The Journal of Neuroscience, November 15, 2002, 22(22):9997–10008

Synchronization of Motor Neurons during Locomotion in the Neonatal Rat: Predictors and Mechanisms

Matthew C. Tresch and Ole Kiehn

Department of Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden

We describe here the robust synchronization of motor neurons at a millisecond time scale during locomotor activity in the neonatal rat. Action potential activity of motor neuron pairs was recorded extracellularly using tetrodes during locomotor activity in the *in vitro* neonatal rat spinal cord. Approximately 40% of motor neuron pairs recorded in the same spinal segment showed significant synchronization, with the duration of the central peak in cross-correlograms between motor neurons typically ranging between ~30 and 100 msec. The percentage of synchronized motor neuron pairs was considerably higher for pairs with similar locomotor-related activity and strong rhythmic modulation. We also found synchronization between the activities of different motor pools, even if located several segments apart. Such distant synchronization was abolished in the absence of chemical synapses, although local coupling between motor neurons persisted. On the other hand, both local and distant coupling between motor neurons were preserved after antagonism of gap junction coupling between motor neurons. These results demonstrate that motor neuron activity is strongly synchronized at a millisecond time scale during the production of locomotor activity in the neonatal rat. These results also demonstrate that chemical synaptic inputs, in addition to electrical synapses, contribute to this synchronization, suggesting the existence of multiple mechanisms underlying motor neuron synchronization in the neonatal rat. The fast synchronization described here might be involved in activity-dependent processes during development or in the coordination of individual motor neurons into a functional population underlying behavior.

Key words: motor neuron; synchronization; gap junction; locomotion; development; pattern generation

Action potential synchronization has been described between neurons in many systems. This prevalence has led several investigators to assign to synchronization an important role in basic neural function. At a network level, synchronization has been suggested to link the activity of disparate neurons into a coordinated, functional population, binding together the features represented by individual neurons into a unified whole (Engel et al., 1992; Welsh and Llinas, 1997; Farmer, 1998; Baker et al., 1999). At a synaptic level, synchronization has been suggested to amplify the effects of single spikes on a postsynaptic neuron, with the close temporal association of presynaptic spikes allowing for complex synaptic integration (Softky and Koch, 1993; Stevens and Zador, 1998) or efficient synaptic plasticity during learning and development (Katz and Shatz, 1996; Markram et al., 1997; O'Donovan et al., 1998; Feller, 1999; Bi and Poo, 2001).

In the developing mammalian spinal cord, electrical gap junction coupling (GJC) between motor neurons (MNs) (Fulton et al., 1980; Walton and Navarrete, 1991; Chang et al., 1999; Tresch and Kiehn, 2000a) has been commonly suggested to mediate synchronization of MN firing during the production of movement. Moreover, because the electrical GJC between MNs seems to disappear over the course of development (Walton and Navarrete, 1991; Chang et al., 1999), the synchronization between MNs is expected to also disappear, and a recent set of experiments has provided evidence in support of this hypothesis (Personius and Balice-Gordon, 2001). Such synchronization has been suggested to be involved in the activity-dependent process of synapse elimination at the developing neuromuscular junction (Busetto et al., 2000; Chang and Balice-Gordon, 2000).

The potential contribution of other mechanisms, however, both synaptic and extrasynaptic, to any synchronization between MNs in this preparation has often not been considered. Many experiments have shown synchronization between MNs in normal adults (Nordstrom et al., 1992; Farmer, 1998; Baker et al., 1999; Hansen et al., 2001), at ages well beyond the time when electrical GJC between MNs is demonstrable in the mammalian spinal cord. This synchronization is generally considered to reflect the presence of a common presynaptic input to each neuron, mediated by classic chemical synapses. The relative contributions of gap junctional coupling and of presynaptic chemical synaptic inputs to the synchronization of MNs in the neonatal rat, in which both mechanisms may shape the activity patterns of MNs, is therefore not obvious.

The experiments described here demonstrate that MNs are robustly synchronized at a millisecond time scale during the production of locomotor activity, as had been predicted. These experiments also show that although gap junction coupling between MNs can make a substantial contribution to the local synchronization of motor pools, it is not the only mechanism mediating such synchronization. In particular, synaptic drive to MNs from spinal interneurons clearly plays a large role in the synchronization of motor pools during the production of locomotor activity in the neonatal rat.

These results have been presented previously in abstract form (Tresch and Kiehn, 2000b).

Received July 1, 2002; revised Aug. 19, 2002; accepted Sept. 6, 2002.

This work was supported by the Danish Medical Research Council, the Novo Nordisk Foundation, and the Karolinska Institute to O.K. M.C.T. was supported by a postdoctoral fellowship from the Lundbeck Foundation.

Correspondence should be addressed to Dr. Ole Kiehn, Department of Neuroscience, Karolinska Institutet, Retzius vag 8, 17177 Stockholm, Sweden. E-mail: ole.kiehn@neuro.ki.se.

M. C. Tresch's present address: Department of Brain and Cognitive Sciences, Massachusetts Insitute of Technology, E25-526, 45 Carleton Street, Cambridge, MA 02139.

Copyright © 2002 Society for Neuroscience 0270-6474/02/229997-12$15.00/0

MATERIALS AND METHODS

Preparation. The dissection procedures and experimental preparation were as described elsewhere (Kiehn and Kjaerulff, 1996; Tresch and Kiehn, 1999; Raastad and Kiehn, 2000). Briefly, rats (postnatal day 0–2; $n = 29$) were anesthetized under ether and decapitated. The spinal cord was exposed by ventral laminectomy and then removed and placed ventral side up in a chamber with continuously circulating oxygenated Ringer's solution containing (in mM): 128 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.25 $MgSO_4$, 2.5 $CaCl_2$, and 20 glucose, pH 7.4, at room temperature. In experiments in which calcium was removed from the Ringer's, $CaCl_2$ was replaced with equal molarity $MgSO_4$. Motor activity was monitored by suction electrodes placed on ventral roots (L5, usually with L2 or L3, sampled at 1000 Hz). In some rats we dissected the peripheral nerves innervating iliopsoas (IP) and quadriceps (Q) muscles intact with L2 and L3 ventral roots. Locomotor activity was induced by bath application of a combination of serotonin (5-HT; 2–8 μM) and NMDA (2–7 μM), by application of 5-HT alone (6–30 μM), or by application of dopamine (DA; 1–3 mM). These agents evoke motor patterns that are similar to the rhythmic, alternating muscle activations observed during normal locomotion in intact animals (Kiehn and Kjaerulff, 1996). For simplicity of presentation, we refer to these motor patterns throughout the present study as "locomotor activity," although no actual behavior was produced. The quality of such locomotor activity was assessed using the modulation depth measure described previously (Kjaerulff and Kiehn, 1996), and locomotor frequency, period, and period variability were calculated.

MN recordings. Along with ventral root activity, the extracellular activity of neurons was recorded using tetrodes (sampled at 20 kHz), as described previously (Tresch and Kiehn, 1999). Tetrodes were inserted through a small slit made in the ventral surface of the cord overlying motor pools. For recordings made within a single spinal segment, multiple tetrodes were placed in the same slit, generally within <500 μm of one another. Most of the recordings were made in L5, occasionally with simultaneous recordings in L2 or L3. The action potentials of multiple neurons were recorded during locomotor activity and saved for off-line analysis. The action potentials of different neurons were separated by clustering analyses performed on features of the recorded waveforms, usually the peak voltages on each channel of the tetrode (Tresch and Kiehn, 1999, their Fig. 1). Once separated, the arrival times of each recorded neuron in 1 msec bins were used for spike-triggered averaging with the raw recorded ventral root activity to determine whether the recorded neuron was an MN. Only those neurons for which the spike-triggered averaging showed an orthodromic action potential in the ventral root were included in subsequent analyses. On average, 297 ± 207 (mean ± SD) action potentials were recorded for each neuron.

The locomotor-related activity of each MN was quantified in terms of its mean phase and mean resultant length, or *R* value (Mardia, 1972; Tresch and Kiehn, 1999). The mean phase characterizes the portion of the locomotor cycle in which a neuron is active, whereas the *R* value of a neuron characterizes the modulation strength of the neuron by the locomotor cycle. The significance of the *R* value was assessed using the Rayleigh test (Mardia, 1972).

In a small number of recordings to examine the effects of carbenoxolone on MN properties, whole-cell tight-seal intracellular recordings of MNs were made in current clamp (5000 Hz; Axopatch-1D, Axon Instruments, Foster City, CA) with glass pipettes [3–6 MΩ, filling solution (in mM): 138 K-gluconate, 10 HEPES, 0.0001 $CaCl_2$, 5 ATP-Mg, 0.3 GTP-Li) (Kiehn et al., 1996; Raastad et al., 1996; Tresch and Kiehn, 2000a). Electrical coupling between MNs was monitored using a collision protocol described previously (Walton and Navarrete, 1991; Chang et al., 1999).

Evaluation of MN synchronization. To quantify the synchronization of MN action potential activity, we performed a cross-correlation analysis. This analysis was complicated by the fact that we were recording the activity of MNs during locomotor activity. The activity of MNs is clearly expected to be modulated during locomotion, and this nonstationarity of neuronal activity makes it difficult to assess the significance of cross-correlations. We therefore used a randomization procedure, similar to a shuffle predictor (Perkel et al., 1967), to determine whether two MNs were correlated with one another more than would be expected simply because of their slow modulation during locomotor activity.

