Supplement 1. Conductance injection vs. current injection

In experiments that measure integrative properties of neurons using postsynaptic electrical stimulation, it is much more natural to use conductance waveforms than the traditional fixed waveforms of current. In addition, the conductance may have its own voltage-dependence (as we have used to model NMDA receptor gating), or voltage-controlled time dependence as in a Hodgkin-Huxley formulation. Conductance stimulation by real-time digital computation to synthesize the current command input for a current-clamp amplifier, also called dynamic clamp, was described over a decade ago (Robinson, 1991; Robinson and Kawai, 1993; Sharp et al., 1993). With the advent of slice-patch recording and improved computational descriptions of synaptic input, the technique is being increasingly adopted as the best way of allowing direct measurement of synaptic integration (e.g. Awatramani et al., 2004; Williams and Stuart, 2003; Cathala et al., 2003; Mitchell and Silver, 2003; Harsch and Robinson, 2000). Since it allows precise, repeated stimulation with the same synaptic conductance waveform, the technique is ideally suited for measuring the variability of postsynaptic spike generation in ensemble experiments, which is essential for assessing information rates. This variability cannot be distinguished properly when stimulating the actual synaptic inputs to a neuron, owing to presynaptic variability.

The traditional (technically-simpler and therefore still widely-used) approach to measuring the integrative properties of neurons is to stimulate with a fixed current pattern specified as a predetermined command signal in a current-clamp recording. The problem with this is that the stimulus fails to react to the changing membrane potential in a realistic way. As a simple example, synaptic input sufficient to drive a cell to fire greatly shortens the time constant of the cell because of the extra conductance of the membrane. This effect is produced by conductance injection but not by current injection. Even in a completely passive membrane, the relationship between the amount of input (conductance) to the output (voltage deflection) can be highly nonlinear, as the membrane approaches the reversal potential. The response of passive membrane is, however, related linearly to input current. During the integration of a spike, the current flowing through the excitatory conductance is damped down to zero and then reversed, shunting the inward sodium current which is driving the spike depolarization, and consequently also slowing the upstroke, leading to a correlated variations in spike amplitude and spike width, which are directly related to the size of the suprathreshold conductance input.

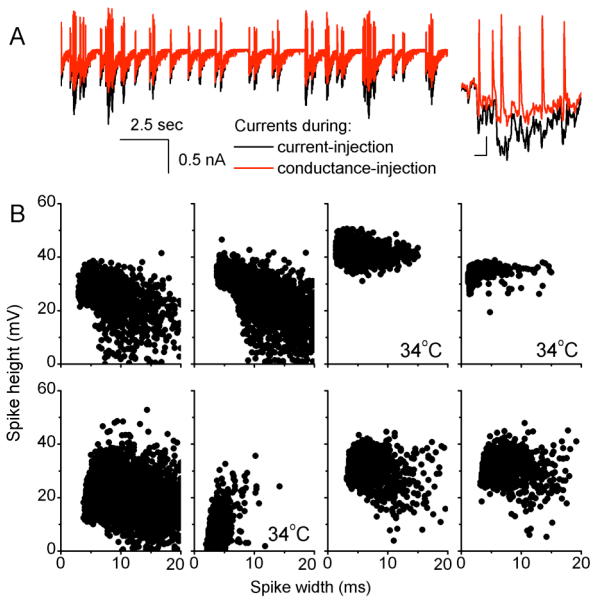

Figure S1-1: Voltage responses of neurons differ between current injection and conductance injection. (A) Recorded traces of the injected current during current clamp (black) and dynamic clamp (red) to the same fluctuating input pattern. The insect highlights the differences; 20 ms and 0.2 nA scale bars. (B) The heights and widths of action potentials (black circles) during fluctuating current stimulation occupy large ranges, and often show less obvious correlations than similar conductance injection / dynamic clamp experiments (cf. Fig. 1 and Fig. 3 in the main Article). The subfigures show experiments from eight different neurons. The widest action potentials are likely to be calcium spikes evoked by large current inputs. These experiments were done in room temperature if not indicated otherwise.

