Characterization of Reliability of Spike Timing in Spinal Interneurons During Oscillating Inputs

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Beierholm, Ulrik, Carsten D. Nielsen, Jesper Ryge, Preben Alstrøm, and Ole Kiehn. Characterization of reliability of spike timing in spinal interneurons during oscillating inputs. J Neurophysiol 86: 1858–1868, 2001. The spike timing in rhythmically active interneurons in the mammalian spinal locomotor network varies from cycle to cycle. We tested the contribution from passive membrane properties to this variable firing pattern, by measuring the reliability of spike timing, in interneurons in the isolated neonatal rat spinal cord, using intracellular injection of sinusoidal command currents of different frequencies (0.325–31.25 Hz). $P$ is a measure of the precision of spike timing. In general, $P$ was low at low frequencies and amplitudes ($P = 0–0.6$; $0–1.875$ Hz; $0–30$ pA), and high at high frequencies and amplitudes ($P = 0.8–1$; $3.125–31.25$ Hz; $30–200$ pA). The exact relationship between $P$ and amplitude was difficult to describe because of the well-known low-pass properties of the membrane, which resulted in amplitude attenuation of high-frequency compared with low-frequency command currents. To formalize the analysis we used a leaky integrate and fire (LIF) model with a noise term added. The LIF model was able to reproduce the experimentally observed properties of $P$ as well as the low-pass properties of the membrane. The LIF model enabled us to use the mathematical theory of nonlinear oscillators to analyze the relationship between amplitude, frequency, and $P$. This was done by systematically calculating the rotational number, $N$, defined as the number of spikes divided by the number of periods of the command current, for a large number of frequencies and amplitudes. These calculations led to a phase portrait based on the amplitude of the command current versus the frequency-containing areas [Arnold tongues (ATs)] with the same rotational number. The largest ATs in the phase portrait were those where $N$ was a whole integer, and the largest areas in the ATs were seen for middle to high ($>3$ Hz) frequencies and middle to high amplitudes (50–120 pA). This corresponded to the amplitude- and frequency-evoked increase in $P$. The model predicted that $P$ would be high when a cell responded with an integer and constant $N$. This prediction was confirmed by comparing $N$ and $P$ in real experiments. Fitting the result of the LIF model to the experimental data enabled us to estimate the standard deviation of the internal neuronal noise and to use these data to simulate the relationship between $N$ and $P$ in the model. This simulation demonstrated a good correspondence between the theoretical and experimental values. Our data demonstrate that interneurons can respond with a high reliability of spike timing, but only by combining fast and slow oscillations is it possible to obtain a high reliability of firing during rhythmic locomotor movements. Theoretical analysis of the rotation number provided new insights into the mechanism for obtaining reliable spike timing.

INTRODUCTION

Central neurons reveal discharge patterns with a high degree of variability (Rieke et al. 1997). In the visual cortex, for example, the number and timing of action potentials in spike trains varies between repeated presentations of the same sensory stimulus (Britten et al. 1993; Snowden et al. 1992; Tolhurst et al. 1983). In addition to time-varying global excitability changes (Arieli et al. 1996; Gur et al. 1997), the variations in spike timing may result from either intrinsic noise in the spike-generating mechanism or from variable synaptic activity generated by the same recurrent stimulus (Azouz and Gray 1999; Shadlen and Newsome 1998). Recent in vitro studies have shown that cortical neurons can respond with a high reliability of spike timing when stimulated repeatedly with intracellular time-varying inputs (Mainen and Sejnowski 1995; Nowak et al. 1997; Tang et al. 1997). The reliability of spiking is, however, highly dependent on the temporal pattern of the input command. Thus high-frequency fluctuating events, comparable in frequencies with those observed in vivo, are transmitted more reliably than low-frequency or DC events. Although cortical neurons may exhibit variations in action potential threshold in relation to the membrane fluctuations (Azouz and Gray 1999), these data suggest a low intrinsic noise level in the spike-generating mechanisms and indicate that the variability in spike timing observed in vivo is mainly due to variations in the underlying synaptic activity generated by the sensory stimulus. To what extent these observations generalize to other areas of the mammalian brain is largely unknown. The primary goal of the present study is to explore this in the context of a rhythmic motor behavior. We do this by investigating the reliability of spike timing in spinal interneurons located in laminae X and VIII and the ventromedial part of lamina VII of the isolated spinal cord of the newborn rat. These spinal cord areas contain sufficient neuronal networks for the creation of locomotor rhythms (Kiehn and Kjaerulf 1998; Kjaerulf and Kiehn 1996). Previous studies have shown that many neurons in these areas are rhythmically active during transmitter-induced locomotor activity in the neonatal rat (Kiehn et al. 1996; MacLean et al. 1995; Raastad et al. 1996–1998; Tresch and Kiehn 1999). However, the spike numbers and spike timing of interneurons in these regions varies from locomotor cycle to cycle.

