A spinal source for the synchronous fluctuations of bilateral monosynaptic reflexes in cats

E. Manjarrez¹, Z. Hernández-Paxtián¹ and A.F Kohn²
¹ Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla, 14 Sur 6301, Col. San Manuel. Apartado Postal 406. Puebla, Pue. CP 72570, México.
² Neuroscience Program and Biomedical Engineering Laboratory, Escola Politécnica, University of Sao Paulo, CEP 05424-970 Sao Paulo, SP, Brazil

Running title: Bilateral monosynaptic reflexes in cats

Corresponding Author:
Dr. Elías Manjarrez
Instituto de Fisiología
Benemérita Universidad Autónoma de Puebla.
14 sur 6301, Col. San Manuel
Apartado Postal 406, C.P. 72570
Puebla, Pue., México
Tels.: +5222-22-44-1657
Fax: +5222-22-33-4511
email: emanjar@siu.buap.mx

Acknowledgements:
This work was partly supported by the following grants: CONACyT J36062-N, VIEP-BUAP and P/PIFI 2001-22-FI-17-BUAP (EM), México.
Abstract
Successive stimuli of constant intensity applied to Ia afferents produce spinal monosynaptic reflexes (MSRs) of variable amplitude. We recorded simultaneous MSRs in the left and right L7 (or L6) ventral roots of anaesthetized cats. We analysed the cross-covariance between the amplitudes of bilateral MSRs. Long time series (5 to 8 hours) of these bilateral MSRs exhibited transitory changes in their covariations (as measured by the zero-lag peak of their cross-covariance), thus suggesting the existence of certain neural sources contributing to produce these changes. The aim of the present study was to show that spinal centers producing negative spontaneous cord dorsum potentials (nSCDPs) contribute to maintain correlations in the amplitude of bilateral MSRs. After spinal cord transection at the L1 segment, no significant changes were observed in the correlation between the amplitude of bilateral nSCDPs versus the amplitude of bilateral MSRs. However, this correlation, as well as the peak at zero lag in the cross-covariance between bilateral MSRs, and the cross-covariance between bilateral nSCDPs, respectively, were abolished after a subsequent longitudinal bisection at the L1-S2 spinal segments. These results suggest that lumbar spinal neurons (bilaterally interconnected) contribute to maintain the synchronous fluctuations of bilateral MSRs.

Key words: Bilateral monosynaptic reflexes, fluctuations, spontaneous cord dorsum potentials, dorsal horn, cross-correlation
INTRODUCTION

The central nervous system is continuously active, exhibiting apparently random activity from the neuronal membrane level up to the behavioral level. The extent of this activity depends on a great number of variables, which include the general state of alertness or level of anaesthesia of the animal. In laboratory experiments or in the clinic, the randomness associated with the CNS activity causes variations (fluctuation) in the size of successive evoked responses produced by stimuli of constant amplitude. In this context, an important question in the physiology of the spinal cord is: what are the sources of the fluctuations of spinal responses when the stimulus strength, stimulus position, and recording site are constant?

The observation that stretch reflexes are highly variable, was first reported by Sherrington (Sherrington 1906). Further work demonstrated that ipsilateral spinal monosynaptic reflexes (MSRs) produced by constant afferent stimuli exhibit considerable variations in size (Hunt 1955; Lloyd and McIntyre 1955; Somjen and Heath 1966). Rall and Hunt differentiated a linearly correlated from an uncorrelated component of this variability, showing that the firing probability of individual motoneurons within a population during MSR was only partly correlated with the population response amplitude (Rall and Hunt 1956). These authors suggested that postsynaptic background drive was the main source of both the correlated and uncorrelated fluctuations that they observed. In other studies, the origin of this variability has been attributed to excitability fluctuations within the motor pool, which are introduced either pre- and/or postsynaptically (Gossard et al. 1994; Rudomin and Dutton 1967, 1969a, b; Rudomin et al. 1969; Rudomin and Madrid 1972; Chang et al., 1994). Recently, Manjarrez et al. (2000) demonstrated that in cats there is a
high correlation between amplitude fluctuations of ipsilateral MSRs and amplitude fluctuations of ipsilateral negative spontaneous cord dorsum potentials (nSCDPs), thus suggesting that the main cause for the ipsilateral MSR fluctuations was the variable activity of ipsilateral dorsal horn neurons. All of these investigations employing conditioning by spontaneous potentials or by afferent volleys into the spinal cord have been restricted to ipsilateral actions. Furthermore, there is an absence of information about the possible interconnection between bilateral dorsal horn spinal neurons with spontaneous activity and their common influence on the amplitude of bilateral MSRs elicited by simultaneous trains of stimuli applied on ipsilateral and contralateral Ia muscle afferents. Some recent studies (Edgley et al., 2003; Butt and Kiehn, 2003), about the functional identification of neurons responsible for left-right coordination of hindlimbs, or the crossed reflexes from group II afferents, support this possibility.

The idea that bilateral spinal mechanisms can regulate movements was first introduced by Sherrington (Sherrington 1910), and the existence of a bilateral coordination in man is well documented (Swinnen and Duysens 2004). For example, during stance, unilateral displacements of one leg produce a bilateral response with similar latencies on both sides (Dietz et al. 1989). In other studies, alternate leg movement amplifies locomotor-like muscle activity in spinal cord injured persons (Kawashima et al., 2005).

