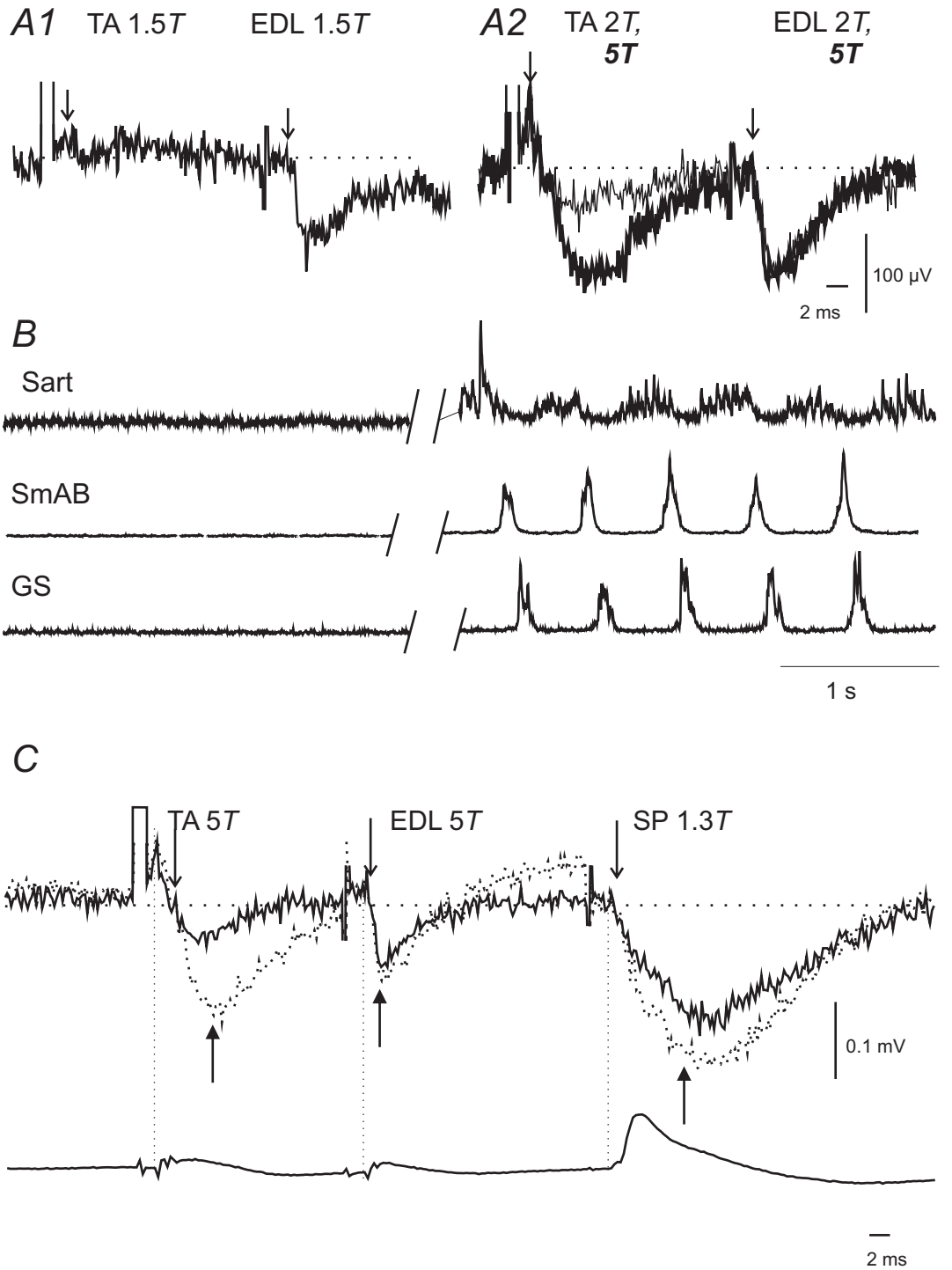
A1
TA 1.5T
EDL 1.5T
A2
TA 2T,
5T
EDL 2T,
5T
100 µV
2 ms
B
Sart
SmAB
GS
1 s
C
TA 5T
EDL 5T
SP 1.3T
0.1 mV
2 ms

Figure 1

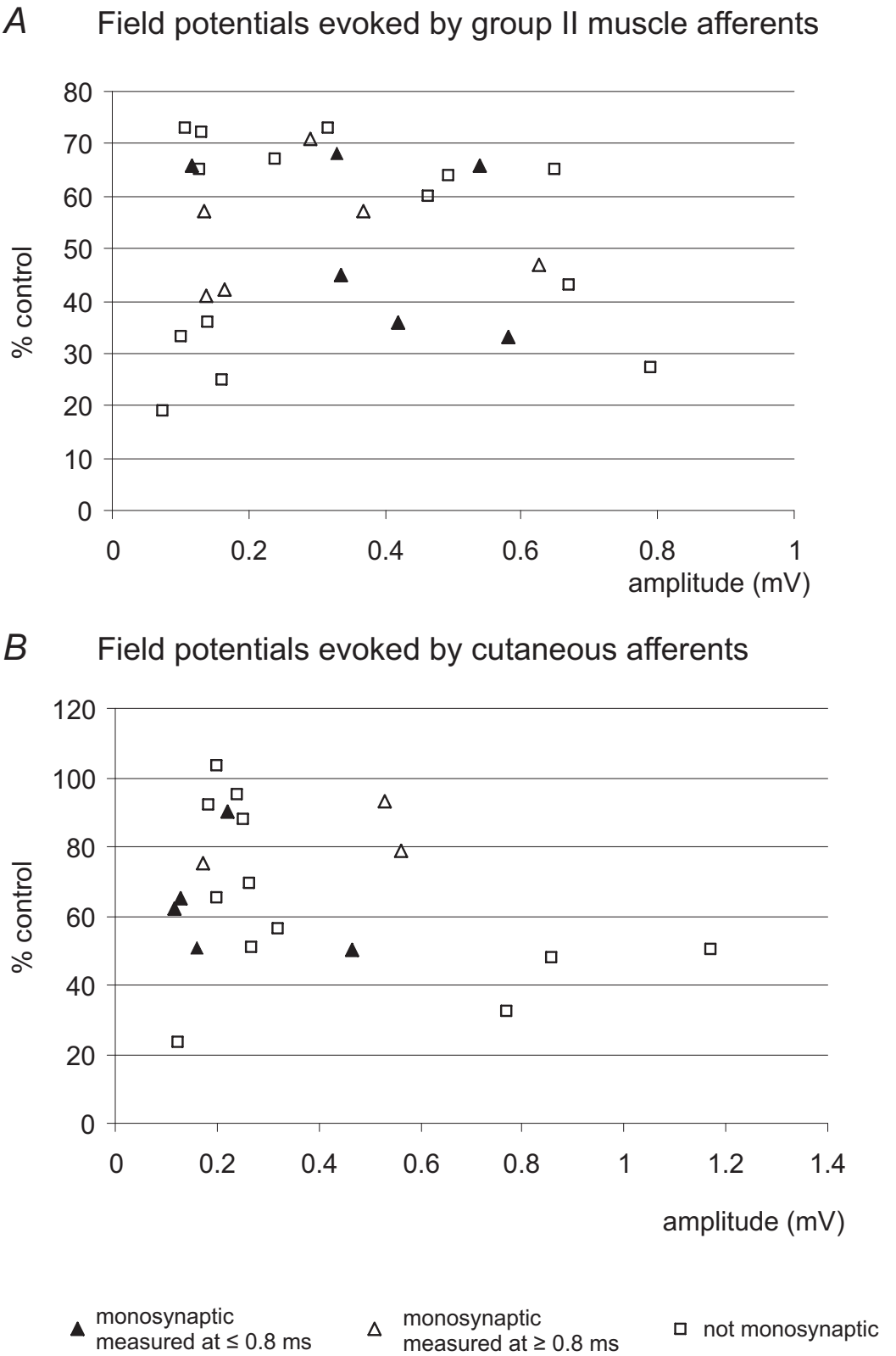
A
Field potentials evoked by group II muscle afferents
% control
amplitude (mV)
B
Field potentials evoked by cutaneous afferents
% control
amplitude (mV)
monosynaptic measured at ≤ 0.8 ms
monosynaptic measured at ≥ 0.8 ms
not monosynaptic

Figure 2

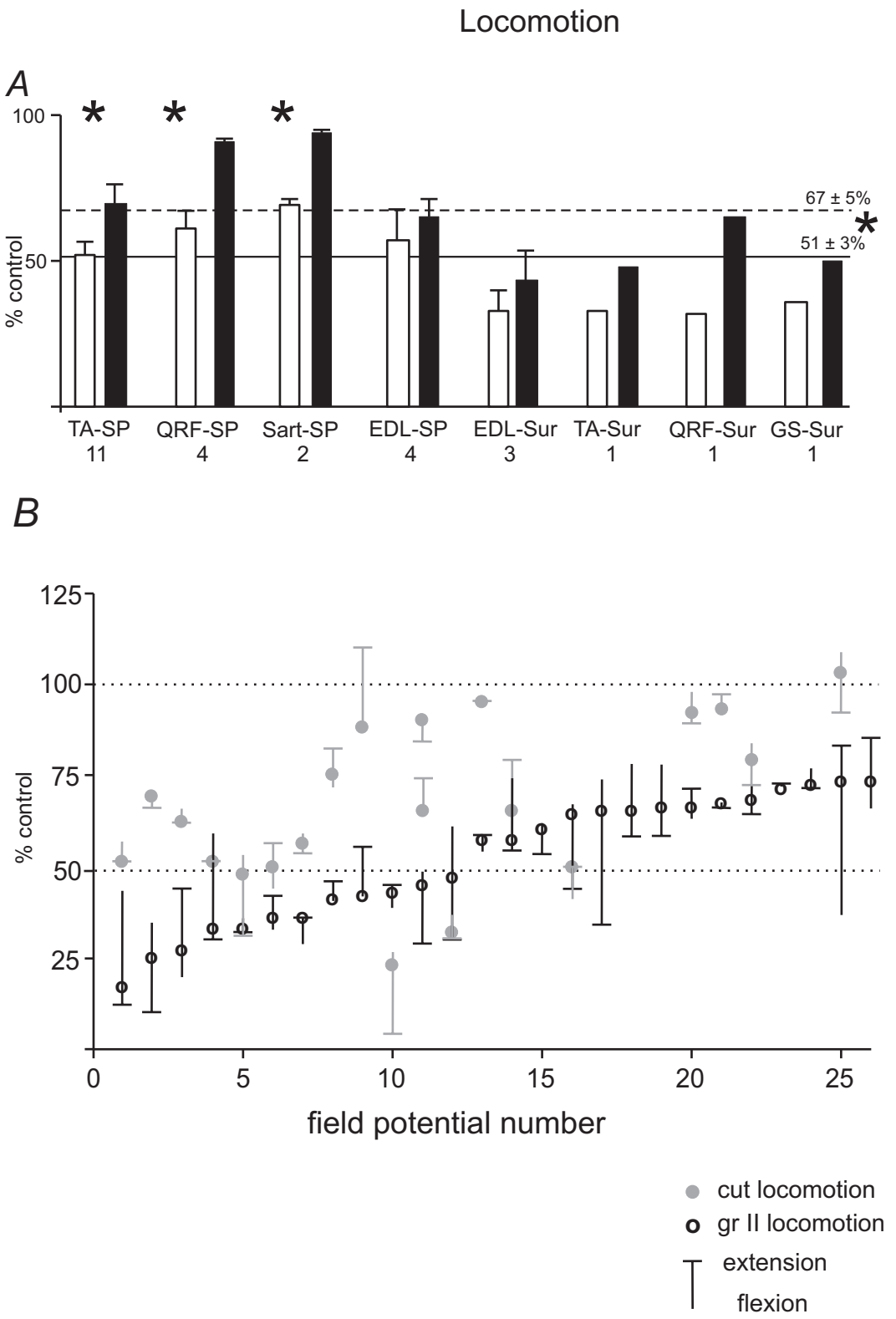
Locomotion
A
100
50
% control
*
*
*
67 ± 5%
51 ± 3%
*
TA-SP 11
QRF-SP 4
Sart-SP 2
EDL-SP 4
EDL-Sur 3
TA-Sur 1
QRF-Sur 1
GS-Sur 1
B
125
100
75
50
25
% control
0
5
10
15
20
25
field potential number
cut locomotion
gr II locomotion
extension
flexion

Figure 3

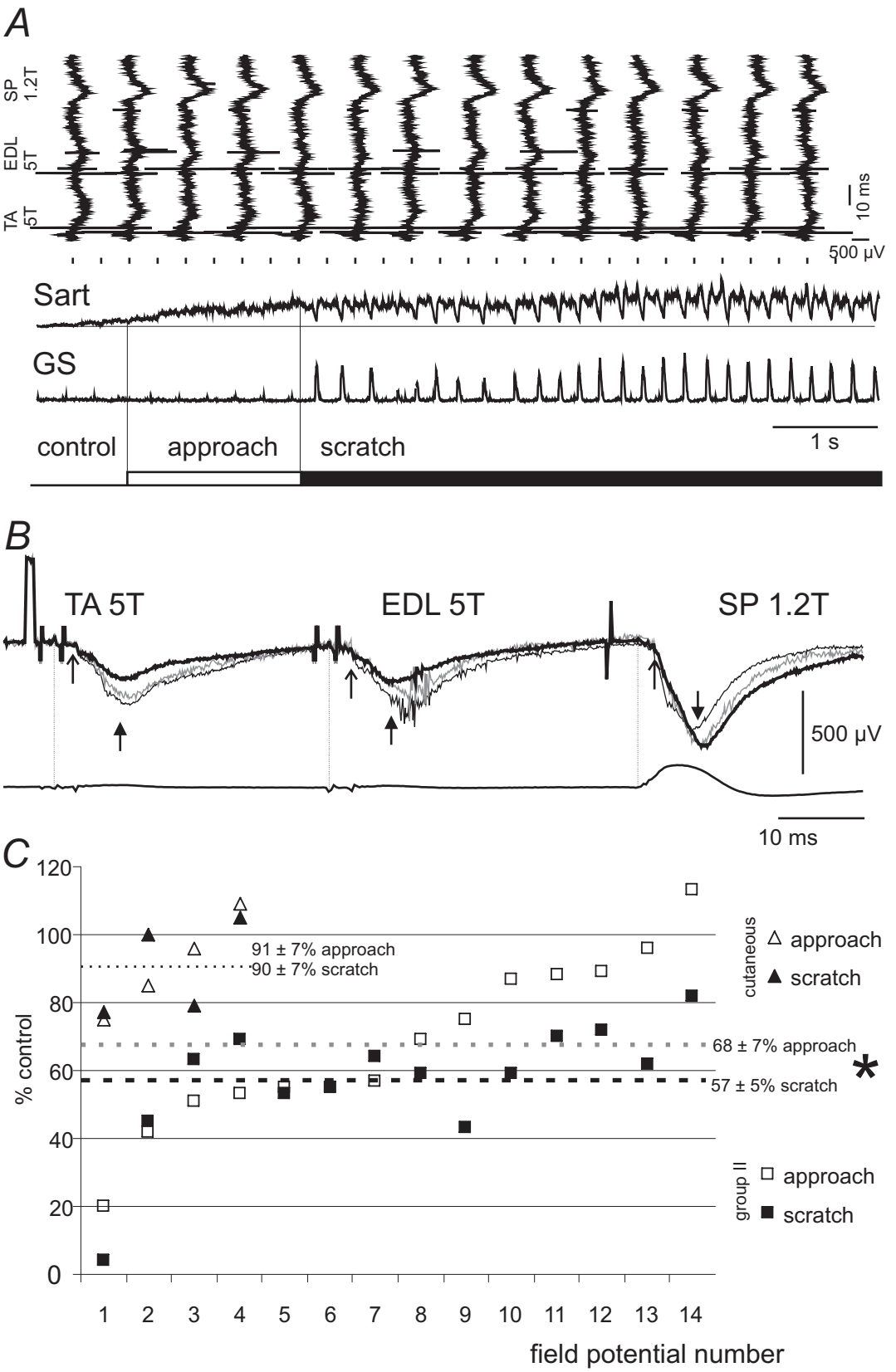
A
SP 1.2T
EDL 5T
TA 5T
10 ms
500 µV
Sart
GS
control
approach
scratch
1 s
B
TA 5T
EDL 5T
SP 1.2T
500 µV
10 ms
C
120
100
80
60
40
20
0
% control
91 ± 7% approach
90 ± 7% scratch
68 ± 7% approach
57 ± 5% scratch
*
cutaneous
approach
scratch
group II
approach
scratch
1 2 3 4 5 6 7 8 9 10 11 12 13 14
field potential number

Figure 4

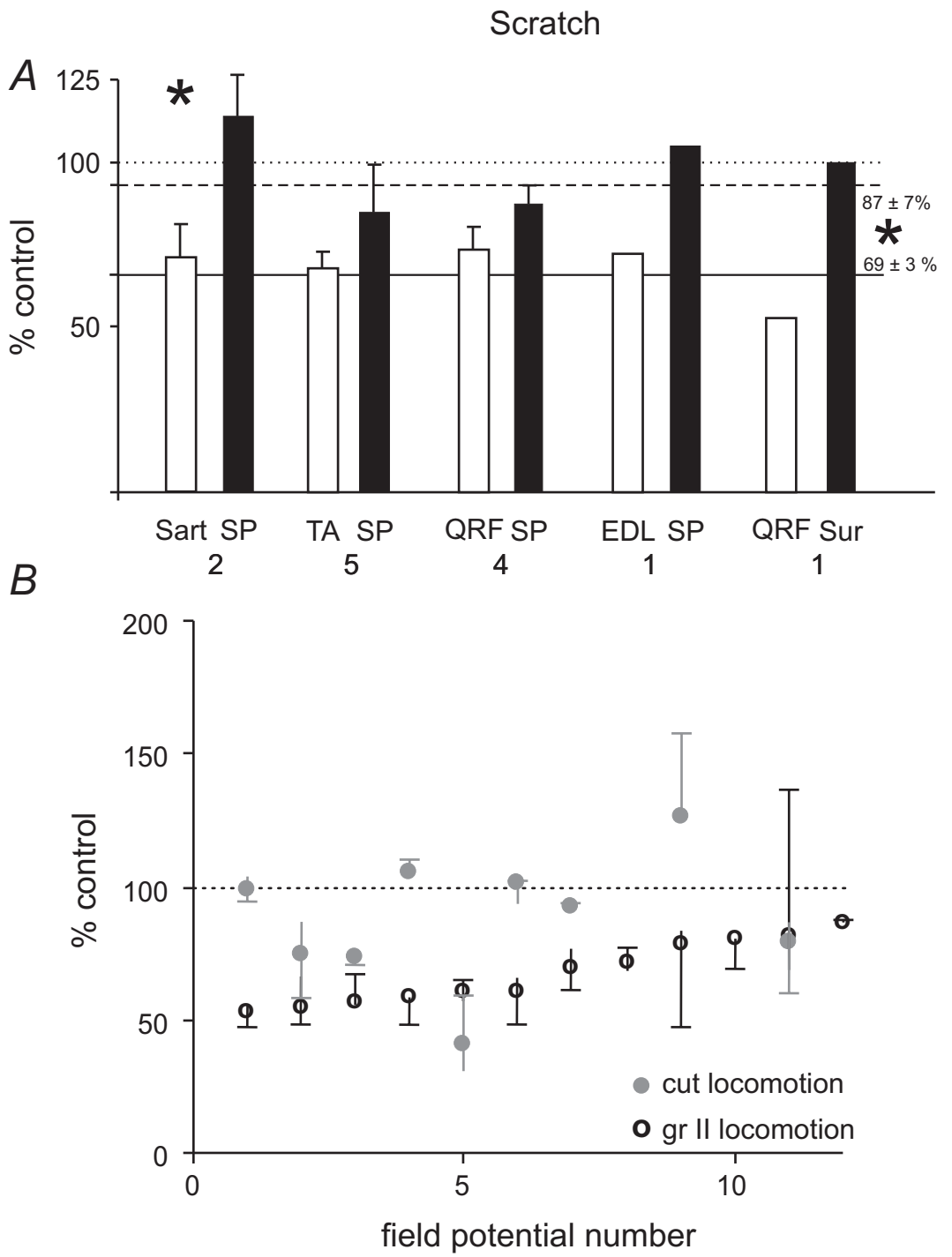
Scratch
A
125
100
50
% control
*
87 ± 7%
*
69 ± 3 %
Sart SP
2
TA SP
5
QRF SP
4
EDL SP
1
QRF Sur
1
B
200
150
100
50
0
% control
cut locomotion
gr II locomotion
0
5
10
field potential number

