Near-optimal information relay by the neuronal population of layer 4 barrel cortex

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Abstract: Cortical function reflects the coordinated activities of populations of neurons, which, in turn, depend on the speed with which each neuron can respond to input, as revealed by dynamic gain analysis. In Layer 4 of the rodent barrel cortex, a finite population of interconnected, small, excitatory neurons rapidly and briefly relays input from the specific thalamus to the rest of the cortical column. Theory predicts that the determinants of a population's dynamic gain - cell number, cell size and the correlation time of the background noise - control the speed with which the population can respond to input. Here, we demonstrate how these parameters are optimized such that a single thalamocortical input spike is reliably reflected in the output population response of layer 4. We show that the synaptic receptor dynamics that dominate the background noise in layer 4 are slower than in other layers. We further show that the speed with which the spike-generation machinery can respond depends on the activity of KV7 channels, suggesting that the relay function of layer 4 is under muscarinic control.

One-Sentence Summary: In layer 4 of the somatosensory cortex, the neuronal population is able to perform its precise temporal function because it is tuned by a set of disparate parameters.

A major challenge of modern neuroscience is to understand how information is encoded and transferred within and between neuronal circuits, given the circuit infrastructure, including the morphology, connectivity, molecular properties and physiological characteristics of the constituent neurons. In cortex, individual neurons fire no more than a few action potentials (APs) each second (*1–4*), so information is encoded as the combined spike output of subpopulations of neurons responding to common inputs (*5*, *6*). The encoding capability of a population may be inferred from the dynamic gain curve, which represents the temporal precision with which the combined firing rate of the neurons can track changes in the common input stream (*6*). Numerous theoretical studies investigated the basic principles of population coding. Early work by Knight (*7*) demonstrated that the information present in the common input can be perfectly encoded by an infinitely large population of noisy, instantaneously responding neurons. However, the size of cortical populations is finite and actual neurons are not perfect encoders. Subsequent studies that implemented more realistic, biophysically inspired neural models have identified a number of features that constrain the quality of encoding by a finite population of neurons. These include the correlation time of the background noise (*8*), the dynamics of action potential generation (*8*, *9*), and the dendritic size of the of the neurons involved (*10*, *11*). Taken together, these studies predict that the encoding performance of a neuronal population reflects multiple factors, including the number of neurons involved, their morphology, the kinetic properties of the synaptic activity responsible for the background noise, and the ionic mechanisms governing AP generation. However, the relative importance of each of these elements can only be determined experimentally.

Experimental assessment of these theoretical concepts requires a stable population of well-defined neurons, in which each of the above factors can be determined. However, a cortical neuron may participate in many functional networks, and at any moment, a relevant subpopulation of neurons is sparsely embedded within a larger network (Fig. 1A). Moreover, the characteristics of the common input are usually unknown. Here, we overcome these problems by studying encoding in a stable population of well-described cortical neurons: the excitatory cells of Layer 4 (L4) of the mouse somatosensory cortex (*12–16*). Numbers of neurons in this population, and their input and output are known (*17*, *18*). The common input is via projections of thalamocortical relay cells (*18*, *19*), the background noise reflects activities of recurrent connections among other L4 cells of the same barrel (*15*, *19*), and the population output converges onto pyramidal cells in Layers 2, 3 and 5 (*15*). L4 is thus a relay layer, rapidly informing the rest of the cortical column about changes in tactile input. However, because L4 neurons have extremely short dendrites that span no more than a few hundred microns (*20–22*), we expect their encoding capacity to be limited. Here, we have experimentally compared the encoding bandwidth of L4 neurons to that of the much larger Layer 5 (L5) pyramidal neurons and found that, as predicted by theory, it is reduced, given the same noise parameters and population size. However, we now demonstrate that synaptic receptor kinetics, axonal potassium channel properties and population size combine to compensate for the morphological constraints. Thus, all these factors appear to be matched, such that a single thalamocortical AP is reliably reflected in the L4 population output.

Results

Identification of the factors that influence coding capabilities, as predicted by theory.

We created a simple model that examines the influence of cellular parameters on a neuronal population's coding capability. It captures how diverging feedforward input is relayed through a population of neurons whose output converges onto readout neurons (Fig.1A, black arrows onto blue relay neurons and blue arrows onto red readout neuron). Each neuron consists of a soma and

a dendrite of length L. Each neuron has two distinct sources of input: thalamocortical projections and massive local, recurrent connectivity. In cortical networks, the postsynaptic effect of local connectivity reflects a balance of recurrent inhibition and excitation, and it is largely asynchronous from neuron to neuron. We represented this input to each neuron as independent, stochastically fluctuating current with correlation time τ. Its amplitude was chosen to evoke irregular AP firing at a background rate ν_0=3 Hz (Fig. 1B).

The relevant information enters into a subpopulation of N neurons through diverging feedforward input where it evokes synaptic currents that appear in synchrony at each neuron in the subpopulation (Fig.1A, blue neurons). When the N neurons receive a presynaptic AP, the firing rate of the population momentarily increases ($\Delta\nu$ in Fig.1B). The salience of the minimal input for the readout neuron depends on the size and duration of $\Delta\nu$. We analyzed this using an "optimal decoder" (see methods), which compares the rate increase during a 3 ms time window against the rate fluctuations that precede the common input. The decoder returns a detection probability (pDetect, see methods). This analysis reproduced the dependencies expected from previous experimental and theoretical studies: larger dendrites (L) and longer noise correlation times (τ) improved the dynamic gain at high frequencies (insets in Fig. 1 C and 1 D). This in turn increases $\Delta\nu$ (see also Fig. S3G-J), which improves the detectability of the single afferent AP. Enlarging the population size (N) was also beneficial, because it reduces the fluctuations in population rate (Fig. 1B, E, F). In an actual neural population, the coding efficiency will reflect some combination of these three parameters (Fig. 1 G). Note that with a correlation time of 5 ms, populations of neurons with short (200 μm) dendrites require 1200 cells to achieve 90% detection probability, whereas only 700 are required when dendrites are long (2000 μm).

For a given τ, L4 neurons are less able than L5 neurons to code high frequencies

We sought to understand how these theoretical concepts of population encoding are implemented by actual cortical neurons and cortical circuits. Excitatory neurons in L4 and L5 differ dramatically in size (Fig. 2A). Most excitatory L4 neurons are spiny stellate cells; their dendritic arbors are short and limited to the same layer and cortical column (*14*, *17*, *18*, *21–24*). The L5 pyramidal cells, by contrast, have broad basal dendritic skirts and large apical dendrites that extend throughout the cortical depth (*25*, *26*). To determine how these two cell types compare electrophysiologically, we performed whole cell patch clamp recordings from identified L4 and regular firing L5 neurons in acute brain slice of the mouse somatosensory cortex. Table 1 summarizes the passive and active membrane properties of the cells. L4 neurons had approximately 2.5-fold lower membrane capacitance, 6-fold higher input resistance and 2-fold longer membrane time constant, consistent with their compact morphology (*24*). As seen in the inset in Fig. 1D, our theoretical model predicts that the smaller L4 neurons should be less able to encode high frequencies. To test this experimentally, we determined the encoding bandwidths of L4 and L5 neurons by analyzing the timing of their AP output during current noise injections. Injected currents were composed of small sinusoidal currents of various frequencies embedded in randomly fluctuating background noise with a correlation time τ of 5 ms (Fig. 2F, methods). Constant DC current adjusted the mean membrane potential such that cells fired irregularly at 5 Hz. The relation between AP timing and the embedded sinewave was determined for each frequency (Fig. 2D, E). The raster plots and corresponding AP timing histograms demonstrate AP locking of L4 and L5 neurons to slower (50 Hz) and faster (200 Hz) sine wave frequencies. At 50 Hz, APs in both L4 and L5 neurons clustered to the depolarizing phase of the sine wave; at the higher frequency, phase-locking was much weaker in the L4 neurons (Fig. 2D, E, G). Statistical analysis revealed no significant difference in vector strength (*6*, *27*) at 50 and 100 Hz, whereas, at 200, 500 and 750 Hz

the vector strength was significantly lower in the L4 neurons (Fig. 2G). These data thus confirm the theoretical expectation that the compact L4 neurons have a narrower encoding bandwidth than the L5 pyramidal cells with their extensive dendritic trees. However, actual spiny stellate neurons *in vivo* respond quite rapidly to weak afferent stimuli (*28–30*), and their functional bandwidth must actually be broader. We therefore tested the possibility there is a difference in another parameter which determines the encoding bandwidth, namely the temporal characteristics of the fluctuating background current. The actual background current of cortical neurons has never been experimentally determined.

