Spike Coding During Locomotor Network Activity in Ventrally Located Neurons in the Isolated Spinal Cord From Neonatal Rat

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Raastad, Morten and Ole Kiehn. Spike coding during locomotor network activity in ventrally located neurons in the isolated spinal cord from neonatal rat. J. Neurophysiol. 83: 2825–2834, 2000. To characterize spike coding in spinal neurons during rhythmic locomotor activity, we recorded from individual cells in the lumbar spinal cord of neonatal rats by using the on-cell patch-clamp technique. Locomotor activity was induced by N-methyl-D-aspartate (NMDA) and 5-hydroxytryptamine (5-HT) and monitored by ventral root recording. We made an estimator based on the assumption that the number of spikes arriving during two halves of the locomotor cycle could be a code used by the neuronal network to distinguish between the halves. This estimator, termed the spike contrast, was calculated as the difference between the number of spikes in the most and least active half of an average cycle. The root activity defined the individual cycles and the positions of the spikes were calculated relative to these cycles. By comparing the average spike contrast to the spike contrast in noncyclic, randomized spike trains we found that approximately one half the cells (19 of 42) contained a significant spike contrast, in noncyclic, randomized spike trains we found that approximately one half the cells (19 of 42) contained a significant spike contrast, averaging 1.25 ± 0.23 (SE) spikes/cycle. The distribution of spike contrasts in the total population of cells was exponential, showing that weak modulation was more typical than strong modulation. To investigate if this low spike contrast was misleading because a higher spike contrast averaged out by occurring at different positions in the individual cycles we compared the spike contrast obtained from the average cycle to its maximal value in the individual cycles. The value was larger (3.13 ± 0.25 spikes) than the spike contrast in the average cycle but not larger than the spike contrast in the individual cycles of a random, noncyclic spike trains (3.21 ± 0.21 spikes). This result suggested that the important distinction between cyclic and noncyclic cells was only the repeated cycle position of the spike contrast and not its magnitude. Low spike frequencies (5.2 ± 0.82 spikes/cycle, that were on average 3.5 s long) and a minimal spike interval of 100–200 ms limited the spike contrast. The standard deviation (SD) of the spike contrast in the individual neurons was similar to the average spike contrasts and was probably stochastic because the SDs of the simulated, noncyclic spike trains were also similar. In conclusion we find a highly distributed and variable locomotor related cyclic signal that is represented in the individual neurons by very few spikes and that becomes significant only because the spike contrast is repeated at a preferred phase of the locomotor cycle.

INTRODUCTION

The nervous system relies on differences in the pattern of action potentials to distinguish between events. Which differences are important for network function is largely unknown, but the number of spikes can almost certainly be used in a neuronal code. Thus two events can be distinguished because a different number of spikes occur during those two events. Here we investigate the ability to distinguish between two halves of a locomotor cycle based on the number of spikes in each half.

The spike patterns we study are produced by neurons located in laminae X and VIII and the ventromedial part of lamina VII of the isolated spinal cord of the newborn rat. Many neurons in these areas are rhythmically active during transmitter-induced locomotor activity (Kiehn et al. 1996; McLean et al. 1996) and lesion studies have shown that these areas are necessary and sufficient for the creation of locomotor rhythms (Kiehn and Kjaerulf 1998; Kjaerulf and Kiehn 1996). The rhythm-generating mechanisms, however, are unknown for the neonatal rat as well as other mammals. The neurons we study can therefore not be assigned to functional groups, but the cyclic modulation of their spike patterns implies that they could participate in rhythm generation or relay information about the locomotor activity to supraspinal areas.

The neonatal rat preparation is well suited to investigate locomotor activity because it can survive in vitro for extended periods. Moreover, the pattern of locomotion induced by neuro-active drugs closely resembles the pattern of motor activity in the intact animal, with alternation between left and right side and a precise timing of different extensors and flexors in the hindlimb (Cowley and Schmidt 1994; Kiehn and Kjaerulf 1996). Therefore it is likely that the network activity in this still immature and isolated spinal cord has many features in common with the activity that actually controls the motor behavior in intact adult animals.

