

Supplementary Information

Methods

We recorded from 62 cells in primary auditory cortex of anesthetized (ketamine-medetomidine) rats aged 17-24 days post-natal using standard blind *in vivo* whole cell methods. All procedures were in strict accordance with the National Institutes of Health guidelines as approved by the Cold Spring Harbor Laboratory Animal Care and Use Committee. We recorded from all subpial depths (range: 140 – 1157 μm , as determined from micromanipulator travel, but 80% of cells were $<500 \mu\text{m}$). Internal solution contained, in mM, K-gluconate 140, HEPES 10, MgCl_2 2, CaCl_2 0.05, MgATP 4, NaGTP 0.4, $\text{Na}_2\text{Phosphocreatine}$ 10, BAPTA 10, QX-314 5, Alexa-594 (a fluorescent dye) 0.1, pH 7.25, diluted to 290 mOsm, producing a calculated reversal potential of -85 mV for both K^+ and Cl^- conductances. QX-314 was omitted from the internal solution for 31 cells (e.g. Figs. 1d, 2g). Note that in addition to blocking fast sodium channels (and thereby blocking action potentials), QX-314 also blocks some other activity-evoked conductances^{1,2}. Mean input resistance was $66 \pm 57 \text{ M}\Omega$ (range: 21 – 162 $\text{M}\Omega$), median series resistance was $83 \pm 90 \text{ M}\Omega$ (range: 11 – 222 $\text{M}\Omega$), and mean resting membrane potential was $-72 \pm 14 \text{ mV}$ for the 62 cells which met our inclusion criterion of a resting potential $< -50 \text{ mV}$. All errors and error bars are standard deviations unless noted otherwise.

Holding potentials were stepped to a pseudorandom sequence of 3-5 values using an Axopatch 200b amplifier. At each potential, after a 1 second equilibration period, ten 10 mV voltage pulses were delivered to monitor series resistance and input conductance, followed by 25-50 tones of pseudorandomly interleaved sound frequency or intensity, at a rate of 1-2 per second. Pure tones had a duration of either 25 ms with 5 ms 10-90%

cosine-squared ramp, or 70 ms with 20 ms ramp, and were delivered free-field with a calibrated electrostatic speaker in a double-walled sound booth.

Series resistance was computed from the peak current transients, by taking the average across each group of 10 pulses, and taking the median of those averages over an entire stimulus protocol. No online series resistance compensation was used. There was no significant correlation between R_s and peak synaptic conductance across cells, suggesting R_s had no systematic effect on conductance estimates. Holding potentials were corrected for a calculated liquid junction potential³ of 12 mV.

Total synaptic conductance, corrected for series resistance, was computed assuming an isopotential neuron as follows (Fig. S1a). The total synaptic current $I_{syn}(t)$ is given by

$$I_{syn}(t) = g_{syn}(t)[V(t) - E_{syn}(t)], \quad (1)$$

where the total synaptic conductance $g_{syn}(t) = g_e(t) + g_i(t)$ is the sum of the inhibitory and excitatory components, $E_{syn}(t) = [g_e(t) E_e + g_i(t) E_i] / [g_e(t) + g_i(t)]$ is the weighted net synaptic reversal potential, and $V(t)$ is the holding potential corrected offline for somatic voltage escape. The corrected voltage $V(t)$ is related to the command potential V_{hold} by

$$V(t) = V_{hold} - I(t) R_s, \quad (2)$$

where R_s is the series resistance and $I(t)$ is the total recorded current. The total current $I(t)$ can be expressed as the sum of the synaptic current and the additional holding current required to voltage clamp the soma away from its resting potential E_{rest} :

$$I(t) = I_{syn}(t) + \frac{[V(t) - E_{rest}]}{R_{IN}}. \quad (3)$$

Here R_{IN} is the input resistance (not including the series R_s), estimated from the short prepulses delivered at each holding potential, and E_{rest} is the X -intercept of the steady-state current voltage relation. Defining the change in the recorded currents following a

tone-evoked response starting at time $t=0$ as $\Delta I(t) = I(t) - I(0)$, and using eqs. (2) and (3), the synaptic current can be expressed in terms of the measured current,

$$I_{syn}(t) = \frac{R_{IN} + R_s}{R_{IN}} \Delta I(t). \quad (4)$$

The term $(R_{IN} + R_s)/R_{IN}$ corrects for the current divider effect of the series resistance.

