Supplement 2. Calculation of information rates

The main problem in the estimation of the rate of information transfer for finite data sets is whether the available amount of data gives a reasonable statistical description for a reliable calculation. We have performed three types of tests of the quality of our statistical description. Tests 1 and 2 are based on an improvement of the statistics of the data by reducing the dimensionality of the representation of action potential waveforms to check that they contain more information than stereotyped spikes. Test 3 is based on a reduction of the amount of data to check that the calculations do not worsen.

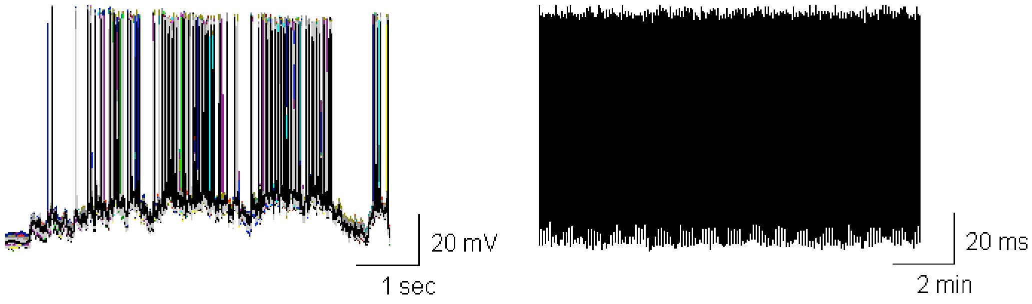
Test 1. We reduce the dimensionality of the representation of action potentials by considering fewer voltage levels. This procedure can reduce the amount of information they contain but can also improve the statistics of the possible patterns. We test that the triple extrapolation method gives comparable results for these representations of reduced dimensionality.

Test 2. We further reduce the dimensionality of data by simply representing the action potential waveforms as rectangular pulses of variable height and width. This reduction of the dimensionality of the representation reduces the values of the information transfer, but as in *Test 1*, the statistics of the possible patterns can be improved. With this representation we find that the waveforms have significantly more information that stereotyped action potentials.

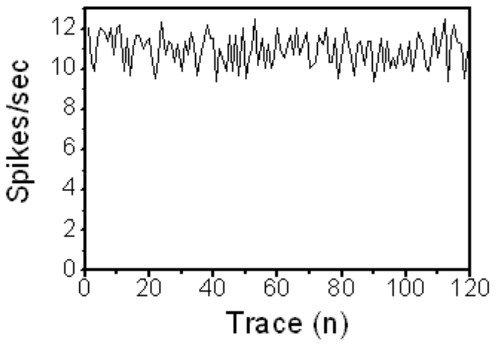
Test 3. We divide a large data file into two sub-blocks and show that the rate of information transfer calculated for each of these blocks is comparable to the one using all the data.

Below we give the details of these three tests after a short discussion of how we start by checking the quality of the data.

Tests for quality of data. All data selected for this paper was inspected for nonstationary behavior. S2-Figure 1 illustrates the quality checks on the raw data. In this example we examine 120 voltage responses (120 superimposed traces) to a virtually identical conductance stimulus resulting from the sum of AMPA events with random arrival times. This recording is from a pyramidal neuron in layer 3 of a 13-days old rat (S2-Fig. 1A).



S2-Figure 1: Quality checks on data. (A, upper left) 120 superimposed traces to a repeated naturalistic input. (B, upper right) The same 120 traces in sequence. (C, lower right) Firing rate of each trace.



Each conductance stimulus sequence was followed by a 20-second long silence to minimize memory effects. Experiments lasted 1-2 hours. Recordings with drifts > 5mV are not considered for analysis. Even drifts of < 5mV could in principle affect the extrapolation to infinity size of data in our calculations. We eliminate the effect of these small drifts on the calculations by randomly shuffling the traces (Juusola and de Polavieja, 2003).