Figure 5

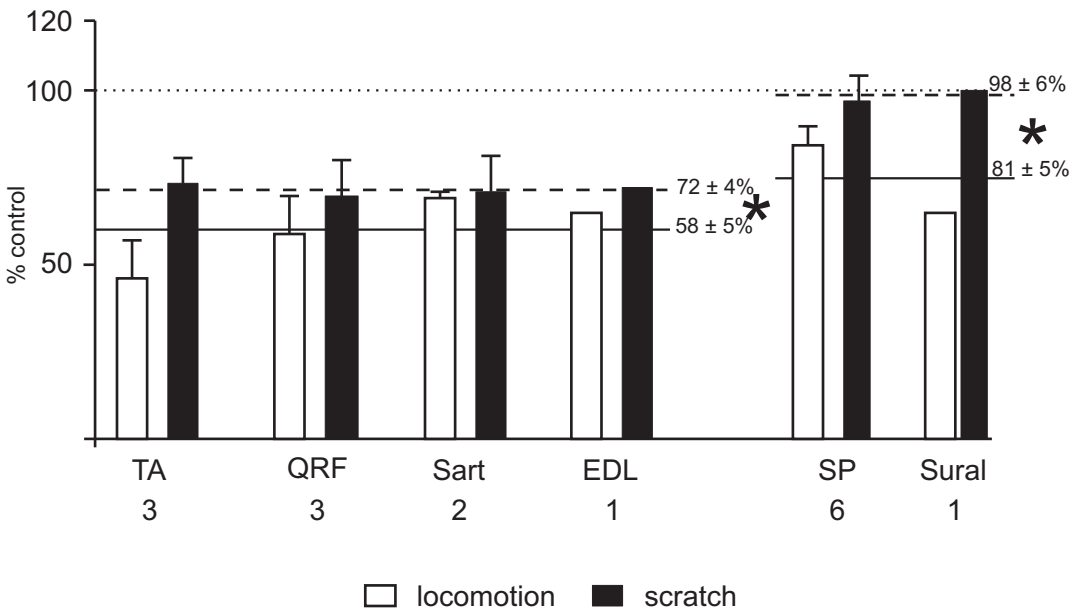
120
100
50
% control
98 ± 6%
72 ± 4%
58 ± 5%
81 ± 5%
*
*
TA
3
QRF
3
Sart
2
EDL
1
SP
6
Sural
1
locomotion
scratch

Figure 6

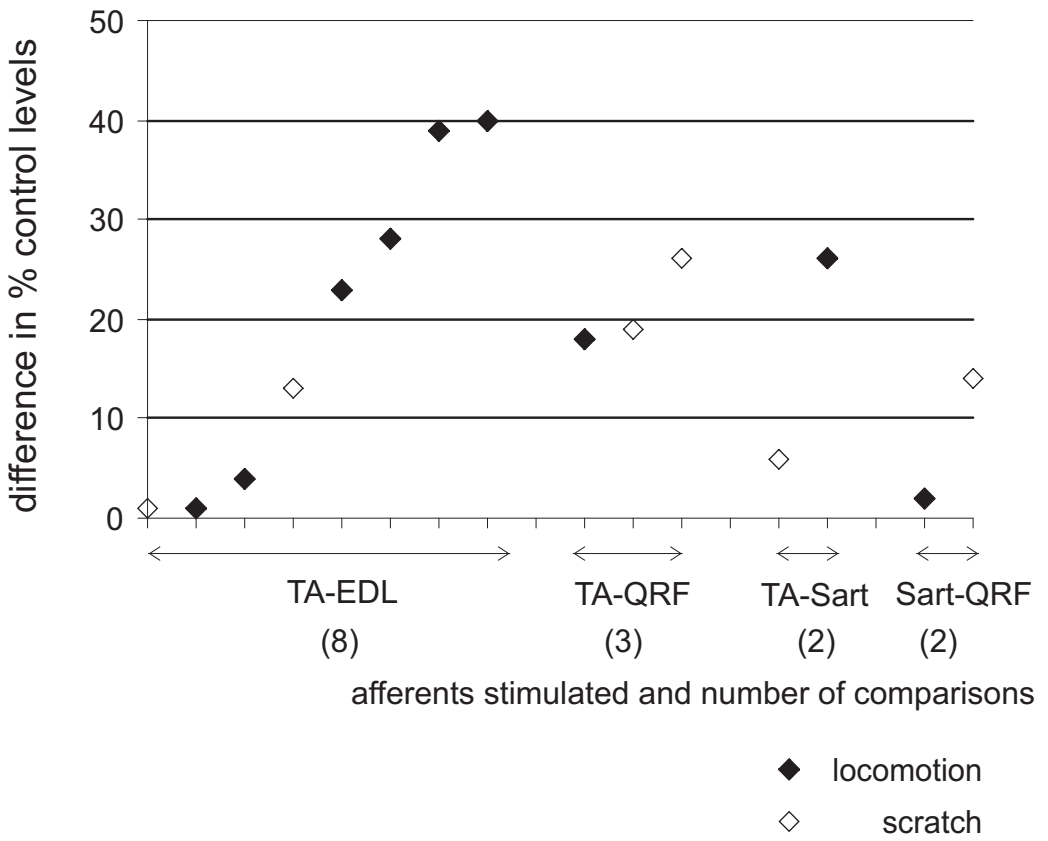
50
40
30
20
10
0
difference in % control levels
TA-EDL
(8)
TA-QRF
(3)
TA-Sart
(2)
Sart-QRF
(2)
afferents stimulated and number of comparisons
locomotion
scratch

Figure 7

Paper 2

Candidate spinal neurones mediating reflex actions of group II muscle afferents during fictive locomotion and scratch

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Key words: spinal cord, sensory system, cat

Abbreviated title: Group II interneurone activity during fictive locomotion

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Summary

Field potentials evoked by electrical stimulation of group II afferents in hindlimb muscle nerves are suppressed during fictive locomotion and scratch in decerebrate cats. Using extracellular recordings from neurones within the lumbar spinal segments, we show that this suppression of group II afferent input during fictive motor activity also manifests in a powerful reduction of the short-latency activation of spinal neurones receiving muscle group II input. In regions of the spinal cord with both cutaneous and group II input, action potentials evoked by stimulation of group II muscle afferents with 1-3 stimuli were reduced in 28/30 and in 6/7 neurones examined during fictive locomotion and during fictive scratch, respectively. There was more reduction of firing evoked by muscle group II afferents in 13/28 cases and in 3/6 cases, similar to the preferential reduction of field potentials evoked by group II afferents as reported previously. However, when trains of 22-52 stimuli at 200 Hz were used for the activation of muscle group II afferents, only 6/18 neurones were found to have reduced firing during fictive locomotion. Despite the strong preferential suppression of neuronal recruitment by muscle group II afferent input during fictive locomotion and scratch, here we show evidence that some neurones may be effectively recruited by subsequent shocks in longer duration stimulus trains during locomotor activity. Of the 12 neurones that remained responsive to muscle group II afferent input during fictive locomotion when long stimulus trains were used, 7 also had spontaneous and often phasic activity. Furthermore, 4 of these 7 neurones had axonal projections to more rostral segments of the spinal cord or to supraspinal areas. The role of group II activated and spontaneously active long projecting neurones in mediating information from segmental afferents and from the spinal central pattern generator is discussed.

Introduction

Sensory afferents arising from secondary muscle spindle endings (group II fibres) were originally described as being part of the flexion reflex afferent (FRA) system as these fibres evoke flexion reflexes in barbiturate anaesthetised, spinalized cats (Eccles and Lundberg 1959). In these preparations stimulation of any of the FRA produces reflexes that are excitatory to ipsilateral flexors and contralateral extensors and inhibitory to ipsilateral extensors and contralateral flexors. A characteristic feature of the flexion reflex system is that a variety of sensory afferents evoke similar reflexes, due to strong convergence from various sensory afferents onto the spinal flexion reflex pathways. Under some conditions, however, muscle group II afferents can evoke other reflex actions. For example, following low-pontine lesions in un-anaesthetized preparations, muscle group II afferents evoke inhibition of ipsilateral flexor motoneurones (Holmquist and Lundberg 1961). These observations indicated the existence of parallel reflex pathways that can be accessed by subsets of FRA fibres.

Perhaps the most striking change in the operation of the FRA system is that observed following intravenous L-DOPA infusion in spinal, anemically decerebrate cats (Anden et al. 1966, Jankowska et al. 1967). After administration of this monoamine precursor, there is a suppression of short-latency reflexes and longer latency reflex actions appear. Slow rhythmic discharges in peripheral nerves also appear to alternate between extensors and flexors (Jankowska et al 1967). During L-DOPA-induced fictive locomotion in unanaesthetized spinalized cats, stimulation of muscle group II afferents evokes flexion reflex-like actions (Schomburg et al. 1998). These reflexes promote ongoing flexor motoneurone activity during the flexor phase and their actions are similar to those evoked by cutaneous nerve stimulation (Schomburg et al. 1998). However, actions of the group II muscle afferents are very different in spinal cord intact cats in which locomotion is evoked by electrical stimulation of the midbrain. During both fictive (Perreault et al. 1995, Stecina et al. 2005) and treadmill locomotion (Hiebert et al. 1996, Lam and Pearson 2002) the common reflex actions evoked by group II and cutaneous components of the FRA are replaced by reflex actions that are more specialized. For example, during fictive locomotion stimulation of group II afferents in the tibialis anterior

(TA) or the posterior biceps-semitendinosus (PBST) nerve usually terminates the ongoing flexor phase and initiates a new extension phase (i.e. evokes a resetting to extension) (Perreault et al 1995). In the same preparations, stimulation of the extensor digitorum longus (EDL) and other flexor nerves enhances ongoing flexion (Stecina et al 2005). These specialised reflex actions of muscle group II afferents seem to be evoked similarly during fictive scratch (Stecina et al. 2005). However, in some decerebrate fictive locomotion preparations these actions can be reversed and opposite reflex effects can be elicited (Stecina et al. 2005). Factors controlling the selection of particular group II reflex pathways on a step-by-step basis are yet unknown but descending and/or centrally-evoked presynaptic modulation of excitability of muscle group II afferent terminals (Perreault et al. 1999, Stecina et al. paper 1 of thesis) and postsynaptic mechanisms regulating the excitability of spinal interneurones with input from group II muscle afferent (Shefchyk et al. 1990 and paper 1 of thesis) could be involved.

Before identifying the factors that control the selection of group II reflex pathways on a step-by-step basis, the neuronal elements relaying these reflex actions have to be established. Despite progress in identifying new classes of group II activated lumbar spinal neurones in anesthetized preparations (reviewed in Jankowska 1992) there is little information about which neurone populations mediate group II reflexes during motor activity. One candidate population consists of neurones located in the 4th lumbar segment with input from muscle group II afferents from quadriceps (Q), sartorius (Sart) and deep peroneal (i.e. TA and EDL) muscle nerves. Some of theses neurones also receive input from the cuneiform nucleus and project monosynaptically to lumbar motoneurones (Edgley and Jankowska 1987, Edgley et al. 1988). A fraction of these neurones has been found to be rhythmically active in the flexion phase of MLR-evoked fictive locomotion (Shefchyk et al. 1990) but despite their rhythmic activity, their short latency sensory-evoked activity seemed to be either phasically or tonically suppressed. Because of this suppression, these interneurones were thought to be unlikely candidates for mediating group II muscle afferent-evoked reflexes during fictive locomotion. We are unaware of any information on the activity of group II-activated spinal neurones during fictive scratch.

Since the report of suppression of muscle group II afferent evoked firing of midlumbar premotor neurones with group II input (Shefchyk et al. 1990), the existence of a powerful suppression of monosynaptic field potentials evoked by group II afferents during fictive locomotion (Perreault et al. 1999, Stecina et al. paper 1 of thesis) has been recognized that is partly the result of presynaptic mechanisms reducing the excitability of group II muscle afferents. Such mechanisms would also account for the failure of group II stimulation to recruit the interneurones studied by Shefchyk et al. (1990) during locomotion. The suppression of not only the monosynaptic but also of the longer latency components of these field potentials, however, suggest that postsynaptic modulation of excitability in reflex pathways from group II afferents may also contribute to the reduction of group II afferent actions.

The fictive locomotor-related reduction of muscle group II afferent input has been shown to have the following three important features. (1) It is preferential as the field potentials evoked by muscle group II afferents are reduced more than those evoked by group I muscle or cutaneous afferent in the same regions of the spinal cord (see paper 1). (2) It is non-uniform as the field potentials evoked by muscle group II fibres originating from different muscles have been found to be reduced to different levels (see paper 1). (3) It also develops during fictive scratch (see paper 1) suggesting that electrical stimulation of the brainstem is not required for it.

In the studies reported here, we further examined this motor-activity related suppression of muscle group II input. The first aim was to determine whether there is a preferential and non-uniform reduction of action potentials evoked by electrical stimulation of muscle group II afferents during fictive motor activity as suggested by the field potential changes (see paper 1). To this end, extracellular recordings from lumbar spinal neurones excited by electrical stimulation of muscle group II and cutaneous hindlimb nerves were collected during no motor activity evoked (i.e. control) and during fictive motor activity (locomotion and scratch) in decerebrate cats. Fictive locomotion was evoked by stimulation of the mesencephalic locomotor region and fictive scratch was evoked by curare application to cervical dorsal roots followed by mechanical stimulation of the skin covering the ear. The effectiveness of neuronal firing evoked by short duration

(1-3 stimuli at 200 Hz) trains was determined by the number of action potentials per stimulus (i.e. firing index) evoked during control and motor activity periods. The second aim was to examine which populations of neurones mediate reflex actions of muscle group II afferents during fictive motor activity. This was difficult to undertake, as at all of the intraspinal locations examined to date, there is a substantial suppression of neuronal activity evoked by muscle group II afferents (Perreault et al. 1999, paper 1). This controversy, that muscle group II afferent input is strongly suppressed and yet powerful reflex actions are evoked by these afferents during both fictive locomotion and scratch has been somewhat elucidate by findings we will present in the results. The use of long stimulus trains (22-52 stimuli at 200 Hz) has been an important tool for finding spinal neurones showing not only sensory-evoked activity from group II muscle afferents but also displaying spontaneous firing that is often coupled to the locomotor cycle. Interestingly, the majority of these neurones (i.e. with sensory-evoked and spontaneous activity) had long axons projecting to rostral spinal or supraspinal areas. Preliminary data have been reported previously (Stecina et al. 2004).

Methods

Animal preparation and surgery

Data presented in the thesis were collected from 7 cats (2-4 kg), 2 of which were also used for studies reported in paper 1 of the thesis. Canadian Council on Animal Care guidelines were followed in all experimental protocols. Halothane-nitrous oxide anaesthesia was applied during the surgery which began with a tracheotomy and cannulation of the carotid artery for blood pressure monitoring and of the jugular and femoral veins for administration of fluids and drugs. At the start of the surgery, subcutaneous atropine (0.05 mg kg^{-1}) and intravenous dexamethasone (2 mg) were given. A buffer solution of 5% glucose and 0.5% $NaHCO_3$ was infused intravenously (5 mL h^{-1}) throughout surgery and the experiment.

The following nerves in the left hindlimb were dissected and placed on bipolar hook electrodes for recording or for stimulation: posterior biceps and semitendinosus (PbSt), semimembrinosus and anterior biceps (SmAB), medial gastrocnemius (MG),

lateral gastrocnemius and soleus (LGS), sometimes MG and LGS were together as GS, plantaris (PL), flexor digitorum longus, flexor hallucis longus, often together as FDHL, tibial (Tib), peroneus longus (PerL), tibialis anterior (TA), extensor digitorum longus (EDL), and superficial peroneal (SP) nerves. Cuff electrodes were used to mount saphenous (Saph), sartorius (Sart), the vasti (Vas) and rectus femoris nerves and often mounted together as quadriceps (Q). Remaining ventral and dorsal nerve branches of the hindlimbs on the left side as well as on the right were cut and tendons around the hip were sectioned bilaterally.

Laminectomies were performed to expose the lumbar spinal cord, the 13th thoracic segment and the first few cervical segments. The head was positioned in a stereotaxic holder and the animal was fixed in place at the hips and at the lumbar and thoracic vertebrae in a rigid frame. The skin of the left hindlimb was used to create a pool filled with mineral oil to suspend the nerves on silver hook electrodes. A precollicular-postmammillary decerebration was performed and the brain tissue rostral to the transection was removed. Anaesthesia was discontinued and the animal was paralysed with pancronium bromide (1.2 mg initially then 0.4 mg hr^{-1}). During the experiment, the CO_2 level was maintained between 3 and 5% by artificial ventilation, the blood pressure was kept above 80 mmHg by dextran injections when needed. Core body temperature was maintained near 38 ^{0}C by infrared lamps and a heating pad. In 2 cats, 4-aminopyridine (4-AP, initial dose 100 µg kg $^{-1}$, total doses 400 µg kg $^{-1}$) was given intravenously to improve locomotor activity. In 2 other animals, diazoxide was administered intravenously for the lowering of blood pressure (1.5 - 4.5 µg/kg). At the termination of the experiments, the animals were euthanised by a lethal injection of pentobarbital.

Stimulation and recording

Fictive locomotion was evoked by monopolar stimulation (30-400 uA, 0.5 - 1 ms pulses, 10-30 Hz) of the mesencephalic locomotor region (MLR). Fictive scratch was evoked by applying curare (0.01 - 1%) on the dorsal roots of the first cervical segment and mechanically stimulating the pinna or the skin of the face and neck area.

Bipolar ball silver electrodes were used for stimulation and recording from

selected hindlimb muscle and cutaneous nerves. Peripheral nerves were stimulated with single shocks or trains of stimuli (usually at 200 Hz) at intensities expressed in multiples of threshold (T) for the most excitable fibres as measured at the cord dorsum. Muscle nerves were stimulated at 2-5 times threshold (T) of firing of the most excitable afferents while cutaneous nerves were usually stimulated at 2 and sometimes at 5T. Bipolar stimulating electrodes were posited bilaterally on the dorsal-lateral surface of the spinal cord with the dura closed at the 13th thoracic level and only ipsilaterally at the first cervical level (dura open) to evoke antidromic activation of the lumbar neurones examined. Rectified, integrated and digitized electroneurogram (ENG) activity was used to monitor fictive motor output. Data capture and analysis was done by software developed at the Winnipeg Spinal Cord Research Centre using Pentium PCs running Linux.

Recordings of interneurone activity and analysis

Glass micropipettes (1-2 μm tip diameter) filled with sodium-citrate were used to record extracellular field potentials and action potentials from lumbar interneurones. Most often several electrode tracts were examined within the segments from L4 to L7. Experiments began by mapping the group II afferent volley recorded at the cord dorsum to locate the regions with the largest cord dorsum potential from a particular nerve. Small openings were made in the pia in this region to allow entry of the microelectrode. Recordings were made at depths between 1.2 and 2.8 mm from the surface of the spinal cord. Neurones were selected for recordings on the basis of short latency (< 5 ms) synaptic responses evoked by electrical stimulation of muscle group II afferents. Sensory input during control (i.e. prior to locomotion) periods was assessed by a continuous 4 Hz stimulation of selected cutaneous and muscle afferents of the ipsilateral hindlimb with short trains (1-3 shocks) or longer trains consisting of up to 52 shocks. Rostral axonal projections were assessed by stimulation applied to the dorso-lateral funiculi at the 13th thoracic segment (T13). Spikes evoked at short (≤0.9 ms) latency following 3-4 shocks at 400 Hz were considered as antidromic. In some experiments, stimulation applied to the dorso-lateral funiculi at the first cervical (C1) segment was also tested. Neurones

antidromically activated by T13 but not C1 stimulation were classified as propriospinal neurones. No tests for caudal axonal projections were carried out. Firing evoked by muscle group II and cutaneous afferent input was considered monosynaptic when the latencies were less than 2 and 1.5 ms, respectively (see Edgley and Jankowska 1987). The latency of firing evoked by muscle and cutaneous afferents was measured from the afferent volley evoked by the most excitable fibres in each nerve by the first stimulus unless otherwise specified.

Comparison of sensory-evoked activity of neurones was done by assessing the changes in the number of spikes evoked by the same stimuli delivered during control, locomotion and recovery periods. Action potentials in response to 15-30 consecutive stimuli were summed off-line following data collection. A normalized firing index was calculated for each neurone during each of the periods by dividing the number of evoked spikes by the number of stimuli and the percent change in firing was calculated between control and locomotor periods by using the normalized firing indexes. Data are expressed as means ± SEM. Statistical significance was calculated by using Student's t-test.