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locomotor cycle. This variability of spike timing could be due to a cycle-to-cycle variability in the synaptic drive to the rhythmically active cells or due to noise in the transfer of presynaptic activity to postsynaptic spiking. Here we assess whether part of the observed variability of firing rests in the interneuron response properties. To investigate this, we injected sinusoidal command currents of different frequencies and amplitudes and measured the reliability of spike timing in response to these commands. We show that the reliability of spike timing can be very high in these interneurons, but that the reliability depends on frequency and amplitude of the stimulus command. By comparing our experimental data with modeling data and introducing a new way of looking at reliability of spike timing, we can make precise predictions about the presynaptic activity that produces a maximal reliability of spike timing in these neurons. A high reliability of spike timing will synchronize activity in large groups of cells leading to an increased motor output.

METHODS

Dissection

The spinal cord was isolated from newborn Wistar rats, generally 1 day old (range 0–2 day). The rats were anesthetized with ether, quickly decapitated, and eviscerated. The spinal cord was split mid-sagittally from L₁ to L₆. Both halves of the cord were pinned in the experimental chamber with the cut surface facing up, and perfused with standard Ringer solution containing (in mM) 128 NaCl, 4.7 KCl, 25 NaHCO₃, 1.2 KH₂PO₄, 1.25 MgSO₄, 2.5 CaCl₂, and 20 glucose, which was oxygenated with 95% O₂-5% CO₂, keeping the pH at 7.4.

To prevent spontaneous synaptic activity during recordings, D,L-2-amino-5-phosphonovaleric acid (AP-5, 40 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) and occasionally strychnine (0.3–0.5 μM) and bicuculline (20 μM) were added to the Ringer solution. All experiments were performed at room temperature.

Recordings

Patch electrodes were pulled on a two-stage Narishige Vertical Puller from 1.5-mm Borosilicate glass without filaments (Clark Instruments, Pangbourne, UK) to a final resistance of 5–10 MΩ. The pipette solution contained 128 mM K-glucuronate, 10 mM HEPES, 0.1 mM CaCl₂, 0.3 mM GTP, 5 mM Li-ATP, and 1 mM glucose (pH 7.3). Whole cell current-clamp recordings were performed with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA) and controlled from a PC using pCLAMP software (Axon Instruments). Interneurons were recorded in laminae X, VIII, and the ventromedial area of lamina VII. The recordings were sampled at 6.25 kHz and filtered at 1 kHz. The current tracings were injected as either a square pulse or sine waves, repeated 10 times with 1-s intervals. This was done for various frequencies and amplitudes for each cell. To estimate the time constant of the membrane, τ, a zap protocol was run with amplitude yielding subthreshold membrane potential fluctuations (see Fig. 3).

Analysis

The reliability, P, of firing has been used as a quantitative measurement of the neural response to repeated identical stimuli (Mainen and Sejnowski 1995; Nowak et al. 1997). Here we have calculated P in a way very similar to that described by Nowak et al. (1997). The exact spike times from all 10 stimuli in a run were projected onto the time axis. This axis was divided into 3-ms bins. The number of spikes in each bin was then used for calculations. P is the total number of spikes in all bins containing more than 1 spike, divided by the total number of spikes in all 10 stimuli. In this way, P is a dimensionless number between 0 and 1, where 0 indicates a random response and 1 a nonrandom and entrained response. Contrary to Nowak et al. (1997), we did not include the immediately adjacent bins in our calculations of an event, unless it contained more than one spike. The bin size we used was chosen to be compatible with the slow firing and relative broad action potentials of neurons in the neonatal rat spinal cord (the width at ½ the peak amplitude of the action potential is 1.67 ± 0.39 (SE) ms for neonatal motor neurons (Fulton and Walton 1986) and 3.2 ± 1.5 ms for the interneurons located in the ventromedial area of the neonatal spinal cord (Hochman et al. 1994)). All calculations and generation of stimulus files were done using Origin 6.0 (Microcal) and IDL (Interactive Data Language version 5.2.1: Research Systems) software.

Model

To examine our experimental findings we used the simple leaky integrate and fire (LIF) neuronal model, with a few modifications. The original model considers a cell consisting of an isolated membrane with resistance, R, and capacitance, C. The current through the membrane is denoted I, and the potential across the membrane, V_m. When no current is injected, the cell will have a natural resting potential, V_rest. When a current is injected at time t = 0, the potential of the cell membrane, relative to the resting potential V(t) = V_m - V_rest, can be described according to

\[ RC \frac{dV(t)}{dt} + V(t) = RI \]

(1)

(see e.g., Lapique 1907).