There were two main motivations to investigate spike coding during locomotor rhythm. First, the spike patterns representing motor acts, like the locomotor rhythms, are less well understood and studied than the spike representations of sensory signals (Borst and Theunissen 1999; Rieke et al. 1997).

Second, a locomotor cycle in mammals usually has longer duration than most signals for which spike codes have been studied. Typically, spikes have been recorded during 10–100 ms for sensory signals of similar duration (Rieke et al. 1997). These time windows are in the order of a typical membrane time constant, making integration of the spike-elicited postsynaptic potentials feasible by the passive membrane properties alone. A common conclusion from such experiments is that relevant signals are represented by small variations in spike counts in a large population of neurons (Georgopoulos et al. 1986; Wilson and McNaughton 1994).

However, the long duration of the locomotor-related cyclic signal (in our preparation 1–3 s) challenges these integration mechanisms. The postsynaptic effect of the spikes will be remembered for only a small fraction of the cycle by the
passive membrane properties alone. Slow membrane currents may in some animals solve this integration problem and even help discriminate between rhythmic patterns (Hooper 1998). Although neurons with long-lasting intrinsic membrane currents are present in the neonatal rat (Kiehn et al. 1996), they constitute a small proportion of the rhythmically active neurons.

Another possibility is that more spikes are used to represent long-lasting compared with a short-lasting signals, to fill the information gaps that would otherwise occur. However, previous results from the neonatal spinal cord showed that the cyclic locomotor-related frequency modulation of spike trains is typically weak and distributed over a large proportion (40–60%) of the cells (Kiehn et al. 1996; Tresch and Kiehn 1999). We therefore decided to quantify the locomotor-related spike modulations to find how many spikes were used in the individual cells to make one half cycle different from the other. The difference between the average number of spikes in the most and least active half cycle, which we term the average spike contrast, was compared with the spike contrast that would appear by chance at a fixed position in the cycle and at all positions in the cycles.

**METHODS**

**Preparation**

The preparation has been described previously in detail (Kiehn et al. 1996; Kudo and Yamada 1987; Smith and Feldman 1987). Briefly, neonatal rats (age, 0–3 days) were deeply anesthetized with ether, decapitated, and eviscerated. The spinal cord extending from C1 to L6, including ventral and dorsal roots, was isolated. The preparation was transferred to a recording chamber and superfused with oxygenated (95% O2–5% CO2) Ringer solution composed of (in mM) 128 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.25 MgSO4, 2.5 CaCl2, and 20 glucose (pH 7.4). Experiments were performed at room temperature.

**Induction of rhythmic activity**

Locomotor-related rhythms are readily induced in the isolated neonatal rat spinal cord by a variety of transmitter agonists (Cazalets et al. 1992; Cowley and Schmidt 1994; Kiehn and Kjaerulff 1996; Kudo and Yamada 1987) and can be monitored by recording the spike activity from the ventral roots. We induced locomotor rhythm by bath application of N-methyl-D-aspartic acid (NMDA; 4–9 μM) in combination with 5-hydroxytryptamine (5-HT; 4–10 μM).

**Recordings**

The L2 and L4 ventral root activities, corresponding to flexor and extensor activity respectively (Kiehn and Kjaerulff 1996), were recorded by using suction electrodes. The experiments were accepted only if there was an alternating activity between these roots. Because small neurons and neurons with very low activity may be missed by using the traditional extracellular single unit recording methods, we recorded spikes with the on-cell patch clamp technique. The patch electrodes had a resistance of 5–10 MΩ. The pipette solution contained (in mM) 128 potassium gluconate and 10 HEPES. This pipette solution was used to have the opportunity to record intracellularly after the extracellular recording period (data not included here). The pH was adjusted to 7.3 with KOH.

The recorded cells were located in segment L2 or L4 in the two ventromedial quadrants of the gray matter <300 μm below the ventral surface (lamina X and VIII and ventromedial part of lamina VII). The density of motor neurons is low in this region, which makes it unlikely but not impossible that some neurons recorded from were motor neurons. Before advancing the electrode into the cord a slit was made with a fine needle over the segment recorded from. The slit usually extended from the surface to the gray matter.