Phasic relationship between neuronal activity and locomotor activity was assessed by circular statistics.

Results

The sensory-evoked activity of 50 lumbar spinal neurones was examined during fictive locomotion and/or during fictive scratch using electrical stimulation of ipsilateral hindlimb muscle and cutaneous nerves. The main finding was that the activation of interneurones by short-trains of stimuli delivered to muscle group II afferents was greatly suppressed during fictive locomotion (of 28/30) and fictive scratch (of 6/7) and usually to a greater extent than the cutaneous afferent-evoked activity. The sensory-evoked activity of the neurones tested with long stimulus trains delivered to peripheral nerves during fictive locomotion, however, was suppressed in only a third of the cases examined (6/18). Of the 12 neurones remaining responsive to later stimuli in the stimulus trains delivered to muscle group II afferents, 7 neurones also had spontaneous firing that was often phasically coupled to locomotion. Four of these spontaneously active cells had axonal projections to

more rostral segments of the spinal cord or to supraspinal areas.

Preferential reduction of muscle group II afferent-evoked firing during fictive locomotion

Extracellular recordings from lumbar spinal neurones activated following electrical stimulation of muscle and cutaneous hindlimb nerves have been collected prior to (control) during and post locomotor (recovery) periods, similar to the illustration in Fig. 1. Action potentials were recorded from this interneurone located in the rostral L6 segment, 1.96 mm deep following the electrical stimulation of the Sart, the TA and the EDL muscle nerves at 5T and the cutaneous SP nerve at 2T in the absence of fictive locomotor activity (A1-A4). During control, stimulation of all of these nerves evoked action potentials with minimal latencies of 2.1, 2.6, 3.4, and 1.3 ms for Sart, TA, EDL and SP, respectively, as measured from the arrival of the fastest conducting afferent volleys recorded on the cord dorsum (evoked by the first stimulus) as indicated in the bottom traces and by the vertical dotted lines in panels A1-A4. Integrated and rectified recordings from the hip flexor Sart and the hip extensor SmAB nerves during MLR-evoked fictive locomotion is shown in panel B with alternating flexor and extensor motoneurone activity developing at about 30 s after the start of continuous MLR stimulation. The average cycle duration as measured between consecutive onsets of Sart ENG activity was 581 ms (n=32 step cycles in this 20 s long bout of locomotion). In panel 1C, single records on top of overlays of 9 other records are shown for each of the corresponding periods. The vertical dotted lines indicate the arrival of the afferent volley recorded at the dorsal surface of the cord. During the control and the recovery periods, TA and EDL nerve stimulation (at 5T, two stimuli at 200 Hz) and SP nerve stimulation (at 2T, single stimuli) evoked action potentials in this neurone. During locomotion, however, stimulation of the TA and the EDL nerves failed to evoke any action potentials (arrows in panel B) while stimulation of the cutaneous SP nerve remained effective in evoking firing. The number of action potentials and their latencies are shown in panel 1D in response to 30 consecutive stimuli during control and post locomotion and 15 consecutive stimuli during locomotion. The firing index (the number of spikes divided by the number of stimulus presentations) was 0.5, 1.0, and 1.3

from TA, EDL and SP nerve stimulation, respectively, during control as shown in panel 1E. During locomotion, the firing indexes from TA and EDL were reduced to zero, but the firing index from the SP nerve was increased to 1.9. When locomotor activity ceased after the termination of MLR stimulation, TA and EDL nerve stimulation was effective again in evoking action potentials in this neurone and the firing index returned to 0.5, 1.1, and to 1.4.

-Figure 1 here-

Note that the field potentials evoked by group II afferents from the TA and the EDL nerves at this location were also suppressed during fictive locomotion but the field potential evoked by the cutaneous SP nerve slightly changed (Fig 1C). This observation is similar to those reported in paper 1 of the thesis. The peak amplitude of field potentials recorded here was measured during control and locomotor conditions before the occurrence of the action potentials (i.e. to avoid contamination of the amplitude measurements by the interneurone spikes). The average group II field potential amplitude evoked by the TA and the EDL stimulation during locomotion fell to 34 and 40% of control values while the SP-evoked field potential remained at 95% of its control amplitude. The new observation described here is that such locomotor-related field potential reductions seem to be associated with reductions of actions potentials evoked in interneurones with input from group II muscle and cutaneous afferents.

Direct comparisons between changes in the firing evoked by group II and cutaneous afferents was assessed in 30 cells during fictive locomotion. As expected based on a previous study (Shefchyk et al. 1990) during fictive locomotion, the majority (28/30) of the neurones showed a reduction and only 2 neurones showed an increase or no change in firing evoked by muscle group II afferents. Perhaps the most prominent evidence for preferential suppression of muscle group II input was seen in 5 cells in which there was a reduction of muscle group II evoked firing but no change (2) or an increase (3) in firing evoked by cutaneous input. One of these neurones is the cell illustrated in Fig.1. The comparison of the overall reduction of firing evoked by group II and cutaneous input in these 5 neurones is shown in Fig. 2D by the first set of bars. The height of the bars represents the percent change in firing evoked by muscle group II (open) and cutaneous

(black) afferents with the number of neurones shown under the graph. Significant differences between means are indicated by the stars. There was a complete reduction (100%) of firing evoked by muscle group II input but only a partial reduction of firing evoked by cutaneous input in 8 other neurones. Figure 2A illustrates the firing indexes of one of these cells and the second pair of bars in panel 2D shows the averaged data. The mean reduction of firing evoked by cutaneous input was 58 ± 11%.

In 4 other neurones, there was a partial reduction of firing evoked by both kinds of afferents. Panel 2B illustrates an example with the firing indexes being reduced to different levels and not completely to zero. As shown in panel 2D by the third pair of bars, there was more reduction of firing evoked by muscle group II input (59 ± 12%) than by firing evoked by cutaneous afferents (37 ± 12%) in this group of cells but without a significant difference between the means ($p \leq 0.23$).

The firing evoked by both muscle group II and cutaneous input was completely suppressed in 10/29 neurones during fictive locomotion. One example is illustrated in panel 2C. Of the remaining 1 cell (from 28), the firing evoked by muscle group II input was reduced by 50% while the cutaneous afferent-evoked activity was reduced by 90% (not illustrated). This was the only case where there was a larger reduction of firing evoked by muscle group II than that by cutaneous input.

Preferential reduction of muscle group II afferent-evoked firing during fictive scratch

Similar to the recordings during fictive locomotion, interneuronal activity following stimulation of muscle group II and cutaneous afferents was also recorded in 7 neurones during fictive scratch. The typical pattern of motoneurone activity in flexor (Sart, EDL) and extensor (Vasti, PerL and GS) muscle nerves developing with the mechanical stimulation of the skin covering the ears is shown in Fig. 3A. Note that the activity of the PerL muscle, which acts as an ankle flexor during fictive locomotion, is synchronized with the extensor motoneurone pools (see Vasti and GS) following the approach phase in which a tonic flexor activity can be seen. Panel 3B shows overlays of 5 single traces recorded from the same neurone as illustrated in Fig. 1. Note that the

stimulation of the TA and the EDL muscle nerves at 5T and the SP cutaneous nerve at 2T during the control period evoked similar action potentials as shown in Fig. 1. Panel C illustrates the number and the latency of the action potentials measured in 15 consecutive stimulus presentation during the control, the scratch and the recovery periods. The firing evoked by the TA and the EDL nerve stimulation was suppressed by 100% and by 80%, respectively. This was a similar observation as during locomotion (see Fig. 1D). There was also a reduction by 79% and a shift in the latency of the action potentials evoked by the SP nerve stimulation. The second group of bars in Fig. 3D illustrates the percentage of the suppression of the action potentials observed during fictive scratch in this cell. The other groups of bars in Fig. 3D illustrate the changes in the number of action potentials evoked by the stimulation of muscle group II (white bars) and cutaneous (black bars) during fictive scratch in the other 6 cells examined. There were several different patterns observed even with this small sample size (see Fig. 3D from left to right): in one case, firing evoked by both types of input was suppressed completely, in 3 cases there was more suppression of group II afferent evoked firing than that of cutaneous afferent evoked firing, in one case there was hardly any change (2% increase) of both types of responses, in one case the cutaneous afferent input was suppressed while the muscle group II-evoked responses increased and in the final case both types of responses were increased. *(I thank Brian Schmidt who made me re-think and include the data set on the sensory-evoked firing of neurones during fictive scratch. Without his honest comment - that it should be scratched- I would have not included such a detailed description of the data we had.)*

-Figure 3 here-

Combining observations in scratch and locomotion, in 17/28 and 3/6 neurones the reduction of firing evoked by group II muscle afferents was greater than the reduction of the responses to cutaneous input. In 4/17 and 2/3 cases, the latency of the action potentials evoked by both muscle group II and cutaneous afferents was compatible with monosynaptic coupling (< 2 ms from group II afferents and < 1.5 ms from cutaneous afferents, see Edgley and Jankowska 1987) suggesting that presynaptic inhibitory mechanisms contribute to the fictive motor activity-related reduction of muscle group II afferent input as suggested by previous observations (Perreault et al. 1999).

Long trains of stimuli are most successful in recruiting spinal neurones with muscle group II input during fictive locomotion

The strong indications for the preferential suppression of neuronal firing evoked by muscle group II afferent input seem controversial with the findings that long stimulus trains delivered to flexor group II afferents evoke powerful reflex actions that modify the ongoing fictive locomotor rhythm (e.g. Perreault et al. 1995, Stecina et al. 2005). Less than 10% (2/30 as described above) of the neurones with group II afferent input remained responsive to muscle nerve stimulation of 1-3 shocks during fictive locomotion and this gave small hopes for locating spinal neurones that mediate the actions of these afferents during fictive locomotion. However, when longer trains of stimuli (usually 22-52 stimuli at 200 Hz) were used during fictive locomotion, the number of cells recruitable by afferent input seemed to increase. Figure 4 illustrates recordings from a neurone (located at a depth of 1.6 mm in the rostral L4 segment) while stimulating muscle afferents with short (1-2) as well as long (32 stimuli) trains during control, locomotor and recovery periods. Action potentials were evoked by double shocks delivered to the Sart nerve at 5T, 200 Hz and single shock to the Saph nerve at 1.5T during the control period (Fig. 4A). The minimal latency of the Sart and the Saph evoked actions potentials was 2.7 and 1.4 ms, respectively. Stimulation of the Vasti, the RF and the TA nerve also evoked firing in this neurone (not illustrated) with a latencies of 3.8, 4.3 and 5.0 ms, respectively. There was a complete and a partial suppression of firing evoked by the Sart and the Saph nerve stimulation respectively, during fictive locomotion (Fig. 4B). Thus it is another example of the preferential suppression of muscle group II input during fictive locomotion. However, when using a train of 52 stimuli during fictive locomotion, the cell responded to Sart stimuli as illustrated in Fig 4C. When examined closely (Fig. 4D) it became evident that action potentials were evoked nearly after all stimuli except the first three.

-Figure 4 here-

The latency (measured from the onset of the first stimulus) and the number of spikes evoked by the stimulation of the Sart nerve is illustrated during control and the locomotor activity. In another neurone from a different preparation, similar observations were evident. Fig 4F illustrates the responses of this neurone located in caudal L4, 1.66

mm deep following the stimulation of the TA muscle nerve with a train of 22 stimuli during control and during fictive locomotion. There were no responses to the first stimuli, or the responses were much later than during control, but firing was evoked when the stimuli presentation was continued. Observations from these 2 neurones suggest that the lack of response to short trains of stimuli during fictive locomotion may not reflect a complete suppression of sensory-evoked activity. Thus, in order to assess the possible role of spinal neurones with input from group II muscle afferents, trains of at least 3 or more stimuli should be used during fictive locomotion.

Overall, muscle group II afferent-evoked firing by long trains of stimuli (22-52 stimuli at 200 Hz) was examined in 18 spinal neurones located within the mid and the lower lumbar segments. In 12/18 cases, the neurones followed most stimulus presentations. It is important to point out, that most (16/18) of these neurones fired at similar latencies during fictive locomotion than during control. Table 1 describes the location, the sensory input evoking spikes during control in these neurones, as well as the effects of the stimulated nerves during the perturbations. When using the long stimulus trains, the delivery of the stimuli were timed by the activity of flexor muscle nerves (i.e. ENG recordings) so the stimulation would be evoked in early or mid flexion. The effects of these stimuli on the locomotor cycle were assessed in parallel with the neuronal responses.

-Table 1 here-

During resetting to extension (RE, i.e. cessation of activity in flexor motoneurones and initiation of activity in extensors) evoked by Sart or TA nerve stimulation at 5T, there were 8 neurones that followed the stimulus train. Figure 5 illustrates the activity of a long projecting cell located in caudal L4, 1.66 mm deep during control and during another perturbation of the fictive locomotor activity. Panel 5A1-A2 illustrates the control responses evoked by muscle group II afferent stimulation at 5T, from Sart and TA, nerves. The latency of the evoked spikes illustrated is 3.8 ms (5A1) and 3.5 ms (5A2). The minimum latency of Sart-evoked activity was 3.3 ms, and of TA-evoked activity was 3.5 ms from the group I afferent volley evoked by the first shock. Other muscle nerves evoking action potentials in this neurone were Vasti (1.4 ms), PbSt (3.8 ms), PerL (1.9

ms), the mixed Tib nerve (3.6 ms), and the cutaneous Sural nerve (2.7 ms). This cell was antidromically activated by stimulation of the dorsal funiculus at the T13 segment, as shown by 3 single recordings in panel 5A3, hence the name long projecting. The latency for the spikes evoked is 0.7 ms (400 μA stimulus). Collision between Sart 5T and T13 stimulation evoked firing verified the antidromic activation (not illustrated). Responsiveness to stimulation delivered to the dorso-lateral funiculi at the first cervical (C1) level was not tested therefore both possibilities, that this neurone was an ascending tract cell or a propriospinal cell remain undecided.

Panel 5B illustrates overlaid averages of several step cycles with (solid traces) and without (dotted traces) stimulation of the Sart and TA nerves during fictive locomotion evoked by MLR-stimulation. ENG recordings from the hip extensor (SmAB), the bifunctional PBSt, two ankle flexors (PerL and EDL) and an ankle extensor (GS) nerve are shown over an 800 ms period. About 100 ms after the onset of activity in the PerL nerve, a 160 ms long train of 5T stimuli (32 shocks at 200 Hz) was delivered to the Sart and TA nerves that evoked a resetting of the ongoing flexion phase to extension. Note that the earlier onset of SmAB activity is seen with both Sart and TA 5T stimulation. This long projecting neuron followed almost every shock of the stimulus train delivered to both Sart and TA muscle nerves as illustrated in the bottom traces (ME). Extracellular action potentials were evoked after the first shock following Sart stimulation but only after the third shock delivered to TA.

-Figure 5 here-

During prolongation of ongoing flexor activity (PF) by Sart, RF or EDL nerve stimulation, 3 neurons followed the stimulus trains. There was 1 neuron that followed the stimulus train delivered to Sart at 5T, while the stimulation evoked no effects (NE) on the timing of the locomotor cycle. Figure 6 illustrates the activity of a long projecting neuron located in the caudal L4 segment, 2.56 mm deep while PF was evoked when the Sart nerve was stimulated. Panel 6A shows superimposed traces (n=5) of the response of this neuron to 5T stimulation of the Sart (6A1) and TA (6A2) nerves. The minimal latencies of the evoked spikes were 1.9 ms from Sart and 3.6 ms from TA during control (note that the latency of the spikes shown in panel 6A1 is 2.8 ms). Stimulation of the Vasti and the RF

nerves at 5T also evoked action potentials at 2.6 ms and at 2.7 ms, respectively while simulation of the PbST nerve at 5T and the Saph nerve at 2T evoked spikes at onset latencies of 4.7 and 3.2 ms (not illustrated). With 2T stimulation, TA and RF nerves evoked occasional excitation of this cell at latencies of 3 and 4.4 ms, respectively. Panel 6A3 illustrates the activation of this cell by stimulation applied to the dorso-lateral funiculi at T13. This cell also displayed some spontaneous action potentials during the control period. In 2 of the 3 illustrated traces, there was an 0.8 ms latency (i.e. antidromic) activation of the cell with 100 μA stimulation at T13. In the second trace from the top, it is likely that the spontaneous activity occurring at 2 ms before the expected antidromic spike caused an occlusion (arrow). Responsiveness to stimulation delivered to the dorso-lateral funiculi at the C1 level was not tested.

Figure 6B illustrates the effects of stimulation of the Sart and the TA nerves at 5T (32 shocks, 200 Hz) delivered during the flexion phase every 6th step. Stimulation of the Sart nerve (Fig. 6B1) prolonged ongoing flexion (see increased duration of Sart activity) and delayed the following extensor burst (see GS activity delayed). Stimulation of the TA nerve (Fig. 6B2) had similar effects to that of the Sart nerve. The average effects of both Sart (Fig 6C1) and TA (Fig 6C2) stimulation (solid traces) show increased amplitude and duration of flexor (TA and Sart) activity and delayed onset of extensor (GS) activity when compared to averaged step cycles during non-stimulated (i.e. control) steps (dotted traces).

-Figure 6 here-

The bottom traces in Fig. 6C1 and 6C2 are microelectrode (ME) recordings from 3 presentations of the stimulus train to the Sart and TA nerves. While this neuron followed most shocks to the Sart nerve, it rarely responded during TA stimulation. Thus the sensory evoked activity was reduced during fictive locomotion and the responses to TA stimulation were more affected than those to Sart stimulation. This example of a differential depression of neuron recruitment is similar to the non-uniform reduction of field potentials evoked by the stimulation of group II muscle afferents (see paper 1 of thesis) and to the differential reduction of group II afferent firing seen in neurones when examined with short stimulus trains delivered to muscle afferents (see Figs. 2B during fictive locomotion and Fig. 3C during fictive scratch).

It is important to point out, that all of the neurons that followed muscle group II afferent stimulation during prolongation of flexion (n=3), and 3/8 neurons that followed trains during resetting to extension were antidromically activated from T13 (i.e long projecting neurons).

The activity of the last two neurons shown in Table 1 was not assessed during the sensory-perturbation of locomotor activity however they were propriospinal neurones that remained responsive to muscle group II afferent input (tested with short trains of stimuli) during fictive locomotion and their possible contributions to muscle group II afferent-evoked reflexes is discussed later (see Fig. 10).

Evidence for segregation of reflex pathways mediating actions of group I and group II muscle afferents during fictive locomotion

In 2/12 cases in which long stimulus trains evoked firing of group II activated cells, we examined the neuronal responsiveness to both 2T and 5T stimulus strength. In both cases, the neurons responded to stimulation at 5T but not at 2T strength during both control and locomotor activity, although, both 2T and 5T stimulation perturbed the locomotor rhythm. The activity of one of these neurons located in the caudal L4 segment, 1.4 mm deep is illustrated in figure 7. This cell was antidromically activated by stimulation at T13, as shown in Fig. 7A1. Stimulation of the dorsolateral funiculus (400 μA) evoked a spike at a latency of 0.4 ms from the volley recorded by the cord dorsum electrode at L6. Stimulation of the Sart nerve at 5T also evoked spikes at a minimal latency of 2.7 ms (spikes illustrated in 7A2 with latencies of 3.5 and 3.7 ms). When the Sart 5T evoked action potential occurred within 0.8 ms of the T14 stimulation, likely a collision with the T13-evoked spike abolished antidromic firing (arrow in panel A2).

-Figure 7 here-

The raw (Fig. 7B) and the averaged (Fig. 7C) responses of the effects evoked by 5T and 2T stimulation of the Sart nerve triggered from the PerL ENG and delivered every 8th step were the prolongation and an increased of the ongoing flexor activity (see PerL and TA ENGs) as well as the delay of the extensor bursts in SmAB and GS nerves. Note

that the prolongation of the flexor phase was greater with 5T stimulation. Panel 7C illustrates averaged step cycles without (dotted) and with (solid) the stimulation of the Sart nerve. The microelectrode (ME) recordings show that this cell responded to the 5T train but not to the 2T train. The other neuron that showed a similar responsiveness to 5T but not to 2T stimulation (not illustrated) was not antidromically activated from the T13 region. These two neurons active during perturbations of the step cycle only when 5T stimulation was applied suggest that reflex pathways serving group I and group II afferent actions on the CPG may be different parallel pathways as proposed in recent studies in our laboratory (Stecina et al. 2005).