When injecting a constant current, I_inj, the equation has the solution

\[ V(t) = LR[1 - \exp(-t/RC)] \]

(2)

The cell membrane will be charged to its new potential V = I_injR and will stabilize at that point. The speed with which the potential approaches this value is determined by RC, also referred to as the time constant, τ, of the system. Values for τ and R in the model was in the range of the τ and R_m found in spinal interneurons in the ventromedial area (τ = 37.5 ± 31 ms and R_m = 568 ± 368 MΩ) (Kiehn et al. 1996; see also Hochman et al. 1994).

To mimic action potential firing in the model, we added a threshold for firing, at a value of the potential V_thres (12 mV above resting membrane potential; average value measured in real neurons). On reaching this V_thres = V_m, the membrane potential momentarily resets to the resting potential V(t) = 0, and the neuron is considered to have generated an action potential.

Thus the classical LIF model does not explicitly contain the form of the action potentials. To compensate for this, we made a small modification to the LIF model. The modification can be summarized in two steps as follows.

1) We introduced a refractory period where the neuron is unable to create a new action potential. This short time interval, d, was set to 2 ms.

2) An exponential function, V₂, with a time constant γ so large that \[ V₂ \approx 0 \] after the time interval d was added to the solution. The exponential function was multiplied with a factor B, chosen to give realistic amplitude of the spike. We use a value of 90 mV for B and γ of 6.25 ms

\[ V(t) = +B \exp(-t/\gamma) \]

(3)

This term is then added to the model (Eq. 2).

With these two additions to the LIF model, more realistic spiking was observed. It should be stressed, however, that the additions do not change the quantitative results of the simple LIF model in any way, as long as the average firing frequency does not exceed 100 Hz.
This now gave us a neuron model that responded with a regular firing pattern if a depolarizing current injection was large enough and with increasing firing rate for increasing current.

The LIF model is initially a deterministic model that always gives the same response to a given stimuli, in contrast to a live neuron. Owing to the many internal processes governing the behavior of a real cell, a neuron will fire with some variation. To include this behavior in our model, we added Gaussian distributed white noise with zero mean and deviation, \( \sigma_n \). The noise term was added to the potential \( V \) of the model as a sum of 100 sine waves

\[
V_n = C \sum \sin(2\pi f t)
\]

where \( f_s \) is a Gaussian distributed random number with standard deviation of 1,000 Hz. \( f_s \) was generated using a random number procedure in IDL.

The magnitude of this noise, the prefactor \( C \), was found by comparison with the experimental data (see RESULTS; Comparison of model and real neurons). Eventually we ended up using noise \( V_n \) with a standard deviation, \( \sigma_n \), of 0.9 mV.

As a final implication, we note that rhythmically active interneurons are subject to an alternating synaptic input, which causes the membrane potential to oscillate between periods of depolarization and hyperpolarization (Kiehn et al. 1996; Raastad et al. 1996, 1998). We therefore introduced time-varying command currents in our model as opposed to the original square pulse. The simplest time-varying command for our purpose is the sine wave current 

\[
I(t) = A \sin(2\pi \omega t)
\]

of frequency \( \omega \) and amplitude \( A \). We also choose to change the resetting of the membrane potential at the time of firing, so that \( V(t_0) = V_{thres} - A_0 AR \) just after firing. Equation 1 now has the solution

\[
V(t) = -V_0 \exp\left[-(t-t_0)/RC\right] + A_0 AR \sin\left[2\pi \omega (t + \varphi)\right]
\]

with

\[
A_0 = \frac{1}{\sqrt{1 + (2\pi \omega RC)^2}}
\]

\( \varphi = \arctan(2\pi \omega)/2\pi \omega \) and \( V_0 = A_0 AR \).

\( A_0 \) characterizes the well-known low-pass ability of the cell (see, for example, Carandini et al. 1996), as the denominator attenuates the higher frequencies (see RESULTS).

This sum of the potentials \( V_1, V_2, \) and \( V_n \), together with the threshold for firing and the 2-ms refractory period (see condition 1 above) now constitute the model.

RESULTS

Reliability of spike timing in lumbar spinal interneurons

The membrane potential of rhythmically active interneurons during locomotion displays a range of different frequencies. In general, fast postsynaptic potentials with frequencies ranging from 5 to 30 Hz are superimposed on slower oscillations with frequencies of approximately 0.3–1 Hz (Kiehn et al. 1996; Raastad et al. 1996–1998). As a first attempt to mimic this synaptic profile and thereby investigate the relationship between input parameters and reliability of spike timing, we injected sinusoidal command currents of different amplitudes and frequencies in the absence of locomotion. We compared these results with the response properties obtained by using DC command currents. In this section, we will give a brief account of the general observations made with these two stimulus paradigms.

