

Supporting material for the article:

Intense synaptic activity enhances temporal resolution in spinal motoneurons

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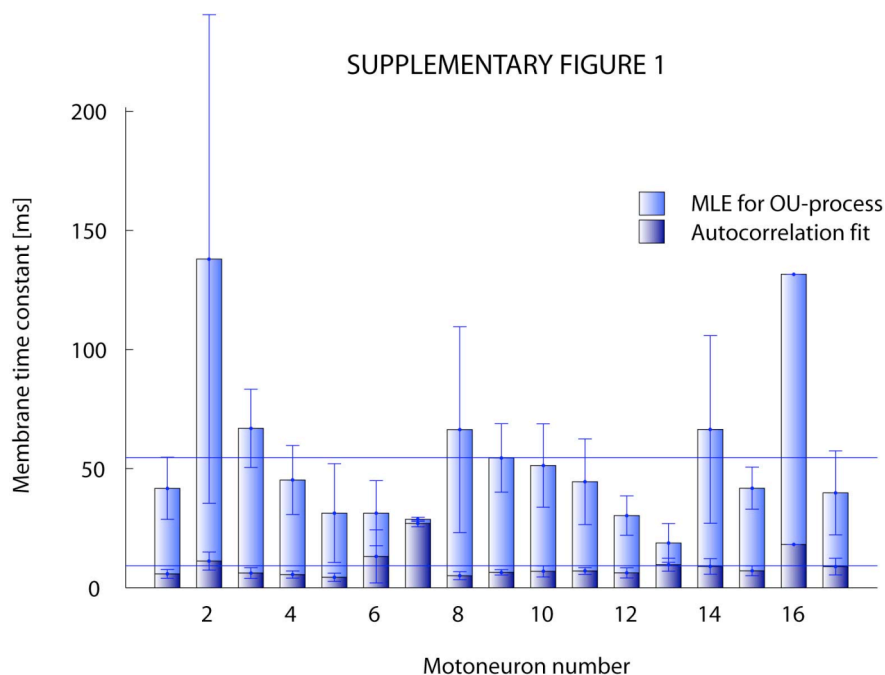
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Supporting data.

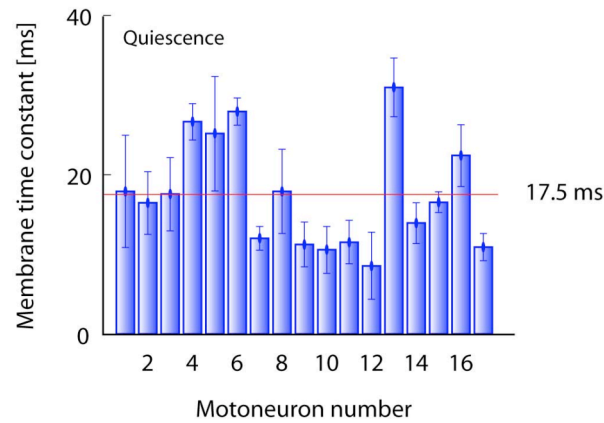
Tau from MLE during ON-cycle. We estimated the membrane time-constants using the Maximum Likelihood estimation (MLE) procedure in addition to using the auto-correlation relaxation fitting (figure 4F). The results for both procedures during ON-cycles are plotted together for comparison in Figure S1. The horizontal lines are the population mean for the MLE and the autocorrelation exponential fit, respectively. The MLE values are roughly 1 order of magnitude larger than the autocorrelation fitting values. The mean value of the

autocorrelation fit was 9.3 ± 1.4 ms (mean \pm standard error) and the median was 7.0 ms.