The present work is a counterpart of experiments done in awake humans by (Mezzarane and Kohn 2002). They studied the correlation between H reflex amplitudes recorded bilaterally to simultaneous stimulation of the right and left tibial nerves. The experiments showed that for 50% of the subjects there was a statistically significant correlation between the H-reflex amplitudes recorded bilaterally. This finding indicates
that, in 50% of the cases, H-reflex amplitude variations tend to fluctuate synchronously in both legs. Mezzarane and Kohn (2002) also reported the case in which a subject changed from a nonflat (synchronous fluctuations) to a flat (absence of synchronous fluctuations) cross-covariance between bilateral H-reflexes on the second day of recording. However, because experiments in humans offer certain limitations, no experimental evidence was provided about the sources modulating the synchronous fluctuations of such bilateral H-reflexes.

The purpose of the present research was to extend the study of Mezzarane and Kohn (2002) to examine the correlation between the amplitude of the bilaterally synchronous nSCDPs and the amplitude of the bilateral MSRs in cats. Analysis of this correlation, before and after spinal sections could be important to identify the sources contributing to this synchrony. Mechanisms that correlate random fluctuations in the activity of spinal neurons may prove to be significant to our understanding of the organization of spinal circuits and of motor control (Newell and Corcos 1993). The present study also could be important to uncover the sources of the high variability of MSRs, which usually affect the performance of intraoperative monitoring during selective partial dorsal rhizotomy (Barolat 1991; Rivera et al. 1994; Weiss and Schiff 1993).

MATERIALS AND METHODS

Preparation

Experiments were carried out around noon in 17 adult cats (weight range, 2.4-3.8 kg) initially anaesthetised with pentobarbitone (35 mg/kg of weight, intraperitoneally). The blood pressure was monitored through the carotid artery. The left radial vein was also
cannulated to administer additional doses (10 mg/kg, intravenously) of pentobarbital to maintain (after induction) the animals in deep anaesthesia. At the end of the experiment each animal was euthanized with an overdose of pentobarbital. Guidelines contained in National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (85-23, revised in 1985) were strictly followed.

The lumbo-sacral and low thoracic spinal segments were exposed and the dura mater was removed. After the surgical procedures, the animal was mounted in a stereotaxic apparatus using spinal and pelvic clamps. The left and right ventral roots L5-S2 were dissected and sectioned. Pools were formed with the skin around the exposed tissues, filled with mineral oil (after placement of the electrodes) and maintained at a constant temperature (37 °C). Adequacy of anaesthesia was assessed by verifying that the pupils were constricted, and that blood pressure was stable (usually between 100-120 mmHg). The animals were paralysed with pancuronium bromide (Pavulon, Organon), and artificially ventilated.

*Stimulation*

Left and right gastrocnemius plus soleus (GS) or posterior biceps and semitendinosus (PBSt) nerves were stimulated with single pulses of approximately 1.2-1.4 times the threshold level (xT) of the afferent volleys recorded on the surface of the spinal cord. The frequency of stimulation was adjusted to 0.5 Hz. We used TTL pulses and a Master-8 system to produce simultaneous pulses of stimulation as is illustrated in Figure 1G. The stimulus intensity was adjusted to evoke MSRs that were between 20 and 30 % of the maximal ventral root discharge since it is known that the susceptibility of the reflex to
facilitation and to inhibition depends on its size (Crone et al. 1990). We adjusted the intensity of the stimulus as necessary to keep equivalent mean MSR sizes after different spinal sections. We excluded experiments in which the spinalization abolished the bilateral MSRs.

Electrophysiological recordings

We recorded bilateral monosynaptic reflexes simultaneously from the central ends of the sectioned L7 (or L6) ventral roots (Figures 1G-I, and 1J-K). We observed that when the stimulus was applied only on one nerve no monosynaptic reflexes were evoked on the contralateral ventral root (Figures 1A-F). These observations imply that muscle spindle afferents do not activate contralateral motoneurones (Harrison and Zytnicki 1984), thus indicating that there are no crossed reflexes. In our experiments, we applied the electrical stimuli simultaneously (see Fig. 1G) to avoid bilateral interactions. We observed that the afferent volleys from the two sides arrived at the cord simultaneously. We verified that MSRs on each side of the spinal cord were evoked simultaneously (i.e., the time interval between left and right MSRs was zero ms). The MSR size was expressed as a percentage of the maximal ventral root discharge. The size of the MSR was continually monitored during the course of an experiment to assess stability of stimulation and recording conditions.