Figure 1A shows an intracellular recording from an interneuron (middle panel) when stimulated with a DC square current pulse (amplitude 75 pA). Even though the cell might seem at first sight to fire at regular intervals, the raster plot (Fig. 1A, top panel) shows that the firing pattern in repeated trials was actually irregular with a low reliability (\( P = 0.136 \)). Characteristically the first spikes following stimulus initiation in every run appear very close in time. This high reliability decreases, however, after only two or three action potentials, confirming previous observations that neurons respond with low reliability when stimulated with DC currents (Mainen and Sejnowski 1995; Nowak et al. 1997).

Figure 1B (middle panel) shows the firing pattern of an interneuron stimulated with a sine wave of low current amplitude (57 pA, 3.125 Hz). The cellular responses are not identical in the repeated runs of stimuli, but the raster plot (Fig. 1B, top panel) shows a much better alignment of the firing times (\( P = 0.331 \)) than for the pure DC stimulus. In this example, the membrane voltage lingered around the threshold for firing, making the cell fire only occasionally at the peak. This gave a relatively low \( P \) value. However, when the amplitude of the command sine wave was increased (85 pA, 3.125 Hz; Fig. 1C, bottom and top panels), the cellular responses were almost identical for repeated runs, with high reliability (\( P = 0.868 \)).

Therefore interneurons in the neonatal rat spinal cord seem to have the capability to entrain to an alternating command, here a simple sine wave current, and to produce a very reliable response. This implies the possibility of precise spike timing when exposed to synaptic currents of certain amplitudes and frequencies.

Measurements were made on 34 interneurons for various frequencies between 0.625 and 31.25 Hz and current amplitudes between 20 and 135 pA. The time constants of the cell membranes varied between 50 and 100 ms, and the input resistance was usually 500–1,000 M\( \Omega \), although a few cells had resistances as low as 215 M\( \Omega \). These values are similar to those previously observed in interneurons in the neonatal rat spinal cord (Kiehn et al. 1996; Raastad et al. 1998).

The general observation from these experiments was that the reliability of spike timing in response to slowly alternating command currents in general was lower than with sinusoidal command currents at higher frequencies. Likewise, reliability increased with the amplitude of the command current. Thus when injecting sine wave commands at high frequencies (6.125–18.375 Hz) and amplitudes (80–130 pA), very high reliability could be observed (\( P > 0.9, 7 \) neurons). Finally, the reliability was low for neurons exposed to a pure DC current (\( P < 0.3, 5 \) neurons).

These general statements are, however, contradicted by the observation that the reliability decreased as the amplitude increased beyond a certain limit. Further increase of the amplitude could then increase the reliability again. This relationship between amplitude and the transient drop in reliability was frequency dependent. Another confounding factor is that the amplitude of the voltage response decreased when the frequency of the command current increased. To investigate these relationships in further detail, we used the LIF model and compared it with the experimentally obtained data.

Spike response in the LIF model

The first set of experiments in the LIF model was to test how well it reproduced the general experimental observations.
Figure 2 shows the intracellular response of the LIF model to a DC square current pulse (Fig. 2A) and sine wave commands (Fig. 2, B and C, using the same frequency and amplitudes as the real cell was stimulated with in Fig. 1). As in spinal interneurons, the DC current (75 pA) gave low reliability of spike timing ($P = 0.168$). Changing to a low-amplitude (57 pA, 3.125 Hz) sine wave increased the reliability (Fig. 2B; $P = 0.425$), although the responses were not identical for repeated runs, as in the experimental situation (Fig. 1B). Again, the amplitude was too small to evoke an action potential in each cycle. The noise in the model is responsible for these irregularities. Raising the amplitude (85 pA) of the command current increased the reliability further ($P = 0.868$) with the sine wave command current was increased to 85 pA.
markedly increased the reliability of the response of the neuron model (Fig. 2C; \( P = 0.892 \)). As in the experimental situation, the alignment of the firing times in the raster plot (Fig. 2C; top trace) shows that the spike timing is reproducible from trial to trial, thereby giving a reliable response.