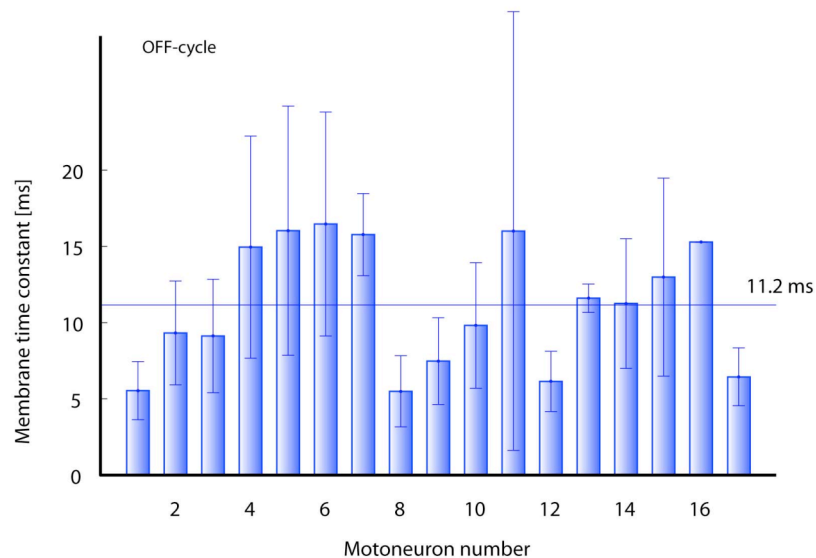
Tau during quiescence. For comparison we calculated the time-constants without synaptic inputs. Current pulses were injected into the cells and the corresponding V_m deflections were analyzed by fitting an exponential decay with constant off-set. The time-constant of this fit was the passive time-constant during quiescence and they are plotted in supplementary figure 2 with a mean of 17.5 ± 1.7 ms (mean \pm standard error) across the population and a median of 16.5 ms.

SUPPLEMENTARY FIGURE 2



Tau during OFF-cycle. For comparison we calculated the time-constants via the autocorrelation fits for the OFF-cycles across a population. The values are plotted in supplementary figure 3. The sample mean was 11.2 ± 1.0 ms (mean \pm standard error) and the median was 11.3 ms.