Bilateral evoked afferent volleys were recorded at lumbar segment L6 by means of two silver ball electrodes placed on the cord dorsum against an indifferent electrode inserted in the back muscles. Low noise, high gain differential amplifiers (Grass model P511) were used to amplify the potentials.
In other series of experiments, nSCDPs were monopolarly recorded on a Synamps electroencephalographic (EEG) amplifier (NeuroScan, Inc. Sterling, VA) using a system of 30 Ag-AgCl electrodes (200 µm diameter) positioned on the surface of the lumbar L5-L7 spinal cord (Figure 5A) against an indifferent electrode placed on the paravertebral muscles. The distance between electrodes was 5 mm along the rostrocaudal axis and 1.5 mm along the mediolateral direction. Topographic maps were created using Scan 4.2 Software from NeuroScan (Inc. Sterling, VA). We recorded nSCDPs (bandpass 0.15 Hz to 70 Hz) and bilateral monosynaptic reflexes simultaneously (Figure 5A) with a sampling rate of 10 kHz.

Conditioned stimulation

We used a window discriminator to select nSCDPs from electrode 12, or electrode 20, from the system of multielectrodes illustrated in Figure 5A. Channel 12 was located on the left side of the spinal cord, whereas the channel 20 was positioned on the right side of the spinal cord (Figure 5). We selected these electrodes (12 and 20) because in the regions in which they are positioned we have observed the largest bilateral nSCDPs (Manjarrez et al., 2002, 2003). The trigger output pulses from the window discriminator were used to produce single trigger pulses only when the maximum amplitude of the left or right nSCDPs occurred. These trigger pulses were used to apply simultaneous stimulation to the left and right GS nerves. The trigger output pulses also were used to synchronize the averaging of the evoked bilateral MSRs, as is illustrated in Figures 1G-I and 1J-K.

Figure 5 shows bilateral GS monosynaptic reflexes preceded at a fixed time interval by nSCDPs (black arrow). The red arrow in Figure 5B illustrates the time at which the
stimuli to the left and right GS (1.4xT) nerves were applied. Note that these stimuli were
applied 2 ms after the occurrence of one nSCDP recorded by the channel 12 of the
multielectrode system. With this protocol, we examined the interaction between negative
nSCDPs and bilateral GS evoked MSRs (“nSCDP + MSR”). We demonstrated that the
spontaneous potentials (the nSCDPs) were not a single event detected (by volume
conduction) on both sides of the spinal cord (see maps in Figures 6E and 6G).

*Signal processing and data analysis*

*Cross-covariance*

We measured peak amplitudes of the nSCDPs using the Neuroscan software, and
the peak amplitudes of the monosynaptic reflexes using the Axoscope software (Axon
Instruments). The procedure used in the cross-covariance analysis of bilateral reflexes was
similar to that employed in (Mezzarane and Kohn 2002) and is briefly described here.

The cross-covariance (CCV) measures the correlation between a sample of the first
signal and a sample of the other signal occurring with a lag K away from the first. If both
signals were generated independently, then the CCV will be zero for all K lag values. If the
two signals have a common source of variability, then there will be a peak in the CCV at
lag K=0. If one signal is a delayed version of the other, the CCV will have a peak at a lag
equal to the delay. Each time series of MSR amplitudes was detrended by subtraction of the
best straight line fit.

Before computing the cross-covariance sequence (CCV) each MSR time series was
whitened by an inverse filtering. This whitening is necessary because spurious peaks may
appear in a cross-covariance even if the two signals are independent (Brockwell and Davis
After the two signals were whitened a confidence interval was used to evaluate the significance of any peak in the cross-covariance of the whitened signals (Brockwell and Davis 1991). A 95 % CI given by ±1.96/\sqrt{N}, N being the number of reflex responses, was employed in the lag range between −100 s to + 100 s. The whitening was achieved by passing each signal through an inverse filter whose coefficients were obtained from an auto-regressive model fitted to the signal (Rangayyan 2002). Cross-covariance samples out of the CI would suggest a correlation between the two series of monosynaptic reflex-amplitudes at the corresponding lags. The same algorithms described above were employed to compute the CCV between continuous recordings of bilateral nSCDPs (Figure 1L-M).

In the series of experiments illustrated in Figure 6, we normalized the data as in a previous study (Manjarrez et al., 2000). Data in the horizontal axis were normalized with respect to the amplitude of the largest averaged nSCDPs in each particular series. The MSR amplitudes in the vertical axis were expressed as a percentage of control (100%). Then the Spearman's rank correlation method was used to test for significant correlations (p<0.001, 22 degrees of freedom (d.f)). Every point in Figures 6B, D, F and H was obtained from the averaging of 32 samples.

RESULTS

Fluctuations of bilateral monosynaptic reflexes in the cat spinal cord

The results presented in this section were derived from 12 cats in which a peak at zero lag in the cross-covariance between bilateral MSRs was observed. In 10 animals the bilateral MSRs were evoked by stimulation to the GS nerves, and in 2 other the MSRs were evoked by stimulation to the PBSt nerves. Figure 2 illustrates the results obtained from one
experiment. We applied simultaneously 2000 successive stimuli (1.2xT) of 100 µs with time intervals of 2 seconds (0.5 Hz) to the left and right PBSt nerves. The duration of continuous recordings of bilateral MSRs was 1.11 hours. Figure 1K, shows three successive pairs of bilateral L7-MSRs. Figure 2A shows the time course (from 3000 to 4000 seconds) of the bilateral MSR fluctuations obtained from the same experiment. Figure 2B represents a zoom of the graph 2A from 3800 to 4000 seconds of recording. The dots represent the amplitudes of successive bilateral MSRs. It is clear that left and right MSR amplitudes have a high tendency to fluctuate in a synchronized form. Figure 2C shows a graph for the left versus right MSR amplitudes. We obtained a correlation coefficient of 0.8, which also suggests that the MSRs evoked in the left and right ventral roots have a high degree of correlation in their fluctuations.