**Cells act as low-pass filters**

Our experiments on spinal interneurons and in the LIF-model using sine wave command currents with the same amplitude and different frequencies revealed a nonlinear connection between the amplitude of the command current and the amplitude of the recorded voltage. Thus the amplitude of the recorded voltage decreased with increasing frequencies (see Figs. 1 and 2). To investigate the nature of this effect, we constructed a “zap-command” protocol, which was a sine wave with discretely increasing frequency (Fig. 3B). The zap command was scaled so it evoked subthreshold voltage responses. From Fig. 3A it can be seen that running the zap command in

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**Fig. 2.** Reliability of spike timing in a leaky integrate and fire (LIF) model. The arrangement of the panels and nature of the command currents in A–C is identical to Fig. 1. The parameters of the LIF model were \( V_{\text{rest}} = -60 \) mV; \( V_{\text{thres}} = -48 \) mV, \( \tau = 50 \) ms; and 0.9 mV for the SD of the noise (see text for further details). A: simulation run with a DC current pulse with a 75-pA amplitude. \( P = 0.168 \). B: sinusoidal command with a frequency of 3.125 Hz and 57-pA amplitude. \( P = 0.425 \). C: sinusoidal command with a frequency of 3.125 Hz and an 85-pA amplitude. \( P = 0.892 \). Note the similarity between the firing responses and reliability of spike timing in Figs. 1 and 2.
an interneuron evoked a voltage response that decreased in amplitude with increasing frequency. This low-pass effect to an external stimulus has also been described in other neurons (see Carandini et al. 1996) and was seen in all the 34 interneurons recorded.

In Fig. 3C (+), the amplitude of the voltage response is plotted as a function of the zap-command frequency. It is clear from this figure that it is only above a certain frequency, the cutoff frequency, that the low-pass effect becomes prominent. In the model, this can also be deduced directly from Eq. 6 (METHODS), where the two ranges of frequencies (low and high) and the splitting between them occurs at the cutoff-frequency \( \omega = 1/(2\pi RC) \). The amplitude of frequencies below this value are not changed, while the amplitudes of frequencies above are attenuated.

When the theoretical estimate \( A_0 \) (see METHODS, Eq. 6) was superimposed on the normalized experimental curve (Fig. 3C) obtained from the zap protocol, a clear correspondence was observed, except for higher frequencies, where the two curves deviate. The theoretical decrease of the amplitude with a factor \( \omega^{-1} \) is only maintained until about 10 Hz, after which the experimental voltage response amplitude decreases with a slower rate, namely \( \omega^{-3/4} \). This relationship was seen in five of six interneurons. The reason for this deviation between experiments and theory is not known. The important point, however, is that there is a good correspondence between the real neurons and the LIF model.

The prominent low-pass effect at higher frequencies made it difficult to compare the reliability of firing at different frequencies of the command current. We therefore turned to a mathematical analysis of the relationship between frequency, amplitude, and reliability of firing.

**Mathematical analysis of the relationship between firing behavior, amplitude, and frequency**

The LIF model enables us to use the mathematical theory of nonlinear oscillators to analyze the firing behavior (see, for...
example, Alstrøm et al. 1988, 1990; Jensen 1998). Using a pure sine wave as stimulus current further lightens the task of analyzing the problem. We will start by considering the LIF model in the absence of noise.

Instead of using \( P \) as a measurement of reliability, we now investigate the entrainment between the incoming command (the sine wave) and cell firing. We use the rotation number \( N \) to describe this relationship. \( N \) is defined as \( N = J/K \), where \( J \) is the sum of all action potentials in a given trial and \( K \) is the number of cycles in the sine wave command. In other words, \( N \) defines the number of spikes per cycle of the input command. As we shall see later, there is a clear connection between the degree of entrainment to a command and the reliability of the response.

The rotation numbers for the different values of sine wave frequency \( \omega \) and voltage fluctuations \( RA0 \) in the LIF model are plotted in Fig. 4. This phase-plot defines areas with constant rotation numbers. The areas with integer rotation number (e.g., 1, 2, 3, etc.) will generally be the largest, but with decreasing area as the rotation number increases. The noninteger areas (e.g., \( 1/2 \), \( 3/2 \), etc.) are interposed between the integer \( N \)'s and are generally very small.

The good relationship between the spike response in the interneurons and the LIF model allows us to use the plot in Fig. 4 to predict the reliability of the firing of a neuron when exposed to a specific sine wave command. The sizes of the areas, also called Arnold tongues, determine the reliability with which a sine wave command is transmitted. Thus a cell responding with \( N = 1 \) (that is 1:1 phase locking) will be more reliable than a cell responding with \( N = 1/2 \), as the area of the parameter space (amplitude and frequency) for which the rotation number is consistent is larger for \( N = 1 \) than for \( N = 1/2 \).

From these considerations, we conclude that the larger the area in the phase plot a neuron occupies within an Arnold tongue, the more reliably it will fire (see DISCUSSION). The largest areas in the phase plots occur for middle to high \( (\omega > 3 \text{ Hz}) \) frequencies and middle to high amplitudes of the command. This corresponds with the increase in reliability seen in the experiments when frequency and amplitude were increased. These areas also correspond to areas where the derivatives of the input commands, \( dI/dt \), are high (the derivative increases with amplitude and frequency).

It should be noted that the lower amplitude limit in the graph is determined by the model parameters. This amplitude limit will shift to lower values if the distance between \( V_{\text{rest}} \) and \( V_{\text{thres}} \) decreases.

