Supplementary Figure S1. Representative learning curve for the microstimulation report task. Microstimulation detection performance over the time course of a representative experiment. Circles indicate the lowest microstimulation currents to which the animal responded consistently in microstimulation-only training sessions. Squares indicate the average microstimulation currents used in single cell stimulation experiments (as explained in Figs. 1a and b) or extracellular current injection control experiments.
Supplementary Figure S2. Neuronal responses during and after microstimulation.

a, Top: trace of a microstimulation trial during a single cell stimulation experiment. Middle: AP (black ticks) raster plot. Bottom: Peristimulus time histogram of AP activity during microstimulation trials (stimulation current: 4.5 µA).

b, Top: trace of a microstimulation trial during another single cell stimulation experiment. Middle: AP (black ticks) raster plot. Bottom: Peristimulus time histogram of AP activity during microstimulation trials (stimulation current: 2.5-3 µA).

Across the population of neurons AP firing during microstimulation pulse trains fell into the following categories: ~ 26% of neurons showed > 20 APs per train (as the neuron shown in a); ~ 41% showed 1-20 APs per train; ~ 33% showed no APs or only sporadic APs (as the neuron shown in b). The percentages given apply to the response pattern observed in the majority of trials.

Across the population of neurons AP firing directly after microstimulation pulse trains fell into the following categories: ~ 61% of neurons showed a transient reduction compared to baseline AP firing (as the neuron shown in a); ~ 12% showed a transient reduction compared to baseline firing followed by a transient increase (as the neuron shown in b); ~ 10% showed a transient increase compared to baseline AP firing; ~ 3% showed no deviation from baseline AP firing; the remaining ~ 14% of neurons showed close to zero AP firing both before and after microstimulation.

Microstimulation pulses induced large transients in the juxtacellular recording which complicated the detection of APs. In some neurons with large APs a thresholding procedure could be used but in most cases APs were detected by visual inspection.
Supplementary Figure S3. Response rates for single cell stimulation trials vs. catch trials in 14 individual animals. Each panel shows the data from one animal. Response rates for single cell stimulation trials (hits) are plotted against false positive rates (response rates in no-current-injection catch trials or subthreshold current injection catch trials). Same data as depicted in Figs. 3a and b. One further experimental animal did not contribute single cell stimulation data.
**Supplementary Figure S4.** Large (> 0.5 mV) secondary action potentials occur only rarely in single cell stimulation experiments.

**a.** Left: trace of a juxtacellular current injection trial. In addition to the APs of the primary neuron that was approached for juxtacellular stimulation, large secondary APs were observed. Triangles indicate onset and offset artifacts of the current injection. Right: Quantification of AP rates of the primary and the secondary neuron in the 1 s prior to each stimulation (Spont.) and during juxtacellular current injection (Stim.). The activity of the primary neuron was more strongly modulated by juxtacellular stimulation than that of the secondary neuron.

**b.** From left to right: fraction of single cell stimulation experiments in which one or more large secondary APs were observed during juxtacellular current injection; average fraction of trials for which large secondary APs were observed during juxtacellular current injection; average fraction of APs accounted for by large secondary APs during juxtacellular current injection.

To obtain these numbers all stimulation trials were visually inspected for large (> 0.5 mV) AP waveforms that deviated from the AP waveform of the primary neuron. Error bars indicate the s.e.m.
Supplementary Figure S5. Small (0.25-0.5 mV) secondary action potentials are not modulated by juxtacellular stimulation.

a, Left: trace of a juxtacellular current injection trial. In addition to the APs of the primary neuron that was approached for juxtacellular stimulation, small secondary APs were observed. Triangles indicate onset and offset artifacts of the current injection. Right: Quantification of AP rates of the primary and the secondary neuron in the 1 s prior to each stimulation (Spont.) and during juxtacellular current injection (Stim.). The activity of the small secondary neuron was not modulated by juxtacellular stimulation (p > 0.05, chi-square test).

b, AP rates of three additional small (0.25-0.5 mV) secondary neurons in the 1 s prior to stimulation (black bars) and during juxtacellular stimulation (white bars). Spontaneous and stimulation AP rates were not significantly different for any secondary unit (p > 0.05, chi-square test), indicating that these units were not modulated by juxtacellular stimulation.