Neurons with muscle group II afferent-evoked and spontaneous activity during locomotion

During fictive locomotion, 7/12 neurons responsive to muscle group II afferent input while perturbing the step cycle were also spontaneously active during locomotion. Figure 8 illustrates the spontaneous (i.e. non-sensory evoked) activity of the long projecting neuron illustrated in figure 6 during fictive locomotion. Alternating activity of the ankle extensor (GS) muscle nerves with hip (Sart) and ankle (EDL) flexors with is shown in panel A. The microelectrode (ME) recordings below illustrate the spontaneous activity of this neuron. The onset of activity in this cell began in late flexion and continued throughout the extension phase. This segment was selected for illustration because it contained several missing bursts of extensor activity during rhythmic hip flexor activity and rhythmic or strongly modulated ankle flexor (EDL) activity. This in an example of extensor deletions (see Lafreniere-Roula & McCrea 2005). Note that this neuron continued to be rhythmic during the deletion of extensor activity and that the number of spontaneous spikes per burst was larger when there was strong activity in the GS ENG (see arrows). In the bout of locomotion illustrated here, the average step cycle (measured as the time between consecutive onsets of EDL ENG activity) was 504 ms. The dots in Panel B are a raster of the occurrence of locomotor-related spiking during 18 step cycles. These raster plots are normalized to the activity of the EDL nerve (thin trace). The circular plot in panel C shows the same spikes taken together from all 18 cycles. There is a

significant directionality of firing as the majority occurred from late flexion to mid extension (at 238^0, $p<0.01$). Note, that this measure of directionality, while providing a distinct point during the phase when most spikes occurred is not very useful for describing the extent of phasic activity in relation to the step cycles.

-Figure 8 here-

The spontaneous activity of the 6 other neurons that were responsive to muscle group II input and showed spontaneous activity during fictive locomotion are shown in figure 9. Interestingly, 4/7 neurons displaying spontaneous and sensory-evoked activity during fictive locomotion were long projecting neurons (i.e. antidromically activated from stimulation at the T13 region). There was one interneurone (i.e. without rostral axonal projections) shown in Panel 8A with a short period of activation during the transition from extension to flexion. There were 2 cells with phasic activity coupled to flexion (9B and 9C), one of these, an interneurone, firing throughout the flexor phase (9B) and the other, a long projecting cell with activity mainly in the first half of flexion (9C). Flexion-coupled activity of caudally projecting, group II activated neurons has been reported previously for mid-lumbar, last-order interneurons (Shefchyk et al. 1990) but no tests for caudal projections were carried out in the present series of experiments.

-Figure 9 here-

Panels 9D and 9E illustrate two neurons that were active in a tonic manner. The neuron illustrated in panel 9F and another long projecting neuron illustrated in figure 8 showed spontaneous activity mainly during the extension phase. However, there was a difference between the overall activity pattern of these two cells. While the cell illustrated in panel 9F seemed to be active mainly in late-extension, the neuron illustrated in figure 8 seemed to fire more during early-extension.

In addition to the neurons described in Fig 9, there were 2 other cells with input from group II muscle afferents that showed rhythmic, flexor-coupled firing during fictive locomotion. These cells were antidromicaly activated by stimulation at T13 but not by stimulation at the C1 spinal segment. Thus, these neurons are considered as propriospinal cells. The activity of one is illustrated in figure 10. Panels A1, A2 show the sensory-evoked firing of this neuron prior to locomotor activity by the simulation of the muscle

nerve Sart at 5T and the cutaneous nerve SP at 2T. The minimal latency of the spikes evoked by these nerves was 0.9 ms (from the group I afferent volley evoked by the first shock) and 3.3 ms, respectively. The illustrated spikes have a minimal latency of 3.5 and 4.8 ms. Panel 10A3 shows the antidromic activation of this neuron. It follows each of the 4 shocks (200 Hz) at a latency of 0.8 ms evoked by 400 μA stimulation of the dorsolateral funiculus at T13. Panel 10A4 illustrates the firing evoked by stimulating at the C1 with 1 mA strength, evoking a spike at 5.6 ms on average.

-Figure 10 here-

This neuron was also spontaneously active prior to locomotor activity. Panel 10C shows all the spikes collected from this cell prior to and during a 30 s period of MLR stimulation. The first 14 s are without MLR stimulation. During this period and during MLR stimulation, the Sart and SP nerves were stimulated at 4 Hz with an interval of 44 ms between them. The vertical axis in panel C illustrates the latency of the spikes fired (diamonds) with respect to the beginning of each trace recorded. From the onset of each trace, the clustering of spikes following the stimulation of the SP nerve (filled arrow marking the afferent volley evoked by the SP nerve stimulation) was less regular than Sart-evoked firing (open arrow marking the arrival of the group I afferent volley following Sart 5T stimulation). Approximately 5 s after the onset of MLR stimulation, with the development of rhythmic flexor and extensor motoneuron activity, the response to the Sart nerve stimulation became suppressed, while the weak response to SP stimulation was slightly altered. The spikes occurring at other than around 18 or 60 ms in each trace were likely due to spontaneous activity. Note that following the onset of MLR stimulation, spontaneous firing increased. Panel 10B shows the alternating TA (flexor) and Plant (extensor) motoneuron activity during fictive locomotion from the last 10s illustrated in panel 10C. The vertical dotted lines indicate the onset of peripheral nerve stimulation (short line: SP 2T, long line: Sart 5T).

Discussion

Extracellular recordings from lumbar spinal neurons with input from muscle group II afferents revealed that there is a strong suppression of short-latency sensory-evoked

activation during fictive locomotion and scratch. When considering the responses to one or two shocks delivered to hindlimb muscle nerves, the onset of locomotion or scratch resulted in a reduced recruitment of 28/30 and 6/7 neurons, respectively. In the majority of cases firing evoked by muscle group II input was more suppressed than that evoked by cutaneous input. These observations further support the idea of a preferential suppression of muscle group II input operating during fictive locomotion as previously suggested by field potential recordings (Perreault et al. 1999, paper 1 of thesis). The results presented here provide indications that the functional correlate of centrally-evoked suppression of field potentials evoked by muscle group II afferents during fictive locomotion and scratch could be the reduction of neuronal activation evoked by short-duration (single or double shocks) stimulation of peripheral nerves.

The cases where there was a suppression of firing evoked by both muscle and cutaneous afferents at monosynaptic latencies (6 neurons total) further support the idea that there is centrally evoked presynaptic suppression of transmitter release from sensory afferents. This was also suggested previously by the suppression of the monosynaptic component of some field potentials (Perreault et al. 1999, Stecina et al. paper 1 of thesis). In contrast, the suppression of the group II input could have been occurring at postsynaptic sites as the latencies of the action potentials evoked by group II input were longer than what is characteristic of monosynaptic coupling in other neurones. Phasic synaptic inputs from the CPG could cause an increase in the postsynaptic conductance of the interneurones and thereby decrease their responsiveness to gr II input. This would explain why longer trains of muscle group II stimuli "break through" during fictive locomotion, as there could be increased temporal facilitation of gr II EPSPs. It is also important to consider the possibility that stimulation of group II afferents results in smaller EPSPs in these neurones than stimulation of cutaneous afferents and therefore the muscle group II inputs are more susceptible to being shunted to a subthreshold depolarization by the "locomotion-induced" conductances. *(I thank Brent Fedirchuk for the suggestion to include these possibilities in the discussion)*. Therefore, intracellular recordings from these neurones are critical in further elucidating the actual mechanisms that control the excitability of these reflex pathways during fictive motor activity, even

though they require a very challenging set of experimental procedures.

While initial responses to peripheral nerve stimulation were greatly reduced and often eliminated during fictive locomotion, neuron recruitment by longer duration trains was found to remain in 12 of the 18 cases examined. Thus the present results suggest that the suppression of sensory input during locomotion and scratch would serve mainly to moderate the effects of spurious or weak sensory input and would not eliminate reflex control of the step cycle with a prolonged barrage of proprioceptive input during the step cycle. Thus, even with a (presumably presynaptic) suppression of group II afferent activity, the net effects of stimulation of muscle group II afferents, can remain powerful and control motoneuron activity across several joints by regulating the CPG itself (e.g. fig. 5,6, 7 here and see Stecina et al. 2005).

A differential presynaptic control of transmission from muscle group II afferents ending in different spinal regions has been shown for sensory-evoked presynaptic inhibition in anaesthetized cats (Jankowska et al. 2002). In those preparations, sensory-evoked presynaptic inhibition of muscle group II afferent input is strong in the intermediate zone where interneurons receive monosynaptic input but the longer latency input via dorsal horn interneurons to the intermediate zone is less suppressed (Riddell et al. 1995, Jankowska et al. 2002). Perreault and colleagues (1999) have also shown a stronger centrally-evoked suppression of muscle group II input in the intermediate zone compared to the dorsal horn. It is feasible that the presynaptic suppression can have different effects on responses evoked though different (i.e. disynaptic vs. trisynaptic or longer latency) pathways (cf. Jankowka et al. 2002) but at this stage we have no information about this idea being applicable to conditions during fictive motor activity.

More than half (7/12) of the neurons recruited by long duration trains activating flexor group II afferents were spontaneously and often phasically active during fictive locomotion. The majority (4/7) of the group II activated and spontaneously firing cells were antidromically activated by the stimulation of the dorsolateral funiculus at the T13 segment. Since we did not test whether these neurons project to supraspinal centres, we consider them simply as long projecting cells. There were 2 additional neurons with input from muscle group II afferents, that were antidromically activated by stimulation at T13

but not at C1 level. These 2 neurons were classified as propriospinal neurons (i.e. projecting to segments between T13 and C1) and they also showed rhythmic spontaneous activity during fictive locomotion. Thus overall, 6/9 neurons responsive to group II input with spontaneous activity during fictive locomotion had ascending projections.

Short vs. long latency pathways mediating muscle group II reflexes

Electrical stimulation utilizing less than 3 shocks to muscle nerves may not be sufficient to determine whether a neuron remains responsive to muscle group II afferent input during fictive locomotion. In the experiments reported here, only 2/30 neurons were recruitable by muscle group II input when examined with a short train. However, when longer trains were applied, 12/18 neurons remained responsive to muscle group II input. In the light of these results, it is important to use long stimulus trains to assess which neurons are candidates for mediating group II afferent reflexes during locomotor activity. The only other study concerning group II activated neurons during fictive locomotion (Shefchyk et al. 1990) utilized mainly 1-3 shocks to assess the sensory-evoked activity of mid-lumbar group II-activated interneurons. In the light of the present results, their finding, that two-thirds of the neurons examined showed a reduction in sensory-evoked firing, cannot be interpreted as proof that such neurons with caudal projections to motoneurons are not involved in group II reflexes during locomotion. Repeating that study with multi-shock (i.e. at least 3 shocks) trains would provide a more realistic estimate on the proportion of last order group II interneurons that remain responsive to segmental input during fictive locomotion. Longer trains of stimuli also could reflect more physiological activation of muscle group II afferents during motor activity and therefore the pathways and functions of neurones revealed by them are in fact more important and not just an artifact. However, when longer stimulus trains "broke through" the suppression and evoked firing (in 2 neurones, see fig 5) no analysis was done to correlate the train duration required to break through the inhibition and the train duration required to be effective in perturbing the locomotor step cycle. This type of analysis would be necessary in future experiments to further elucidate the role of the centrally-evoked suppression of muscle group II input. *(I thank Steve Edgley for suggesting to make these considerations.)*

The strong suppression of responsiveness to short-trains but powerful activation evoked by long stimulus trains during fictive locomotion have been also observed in another series of studies conducted in our laboratory. The stimulation of the cutaneous SP nerve evokes the stumbling corrective reflex when activated during the flexor phase of fictive locomotion (Quevedo et al. 2005a). In general terms, the net effect of SP stimulation is inhibition of ankle flexors and excitation of ankle extensors and PBSt motoneurons (Quevedo et al. 2005b). Often, the net effect in a given motoneuron pool is different from the initial, short latency effects evoked by stimulation with 1-2 shocks. Thus, the common feature between SP and muscle group II afferent reflex pathways is that there is a great qualitative difference between the early onset actions and those arising from subsequent shocks in the long-duration trains. Similar discrepancies between early and late effects have been reported in anaesthetized preparations for Sural nerve stimulation (Heckman et al. 1992).

Rhythmically active neurons during fictive locomotion

The spinal neuronal populations that have been examined during fictive locomotion and display rhythmic activity include Ia inhibitory interneurons (Feldman and Orlovsky 1975, Jankowska et al. 1975, McCrea et al. 1980, Pratt and Jordan 1987), Renshaw cells (McCrea et al. 1980), lumbar neurons with excitation or inhibition from unspecified ipsi or contralateral FRA pathways (Baev et al. 1978a,b), and the group II activated midlumbar last order interneurons (Shefchyk et al. 1990). All of these populations have been described to be active in either the flexion or the extension phase. Some of the FRA-activated (Baev et al. 1978b) neurons as well as midlumbar group II activated (Shefchyk et al. 1990) interneurons have also been found to be tonically and transitionally (i.e. peak firing occurs during transitions between phases) active during fictive locomotion.

There were 12 neurons reported in this paper that were located within the 4th and 6th lumbar segments from 1.6 to 2.8 mm deep responding to muscle group II input during locomotion. Seven of these neurons also displayed spontaneous (non-sensory evoked) activity coupled to the locomotor rhythm. Four were antidromically activated from the

T13 level but not tested for antidromic activation from the C1 level. Therefore, these cells are considered as long projecting neurons. The other 3 cells are considered as interneurons.

To which population of previously described group II activated cells could the spontaneously active neurons belong? The interneurons could have been intermediate last order cells and essentially represent the same population as those reported by Shefchyk et al. (1990). In future studies, intraspinal stimulation in selected ipsilateral motor nuclei would allow for identifying whether the spontaneously firing group II activated cells are last order neurons to motoneurons.

All four of the long projecting neurons were located within the caudal L4 and rostral L5 segments and these could also overlap with some of the previously described group II activated neuronal populations. Dorsal horn spinocerebellar tract (DSCT) neurons convey muscle group II input from the mid-lumbar segments to the cerebellum (Edgley and Jankowska 1988, Edgley and Gallimore 1988), and some of these neurons have axons collaterals terminating in the region of nucleus Z (Asif and Edgley 1992). Both electrophysiological (Edgley and Jankowska 1988, Riddel et al. 1994) and anatomical (e.g. Matsushita et al. 1979) studies showed that group II-activated DSCT neurons are more commonly encountered in the midlumbar segments than in the sacral segments. DSCT neurons have been found to be rhythmically firing during fictive locomotion evoked by midbrain stimulation (Fedirchuk et al. 1995) and during passive limb movements that resemble stepping in both anesthetized and decerebrate cats (Poppele et al. 2003). There is a sub-population of DSCT neurons that has no group I but only group II and cutaneous input (Edgley and Jankowska 1988). These spinocerebellar neurons are different from others as their cell bodies are located in the dorsal horn of the mid-lumbar segments outside of Clarke's column. Thus, it is likely that some of the rhythmically active neurons that remained responsive to muscle group II input belong to this non-Carke's column sub-population of DSCT neurons. In future experiments, it would be necessary to identify whether the long projecting neurons can be antidromicaly activated form the C1 level and from the cerebellum. Those long projecting cells that were responsive to both group I and group II input are likely to belong to the ventral

spinocerebellar (VSCT) neuronal pool. This population has also been shown to display rhythmic activity during locomotion (Orsal et al. 1988). In the light of these facts, it will be important to assess the antidromic activation of the rhythmically active group II activated neurons from the cerebellum in future studies.

One of the best studied group of ascending tracts originating in the lumbosacral area is the spinocervical tract (SCT). Electrophysiological investigations showed that some of the SCT neurons in the midlumbar and in the sacral segments receive both group I and group II afferent input via short latency pathways. Monosynaptic activation of SCT neurons by group II afferents in the mid-lumbar (Hammar et al. 1994) and sacral (Riddell et al. 1994) segments is present, but in the lumbosacral enlargement (i.e. in the lower lumbar region) only disynaptic pathways have been found (Harrison and Jankowska 1984). There is no information, to our knowledge, on the activity of SCT during locomotion. Based on the findings that some neurons clearly responded to 5T but not to 2T stimulation of the same muscle nerves (see fig. 6), we hypothesize that these neurons belong to the SCT pool and partake in the segregation of group I and group II afferent pathways.

Ipsilateraly projecting propriospinal neurons with input from muscle group II afferents have been described in the sacral segments in anaesthetized cats (Riddell et al. 1994). These neurons receive convergent (monosynaptic) input from both muscle group II and cutaneous afferents and they are antidromicaly activated from the T13 segment but do not respond to stimuli applied at the C3 level. In the present experiments, 2 neurons were identified as propriospinal cells and showed rhythmic spontaneous firing coupled with flexion. Propriospinal neurons are important for the coordination of forelimb-hindlimb activity and presumably for informing the cervical spinal cord about switching the locomotor rhythm during sensory perturbations (for review see Jordan and Schmidt 2002). During fictive locomotion, a population of commissural propriospinal neurons have been reported to be phasically active (Huang et al. 2000, Matsuyama et al. 2004). Although in these studies the sensory input of the examined propriospinal neurons have not been examined, there is a pool of lumbar commissural neurons with input from muscle group II afferents (Jankowska et al. 2005). The neurons described in the present paper, however,

could not overlap with this pool because there were no commissural projections of the neurons as examined by stimulation of the dorsolateral funiculus on the contralateral side. However, it cannot be excluded that the neurons encountered in the experiments described here have been commissural cells reaching segments below T13.

In spite of the incomplete survey of sensory input to the examined neurons and the incomplete electrophysiological characterization of axonal projections done in the experiments presented in this paper, we provide strong evidence that propriospinal and long projecting neurons are viable candidates for mediating reflex actions of muscle group II afferents during fictive locomotion. A systematic survey of the group II activated spinal neuronal populations would be required to assess whether long projecting neurons or interneurons are more likely to remain recruitable by muscle group II input and/or display rhythmic activity during fictive locomotion. This assessment, however, must be done by using long trains of stimuli.

	location		latency of evoked firing during control (ms)			sensory perturbation of locomotion		
cell type	segm.	depth (mm)	Sart 5T	TA 5T	SP/ Saph*/Sur**	long train stim	reflex evoked	spont. activity
interneurone	rL4	1,6	1,4	4,9	2,7	Sart	NE	T
long projecting	cL4	1,4	4,4	7,3	8.7*	Sart	PF	---
long projecting	cL4	1,6	3,9	3,5	2.6**	Sart, TA	RE	F
long projecting	cL4	1,7	6,7	3,3	1.4**	Sart, TA	RE	T/F
long projecting	cL4	2,56	2,8	4,4	3,2	Sart	PF	E
long projecting	rL5	2,1	---	5	3,6	RF, TA	PF	E
interneurone	cL5	1,6	3,1	3,4	2.8*	Sart	RE	---
interneurone	cL5	2,2	---	3,6	4.8**	TA	RE	---
interneurone	rL6	1,92	---	---	---	EDL	PF	---
interneurone	rL6	1,92	---	1,2	0,9	TA	RE	TR/F
interneurone	rL6	2,5	4,8	5	3,6	Sart	RE	---
interneurone	rl6	2,8	1,6	---	2,6	Sart	RE	F
propriospinal	rL4	1,88	4	14	---	na	na	F
propriospinal	rL4	2,56	3,3	---	---	na	na	F

Table 1. Location, sources of sensory input and evoked activity of neurons with group II input during fictive locomotion

The type of the neuron (interneuron: not projecting to thoracic segments, long projecting: reaching to T13 or higher, propriospinal: reaching to T13 but not higher than C1); the spinal segmental location (r: rostral, c: caudal, L: lumbar); the depth (mm); the latency of sensory evoked firing by muscle group II afferents (Sart, TA) and cutaneous afferents (SP, Saph *, and Sur **) during control; the type of muscle afferent stimulated by long trains of stimuli when locomotor activity was perturbed; the type of the reflex evoked (NE: no effects, PF: prolongation of flexion, RE: resetting to extension); and the spontaneous

activity (T: tonic, F: flexion coupled, E: extension coupled, TR: coupled to transitional periods) displayed by the examined neuron is shown for all cells remaining recruitable by muscle group II afferent input during fictive locomotion.

Figure Legends

Figure 1. Reduction of muscle group II but not cutaneous afferent-evoked firing in a lumbar spinal interneuron

A. Extracellular interneuron recordings following the stimulation of Sart (A1), TA (A2), EDL (A3) muscle nerves at 5T with 2 pulses at 200 Hz and the stimulation of the SP cutaneous nerve at 2T (A4) prior to fictive locomotor activity evoked by MLR stimulation. The vertical dotted lines mark the onset of the group I afferent volley as recorded by the cord dorsum potential (bottom traces). The stimulus artefacts were truncated for clarity.