The procedure for finding cells was identical to that described in Kiehn et al. (1996), except that here we accepted seal resistance down to ~300 MΩ. Cells were searched for after the induction of locomotion activity and spike activity was recorded in “on-cell” voltage-clamp configuration. All cells were tested by comparing the average spike frequencies when the (external) holding potential was changed from 0 to ~50 mV. Only cells without significant change in spike frequency during this procedure were included in the study. In this way we ensured that the natural firing was not influenced by the recording procedure.

Recordings were performed with an Axopatch 1-D amplifier and data were stored on a digital tape recorder (Biologic 1800) for offline analysis. The signals were low-pass filtered at 2–5 kHz and digitized at 5 kHz.

**Detection of significant cyclic signals**

The average cycle-related spike pattern was estimated in the following way. First, the time position of the maximal root activity in each cycle was determined after the root records had been rectified and integrated. Second, the time positions of the spikes were measured relative to the preceding and following root bursts.

**Statistics**

If nothing else is indicated values are given as means ± SE and means compared using Student t-test. We considered the difference significant if P < 0.05.

**RESULTS**

In 11 rats we recorded 42 cells fulfilling the criteria listed in METHODS.

**Cyclic locomotor related signal**

The bottom trace in Fig. 1A shows the rhythmic spike activity in the L2 ventral root induced by bath application of NMDA and 5-HT (Kiehn and Kjaerulff 1996). The top trace shows an on-cell voltage-clamp recording from a neuron where the spike activity is seen as fast downward deflections. Because we knew that some neurons fired infrequently (Tresch and Kiehn 1999) and could therefore easily be missed by conventional extracellular unit recordings, we used the on-cell patch-clamp technique. Thereby we also eliminated the possibility of recording from more than one neuron at a time. Between one half and one third of the membranes with which the electrode formed a seal showed online detectable spikes. The identity of the nonspiking membranes remains unknown but they could represent silent neurons, glia cells, or nonneuronal structures.

The cell in Fig. 1A had a rhythmic pattern with a concentration of spikes in periods with low root activity in the ipsilateral L2 ventral root (bottom trace). The spike positions in consecutive cycles can be better seen in a raster plot (Fig. 1B) for the cell in Fig. 1A and three additional cells. Each small line in the raster plot marks the time of occurrence of a spike. Each cycle is normalized so each spike is plotted at its relative position between the maxima of two consecutive root bursts. The cycle length was relatively constant in all experiments [coefficient of variation (CV): 9 ± 2%] so the normalization had only a small effect on the density of
spikes in the individual cycles. The spike activity in these four cells was representative for the population of recorded cells. Some cells showed a cyclic signal that was obvious (Fig. 1B, 2 leftmost cells), whereas others had no clear cyclic signal (Fig. 1B, 2 rightmost cells).

We estimated the number of spikes that could be used to distinguish a neuron’s most and least active halves over a time window determined by the locomotor period recorded in the root. The resulting spike contrast will therefore not depend on whether spikes were added or subtracted from a background activity. This property of the estimator was intended because an earlier investigation showed that the cyclic signal could be mediated both through inhibitory and excitatory synaptic transmission (Raastad et al. 1997).

Figure 1C shows histograms of the average spike count during locomotion in 10 time bins of equal duration for the same cells as in Fig. 1B. The most and least active cycle halves used to estimate the spike contrast are illustrated below the histograms. The transition between the least and most active cycle halves was found simply by trying out all 10 possibilities thereby finding the maximal spike contrast. The resulting transition points are marked $\theta$ in this figure and referred to as the