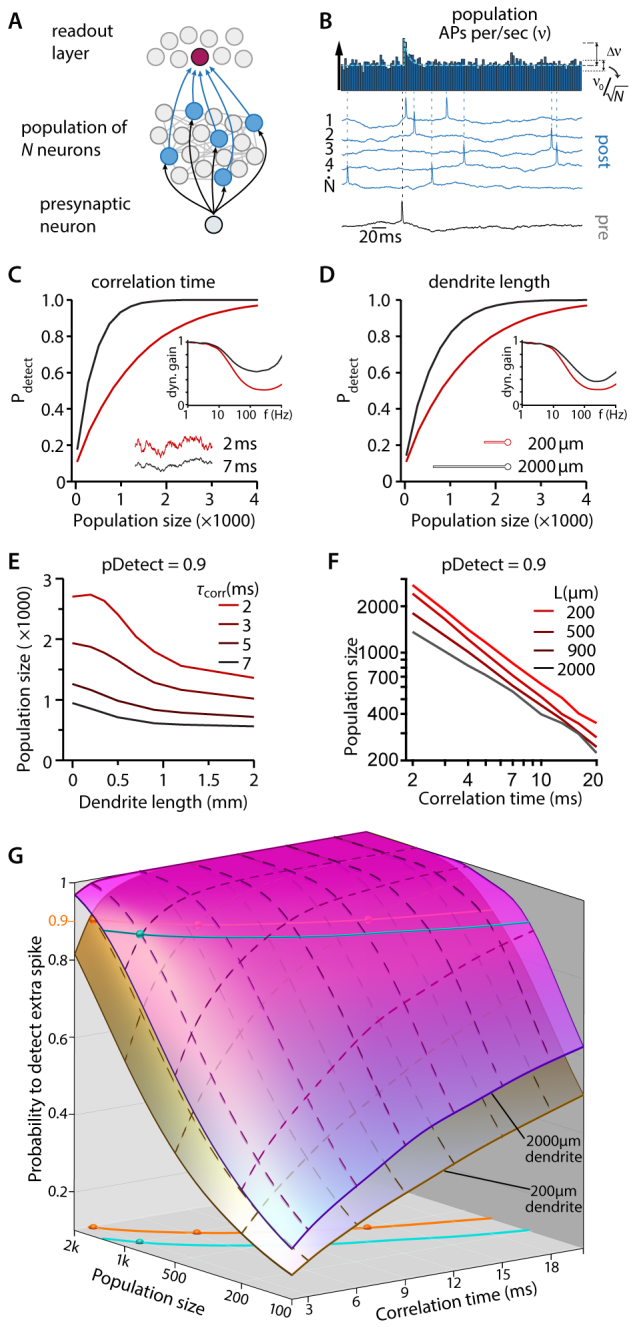

Fig. 1. Encoding and decoding in a model population of neurons (**A**) Schematics showing a simplified neural network comprised of a single presynaptic neuron connected to 'N' postsynaptic neurons firing asynchronously at about 5Hz, and a readout layer which detects firing rate changes in the postsynaptic population. (**B**) Brief increases in the population firing rate (Δν) occur when a single AP-induced excitatory synaptic current is added to the total input. The probability of detection (pDetect) by an ideal decoder (methods) in the readout-layer depends on the relationship between Δν and the basal firing rate fluctuations ($\nu_0/\sqrt{N}$). (**C**) The effect of background synaptic noise correlation time on pDetect and on dynamic gain. (**D**) The effect of dendrite length on pDetect and on dynamic gain. **(E-F)** pDetect reflects relationships between population size, dendrite length and synaptic noise correlation time. (**G**) Summary of the relationship between properties of recurrent synaptic connections (correlation time), size of the encoding population N, and the size of the dendrites L. Shown are surfaces for 200 and 2,000 micrometer long dendrites. Combinations of the three parameters that allow for a 90% detection probability are indicated by colored lines (blue for L=2,000 micrometer, orange for L= 200 micrometer).

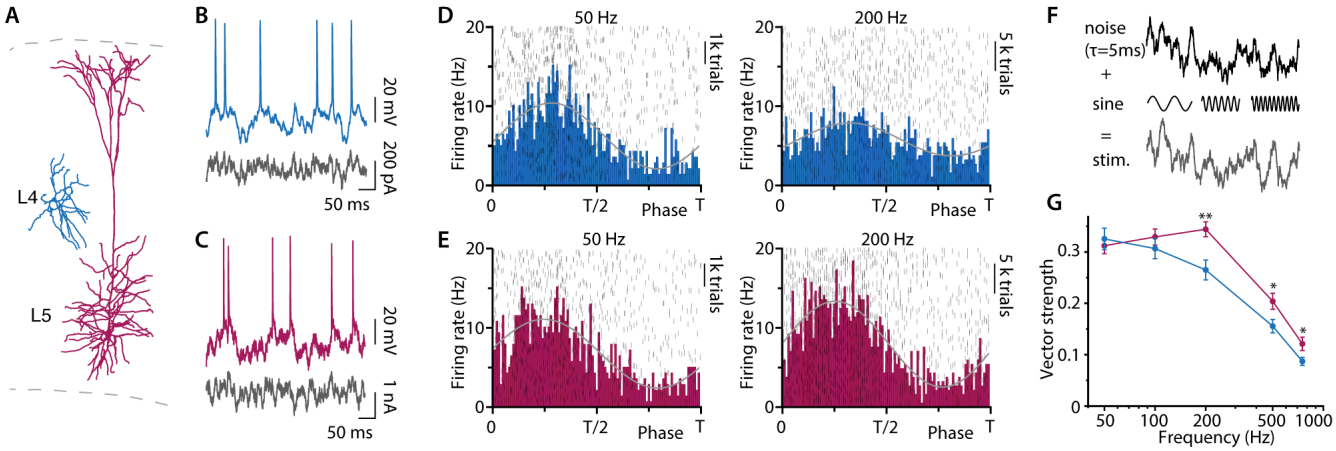

Fig. 2. When temporal characteristics of the noise are the same, L4 neurons are less effective than L5 neurons at encoding high frequencies. (**A**) Laminar position and morphology of L4 (red) and L5 (blue) excitatory neurons in the somatosensory cortex. (**B**) and (**C**)Voltage fluctuations and neuronal firing in response to current noise injections (black) in a L4 cell (B) and a L5 pyramidal cell (C). (**D**) and **(E)** Raster plot (grey) and histogram of AP times as a function of phase of the embedded sine wave (50 and 200 Hz) for L4 cells (D) and L5 pyramidal cells (E). Data in raster plots and histograms pooled from 3 neurons for each group. **(F)** For both cells, stimuli are the sum of a fluctuating waveform with 5 ms correlation time and a small sinewave of 50 to 750 Hz frequency. (**G**) Vector strength as a function of input frequency (50 Hz, n = 12 L4, n = 13 L5, p=0.61; 100 Hz, n = 13 L4, n = 13 L5, p=0.38; 200 Hz, n = 13 L4; n = 13 L5, p= 0.003; 500 Hz, n = 13 L4; n = 13 L5, p= 0.026; 750 Hz, n = 12 L4; n = 13 L5, p= 0.038). Shown are means ± SE.

Parameter / Cell type	V_{rest} (mV)	τ_{mem} (ms)	R_{input} (MΩ)	C_{mem} (pF)	AP Threshold (mV)	AP Amplitude (mV)	AP max. dV/dt (V/s)	AP min. dV/dt (V/s)	Bursting (%)
Layer 4 (n=12)	-74±1.2	31.3±4.0	450.4±68.1	75±7.5	-44.0±1.4	69.3±2.9	310.4±23.7	60.3±2.4	0
Layer 5 (n=13)	-66.8±1.1	17.3±1.2	90.1±10.6	212.5±20.5	-43.5±1.7	83.4±2.5	367.8±20.1	78.2±3.8	46
p value	<0.001	0.002	<0.001	<0.001	0.83	0.0012	0.07665	<0.001	

Table 1. Properties of the excitatory cells studied in tangential slices (layer 4) and coronal slices (layer 5) of somatosensory cortex. Values represent mean and standard error, p-values are uncorrected results of t-Tests.

Fluctuating background current is slower in L4 neurons

During ongoing cortical activity, the background synaptic noise in a neuron reflects the relatively uncorrelated synaptic bombardment by excitatory and inhibitory local inputs (*4*, *28*, *29*). To preclude inputs from other brain areas and maximize the spatial effectiveness of the voltage clamp, we recorded in brain slices. Because neocortical neurons maintained in brain slices are mostly quiescent, we induced reverberating synaptic activity by briefly uncaging glutamate (MNI-glutamate) several hundred microns away from the recording site (Fig. 3A, B). Care was taken to ensure that the uncaged glutamate did not come into direct contact with the cell under study, such that the subsequent synaptic barrage that resulted tens of milliseconds later reflected the activity of the local circuitry. Because we were interested in the effect of background noise characteristics on AP firing, we held the neuron at -50 mV, close to AP threshold. In these voltage clamp recordings, space clamp was maximized by replacing intracellular K^+ with Cs^+ while intracellular Cl^- was maintained at physiological level. As shown in Fig. 3B, UV flashes (50 ms) elicited subsequent network activity lasting up to 0.5 sec. The consequent synaptic noise consisted of a mixture of inhibitory and excitatory synaptic events (Supp Fig. 1A). However, since the voltage was held near the IPSC equilibrium potential, the recorded current was almost entirely excitatory (Supp. Fig. 1A, B).

We measured the temporal dynamics of the background activity in L4 neurons and found them to be significantly different than those of L5 neurons. The autocorrelation of the fluctuating background activity revealed that fluctuations in L4 decayed more slowly (Fig. 3C). In Fig. 3D, the plots were quantified by fitting a single exponential to the averaged autocorrelation function (10-20 trials per neuron); correlation time was 3-5 times slower in the L4 neurons. In order to determine how this difference in correlation time of the synaptic noise relates to the kinetics of the underlying synaptic events, we examined spontaneous excitatory post-synaptic currents (EPSCs) recorded at a holding potential of -70 mV, and inhibitory post-synaptic currents (IPSCs) at a holding potential of 0 mV in the two cell types (Supp. Fig. S1A and B). While the time course of IPSCs in L4 and L5 cells was not significantly different, EPSC decay in L4 neurons was 2-3 times longer (Supp. Fig. S1C-F). In addition, we found that the decay time constant of EPSCs was linearly related to the correlation time of the noise (Fig. 3D) Inhibitory time constants have minimal if any effect because the measurements were made close to the IPSC reversal potential (Fig.S2).