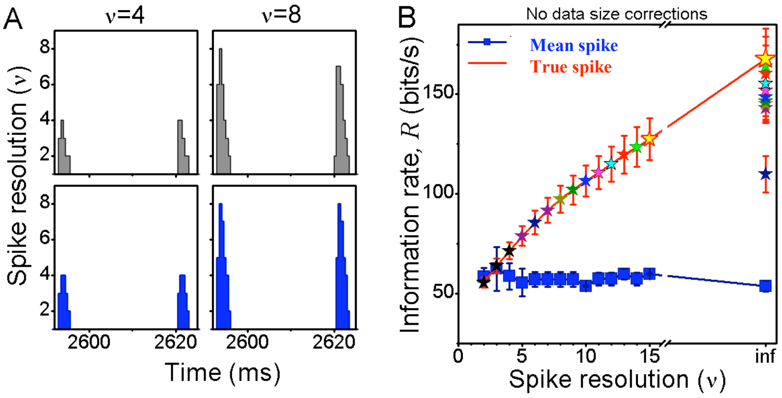
S2-Figure 1B shows the same 120 responses as in S2-Figure 1A but now the trials have been randomly shuffled and are plotted in sequence without the intervals of silence. This shows that there were no progressive changes in spike height or resting potential. We also checked that the firing rate of a neuron did not significantly change across trials. S2-Figure 1C shows the average spike rate for each trial in our test data. Data sets with a variability, measured by the standard deviation, >10% were not considered for analysis. For the data in S2-Figure 1 the variability is 7.3% (the spike rate is 10.9 ± 0.8, mean ± SD) and the data was selected for analysis.

Test 1. In the calculations of the information transfer used in the paper, we extrapolated the information rate using the trends obtained in the entropies from 5 to 15 voltage levels. To test that the statistical description with this many levels is reasonable, we have performed calculations with lower number of voltage levels.

First we describe how we digitize the data and give details of the calculation of the information rate that are relevant for the calculations with lower dimensionality. The data from S2-Figure 1 is digitized at different levels of voltage resolution ν (from 2 to 15) in the following way. We cut the voltage responses 33 mV above the resting potential, effectively discarding any subthreshold activity from the dataset. The action potentials are then digitized using ν voltage levels using 2 kHz sampling (0.5 ms bin size). The sampling rate is selected so that action potentials fall within 10 ms long patterns (i.e. 20 letters long words).

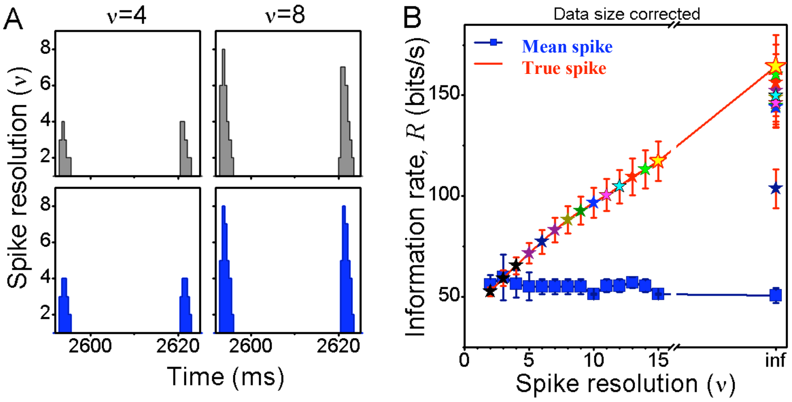
Two consecutive action potentials are shown for 4 and 8 voltage resolutions (S2-Fig. 2A, gray and blue for true and stereotyped spikes, respectively). S2-Figure 2B (colored stars) gives the information rate calculated using different numbers of voltage levels ν and without the extrapolation in the voltage levels or the data size. Increasing the number of voltage levels increases the information rate. In the calculation of entropies, we used trends in the range 5-15 voltage levels for the extrapolation to infinite number of voltage levels. For this data the extrapolation results in an information rate of 167.6 ± 15.4 bits/sec (big yellow star). An analogous calculation for stereotyped spikes, made by substituting each spike by the average spike, results in an information rate of 57.1 ± 2.5 bits/sec independently of the number of voltage levels used (S2-Figure 2B, blue squares).

S2-Figure 2: The effect of voltage resolution, ν, on the information transfer rate, R, of action potential waveforms (gray) and stereotyped, or mean, spikes (blue) using the data of S-Fig1. (A) Spikes digitized using 2 kHz sampling at 4 and 8 voltage levels. (B) Increasing the voltage resolution does not increase the information transfer rate of the mean spikes (~50 bits/s) but converges to a more than 3 times larger value for the true spikes.