This analysis of small secondary background units could only be performed in four juxtacellular stimulation experiments. In other experiments we either did not observe small (0.25-0.5 mV) secondary APs or juxtacellular stimulation was associated with increased high-frequency noise, which prevented us from quantifying AP rates of the small background units during stimulation. Error bars indicate the s.e.m.
Supplementary Figure S6. Detection of microstimulation and single cell stimulation in barrel cortex occurs in the absence of stimulation evoked whisker movements.

a. Microstimulation near the sensory threshold does not evoke whisker movements. Left: averaged traces of whisker position in microstimulation trials at different current strengths. Dashed lines indicate the onset and offset of the microstimulation (40 cathodal pulses at 200 Hz, 0.3 ms pulse duration). Microstimulation at 5 and 10 µA evoked backward whisker movements (top two traces), whereas microstimulation at 3 and 3.5 µA did not evoke whisker movements (bottom two traces). Whisker movements near and after microstimulation offset (top three traces) are related to the animal’s licking response. Measurements relate to whisker C2, which showed microstimulation evoked movements that were as strong as or stronger than those of other whiskers of the animal. Right: The animal detected microstimulation currents of 3.5 µA and above, whereas performance dropped to chance value at 3 µA. Thus, at 3.5 µA, the animal reported a large fraction of microstimulation events (54%) in the absence of evoked movements. The same observation (large fraction of reported microstimulation events in the absence of evoked movements) was made in 12 further microstimulation / whisker tracking experiments.

b. Single cell stimulation in barrel cortex does not evoke whisker movements, even when it leads to a bias towards responding. Dashed lines indicate the onset and offset of the single cell stimulation (top two traces) or microstimulation (bottom trace). Stimulation of the cell led to a bias towards responding (single cell stimulation hit rate 18.2% vs. false positive rate 10.7%). Top: trace of whisker position in a single cell stimulation trial that led to a hit. Middle: averaged trace of whisker position over all single cell stimulation trials (n = 101) for this cell. No whisker movements were evoked. The same result was obtained when averaging was restricted to the subset of hit trials. Bottom: Microstimulation at 15 µA evoked movements of the tracked whisker D1. The same observation (an absence of single cell stimulation evoked whisker movements) was made in 8 further single cell stimulation / whisker tracking experiments.

Whisker movements were measured with a high-precision tracking system (ISCAN, Burlington, MA, USA). Whiskers constitute a minimal load and were free in the air, which makes it unlikely that non-overt / isometric whisker muscle contractions were evoked.
Supplementary Figure S7. Sensory effects depend on the overall responsiveness of the animal. Response rates for single cell stimulation trials (hits) are plotted against false positive rates (response rates in no-current-injection catch trials or subthreshold current injection catch trials) for barrel cortex neurons (n = 70; same data as depicted in Figs. 3a and b). The data set was divided into two equal subsets (n = 35) depending on the overall response rate obtained in each experiment (pooled number of responses in single cell stimulation trials and catch trials divided by pooled number of single cell stimulation trials and catch trials). When animals were conservative (filled squares; overall response rate ≤ 15%) the sensory effect (hit rate - false positive rate) was small (and not significant); at high overall response rates (> 15%; open squares), however, the effect size was ~ 9% (and significant). Extracellular current injection control experiments did not show a dependence of effect size on overall response rate (data not shown).
**Supplementary Figure S8.** Maximum discharge patterns of identified excitatory neurons and fast spiking, putative interneurons. All data relate to the juxtacellular current injection trial for which the maximum number of spikes were evoked in a single cell stimulation experiment.

**a.** Maximum discharge patterns of identified excitatory neurons. Stimulation currents were from top to bottom: 8, 9, 20, 8 nA. Some cells show massive loss of spike height during juxtacellular current injection. Such loss of spike height was frequently observed for the maximum discharge patterns of identified and putative excitatory neurons (in about one third of cells), but it occurred only rarely in the average stimulation trial. Note that the layer 4 pyramidal neuron did not enter our data set because not enough trials were collected.

**b.** Maximum discharge patterns of fast spiking, putative interneurons. Stimulation currents were from top to bottom: 22.5, 8, 22, 18 nA. The top three maximum discharge patterns relate to the three putative interneurons that induced the strongest biases towards responding in the data set (see Fig. 3e), whereas the fourth putative interneurons induced an opposite bias.