However, in order to have a more detailed analysis of the degree of correlation for these synchronized fluctuations we calculated the CCV from the time series of amplitudes of bilateral MSRs. Figure 2D shows a clear peak at zero delay in the CCV after whitening the two MSR series. The peak (CCV peak = 0.48) is statistically significant as it is well outside the CI. Similar results were obtained for bilateral MSRs (CCV peak = 0.5 ± 0.1; mean ± SD) evoked by stimulation to the left-right GS nerves (n = 10 cats), or the left-right PBSt nerves (n = 2 cats).

Is there a link between MSR size and CCV peak? Figure 2E shows a graph of the MSR amplitude versus CCV-peak for 12 different experiments. We did not observe evidence of a linear relation between the MSR size and the CCV-peak at zero lag obtained from bilateral MSR (correlation coefficient = 0.1).
Analysis of cross-correlation from long time series of bilateral monosynaptic reflexes

The results presented in this section were derived from four cats in which long duration recordings (up to 8 hours) of bilateral MSRs (evoked by stimulation to the left and right GS nerves) were obtained. Figure 3 shows sets of four graphs (MSR amplitude, MSR-CCV peak at zero lag versus time, nSCDP amplitude and nSCDP-CCV peak at zero lag versus time) for each one of the cats. Each symbol represents the corresponding measurements computed from time series of 500 bilateral MSRs. In order to avoid fatigue after every sequence of 500 MSRs the stimulus train was not applied during a time interval of 5 min.

Figures 3E to H show the peak values of the CCVs of long time series (5 to 8 hours) of bilateral MSRs. Note the transitory changes to near zero CCV peak. However, these transitory changes were not observed in the amplitude of the corresponding bilateral MSRs (Figures 3A to D). The MSR size was expressed as a percentage of the maximal ventral root discharge. Figures 3E to H also show that the MSR-CCV (at zero time lag) tends to remain for larger time intervals above the confidence interval (up to 5 hours). In contrast, the MSR-CCV tends to remain for shorter time intervals (less than 1 hour, or less than 30 min) inside the confidence interval. Figures 3I to L show the corresponding changes in amplitude of the nSCDPs. These bilateral nSCDPs exhibited CCV peaks at zero lag above 0.4 (Figures 3M to P). No transitory changes in the nSCDP-CCV were observed as in the MSR-CCV.
Effects of spinalization and longitudinal bisection on the amplitude and synchrony of bilateral monosynaptic reflexes and bilateral negative spontaneous cord dorsum potentials

In five other cats, we recorded simultaneously bilateral MSRs and bilateral nSCDPs. We analyzed the changes in: the peak amplitude of the CCV between bilateral MSRs, the peak amplitude of the CCV between bilateral nSCDPs, and the amplitude of the MSRs and nSCDPs following spinalization (at L1) and longitudinal bisection (from L1 to S2 segments). The integrity of the spinal cord caudal to the transection (L1) was monitored (on the basis of the size of MSRs and nSCDPs). We have included experiments in which the amplitudes of the MSRs were depressed less than 40% with respect to control and exhibited a clear stability in their fluctuations. In cases where the MSR amplitude was depressed after spinalization the stimulus intensity was adjusted to evoke an equivalent MSR response to that of the control MSR.

Figures 4A, D and G illustrate MSR-CCV graphs obtained from one cat. Note that the peak amplitude of the MSR-CCV peak was not significantly altered after the spinalization (Figure 4D). However, the MSR-CCV peak was abolished after a subsequent longitudinal bisection from the L1 to S2 segments (figure 4G). Similar results were obtained in four other cats (control, spinalization and subsequent longitudinal bisection). Figures 4B, E and H show the mean amplitude of the bilateral MSR for the five cats in the conditions indicated above. Figures 4C, F and I show the mean changes in the corresponding MSR-CCV peaks for the same five animals. After the spinalization the mean MSR-CCV peaks for bilateral MSRs were similar in both conditions (control and
spinalization) (Figures 4C and 4F). However, after a subsequent longitudinal bisection the mean MSR-CCV peak was abolished (Figure 4I).

Figures 4J, M and P illustrate nSCDP-CCV graphs obtained from the same cat (as in Figures 4A, D and G). Note that the peak amplitude of the nSCDP-CCV was not significantly altered after the spinalization (Figure 4M). However, the nSCDP-CCV peak was abolished after a subsequent longitudinal bisection from the L1 to S2 segments (figure 4P). Figures 4K, N and Q show the changes in mean amplitude of the bilateral nSCDPs for the five cats in the same conditions indicated above. Figures 4L, O and R show the mean amplitude of the nSCDPs-CCV peak in control conditions, following the spinalization, and after the subsequent longitudinal bisection for the same five animals, respectively. Note that the amplitude of the bilateral nSCDPs was not significantly affected after the spinalization or the longitudinal bisection, but the nSCDP-CCV was abolished after the longitudinal bisection (figure 4R).