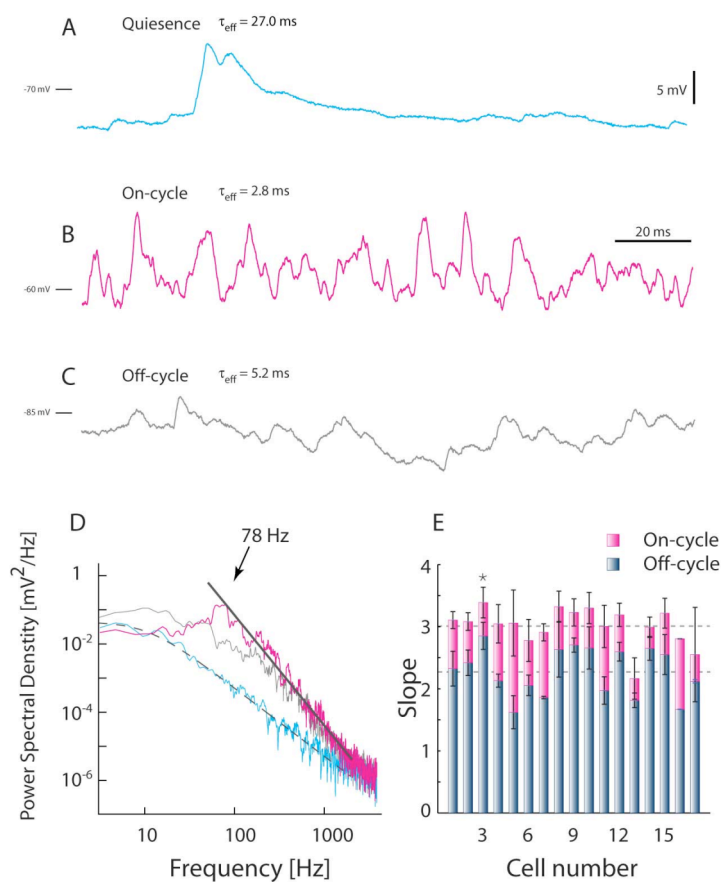
SUPPLEMENTARY FIGURE 3



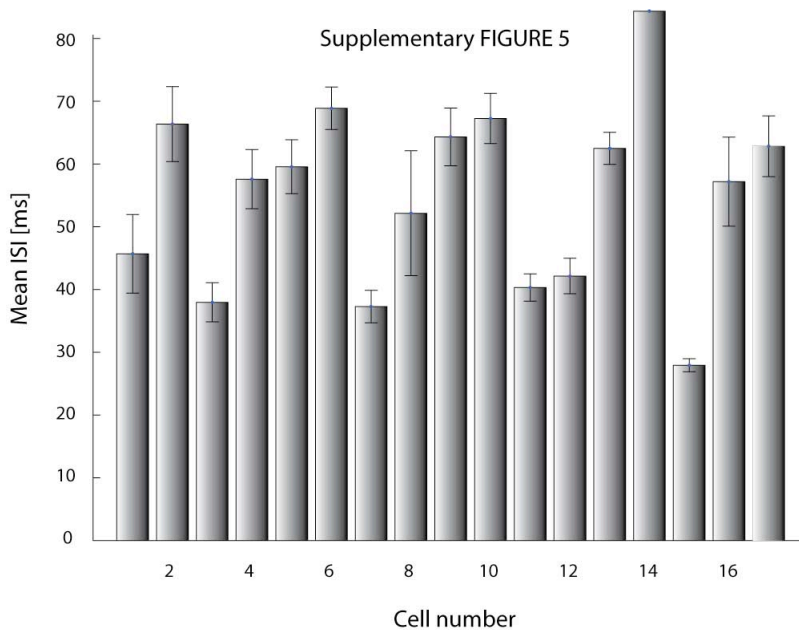
Spectral features of fluctuations

The OU-process, the most commonly assumed and applied stochastic process to describe the synaptic V_m fluctuations, is a smoothed version of Steins model where inhibitory and excitatory synaptic input is modeled as Poisson processes, and the membrane potential decays towards a resting potential in absence of input (a leaky term). The estimated values of τ_{eff} with this technique are listed above in the supplementary figure 1. It turned out that V_m in motoneurons were poorly described by an OU-process. The roll-off slopes in the power spectrum of the sample data was about -3.2 during on-cycle (supplementary figure 4B) and -2.8 during off-cycle (Figure S4C), which was substantially higher than the anticipated -2 (Figure S4D). Notice the high frequency peak in power, which was common, but not always present. The roll-off slopes for the entire population of MN clustered around -3 for on-cycle and -2.3 for off-cycle (Figure S4E). We attribute the difference in slope to the dendritic arbor of MNs which deviates from the one-compartment assumption and causes a steeper power spectrum. Further, the deviation from the OU-process was also reflected in the fact that estimates of τ_{eff} were an order of magnitude higher than the empirical eRT. Therefore, τ_{eff} was instead estimated by fitting an exponential decay to the initial part of the auto-correlation sequence (main manuscript Figure 4F).

SUPPLEMENTARY Figure 4.



Inter-spike intervals. We measured the mean inter-spike intervals for each cell, while ignoring the inter-burst intervals. The values are shown in supplementary figure 5 with the sample standard deviation. The average value is approximately an order of magnitude higher than the average time constants (Figure 5 A).



Supplementary Method.

Estimating V_m time-constants for NON-OU-processes. An OU-process has a power spectrum with a roll-off slope of -2 on the log-log scale (see Figure S4 above). Our data had a steeper roll-off slope of about -3, which indicates that the one-dimensional process probably is a too simple description of these data and therefore gave poor estimates of the time-constant. A good indicator of the temporal process is either the location of the roll-off in the power-spectrum, or the decay of the auto-correlation function. For slow processes, the auto-correlation has a long decay time and vice versa for fast processes. Thus, we used the initial segment of the empirical autocorrelation function and fitted an exponential decay. The autocorrelation estimate is only reliable up to some lag value \ll the number

of observations in the sample (which is 250 ms at 10 khz = 2500). The recommended maximum lag value differ among authors, but 20-40 lags are generally accepted as long as $N_{\text{observations}} > 160$. Here, we used 30 lags of the sampling interval of 0.1 ms corresponding to 3 ms of the autocorrelation to fit the mono-exponential fit (Figure 4F). Another guideline for estimating this critical lag time is the general formula used in the computer languages R and S-plus:

$$\tau_{\text{lag}} \leq 10 \log_{10}(N_{\text{observations}}) * \Delta$$

where $N_{\text{observations}}$ is the number of samples that the the autocorrelation estimate is based on, and Δ is again the sampling interval (R Development Core Team, 2007). This also suggest use up to $10 * \text{Log}(250 \text{ ms} / .1 \text{ ms/sample}) * 0.1 \text{ ms} = 3.3 \text{ ms} \approx 3 \text{ ms}$ of the autocorrelation to fit the mono-exponential fit (Figure 4F).

Maximum likelihood estimation of effective time constant.

Membrane time constants during quiescence can be estimated from V_m -decay times in response to injected current pulses. In the dynamic situation of intense synaptic input this method is less expedient. Instead, we use maximum likelihood estimation of the effective membrane time constant by assuming V_m is governed by an Ornstein-Uhlenbeck process. The subthreshold membrane dynamics during massive synaptic input (synaptic fluctuations) can be approximately characterized by the Langevin equation i.e. by a passive membrane potential equation with a stochastic input, representing random synaptic input. This is referred to as an Ornstein-Uhlenbeck (OU) process. The Fokker-Planck equation describes the evolution of the probability distribution of V_m , which is Gaussian.

The transition probability turns out to be particularly useful for our analysis, in which each action potential acts as a reset of the V_m distribution, and our task is to follow the evolution of the distribution from this point. If the decay of P_V back to a steady state takes longer after the action potential, than expected from an OU process, the extra time delay must be due to spike after-effects. The power spectra were calculated for windowed data and their slopes were found by linear fitting in the roll-off region (30-500 Hz).

Details of the model neuron. The complete principal model equation of figure 1 including the fast conductances was

$$C \frac{dV_m}{dt} = G_{leak} \cdot (E_{leak} - V_m) - I_{Na} - I_{AHP} - I_K - I_{Ca} - I_{Syn}$$

Where we set $C=1 \cdot 10^{-15}$ F, $G_{leak} = 0.3 \mu S$, $E_{leak} = -54$ mV. During the conductance noise the total conductance was $8 \mu S$.

Na⁺ current. The Na⁺-current was modeled as $I_{Na} = G_{Na,peak} \cdot m_{Na}^3 \cdot h_{Na} \cdot (V_m - E_{Na})$, with activation parameter m_{Na} , from $dm_{Na}/dt = (m_{Na-inf} - m_{Na})/\tau_{mNa}$, where $\tau_{mNa} = 1/[\alpha_m + \beta_m]$, $\alpha_m = (V_m + 40)/[10 \cdot (1 - \exp(-(V_m + 40)/10))]$, $\beta_m = 4 \cdot \exp(-0.0556 \cdot (V_m + 65))$, $m_{Na-inf} = \alpha_m/(\alpha_m + \beta_m)$, and with inactivation parameter, $dh_{Na}/dt = (h_{Na-inf} - h_{Na})/\tau_{hNa}$, $\tau_{hNa} = 1/[\alpha_h + \beta_h]$, $\alpha_h = 0.07 \cdot \exp(-0.05 \cdot (V_m + 65))$, $\beta_h = 1/\{1 + \exp(-0.1 \cdot (V_m + 35))\}$, $h_{Na-inf} = \alpha_h/(\alpha_h + \beta_h)$. $G_{Na,peak} = 480$ nS and $E_{Na} = 50$ mV.

K-current. The K-current was modeled as $I_K = G_{K,peak} \cdot m_K^4 \cdot (V_m - E_K)$, with activation parameter m_K , from $dm_K/dt = (m_{K-inf} - m_K)/\tau_{mK}$, where $\tau_{mK} = 1/[\alpha_{mK} + \beta_{mK}]$,

$\alpha_{mK} = (V_m + 55) / [100 * (1 - \exp(-(V_m + 55) / 10))]$, $\beta_m = 0.125 * \exp(-0.0125 * (V_m + 65))$, $m_{K-inf} = \alpha_{mK} / (\alpha_{mK} + \beta_{mK})$. $G_{K,peak} = 100$ nS and $E_K = -77$ mV.