We first described the modulation of each neuron with respect to the ventral root activity. The rectified and filtered ventral root recording was used to define a locomotor phase, expressed in angular coordinates from 0 to 360° (Tresch and Kiehn, 1999). This range was divided into 50 bins of equal phase, and the number of action potentials produced by the neuron in each bin was counted. We then used this observed modulation of spike count to generate a random spike train. For each spike from the original spike train we randomly chose a new time of arrival, with the condition that the locomotor phase in which the new spike occurred was the same as that of the original spike. We further ensured that for each locomotor cycle, the random spike train contained the same number of spikes in that cycle as the original spike train. This latter condition ensured that any cycle-to-cycle variation or slow drift in neuronal excitability through the locomotor run was reproduced in the random spike train, thereby taking into account any features of cross-correlations that might be caused by such changes in excitation (Brody, 1998, 1999). We did not directly attempt to account for latency covariations, which in the present case would be reflected in a covariation between the onsets of a pair of neurons from cycle to cycle (Brody, 1998, 1999). This randomization produced a spike train that preserved the slow modulation of neuronal activity related to the locomotor cycle but abolished the precise temporal details of the original spike train. The same procedure was performed for the other neuron in the pair, and a cross-correlogram was performed between the two random spike trains. We generated 100 such random cross-correlograms for each pair of neurons using a bin size of 10 msec. The mean cross-correlogram between these random spike trains represents the null hypothesis that the cross-correlogram observed between two neurons resulted only from their common slow modulation during locomotor activity. This null hypothesis was rejected, and the pair of neurons was considered significantly synchronized at a short time scale if the actual observed cross-correlation had a peak that was >3 SDs, calculated from the distribution of random cross-correlations, from the mean of the random cross-correlograms within the central ± 100 msec. This procedure generally agreed with a qualitative examination of the activity in the pairs but provided an objective means of assessing this issue.

Synchronization between groups of MNs, if present, should also be evident in the relationship between the activity in an MN and the gross ventral root recording. Such a synchronization should be seen as a correlation between a spike in a recorded MN and a cluster of action potentials recorded in the ventral root, corresponding to the action potential of the MN along with action potentials of synchronized MNs firing in close temporal proximity (usually approximately ± 100 msec; see Results and Figs. 2*A*, 3, and 7). We therefore also performed a cross-correlation analysis between the action potentials in each MN and the rectified activity of each ventral root. We performed an analysis analogous to that described above to determine the significance of the cross-correlation. We generated random spike trains that preserved the activity relationship of the MN to the locomotor-related modulation of the ventral root and the cycle-to-cycle variations in spike count but that abolished the fine temporal aspects of this relationship. The rectified ventral root recordings were smoothed by convolution with a Gaussian kernel (10 msec SD). Cross-correlations >3 SDs greater than the mean of the random cross-correlation were considered to be significant. Although the action potential of the recorded MN was also present in the recorded ventral root, this orthodromic spike only contributed a narrow correlation near zero lag. Any cross-correlations with such a narrow peak were not considered to be significant. All cross-correlations between a neuron and a ventral root are shown as correlation coefficients.

We also performed cross-correlations between ventral root recordings to examine coupling between the motor outputs of different spinal segments. The randomization procedures described above for neuronal spike trains are not applicable to such cross-correlations, and we were therefore unable to develop a clear statistical evaluation of these ventral root cross-correlations. The presence of coupling between ventral roots was therefore assessed by qualitative inspection of the cross-correlations.

Evaluation of synchronization strength. We estimated synchronization strength using several previously described measures: k'-1, the index S, the synchronization index (SI), the common input strength (CIS) (Nordstrom et al., 1992), and the correlation coefficient (CC) (Eggermont, 1992). The beginning and end of the central peak in the cross-correlograms with significant synchronization were identified visually. For cross-correlograms that were not significant, the correlation strength measures were calculated over the mean period of the significantly correlated correlograms. Table 1 shows the relationships between these measures of correlation strength calculated from the MN cross-correlograms. As can be seen in Table 1, there is a range of relationships between these measures [see also Molotchnikoff et al. (2001)], suggesting that they are not interchangeable. A lack of dependence on overall levels of neuronal activity, as indicated by interspike interval or firing rate, has

Table 1. Correlations between synchronization strength measures

	k′-1	S	SI	CIS	CC
k′-1	1	0.57	0.69	0.15	0.58
S		1	0.84	0.63	0.996
SI			1	0.18	0.85
CIS				1	0.62
CC					1

The correlation between the measures of synchronization strength calculated for each motor neuron pair. See Materials and Methods, Evaluation of synchronization strength, for description of the different measures.

Table 2. Dependence of synchronization strength measures on activity level

Geometric mean of firing rate for MN pair				Geometric mean of interspike interval for MN pair			
	r^2	p	b		r^2	p	b
k′-1	0.09	0.0002	−0.37	k′-1	0.25	0.0000	1.16
S	0.004	0.3971	−0.003	S	0.05	0.0035	0.01
SI	0.11	0.0000	−0.4	SI	0.28	0.0000	1.14
CIS	0.22	0.0000	0.07	CIS	0.10	0.0000	−0.09
CC	0.007	0.2661	−0.01	CC	0.05	0.0019	0.04

The results obtained from the linear regression of synchronization strength measures to either the geometric mean of interspike interval or of firing rate for each pair of motor neurons. r^2 is the amount of variance explained by the regression, p is the significance level of the regression, and b is the slope of the regression. See Materials and Methods, Evaluation of synchronization strength, for description of the different measures.

been taken as a criterion of a good measure of correlation strength (Nordstrom et al., 1992). Table 2 shows the relationship between these measures and the activity level of the neuron pairs, as reflected in the geometric mean of the interspike interval and the firing rate of each neuron (Nordstrom et al., 1992). As can be seen in Table 2, only the index S and the CC were unrelated to the mean firing rate. All measures were significantly related to the mean interspike interval, although the relationship for the index S and CC was weak ($r^2 = 0.05$ for both). These two measures were also highly correlated to one another as shown in Table 1, resulting from the strong correlation in the present data set between their normalization terms ($r = 0.96$). Because the CC is a standard measure of correlation strength and because of its relative lack of dependence on activity levels for this data set, we use this measure to characterize the strength of interactions between neurons in the present study. Note, however, that our assessment of the significance of the cross-correlations using the randomization procedures described above was not directly dependent on any measure of correlation strength.

To examine whether correlation strength depended on the difference between mean phases of each neuron in the pair, we performed a linear–circular correlation analysis using a rank correlation test (Mardia, 1976). The test statistic, D_n, ranges between 0 and 1, with a value of 1 indicating a strong relationship, and its significance was determined as described in Mardia (1976). Note that because the ordering of neurons within a pair is arbitrary, the sign of the difference in mean phases (positive or negative) is also arbitrary. However, for the data sets examined here, D_n did not vary considerably when the sign of the difference was randomized, and none of the significance values changed.

All values in the text are reported as mean ± SD unless noted otherwise.

RESULTS

MN action potentials are synchronized during locomotor activity in the neonatal rat

We recorded the activity of 142 MNs during locomotor activity. In these recordings, the activity of 176 MN pairs within the same segment was recorded simultaneously. An example of the spike trains of two L5 MNs after application of 5-HT/NMDA (6/6 μM) is illustrated in Figure 1*A*. As can be seen in the two illustrated cycles, action potentials in the two MNs were often temporally very close to one another. Figure 1*B* shows the cross-correlogram between the two spike trains illustrated in Figure 1*A*. The two neurons show a common slow modulation in their firing rate, related to the locomotor activity in the ventral root. In addition to this slow modulation, however, there is a sharp peak in the cross-correlation centered near zero lag. This peak was >3 SDs from the correlation level expected from a simple slow comodulation of the neurons by the locomotor cycle. The correlation attributable to slow, locomotor-related modulation was estimated from the mean of the randomized cross-correlograms (see Materials and Methods), indicated in Figure 1 by the middle superimposed line, shown along with lines indicating 3 SDs above and

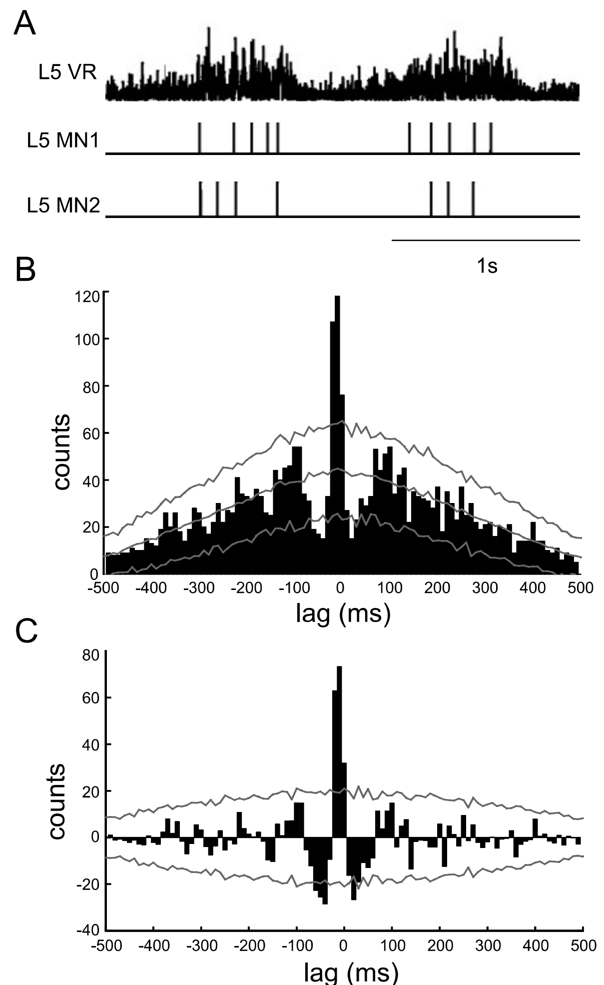

Figure 1. Action potential synchronization in MNs during locomotor activity. *A*, Spike trains of two L5 MNs (*bottom* and *middle traces*) and simultaneous activity in the L5 ventral root (*top trace*) during the production of locomotor activity evoked by NMDA and 5-HT. Both neurons were activated in phase with the burst in the L5 ventral root (*VR*). Within each burst, the arrival times of individual action potentials from each neuron were often close to one another. *B*, The cross-correlogram between the spike trains of the MNs shown in *A*. The *middle thin line* shows the mean of the random cross-correlograms representing the null hypothesis of comodulation of the neurons attributable to the locomotor cycle. The *other two thin lines* show ±3 SDs from this expected level of comodulation. *C*, The covariogram of the two neurons shown in *A*, obtained by subtracting the expected level of correlation from the observed cross-correlogram. The *thin lines* show ±3 SDs of the distribution of expected cross-correlograms.