**Correlation between the amplitude of bilateral negative spontaneous cord dorsum potentials (nSCDP) and the amplitude of nSCDP - conditioned bilateral MSRs.**

We have obtained simultaneous recordings of the nSCDPs and of the bilateral GS-MSRs. Figure 5A shows the recordings of the nSCDPs (electrodes 1 to 30) and of the left and right MSRs (electrodes 31 and 32, respectively). With the method of conditioned stimulation shown in Figure 5 (see details in methods section), we examined the effects of different bilateral nSCDPs amplitudes (five different amplitudes) on bilateral MSRs. Figure 6 shows the results obtained from four cats in three different conditions (control,
spinalization and longitudinal bisection) and in two different types of nSCDP-triggered averaging (left nSCDP or right nSCDP).

Figure 6A shows averages from n=32 samples of the nSCDP-conditioned bilateral nSCDPs (upper records) and of the corresponding nSCDP-conditioned bilateral MSRs (lower records), obtained from one animal in control conditions. The white circle on the map of Figure 6A indicates the electrode from which the left nSCDPs were recorded and used to trigger the averaging of the bilateral nSCDPs and MSRs. Bilateral MSRs were produced by stimulation to the GS nerve 2 ms after the peak of the left nSCDPs, as was described in Figure 5. The map in Figures 6A illustrates the topographic distribution of the averaged nSCDPs. Figure 6B (Left), shows superimposed graphs of amplitude of the nSCDP-conditioned left MSRs versus the amplitude of the left nSCDPs (Spearman's rank correlation coefficient ($r_s$), $r_s = 0.9$, $p<0.001$, $t = 9.7$ with 22 d.f) in control conditions for four animals. Note that the larger the left nSCDP amplitude the larger was the nSCDP-conditioned left MSR amplitude (i.e. the corresponding time-locked MSR). Figure 6B (Right), shows superimposed graphs of amplitude of the nSCDP-conditioned right MSRs versus the amplitude of the left nSCDPs ($r_s = 0.7$, $p<0.001$, $t = 5.4$ with 22 d.f) in control conditions for the same four animals. Similar correlations were found after spinalization ($r_s = 0.9$, $p<0.001$, $t = 16.4$ and $r_s = 0.9$, $p<0.001$, $t = 9.9$, with 22 d.f; Figures 6D (Left) and 6D (right), respectively). However, after the complete longitudinal bisection (from L1 to S2 segments) the larger the left nSCDP amplitude the larger was the nSCDP-conditioned left MSR amplitude, but not the nSCDP-conditioned right MSR amplitude ($r_s = 0.8$, $p<0.001$, $t = 9.4$ and $r_s = -0.2$, $p=0.3$, $t = -1.0$, with 22 d.f; Figures 6F (Left) and 6F (right), respectively). In contrast, when the right nSCDPs were used to trigger the averaging we
observed the opposite effect ($r_s = -0.1$, $p=0.4$, $t = -0.7$ and $r_s = 0.8$, $p<0.001$, $t = 9.2$, with 22 d.f; Figures 6H (Left) and 6H (right), respectively). Figures 6F and 6H, suggest that after the longitudinal bisection the dorsal horn neurons producing nSCDPs were separated on both sides of the spinal cord to act independently on left and right monosynaptic reflex pathways. These observations were consistent for the same four animals and indicate that in control conditions bilateral fluctuations of nSCDPs are correlated with the fluctuations of bilateral MSRs.

**DISCUSSION**

Since Sherrington (1906) the studies about the origin of variability of monosynaptic reflexes have been restricted to ipsilateral actions. Here we show that such studies should consider the bilateral actions from spinal sources.

**Synchronous fluctuations of bilateral MSRs in humans and cats**

The present work is a counterpart of previous experiments done in awake humans (Mezzarane and Kohn 2002). The approach was to study the correlation between H reflex amplitudes recorded bilaterally to simultaneous stimulation of the right and left tibial nerves.

In the present study, we observed that bilateral MSRs exhibit “spontaneous” transient changes in the amplitude of the MSR-CCV peak at zero lag. Figures 3E-H show this phenomenon: the MSR amplitudes on the two sides covary during long periods, but in shorter intervals the MSR amplitudes from the two sides seem to vary independently from each other.
The results illustrated in Figures 3E-H are consistent with the observations of Mezzarane and Kohn (2002), who reported the case in which a subject changed from a nonflat (synchronous fluctuations) to a flat cross-covariance on the second day of experiment. Further work in cats and humans will be necessary to understand the mechanisms involved in the activation or deactivation of the sources producing the synchronous fluctuations of bilateral MSRs.

As all the experiments started at noon, the potential effects of circadian rhythms on the degree of synchrony between bilateral MSRs were minimized (Chen et al. 2002).