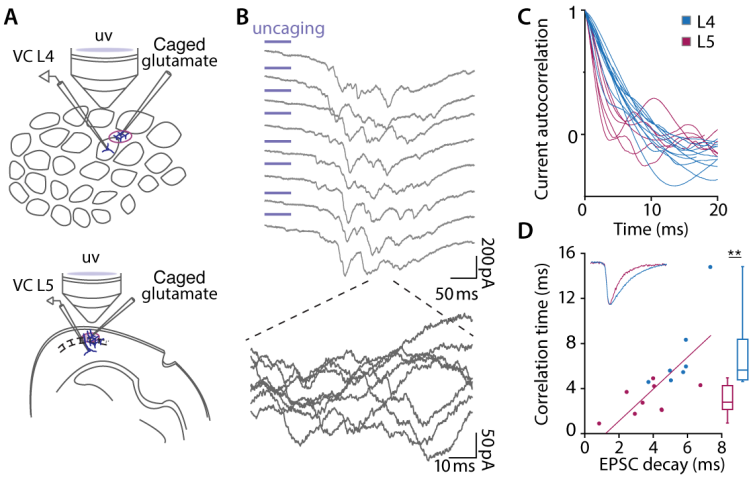

Fig. 3. Synaptic noise is slower in L4 neurons. (**A**) Experimental setup for synaptic current noise recordings *in vitro*. Whole cell voltage clamp measurements in tangential slices (L4) and in coronal slices (L5). A second pipette carrying caged (MNI)-glutamate is situated 200-400 µm from the recorded cell and UV flashes (380 nm, 50 ms) release glutamate to activate the local synaptic network. (**B**) Current traces obtained in voltage clamp mode (V_{hold} = -50 mV) showing synaptic fluctuations in response to 8 consecutive UV flashes. Note that each flash results in a burst of synaptic input with a waveform that differs from trial to trial. (**C**) Autocorrelation functions. (**D**) Correlation time of the synaptic noise plotted as a function of the decay time constant of spontaneous EPSCs. (Pearson Corr = 0.72 , n = 7 L4 cells and n = 9 L5 cells for EPSC vs. correlation time). Box plots summarize the correlation times of synaptic noise. Correlation times of synaptic noise (two-sided t-test, $^{**}p < 0.01$)

Slow background noise enables L4 neurons to code high frequencies

We next determined, how different background correlation times affect the dynamic gain of the L4 cells. The raster plots and corresponding AP time histograms in Figure 4 (A, B) compare the ability of 3 representative L4 neurons to follow a 200 Hz input signal when the noise was fast (τ =2 ms) and when the noise was slow (10 msec). It can be seen that the high frequency tracking performance of the neurons was greatly enhanced when the time correlation of the noise was slower. Thus, both dynamic gain analysis and vector strength analysis showed that the slower background fluctuation is associated with a broadened bandwidth and a significant increase in the encoding abilities of the L4 neurons at higher frequencies (Fig. 4E and G). When we quantitatively compared the two methods, we found close agreement in the results, with a more efficient use of the recording time in the dynamic gain analysis (Fig. S3A-F).

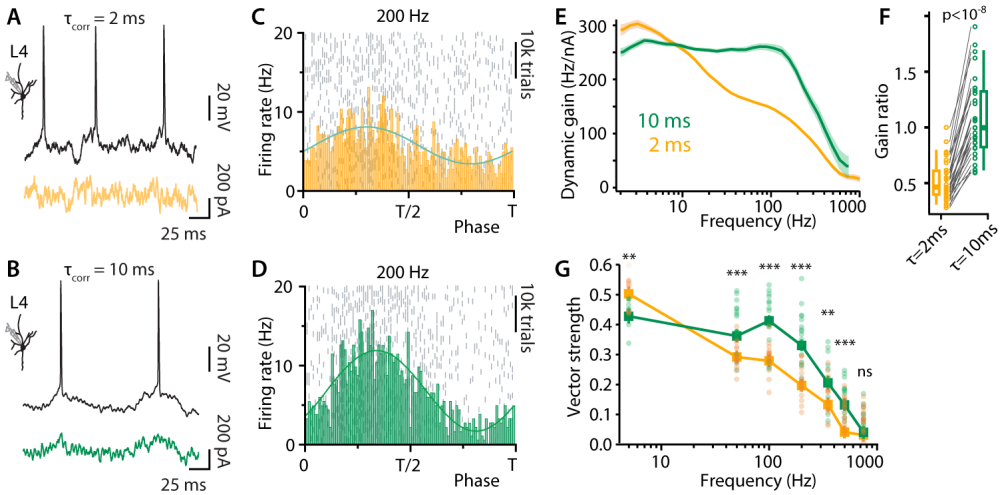

Fig. 4. Slow noise improves the encoding performance of L4 neurons. Voltage fluctuations and neuronal firing in a L4 neuron in response to a 200 Hz sinusoidal modulation buried in synaptic noise with a correlation time of either 10 ms (**A**) or 2 ms (**B**). Shown are brief sections of 45 second-long trials. (**C-D**) Raster plots and population rates showing the effect of the correlation time on the locking performance of the neurons to 200 (Hz) (APs were pooled from n = 3 L4 neurons). (**E**) Dynamic gain of L4 neurons as a function of input frequency. Pooled data from n = 30 L4 neurons are shown, each was stimulated with both correlation times. Solid lines and shaded areas represent the medians and confidence intervals of bootstrap statistics (see methods). (**F**) An individual dynamic gain curve |G(f)| was calculated for each of the 30 neurons from (E). Shown are the ratios |G(100Hz)|/|G(2Hz)| for the two correlation times. Paired-data, two-sided Wilcoxon rank test indicated a significant difference between the data for the different correlation times with p=1.9e-9. (**G**) Vector-strength as a function of sine wave frequency with fast and slow correlation times. Shown are results for individual trials (circles), medians (squares) and confidence intervals (vertical lines). The latter were calculated as described in methods. For each frequency, both correlation times (2 and 10 ms) were tested in the same cell. The significance of the effect of correlation time was determined with a paired, two-sided t-Test. Sample size, uncorrected p-values and Cohen's D are, in sequence of increasing frequency: n=8, p=0.003, D=1.8; n=15, p=4e-4, D=2.3; n=16, p=2e-4, D=2.5; n=16, p=6e-5, D=3.0; n=14, p=9e-4, D=1.3; n=16, p=0.002, D=1.5; n=15, p=0.89, D=0.13.

Population encoding fidelity depends on the number of neurons and the background correlation time

We next sought to determine how large the L4 population must be for its output to reliably reflect brief, correlated changes in its input. Our approach was to embed a time-locked artificial EPSC (aEPSC) in the random noise (as generated above), and to determine how many sweeps are required to reliably detect this input in the output population spike train. In these experiments, since the noise is uncorrelated, each sweep may be considered the equivalent of a differrent neuron. An aEPSC (20 pA), mimicking the effect of a single thalamocortical AP (*31*, *32*) was superimposed on either 'slow' or 'fast' background fluctuations and injected into single cells every 50 ms. As can be seen in the traces in Fig. 5A, AP clustering just after the aEPSC was most prominent when the background noise was 'slow'. This is a direct consequence of the wider bandwidth of the dynamic gain, in fact, the waveform and amplitude of the response can be quantitatively predicted from the dynamic gain (Fig.S3G-J). In the peri-stimulus time histograms of Figs. 5 B and C, it can be seen that for an 'infinitely' large population (n = 25,200), the aEPSC is readily detected at both slow and fast noise correlation times. However, when the population consists of fewer neurons, it is much harder to detect with the faster time correlation of the noise. Analysis of the detection probability as a function of the population size (see methods) reveals that 'slow' fluctuations improve the reliability of detection by 70%-100% for firing rate changes occurring within 1 ms (Fig. 5D). In a single barrel, the neuronal population of L4 contains no more than a few thousand excitatory neurons (*18*). Because a thalamocortical volley is immediately followed by strong feed-

forward and feedback inhibition (*33*, *34*), these data indicate that the slower correlation time of the noise is essential for the fast encoding that is actually achieved by L4.

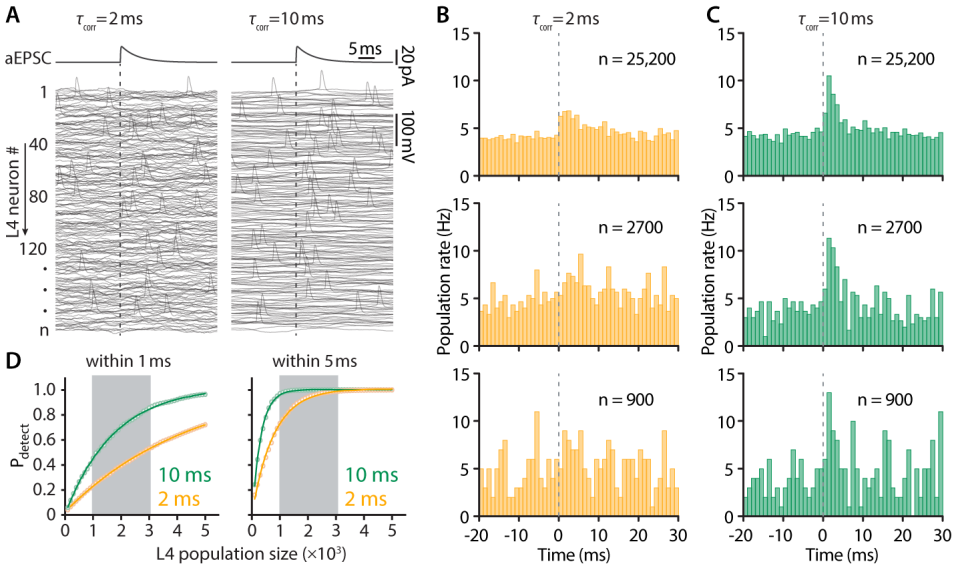

Fig. 5. Slow noise improves coding performance. (**A**) A single artificial EPSC, embedded in either fast (left) or slow (right) noise, was injected into a population of 'n' L4 excitatory neurons. Example voltage waveforms are shown. (**B**) and (**C**) Example peri-stimulus time histograms for population size 'n' of >25,000, 2700 and 900. (**D**) The effect of synaptic noise correlation time on detection probability of input within 1 ms and within 5 ms. Circles denote the data points and solid lines denote exponential fits (pooled from n = 13 L4 neurons).