In current-clamp with a fixed pattern of current, on the other hand, the fluctuation of current which finally triggers the spike is undiminished by the spike, and there is no shunting – the spike is then driven to have an amplitude and width which bear very little relation to the prevailing input level or its recent history. Generally, spikes in current-clamp are taller and wider – the dynamic time constant of the membrane is longer in current-clamp. This is illustrated in S1-Figure 1 which shows the differences between the current injected through the conductance stimulus and the current injected as a fixed pattern in conventional current-clamp, obtained from the conductance pattern by assuming a steady resting-potential. There are large differences in the form, and even the sign, of the two stimuli. S1-Figure 1B shows scatter plots of spike height vs. width for 8 different current-clamp experiments. There is very little correlation between the two quantities, in contrast to the plots in Figures 1, 3 and 4, obtained with conductance injection. Waveform signaling thus operates differently for current injection than it does for the more natural conductance injection.

Average stimulus for current clamp data

In the main text, we have given the average stimulus before action potentials of different widths for conductance injection / dynamic-clamp data Here we give an analogous analysis for current-clamp data.

S1-Figure 2A shows the width-height relationship of action potentials generated by a predetermined current pattern. In general, these action potentials are taller (15-20 mV) than those evoked during dynamic clamp. Again the thin spikes are produced by transient stimuli rapidly rising from silence and the fat spikes correlate with high stimulus levels (S1-Fig. 2B). However, the variances of the average stimulus at 5 and 40 ms (S1-Fig. 2C) before the spikes are larger than in conductance injection for the same neuron, indicating a worse encoding. The widest action potential are likely to be calcium spikes evoked by the large current inputs (see also Fig. 7 in the main paper).

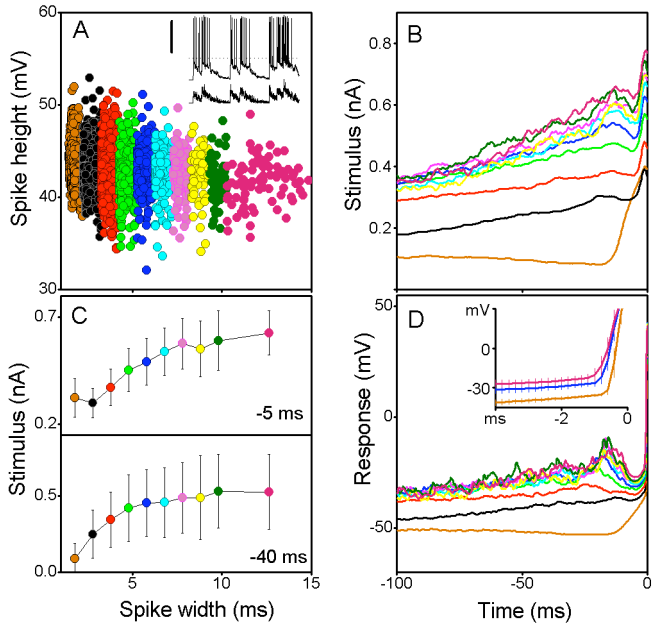

Figure S1-2: Action potential waveforms of pyramidal neurons responding to naturalistic input during a current-clamp experiment. (A) The heights and widths of action potentials occupy a large fuzzy range (16-day-old rat at 34°C). Action potential waveforms (top inset; black trace) vary with synaptic input patterns of a sum of AMPA unitary events. Scale bars: 30 mV and 0.5 seconds. The thin horizontal line indicates the voltage (-30 mV) where the action potential widths were measured. The widths are color-coded into 10 groups (lower inset, 9 equally-spaced with the 10th including the remainder), from narrow to wide as: orange, black, red, green, cyan, blue, magenta, yellow, purple and olive. (B) Average stimulus (current) history preceding the peak of the action potentials for each group (same coloring). (C) Mean and SD of the current histories for each action potential group at 5 ms (upper window) and 40 ms (lower window) before firing. (D) Average voltage responses preceding the action potential waveforms (same grouping and coloring). The inset shows the mean and SD of the voltage response 4 ms before firing for three action potential groups.

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