**Spinal sources contributing to the synchronous fluctuations of bilateral MSRs**

It has been established that there is a strong functional link between sensory neural circuits on the two sides of the spinal cord. Recently Petko et al. (2004) provided a morphological confirmation of this functional phenomenon, presenting evidence for the presence of a direct commissural connection between the lateral aspects of the dorsal horn on the two sides of the lumbar spinal cord of the rat. These authors found that the cells of origin of commissural fibers in the lateral aspect of the dorsal horn were confined to laminae III-IV and projected to the corresponding area of the contralateral gray matter. Most of the commissural axon terminals established synaptic contacts with dendrites. They demonstrated that there is a substantial reciprocal commissural synaptic interaction between the lateral aspects of laminae III-IV on the two sides of the lumbar spinal cord and that this pathway may transmit both inhibitory and excitatory signals to their postsynaptic targets. Because dorsal horn neurons producing the nSCDPs are located within laminae III-VI of the lumbar spinal cord (Manjarrez et al. 2000), we suggest that probably the neurons
described by Petko et al (2004) are a subset of the neurons responsible for the production of nSCDPS.

The results obtained in (Garcia et al. 2004) suggest the existence of a system of spontaneously active dorsal horn neurons that is bilaterally distributed along the lumbosacral segments (Manjarrez et al. 2002; 2003; Vazquez et al., 2004) that affects, in a synchronized manner, impulse transmission in the spinal cord. However, in the study performed in (Garcia et al. 2004) no evidence was provided about the influence of bilateral dorsal horn neurons producing nSCDPS on bilateral MSRs. Here we show (Figure 6) that MSRs recorded on either side of the spinal cord are correlated with left nSCDPS until a longitudinal bisection is made.

Figures 6E-H extend the findings of Manjarrez et al. (2000) by showing that after spinalization and longitudinal bisection separated groups of neurons on both sides of the spinal cord can exert an independent modulation in the amplitude of MSRs.

Based on the evidence that MSR fluctuations at a given ventral root are generated in part by the spontaneous activity of lamina III-VI ipsilateral dorsal horn neurons (Manjarrez et al. 2000), and on the results of our spinalization and longitudinal bisection experiments (Figure 4), we suggest that bilaterally interconnected dorsal horn neurons contribute in part to the synchrony of the bilateral fluctuations of MSRs. This is what should be expected on the basis of early anatomical observations of Ramon y Cajal (Ramon y Cajal 1909), who found that in the dog spinal cord there are posterior commissural neurones located in bilateral zones of the dorsal horn.

The results illustrated in Figure 6 also provide support to the hypothesis that dorsal horn neurons producing nSCDPS are involved in the synchronous fluctuations of bilateral
MSRs. We suggest that if bilaterally situated dorsal horn neurons (lamina III-VI) mutually excite or inhibit each other by crossing axons, then this could be a source contributing to the bilateral reflex covariance in cats and humans. However, based on the evidence that there are also anterior commissural neurons (Jankowska et al. 2003) in the spinal cord, these could also contribute to the correlated fluctuations of bilateral MSRs.

The idea that other possible unilateral sources could contribute to the fluctuations of the corresponding ipsilateral MSRs but not to the correlation between bilateral MSRs is justified by the evidence that there is a spontaneous activation of groups of neurons in different zones of the spinal cord (Vazquez et al., 2004); however it is not as frequent (or as potent) as the synchronous nSCDPs occurring in bilateral zones of the spinal cord. We suggest that such unilateral spontaneous activation of dorsal horn neurons in different zones of the spinal cord contribute to the fluctuations of MSRs but not to the correlations of bilateral MSRs.

Other possible sources of common random inputs to both sides of the spinal cord in the intact animal and in humans are the supraspinal centers and the long propriospinal systems above the L1 segment. These possibilities also merit a discussion in this paper.

**Propriospinal centers could contribute to the synchronous fluctuations of bilateral MSRs**

The propriospinal interneuron system with their cells in the gray matter and their axons in the white matter of the spinal cord conduct activity between different spinal cord segments. This system, together with mono- and oligo- synaptic reflex arcs and poly-synaptic pathways participate continuously in the generation of spinal cord reflex output
activating muscles. There is some evidence that suggests that a long propriospinal system could act on lumbo-sacral neurons (Jankowska et al., 1974), which could then contribute to the synchronous fluctuations of bilateral MSRs. Bolton et al. (1991) identified commissural neurons projecting to the contralateral ventral horn of the cat upper cervical spinal cord. Furthermore, Jankowska et al. (1983) demonstrated that propriospinal neurons originating in the forelimb segments have direct excitatory connexions with inter-neurons of Ib reflex pathways to hindlimb motoneurons. Recently, Krutki and Mrowczynski (2004), showed a bilateral projection of cervical propriospinal neurons to sacral segments of the cat spinal cord. They suggested that cervical neurons transmit information to motor centers controlling hindlimb muscles, forming a part of the system contributing to the process of coordination of movements of forelimbs and hindlimbs. This putative link could cause correlations between forelimb and hindlimb MSRs as well as between forelimb MSRs. It is tempting to suggest that in some motor tasks bilateral MSRs of hindlimbs may be synchronized with bilateral MSRs of forelimbs, with the participation of propriospinal systems and of dorsal horn neurons that produce bilateral nSCDPs.

In humans there are corticospinal projections to lower limb motoneurons (Brouwer and Ashby, 1992). Additionally, there are also indirect corticospinal connections via propriospinal neuronal circuits (Pierrot-Deseilligny, 1996; Dietz, 2004). For example, the interlimb coordination during stance and gait is mediated by propriospinal neurons, which themselves are under supra-spinal control (Dietz, 2004).