B. Filtered and rectified electroneurograms (ENGs) of a hip flexor (Sart,) and an extensor (SmAB) showing motoneuron activity evoked by electrical stimulation of the MLR.

C. The top traces in each panel are single recordings of sensory-evoked firing and beneath them there is a superimposition of 9 recordings collected during control (left), fictive locomotion (middle) and post locomotor activity (rigth) following stimulation of TA and EDL nerves at 5T with two pulses at 200 Hz,and the SP cutaneous nerve at 2T. The vertical dotted lines show the onset of the group I afferent volley evoked by the second shock. During fictive locomotion, the TA and EDL-stimulation evoked short-latency responses are missing (arrows), but the SP-evoked responses are present. The with a single shock.

D. Histograms showing the number of spikes and their onset latencies evoked by the stimulation of the peripheral nerves indicated in response to 15 consecutive stimulus presentations during control, locomotion and recovery.

E. The change in the firing normalized, i.e. the firing index, is shown following stimulation of the TA(♢), the EDL (□) and the SP (●) nerves in each condition.

Figure 2. More reduction of group II afferent-evoked than cutaneous afferent-evoked interneuron firing during fictive locomotion

A-C. The firing index of 4 neurons with input from both muscle group II and cutaneous

afferents is shown (vertical axes) during control, locomotion and recovery. An example of reduction of muscle group II-evoked and increase of cutaneous-evoked firing (**A**), of complete reduction of muscle group II-evoked and partial reduction of cutaneous-evoked firing (**B**), and of partial reduction of both (**C**). The firing indexes were calculated from 15-30 number of stimuli delivered to the peripheral nerves indicated by the symbols (TA ♢, EDL □, SP ●) in each condition.

D. Summary of percent changes in firing evoked by muscle group II and cutaneous afferents during fictive locomotion. The bars represent the percent change in action potentials evoked by muscle group II (open bars) and cutaneous (black bars) afferents between the control and locomotor conditions. Each pair of bars illustrates the mean reduction with SEM indicated in different categories with the number of neurones in each category indicated below the bars. Statistical differences are indicated by an asterisk ($p<0.01$).

Figure 3. Suppression of firing evoked by muscle group II afferent input during fictive scratch

A. Rectified and integrated ENG recordings from hip (Sart, Vasti) and ankle (PerL, EDL GS) muscle nerves prior to and during fictive scratch activity evoked by mechanical stimulation of the skin covering the ears following topical application of curare onto the first cervical dorsal root. Note tonic flexor activity developing during the approach phase that is followed by cyclic scratch.

B. Overlay of 5 traces recorded from the same interneurone as shown in Fig. 1 prior to fictive scratch activity showing the action potentials evoked by the stimulation of the TA and the EDL muscle nerves at 5T with 2 stimuli at 200 Hz and of the SP nerve at 2T. Averaged cord dorsum recording (bottom) and the vertical lines indicate the onset of the fastest conducting afferent volleys from which the latency of the responses shown in C were measured.

C. Histograms showing the number of spikes and their onset latencies evoked by the stimulation of the peripheral nerves indicated in response to 15 consecutive stimulus

presentations during control, locomotion and recovery.

D. Summary of percent changes in firing evoked by muscle group II and cutaneous afferents during fictive scratch in each of the 7 neurones tested. The bars represent the percent change in action potentials evoked by muscle group II (open bars) and cutaneous (black bars) afferents between the control and locomotor conditions in response to 15-30 consecutive stimuli.

Figure 4. Firing evoked by long trains of stimuli delivered to muscle afferents during fictive locomotion

A. Extracellular interneuron recordings (5 traces overlaid) from an interneuron located in the rostral L4 segment, 1.61 mm deep evoked by the stimulation of the Sart (5T, 2 pulses at 200 Hz), and the Saph (1.5T) during control (left) and locomotion (right). Note that the Sart 5T evoked response was (see arrows in panel B2) while the SP evoked firing was not completely suppressed during fictive locomotion.

B. The trace illustrates a 150 ms long recording with a long stimulus train (42 pulses at 200 Hz at 5T) delivered to the Sart nerve during fictive locomotion. Note that firing occurs frequently later during the train.

C. The trace is the same recording as in panel **B** but expanded on a different time scale. Arrows represent the onset of each pulse (1 ms long square pulses) applied during stimulation. Note that firing was evoked approximately after the third shock of the train delivered or with a longer latency in response to the 2^{nd} shock. The artefacts evoked by the pulses were blanked (over a 1.2 ms window).

Figure 5. Sensory-evoked activity of a long projecting neuron during resetting to extension

A. Extracellular interneuron recordings taken during control from a long projecting neuron located in the caudal L4 segment, 1.66 mm deep. Firing evoked by the stimulation of the Sart (A1) and TA (A2) muscle nerves (5T) and of the dorsolateral funiculus at the 13^{th} thoracic segment (A3) is shown during control.

B. Averaged ENG recordings during steps without (dotted, n=6, 6) and with (solid, n=3, 4) the stimulation of the Sart (C1) and TA (C2) muscle nerves at 5T, 42 shocks at 200 Hz showing resetting to extension (see text for details). The bottom traces illustrate a single microelectrode (ME) recordings during one stimulated step. Note that the actions potentials follow the stimuli presented.

Figure 6. Sensory-evoked activity of a long projecting neuron during prolongation of flexion and a non-uniform suppression of firing evoked by muscle group II afferents

A. Extracellular recordings from a long projecting neuron in the caudal L4 segment, 2.56 mm deep during control, following the stimulation of Sart (A1) and TA (A2) muscle nerves at 5T with single shocks. The spontaneous firing of this neuron during control has likely collided with the spike evoked by the stimulation of the dorsolateral funiculus at T13 (see arrow in panel A3).

B. ENG activity of the ankle flexor TA and extensor GS muscle nerves following midbrain stimulation. The Sart nerve was stimulated (at 5T, 52 shocks at 200 Hz) during the flexion phase in every 6^{th} step (B1) and in a different bout of activity, the TA muscle nerve was stimulated (5T, 52 shocks at 200 Hz) during the flexion phase in every 5^{th} step cycle (B2). Both Sart and TA nerve stimulation during flexion enhanced ongoing flexor motoneuron activity.

C. Averaged ENG recordings from non-stimulated (dotted traces, n=6,13) and perturbed (solid traces, n=4, 13) step cycles. Sart 5T stimulation prolonged ongoing flexion and delayed the subsequent extensor burst in GS (C1), while TA 5T stimulation enhanced the amplitude and slightly prolonged flexor activity in Sart and ED (C2)L. The bottom traces show the microelectrode (ME) recordings during three stimulated steps.

Figure 7. Segregation of group I and group II afferent reflex pathways during fictive locomotion

A. Extracellular recordings from a long projecting neuron in the caudal L4 segment, 1.4 mm deep during control following the stimulation of the dorsolateral funiculus at T13,

with 400 μA strength (A1) and the stimulation of the Sart muscle nerve at 5T (A2). The spike evoked by the thoracic stimulation have likely collided with the spike evoked by the Sart 5T stimulation (arrow in A2).

B. MLR-evoked fictive locomotor activity was perturbed by the stimulation of the Sart nerve at 2 and 5T strength alternating in every 8th steps during the early flexion phase. ENG recordings from the hip and ankle, nerves show that stimulation of Sart at 5T evoked a prolongation of the ongoing flexor activity and a delay of the subsequent extensor bursts (SmAB, GS). Sart 2T stimulation evoked similar actions. The bottom trace shows the extracellular microelectrode (ME) recordings collected during the locomotor activity.

C. Averaged ENG recordings from non stimulated steps (dotted, n=5) and stimulated steps (solid, n=2,3) showing the perturbations evoked by Sart 5 and 2T stimulation during the flexion phase of fictive locomotion. Note that both 5 and 2T stimulation delayed activity in SmAB and GS nerves while prolonging the activity of PerL and TA nerves, except 5T stimulation evoked longer prolongation than 2T stimulation. The bottom traces illustrate a single microelectrode (ME) recording during a stimulated step.

Figure 8. Sensory-evoked and rhythmic spontaneous activity of a long projecting neuron during fictive locomotion

A. Extracellular recordings (ME) during fictive locomotion from the same long projecting neuron as illustrated in figure 5. ENG recordings from the Sart, EDL and GS nerves following MLR stimulation show locomotor activity with deletions of extensor activity. Note that the larger the activity in the ankle extensor GS nerve, the more this neuron seemed to fire (arrows).

B. Normalized activity of the EDL nerve (thin trace) is shown from 18 step cycles (some illustrated in panel 7A). The white bar represent flexion and solid bar represents extension. In each step cycle, the spiking of this neuron is shown by the filled diamonds.

C. Circular representation of neuronal activity during flexion (F, 0-180°) and during extension (E, 180-360°) combined from all 18 steps cycles shown in panel B. The small circle in the middle represents the level of statistical significance ($p \leq 0.0001$). The length

of the line originating in the centre of the circle illustrates "directionality" of the averaged activity: 238°. The length of the line denotes the significance interval by which the phasic activity is coupled to the cycle period (i.e. the longer the line the more probable that the neuronal activity was coupled to the direction indicated by the line and $p \leq 0.001$ applies when the line crosses the small circle).

Figure 9. Phasic spontaneous activity of neurons responsive to muscle group II-afferent input during fictive locomotion

A-F. Normalized, averaged ENG recordings from flexor muscle nerves indicated following MLR stimulation (thin traces) overlaid with the extracellularly recorded spikes (filled diamonds) in each cycle from bouts of activity when no peripheral nerve stimulation was applied. The number of cycles are indicated on the vertical axes. Each panel represents a different neuron. Circular statistics on the spontaneous activity are shown in each panel on the right. Same format as in Fig. 8C.

Figure 10. Sensory-evoked and spontaneous activity of a propriospinal neuron

A. Overlay of 4 extracellular recordings from a propriospinal neuron in rL4, 1.88 mm deep, following the stimulation of the Sart nerve at 5T with two shocks (A1), the cutaneous SP nerve at 2T (A2), the dorsolateral funiculus at the ipsilateral T13 segment with 0.4 mA, and at the C1 segment at 1 mA prior to fictive locomotor activity. Note that antidromic firing was evoked by stimulation at T13 but not at C1.

B. Integrated, rectified ENG recordings following the stimulation of the MLR illustrate a 10 s long bout of fictive locomotor activity with alternating flexor (TA) and extensor (Plant) motoneurone activity. Microelectrode recording (ME) illustrates the firing of the propriospinal neurone during fictive locomotion. The vertical dashed lines mark the onset of the peripheral nerve stimulation delivered to SP (short) and Sart (long) nerves.

C. The firing of the propriospinal neurone in relation to the onset of the recordings taken. Traces were collected at a 4 Hz rate continuously prior to and following MLR-stimulation. The onset of the volley evoked by the most excitable afferent fibres following stimulation

of the SP nerve was at 9 ms (filled arrow) and of the Sart nerve was at 53 ms (open arrow). Note that after MLR stimulation, the sensory-evoked activity by Sart 5T stimulation was reduced while the spontaenous activity was increased.

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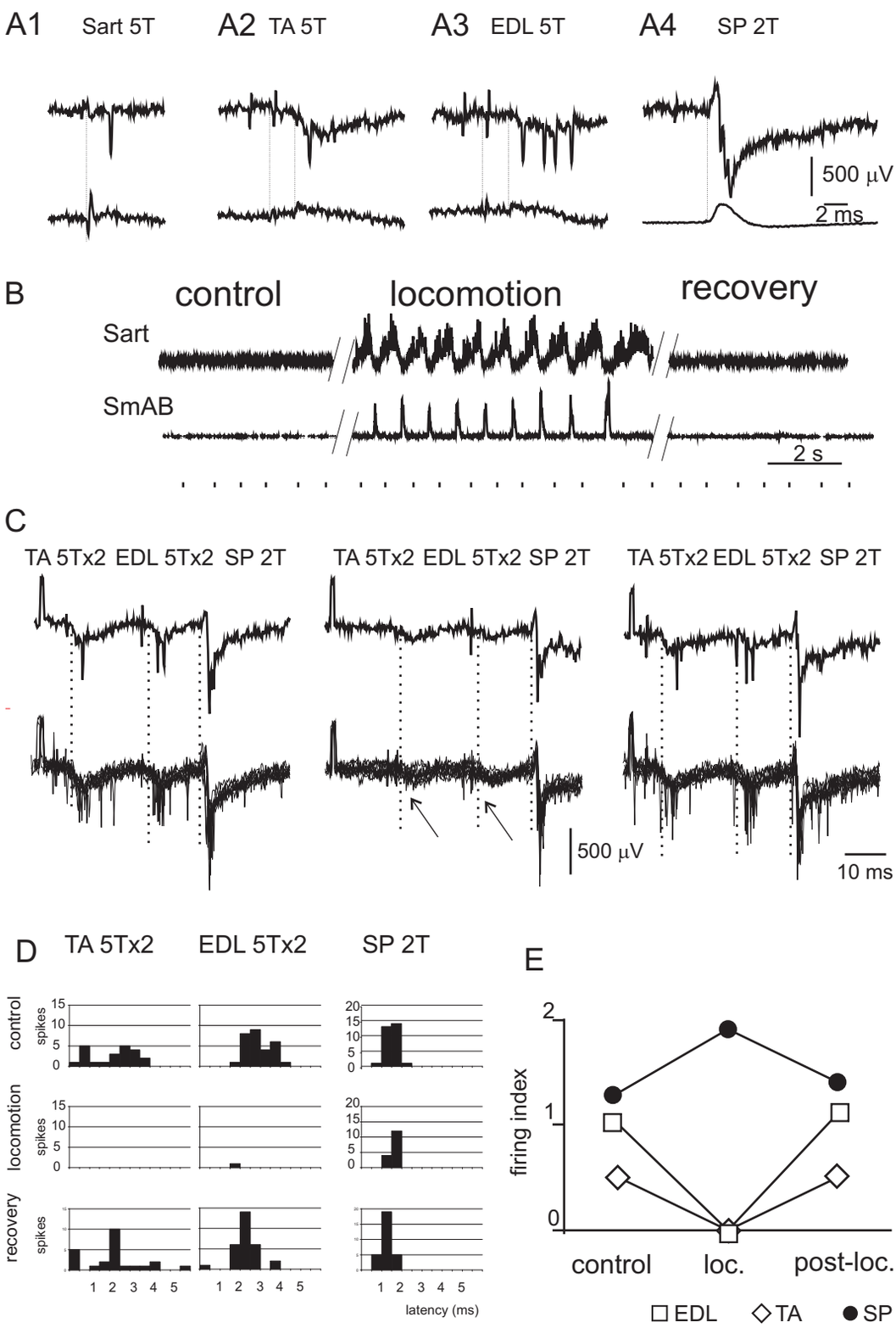
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A2 TA 5T
A3 EDL 5T
A4 SP 2T
500 μV
2 ms
B
control
locomotion
recovery
Sart
SmAB
2 s
C
TA 5Tx2 EDL 5Tx2 SP 2T
TA 5Tx2 EDL 5Tx2 SP 2T
TA 5Tx2 EDL 5Tx2 SP 2T
500 μV
10 ms
D
TA 5Tx2
EDL 5Tx2
SP 2T
control
locomotion
recovery
spikes
latency (ms)
E
firing index
control
loc.
post-loc.
EDL
TA
SP

Figure 1

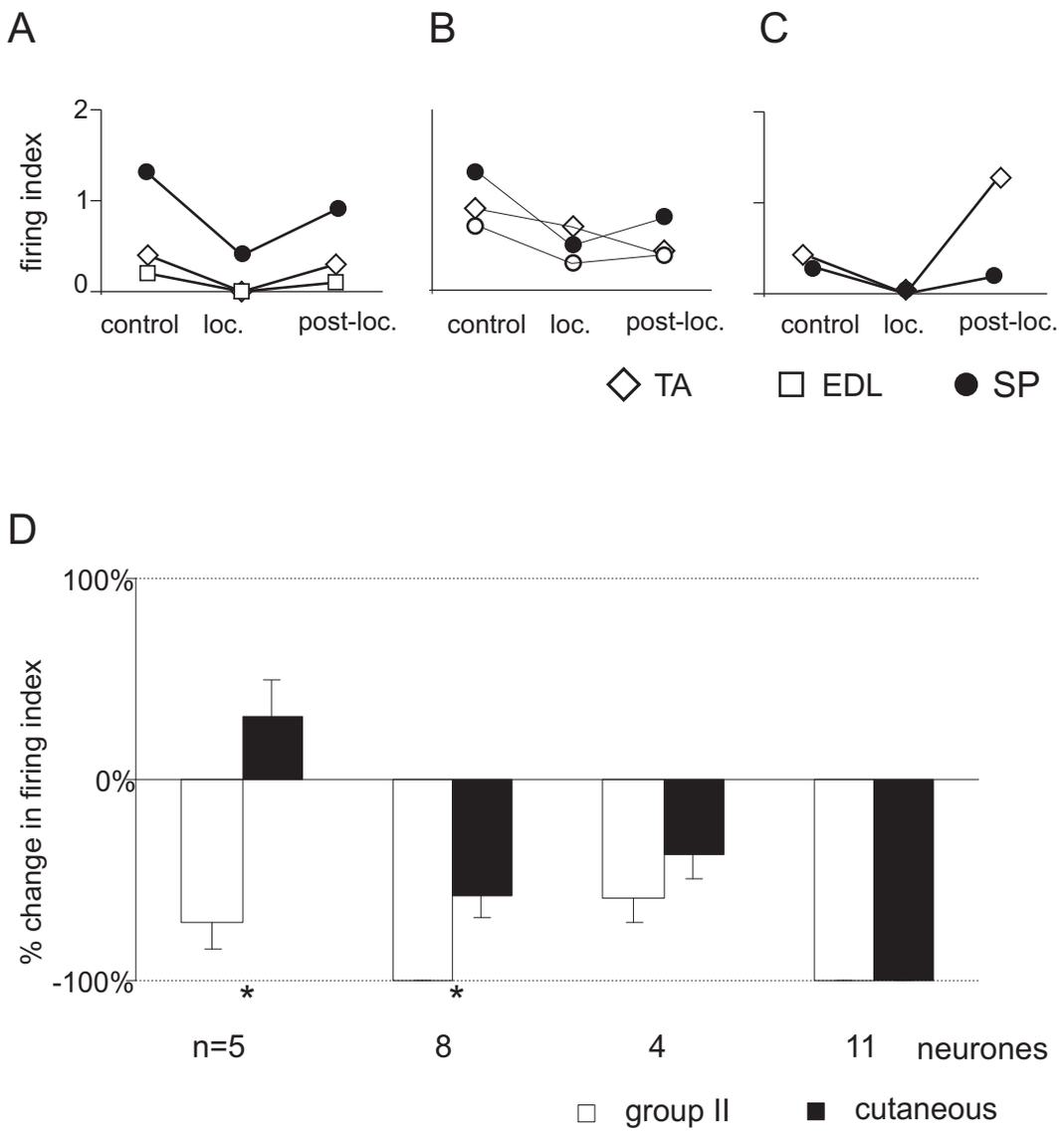

A
B
C
firing index
2
1
0
control
loc.
post-loc.
TA
EDL
SP
D
100%
0%
-100%
% change in firing index
*
*
n=5
8
4
11
neurones
group II
cutaneous