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**FIG. 1.** A: action potentials recorded with on-cell tight-seal technique from an interneuron in L2 lumbar segment (top) and simultaneous recording of spike activity in L2 ventral root (bottom). Firing frequency of neuron was modulated in correlation with spike activity in root. B: raster plots of spike times relative to preceding and following maximal root activity in 4 interneurons. Each small line represents a spike and each row of spikes represents 1 normalized cycle. C: histograms of number of spikes in 10 time bins, each representing 1/10 cycle. Total number of spikes in each bin was divided by number of sampled cycles. SE is added as a vertical bar on top of bins. Spike contrast was calculated as difference in spike count in least and most active cycle halves. Most and least active cycle halves are indicated below histograms. Transition from low to high activity ($\theta$) is considered cycle position where maximal spike contrast in average cycle occurred. D: expanded section (16 s) of 5 locomotor cycles from same neuron as in A, illustrating that individual cycles contain relatively few and variable number of spikes and that information about the root activity (dotted lines) is important for evaluation of cyclic activity.
cycle position of the spike contrast in the rest of this article. Graphic illustrations of the spike contrasts and the cycle positions are shown below the histograms.

Figure 1D shows an expanded section of the record in Fig. 1A with the maximal root activity marked with dotted lines and is made to illustrate two important points. First, the information about the cycle is not clear when only one or a few cycles are observed. Second, knowing the frequency of the root activity (dotted lines) is important for the evaluation of the cyclic information. We will address these issues in the next paragraphs.

Quantification of the cyclic signal

We first estimated the spike contrast by determining the difference between the most and least active average cycle and comparing it with the spike contrast appearing by chance in a spike train with the same interspike intervals (ISIs) in random order. The spike contrast in the individual cycles was measured at the cycle position that divided the average cycle (θ in histograms Fig. 1C) in the most and least active halves. It is seen in Fig. 2A that the cell (same as left cell in Fig. 1, B and C) has a spike contrast in the individual cycles (black bars) that fluctuates mainly between one and four spikes. When the ISIs appear in random order, the spike contrast fluctuates around zero but with a small predominance of positive values.

The distribution of spike contrasts can be better evaluated in the cumulative plots in Fig. 2B, showing data from the same four cells as shown in Fig. 1, B and C. The difference between the random (thin line) and the original spike contrasts (circles)

FIG. 2.  A: spike contrast in 70 consecutive cycles of leftmost cell from Fig. 1B and (bottom) spike contrast in 70 cycles when interspike intervals (ISIs) were randomized, abolishing the cyclic signal (see text for details). B: cumulative plots of spike contrast obtained in original recordings (thin lines, corresponding to the 4 cells in Fig. 1B) and spike contrast after randomizing the ISIs (circles). A small bias toward positive values appear in randomized spike trains (explained in text) which is subtracted from original spike contrasts to give net spike contrast shown as filled bars in C. Arrow: division between most and least modulated cells used in analysis in text. Asterisk: those that were significant on a Kolmogorov-Smirnov test with P < 0.05. Some of spike contrasts to right of this arrow were positive (13) and some negative (9) as expected in a population with mean close to zero. Distribution is well described by an exponential function (dotted line). D: exponential function is indicated by a continuous line together with SDs of spike contrasts. E: SD in the 20 most modulated cells correlated with SD obtained in the same cells when spike intervals were randomized. Mean SD from randomized spike trains was not significantly different from mean SD of original spike trains, but correlation plot uncovered a systematically lower SD in original spike trains because only 2 of 20 SDs occurred below identity line (dotted line).
is decreasing in the cells shown from left to right in the figure. We note that the random spike contrasts have a small bias toward positive values, which is a result of our decision to always subtract the least from the most active average cycle half. If we consider the random spike contrast a “bias” it is logical to subtract it from the spike contrast measured in the individual cells.