At present, limited information is available regarding the anatomy of the propriospinal system in humans (Nathan et al., 1996). However, there are several
electrophysiological studies suggesting the existence of propriospinal neurons in humans (Delwayde et al., 1977; Baldissera et al., 1998; Hiraoka and Nagata, 1999; Meinck and Piesiur-Strehlow, 1981; Faganel and Dimitrijevic, 1982; Zehr et al., 2001; Dimitrijevic et al., 2005). Some of these studies have suggested that propriospinal neurons provide a linkage between cervical and lumbar neuronal circuits of the spinal cord that act in the interlimb coordination of leg and arm movements during human locomotor activities (Dietz, 2004; for review see Swinnen and Duysens, 2004). On the basis of these studies we suggest that a propriospinal system could also constitute another important source for the bilateral MSR fluctuations.

**Supraspinal centers could contribute to the synchronous fluctuations of bilateral MSRs**

We suggest that either there are common bilateral inputs originating from some nucleus, e.g., pontine reticular system (Matsuyama et al. 1999), or there is a correlation between bilaterally descending drives; or still, a specific descending drive acts on spinal interneurons that influence bilaterally the homonymous motoneuron pools. These possibilities receive some support from reports of reflex depression modulation by raphe nuclei stimulation in frogs (Cardona and Rudomin 1983). In this context, recently, Jankowska et al., (2003) demonstrated that although direct actions of reticulospinal fibers are much more potent on ipsilateral motoneurons, interneuronally mediated actions are as potent contralaterally as ipsilaterally, and midlumbar commissural neurons are likely to contribute to them. Such interneurons mediating crossed actions evoked from the reticular formation have been found in Rexed’s lamina VIII. According to Jankowska et al. (2003), these are interneurons that are powerfully monosynaptically excited by stimuli on the
reticular formation and project to contralateral motor nuclei through the anterior commissure. Therefore, posterior and anterior commissural neurons could be potential spinal sources for the correlated variations of bilateral MSRs.

In humans, a report on spinal cord injured subjects (Nozaki et al. 1996) showed a desynchronization between the H-reflex time series on the two sides, with the suggestion of a supraspinal origin for the correlation between two-sided H reflexes in normal subjects. It is not clear if their method (coherence analysis) was sensitive enough to detect spinally generated two-side correlation, as we found in cats, or in spinal cord injured humans some mechanism inhibits or decreases the effects of such spinally originated bilateral reflex variabilities.

It has been reported (Ellaway et al., 1998) that bilateral transcranial magnetic stimulation causes correlated variation in the bilaterally recorded upper limb muscle responses. These authors suggested the corticospinal pathways as possible sites for the fluctuations. However, Figures 4 and 6 also suggest that the spinal cord is itself another important putative source of bilateral correlations. Further work will be necessary to determine the relative contributions of lumbar and propriospinal/supraspinal systems to these correlations.

**Sensory inputs could affect correlations between bilateral MSRs**

In humans and animals the afferent input plays a major role in shaping the interlimb coordination (for review see Duysens et al., 2004; Dietz, 2004; Zehr and Duysens, 2004; Swinnen and Duysens, 2004). For example, during gait the interlimb coordination depends on sensory input, mainly from Ib afferents (Duysens et al., 2004).
Delwayde et al. (1977) reported that stretch reflexes in the lower limbs of humans can be affected by alterations in arm postures, thus suggesting that there may be “interlimb reflex” modulation with movement. In this context, Zehr et al. (2001) investigated directly the existence of “interlimb reflexes” in neurologically intact humans. Zehr et al. (2001) showed that electrical stimulation of a cutaneous nerve in the hand evoked large bilateral reflexes in the legs and contralateral arm. Their study supports the idea that interlimb reflex pathways connecting distant cutaneous receptive fields could be important to access relevant exteroceptive information for the reflex coordination of movement. We suggest that bilateral MSRs, in humans and cats, also could be modulated by the activation of such interlimb reflex pathways. In this context, it is tempting to propose that the CCV between bilateral MSRs in lower limbs could be affected by the simultaneous activation of a cutaneous receptive field on the upper limb of neurologically intact humans. A study of this kind in spinal cord-injured humans could be important to assess the degree of lesion of the proprioceptive system mediating the modulation of interlimb reflexes. Nevertheless, the extent to which the CCV between bilateral MSRs can be affected by the activation of a cutaneous receptive field of an upper limb in humans (and forelimb in cats) remains to be demonstrated in future research. This possibility is supported by recent studies (Zehr et al., 2004; Frigon et al., 2004) showing that there is an influence of cutaneous nerve stimulation from the upper limb on transmission through the soleus H-reflex pathway in the leg during static contractions and during rhythmic arm movements.