Figure 2

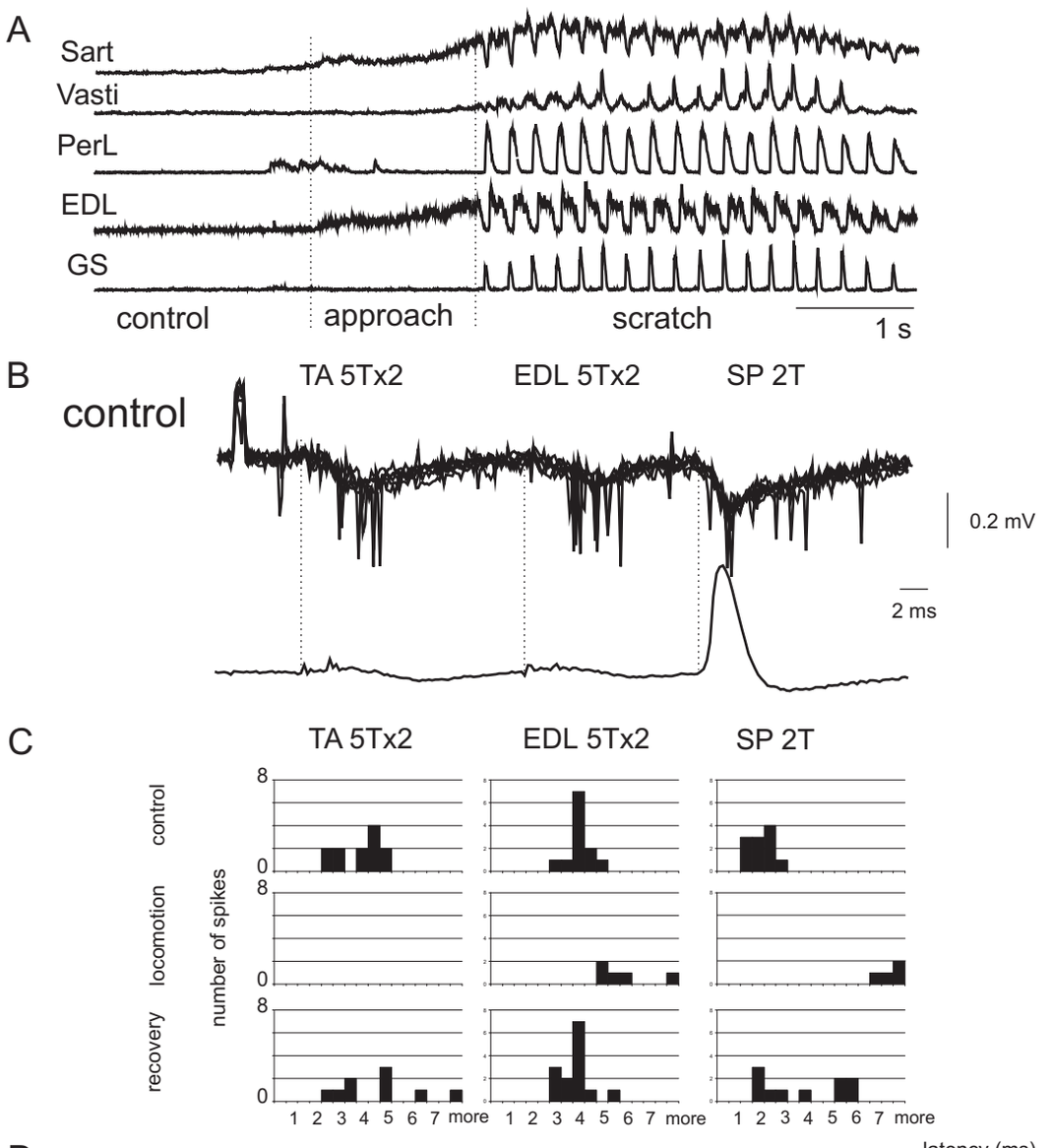

A
Sart
Vasti
PerL
EDL
GS
control
approach
scratch
1 s
B
control
TA 5Tx2
EDL 5Tx2
SP 2T
0.2 mV
2 ms
C
TA 5Tx2
EDL 5Tx2
SP 2T
control
locomotion
recovery
number of spikes
8
0
1 2 3 4 5 6 7 more
latency (ms)
D
% change in firing index during fictive scrahct
200%
100%
0%
-100%
gr II
cut

Figure 3

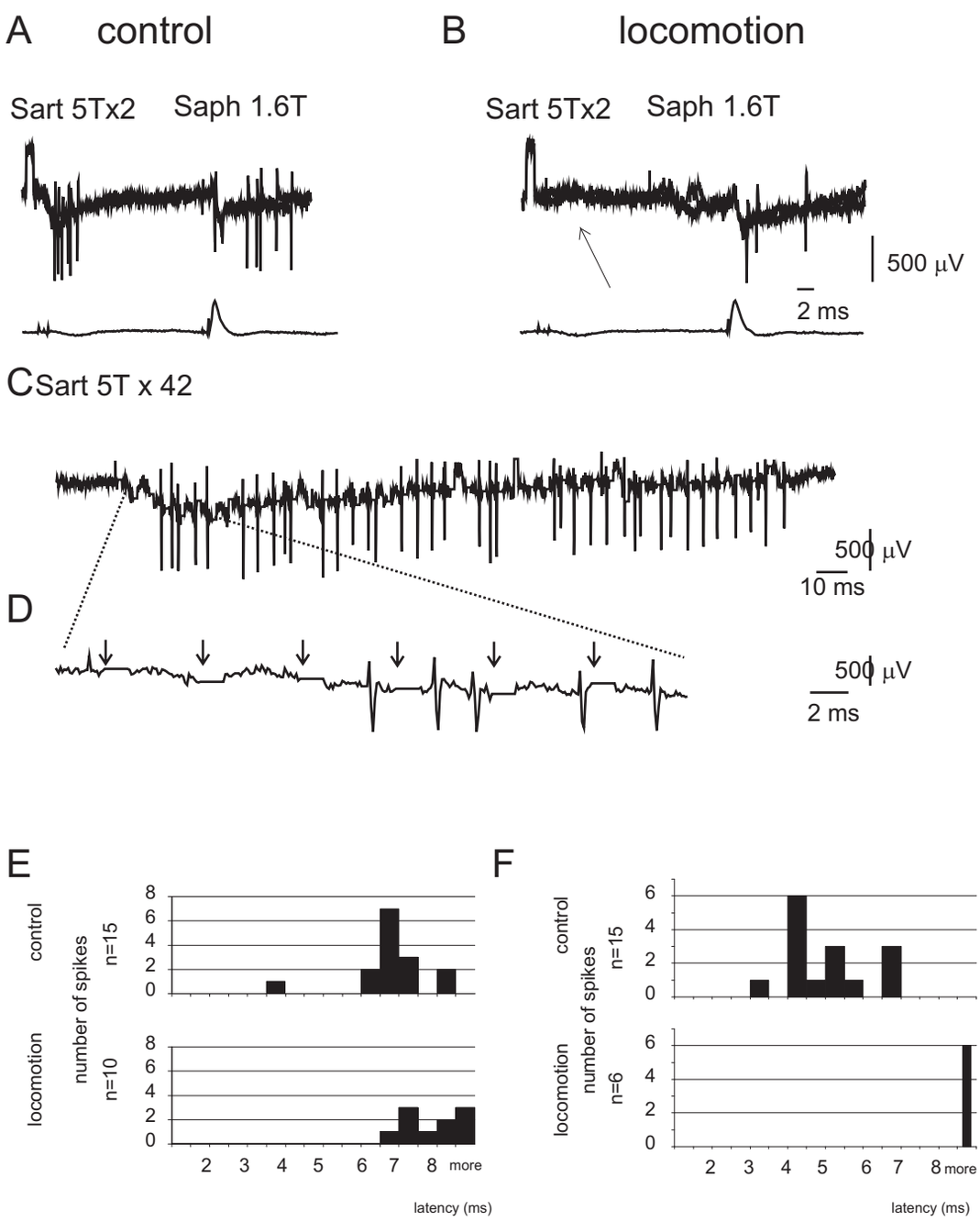
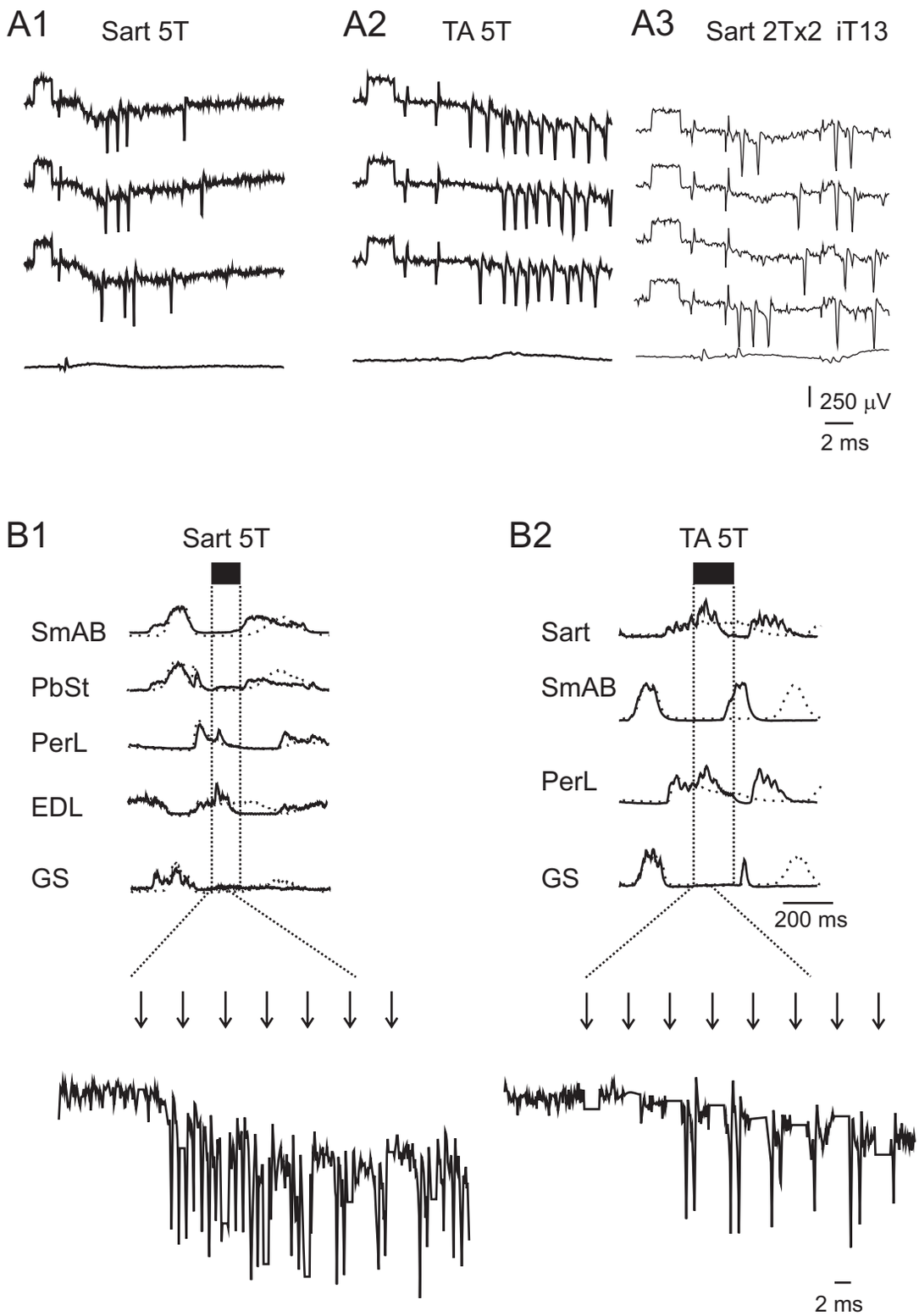

A control
B locomotion
Sart 5Tx2
Saph 1.6T
Sart 5Tx2
Saph 1.6T
500 μV
2 ms
C Sart 5T x 42
500 μV
10 ms
D
500 μV
2 ms
E
F
control
locomotion
number of spikes
n=15
n=10
n=6
latency (ms)
more

Figure 4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Sart 5T
A2
TA 5T
A3
Sart 2Tx2 iT13
250 μV
2 ms
B1
Sart 5T
SmAB
PbSt
PerL
EDL
GS
B2
TA 5T
Sart
SmAB
PerL
GS
200 ms
2 ms

Figure 5

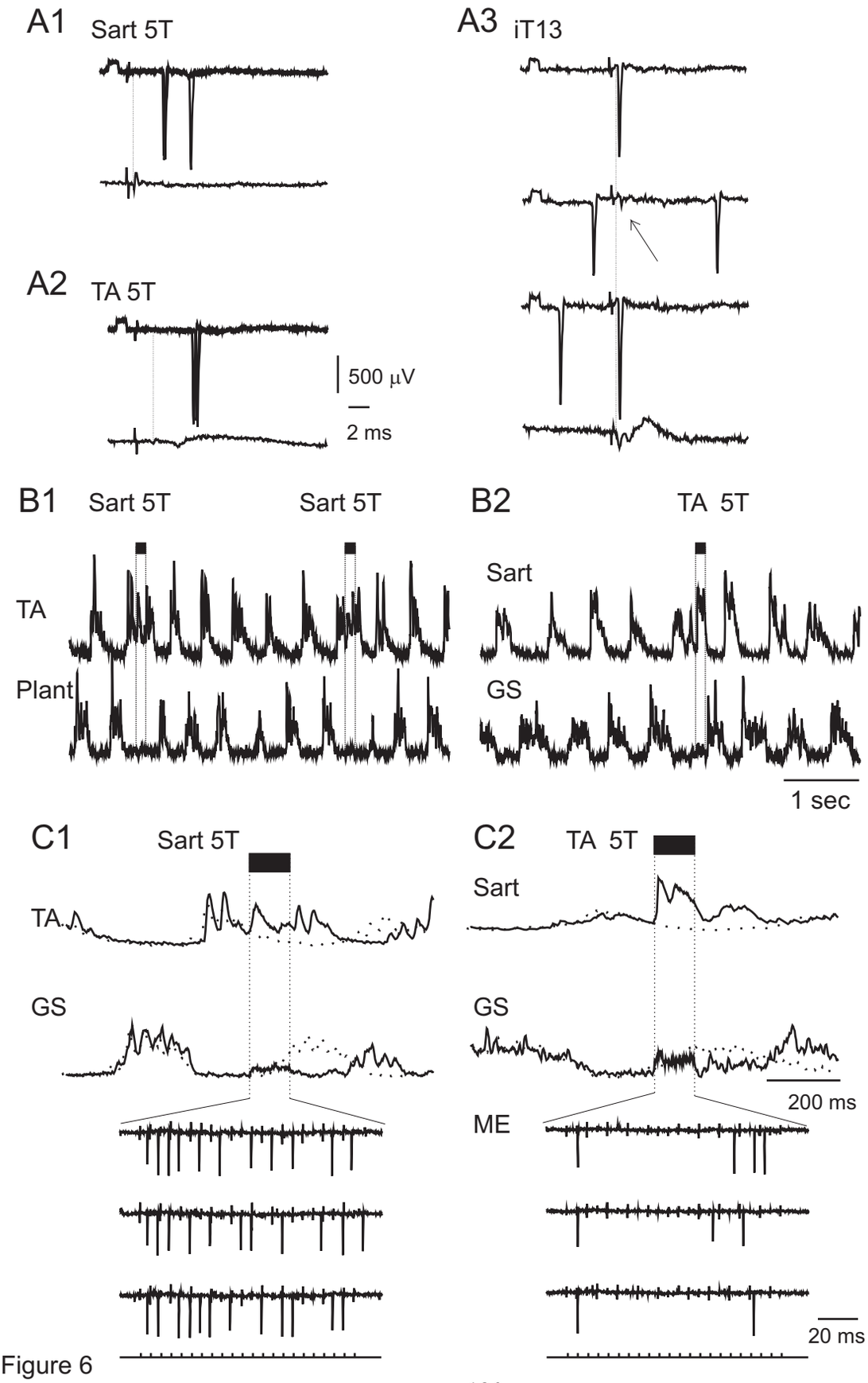

A1
Sart 5T
A2
TA 5T
500 μV
2 ms
A3
iT13
B1
Sart 5T
Sart 5T
TA
Plant
B2
TA 5T
Sart
GS
1 sec
C1
Sart 5T
TA
GS
C2
TA 5T
Sart
GS
200 ms
ME
20 ms

Figure 6

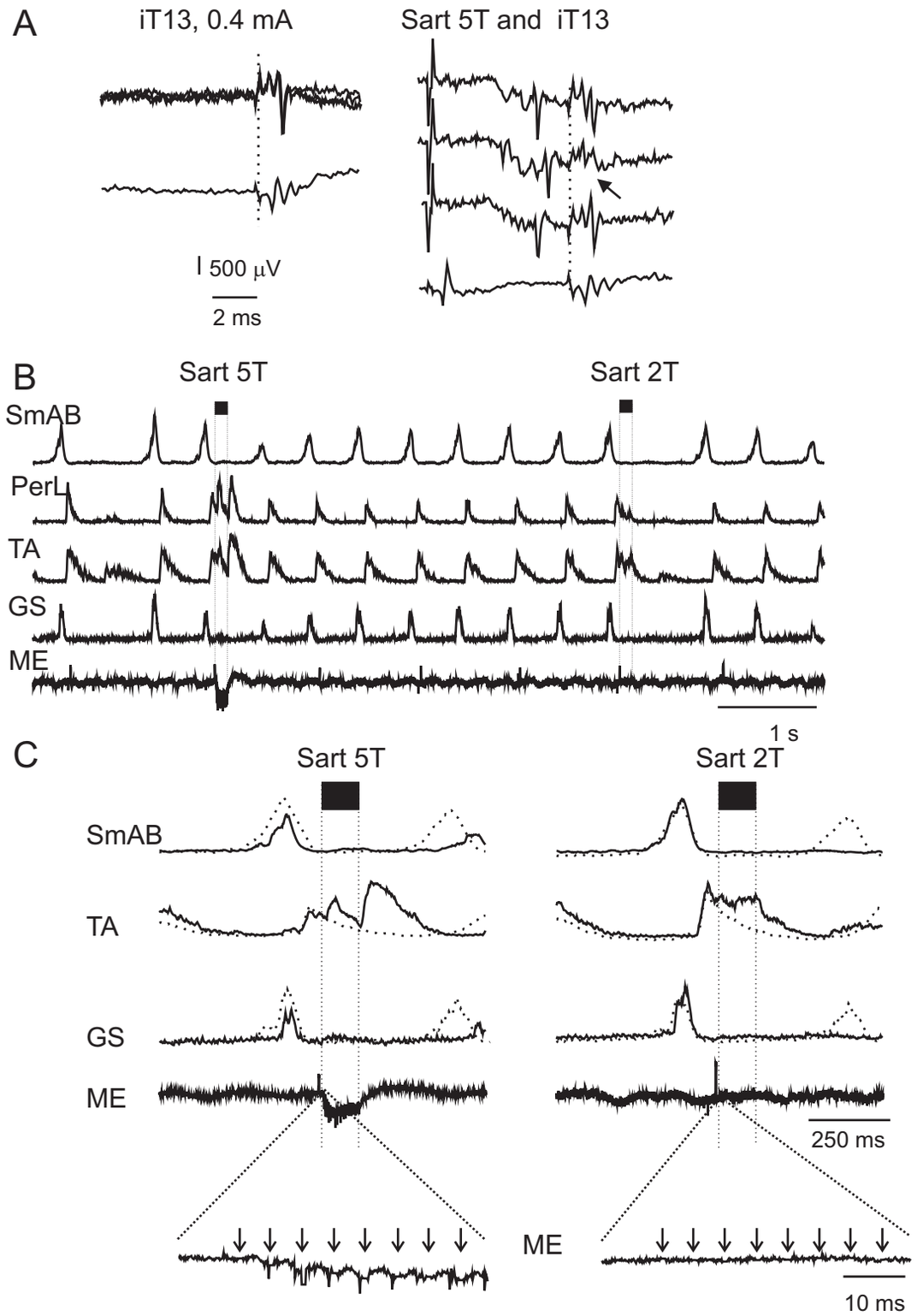
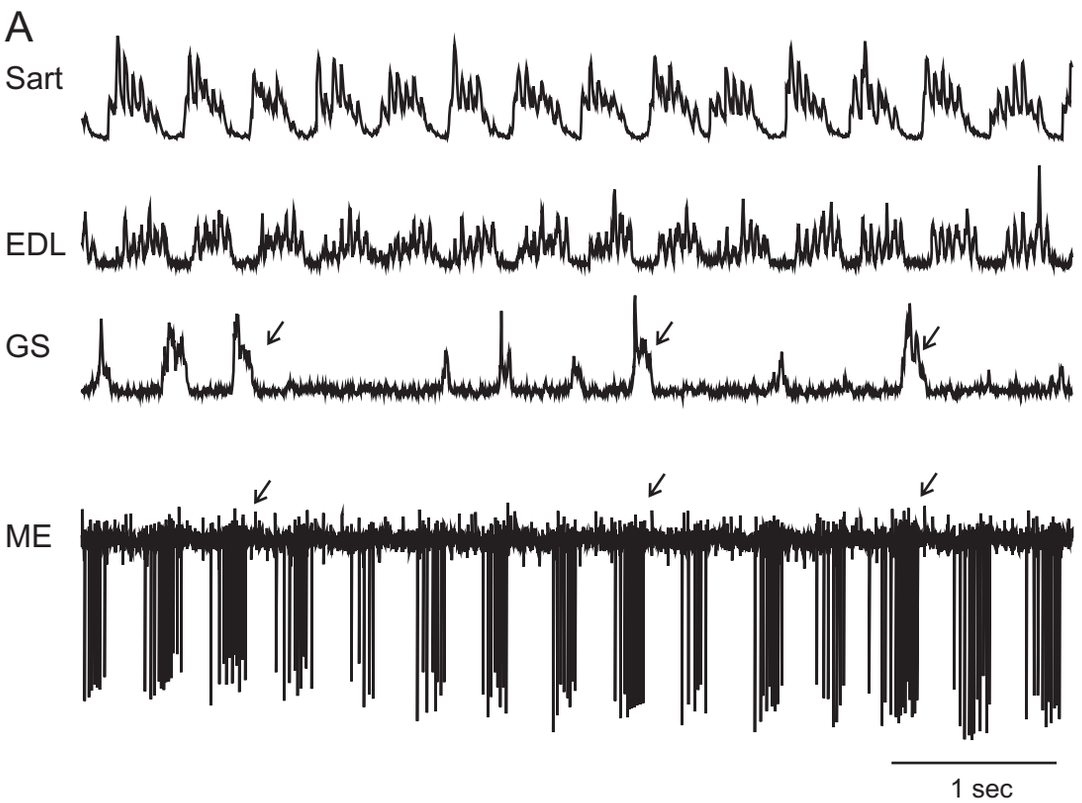
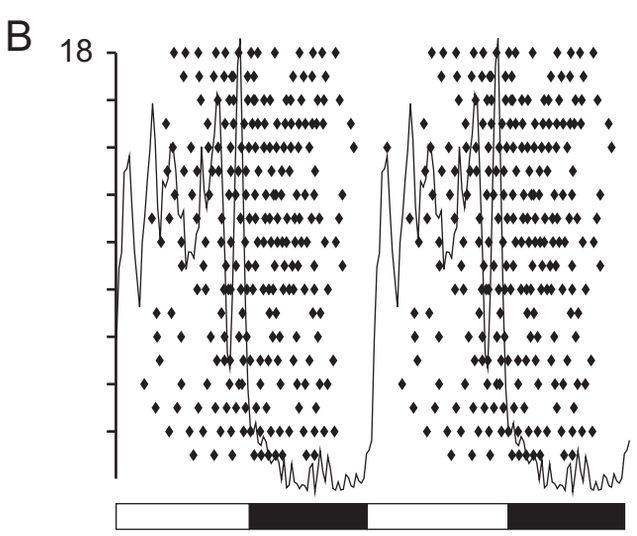