Equivalently, this correction can be expressed in terms of the somatic voltage escape

$$\Delta V(t) = V(t) - V(0),$$

$$I_{syn}(t) = \Delta I(t) - \Delta V(t)/R_{IN}. \quad (5)$$

With $V(t)$ thus obtained from eq. (2), and $I_{syn}(t)$ from eq. (4), we can then solve eq. (1) for $g_{syn}(t)$ and $E_{syn}(t)$ by linear regression. The total synaptic conductance $g_{syn}(t)$ is given by the slope, and the net synaptic reversal potential is obtained from the intercept - $g_{syn}(t)E_{syn}(t)$, as in Fig. 1b. Note that this method differs slightly from previous work⁴⁻⁶, in that we compute synaptic conductance, rather than total conductance. In this way, by basing our estimates on the transient synaptic component of the I-V curves, we minimized the potential distortions introduced by any additional nonlinearities in the steady-state I-V curves.

The total synaptic conductance $g_{syn}(t)$ estimated by regression is then decomposed into its excitatory and inhibitory components (Fig. S1b) by assuming $g_{syn}(t) = g_e(t) + g_i(t)$ and $E_{syn}(t) = [g_e(t) E_e + g_i(t) E_i] / [g_e(t) + g_i(t)]$ and solving for $g_e(t)$ and $g_i(t)$:

$$g_i(t) = \frac{g_{syn}(t)[E_e - E_{syn}(t)]}{E_e - E_i},$$

$$g_e(t) = g_{syn}(t) - g_i(t),$$

where $E_e = 0$ mV and $E_i = -85$ mV were set by our internal solution.

For cells in which action potentials were not blocked, tone-evoked synaptic conductances were so large that they could sometimes evoke spikes due to escape from voltage clamp. The active conductances involved in spike generation obscured synaptic conductances and therefore precluded their precise estimation. Nevertheless, by discarding trials on which spikes were evoked, we were able to estimate conductances which were uncontaminated by action potentials (Fig 1d, 2g). This culling procedure introduces a bias by discarding trials on which responses were presumably more depolarizing, and these conductance estimates are therefore less reliable than those for which spikes were blocked with QX-314 (although they allow the direct comparison of excitatory and inhibitory inputs with spike responses in the same cell). Spiking cells were therefore excluded from population analysis of conductance magnitudes and relative timing. We measured the rms error between actual voltage measured in current clamp ($V(t)$) and that predicted from the excitatory and inhibitory conductances

$$(\hat{V}(t) = \frac{g_e(t)E_e + g_i(t)E_i + g_{rest}E_{rest}}{g_e(t) + g_i(t) + g_{rest}}, \text{ where } g_{rest} = \frac{1}{R_{IN}}). \text{ Rms error was calculated as}$$

$$rms = \left\langle \sqrt{\frac{1}{T} \int_0^T (\bar{V}(t) - \hat{V}(t))^2 dt} \right\rangle, \text{ where the angle brackets indicate averaging across}$$

stimulus conditions, $\bar{V}(t)$ is the trial average of $V(t)$, and baselines were subtracted from $\bar{V}(t)$ and $\hat{V}(t)$. Spikes were digitally removed from $V(t)$ for rms error calculation and for display (Figs. 1d, 2g).

In cells for which action potentials were blocked with QX-314, *best frequency* was defined as the frequency that evoked maximal depolarization, obtained by repeating the stimuli in current clamp mode at rest ($I=0$) when sufficient time was available, or by estimating $\hat{V}(t)$ from the conductance measurements in voltage clamp. For spiking cells, best frequency was defined as the frequency which evoked the maximal spike count. We used these definitions of best frequency even if the maximal response occurred at the lowest or highest frequency tested for that cell (e.g. Fig. 3d), although responses to other

(unpresented) frequencies might have been greater. Best intensity was defined equivalently.