The result is shown in Fig. 2C where the cells are ordered with respect to the spike contrasts remaining (filled bars) after subtracting the spike contrast expected by chance (open bar) in the 42 cells. The error bars for the original spike contrast are added as vertical lines on top of the bars. The SE of the chance estimate is small relative to the SE of the original spike contrast because of repeated simulations (10) and is therefore not shown. Nineteen of 42 cells gave a spike contrast that was significantly different from what would be expected by chance (using a Kolmogorov-Smirnov test with \(P < 0.05\), marked with *). The significance level is a function of the average magnitude of the spike contrast, the variability around the mean and the number of cycles averaged. In this figure we see that at least the average magnitude was important for the significance because most cells with large spike contrast also were significant. Furthermore, the distribution of average spike contrasts was well described by an exponential function (dotted line) without any clear transition between significantly and not significantly modulated cells. There is therefore no reason to believe that either significance level or magnitude of the spike contrast can be used to distinguish between underlying populations of neurons. For subsequent analysis we chose, however, a magnitude limit (arrow) to study the most modulated neurons left of this arrow, including 18 significantly and 2 nonsignificantly modulated cells.

The average spike contrast among these 20 most modulated cells was \(1.25 \pm 0.23\) spikes. This may seem like a very weak and therefore unreliable signal, but the reliability depends on the variability of the spike contrast. If exactly one spike in difference appeared in every cycle it would represent very reliable information. We will therefore compare this average spike contrast with its variability.

Quantification of spike contrast variability at one repeated cycle position

The standard deviation (SD) of the 20 most modulated cells was on average \(1.82 \pm 0.12\) spikes, larger than the average spike contrast. In Fig. 2D, where the cells are ordered as in Fig. 2C, only one cell had an average spike contrast (indicated by the exponential curve) larger than the SD of the spike contrast. This variability does not necessarily express unreliable spiking. We compared this variability in the 20 most modulated cells with the variability that we knew was of stochastic origin, namely that in the randomized spike trains (Fig. 2E). The original variability was almost as high as the average stochastic variability obtained by randomizing the ISIs (\(2.09 \pm 0.10\) spikes) and the average SD of the spike contrast in the unmodulated neurons (\(1.71 \pm 0.18\) spikes). This result is therefore compatible with the theory that the spike contrast variability was mainly a stochastic variability. The correlation in Fig. 2E shows, however, that only 2 of 20 cells lie below the identity line (\(P < 0.01\)), which means that the underlying locomotor related signal slightly decreased the variability in the spike contrast from what would be expected by chance.

Quantification of the maximal spike contrast in individual cycles

In the previous section we analyzed the spike contrast and its variability at only one cycle position, the one chosen by our analysis to give the largest spike contrast in the average cycle. However it might be possible for larger values for the spike contrast to appear at other positions within the cycle because the cyclic signal shifted back and forth relative to the cycle.

To evaluate this possibility we estimated all spike contrasts that were possible in the spike train and afterwards determined the maximal spike contrast and its position within each individual cycle. The estimation procedure is illustrated in Fig. 3A. The top trace shows the rectified current trace from an on-cell recording; the same cell as analyzed in the second plot from left in Fig. 2B. The trace was rectified and integrated over 5 ms to improve the signal-to-noise ratio. The middle trace is the root (\(L^2\)) recording, also rectified. We used two adjacent windows, each one half of an average cycle length, called \(a\) and \(b\) in this figure. The difference between the number of spikes in window \(b\) and \(a\) was used as the estimate for the spike contrast, similar to the analysis described above. Different from the previous analysis was that the two windows were moved in small steps (1 ms, small enough to not miss any spikes) from the beginning to the end of the recording giving all available spike contrasts.

The result of this analysis is the third trace in Fig. 3A, which contains all possible spike contrasts varying between +5 and −5 spikes. We see that the spike contrasts were higher than the spike contrast measured at one particular cycle position. This analysis is blind for the position of the root cycle and therefore gave the opportunity to study how well the spike contrast contained information about the phase without using information from the average of all the recorded cycles. This could be an important feature because the average spike contrast over many cycles is not a quantity the neurons have available during the individual cycles. The maximal spike contrast within each cycle is marked with arrows in Fig. 3A. These points are plotted for all cycles relative to the normalized cycle in Fig. 3B. For this cell, most maxima lie toward the end (or beginning) of the cycle and range from 1 to 10 spikes. The average spike contrast obtained with this method was 3.5 for this cell compared with 2.5 when the contrast was estimated based on the average modulation (Fig. 2). The maximal spike contrast in the individual cycles for the 20 most modulated cells was \(3.13 \pm 0.25\). As expected, this value was higher than the previously measured spike contrast in the average cycle (\(1.82 \pm 0.12\)).