Fast encoding depends on Kv7 potassium currents

Our experiments show that when background noise fluctuates slowly, the population of L4 neurons rapidly encodes thalamocortical input volleys. This is in agreement with predictions of simple theoretical models (Fig. 1). Recent physiological as well as theoretical evidence (Lazarov, Revah, Zhang 2024) indicate that this depends on the sodium and potassium currents that underly AP initiation. In the axon initial segment (AIS), sodium channels and Kv7.2/3 potassium channels are arranged in a highly specific organization (*35*), and these Kv7.2/3 channels modulate the AP initiation (*36*, *37*). We sought to determine whether these specific potassium channels may regulate the dynamic gain of the L4 neurons. Fig. 6 shows the effect of blocking Kv7 channels with the specific antagonist XE991 (*38*), on AP initiation and dynamic gain. Fig. 6A shows average APs during 10 ms and 2 ms background activity with and without application of the Kv7 blocker. Under control conditions, the slower background is associated with a far more gradual voltage incline to AP threshold than the faster background. This difference is nearly abolished by XE991. The finding is quantified in Fig. 6B, which plots the difference between the voltage recorded 5 ms before AP initiation at the two background correlation times. Fig. 6C shows that, following addition of XE991 to the bath, the expanded bandwidth of L4 neurons completely disappeared despite their characteristically slow background noise. Thus, blockade of these K channels removed the ability of the L4 neurons to faithfully respond to a brief thalamocortical volley. Because Kv7 channels generate the acetylcholine-sensitive M-current (*39*), and acetylcholine is a prominent neuromodulator in the cortex (*40*), we conclude that the coding ability of L4 may vary as a function of brain state.

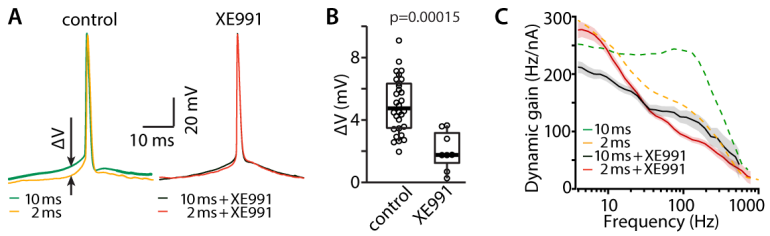

Fig. 6. Kv7 activity is required for more precise AP firing under slow background correlations (**A**) Average AP waveforms of for one neuron in control conditions (left) and one neuron under XE991 application (right). The membrane voltage 5 milliseconds before AP threshold differs in fast and slow background noise (ΔV, arrows). (**B**) The voltage difference, defined in (A), is shown for the 30 cells from Fig. 4 (control) and 8 cells under XE911 block. p=0.00015, two-sided Wilcoxon rank test. (**C**) Slow background improves the dynamic gain bandwidth, when K_V7 channels are active (orange to green). This 'high-frequency boost' is largely abolished, when Kv7 channels are blocked (red to black).

Discussion

We show that in layer 4 of the somatosensory cortex, the temporal properties of background noise, axonal potassium channels, and neuronal population size, offset the small size of the excitatory neurons, so as to assure faithful transmission of even the weakest thalamocortical input. This study provides a direct experimental assessment of information encoding in a population of cortical neurons using theoretical concepts proposed 50 years ago (*7*), and for the first time relates dynamic gain to a specific cortical function. We demonstrate that 1) L4 neurons generate synaptic noise of very slow correlation time, which increases their encoding bandwidth, 2) broad bandwidth enables populations of only hundreds of neurons to reliably reflect single thalamocortical APs with millisecond precision and 3) changes in L4 neuron properties that appear to have only a minor influence at the single cell level, strongly impact coding by the population as a whole. Although our study is focused on the rodent barrel cortex, we expect that our findings are broadly applicable to the mechanisms of cortical neuronal population coding in general.

Almost all neurons in a cortical column receive direct thalamocortical input. The neurons of layer 4 are unique, however, in that they only receive direct input from the specific thalamus and other L4 neurons of the same barrel (*15*). Thus, the output of the L4 neuronal population, which projects throughout the column (*15*), constitutes a beam of exclusively sensory information. Strong feed-forward and feedback inhibition leaves only a narrow window of about 2 ms during which these cells must respond (*33*, *41*, *42*), such that the relayed output from L4, by amplifying the thalamic input, serves to focus the activity of the entire cortical column. Our findings explain how these neurons, despite their small size, are able to respond within this extremely brief window. Because the correlation time of the background noise is slow in L4 (Fig. 3), less than 1000 L4 neurons are sufficient to allow detection of a single thalamic EPSC with 90% confidence within the time constraints imposed by inhibition (Fig. 5). Remarkably, this corresponds well to the number of L4 neurons (700) contacted by a single thalamocortical axon (*43*).

We provide the first direct measurements of the kinetics of background current noise and find that, whereas it is fast in the L5 neurons, it is slow in the much smaller L4 neurons (Fig. 3). We further demonstrate that, as theoretically predicted, the slow kinetics of the noise is due to long EPSC decay time constants. This difference between L4 and other layers may be explained by previous evidence that, although fast AMPA-receptors are the predominant mediators of excitatory synaptic activity in all other cortical layers, in L4 of the mouse barrel cortex, a slower NMDA-receptor with minimal Mg^{2+}-sensitivity (NR2C) is mainly responsible for ongoing synaptic activity, even at

resting potential (*23*, *44*). Because encoding capacity critically depends on background correlation time (Fig. 4), the unique synaptic physiology in L4 is crucial to its function.

Ultimately, the bandwidth of dynamic gain must reflect the ionic mechanisms underlying AP generation (*45*). Briefly, wide bandwidth requires that the voltage dependence of inward current at threshold be steep. In cortical neurons, APs are generated in the proximal part of the axon, the AIS, where an elaborate cytoskeleton anchors both voltage dependent Na channels and voltage-dependent Kv7 channels in a precise spatial pattern (Cooper E.D., Letterier 2018). We suggest that, because the slower background noise is associated with a much more gradual incline of the pre-AP membrane potential towards threshold, the local K channels are activated, the Na current is opposed, and by the time threshold is reached the membrane voltage is at a much steeper point on the Na activation curve. This would explain our finding that blocking the Kv7 channels, by removing the difference in pre-AP voltage trajectory, narrowed the bandwidth. This result predicts that the relay function of the L4 population may vary as a function of brain state, since Kv7 channels are regulated by the neuromodulator acetylcholine (*46*).

Fundamentally, cortical function entails the passage of information between neuronal ensembles composed of neurons whose output collectively represents common input at a given point in time. However, a given neuron joins different populations at different moments, and the specific function that is served is usually not known. By measuring the bandwidth of the dynamic gain of a single neuron, we obtain insight into how this neuron and others like it participate in the dynamics of cortical function. We note that the dynamic gain of a given neuron is not necessarily a fixed parameter, but may be sensitive to physiological or pathophysiological states. Merino et al. (*47*) reported that in L2/3, when slow oscillatory brain rhythms appear, they are reflected in the background noise, and the dynamic gain function of certain inhibitory neurons is thereby dramatically broadened. Revah et al. (*48*) showed that dynamic gain is modified due to changes in the AP initiation machinery during recovery from brief epochs transient cortical anoxia and consequent spreading depolarization. The findings illustrated in Fig. 6 suggest that dynamic gain of some cortical neurons may be modified during normal behaviors that are associated with changes in Kv7 potassium channel activation.

References and Notes

1. T. W. Margrie, M. Brecht, B. Sakmann, In vivo, low-resistance, whole-cell recordings from neurons in the anaesthetized and awake mammalian brain. *Pflüg. Arch.* **444**, 491–498 (2002).

2. A. K. Lee, I. D. Manns, B. Sakmann, M. Brecht, Whole-cell recordings in freely moving rats. *Neuron* **51**, 399–407 (2006).

3. A. K. Lee, J. Epsztein, M. Brecht, Head-anchored whole-cell recordings in freely moving rats. *Nat. Protoc.* **4**, 385–392 (2009).

4. S. Crochet, C. C. H. Petersen, Correlating whisker behavior with membrane potential in barrel cortex of awake mice. *Nat. Neurosci.* **9**, 608–610 (2006).

5. F. Wolf, R. Engelken, M. Puelma-Touzel, J. D. F. Weidinger, A. Neef, Dynamical models of cortical circuits. *Curr. Opin. Neurobiol.* **25**, 228–236 (2014).

6. T. Tchumatchenko, A. Malyshev, F. Wolf, M. Volgushev, Ultrafast population encoding by cortical neurons. *J. Neurosci.* **31**, 12171–9 (2011).

7. B. W. Knight, Dynamics of Encoding in a Population of Neurons. *J. Gen. Physiol.* **59**, 734–766 (1972).

8. N. Brunel, F. S. Chance, N. Fourcaud, L. F. Abbott, Effects of Synaptic Noise and Filtering on the Frequency Response of Spiking Neurons. *Phys. Rev. Lett.* **86**, 2186 (2001).

9. B. Naundorf, T. Geisel, F. Wolf, Action potential onset dynamics and the response speed of neuronal populations. *J. Comput. Neurosci.* **18**, 297–309 (2005).