A
iT13, 0.4 mA
Sart 5T and iT13
500 μV
2 ms
B
Sart 5T
Sart 2T
SmAB
PerL
TA
GS
ME
1 s
C
Sart 5T
Sart 2T
SmAB
TA
GS
ME
250 ms
ME
10 ms

Figure 7





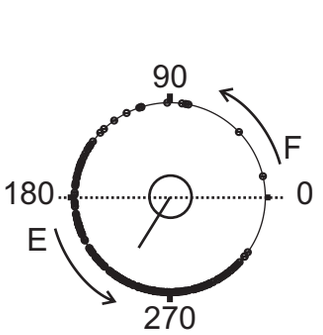


Figure 8

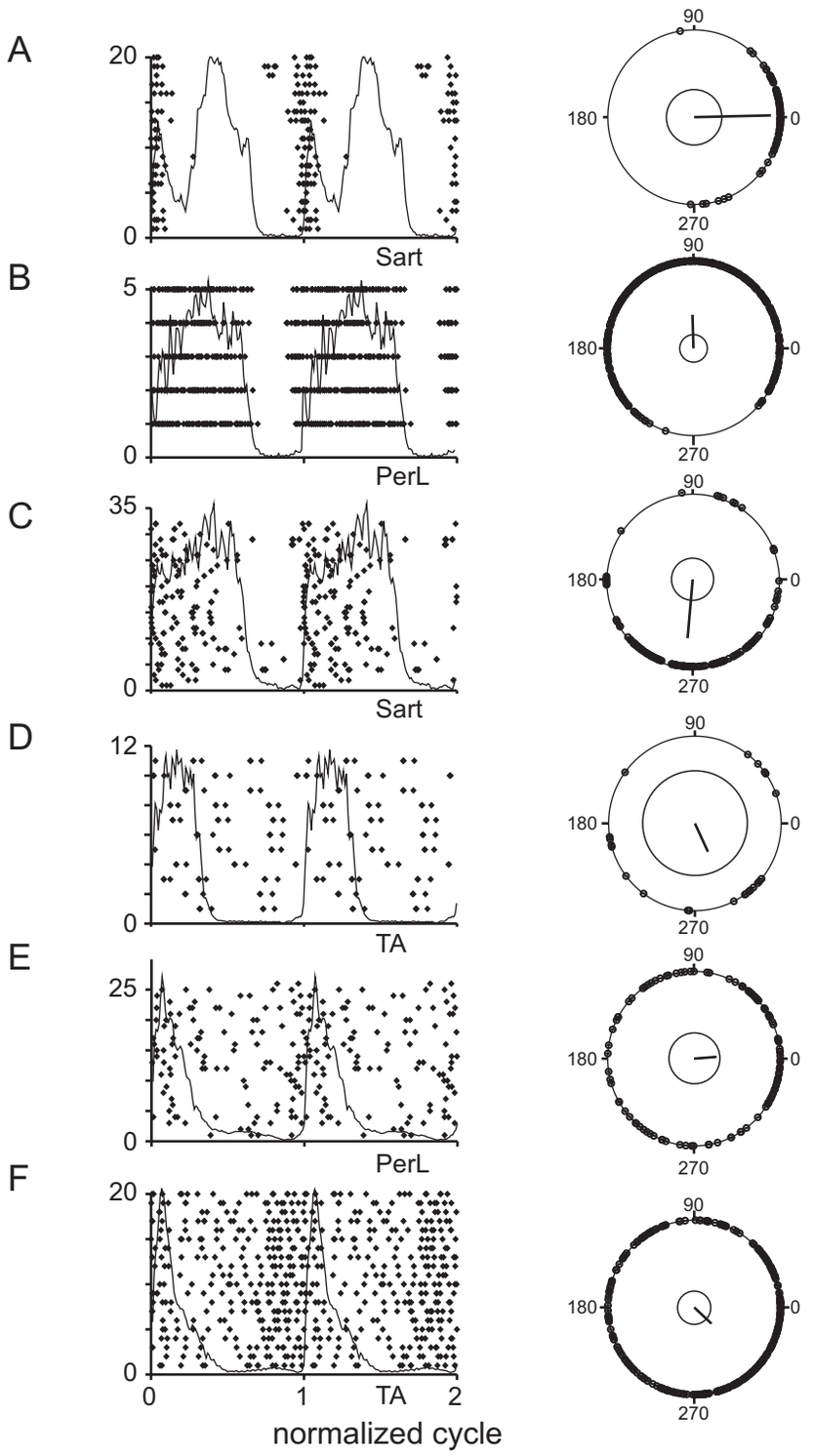

A
20
0
Sart
90
180
0
270
B
5
0
PerL
C
35
0
Sart
D
12
0
TA
E
25
0
PerL
F
20
0
0
1
TA
2
normalized cycle

Figure 9

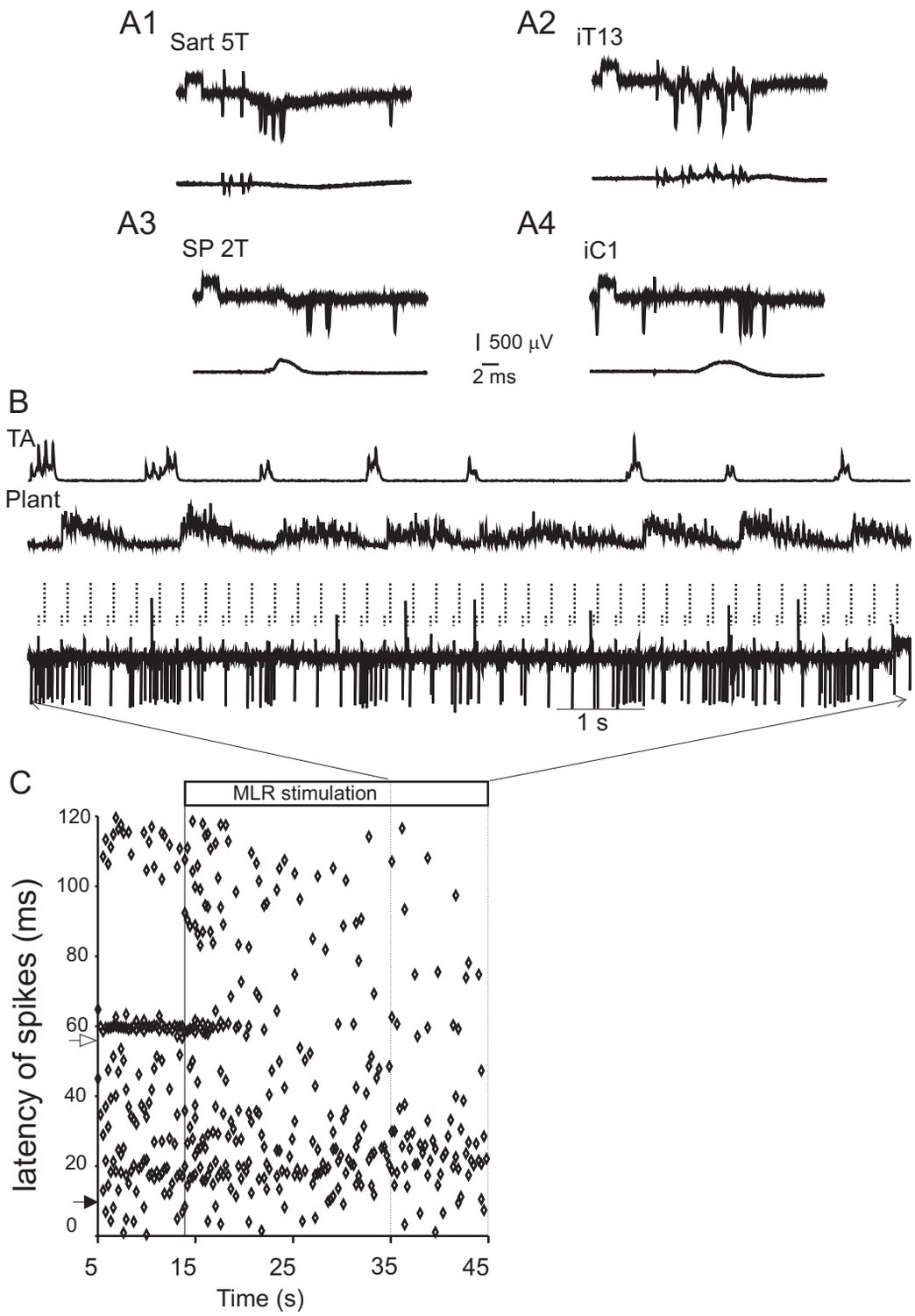

A1
Sart 5T
A2
iT13
A3
SP 2T
A4
iC1
500 μV
2 ms
B
TA
Plant
1 s
C
MLR stimulation
latency of spikes (ms)
120
100
80
60
40
20
0
5
15
25
35
45
Time (s)

Figure 10

General Discussion

1. Overview of results

It has been previously shown that rhythmic motor activity evoked by midbrain stimulation in decerebrate cats results in a centrally-generated, partly presynaptic suppression of transmission from myelinated cutaneous and muscle hindlimb afferents (reviewed in Rossignol et al. 1998, McCrea and Perreault 1998). Studies reported in Paper 1 examined whether this suppression exerts a preferential control of transmission from muscle group II afferents in regions of the spinal cord receiving input from both group II and cutaneous afferents. Studies presented in Paper 2, further elucidate the functional role or consequence of this centrally-generated suppression of transmission from cutaneous and muscle hindlimb afferents by showed that the suppression of group II afferent input during fictive locomotion results in a powerful reduction of the neuronal recruitment by short trains of stimuli. Overall, during fictive locomotion the field potentials evoked by muscle group II afferents were suppressed more (to 51% of control) than those evoked by cutaneous afferents (to 67% of control). A similar preferential suppression of muscle group II afferent input was also observed during fictive scratch (69% vs. 87%). Comparison of field potential suppression during fictive locomotion and scratch revealed a similar but less pronounced depression during scratch. Thus one of the main conclusions of the studies of this thesis is that electrical stimulation of the brainstem may contribute to, but is not entirely responsible for fictive motor activity-related field potential depression (see also Discussions in Perreault et al. 1999; Gosgnach 2003). Field potentials evoked by group II afferents from different muscle nerves were suppressed non-uniformly during both fictive locomotion and scratch.

Action potentials evoked by stimulation of group II muscle afferents with 1-3 stimuli were reduced in 28/30 and in 7/8 neurones examined during fictive locomotion and during fictive scratch, respectively. These results suggest that the suppression of group II afferent input during fictive locomotion results in a powerful reduction of the neuronal recruitment by short trains of stimuli. There was more reduction of firing evoked by muscle group II afferents in 13/28 cases and in 3/7 cases, similar to the preferential reduction of field potentials evoked by group II afferents. Despite this

suppression, the longer duration (typically 22-52 shocks at 200 Hz) stimulus trains to flexor muscle group II fibres produced strong reflex actions during fictive locomotion and were able to control cycle and phase transitions. The recruitment of 12/18 neurones with long stimulus trains remained possible during locomotor activity and the majority of these neurones (7/12) also showed rhythmic spontaneous activity.

Interestingly and unexpectedly, axonal projections to more rostral segments of the spinal cord or to supraspinal areas were evident in 4 of these 7 cells, hence they were considered as long projecting neurones. Additionally, 2 other group II activated propriospinal neurones projecting to segments between T13 and C1 also showed rhythmic spontaneous activity. These neurones with both, sensory-evoked and spontaneous activity during fictive locomotion are considered as candidates for being the neuronal substrates of muscle group II afferent reflex pathways functioning during locomotion in decerebrate cats.

2. What have we learned by evoking two types of fictive behaviours - locomotion and scratch - during the studies of centrally-evoked modulation of sensor input?

This thesis examined how the activity of spinal neurones is influenced by mechanisms related to the operation of the central patter generator (CPG). The results reported in this thesis provide further evidence for several similarities that occur during these two behaviours- at least fictively, that alter the transmission of synaptic input from specific peripheral afferents. As described in the Introduction of the thesis, specific brainstem areas evoking locomotion in decerebrate cats can also suppress muscle group II afferent input in anaesthetized cats in the absence of locomotion. However, it was unclear that how much to this suppression of the mesencephalic locomotor region contributes, if at all. Therefore, fictive scratch behaviour was used in some of the experiments to further examine the factors involved in the modulation of afferent transmission from muscle group II afferents in the absence of brainstem stimulation.

There are indications that the spinal circuitry generating scratch and locomotion seems to share some common elements in spite of a few differences between these behaviours (for more information on differences see Gelfand et al. 1998, Kuhta and

Smith 1990 and Barajon et al. 1992). All hindlimb muscles (except for peroneus longus) are active during the same phase in both behaviours (Gelfand et al. 1988, Lafreniere-Roula and McCrea 2005). There are lumbar interneurones with similar activity patterns during both behaviours (Bayev and Kostyuk 1981). There is a decrease of hindlimb motoneurone membrane resistance(Perreault 2002) and they seem to undergo similar rhythmic depolarizations and hyperpolarizations during both behaviours (Quevedo et al. 1998). Most importantly for the purpose of the studies presented in the thesis, the reflex actions of muscle group II afferents seem to be also similar during these two behaviours. For example, the stimulation of the TA and the EDL muscle nerves evokes prolongation of ongoing flexor activity during both fictive locomotion and scratch (Stecina et al. 2005).

Since electrical stimulation of the brainstem is not required to evoke fictive scratch, it seemed as a good model to examine whether presynaptic changes occur without direct electrical stimulation brainstem areas during alternating flexor and extensor motoneurone activity. The results presented in paper 1 and paper 2 provide further evidence that these two behaviours share even more common elements. The preferential and non-uniform reduction of muscle group II afferent activity was commonly observed during both behaviours and seemed to be more as a tonic rather a phasic suppression. There was evidence during both behaviours, albeit small in numbers, that mechanisms can be triggered during these motor activities that alter afferent transmission at presynaptic sites. Since these studies are the first to make such an extensive comparison of changes in afferent transmission from different types of fibres, it is difficult to comment on the actual mechanisms underlying the presynaptic modulation. Nevertheless, to understand these mechanisms could be an important step for not only further elucidating the control of sensory information during motor activity but also for disclosing the neuronal components of the circuits that contribute to patterned motor activity during these behaviours.

Although, the focus of the studies here was to examine the modulation of the input originating from muscle group II afferents and less attention was paid to the modulation of afferent input from cutaneous fibres during fictive scratch when long

stimulus trains are used, it is important to continue the investigations on how cutaneous input is modulated during fictive scratch. Although, there is limited evidence on the reflex actions of cutaneous afferents during fictive scratch, they are still considered as afferents with powerful modulatory abilities to modify a given motor output.

There is one ultimate questions that has asked numerous times from us and other who have utilized both fictive locomotion and scratch: is the central pattern generator shared? To answer this most popular question would need to begin with the definition of the central pattern generator. That is an impossible task nearly on its own. Depending on one's world view, a CPG can be comprised of many elements: motoneurones, interneurones, afferent fibres, descending tracts just to mention a few. If we consider the "sort of bare minimum" –that is the motoneurones and the interneurones as the CPG -for the sake of the discussion here, then perhaps some conclusions can be drawn from my thesis work. One conclusion is that interneurones with input from group II muscle afferents are likely to show similar modulation of activity when going from rest to locomotion or to scratch. Another conclusion could be that neurones that evoke presynaptic modulation of muscle group II afferent activity (based on the assumption that it is evoked by neurones and not descending tracts) would also be likely to show similar tonic activity throughout each behaviours. But whether these neurones are actually a component of the CPG or whether their activity is necessary for evoking alternating flexor and extensor motoneurone firing cannot be concluded from the results presented here. One strong indication in support of the arguments that they are part of it is that many of them have rhythmic spontaneous activity that could be the result of the "drive" from the CPG.

Earlier studies that compared the centrally-evoked presynaptic inhibition of afferent input from other (group I) muscle afferents during fictive locomotion and scratch have also described similarities (Gosgnach 2003). For example, the reduction of muscle group I afferent input seemed to be non-uniform based on the source during both behaviours. However, studies that have examined sensory-evoked modulation of transmission in pathways from group I muscle afferents during fictive locomotion and scratch have reported major differences (Cote and Gossard 2001, 2003). For example, the

sensory-evoked depolarization of primary afferents seemed to be much larger during fictive locomotion than during scratch (Cote and Gossard 2003). Although, more studies are needed to clearly understand why such differences can occur between sensory-evoked and centrally-evoked modulation of afferent transmission, but in light of these few reports one may draw the conclusion that there is a fundamental difference in the operation of these two main types of modulation. Perhaps a comparative study of sensory-evoked and centrally-evoked modulation of the activity of the candidate interneurones involved in mediating muscle group II afferent input during fictive locomotion described in this thesis could be a useful study while utilizing these two behaviours again in order to further understand what is and what is not a part of "central pattern generation".

In summary, from using two types of behaviours in the studies reported in my thesis, it can be concluded that the centrally-evoked suppression of input from muscle group II afferents is similarly modulated during these two behaviours. Whether the mechanisms that evoke the suppression of the examined sensory input are part of the activity required for the generation of patterned motor activity in *sensu stricto* remains unknown, but a likely possibility.

(I thank Susan Shefchyk and Brent Fedirchuk for encouraging me to include this entire section in my general discussion and for helping me to understand my work in a much broader perspective than presented before.)

On the centrally-evoked presynaptic inhibition of muscle group II input

The modulation of the non-monosynaptic component of the field potentials and the changes in firing of spikes evoked at longer than monosynaptic latencies (14/17 neurones during fictive locomotion) suggest that the suppression of the group II input could occur at postsynaptic levels. However, the modulation of the monosynaptic component of the field potentials and the changes in firing of spikes evoked at monosynaptic latencies in a subset of the results presented in paper 1 and 2 suggest that presynaptic mechanisms are also involved in modulating sensory transmission. In general, the sensory-evoked presynaptic inhibition of muscle and cutaneous afferents has

been examined in more detail than the centrally-evoked presynaptic inhibition. Sensory-evoked presynaptic inhibition has been studied mainly in anaesthetized preparations in the absence of motor activity. It is commonly accepted that primary afferent depolarization (PAD) is related to presynaptic control mechanisms in action (reviewed in Schmidt 1971). The sensory-evoked suppression of group II field potentials was found to be associated with both PAD of group II fibres and a reduction of the monosynaptic EPSPs evoked by group II afferents in first order interneurones (Riddell et al. 1995).