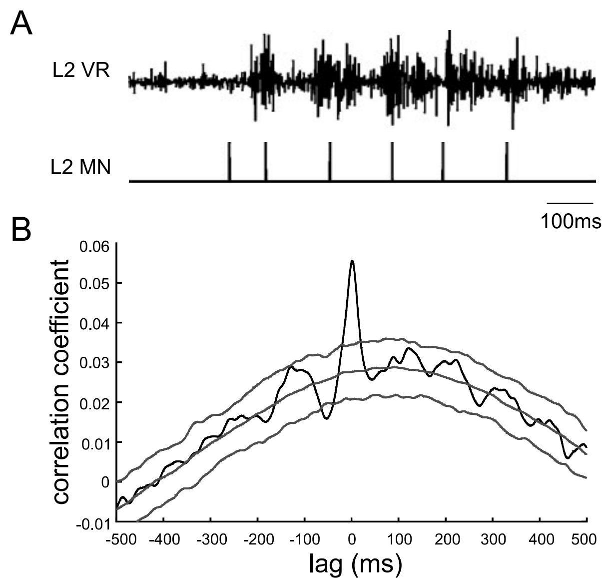

Figure 2. Synchronization of MNs to ventral root clusters. *A*, Ventral root activity in L2 (*top trace*) showing the presence of clusters of motor output. The spike train in a simultaneously recorded L2 MN (*bottom trace*) was closely related to these clusters. *B*, The cross-correlation between the spike activity in the neuron illustrated in *A* and the rectified ventral root is shown in the *thick line*. The *thin lines* represent the mean ±3 SDs of the distribution of random cross-correlations expected if the relationship of the neuron to the ventral root activity were caused only by modulation of its activity by the locomotor cycle.

below this mean level. Figure 1*C* shows the cross-covariogram of these two cells, which was obtained from subtracting the mean of the randomized cross-correlograms from the observed cross-correlogram; the temporal coupling between these neurons is shown clearly. Of the 176 MN pairs recorded within the same segment, 75 (43%) showed a significant cross-correlation peak (L2, 1 of 3; L3, 4 of 9; L5, 70 of 164). For the population of correlated pairs, the correlation lag ranged from 0.2 to 54.4 msec (mean 10.1 ± 11.3 msec). The mean duration of the central peak of the cross-correlation was 59.2 ± 32.7 msec.

Synchronization of MNs could also be observed by cross-correlating MN action potentials with rectified ventral root recordings, as illustrated in Figure 2. Figure 2*A* shows an example of a locomotor burst in the L2 ventral root in which clusters of MN activity were especially clear (Cazalets et al., 1990; Westerga and Gramsbergen, 1993, 1994; Cowley and Schmidt, 1995; MacLean et al., 1997; Hochman and Schmidt, 1998). A simultaneously recorded L2 MN fired action potentials associated with each of the clusters. Figure 2*B* shows the cross-correlation between the MN and the rectified ventral root, along with the mean ± 3 SDs of the distribution of randomized cross-correlations. These plots show the clear tendency of this MN to be associated with a cluster of ventral root activity. Such cross-correlations regularly revealed a significant peak: of 142 MNs recorded, 116 (82%) showed a significant cross-correlation with the ventral root in which it projected its axon. This correlation further demonstrates the prevalent synchronization of MNs during the production of locomotor activity in this preparation.

The clusters of MN activity in the ventral root indicative of MN synchronization were not unique to the locomotor activity evoked by combination of NMDA and 5-HT. Ventral root clusters were observed during the locomotor activity evoked by 5-HT alone (eight of eight runs in four animals) (Fig. 3*A*) or by dopamine (10 of 10 runs in six animals) (Fig. 3*B*). We also observed ventral root clusters in the rhythmic but non-locomotor-like activity (with ipsilateral L2–L5 synchrony and alternating segmental motor discharge) (Cowley and Schmidt, 1994b) evoked by muscarine (one of one run in one animal). These results suggest that MN synchronization is found during many rhythmic motor acts in the neonatal rat spinal cord.

Examination of the cross-correlations in Figures 1–3 also suggests that there was a substantial oscillatory component to the cross-correlations between MNs, as illustrated in the peaks to the right and left of the central peak. Such oscillatory behavior was commonly observed in these experiments and will be discussed in more detail in a later section (see Oscillatory features of MN synchronization). In the subsequent analyses, however, we examine the characteristics of MN synchronization as reflected in the central peak of the cross-correlograms centered near zero lag.

Predictors of MN synchronization

We next considered whether the synchronization described above could be predicted by the component of motor neuronal activity that was modulated in relation to the ongoing locomotor pattern. A relationship between features of such locomotor-related activity of MNs and MN synchronization would suggest that the synchronization observed here is not distributed randomly between neurons but is functionally related to the motor production in this preparation.

Similarity between the locomotor-related activity of neurons predicts synchronization

We first examined the relationship between the synchronization of a MN pair and the similarity between the locomotor-related activity of each neuron in the pair. We found that the percentage of synchronized MN pairs was considerably higher between neurons with similar locomotor-related activity. Figure 4*A* shows the percentage of synchronized MN pairs as a function of the difference between the mean phases of each cell in the pair: a small difference of mean phases indicates that the two neurons were activated in a similar portion of the locomotor cycle. Because the mean phase is only well defined when the locomotor-related activity of a neuron has a significant unimodal component, only neuron pairs in which both neurons had a significant R value (Rayleigh test; $p < 0.05$) were included in this analysis. The large majority of pairs met this condition (155 of 176, 88%). MN pairs that were activated in different portions of the locomotor cycle were rarely synchronized (Fig. 4*A*), whereas MN pairs with similar locomotor-related activations were correlated very often, with nearly 70% of such pairs having a significant correlation. This percentage of synchronized MN pairs with similar locomotor-related activity was close to the percentage found by correlation of MN spikes with rectified ventral root recordings. The lower figure of ~40% for MN synchronization reported in the previous section therefore likely reflected the difficulty in randomly sampling two MNs with similar locomotor-related activity.

Similarly, the strength of synchronization also depended on the similarity between the locomotor-related activity of the neurons. Figure 4*B* shows the scatter plot of the correlation strength, measured as the correlation coefficient between the two spike trains (see Materials and Methods), as a function of the difference between mean phases of the neurons. Neuron pairs with a small difference in mean locomotor phase tended to have high correlation strengths, whereas pairs with larger differences tended to

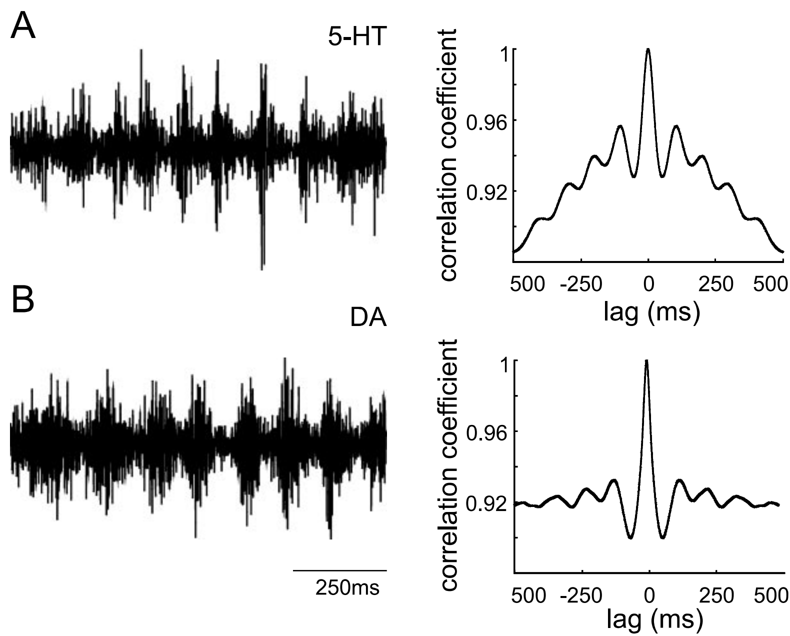

Figure 3. Synchronization of MNs during 5-HT- and DA-evoked locomotor activity. *A*, The activity of a L5 ventral root in one burst of locomotor activity evoked by serotonin (*5-HT*), showing clusters of MN output. The autocorrelation to the *right* shows the robustness of these clusters, with clear off-center peaks as well. *B*, The activity of a L5 ventral root during one burst of locomotor activity evoked by dopamine (*DA*) and its autocorrelation to the *right*, similarly showing clusters in the motor output.