10. S. Ostojic, G. Szapiro, E. Schwartz, B. Barbour, N. Brunel, V. Hakim, Neuronal Morphology Generates High-Frequency Firing Resonance. *J. Neurosci.* **35**, 7056–7068 (2015).

11. G. Eyal, H. D. Mansvelder, C. P. J. de Kock, I. Segev, Dendrites Impact the Encoding Capabilities of the Axon. *J. Neurosci.* **34**, 8063–8071 (2014).

12. T. A. Woolsey, H. Van der Loos, The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res.* **17**, 205–242 (1970).

13. H. Van der Loos, T. A. Woolsey, Somatosensory cortex: structural alterations following early injury to sense organs. *Science* **179**, 395–398 (1973).

14. C. C. H. Petersen, The functional organization of the barrel cortex. *Neuron* **56**, 339–355 (2007).

15. S. Lefort, C. Tomm, J.-C. Floyd Sarria, C. C. H. Petersen, The excitatory neuronal network of the C2 barrel column in mouse primary somatosensory cortex. *Neuron* **61**, 301–316 (2009).

16. D. Feldmeyer, M. Brecht, F. Helmchen, C. C. H. Petersen, J. F. A. Poulet, J. F. Staiger, H. J. Luhmann, C. Schwarz, Barrel cortex function. *Prog. Neurobiol.* **103**, 3–27 (2013).

17. M. Oberlaender, C. P. J. de Kock, R. M. Bruno, A. Ramirez, H. S. Meyer, V. J. Dercksen, M. Helmstaedter, B. Sakmann, Cell type-specific three-dimensional structure of thalamocortical circuits in a column of rat vibrissal cortex. *Cereb. Cortex N. Y. N 1991* **22**, 2375–2391 (2012).

18. H. S. Meyer, V. C. Wimmer, M. Oberlaender, C. P. J. de Kock, B. Sakmann, M. Helmstaedter, Number and laminar distribution of neurons in a thalamocortical projection column of rat vibrissal cortex. *Cereb. Cortex N. Y. N 1991* **20**, 2277–2286 (2010).

19. G. Benshalom, E. L. White, Quantification of thalamocortical synapses with spiny stellate neurons in layer IV of mouse somatosensory cortex. *J. Comp. Neurol.* **253**, 303–314 (1986).

20. V. Egger, T. Nevian, R. M. Bruno, Subcolumnar dendritic and axonal organization of spiny stellate and star pyramid neurons within a barrel in rat somatosensory cortex. *Cereb. Cortex N. Y. N 1991* **18**, 876–889 (2008).

21. D. Feldmeyer, V. Egger, J. Lubke, B. Sakmann, Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single "barrel" of developing rat somatosensory cortex. *J. Physiol.* **521 Pt 1**, 169–190 (1999).

22. J. F. Staiger, I. Flagmeyer, D. Schubert, K. Zilles, R. Kötter, H. J. Luhmann, Functional diversity of layer IV spiny neurons in rat somatosensory cortex: quantitative morphology of electrophysiologically characterized and biocytin labeled cells. *Cereb. Cortex N. Y. N 1991* **14**, 690–701 (2004).

23. I. A. Fleidervish, A. M. Binshtok, M. J. Gutnick, Functionally Distinct NMDA Receptors Mediate Horizontal Connectivity within Layer 4 of Mouse Barrel Cortex. *Neuron* **21**, 1055–1065 (1998).

24. D. Feldmeyer, A. Roth, B. Sakmann, Monosynaptic connections between pairs of spiny stellate cells in layer 4 and pyramidal cells in layer 5A indicate that lemniscal and paralemniscal afferent pathways converge in the infragranular somatosensory cortex. *J. Neurosci. Off. J. Soc. Neurosci.* **25**, 3423–3431 (2005).

25. D. Schubert, J. F. Staiger, N. Cho, R. Kötter, K. Zilles, H. J. Luhmann, Layer-specific intracolumnar and transcolumnar functional connectivity of layer V pyramidal cells in rat barrel cortex. *J. Neurosci. Off. J. Soc. Neurosci.* **21**, 3580–3592 (2001).

26. S. Ramaswamy, H. Markram, Anatomy and physiology of the thick-tufted layer 5 pyramidal neuron. *Front. Cell. Neurosci.* **9** (2015).

27. J. M. Goldberg, P. B. Brown, Response of binaural neurons of dog superior olivary complex to dichotic tonal stimuli: some physiological mechanisms of sound localization. *J. Neurophysiol.* **32**, 613–636 (1969).

28. J. Yu, D. A. Gutnisky, S. A. Hires, K. Svoboda, Layer 4 fast-spiking interneurons filter thalamocortical signals during active somatosensation. *Nat. Neurosci.* **19**, 1647–1657 (2016).

29. J. F. A. Poulet, C. C. H. Petersen, Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. *Nature* **454**, 881–885 (2008).

30. S. A. Hires, D. A. Gutnisky, J. Yu, D. H. O'Connor, K. Svoboda, Low-noise encoding of active touch by layer 4 in the somatosensory cortex. *eLife* **4** (2015).

31. Z. Gil, B. W. Connors, Y. Amitai, Efficacy of Thalamocortical and Intracortical Synaptic Connections: Quanta, Innervation, and Reliability. *Neuron* **23**, 385–397 (1999).

32. N. Urbain, P. A. Salin, P.-A. Libourel, J.-C. Comte, L. J. Gentet, C. C. H. Petersen, Whisking-Related Changes in Neuronal Firing and Membrane Potential Dynamics in the Somatosensory Thalamus of Awake Mice. *Cell Rep.* **13**, 647–656 (2015).

33. D. A. Gutnisky, J. Yu, S. A. Hires, M.-S. To, M. R. Bale, K. Svoboda, D. Golomb, Mechanisms underlying a thalamocortical transformation during active tactile sensation. *PLOS Comput. Biol.* **13**, e1005576 (2017).

34. R. M. Bruno, D. J. Simons, Feedforward Mechanisms of Excitatory and Inhibitory Cortical Receptive Fields. *J. Neurosci.* **22**, 10966–10975 (2002).

35. Z. Pan, T. Kao, Z. Horvath, J. Lemos, J.-Y. Sul, S. D. Cranstoun, V. Bennett, S. S. Scherer, E. C. Cooper, A Common Ankyrin-G-Based Mechanism Retains KCNQ and NaV Channels at Electrically Active Domains of the Axon. *J. Neurosci.* **26**, 2599–2613 (2006).

36. M. M. Shah, M. Migliore, I. Valencia, E. C. Cooper, D. A. Brown, Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 7869–7874 (2008).

37. H. Kuba, R. Yamada, G. Ishiguro, R. Adachi, Redistribution of Kv1 and Kv7 enhances neuronal excitability during structural axon initial segment plasticity. *Nat. Commun.* **6**, 8815 (2015).

38. H. S. Wang, B. S. Brown, D. McKinnon, I. S. Cohen, Molecular basis for differential sensitivity of KCNQ and I(Ks) channels to the cognitive enhancer XE991. *Mol. Pharmacol.* **57**, 1218–1223 (2000).

39. H.-S. Wang, Z. Pan, W. Shi, B. S. Brown, R. S. Wymore, I. S. Cohen, J. E. Dixon, D. McKinnon, KCNQ2 and KCNQ3 Potassium Channel Subunits: Molecular Correlates of the M-Channel. *Science* **282**, 1890–1893 (1998).

40. M. R. Picciotto, M. J. Higley, Y. S. Mineur, Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron* **76**, 116–129 (2012).

41. D. J. Simons, G. E. Carvell, Thalamocortical response transformation in the rat vibrissa/barrel system. *J. Neurophysiol.* **61**, 311–330 (1989).

42. L. Gabernet, S. P. Jadhav, D. E. Feldman, M. Carandini, M. Scanziani, Somatosensory integration controlled by dynamic thalamocortical feed-forward inhibition. *Neuron* **48**, 315–327 (2005).

43. H. S. Meyer, V. C. Wimmer, M. Hemberger, R. M. Bruno, C. P. J. de Kock, A. Frick, B. Sakmann, M. Helmstaedter, Cell Type–Specific Thalamic Innervation in a Column of Rat Vibrissal Cortex. *Cereb. Cortex* **20**, 2287–2303 (2010).

44. A. M. Binshtok, I. A. Fleidervish, R. Sprengel, M. J. Gutnick, NMDA Receptors in Layer 4 Spiny Stellate Cells of the Mouse Barrel Cortex Contain the NR2C Subunit. *J. Neurosci.* **26**, 708–715 (2006).

45. N. Fourcaud-Trocme, D. Hansel, C. van Vreeswijk, N. Brunel, How Spike Generation Mechanisms Determine the Neuronal Response to Fluctuating Inputs. *J. Neurosci.* **23**, 11628–11640 (2003).

46. P. Delmas, D. A. Brown, Pathways modulating neural KCNQ/M (Kv7) potassium channels. *Nat. Rev. Neurosci.* **6**, 850–862 (2005).

47. R. M. Merino, C. Leon-Pinzon, W. Stühmer, M. Möck, J. F. Staiger, F. Wolf, A. Neef, Theta activity paradoxically boosts gamma and ripple frequency sensitivity in prefrontal interneurons. *Proc. Natl. Acad. Sci.* **118**, e2114549118 (2021).

48. O. Revah, O. Stoler, A. Neef, F. Wolf, I. A. Fleidervish, M. J. Gutnick, Dynamic Gain Analysis Reveals Encoding Deficiencies in Cortical Neurons That Recover from Hypoxia-Induced Spreading Depolarizations. *J Neurosci* **39**, 7790–7800 (2019).

49. I. A. Fleidervish, N. Lasser-Ross, M. J. Gutnick, W. N. Ross, Na+ imaging reveals little difference in action potential-evoked Na+ influx between axon and soma. *Nat Neurosci* **13**, 852–860 (2010).