In Fig. 5, we have incorporated the low-pass effect in the phase plot. The areas of individual tongues are compressed and stretched. This is because points in the phase plots above the cutoff frequency will be dislocated to the right, because a higher amplitude is needed to evoke the same response as without the low-pass effect. The consequence of this effect is an upper limit for frequencies that evoke spiking (see DISCUSSION). In the real experiments, we were able to observe 1:1 phase locking for input commands up to 31.25 Hz. It can also be seen that increasing the frequency for fixed amplitude will make the cell fire with lower rotation number until firing eventually stops.
integer rotation number $N$ to a noninteger $N$ will actually lower the reliability.

In summary, a high reliability of spike timing requires a high derivative of the input command and a constant rotation number in the phase plane. This is obtained when a cell fires at a point in the phase plane where it is situated in a large area of an Arnold tongue, and at a minimum distance in the order of the standard deviation (SD) of the noise away from other Arnold tongues. In this way, the noise is unable to move it sufficiently in the phase plane to cause it to shift between rotation numbers. The ideal situation is therefore 1:1 phase locking.

Relationship between reliability and rotation number

One of the analytical predictions of the preceding section was that there should be a connection between integer rotation numbers and reliability. To examine this closer, we compiled the data of 124 sine wave current stimulations performed on 7 different neurons and plotted the reliability, $P$, of each stimulation as a function of the rotation number, $N$ (Fig. 6, ●). Because of the pronounced low-pass characteristic of the neurons, we were not able to obtain rotation numbers larger than two in the real neurons.

As predicted from the model, the reliability slowly increases until $N = 1$. At $N = 1$ the reliability can be high because of the large area in the phase plot, but the reliability is still dependent on the derivative of the stimulus. Both high and relatively low reliability can therefore be seen in Fig. 6 for $N = 1$. As $N$ moves from $N = 1$ to $N = 2$, the reliability drops due to the small area of the Arnold tongue. At $N = 2$ high reliability can again be achieved.

When increasing the amplitude for frequencies above 1 Hz, the area between rotation number $N = 1$ and $N = 2$ is very small. This observation also fits perfectly with the nature of the Arnold tongues, as can be seen by making a horizontal cut through Figs. 4 and 5 (not shown). Around $N = \frac{1}{2}$ (Fig. 6) there is a small increase in reliability. This local increase in reliability corresponds to stimulation frequencies above 15 Hz and can be explained as an increase of the Arnold tongue area $N = \frac{1}{2}$ starting at $1.25$ and $18.75$ Hz and amplitudes between $20$ and $135$ pA. $P$ increases until $N = 1$. It then drops when $N$ moves through noninteger numbers until it reaches $N = 2$. See text for further details.

Comparison of response of LIF model and real neurons

To evaluate the response of the LIF model more directly, we ran simulations using membrane parameters obtained in the intracellular recordings and compared the results of the simulations and the experimental values for reliability and rotation numbers. An example of this is shown for one cell in Fig. 7.

An estimate of the membrane time constant, $\tau = 50$ ms, was obtained from the theoretical curve (Eq. 6) that was fitted to the actual data (see Fig. 3C). The resting potential, $V_{rest}$, was measured directly, whereas the voltage threshold for spiking, $V_{thres} = -48$ mV, was measured by depolarizing the cell sufficiently for the cell to start firing. Finally, we needed to determine the magnitude of the intrinsic noise, $C$, in the real neurons. This required a comparison between the numerical and experimental data. To do this we ran simulations of the model with different magnitudes of noise, $C$, for fixed frequency, $\omega$, but varied the sine wave amplitude, $A$, and calculated the reliability for each set of parameters. These simulations showed that the magnitude of noise in the model determined the maximally achievable reliability. A high magnitude (SD $> 1.5$ mV) of noise made it impossible to obtain high reliability ($P > 0.9$). The simulations also allowed us to fit the numerical data to the experimental and thereby get an estimate of the magnitude of noise. Thus good fits were obtained using noise with SD of 0.9 mV. This seems to confirm...
the theoretical calculations of Manwani and Koch (1999), who estimated that the intrinsic noise of the neurons should have a SD of 1.01 mV.