As shown in Fig. 3C the maximal spike contrast in the individual cycles correlated well with, but were in all cases greater than, the spike contrast determined in the average cycle.

We now have two types of analysis determining the cycle position that gave the largest spike contrast. In the first method (Fig. 3A) the average activity in all cycles was estimated first and the position in this average cycle giving the largest spike contrast was chosen. In the second method the cycle positions giving the maximal spike contrast in the individual cycles was chosen first and the average of these positions was calculated. Figure 3D compares the cycle positions determined in these
two ways. In this plot some of the maximal spike contrasts occurred in the beginning of the cycle (open circles) and were repositioned to the beginning of the next cycle to make the correlation more obvious (Pearson’s $r = 0.94$, $P < 0.01$). Whereas this correlation is not particularly surprising, it demonstrates that the position of the maximal spike contrast contained information about the root activity.

The magnitude of the maximum spike contrast, however, contained very little information because maximal spike contrasts of similar magnitude could be found among both the most and least modulated cells. This can be seen in Fig. 3E, which shows the average maximal spike contrast measured in the individual cycles for all cells ordered as in Fig. 2C. The distribution of the spike contrast estimated in the average cycle (as in Fig. 2C) is indicated as an exponential curve. Only the two most modulated cells had a maximal spike contrast slightly larger than the rest of the population. Also when the cyclic signal was abolished in the 20 most modulated cells by randomizing the ISIs, the maximal spike contrast in individual cycles was on average as high (3.21 ± 0.21) as in the originals (3.13 ± 0.25).

Figure 3F shows that the magnitude of the maximal spike contrast...
contrast in individual cycles was determined mainly by features in the spike trains because there was a strong correlation (Pearson’s $r = 0.93$) between the magnitude of this estimator in the original and the randomized spike trains. This led us to investigate which features that might determine the magnitude of the spike contrast.

**Factors that contributed to low spike contrast**

The average number of spikes/cycle was $5.2 \pm 0.82$ in the 20 most modulated cells and $7.8 \pm 1.18$ in the least modulated cell. These numbers can be compared directly because the average cycle length was not significantly different in the two groups ($3.40 \pm 0.17$ and $3.66 \pm 0.23$ s, most and least modulated, respectively). We can conclude that the activity was relatively similar in cells with and without a cyclic signal, and that the absence of a cyclic signal was not caused by a general lack of spiking activity.

Could the low frequencies of spikes actually limit the spike contrast? It is obviously not an absolute limit because the average number of spikes/cycle ($5.2$) was considerably higher than the spike contrast ($1.25 \pm 0.23$). Nevertheless, a weak but significant correlation between the number of spikes/cycle and the spike contrast in the 20 most modulated neurons (Fig. 4A, Pearson’s $r = 0.54$, $P < 0.02$) suggests that more spikes allowed a higher spike contrast.

Regular intervals in the spike trains might also decrease the spike contrast by limiting the number of spikes that could occur over a certain period. This may contribute to keeping the spike contrast low because more spikes/cycle was correlated with lower CV of the ISIs (Fig. 4B).

Another interesting regularity we observed in all cells was a minimal ISI of 100–200 ms. Figure 4C shows the ISI distributions from the same four cells shown in Fig. 1C. Very few cells had ISIs shorter than 100 ms and the most frequent (modal) value for all 42 cells was $177 \pm 13$ ms, marked as a dotted line through the histograms. Most distributions were skewed toward longer intervals. Figure 4D shows the 10% shortest and the 10% longest intervals as filled and open circles, respectively. We see that a minimal interval of at least 100 ms was typical for cells both with and without a cyclic modulation.