50. M. H. Higgs, W. J. Spain, Conditional Bursting Enhances Resonant Firing in Neocortical Layer 2-3 Pyramidal Neurons. *J. Neurosci.* **29**, 1285–1299 (2009).

51. T. Tchumatchenko, F. Wolf, Representation of Dynamical Stimuli in Populations of Threshold Neurons. *PLoS Comput Biol* **7**, e1002239 (2011).

52. F. Aspart, J. Ladenbauer, K. Obermayer, Extending Integrate-and-Fire Model Neurons to Account for the Effects of Weak Electric Fields and Input Filtering Mediated by the Dendrite. *PLOS Comput. Biol.* **12**, e1005206 (2016).

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Materials and Methods

All experiments were approved by the Animal Care and Use Committee of The Hebrew University of Jerusalem. Recordings were made in 300-400 µm thick coronal slices (L5) and tangential slices (L4) of somatosensory cortex of CD-1 mice of either sex that were 14–24 postnatal days of age. Procedures for preparation and maintenance of slices were similar to those described previously (49). Animals of either sex were deeply anesthetized with Nembutal (60 mg/kg) or isoflurane and killed by decapitation; their brains were rapidly removed and placed in cold (4°C), oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (aCSF). Coronal and tangential, barrels, slices were obtained from a region corresponding to the primary somatosensory cortex were cut on a vibratome (Series 1000; Pelco International, Redding, Canada), as previously shown (23).The slices were then placed in a holding chamber containing aCSF at room temperature for >30 minutes of recovery and transferred to the recording chamber for recordings and analysis.

Electrophysiology

Whole-cell patch clamp recordings were made from visually identified L4 and L5 neurons under infrared differential interference contrast (IR-DIC) microscopic control. Slices were held submerged in a chamber on a fixed stage of an Axioskop FS microscope (Carl Zeiss, Oberkochen, Germany). Voltage and current were recorded in whole-cell configuration using an Axoclamp 2B (Molecular Devices, Foster City, CA) connected to an MU-type head stage (Molecular Devices, Foster City, CA). Patch pipettes were manufactured from thick-walled borosilicate glass capillaries (outer diameter, 1.5 mm; Hilgenberg, Malsfeld, Germany) and had resistances of 5–7 MΩ for somatic and extracellular recordings. All recordings were made at 30 ± 2°C. Command voltage protocols were generated and whole-cell data were acquired on-line with a Digidata 1320A analog-to-digital interface. Data were low-pass filtered (-3 dB, one-pole Butterworth filter) and digitized at 20 kHz, except for spike shape analysis which was oversampled at 250 kHz. For each neuron, bridge correction and capacitance compensation were performed manually online.

Assessing passive and active membrane properties

The subthreshold membrane properties and the characteristics of the action potential were obtained by injecting a series of increasing 500 ms current steps, separated by 2-5 s intervals during which the cell was held at either resting or at -77 mV.

Synaptic noise simulation in brain slices.

To achieve periods of reverberating network activity, in addition to the recording electrode, a second borosilicate glass electrode, pulled to a resistance of 2-4 MΩ, containing caged glutamate (MNI-caged-L-glutamate, 10 mM, Tocris), and connected to Picospritzer II device (PARKER HANNIFIN CORPORATION), was placed 100-200 µm away from the recorded neuron. Caged glutamate was puffed locally for 1 second, and, following, a short, 50-100 ms long uv light-pulse was delivered onto the tissue, through a 40X objective (~25 mW/mm^2). Successful network activations resulted in incoming synaptic barrages impinging on the recorded cell after a considerable delay (see Fig.3). Special care was taken to prevent glutamate from exciting the cell directly.

Assessing the frequency response function of L4 and L5 neurons

To assess the frequency response of neuronal populations, we injected stochastically fluctuating currents in the whole cell configuration. This analysis aims to achieve an *in vivo*-like operating point, mimicking a situation in which a high rate of synaptic inputs provides a continuously changing net background current, and AP firing is driven not by the average input but by its transient depolarizing excursions. The injected currents were composed of a stochastic signal, and a sinusoid signal of frequency f_s (between 5 and 750 Hz as indicated). The stochastic signal was based on an Ornstein–Uhlenbeck (OU) process, i.e. low-pass filtered white noise with a Gaussian amplitude distribution. Its correlation time was either 2, 5, or 10 ms as indicated. While 5 ms were chosen because this was previously assumed to be representative of glutamatergic synaptic decay time constants, the 2 and 10 ms were chosen based on our measurements (Fig. 3). We adjusted the input's standard deviation, σ_I, based on the input resistance of the neuron, to assure that the voltage fluctuations were similar in all neurons. Along with σ_I, we also scaled the amplitude of the sinusoidal component $A_{sin}=0.37\cdot\sigma_I$. A constant, direct current (DC) $\langle I \rangle$ was added and adjusted to maintain a target firing rate of ~5 Hz. Currents were injected in 46 s episodes, with ~30 s intervals between the injections. APs were detected as crossings of zero membrane voltage.

Vector strength

A neuron's ability to encode signals can be evaluated with different methods. We used two methods to assure precise results. Results for both are obtained with code available at https://github.com/Anneef/AnTools in the subfolder 'Dynamic Gain Code'.

The first method relies on the presence of a sinusoidal component in the input and uses the vector strength r to characterize how the AP times t_j lock to the phase of the periodic stimulus:

$$r = \mathrm{abs}\left(\sum_{j=1}^{N} \exp\left(i \cdot 2\pi \cdot f \cdot t_j\right)\right)/N \qquad \text{Eq:S1}$$

In the experiments underlying figure 2, where stimuli of a single correlation time were used (5 ms), each cell (n=10 L4 cells, n=9 L5 cells) was typically studied with all 5 frequencies (f = 50, 100, 200, 500, and 750 Hz) twice (for L4 cells) or thrice (for L5 cells). In the experiments underlying figure 4, 30 L4 neurons received fluctuating stimuli of 2 and of 10 ms correlation time. For both correlation times, typically 3 to 4 of the 7 frequencies (5, 50, 100, 200, 350, 500 and 750 Hz) were applied once to each cell. For the number of stimuli applied, the cell in Fig.S3 A-F is not typical, because there a total of 11 trials were successfully recorded. In terms of results, it is a typical cell. Out of the 204 trials recorded from these 30 cells, 200 trials were part of a 2ms -10ms pair, i.e. the trial stimuli contained the same sine frequency for both correlation times, recorded in the same cell. In Fig. 4F, the vector strengths calculated for each of these 200 trials are shown in circles. The average vector strengths, calculated for all trials with a given sine frequency, are shown as squares.

Note that the average values are not simply the averages of the individual vector strength values because complex numbers are averaged and only their absolute values are reported. Specifically, the average absolute value is at most equal to the average of the individual absolute values of the trials. This case occurs only, if the phases of all trial vector strengths are identical, i.e. if $r_{trial} = \frac{1}{N_{trial}}\sum_{j=1}^{N_{trial}}\left(2\pi \cdot f \cdot t_j\right)$ is the same for all trials.

Furthermore, note that each of the 104 trials contributes to the calculation of the dynamic gain in Fig. 4E.

To determine the confidence intervals and statistical significance of phase locking (vector strength r) of recorded APs to periodic input current stimulation, we used 10,000 bootstrap

samples of the actual spike times and of randomized spike times similar to the procedure described below for the dynamic gain. Because the contributions of individual APs are points on the unit circle, the average vector of the kth bootstrap sample is a point somewhere inside that unit circle, characterize by its magnitude and phase: $m_k \cdot e^{i\phi_k}$. To obtain the confidence interval of the magnitudes, it is not correct to simply study the distribution of all m_k. Instead, the bootstrap results first have to be projected onto the vector of the average $\frac{1}{N}\sum_{j=1}^{N} \exp\left(i \cdot 2\pi \cdot f \cdot t_j\right) = m_{\text{avg}} \cdot e^{i\phi_{\text{avg}}}$. The central 95 percentiles of these projected vectors' magnitudes, i.e. of $m_k \cdot \cos(\phi_k - \phi_{\text{avg}})$, corresponds to the confidence interval of the vector strength as shown in Fig. 2, Fig. 4 and Fig.S3.

Dynamic gain calculation

The second method to characterize the encoding ability of neurons is the dynamic gain function $G(f)$. It represents the frequency-domain, linear transfer function (or susceptibility) of a population of neurons that receive a common feed-forward input ΔI. In other words, in the frequency domain, the neurons' common population rate changes as follows: $\Delta\tilde{\nu}(f) = \tilde{I}(f) \cdot G(f)$ $(Eq. S2)$. The tilde represents the Fourier-transformations of the respective functions. The calculation of the dynamic gain, as performed here, based on (*50*), does not rely on an embedded sinusoidal signal. Instead, all the frequency components that are present in the stochastic signal are used as reference to calculate phase-locking. We described the rational and calculations in detail earlier (*47*), it is summarized here. A spike-triggered average (STA) input current was obtained by summing up 1-s-long stimulus segments centered on the AP times for all cells of a given condition, and dividing by the total number of APs.