The result of the simulation (averaged over 50 runs) and the experimental values for reliability and rotation numbers for a fixed frequency (ω = 3.125 Hz) but different amplitudes are shown in Fig. 7. For fixed frequency, there was a linear relation between the input current and the measured voltage amplitude V_{in}(ω) = AA_0 R, so the experimental results were scaled with a factor A_0 R. The solid lines show the rotation number (bottom line; y-axis to right) and the reliability (top line; y-axis to the left) for the simulations, while the crosses shows the corresponding data from the real experiment. There is a clear correspondence between the simulations and the experiments. As for the real data, reliability (P) in the model increased with amplitude to a high value (P > 0.6). This higher value was obtained and stayed relatively stable when N = 1. P then dropped, until it again increased when N moved though non-integer numbers from N = 1 to N = 2. This profile is very similar to that seen in Fig. 6. When N > 2, a comparison between simulations and real data becomes impossible because of a lack of experimental data (see above, Relationship between reliability and rotation number). Similar data were obtained for other cells (n = 7).

DISCUSSION

We have made a series of experiments stimulating spinal interneurons with time-varying command currents and characterized the reliability of spike timing (P). These interneurons are located in a part of the lumbar spinal cord that is involved in generating hind limb locomotion (Kiehn and Kjaerulf 1998; Kjaerulf and Kiehn 1996). Our data demonstrate that the reliability of spike timing in these interneurons depends on the amplitude and/or frequency of the command input. In general, P was low at low frequencies and amplitudes, and high at high frequencies and amplitudes. These data were then compared with simulations made using a LIF model with an additional noise term added (Eq. 4). Using the LIF model and the quantitative measure of the rotation number (N), we were able to explain the changes in reliability observed when varying the amplitude and frequency of the command current. Fitting the result of the LIF model to the experimental data also enabled us to estimate the standard deviation of the internal neuronal noise. We conclude that the model captures many aspects of the reliability of spike timing in real neurons. The simplicity of the model offers the possibility to use analytical tools to understand the rather complex interactions between input commands and evoked spike activity.

Comparison of theory and experimental data

The LIF model is extremely simple and does not provide an exact biophysical description of neuronal behavior. The LIF model, however, did reproduce the experimentally observed relationship between P and amplitude/frequency, suggesting that this complex relationship can be investigated in a simple neuronal model. With slight modification of the LIF model, it was able not only to represent the firing times but also to mimic the trajectory of the spikes and their afterpotentials, increasing its value for comparing with real recordings.

Reliability of spike timing

It has been suggested that, when stimulated with high frequencies and amplitude, spike timing in the LIF model is too inaccurate to be used in network models (Brown et al. 1999). This suggestion is based on comparisons with the more physiological Hodgkin-Huxley model. The problem of inaccuracies is not pronounced unless the neuron was stimulated with a large proportion of excitatory postsynaptic potentials (EPSPs) with frequencies above 100 Hz, far outside the frequency range used in the present experiments. As a first approximation, we therefore used a modified LIF model, which when stimulated with physiologically realistic input command captures the basic firing behavior of the cell. This enabled us to analyze the system using the concept of phase locking. Moreover, numerical simulations could be compared with real data to fit experimental parameters (input conductance, time constant, and internal noise).

Phase locking

The general idea explored in this paper is the concept of phase locking of neuronal firing to an external command, in this case a current command. We have shown that there is a relationship between the rotation number (N) and the reliability (P). By examining phase locking, we can provide a mechanistic explanation for the relationship between the amplitude and
frequency of the input command and the reliability of spike timing, which is not easily understood otherwise.

The analysis involves plotting the number of firings per period, the rotation number, as a function of the frequency and amplitude of the incoming signal, thereby generating areas of different rotation number. The large plateaus in the phase plot (Figs. 4 and 5), primarily the 1:1 phase locking, provide a robustness to the system that eliminates the influence of noise over a wide range of input commands.

Our findings show the importance of choosing the frequency components and the amplitude of the input command in accordance with the time constant and spike threshold of the neuron, to achieve high reliability. It should be noted that the relevant command amplitude is actually the relation between the resulting amplitude ($\Delta R_AA$) and the threshold for firing above the resting membrane potential ($V_{rest} - V_{thres}$), as can be seen by nondimensionalizing Eq. 5 with $V_{thres}$. A lower threshold for firing would yield the same rotation number if the input command amplitude was equally lowered.

Phase locking is a general phenomenon and can be found in many systems experiencing a fluctuating input (see, e.g., Jensen 1998). These fluctuating inputs may be sine waves as in the present case, but phase locking has also been observed for systems driven by randomly fluctuating forces (Jensen 1998).

The idea of thinking of neuronal firing in terms of phase locking is not new (Keener et al. 1981; Knight 1972). Recently Hunter et al. (1998) applied this approach to Aplysia motor neurons. They defined a ratio called $f/f_{DC}$, where $f_{DC}$ is the mean firing rate resulting from injecting a constant current, and $f$ is the sine wave frequency. By injecting sine wave currents of different frequencies superimposed on the constant DC current used to determine $f_{DC}$, they found that the highest reliability was observed for frequencies equal to $f_{DC}$. Thus 1:1 phase locking between the command frequency and the mean firing rate will optimize the reliability of firing. Numerical calculations using a LIF model gave the same results. These results are very similar to those obtained in the present study. However, the type of phase locking is different in the two studies. A constant suprathreshold depolarization in combination with a sine wave command will create areas in the Arnold plot without phase locking, quasi-periodic states, which will even further decrease the reliability outside of the integer rotation number areas. For a more thorough discussion of this phenomenon, see Alström et al. (1988, 1990).