Presynaptic GABAergic mechanisms are an important component of the modulation of the synaptic effectiveness of muscle and cutaneous afferents. Sensory-evoked presynaptic inhibition of transmission from cutaneous afferents is related to the activation of spinal GABAergic neurones (Jimenez et al. 1987). Axo-axonic contacts in the cat spinal cord from low-threshold cutaneous afferents onto other, large cutaneous fibres were shown to be immunoreactive to $GABA_A$ and are likely to play a role in presynaptic inhibition of cutaneous input (reviewed in Rudomin and Schmidt 1999). Identified group II muscle afferents of the gastrocnemius and semitendinosus muscles have been found to have terminals presynaptic to another single group II axon terminal containing GABA (Maxwell and Riddell 1999). These observations support the idea that GABAergic interneurones contribute to PAD of group II afferents. There is very limited information on the PAD interneurones that contact group II afferents. They are likely to be located in the dorsal horn of midlumbar segments and they are likely to be activated by several different segmental afferents and descending pathways (Jankowska and Riddell 1995, Carpenter et al. 1963).

A considerable proportion of the presynaptic boutons of group II axons examined were found to be components of synaptic triads with a bouton being presynaptic to another group II axon and a dendrite that is postsynaptic to the same group II axon (Maxwell and Riddell 1999). Thus group II PAD interneurones have both presynaptic and postsynaptic inhibitory actions on spinal neurones. Common interneurones mediating presynaptic and postsynaptic inhibition in group I afferent pathways in the feline lumbar spinal cord have been functionally identified previously (Solodkin et al. 1984). For group II afferent pathways, the existence of such neurones is a strong possibility. Future studies

should examine the activity of these 'dual-action' PAD interneurones contacting group II muscle afferents during centrally-evoked motor activity to gain more information about their role in controlling transmission from group II afferents.

Recent electrophysiological studies revealed that the distribution of $GABA_A$ synapses in the intraspinal arborization of muscle spindle and tendon organ afferents is not homogeneous (reviewed in Rudomin 1999). Some afferent collaterals are the targets of one, or more GABAergic interneurone, while other collaterals of the same fibre receive no GABAergic connections. This could allow for de-synchronisation of the information arising from common sensory inputs. Such spatially restricted modulation of PAD could play a significant role in the adjustment of the synaptic effectiveness of muscle afferents during centrally-generated motor activity. Changes in the synchronization of the PAD-mediating interneurones could also have a profound effect on the information transmitted by a given set of afferent fibres (see review by Rudomin 1999). If such evidence could be provided regarding group II muscle afferents, then the variations of group II reflexes evoked during fictive locomotion could be attributed, in part, to variations in the activity of a population of GABAergic neurones. Fluctuations of group II reflexes by means of pre- and postsynaptic mechanisms could represent a fine balance established on a step-by-step basis.

There is strong convergence of muscle and cutaneous input to spinal neurones that are involved in evoking suppression of group I afferent input during fictive locomotion (Menard et al. 2002). Is there a possibility that the same group of spinal neurones mediates presynaptic inhibition of group II muscle afferent and cutaneous input during locomotion? There is evidence that the same pool of interneurones control transmission from perineal cutaneous (innervating the urethra, the sexual organs and the skin covering the perineum and scrotum) and muscle group II afferents during micturition (Angel et al. 1994). During fictive locomotion and scratch, further experiments are necessary to support the existence of common interneurones in regulating transmission from cutaneous and muscle group II afferents. The examples of increases in cutaneous and decreases in group II fields recorded in the same location reported here (e.g. figure 4 in paper 1 and figure 2 in paper 2) argue against this

possibility.

Based on several lines of indirect evidence, PAD alone may not be directly related to the reduction of transmission from muscle and cutaneous afferents during motor activity. One line of evidence comes from studies showing that the excitability changes of group I afferents are poorly and sometimes inversely related to field potential reductions during fictive locomotion and scratch (Gosgnach 2003). Studies in awake primates during a voluntary reaching-task by Seki and colleagues (2003) have shown that cutaneous input to spinal interneurones is inhibited by descending commands during active wrist movements but this descending-evoked presynaptic inhibition seemed to be greater than what was expected based on the excitability changes of cutaneous afferents. These observations suggest caution about making a direct association between PAD and presynaptic inhibition. The extent to which the locomotor-related excitability changes of group II muscle afferents contribute to the reduction of monosynaptic field potentials has to await further studies. It is difficult to assess the excitability of group II muscle afferents compared to group I or cutaneous fibres, but Riddell and colleagues (1995) have succeeded to assess the sensory-evoked excitability changes of identified single group II fibres using electrical stimulation of muscle nerves by utilizing the Wall technique (see Wall 1958). Repeating similar studies during fictive locomotion and/or scratch while simultaneously assessing the field potential changes would provide further insights into the extent to which PAD contributes to reduced synaptic efficacy from these afferents during fictive motor activity.

Several pharmacological agents can also modify transmission from muscle and low-threshold cutaneous fibres. The opioid, D-Ser-Leu-enkephalin-Thr (DSLET), reduces transmission from group II afferents of Quad and deep peroneal muscles and from low threshold cutaneous afferents of Saph and Sur nerves, but it does not reduce transmission from group Ia and Ib afferents (Jankowska and Schomburg 1998). Baclofen, a selective $GABA_B$ agonist, when applied onto spinal neurones of anaesthetised cats, selectively reduces monosynaptic excitation evoked by low threshold cutaneous afferents but does not effect the polysynaptic excitation evoked by higher threshold afferents (Curtis et al. 1981). L-DOPA (a monoaminergic precursor), and clonidine (an alpha-2

agonist) reduces transmission in group II muscle afferent pathways from PbSt, LGS, FDHL and SmAB muscles more than in cutaneous pathways from the Sur nerve in high spinal cats (Schomburg and Steffens 1988). Monoaminergic control of muscle group II input during sensory-evoked presynaptic inhibition has also been described to some extent (see Introduction for more details). It is difficult to comment on which of the above discussed pharmacological agents may play a role in the centrally-evoked reduction of group II afferent transmission. Relatively simple studies could be done in the future to gain further insights about this question. For example, different monoaminergic agonists could be applied to counter the centrally-evoked reduction of muscle group II-evoked field potentials during fictive motor activity.

The amplitude of some cutaneous afferent-evoked field potentials was increased instead of being reduced during fictive scratch. Increased amplitude of sensory-evoked field potentials could reflect decreased excitability of afferent fibres by a process termed "primary afferent hyperpolarization" (PAH) (Mendell 1972). The decreased excitability of afferents is thought to be related to a reduction in the activity of spinal neurones that cause PAD (Mendell 1972). Accordingly, the locomotor-related inhibition of PAD interneurones tonically active during rest would result in increased synaptic transmission during locomotion. Direct measurement of afferent excitability have shown that there can be a PAH of group I afferents during fictive locomotion (Baev and Kostyuk 1981; Gosgnach 2003) and of pudendal afferents during micturition (Angel et al. 1994). However, as for the role of PAD in decreased presynaptic synaptic transmitter release during locomotion, it is quite possible that increases in field potential amplitude during locomotion reflect increased transmitter release by mechanisms not associated with PAH.

Positive spatial facilitation from both muscle and cutaneous input or sensory and CPG input could also result in increased firing of neuronal activity, which is one plausible explanation for the increased firing seen when longer than monosynaptically evoked actions were facilitated. This could relate to the functional role of specific afferents, for example in an analogous way as it has been described by studies examining the modulation of urethral afferent excitability (Buss and Shefchyk 1999). Since urethral afferents facilitate bladder reflexes as well as evoke sphincter reflexes, the variations of

their excitability (i.e. some with PAD while others with PAH) has been thought to be related to the subgroups of which they belong--see Discussion in paper 1 for more detail. *(I thank Susan Shefchyk for recommending this possibility to be included in the discussion)*.

Given the above discussion on some of the known or hypothesized mechanisms regulating afferent transmission from muscle and cutaneous fibres during fictive locomotion, I hope to have made it clear that multiple control mechanisms exist. Some of these mechanisms could also involve alterations in second messenger pathways at the presynaptic terminals. The possibility that second messenger systems are activated through $GABA_B$ receptors and result in the depression of group I synaptic transmission during fictive locomotion has been raised (see more in McCrea 2001, Gosgnach et al. 2000, Gosgnach 2003). There is, however, little direct evidence about the state-dependent operation of such mechanisms during quiescent conditions or during motor output. Any variability of effectiveness and of time course of these multiple mechanisms could explain the differences seen between the tonic suppression of EPSPs and field potentials and phasic (cycle dependent) variations in their amplitudes.

It is also important to emphasize that much of the available evidence behind our current understanding of the mechanisms described above comes from observations in anaesthetized preparations in the absence of motor activity. Thus in many ways we must break new ground and rethink how state-dependent suppression of muscle group II afferent transmission during fictive locomotion fits into the larger scheme of motor control. The present studies have probably raised more questions than those that have been answered. It is clear, that further insight into the central control of synaptic transmission from sensory afferents requires examining these systems during coordinated and rhythmic motor activity. In this regard, the decerebrate cat preparation has tremendous advantages over more reduced preparations. With more detailed information about the centrally-evoked control of muscle group II afferent reflexes, we may be able to design selective pharmacological therapies to optimize sensory-evoked reflexes during movements for example in humans with spastic conditions.

4. On presynaptic inhibition during fictive locomotion and scratch

Both fictive locomotion and scratch produce similar membrane potential oscillations in dorsal roots (Cote and Gossard et al. 2003). Both behaviours evoke similar reductions in muscle group I field potentials and monosynaptic EPSPs recorded in motoneurones (Gosgnach 2003). Potential differences between presynaptic control of afferent transmission during fictive locomotion and scratch have also been described. The field potential measurements in paper 1 show that transmission from muscle and cutaneous afferents during fictive scratch is less depressed than during fictive locomotion (i.e that there is a larger centrally-evoked presynaptic inhibition during fictive locomotion). In accord with this observation, rhythmic bursts of antidromic firing in primary afferents (i.e. centrally-evoked suprathreshold depolarization of sensory afferent terminals) are common during fictive locomotion but rare during fictive scratch (Cote and Gossard 2001). As determined by potentials recorded in lumbar dorsal roots following peripheral nerve stimulation, there also appears to be a greater sensory-evoked presynaptic inhibition during fictive locomotion than during scratch (Cote and Gossard 2003). There is phasic modulation of transmission in disynaptic pathways from SP afferents to ankle flexor motoneurones during scratch, but these reflexes are strongly depressed and not phasically modulated during fictive locomotion (reviewed in Burke 1999). It is unknown, however, whether the strong reduction of transmission from SP afferents to ankle flexor motoneurones during scratch is related to pre- or postsynaptic mechanisms. Cote and Gossard (2003) concluded that the larger PAD during fictive locomotion made sense since stepping movements are more likely to be perturbed by obstacles than the scratching movements and these perturbations may have more severe consequences during stepping than during scratch. The results presented in the thesis are in line with this suggestion.

5. On propriospinal neurones

In this thesis, neurones were considered as propriospinal if they were antidromically activated from the T13 segment but not from the C1 segment. In cases where antidromic activation was not tested by stimulating at the C1 segment, or a cell

was antidromically activated by C1 stimulation, neurones were considered as a long projecting cells. We tested only for ascending projections and descending ones were not assessed. In previous studies, group II activated midlumbar interneurones projecting caudally to motoneurones were also referred to as 'propriospinal' cells (Edgley and Jankowska1987c). This may lead to some confusion when trying to compare the results presented here with previous findings. Also, the neurones described here as propriospinal or long projecting group II activated cells may overlap with previously described populations in anaesthetized, intact, non-locomoting cats. These populations include 1) ascending tract cells originating in the lumbosacral region described by Harrison and Riddell (1990) that are likely to terminate in the brainstem, 2) dorsal spinocerebellar tract cells originating mainly from the midlumbar segments (Edgley and Jankowska 1988, Edgley and Gallimore 1988), or 3) spinocervical tract cells (Hammar et al. 1994, Harrison and Jankowska 1984). The ascending SCT cells also have axon collaterals that affect lumbar interneurones (Djouhri & Jankowska 1998) suggesting a specialized role for these cells in coordination of sensory and centrally-evoked inputs at different segmental levels.

Based on an examination of forelimb reflexes, a strong control of forelimb flexors was linked to the activity of ascending propriospinal systems (reviewed in Miller and van der Burg 1973). This strong control of forelimb flexors is also evident in high spinal cats (Miller et al. 1973). Group II and III muscle and cutaneous afferents of the hindlimbs were shown to be effective in the facilitation of reflex actions of forelimb segmental afferents in motoneurones innervating the forelimbs (reviewed in Miller and van den Burg 1973). It is not clear which group II activated long projecting or propriospinal neurones may be involved in ascending propriospinal pathways allowing for communication between forelimbs, trunk and hindlimbs, but those that are active during fictive locomotion and respond to muscle sensory and cutaneous input are good candidates. Propriospinal neurones have been described to be rhythmically active also in turtles during fictive scratch (Berkowitz and Stein 1994). In quadrupeds, galloping involves both hindlimbs moving together and more less in phase with back muscles contracting in order to improve the forward thrust of the body (see Miller and van der

Burg 1973). Input from muscle group II afferents via ascending propriospinal pathways could be one component of the factors coordinating back and trunk muscles as well as the forelimbs with hindlimb activity during galloping. Since activity in group II afferents (muscle spindle secondaries) is likely a critical component in signalling current limb position and controlling phase transition, these cells could play two important roles: 1) their cycle-related rhythmic activity can inform the cervical cord about hindlimb flexor and extensor phase durations, and 2) their ability to follow flexor nerve group II activity suggests that sensory-evoked changes in their activity could be used to coordinate front and hindlimb CPG activities.

These propriospinal cells with group II input remain poorly described. For example, we don't know if they also project to targets within the lumbar cord, or the full complement of their synaptic input, or how well their activity reflects reflex-evoked changes in the timing within the hindlimb CPG. Future studies need to assess their 1) contra and ipsilateral afferent inputs 2) axonal projections to regions within the cervical cord 3) potential projections above the cervical cord and 4) changes in their activity during sensory-evoked step-cycle perturbations. It may be difficult to assign a critical role for these propriospinal neurones in interlimb CPG coordination in decerebrate cats since the contribution of other systems cannot be unequivocally ruled out. However, it is important to assess whether this system has the potential to fulfill this role. Until more of the properties of this ascending propriospinal system are known, it will be impossible to address the general hypothesis about how group II (and other) inputs control interlimb coordination.

6. Limitations of the work presented in the thesis

When longer stimulus trains "brake through" the suppression and evoke firing as shown in 2 neurones during fictive locomotion (see fig 5 in paper 2) no analysis has been done in these series of experiments to correlate the train duration required to break through the inhibition or to be effective in perturbing the locomotor step cycle. This type of analysis would include the measurement of the latency of responses evoked by each stimulus presentation in a train. Although, this can be done in future experiments or even

with the data set presented in the thesis, it should be interpreted with caution because spikes could be evoked in relation to the first shock with longer latency rather than the second shock with short latency. *(I thank Steve Edgley for pointing out these limitations.)*

Different muscles have different sizes and different numbers of afferent spindle axons in different categories (see Introduction). Therefore, electrical stimulation will activate a different number of fibres in the group II range depending on the muscle of origin. This could result in muscles with greater number of group II afferent fibres having greater impact on spinal neurones compared to those muscles with smaller numbers of group II afferent axons if the synaptic efficacy is equal. By using a measure as percent of control levels of field potentials, in theory, differences between muscle afferents as a result of varying number of afferent fibres in the group II range should have been prevented from influencing the results. However, the number of group II afferents activated in two different nerves (i.e. TA and EDL) compared has more important implications when it comes to assessing the number of action potentials evoked in spinal neurones. For example, if more group II afferents were stimulated in the TA nerve than in the EDL and the neurone showed a partial reduction of firing evoked by TA but a complete reduction of firing evoked by EDL, would the neurone had also only a partial reduction in firing if there were the same number of afferent fibres activated in the two nerves? There were no attempts that could be made for normalizing the number of group II afferents in the peripheral nerves that were stimulated. *(I thank Tony Szturm for suggesting to address these issues in my general discussion).*

Summary and concluding remarks

Group II muscle afferents evoke flexion reflexes in anesthetized spinal animals. There is a general suppression of such flexion reflexes in decerebrate preparations even in the absence of locomotion (Perreault et al. 1999). One mechanism for the suppression of flexion reflexes may be a tonic presynaptic inhibition of muscle group II afferents by intact descending systems (Noga et al. 1995). In addition, there seems to be an additional level of suppression present during fictive locomotion. This state-dependent reduction of group II-related flexion reflexes during midbrain-stimulation-evoked fictive locomotion

is associated with the reduction of monosynaptic field potentials evoked by these afferents (Perreault et al. 1999). During fictive locomotion (Perreault et al. 1999) and fictive scratch (see results in paper 1), there is a strong depression of transmission from group II muscle spindle afferents in dorsal and intermediate spinal laminae throughout the lumbar segments. The presence of this depression during fictive scratch indicates that it is not simply the result of direct electrical stimulation of the MLR.

Actions of group II afferents in spinal, anaesthetized cats can spontaneously reverse in sign and evoke excitation of ipsilateral extensors instead of inhibition (Eccles and Lundberg 1959, for a review see Baldissera et al. 1981). Such reversals are partly controlled by descending spinal systems and suggest that there are parallel reflex pathways from muscle group II afferents (see Lundberg et al. 1987b,c). As the generation of rhythmic motor activity involves several descending systems (for a review see Jordan 1998), some group II reflex pathways may be released or inhibited with the activation of the central pattern generator. During fictive locomotion, stimulation of group II afferents in specific muscle nerves can prolong ongoing flexion or reset to extension (Perreault et al 1999; Stecina et al. 2005). We have also shown that there are parallel reflex pathways contacted by group II afferents in muscle nerves and that on occasion spontaneous reflex reversals from given muscle nerves can occur during fictive locomotion (Stecina et al. 2005). In a previous discussion we raised the theoretical possibility that a selective presynaptic inhibition of particular group II afferents might be involved in controlling the selection between parallel (alternate) reflex pathways (Stecina et al. 2005). The present results (paper 1) add direct support to this possibility by showing that there is often a non-uniform suppression of muscle group II input from different nerves to interneurones in circumscribed regions of the spinal cord. This suggests that the central organization of the mechanisms that control group II synaptic transmission is detailed enough to permit selective control of transmission from subsets of group II afferents. Selective central control of transmission from particular afferents could thus regulate the type of reflex evoked.

Previously, a group of caudally projecting mid-lumbar group II interneurones was examined during fictive locomotion (Shefchyk et al. 1990). Although, some of these

cells with direct projections to motoneurones were found to be rhythmically active during flexion, their sensory-evoked activity was suppressed. The results presented in the second paper show that in mid-lumbar segments there is also a reduction of firing evoked by short trains of stimuli delivered to flexor muscle afferents at 5T during fictive locomotion. The direct correlation between reduction in monosynaptic field potentials and neurone recruitment is an important observation. It gives the strongest evidence to date that there is a presynaptic component to control of transmission from group II afferents. It also supports drawing inferences about the functional consequences of changes in afferent transmission from the examination of field potentials.

Perhaps the most surprising observation to us was the demonstration in paper 2 that despite the suppression of the initial responses to trains of sensory stimuli, some neurones can be recruited by longer trains of muscle group II input during fictive locomotion. This suggests that central processes controlling sensory transmission may serve more to reduce the effects of low level afferent feedback (i.e. a few spikes from sensory afferents) than to gate out sensory feedback completely. Such a system would seem ideally suited to one in which disruption of the locomotor cycle by occasional afferent activity can be avoided. At the same time, prolonged sensory input during the locomotor cycle could still regulate cycle phase transitions.

It seems appropriate that long projecting and propriospinal neurones are effectively activated by trains of group II stimuli. Such ascending neurones may help regulate the interactions between lumbar proprioceptive activity concerning the length of hindlimb muscles and operation of the cervical CPG. The rhythmic activity of these cells during fictive locomotion in the absence of sensory feedback indicates that they also receive excitatory input from the CPG. They are thus ideally suited to inform cervical segments about the central generation of lumbar locomotion and the sensory consequences of these motor commands. Further studies should be aimed at identifying the projections of these neurones and their connectivity to other neurones in various segments of the spinal cord.

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