The complex dynamic gain function *G(f)* was calculated as the ratio of the complex conjugate of the Fourier transform of the STA, $F(STA_I)^*$, and the Fourier transform of the autocorrelation of the stimulus:

$$G(f) = \frac{F(STA_I)^*}{F(AC_I)} \qquad \text{Eq. S3}$$

In order to improve signal-to-noise ratio, *G(f)* was filtered in the frequency domain by a Gaussian filter *w(f')* centered at frequency *f'=f* and a frequency-dependent window size with standard deviation of $f/2\pi$ as proposed by (*50*):

$$w(f') = \frac{1}{\sqrt{2\pi}\left(\frac{f}{2\pi}\right)} exp\left[\frac{-1}{2}\left(\frac{f'-f}{f/2\pi}\right)^2\right] \qquad \text{Eq. S4}$$

The filtered dynamic gain function $G_w(f)$ thus becomes

$$G_w(f) = \frac{\int G(f')\cdot w(f')\cdot df'}{\int w(f')\cdot df'} \qquad \text{Eq. S5}$$

The magnitude |*G*| of this complex dynamic gain function is shown in figures 4 and 6. To determine the region of the gain curves, where |G| is significantly larger than zero, a noise floor was calculated as the 95th percentile of the bootstrap distribution of dynamic gain functions obtained from AP times for which the temporal relation to the stimulus is destroyed by shifting the original times by an interval, larger than 1 second. The noise floors are shown in Figure 4 E as dashed lines. The results for |G| are only shown for frequencies, where it is above the noise floor. To obtain the confidence interval of |G|, bootstrap-sampling was performed over all AP times and the central 95% of the resulting distribution of |G(f)| was determined. The number of bootstrap samples for noise-floor and confidence interval was 1000.

In Fig. S3A-F, the two methods are compared. Their results are in good quantitative agreement. The analysis there also highlights the great advantage of the STA-based method: each AP is analyzed with respect to all input frequencies and not just a single frequency of a superimposed periodic input component. For correlation times as short as 2 ms, the input contains so much power throughout the interesting frequency range (1 to 800 Hz), that the STA_I-based dynamic gain analysis provides confidence intervals as narrow as the vector-strength method for all relevant frequencies. For such short input times, the vector strength seems to offer no advantage. In the case of longer input correlations (10 ms), the confidence intervals from the STA-based dynamic gain curve are very wide for high frequencies. For a low number of spikes the vector strength provides a more precise estimate. However, that only describes encoding at a single frequency. As soon as vector strength measurements at more than three or four frequencies are conducted, all recorded APs together allow a precise STA-based estimate of the gain at all frequencies and once more the vector strength is outperformed.

Theoretical model

For the neuron model, we had to find a possibility to study the effect of dendrite properties and input correlation times. We implemented a simple Gauss neuron, in which AP firing is not explicitly modelled, but instead each crossing of a voltage threshold from below is detected as an AP. From our earlier characterization of this model, we knew, that its encoding ability depends on the correlation time τ of the input current (*51*).
The electrotonic structure, i.e. the passive properties of the neuron model and its morphology, determine how the current input is transformed into the fluctuating membrane voltage. If the model was only a point neuron with resistance R and capacitance C, the transformation would correspond to a low-pass filter $\tilde{V}(\omega) = R \cdot \tilde{I}(\omega)/\sqrt{(1 + R^2C^2\omega^2 \cdot)}$. When a dendrite is added, low frequencies experience an additional suppression, while very high frequencies are less affected. For the transfer function for currents injected into the soma of this more complex structure (neuron + dendrite), we used an analytical expression derived in (*52*). The soma was represented by a sphere with 10 µm diameter, the dendrite is a cylinder with 5 µm diameter and variable length (as specified in Fig. 1). Intracellular and membrane resistivity were 1.5 Ωm and 0.9 Ωm^2, the specific membrane capacitance was 0.01 F/m^2. For reference, these parameters result in a membrane time constant of 9 ms and an electrotonic length constant of 866 µm.

Statistical tests

Before tests were applied, the individual distributions were tested against the normal distribution with Jarque-Bera tests. If the hypothesis of a normal distribution could not be rejected, the hypothesis of equal variances was tested with F-tests. If not rejected, the distributions were then compared with t-Tests. In Figs. 4F and 6B, those criteria were not passed and therefore a Wilcoxon rank test was used.

Optimal decoder and detection probability

The optimal decoder is set up as described earlier (*6*). It monitors the number of APs n_{detect} fired by a neuronal population during a "measurement window" of a certain width (3 ms Fig. 1; 1 ms and 5 ms in Fig. 5D left and right panel). If the AP count in this window exceeds the threshold

n_{thresh}, defined as the 95th percentile of the distribution of AP counts in all the baseline windows (of equal width), then a significant increase is detected.
Because n_{detect} and n_{thresh} are both discrete random numbers with distributions following count statistics, the detection probability shows abrupt jumps as the population size increases. Every time n_{thresh} is incremented by one, the detection probability drops. These jumps are artifacts of the decoder definition. They overlay the steady increase of the underlying detection probability with population size. We therefore do not show these jumps but only the overall trend of increasing P_{detect}.

Supplementary Text

Dynamic gain accurately describes rate change in response to an artificial EPSC

Here, we provide extended technical descriptions of how the presented results are mathematically connected. We introduced the dynamic gain as the linear transfer function of a population of neurons, with respect to changes in the common (feed-forward) input. This is expressed in the frequency-domain through eq. S2. Its time-domain equivalent is a convolution:

$$\Delta\nu(t) = \int_{-\infty}^{t} \Delta I(\tau) \cdot \tilde{G}(t-\tau) d\tau \qquad \text{Eq. S6}$$

The frequency domain equivalent is a simple multiplication:

$$\Delta\nu(t) = \mathcal{F}^{-1}\left(\mathcal{F}\big(\Delta I(t)\big) \cdot G(f)\right) \qquad \text{Eq. S7}$$

It is not a priori clear, to which degree this linear approximation captures the population's response to a change in the common input ΔI. For very small perturbations, the linearity can be plausibly assumed, but what input magnitude can be considered sufficiently small? We can test this for a concrete realization of ΔI by comparing the predictions of the time-domain equivalent of eq. S2 and the experimentally obtained $\Delta\nu$.
This test is conducted in Fig. S3. We obtained the dynamic gain curves from APs fired at least 25 ms after the EPSC onset (Fig. S3G). Notably, those are very similar to the gain curves in Fig. 4, recorded from cells in the same cortical area and with similar cellular properties. This indicates that the presence of the aEPSC signal did not substantially influence the dynamic gain calculation. In a next step, we took those complex dynamic gain functions (including the phase information) and multiplied it with the Fourier transformation of the waveform $\Delta I(t)$ of the artificial EPSCs (Fig. S3F and Fig 5A) following Eq. S7, the inverse Fourier-transformation results in a prediction for the response $\Delta\nu(t)$, shown in Fig.S3 H. Finally, we compared this theoretical prediction with the experimental results that are shown in the top panels of Fig. 5 B and 5 C. This comparison demonstrates, that the theoretically predicted responses capture all features of the experimental responses, i.e. delay, magnitude, and temporal extend (Fig. S3 I). Importantly, the different dynamic gain curves for background correlations of 2 and 10 ms closely predict the different response waveforms in Fig. 5 B and 5 C respectively. This demonstrates that the dynamic gain, the linear approximation of encoding, faithfully describes the encoding in the conditions tested, i.e. at *a given working point* characterized by input parameters μ_I, σ_I, τ and AP firing statistics. That notwistanding, it is obvious that the system is not *globally* linear. This is evident from the very fact that the temporal statistics of the input has a profound impact on the dynamic gain. This would not be the case, if the system was globally linear.

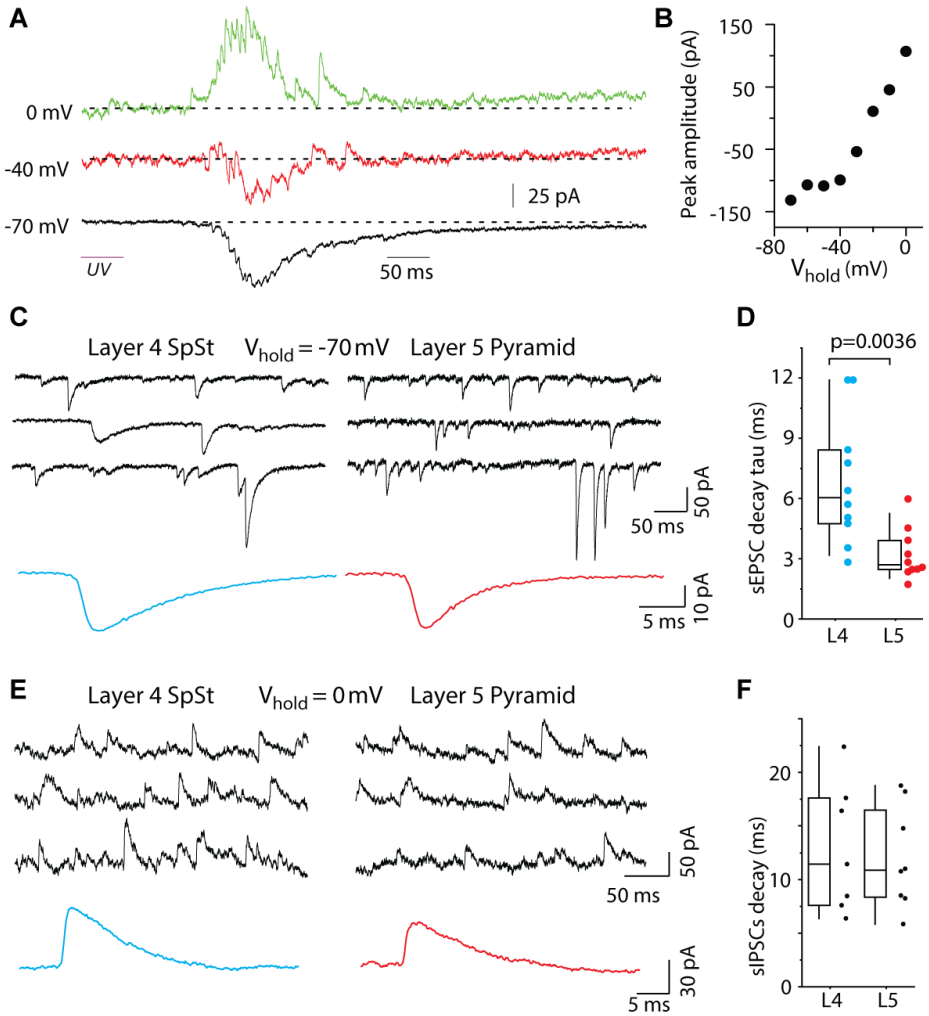

Fig. S1. Temporal characteristics of excitatory and inhibitory synaptic inputs from local networks are revealed by voltage clamp recordings and glutamate uncaging.