Reliability of spike timing during network activity

In our analysis of the reliability of spike timing, we restricted the analysis to evaluating simple input commands: sine waves of different amplitudes and frequencies. These input commands do not directly resemble the synaptic inputs that a spinal interneuron experiences during normal behavior. They do capture, however, some of the basic features of the synaptic network activity that converge onto a rhythmically active interneuron during locomotion. Thus the membrane potential of locomotor-related interneurons in the neonatal rat oscillates between phases of relative hyperpolarization and relative depolarization. These oscillations slow with a frequency corresponding to the step cycle (0.3–1 Hz) (Kiehn et al. 1996; MacLean et al. 1995; Raastad et al. 1996–1998) and with average amplitude of the oscillations in the range of 10–12 mV (Kiehn et al. 1996; Raastad et al. 1996). Distinct fast synaptic potentials with faster frequencies are superimposed on the slow oscillations. It is from the fast synaptic potentials that the spikes seem to be triggered in the depolarizing phase of the locomotor cycle.

Using the phase diagrams (Figs. 4 and 5), we can understand how the different components of the locomotor-related membrane fluctuations influence spike timing and can make predictions about the frequency components of a presynaptic input that will be most reliably transmitted. It is clear that a cell stimulated with sine-wave frequencies around 1 Hz (the leftmost point in Fig. 5) and lower occupies a very small area in the Arnold tongues. With the level of internal noise (SD of 0.9 mV) determined in this and previous studies (Manwani and Koch 1999), the neuron can be moved between different Arnold tongues, thereby decreasing the reliability of firing. Thus the slow membrane oscillations observed during locomotion are bound to cause a very low reliability of spike timing. In contrast, input commands with frequencies of >3 Hz will evoke a high reliability of firing (rightmost point in Fig. 5). To obtain this, very large positive voltage deflections are needed. Such large-amplitude voltage deflections are not observed during natural synaptic activity in spinal interneurons. However, if the neuron is depolarized so that the membrane potential moves closer to spike threshold, the phase diagram is shifted to the left along the x-axis, thereby reducing the voltage fluctuations needed to give a high reliability. The slow membrane voltage oscillations observed during locomotion can serve this depolarizing function. Thus by combining the slow (<1 Hz and below) and the fast voltage (>3 Hz) oscillations it is possible to obtain a high reliability of spike timing during locomotion. In this context, it is worth noting what we call fast oscillations are relatively slow compared with the stimulus commands used in cortex (Mainen and Sejnowski 1995; Nowak et al. 1997). Cortical neurons fire frequencies around 50–100 Hz, while spontaneously active and locomotor-related interneurons fire with frequencies around 1–10 Hz (Raastad and Kiehn 2000; Tresch and Kiehn 1999, 2000a). This suggests that the frequency range for optimal reliability of firing is precisely tuned to the natural firing range of the neuron.

So while the slow oscillations at first sight mainly seem to serve a role as determining the difference between the preferred and nonpreferred phase of firing during locomotion, they are as discussed in the previous section a necessary condition for spinal interneurons to fire with a high reliability. A high reliability of spike timing is, however, only obtained if fast synaptic inputs are superimposed on the slow oscillations as occur during locomotion. It is therefore likely that, although single interneurons seem to fire very unreliably in individual locomotor cycles (Raastad and Kiehn 2000; Tresch and Kiehn 1999, 2000a), they might in fact react with a high reliability of spike timing in response to presynaptic activity. A high reliability of spike timing in individual interneurons will tend to synchronize the activity across a group of functionally related interneurons that are activated by a common synaptic input (Usrey and Reid 1999). Synchronized reliable firing in a group of premotor interneurons will in turn lead to a synaptic output, which may recruit motor neurons more efficiently than an asynchronous synaptic activity. Moreover, model studies have shown that synchronized inputs will increase the force output from motor neurons more than asynchronous inputs for a given
input rate (Baker et al. 1999). While the presence of synchronized firing has not yet been investigated in spinal interneurons, there is in fact indications that a synchronized output is “read out” to motor neurons during locomotion and respiration (Christensen et al. 1998; Kiehn et al. 2000; Kirkwood and Sears 1978; Tresch and Kiehn 2000b). Altogether, this may suggest that a high reliability of firing in spinal interneurons might serve an important role for the motor output generated by the spinal locomotor network.

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