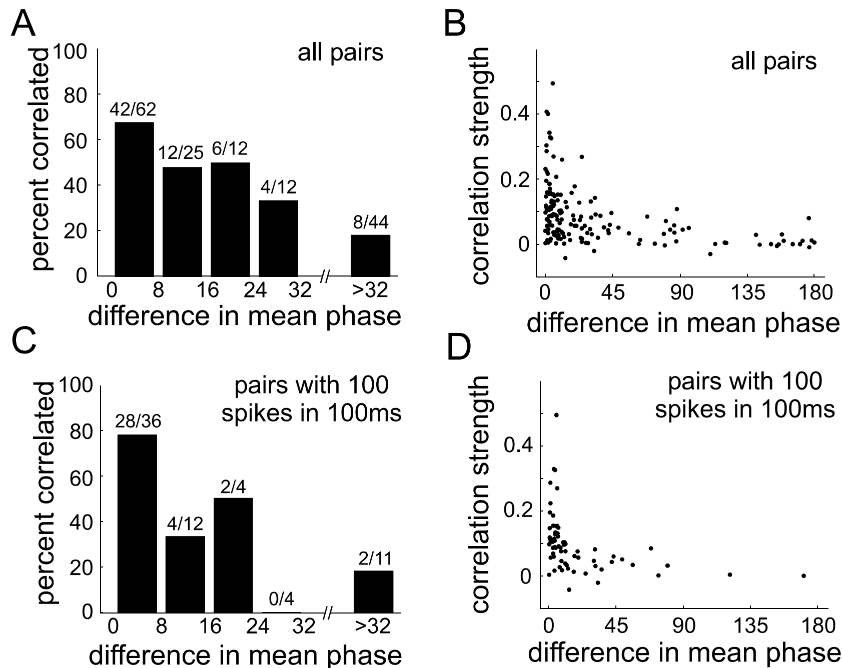

Figure 4. Synchronization is more common between MNs with similar locomotor-related activity. *A*, The percentage of synchronized pairs is illustrated as a function of the difference between the mean phases of the neurons in the pair (bin size, 8°). A small difference indicates that the neurons were activated similarly during locomotor activity. The ratio on *top* of each *bar* indicates the fraction of MN pairs that was synchronized in a particular bin. More MN pairs were synchronized when the neurons had similar mean phases. *B*, The correlation strength, measured as the correlation coefficient, between neurons as a function of difference in their mean phases. *C, D*, The same analyses as *A* and *B* but with only those MN pairs that produced at least 100 action potentials within 100 msec of one another.

have low correlation strengths (linear–circular correlation; see Materials and Methods; $D_n = 0.33$; $p < 0.001$).

Part of this weaker synchronization between out-of-phase neurons might simply reflect the fact that the neurons did not produce any action potentials close to one another, making it impossible to observe any synchronization even if it were present between the neurons. We therefore performed the same analyses but limited the set of neurons to only those pairs for which at least 100 individual action potentials in one neuron were accompanied within 100 msec by an action potential in the other neuron. As would be expected, this condition excluded a large number of those MN pairs that were activated in different portions of the cycle. However, even in the remaining pairs that produced a number of action potentials close to one another, only a small fraction of the pairs with different locomotor-related activity was synchronized (Fig. 4*C*). Similarly, strong correlations were still observed primarily between MN pairs with a similar locomotor-related activity (Fig. 4*D*). This relationship between correlation strength and difference in mean phase was again significant ($D_n = 0.43$; $p < 0.001$).

Neurons recorded on different tetrodes are synchronized less frequently

Synchronization between MNs within the same segment was observed for pairs recorded on the same tetrode as well as for pairs recorded on different tetrodes. However, synchronization between MN pairs on different electrodes was less common than that between pairs recorded on the same tetrode. Of MN pairs recorded on the same tetrode, 35 of 65 (54%) were synchronized, whereas 40 of 111 (36%) of pairs recorded on different tetrodes

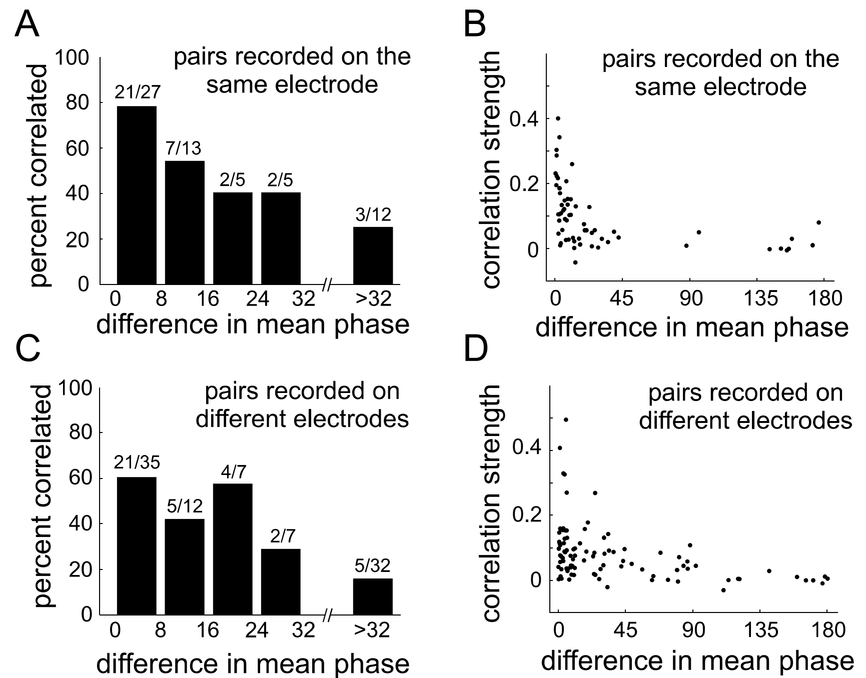

Figure 5. Synchronization is more common with more similar locomotor-related activity for MNs recorded on the same or on different tetrodes. *A*, *B*, Conventions same as Figure 4, *A* and *B*, except that only neuron pairs recorded on the same tetrode are included. *C*, *D*, Conventions same as Figure 4, *A* and *B*, except that only neuron pairs recorded on different tetrodes are included.

were synchronized ($\chi^2_{(1,\ n\ =\ 176)} = 5.3$; $p < 0.05$). However, this decrease was not observed for correlation strength: the correlation strength between neurons recorded on the same electrode was 0.09 ± 0.09, and it was 0.08 ± 0.10 for neurons recorded on different electrodes, an insignificant difference ($p > 0.05$). We also found that MNs recorded on the same tetrode tended to have more similar mean phases than pairs recorded on different tetrodes. The mean absolute phase difference between MN pairs recorded on the same tetrode was 21.4 ± 37.2° (mean ± angular dispersion) (Mardia 1972), whereas the mean phase difference between pairs recorded on different tetrodes was 35.4 ± 18.1°, a significant difference ($p < 0.05$; bootstrap test).

Relationships of synchronization to mean phase difference and to same/different tetrode are independent

This last observation, that neurons recorded on the same tetrode have similar locomotor-related activity, has potential implications for the results described previously. On the one hand, it might imply that the lower percentage of synchronization between neurons recorded on different tetrodes reflected the weaker correlation between neurons with larger differences in mean phase. Conversely, it might imply that the decrease in correlation strength with increasing difference in mean phase reflected the fact that neuron pairs with large differences in mean phase tended to be recorded on different tetrodes. The results shown in Figure 5, however, suggest that neither of these potential implications applied to the present data. First, it can be seen in Figure 5 that for pairs recorded either on the same or on different tetrodes, there was decreasing synchronization with increasing difference in mean phase, as assessed in the percentage of synchronized pairs (Fig. 5*A*,*C*) and in correlation strength (Fig. 5*B*,*D*) (D_n = 0.48 and 0.26 for same and different tetrodes; $p < 0.001$ for each). Second, comparison of Figure 5, *A* and *C*, shows that the percentage of synchronized pairs was generally larger for pairs recorded on the same tetrode across the range of differences in mean phase (Fig. 5*A*, percentages in each bin on the same tetrode: 78, 54, 40, 40, 25; Fig. 5*C*, percentages on different tetrodes: 60, 42, 57, 29, 16), although there was no clear difference in the relationship between correlation strength and mean phase for pairs recorded on the same or different tetrodes (Figs. 5*B*,*D*). Thus, it appears that synchronization strength between neurons is related to the similarity of their locomotor-related activity, independent of whether the neurons are recorded on the same tetrode, and that neurons recorded on different tetrodes are synchronized less commonly than neurons on the same tetrode, independent of the similarity of their locomotor-related activity.

Strong modulation predicts synchronization

In addition to the mean phase of a neuron, another parameter characterizing the locomotor-related activity of a neuron is its modulation strength, reflecting how well a given neuron is related to the ongoing rhythm. The modulation strength of a neuron is commonly characterized by its *R* value (see Materials and Methods) (Mardia, 1972). We found that MN synchronization was more common and stronger for neurons that were strongly modulated by the locomotor cycle. Figure 6*A* shows that increasing percentages of MN pairs were significantly correlated with increasing mean *R* values of the pairs. Similarly, Figure 6*B* shows that correlation strength increased with the mean *R* value of the pair ($r^2 = 0.13$; $p < 0.001$). This relationship between synchronization strength and mean *R* value also held when only neuron pairs for which at least 100 individual action potentials in one neuron were accompanied within 100 msec by an action potential in the other neuron were included in the analysis ($r^2 = 0.11$; $p < 0.01$; data not shown).