Another issue that may be important to examine is the analysis of the CCV and autocovariance of the amplitudes of bilateral interlimb reflexes elicited by cutaneous stimulation of an upper limb. It is probable that in such bilateral interlimb reflexes the
dorsal horn spinal neurons (producing the nSCDPs) also contribute to the bilateral correlations.
REFERENCES


FIGURE LEGENDS

Figure 1. Stimulation of Ia afferents from a nerve does not produce monosynaptic reflexes contralaterally. **A, D** and **G**, Schemes of three different experimental arrangements. Monosynaptic reflexes were recorded bilaterally from the central ends of sectioned L6 ventral roots. **B** and **C**, Afferent volley and monosynaptic reflex (MSR) elicited when a pulse (1.2xT) was applied to the left PBSt nerve. **E** and **F**, The same as **B** and **C**, but the pulse was applied to the right PBSt nerve. **H** and **I**, The same as **B** and **C**, but two stimuli were applied simultaneously to the left and right PBSt nerve. This last protocol was employed in all the experiments to evoke bilateral MSRs, as follows. **J** and **K**, We applied trains of stimuli of constant amplitude on the left and right muscle nerves and a sequence of bilateral MSRs was obtained. **L** and **M**, Experimental arrangement and typical recordings of bilateral negative spontaneous cord dorsum potentials (nSCDPs).

Figure 2. Synchronous fluctuations of bilateral monosynaptic reflex (MSR) amplitudes. MSRs were produced by stimuli (1.2xT) applied every 2 seconds to the left and right PBSt nerves. Results obtained from one experiment. **A**, Graph of monosynaptic reflex peak amplitude versus time. The dots represent the amplitudes of 500 successive MSRs. **B**, the same as **A** but for other time scale. **C**, Graph of the left MSR amplitudes versus the right MSR amplitudes. A positive correlation is suggested but the CCV analysis was used to confirm this. **D**, Cross-covariance computed from the whitened series of 2000 successive bilateral MSRs. The band delimited by the two horizontal lines indicates the 95% confidence interval. Note the clear peak at lag 0, thus indicating a statistically significant correlation between the MSR amplitudes recorded bilaterally. **E**, Graph of the MSR
amplitude (left and right) versus the corresponding CCV obtained from 12 animals. The MSR size was expressed as a percentage of the maximal ventral root discharge.

**Figure 3.** A-D, Amplitude of bilateral MSRs versus time, recorded up to 8 hours. Each of the four graphs was obtained from a different cat. Each symbol represents the mean amplitude computed from time series of 500 bilateral MSRs. The MSR size was expressed as a percentage of the maximal ventral root discharge. After every sequence of 500 MSRs the stimuli were not applied during a time interval of 5 min. Black circles indicate left MSRs, white circles indicate right MSRs. E-H, Changes in CCV-peak values for the bilateral MSRs. I-L, The same as A-D but for amplitudes of the bilateral nSCDPs. M-P, The same as E-H but for bilateral nSCDPs.

**Figure 4.** Effects of spinalization (at L1) and complete longitudinal bisection (from L1 to S2), on the amplitude and CCV peak at zero lag of bilateral MSRS and nSCDPs. A, MSR-CCV graph obtained from one animal. B, Mean amplitude of the left and right MSRs for five animals. The MSR size was expressed as a percentage of the maximal ventral root discharge. C, Mean MSR-CCV peak at zero lag computed from the same five animals. D-M, The same as A-C but after the spinalization. G-I, The same as D-F, but after the complete longitudinal bisection of the spinal cord. J-R, The same as A-I but for the nSCDPs.

**Figure 5.** Scheme of the experimental arrangement used for the conditioning of the bilateral MSRs. A, Typical continuous recordings of nSCDPs and GS-MSRs obtained with
the Neuroscan System of 32 channels. The red arrow illustrates the time in which the stimulus to GS (1.6xT) was applied. Negativity upwards. The number 12 within the circle indicates the electrode from which the nSCDPs were selected to trigger the averaging. B, Topographical map obtained from the nSCDPs at the time indicated by the green vertical line in Fig. 5A. Note that the bilateral MSRs were evoked by the occurrence of the nSCDPs. The blue horizontal line indicates the level of the window discriminator used to trigger the averaging.

**Figure 6.** Correlation between amplitude of nSCDPs and amplitude of bilateral MSRs evoked during the occurrence of nSCDPs detected with electrode 12 (white circle, or blue circle in Fig. 5A) or electrode 20. A, Recordings of cord dorsum potentials with electrode 12 showing the interaction between nSCDPs and GS-evoked nSCDPs (negativity upwards). Map was obtained at the peak of the left nSCDP. B (left), Superimposed graphs obtained from four animals. Amplitude of nSCDPs selected with the electrode 12 (located on the left side of the spinal cord) versus amplitude of the left MSRs. Abscissa, amplitude of the spontaneous nSCDPs, expressed as a fraction of the largest averaged potential in each particular series (100 %). Ordinates, amplitude of MSRs as a percentage of control (100 %). Continuous line represents best linear fit. Correlations were tested with the nonparametric Spearman's rank coefficient method (p<0.001). B (right), The same as B (left), but for MSRs recorded from the right ventral root. C-D, The same as A-B but after spinalization at L1. E-F, The same as C-D but after a subsequent longitudinal bisection from L1 to S2. G-H, the same as E-F but the nSCDPs used to trigger the averaging were recorded from the electrode 20, located on the right side of the spinal cord.
Figure 2
Figure 3
Figure 4