**DISCUSSION**

**Properties of the spike contrast estimator**

An important task for locomotor-related spinal neurons is to discriminate between periods of active and quiescent motor output. Although it is largely unknown which neuronal coding strategies are effective for this discrimination, we assume here that the number of spikes occurring during two cycle halves is a code that is used in the locomotor network. Therefore we measured the difference in spike count in the two cycle halves and the spike contrast, and we analyzed the ability of this simple measure to distinguish between the cycle halves. This estimator was also chosen to be independent of the background activity so that a long spike train lacking one spike in one cycle half and another with only one spike in one cycle half would both come out with a spike contrast of one.

**Average spike contrast**

This study suggests that the difference between two half cycles during locomotor rhythm in the neonatal rat spinal cord is typically represented by one or two spikes (average $1.25 \pm 0.23$).

Although the number of spikes used in spinal motor related network activity has not previously been investigated explicitly, it is clear that the low firing frequency and low spike contrast we describe are different from previously published recordings of cat spinal and brainstem motor neurons (Bianchi et al. 1995; Brownstone et al. 1994), spinal interneurons in cat, rabbit, and turtle (Berkinblit et al. 1978; Berkowitz and Stein 1994; Deliagina et al. 1983; Orlovsky and Feldman 1972; Shefchyk et al. 1990; Viala et al. 1991), and cat reticulospinal neurons (Perreault et al. 1993).
These cells have all been reported to fire high frequencies of spikes over distinct time periods.

There are probably many causes of the differences between these results. One factor may be that neurons with low spike activity are difficult to find using conventional extracellular recording techniques. The on-cell patch-clamp technique that we used is not guided by spikes for cell selection and we may therefore have recorded from a different population of neurons than in previous reports. The neuronal spike activity we recorded could be lower than in the intact animal as a result of factors such as low temperature and the relative immaturity of the preparation. The neuronal activity was, however, sufficient to maintain a rhythmic network function that gave motor neuron activation resembling the activity in the intact animal (Kiehn and Kjaerulff 1996).

There are also many neural networks that use surprisingly few spikes to transfer behaviorally relevant information (see Rieke et al. 1997 for a review). Examples are echo-activated cells in bats (Dear et al. 1993), visual movement sensitive neurons in the fly (Hausen 1984), and cat visual cortex (Reid et al. 1991). These examples have in common that the neuronal activities are responses to sensory stimulation and take place in time windows of 10–100 ms. Our study shows that the use of few spikes by the individual cells may be typical for information transfer over considerably longer time windows (seconds) and for networks that generate a motor output.

**Distribution of the spike contrast**

Approximately one half of the recorded cells (19 of 42) had a larger spike contrast than would be expected by randomness alone. This proportion is similar to the finding in other studies of interneuron activity in neonatal rat using different techniques (Kiehn et al. 1996; Raastad et al. 1996; Tresch and Kiehn 1999) and the adult cat (Shefchyk et al. 1990).

However, there was a smooth transition between the spike contrast of the significantly and not significantly modulated cells. Thus the cells with nonsignificant spike contrasts did not form a population that was clearly separate from the significantly modulated cells. Because the distribution of spike contrasts also showed that there were more cells with small than with large spike contrast among the significantly modulated cells (described by an exponential function), the neurons with low spike contrast may be very important in this spinal locomotor network.

**Noise and variability**

Only by being different from what would be expected by chance can the spike contrast carry information. In individual cycles in individual neurons the magnitude of the spike contrast was rarely outside what would be expected by chance in a noncyclic spike train (Fig. 2B). Two conditions were necessary to obtain a signal that was significant: many cycles were averaged and the cycle position (phase) at which the spike contrast was observed was the same in all cycles. These are the conditions that would occur if the individual cycles were representative for the activity in many, simultaneously active cells and these cells had a common target neuron. Their average and coherent activity is then described by our averages.

Estimating the spike contrast in the average of many cycles corresponds to measuring the spike contrast in the individual cycles at a given and repeated cycle position and taking the average of those individual measures. By this procedure we could also estimate the variability (SD) of the spike contrast. This variability was found to be as large as the spike contrast itself. This finding is similar to other reports (Britten et al. 1993; Miller et al. 1991; Tolhurst et al. 1983; Vogels et al. 1989) that conclude that the signal amplitude at many levels of network processing is similar to the noise amplitude.