Synchronization is not related to characteristics of the ongoing locomotor activity

Finally, we examined the relationship between MN synchronization and various features of the locomotor pattern itself. We found no relationship between MN correlation strength and locomotor frequency ($r^2 = 0.0001$; $p > 0.05$), locomotor period ($r^2 = 0.0004$; $p > 0.05$), the variability in the locomotor period ($r^2 = 0.002$; $p > 0.05$), or the ventral root depth of modulation

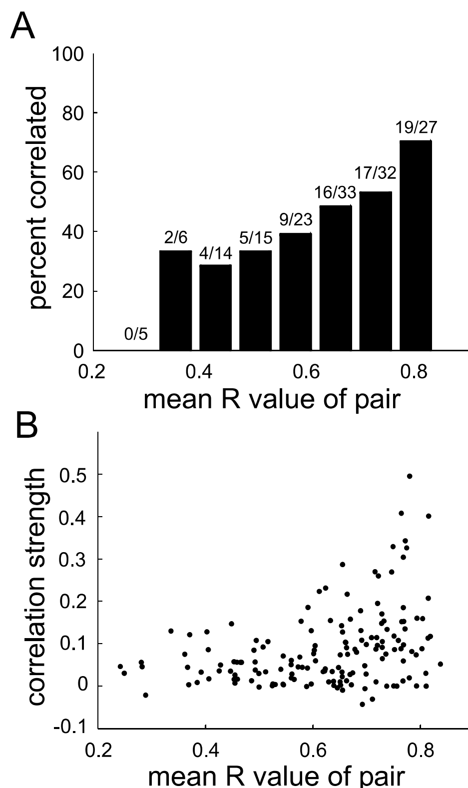

Figure 6. Synchronization is increased between neuron pairs with strong locomotor modulation. *A*, The percentage of MN pairs with significant correlation as a function of the mean *R* value, measuring neuronal modulation strength, of neurons in the pair. *B*, The correlation strength between MNs as a function of the mean *R* value. Only neuron pairs for which both neurons had a significant *R* value were included.

($r^2 = 0.01$; $p > 0.05$). Similarly, there was no clear relationship of any these quantities to the percentages of synchronized MN pairs observed.

Consideration of possible artifacts in cross-correlational analyses

One possible source of artifacts in the cross-correlograms could result from covariation in the excitability of neuron pairs (Brody, 1998, 1999). We addressed this concern by guaranteeing in the randomized spike trains that the number of spikes in each locomotor cycle was the same as in the original spike train. We also observed that synchronization peaks were faster than the locomotor-related modulation of neurons, that synchronization peaks in cross-correlations between neurons were dissimilar to the autocorrelations of neurons, and that integrals of cross-covariograms were generally small, conditions all arguing against effects from either covariation of excitation or of latency (Brody, 1998, 1999). Another source of artifact might result from the "shadowing" of the spike of one neuron by the spike of a second neuron, which can introduce features of the firing statistics of the second neuron to the firing statistics of the first neuron, thereby obscuring correlational analyses (Bar-Gad et al., 2001). However, we believe that this effect is minimal here because (1) synchronization was robustly observed between neurons on different tetrodes, (2) in cases not included here in which the overlapping effect was pronounced, no significant correlation peaks were observed outside of the central region, and (3) this effect is expected to be minimal for neurons with low firing rates such as those examined here. Another possible contaminant to the cross-correlations could result from a misattribution of low-amplitude action potentials, which tend to occur at the end of a burst, from one neuron to another neuron. This effect can introduce temporal correlations between neurons when none exist in reality (Quirk et al., 2001). Although we cannot entirely exclude such an effect here, we do not believe that it contributed substantially. For instance, the effect would be expected to be equally influential when tetrodes were placed in different segments as when different tetrodes were placed in the same segment. However, we observed that neuron pairs recorded in different segments were synchronized much less commonly than pairs recorded in the same segment (see below). More generally, and this applies to all concerns about artifactual contributions to cross-correlograms, the synchronization described here could be observed in individual spike trains and also could be observed directly in the clusters of activity in the raw ventral root recordings.

Synchronization between distant motor pools

Although many different mechanisms might be responsible for MN synchronization, one obvious mechanism for the local synchronization of MNs is the GJC between MNs (Kiehn and Tresch, 2002), which is anatomically restricted (Chang et al., 1999; Tresch and Kiehn, 2000a) and mainly between homonymous MNs (Walton and Navarrete, 1991). Synchronization mediated by gap junctions in the neonatal rat therefore would be expected to be restricted to MNs within the same motor pool. Contrary to this expectation, however, in the present experiments we also observed clear synchronization between the activity of distinct motor pools.

First, we found that the activity of motor pools innervating different muscles was synchronized. Recordings from peripheral nerves innervating the anatomical hip flexor IP and the anatomical knee extensor Q were synchronized regularly. During transmitter-induced locomotor activity, these muscles can fire in phase (Kiehn and Kjaerulff, 1996; Iizuka et al., 1997). Synchronization was observed in 7 of 12 locomotor runs in four animals in which these two muscles were activated in phase with one another. These results show that synchronization was not restricted to MNs innervating the same muscle.

Although these motor pools are not homonymous, the existence of GJC between these motor pools cannot be excluded entirely because the two MN pools are localized closely anatomically (both innervated by the intact L2 and L3 ventral roots) (Nicolopoulos-Stournaras and Iles, 1983). However, we also observed synchronization between these motor pools and the activity of motor pools located in distant segments. Synchronization between activity in the quadriceps muscle and the in-phase, extensor-related activity in the ipsilateral L5 ventral root was observed in 21 of 23 locomotor runs in five animals. Synchronization between activity in iliopsoas and in-phase, flexor-related activity in the ipsilateral L5 root was observed in 11 of 23 runs in five animals. Note that the activity of the L5 ventral root is predominantly extensor related, often making evaluation of the coupling between IP and L5 difficult. This long distance (2–3 mm or several segments) synchronization could be observed whether the locomotor activity was evoked by 5-HT and NMDA (Q-L5, 10 of 12; IP-L5, 5 of 12), by 5-HT alone (Q-L5, 3 of 3; IP-L5, 0 of 3), or by dopamine (Q-L5, 8 of 8; IP-L5, 6 of 8). An example of strong synchronization between Q and ipsilateral L5 is illustrated in Figure 7, *A* and *B*. Figure 7*C* shows that the in-phase activity recorded during locomotor activity (Kiehn et al., 1999, their Fig.

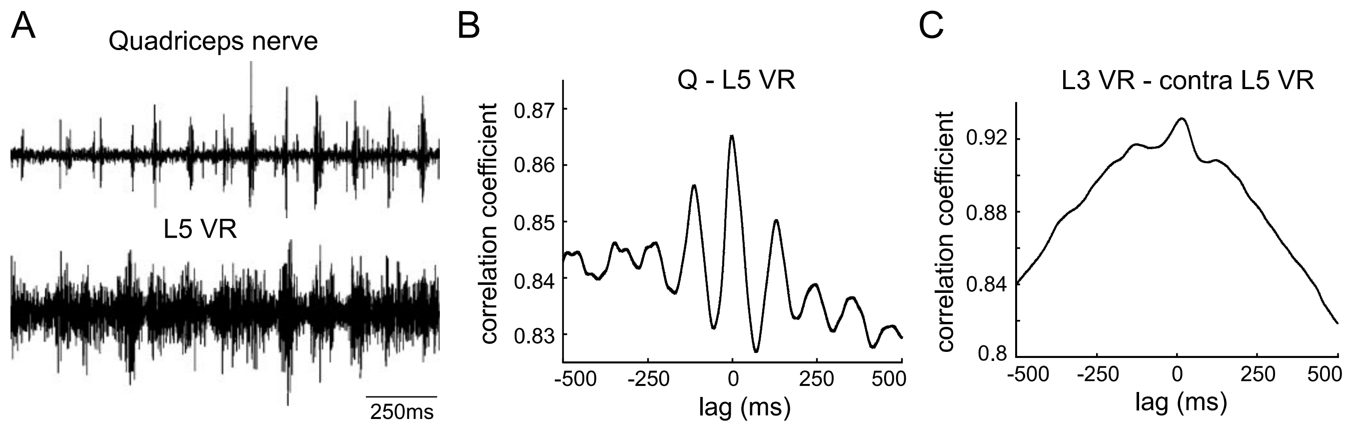

Figure 7. Distant MN pools are synchronized during locomotor activity. *A*, Recordings of activity in quadriceps peripheral nerve and the ipsilateral L5 ventral root (*L5 VR*) during one burst of locomotor activity. In this experiment, the L2 and L3 ventral roots were kept intact with their peripheral nerves to allow muscle nerve recordings. The clusters of discharge in the quadriceps nerve are aligned with clusters in the L5 ventral root. *B*, Cross-correlation between quadriceps (*Q*) and L5 ventral roots (*L5 VR*) shows clearly the coupling illustrated in *A*. *C*, The cross-correlation between L3 and contralateral L5 during locomotion, similarly showing a synchronization above that caused by common locomotor modulation (also see Figs. 8*A*, 9*B*).

1) in L3 and contralateral L5 ventral roots could also be synchronized (see also Fig. 9*B*). Such distant contralateral synchronization (between L2 or L3 and contralateral L5) was observed in 27 of 40 locomotor runs in nine animals. On the basis of existing knowledge of the anatomical and physiological extent of gap junctional coupling in the spinal cord, it seems unlikely that such coupling could be responsible for this distant synchronization of motor output.

Although prevalent during locomotor activity, the distant synchronization described above did not appear to be as robust as the synchronization within the same spinal segment. First, of the 79 MNs recorded at the same time as a distant contralateral ventral root, only 5 showed a significant cross-correlation with the distant ventral root discharge, and of 62 MN pairs recorded between neurons in different spinal segments (L5 and either contralateral L2 or L3), only 2 showed a significant cross-correlation. The tendency for synchronization between MNs to be more readily observable for nearby MNs is also consistent with the finding described previously that neuron pairs recorded on the same tetrode were more likely to be synchronized than pairs recorded on different tetrodes. The ability to observe synchronization in correlations between ventral roots likely results from the fact that such root recordings sample the activity of hundreds of neurons, allowing weak correlations across the population to be observed.