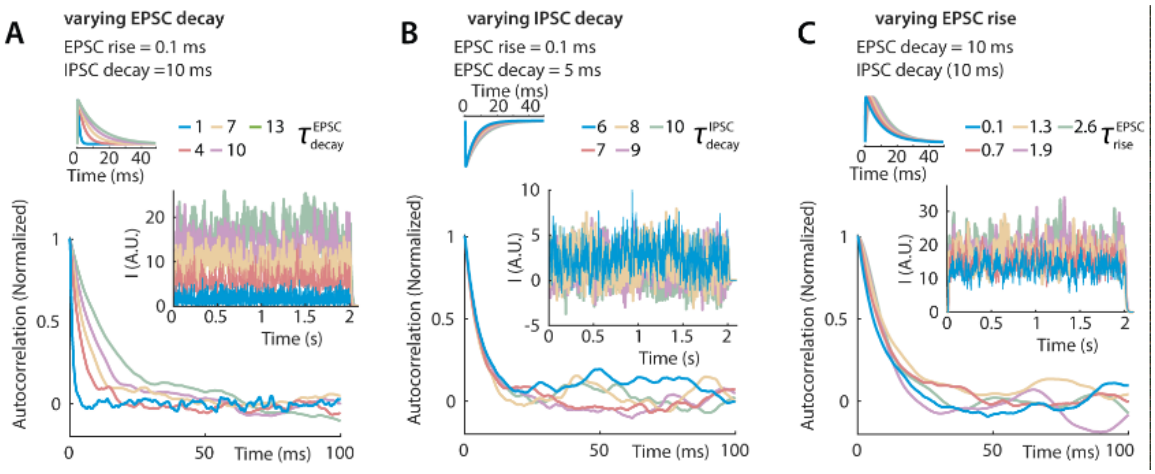

Fig. S2. A computational model in which randomly generated EPSCs with a reversal potential of 0 mV and IPSCs with a reversal potential of -70 mV combine at a holding potential of -50 mV to form fluctuating background noise. In the model, the frequencies and amplitudes of the excitatory and inhibitory synaptic conductances are the same. **A** It can be seen that, as the decay time constant of the EPSCs increases, the time correlation of the noise is markedly slowed. **B** By contrast, similar changes in the decay of the inhibitory synapses have a much smaller effect on the characteristics of the noise because the driving force for inhibition is quite small at -50 mV. **C** Similarly, the noise characteristics is also not very sensitive to the rise time of the EPSCs (but see the minor widening of the autocorrelation at its peak.

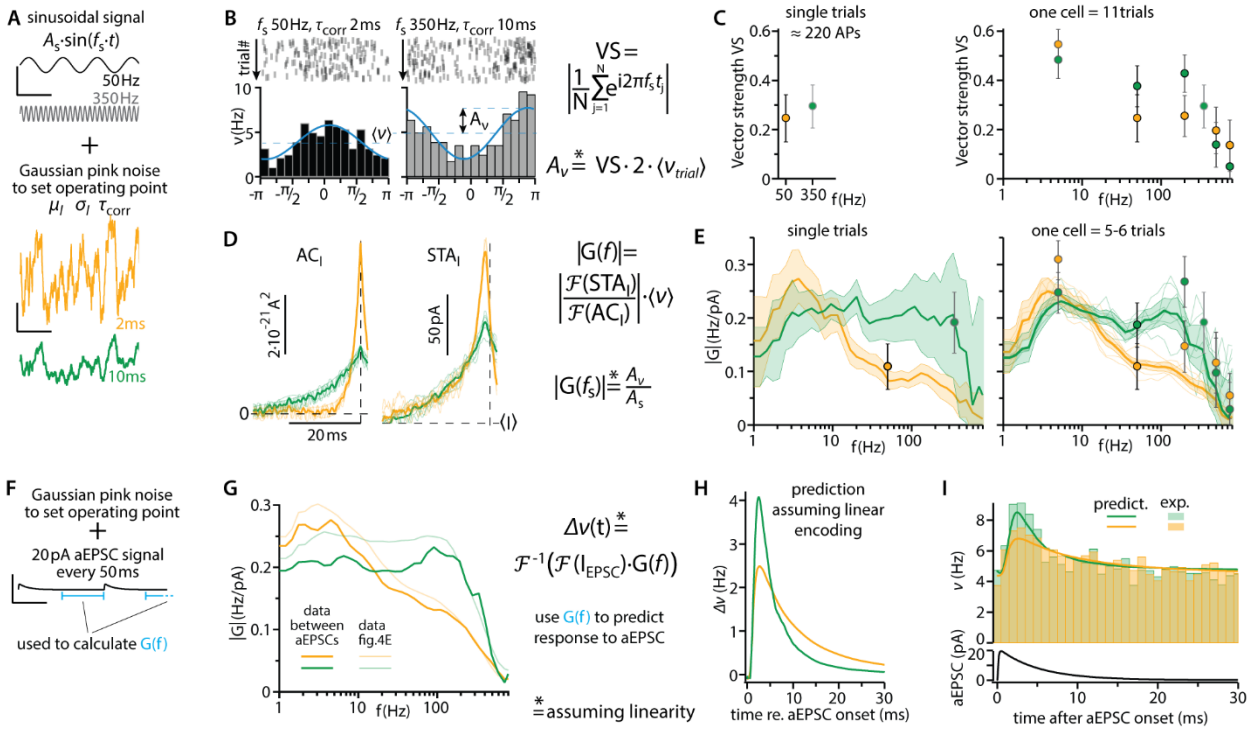

Fig. S3. Linear ansatz to quantify encoding is confirmed by comparison of three different approaches.

A In all cases the neurons' working points are set by fluctuating inputs with a correlation time of either 2 ms (orange) or 10 ms (green). Mean and standard deviation are chosen to achieve irregular firing with an average rate $\langle \nu \rangle$ around 5Hz. (see fig.4A). This stochastic background stimulus (Ornstein-Uhlenbeck-Type, see methods), can be combined with a periodic, sinusoidal input. Here, examples are shown with frequencies f_s of 50 Hz (black) and 350 Hz (grey). **B** When AP times are evaluated only with respect to the phase of the added sine component, the sine-locked firing rate is obtained (bars). It follows the sinusoidal input modulation with a phase shift and a modulation depth amplitude A_ν. Each of the two panels displays data of a single, 45-second-long stimulation trial, evoking about 220 APs. **C** From all AP times t_j the vector strength VS can be calculated. The results for the two example trials from B are shown in the left panel. The right panel contains the results for all 11 trials in this cell (5 different sine frequencies for 2 ms correlated background and 6 frequencies for 10 ms. Shown are average and 95% confidence intervals. **D** AP times can also be evaluated with respect to a11 the frequency components that jointly constitute the noisy background and the sine component. To do so, the input auto-correlation AC_I and the spike-triggered average input STA_I were obtained from the total input and the times t_j, at which action potentials occurred. Shown are results for the 11 individual trials (semi-transparent) and for the average of the trials with the same correlation time (opaque). Note that the AC_I of each trial clearly displays a periodic component, reflecting the sinusoidal current that had been added to the stimulus (see A). **E** The dynamic gain function G(f) can be calculated as the ratio of the Fourier-transformations of STA_I and AC_I, multiplied with the average firing rate. The left panel shows its magnitude |G| for two individual trials, for correlation times of 2 (orange) and 10 (green) milliseconds. Note how already single trials reveal the strong dependence on correlation time. The semi-transparent bands represent confidence intervals obtained by bootstrap-resampling of the approximately 220 action potentials within a trial. The dynamic gain magnitude at the frequency

of the sine component $|G(f_s)|$ can be calculated as the ratio of rate modulation A_v to current modulation A_s. The values obtained from C in that way are overlayed as circles with confidence bands. Note the close agreement of the results obtained with the two different methods and also the difference in confidence intervals. The right panel shows the corresponding results for the 11 individual trials (thin semi-transparent lines) as well as the overall results of this one cell (thicker opaque lines) with their much narrower confidence bands. **F** The data from figure 5 can be used for a critical test of the linearity of neuronal encoding under the conditions of our experiments. The last 25 ms before each aEPSC signal can be considered as approximately stationary. APs fired in these periods were used to calculate the dynamic gain curves for correlation times of 2 and 10 ms. Using these (complex) dynamic gain functions G(f), it should be possible to predict the transient increase in firing rate in response to the artificial EPSC inputs. However, this prediction assumes that the assumption of linear encoding is met. **G** The gain curves derived from the periods between the aEPSCs (opaque lines, see also figure 5) are similar to the gain curves that resulted from the experiments in figure 4, which are shown here semi-transparent as reference. **H** The predicted transient increase in the firing rate is shown. Because slower background fluctuations lead to a dynamic gain curve with a wider bandwidth, they are predicted to lead to a larger but briefer response. **I** The predicted curves from H are offset to the average firing rates and overlayed onto the measured rate increases. This comparison shows a quantitative agreement. Wide bandwidth dynamic gain (orange, 10 ms) leads to a more salient response to an artificial EPSC of 20 pA amplitude. Scale bars in A, and F are 20 ms and 100 pA.