We simulated a conductance-based integrate-and-fire model using the difference equation $V_{t+1} = -\frac{dt}{C} [g_{e_i}(V_t - E_e) + g_{i_i}(V_t - E_i) + g_{rest}(V_t - E_{rest})] + V_t$, where $g_{rest} = 25$ nS and capacitance $C = 0.25$ nF gave a membrane time constant of 10 ms. When the membrane potential V_t reached the spike threshold of 20 mV above E_{rest} , it was set to E_{rest} . Synaptic inputs were modeled as conductances (given by an alpha function, $g(t) = \alpha^2 t e^{-\alpha t}$, with amplitude 3 nS and time constant 5 ms), and the jitter in each of their onset times was drawn from a gaussian distribution with standard deviation 1 ms. Simulations for Fig. 4a and 4b were identical except that for Fig. 4a, inputs (n=10 excitatory and 10 inhibitory) had fixed onset times (with a 2.5 ms inhibitory delay) before jitter was applied, whereas for Fig. 4b, inputs (n=23 excitatory and 23 inhibitory) had onset times uniformly distributed over a 100 ms window before jitter was applied. In each case, reported values for output spike time jitter are standard deviations across 10,000 trials (100 runs each for 100 unique seeds).

Space clamp, cable attenuation, and conductance estimation

To derive conductance estimates from voltage-clamp measurements, we assumed a linear, isopotential neuron. Although neurons are neither linear nor isopotential, the errors that arise due to deviations from these assumptions can be well characterized. Here we show, both analytically and with a compartmental simulation, that these errors cause underestimates of the absolute conductances and bias the decomposition into excitation and inhibition, but do not strongly affect estimates of relative timing. We avoid the effects of these absolute errors by comparing responses across stimuli, within a given cell.

Nonlinearities in the dendritic integration of synaptic inputs would be apparent as deviations from linearity in the synaptic IV curve. Synaptic IV curves were quite linear (Fig. 1b), suggesting that synaptic conductances were not strongly affected by nonlinearities such as NMDA or other voltage-dependent currents. Linearity may be enhanced by our use of QX-314 (which blocks many voltage-dependent currents in addition to those underlying action potential generation¹) and ketamine anesthesia (a low-affinity NMDA receptor antagonist). Note that because we estimate *synaptic* conductances (see *Methods*), steady-state nonlinearities, such as a nonlinear dependence of the input conductance on membrane potential, should have little effect on our estimates.

The electrotonic structure of neurons leads to an underestimation of conductances in three ways. First, although the soma may be clamped to a particular holding potential, distal synapses will be closer to the resting membrane potential due to imperfect steady state space clamp. Second, synaptic inputs will cause local escape from voltage clamp, leading to saturation (a reduction in the local synaptic driving force). Third, synaptic currents will be attenuated as they flow to the soma. Together these effects conspire to decrease synaptic currents compared to the isopotential ideal, thereby producing conductance estimates smaller than the true subsynaptic values. Note, however, that saturation and synaptic current attenuation also reduce the effective excitatory and inhibitory conductances seen from the soma, which presumably are more relevant to neuronal output than subsynaptic events. Thus these factors represent not solely errors in estimation, but reflect functional consequences of dendrites for neuronal integration.

The effects of cable attenuation on synaptic conductance estimates made from the soma can be understood analytically. In the limiting case in which synaptic conductances are so small that they do not perturb the local membrane potential, such that saturation

may be ignored, the estimated synaptic conductance \hat{G} and the estimated synaptic reversal potential \hat{E}_{syn} underestimate the true G and E_{syn} by:

$$\hat{G} = A^2 G, \text{ and}$$

$$\hat{E}_{syn} = \left[E_{rest} + \frac{(E_{syn} - E_{rest})}{A} \right],$$

where $A \leq 1$ is the electrotonic voltage attenuation factor between soma and dendrite, and E_{rest} is the rest potential⁷⁻⁹. Thus the synaptic conductance \hat{G} estimated from a somatic recording underestimates G by a fraction A^2 (Fig. S2a, black line). Furthermore, the estimated synaptic reversal potentials \hat{E}_{syn} will be in error, in the direction away from resting potential. This error in the synaptic reversal potential¹⁰ causes a bias in the decomposition into excitatory and inhibitory inputs. If the true synaptic reversal potential is above rest (which was nearly always true for the balanced excitation and inhibition we observed), the estimated synaptic reversal potential will be too high by the amount $\frac{(E_{syn} - E_{rest})}{A}$. As a result, a corresponding proportion of the estimated synaptic conductance will be misattributed to excitation rather than inhibition. In other words, cable attenuation causes inhibitory conductance to be more underestimated than excitatory conductance (Fig. S2a, green and red lines). The ratio of inhibitory to excitatory conductance, therefore, will also tend to be underestimated.

Although the relative magnitudes of the excitatory and inhibitory components are affected by cable attenuation, the relative timing of these components is much less affected. To extract the delay between excitation and inhibition, we normalized both components (Fig. 3), removing the effects of conductance underestimation on the magnitudes. This can be seen directly in simulations (see below), which show that the attenuation does not affect the relative timing of excitation and inhibition (Fig. S2c).

To illustrate how the attenuation A captures the underestimation due to dendritic cable properties, we performed a 198-compartment NEURON¹¹ simulation of a layer 6 cortical pyramidal cell¹² (with $R_s=60\text{ M}\Omega$, $R_a=200\text{ }\Omega\text{cm}$, $C_m=1\text{ }\mu\text{F}/\text{cm}^2$, $E_r=-70\text{ mV}$, $g_{rest}=39\text{ nS}$). We replicated our voltage clamp experiment, using the same method to estimate conductances, and introduced pairs of excitatory and inhibitory synapses at varying dendritic locations (conductances were small to minimize saturation effects). Figure S2b shows estimates of the synaptic conductance (black line) and its excitatory (green) and inhibitory (red) components as a function of distance from the soma. In agreement with the linear analysis above (Fig. S2a), increasing distance from the soma caused a progressive underestimate of synaptic conductance, and the inhibitory conductance was relatively more underestimated. When a 5 ms delay between excitatory and inhibitory onsets was used, the delay extracted from the estimated conductances at half maximal amplitude was essentially unaffected by dendritic location (Fig. S2c). Thus, although peak conductance values (*e.g.* Fig. 2) must be treated as underestimates, delay values (*e.g.* Fig. 3) are more likely to reflect the true relative timing of excitation and inhibition.

Figure S1 Equivalent circuits used for estimation of synaptic conductances. **a** Circuit used to estimate total synaptic conductance $g_{syn}(t)$. **b** Circuit used to decompose $g_{syn}(t)$ into $g_e(t)$ and $g_i(t)$. The voltage clamp is represented by the ammeter $I(t)$ and the battery V_{hold} . See text for details.

Figure S2 Attenuation due to cable properties causes an underestimate of synaptic conductance magnitudes, but preserves relative timing. **a**, Analytic results for estimation of total conductance (black) and decomposition into excitatory (green) and inhibitory (red) components as a function of the

attenuation between the synaptic inputs and the soma, for a 2 nS synaptic conductance (1 nS excitatory, 1 nS inhibitory). Note that the inhibitory conductance is more underestimated than the excitatory conductance. **b**, Simulation results for decomposition of simultaneous excitatory and inhibitory synaptic conductances of equal amplitude as a function of distance from the soma. Estimates of peak synaptic conductance (black), peak excitatory (green), and peak inhibitory (red) components, using somatically recorded currents, are progressively more underestimated as the synaptic location moves further from the soma. The inhibitory component is more underestimated than the excitatory component. The effect of the electrotonic structure, even on transient conductances, matches the analytic results in **a** for the attenuation factor A . **c**, Estimated delay between excitatory and inhibitory conductances did not vary as a function of distance from the soma. Delays were extracted from normalized somatic conductance estimates at half maximal amplitude (Fig. 3). Estimated delay closely matched the 5 ms delay used between excitatory and inhibitory conductance onsets.

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