Mechanisms of MN synchronization

In a previous study, we showed that in the absence of chemical synapses, GJC between MNs is capable of coordinating the activity of local MN populations (Tresch and Kiehn, 2000a). The distant synchronization described in the previous section, however, suggests that mechanisms other than GJC contribute to the synchronization of MNs. One obvious mechanism is MN coordination by presynaptic spinal pattern-generating interneurons, mediated by chemical synapses. We therefore examined the differential roles of chemical and electrical synapses in the MN synchronization described above.

Distant MN coupling requires chemical synaptic transmission

We blocked chemical synaptic transmission by removing calcium from the perfusing bath (Johnson et al., 1994; Tresch and Kiehn, 2000a). As demonstrated previously, after such chemical synaptic transmission blockade, the local coupling between action poten-

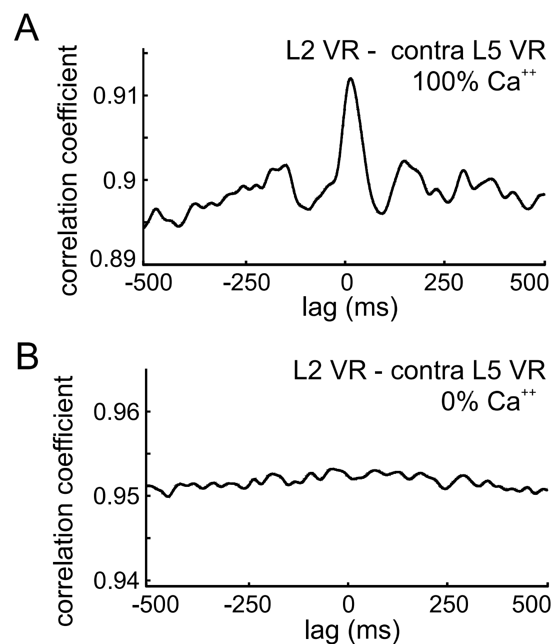

Figure 8. Distant synchronization between motor outputs requires chemical synaptic transmission. *A*, The cross-correlation between the L2 and contralateral L5 ventral roots during intact locomotor activity, showing a clear synchronization. *B*, The same cross-correlation after removal of calcium from the Ringer's solution for 45 min, blocking chemical synaptic transmission and also the synchronization in *A*.

tial activity in MNs within the same segment can persist after application of 5-HT/NMDA (Tresch and Kiehn, 2000a). However, after removal of calcium the fast rhythmic activity evoked on distant ventral roots was uncoupled (26 of 26 runs in eight animals). Figure 8 shows an example of distant coupling between L2 and contralateral L5 (Fig. 8*A*) that was abolished after removal of calcium (Fig. 8*B*). On occasion (3 of 26 runs in eight animals) we observed very slow modulations (period >10 sec) in ventral root activity that appeared to be coupled on different ventral roots, but correlations on the shorter time scales typical of the locomotor activity and synchronization described here were never observed. Thus, these observations suggest that, although not critical for the local synchronization of MNs, chemical syn-

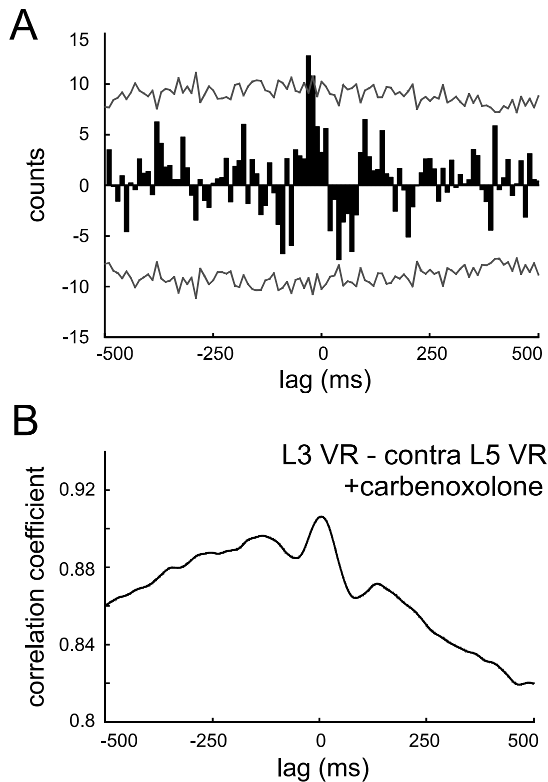

Figure 9. MN synchronization can be maintained after antagonism of gap junctions by carbenoxolone. *A*, An example of a covariogram between MNs during locomotor activity after 60 min of carbenoxolone showing significant synchronization. Conventions are the same as Figure 1. *B*, Cross-correlation between L3 and contralateral L5 ventral roots after 60 min of carbenoxolone, showing the persistent synchronization between these ventral roots. The data in *B* were taken from the same experiment as that shown in Figure 7*C*.

aptic transmission made a strong contribution to the distant coupling between motor outputs observed during locomotor activity.

MN synchronization persists after antagonism of GJC

We next examined whether chemical synapses were sufficient to mediate the synchronization between MNs after application of the gap junction antagonist carbenoxolone. Carbenoxelone is one among several gap-junction antagonists that in other experiments we have shown can uncouple NMDA-induced MN population oscillations observed after blocking action potentials by TTX (Tresch and Kiehn, 2000a). After application of carbenoxolone (100 μM) for at least 45 min and up to 2 hr, the quality of locomotor activity was consistently worse than that in baseline conditions (modulation depth 0.40 $\pm$ 0.13 baseline vs 0.25 $\pm$ 0.07 carbenoxolone; $p < 0.05$) [see also Tresch and Kiehn (2000a)]. This reduction in quality often made it difficult to obtain the long-lasting, stable activity required for the cross-correlation analyses performed here. In 8 of the 14 MN pairs in the same segment that we recorded after carbenoxolone application, significant synchronization was still observed. An example of a cross-correlation between MNs after application of carbenoxolone is shown in Figure 9*A*. The strength of the cross-correlations between MNs after carbenoxolone application was not significantly different from that without carbenoxolone (0.10 $\pm$ 0.07 with vs 0.09 $\pm$ 0.10 without carbenoxolone; $p > 0.05$). Figure 9*B* shows the cross-correlation between the L3 and contralateral L5 ventral root shown in Figure 7*C* after 1 hr of carbenoxolone application, demonstrating that distant coupling between ventral roots was also preserved in the presence of carbenoxlone (four of nine runs in eight rats). Thus, it appeared that after antagonism of GJC by carbenoxolone, both local and distant synchronization of MNs could still be observed.

Although many studies have reported no side effects of carbenoxelone on cellular properties (Kamermans et al., 2001; Kohling et al., 2001; Hughes et al., 2002), there have been reports of substantial effects (Rekling et al., 2000). In three motor neurons recorded for 2 hr in carbenoxolone (100 μM), we did not observe such substantial side effects on basic neuronal function. In particular, all neurons were capable of producing action potential responses to intracellular current injection. Recordings from commissural interneurons in the *in vitro* neonatal rat spinal cord have similarly not shown significant side effects from carbenoxolone (S. Butt and O. Kiehn, unpublished observations). Although we cannot exclude the possibility that carbenoxolone had other effects that would have been revealed with a larger sample of MNs, these data, along with the persistent MN synchronization described above and intrinsic MN oscillations described previously (Tresch and Kiehn, 2000a), suggest that carbenoxolone did not disrupt basic neuronal function in the neonatal rat spinal cord (see also Discussion). Because gap junction antagonists such as carbenoxelone might cause an incomplete block of GJC (Brivanlou et al., 1998), we monitored GJC after carbenoxolone application, using a collision protocol described elsewhere (Walton and Navarrete, 1991; Chang et al., 1999; Kiehn and Tresch, 2002). In all cases, carbenoxolone strongly antagonized the short-latency potential evoked by antidromic ventral root stimulation when it was observed, beginning at latencies of ~10–15 min ($n = 5$) and being maximal (reducing the potential by 50 and 80%) by 1 hr ($n = 2$; a third MN showed no clear coupling potential from ventral root stimulation). After application of carbenoxolone for 2 hr, in one neuron additional application of halothane (10 mM for 20 min), which has been shown to block gap junctional coupling in this preparation (Chang et al., 1999), did not further reduce any residual potential. Corresponding observations were obtained from experiments in commissural interneurons (Butt and Kiehn, unpublished observations). Together these results show that carbenoxolone strongly reduced the gap junctional coupling between MNs without affecting basic properties of individual MNs (see Discussion).

Oscillatory features of MN synchronization

As mentioned previously, the synchronization between MNs was often oscillatory. For instance, in Figure 2*A*, the clusters of MN action potentials were regularly spaced at ~100 msec intervals. Also, the smaller peaks to the left and right of the central correlation peak in Figure 1*C* indicate an ~100 msec interval oscillation in the synchronization between MNs. Such off-center peaks in cross-correlations were observed qualitatively in 32 of 75 (43%) of the synchronized MN pairs. The frequency of this oscillation was typically faster for rostral segments than for caudal segments, as observed in cross-correlations between MNs (L3, 11.51 $\pm$ 2.38 Hz; L5, 8.01 $\pm$ 1.91 Hz; $p < 0.001$) and in ventral root autocorrelations (L2, 10.22 $\pm$ 2.03 Hz; L3, 9.01 $\pm$ 0.60; L5, 7.65 $\pm$ 1.19 Hz; $p < 0.05$). A difference in the frequency of oscillations on different ventral roots has been described previously after blockade of action potential activity by TTX (Tresch and Kiehn, 2000a). Oscillations were also observed after carbenoxolone application, as can be seen from examination of Figure 9.