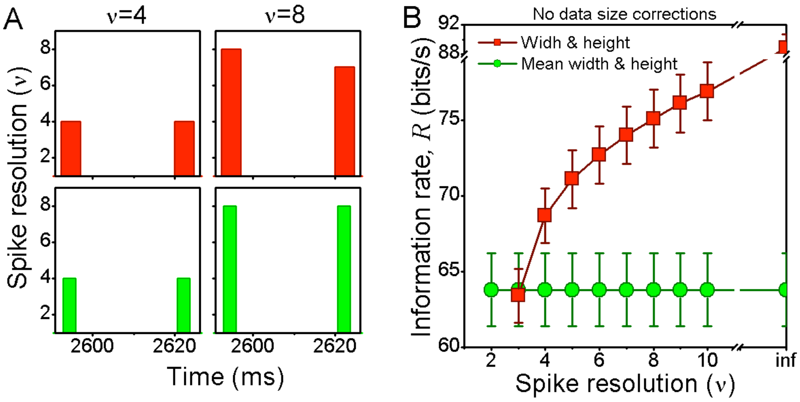
We test whether a representation of lower dimensionality of the action potentials, and therefore with richer statistics, gives similar results. Instead of using 5 to 15 voltage levels in the infinite voltage level limit of the trends in the entropies, we test the results for trends in the interval 5 to ν with ν varying from 6 to 14. S2-Figure 2B shows these 9 extrapolated points (at the right, inf) using the same symbols as for the rates with ν number of voltage levels without extrapolation. The values of the information rates are similar to the original value of 167.6 ± 15.4 bits/sec except when the extrapolation uses only two points, levels 5 and 6. Thus we have shown that for representations of reduced dimensionality the information transfer has similar information rates, for the case in the S2-Figure 2B around 3 times the one for stereotyped spikes.

Results in S2-Figure 2 are given without extrapolation to infinite *size*, which also corrects for defects in size. S2-Figure 3 shows the results including this limit with results very similar to those in S2-Figure 2. Once extrapolated to the limit of infinite data *size* and voltage resolution the information transfer rate of the action potential waveforms and the stereotyped spikes are 164.5 ± 15.5 and 50.8 ± 3.7 bits/sec, respectively. We thus show that for representations of reduced dimensionality the information transfer have similar information rates, always around 3 times the one for stereotyped spikes for this recording.



S2-Figure 3: The effect of voltage resolution, ν, on the information transfer rate, *R*, of action potential waveforms (gray) and stereotyped, or mean, spikes (blue) using data (S-Fig1) that is corrected for infinite size. (A) Spikes digitized at 4 and 8 voltage levels. (B) Increasing the voltage resolution does not increase the information transfer rate of the mean spikes (~50 bits/s) but converges to over 3 times larger value for the true spikes.

Test 2. We now perform a more drastic reduction of dimensionality in the representation of the action potentials. We now replace each action potential with a rectangular pulse of the same width and height (S2-Fig. 4A, red bars) and calculate the information transfer rate for different voltage resolutions as above using all the data. This representation has important loss of information with respect to the digititization in previous figures, but has the advantage of a much richer statistical description. Using solely the widths and heights, our test data was able to transmit at 88.8 ± 1.9 bits/sec. Although this is about 50% less than the true action potential waveforms, it is still roughly 50 % more than the stereotyped action potentials or pulses that have the width and the height of a mean action potential (S2- Figs. 4A and 4B, green bars and circles, respectively).



S2-Figure 4: The effect of width and height on the information transfer rate. (A) Spikes are replaced by bars (red) that have their widths and heights taken at 33 mV above the resting potential, or by bars that have the mean values (green). (B) Increasing the voltage resolution does not increase the information transfer rate of the mean bars but increases it for the bars that have the true width and height values of the spikes.

Test 3. To test that the statistics of the data is large enough for the calculation of information transfer, we have eliminated part of the data and checked that the calculations do not suffer an important change. We have divided one recording into two halves and calculated the rate of information transfer for each half and the total recording. S2-Figure 5 shows that for these three calculations the information transfer for the true spike is three times that of the stereotyped spike.

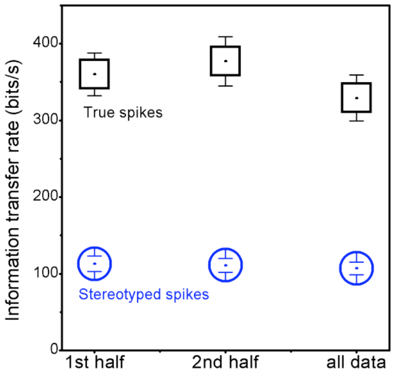


Figure 5. The effect of the size of the recordings on the calculations of the information transfer rate.

References

Juusola M, de Polavieja GG (2003) The rate of information transfer of naturalistic stimulation by graded potentials. J Gen Physiol 122: 191-206.