Ca²⁺-current. The Ca²⁺-current was modeled as $I_{Ca} = G_{Ca,peak} * m_{Ca} * h_{Ca} * (V_m - E_{Ca})$, with activation parameter m_{Ca} , from $dm_{Ca}/dt = (m_{Ca-inf} - m_{Ca}) / \tau_{mCa}$, where $\tau_{mCa} = 7.8 / [\exp((V_m + 6) / 16) + \exp(-(V_m + 6) / 16)]$, $m_{Ca-inf} = 1 / [1 + \exp(-(V_m - 3) / 8)]$ and inactivation parameter $h_{Ca} = 0.01 / [0.01 + [Ca^{2+}]_{in}]$ where $[Ca^{2+}]_{in}$ is the intracellular Ca²⁺-concentration. $G_{Ca,peak} = 0.1$ nS and $E_{Ca} = 141$ mV. The Ca²⁺ removal via buffers, pumps and diffusion was modeled as a simple exponential decay, $d[Ca^{2+}]_{in} / dt = \{ [Ca^{2+}]_{in-inf} - [Ca^{2+}]_{in} \} / \tau_{Ca-removal}$, with $\tau_{Ca-removal} = 10$ ms. $[Ca^{2+}]_{in-inf} = -0.005 * I_{Ca} * \tau_{Ca-removal}$.

AHP-current. The AHP-current is modeled as a non-inactivating Ca²⁺-dependent K⁺-current, $I_{AHP} = G_{AHP,peak} * m_{AHP} * (V_m - E_K)$, where $dm_{AHP}/dt = (m_{AHP-inf} - m_{AHP}) / \tau_{AHP}$, $\tau_{AHP} = 30\,000 / \{ [Ca^{2+}]_{in}^2 * 1.25 * 10^8 + 25\,000 \}$, $m_{Ca-inf} = [Ca^{2+}]_{in}^2 * 1.25 * 10^8 / \{ [Ca^{2+}]_{in}^2 * 1.25 * 10^8 + 25\,000 \}$. $G_{AHP,peak} = 7.99$ nS. These parameters were adapted values published elsewhere (Koch 1999, Gerstner and Kistler 2002).

Synaptic conductance

The synaptic conductances in the high intensity scheme were modeled as Ornstein-Uhlenbeck processes with the stochastic differential equation in compact notation written as

$$dG_t = -\frac{1}{\tau_G}(G_t - G_{mean}) + \sigma_G dW_t$$

and integrated via exact numerical integration (Gillespie 1996) where G is either the inhibitory or excitatory conductance G_i and G_e , and respective relaxation time constants $\tau_i = 2$ ms, $\tau_e = 1$ ms. The values used for both were $\sigma = 0.0005$ nS²/ms and the mean conductances were balanced so $V_m = -68$ mV and $G_{i, mean} = G_{total} - G_{leak} - G_{e, mean}$ and $G_{e, mean} = [G_{tot} * (V_m - E_i) + G_{leak} * (E_i - E_{leak})] / (E_e - E_i)$, and $G_{tot} = 8$ nS. All computer simulations were performed in Matlab (version 7.3, Mathworks) with integration time step of 0.05 ms.

References

Ditlevsen S, Lansky P (2005) "Estimation of the input parameters in the Ornstein-Uhlenbeck neuronal model" *Physical review E*, 71, 011907

Gerstner W, Kistler WM (2002) "Spiking neuron models: Single neurons, populations, plasticity" Cambridge University Press.

Gillespie DT (1996) "Exact numerical simulation of the Ornstein-Uhlenbeck process and its integral" *Physical Review E* 54(2): 2084-2091

Koch C (1999) "Biophysics of computation: information processing in single neurons. Oxford: Oxford university press

R Development Core Team (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.

Tuckwell HC (1988) "Introduction to theoretical neuroscience, Vol. 2" Cambridge, Cambridge University Press