The higher frequency of rostral spinal segments was also preserved after carbenoxolone application (L2, 6.80 ± 1.74 Hz; L3, 6.88 ± 0.89 Hz; L5, 5.33 ± 0.92 Hz; $p < 0.05$). Thus, similar to the short-term synchronization described in previous sections, the presence and features of oscillatory activity in MNs appear not to be uniquely dependent on the coupling of MNs by gap junctions.

DISCUSSION

The results presented here demonstrate the robust synchronization of spinal MNs during locomotor activity in the neonatal rat. This synchronization was stronger and more common between neurons with similar locomotor-related activity and between MNs with strong rhythmic modulation. Synchronization was observed between distinct motor pools, located either in the same segment or in distant spinal segments. This distant coupling was abolished after chemical synaptic blockade, whereas both distant and local MN coupling were maintained after application of the GJC antagonist carbenoxolone. These results suggest that chemical synaptic transmission, in addition to GJC, plays an important role in MN synchronization in the neonatal rat.

Short time-scale synchronization of MNs during locomotor activity

Synchronization of MNs at a millisecond temporal resolution during locomotor activity was observed in fewer than half the MN pairs. This figure, however, approached 80% for pairs with strong locomotor modulation and similar locomotor-related activity. The robustness of this MN synchronization was also evident because it was observed during rhythmic motor outputs evoked by many neuroactive substances and could often be observed in raw MN spike trains (Fig. 1*A*).

There have been several indirect observations suggesting MN synchronization in the neonatal rat, mainly on the basis of observations of clusters of root activity (see Results). We confirmed that such clusters correspond to synchronization of individual MNs. A recent study in neonatal mice also demonstrated MN synchronization (Personius and Balice-Gordon, 2001), although that synchronization was much slower than that described here: the duration of central correlation peaks in mice was 1–3 sec, more similar to slow locomotor comodulation than to the faster ~30–100 msec peaks described here (Fig. 1). Although reasons for these differences are unclear, the results of both studies demonstrate the robust synchronization of MNs in the developing spinal cord.

Predictors of MN synchronization

We found that MN synchronization was not randomly distributed but could be predicted by features of locomotor-related neuronal activity. First, synchronization was likelier and stronger between neurons with similar locomotor-related activity. This observation suggests that synchronization was related to behavioral roles of MNs in locomotion. This result is also consistent with proposals that synchronization plays a role in coordinating functionally related motor pools during behavior or in the developmental specification of motor systems (see below).

Synchronization was stronger between neurons strongly modulated during locomotor activity. One interpretation of this finding is that synchronization is a signature of neurons closely tied to locomotor networks, with reduced synchronization between weakly recruited or modulated MNs. Alternatively, this reduced modulation might reflect a general insensitivity of a neuron to external synaptic inputs, whether they be inputs responsible for locomotor-related slow modulation or for faster synchronization. For instance, a general insensitivity could result from a sustained depolarization, causing the action potential activity of a neuron to be dominated by intrinsic membrane potential dynamics rather than extrinsic synaptic inputs (Mainen and Sejnowski, 1995; Beierholm et al., 2001).

Neurons recorded on the same tetrode and presumably located nearby were more likely to be synchronized than neurons recorded on different tetrodes and therefore presumably anatomically distant. This result is consistent with other findings reported here of weaker synchronization between distant motor pools. The preferential synchronization of nearby MNs could reflect properties of either chemical or electrical synapses to MNs, both of which are focused predominantly on nearby MNs (Puskar and Antal, 1997; Chang et al., 1999). Coupling between MNs, whether mediated by chemical or electrical synapses, would therefore be expected to be weaker for distant MNs.

Finally, we found no relationship between synchronization and characteristics of the locomotor pattern. Although a lack of correlation to locomotor frequency has been reported (Hansen et al., 2001), one might have expected neuronal synchronization to be related to locomotor quality, as measured by modulation depth or period variability, if synchronization contributed to locomotor production. This lack of correlation might suggest that MN synchronization is not uniquely coupled to locomotor networks but is a more basic feature of spinal motor systems. However, our characterization of locomotor quality using ventral root recordings has drawbacks because such recordings combine activity across several motor pools (Cowley and Schmidt, 1994a). Also, variations in these locomotor parameters resulted from uncontrolled differences between locomotor runs or between different animals. Experiments monitoring individual motor pools or systematically inducing variations in locomotor parameters might reveal relationships missed here.

Mechanisms of MN synchronization

An important finding of this study is the existence of multiple mechanisms underlying MN synchronization, with both GJC and chemical synapses contributing. In particular, distant synchronization between motor pools was abolished after chemical synaptic antagonism, whereas local MN coupling persisted. On the other hand, both local and distant MN coupling persisted after GJC antagonism. In previous work we demonstrated that local MN coupling is abolished after antagonism of both chemical and electrical synapses (Tresch and Kiehn, 2000a). These results suggest that chemical synapses are necessary for distant coupling of motor pools, whereas both chemical and GJC contribute to local coupling. The relative contribution of chemical and electrical synapses to local synchronization is unclear. For instance, we observed no change in MN correlation strength after gap junction antagonism, suggesting minimal contributions from GJC. However, after chemical synapse blockade, rhythmic motor activity could still be evoked (Tresch and Kiehn, 2000a), clearly indicating that electrical synapses contribute to local MN synchronization. Determination of the quantitative contributions of chemical and electrical synapses to MN synchronization during normal locomotion will require further research.

The chemical synapses contributing to MN synchronization might come from synchronized activity in presynaptic interneurons that provide a common input to multiple MNs via branching axons (Kirkwood 1995; Matsumura et al., 1996). Such common input need not be especially strong because postsynaptic properties of MNs, such as intrinsic oscillatory properties (MacLean et

al., 1997; Tresch and Kiehn, 2000a), will also help to regularize and synchronize MN spike activity (Mann-Metzer and Yarom, 1999). All of these mechanisms, chemical and electrical synapses along with intrinsic MN properties, might act complementarily to produce the synchronization described here.

This result, of multiple mechanisms underlying MN synchronization in neonatal rats, differs from results in neonatal mice (Personius and Balice-Gordon, 2001), in which systemic carbenoxolone abolished MN synchronization. As mentioned above, however, the characteristics of synchronization described in that study differed substantially from the synchronization observed here, possibly suggesting that the types of synchronization examined were distinct, with distinct mechanisms. Methodological differences or differences between behaviors examined might also have contributed. Although explanations for the differences between these studies are unclear, previous observations also suggest that MN synchronization during locomotion is not exclusively dependent on GJC. In particular, coupling between clusters of activity on distant roots has been described briefly (Cowley and Schmidt, 1995). Furthermore, such clusters are observed during locomotion in 2-week-old animals (Westerga and Gramsbergen, 1993, 1994), when electrical GJC between MNs is undetectable. These results, combined with those described here, strongly suggest a role for chemical synaptic transmission in fast MN synchronization.

Some of the evidence for a role of chemical synapses in MN synchronization relied on carbenoxolone antagonism of GJC. Although carbenoxolone has been reported to affect basic neuronal function (Rekling et al., 2000), we did not observe such substantial effects, in contrast to other gap junction blockers such as heptanol and octanol (Tresch and Kiehn, 2000a). Also, although carbenoxolone has been shown to strongly antagonize GJC (Davidson and Baumgarten, 1988; Rekling et al., 2000), this block appeared to be only partial. However, in previous work such a block was strong enough to abolish MN coupling after TTX application (Tresch and Kiehn, 2000a). The maintenance of MN synchronization after such reduction, even if incomplete, strongly suggests that fast synchronization was not uniquely dependent on GJC. Finally, we emphasize that a role for chemical synapses in MN synchronization is not supported solely by experiments with carbenoxolone but is also supported by observations of distant coupling between motor pools and its abolition after chemical synaptic blockade.

Potential roles of MN synchronization

MN synchronization has been proposed to mediate synapse elimination during neuromuscular junction development (Busetto et al., 2000; Chang and Balice-Gordon, 2000). The fast synchronization described here is clearly amenable to this role, because the mechanisms of synaptic plasticity often proposed to mediate elimination occur between action potentials separated by tens of milliseconds (Markram et al., 1997; Bi and Poo, 2001). MN synchronization has also been proposed to contribute to developmental refinement of central synapses (O'Donovan et al., 1998; Chang and Balice-Gordon, 2000). The present finding of coupling between distant motor pools, although difficult to interpret in the context of neuromuscular junction development, is consistent with this latter role in the development of spinal networks, as is the finding that synchronization was most often observed between MNs with common locomotor-related activity.

This preferential coupling of similarly activated neurons could also reflect a role for synchronization in the production of behavior. Synchronization might mediate the coordination, or "binding," of individual MNs into the global motor patterns underlying behavior (Welsh and Llinas, 1997; Farmer, 1998; Baker et al., 1999). Evaluating such a contribution of MN synchronization will require examination of synchronization patterns between different muscles in this preparation. The present results further suggest that MN synchronization described in adults and normally ascribed to descending systems might have strong contributions from intrinsic spinal mechanisms.

REFERENCES

Baker SN, Kilner JM, Pinches EM, Lemon RN (1999) The role of synchrony and oscillations in the motor output. Exp Brain Res 128:109–117.

Bar-Gad I, Ritov Y, Vaadia E, Bergman H (2001) Failure in identification of overlapping spikes from multiple neuron activity causes artificial correlations. J Neurosci Methods 107:1–13.

Beierholm U, Nielsen CD, Ryge J, Alstrom P, Kiehn O (2001) Characterization of reliability of spike timing in spinal interneurons during oscillating inputs. J Neurophysiol 86:1858–1868.

Bi G, Poo M (2001) Synaptic modification by correlated activity: Hebb's postulate revisited. Annu Rev Neurosci 24:139–166.

Brivanlou IH, Warland DK, Meister M (1998) Mechanisms of concerted firing among retinal ganglion cells. Neuron 20:527–539.