The average SD of the spike contrast in the cyclically modulated cells was, however, similar to that of the randomized, noncyclic spike trains. The variability in the simulated data set was a result of the stochastic ordering of the ISI, suggesting that the variability in the spike contrast in the cyclically modulated cells was mainly of stochastic origin. Because the average spike contrast did not exceed the average noise level (SD) and the noise was compatible with a stochastic process, a possible theory is that one locomotor-related cycle is rarely defined in individual cells, only in the average activity of populations of cells. This conclusion is supported by other methods used on comparable neurons in the neonatal rat (Tresch and Kiehn 2000) and is a well-developed theory for other areas of the brain (Georgopoulos et al. 1986; Wilson and McNaughton 1994).

**Maximal spike contrast in individual cycles**

Could we have underestimated the spike contrast because the maximal spike contrast had a slightly different cycle position in the individual cycles? We addressed this question by determining the maximal value of the spike contrast in the individual locomotor cycles as opposed to the other method that determined the spike contrast in an averaged cycle (Fig. 1C). The difference between these two approaches can also be seen in the way the phase information was used. Determining the spike contrast in an average cycle is equivalent to measuring the spike contrast at the same cycle position in all cycles, whereas determining the spike contrast for each individual cycle does not put constraints on the phase at which it is measured.

Because of this additional free variable, we expected to find that the average maximal spike contrast in the individual cycles was larger than the value obtained at only one repeated cycle position. However, the spike contrasts observed in the individual cycles of original spike trains were not different from those in the noncyclic, randomized spike trains. This means that the magnitude of the maximal spike contrast could not be used to distinguish between cyclic and noncyclic spike patterns. As expected the position of the spike contrast was important, illustrated by the correlation between the position found in the average cycle and the average position found in the individual cycles. It was, however, not necessarily expected that the phase for the maximal spike contrast in the individual cycles alone (i.e., without the magnitude information) could predict the same average phase as that obtained in the average of all cycles.

**Frequencies and ISIs**

The spike activity was characterized by low frequencies, which probably contributed to the low spike contrast be-
cause the lowest average frequencies were also associated with the lowest spike contrast. Furthermore, very few spikes appeared earlier than 100 ms after the previous spike and the average shortest interval was 177 ms giving a maximal firing frequency around 10 Hz and a modal value around 6 Hz. This minimal ISI may limit the number of spikes that occur in one cycle half.

Based on the presented data we cannot determine whether network properties and/or intrinsic membrane properties of the recorded neurons limited the firing frequency. One consequence of the low frequencies is, however, that the postsynaptic potentials from individual neurons with these frequencies decay completely before the next one from the same cell arrives because few neurons in the area recorded from have time-constants longer than the minimal ISI of 100–177 ms (see Raastad et al. 1998 for time-constants of a comparable group of neurons). Many neurons are required to build a locomotor related signal for two reasons: first to fill the information gaps between the spikes and second to obtain a reliable average signal.

Network function

Whether this cyclic spike activity was actually used to build the reliable cyclic root activity is a question outside the scope of this investigation. One crucial question is whether the neurons with the strongest signal or, alternatively, the average activity of a large population of neurons are controlling the network, the “winner-take-all” versus “vector average” hypotheses (Sparks et al. 1976). In this locomotor network the necessity for an averaging network mechanism is fulfilled in that the cyclic locomotor signal is weak and distributed over many neurons.

This investigation shows that the spinal networks in the neonatal rat is capable of producing a rhythmic locomotor output even though there is a very weak modulation of the spike activity in the majority of interneurons. The fact that a significant spike contrast was present in at least one half of the cells, and that low rather than high spike contrasts were typical, suggests that a few spikes can make an important difference in the individual neurons.

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REFERENCES


RAASTAD, M., JOHNSON, B. R., AND KIEHN, O. The number of postsynaptic currents necessary to produce locomotor-related cyclic information in neurons in the neonatal rat spinal cord. Neuron 17: 729–738, 1996.