Brody CD (1998) Slow covariations in neuronal resting potentials can lead to artifactually fast cross-correlations in their spike trains. J Neurophysiol 80:3345–3351.

Brody CD (1999) Correlations without synchrony. Neural Comput 11:1537–1551.

Busetto G, Buffelli M, Tognana E, Bellico F, Cangiano A (2000) Hebbian mechanisms revealed by electrical stimulation at developing rat neuromuscular junctions. J Neurosci 20:685–695.

Cazalets JR, Grillner P, Menard I, Cremieux J, Clarac F (1990) Two types of motor rhythm induced by NMDA and amines in an in vitro spinal cord preparation of neonatal rat. Neurosci Lett 111:116–121.

Chang Q, Balice-Gordon RJ (2000) Gap junctional communication among developing and injured motor neurons. Brain Res Brain Res Rev 32:242–249.

Chang Q, Gonzalez M, Pinter MJ, Balice-Gordon RJ (1999) Gap junctional coupling and patterns of connexin expression among neonatal rat lumbar spinal motor neurons. J Neurosci 19:10813–10828.

Cowley KC, Schmidt BJ (1994a) Some limitations of ventral root recordings for monitoring locomotion in the in vitro neonatal rat spinal cord preparation. Neurosci Lett 171:142–146.

Cowley KC, Schmidt BJ (1994b) A comparison of motor patterns induced by *N*-methyl-D-aspartate, acetylcholine and serotonin in the in vitro neonatal rat spinal cord. Neurosci Lett 171:147–150.

Cowley KC, Schmidt BJ (1995) Effects of inhibitory amino acid antagonists on reciprocal inhibitory interactions during rhythmic motor activity in the in vitro neonatal rat spinal cord. J Neurophysiol 74:1109–1117.

Davidson JS, Baumgarten IM (1988) Glycyrrhetinic acid derivatives: a novel class of inhibitors of gap-junctional intercellular communication. Structure-activity relationships. J Pharmacol Exp Ther 246:1104–1107.

Eggermont JJ (1992) Neural interaction in cat primary auditory cortex. Dependence on recording depth, electrode separation, and age. J Neurophysiol 68:1216–1228.

Engel AK, Konig P, Kreiter AK, Schillen TB, Singer W (1992) Temporal coding in the visual cortex: new vistas on integration in the nervous system. Trends Neurosci 15:218–226.

Farmer SF (1998) Rhythmicity, synchronization and binding in human and primate motor systems. J Physiol (Lond) 509:3–14.

Feller MB (1999) Spontaneous correlated activity in developing neural circuits. Neuron 22:653–656.

Fulton BP, Miledi R, Takahashi T (1980) Electrical synapses between motoneurons in the spinal cord of the newborn rat. Proc R Soc Lond B Biol Sci 208:115–120.

Hansen NL, Hansen S, Christensen LO, Petersen NT, Nielsen JB (2001) Synchronization of lower limb motor unit activity during walking in human subjects. J Neurophysiol 86:1266–1276.

Hochman S, Schmidt BJ (1998) Whole cell recordings of lumbar motoneurons during locomotor-like activity in the in vitro neonatal rat spinal cord. J Neurophysiol 79:743–752.

Hughes SW, Blethyn KL, Cope DW, Crunelli V (2002) Properties and origin of spikelets in thalamocortical neurones in vitro. Neuroscience 110:395–401.

Iizuka M, Kiehn O, Kudo N (1997) Development in neonatal rats of the sensory resetting of the locomotor rhythm induced by NMDA and 5-HT. Exp Brain Res 114:193–204.

Johnson SM, Smith JC, Funk GD, Feldman JL (1994) Pacemaker be-

havior of respiratory neurons in medullary slices from neonatal rat. J Neurophysiol 72:2598–2608.
Kamermans M, Fahrenfort I, Schultz K, Janssen-Bienhold U, Sjoerdsma T, Weiler R (2001) Hemichannel-mediated inhibition in the outer retina. Science 292:1178–1180.
Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133–1138.
Kiehn O, Kjaerulff O (1996) Spatiotemporal characteristics of 5-HT and dopamine-induced rhythmic hindlimb activity in the in vitro neonatal rat. J Neurophysiol 75:1472–1482.
Kiehn O, Tresch MC (2002) Gap junctions and motor behavior. Trends Neurosci 25:108–115.
Kiehn O, Johnson BR, Raastad M (1996) Plateau properties in mammalian spinal interneurons during transmitter-induced locomotor activity. Neuroscience 75:263–273.
Kiehn O, Sillar KT, Kjaerulff O, McDearmid JR (1999) Effects of noradrenaline on locomotor rhythm-generating networks in the isolated neonatal rat spinal cord. J Neurophysiol 82:741–746.
Kirkwood PA (1995) Synaptic excitation in the thoracic spinal cord from expiratory bulbospinal neurones in the cat. J Physiol (Lond) 484:201–225.
Kjaerulff O, Kiehn O (1996) Distribution of networks generating and coordinating locomotor activity in the neonatal rat spinal cord in vitro: a lesion study. J Neurosci 16:5777–5794.
Kohling R, Gladwell SJ, Bracci E, Vreugdenhil M, Jefferys JG (2001) Prolonged epileptiform bursting induced by 0-Mg(2+) in rat hippocampal slices depends on gap junctional coupling. Neuroscience 105:579–587.
MacLean JN, Schmidt BJ, Hochman S (1997) NMDA receptor activation triggers voltage oscillations, plateau potentials and bursting in neonatal rat lumbar motoneurons in vitro. Eur J Neurosci 9:2702–2711.
Mainen ZF, Sejnowski TJ (1995) Reliability of spike timing in neocortical neurons. Science 268:1503–1506.
Mann-Metzer P, Yarom Y (1999) Electrotonic coupling interacts with intrinsic properties to generate synchronized activity in cerebellar networks of inhibitory interneurons. J Neurosci 19:3298–3306.
Mardia K (1972) Statistics of directional data. London: Academic.
Mardia K (1976) Linear-circular correlation coefficients and rhythmometry. Biometrika 63:203–206.
Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence detection of postsynaptic APs and EPSPs. Science 275:213–215.
Matsumura M, Chen D, Sawaguchi T, Kubota K, Fetz EE (1996) Synaptic interactions between primate precentral cortex neurons revealed by spike-triggered averaging of intracellular membrane potentials in vivo. J Neurosci 16:7757–7767.
Molotchnikoff S, Aitoubah J, Bretzner F, Shumikhina S, Tan YF, Guillemot JP (2001) Comparative computations of spike synchronization in visual cortex of cats. Brain Res Brain Res Protoc 6:148–158.
Nicolopoulos-Stournaras S, Iles JF (1983) Motor neuron columns in the lumbar spinal cord of the rat. J Comp Neurol 217:75–85.
Nordstrom MA, Fuglevand AJ, Enoka RM (1992) Estimating the strength of common input to human motoneurons from the cross-correlogram. J Physiol (Lond) 453:547–574.
O'Donovan MJ, Chub N, Wenner P (1998) Mechanisms of spontaneous activity in developing spinal networks. J Neurobiol 37:131–145.
Perkel DH, Gerstein GL, Moore GP (1967) Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. Biophys J 7:419–440.
Personius KE, Balice-Gordon RJ (2001) Loss of correlated motor neuron activity during synaptic competition at developing neuromuscular synapses. Neuron 31:395–408.
Puskar Z, Antal M (1997) Localization of last-order premotor interneurons in the lumbar spinal cord of rats. J Comp Neurol 389:377–389.
Quirk MC, Blum KI, Wilson MA (2001) Experience-dependent changes in extracellular spike amplitude may reflect regulation of dendritic action potential back-propagation in rat hippocampal pyramidal cells. J Neurosci 21:240–248.
Raastad M, Kiehn O (2000) Spike coding during locomotor network activity in ventrally located neurons in the isolated spinal cord from neonatal rat. J Neurophysiol 83:2825–2834.
Raastad M, Johnson BR, Kiehn O (1996) The number of postsynaptic currents necessary to produce locomotor-related cyclic information in neurons in the neonatal rat spinal cord. Neuron 17:729–738.
Rekling JC, Shao XM, Feldman JL (2000) Electrical coupling and excitatory synaptic transmission between rhythmogenic respiratory neurons in the pre-Botzinger complex. J Neurosci 20:RC113(1–5).
Softky WR, Koch C (1993) The highly irregular firing of cortical cells is inconsistent with temporal integration of random EPSPs. J Neurosci 13:334–350.
Stevens CF, Zador AM (1998) Input synchrony and the irregular firing of cortical neurons. Nat Neurosci 1:210–217.
Tresch MC, Kiehn O (1999) Coding of locomotor phase in populations of neurons in rostral and caudal segments of the neonatal rat lumbar spinal cord. J Neurophysiol 82:3563–3574.
Tresch MC, Kiehn O (2000a) Motor coordination without action potentials in the mammalian spinal cord. Nat Neurosci 3:593–599.
Tresch MC, Kiehn O (2000b) Redundant mechanisms underlying synchronization of motor neuron activity in the neonatal rat. Soc Neurosci Abstr 26:689.
Walton KD, Navarrete R (1991) Postnatal changes in motoneurone electrotonic coupling studied in the in vitro rat lumbar spinal cord. J Physiol (Lond) 433:283–305.
Welsh JP, Llinas R (1997) Some organizing principles for the control of movement based on olivocerebellar physiology. Prog Brain Res 114:449–461.
Westerga J, Gramsbergen A (1993) Changes in the electromyogram of two major hindlimb muscles during locomotor development in the rat. Exp Brain Res 92:479–488.
Westerga J, Gramsbergen A (1994) Development of the EMG of the soleus muscle in the rat. Brain Res Dev Brain Res 80:233–243.