BRIEF COMMUNICATIONS

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Instantaneous correlation of excitation and inhibition during ongoing and sensory-evoked activities

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Temporal and quantitative relations between excitatory and inhibitory inputs in the cortex are central to its activity, yet they remain poorly understood. In particular, a controversy exists regarding the extent of correlation between cortical excitation and inhibition. Using simultaneous intracellular recordings in pairs of nearby neurons *in vivo*, we found that excitatory and inhibitory inputs are continuously synchronized and correlated in strength during spontaneous and sensory-evoked activities in the rat somatosensory cortex.

Inhibitory neurons comprise only a small fraction of all of the cortical neurons, yet they are believed to be important in regulating the excitatory activity in the cortex. In a widely accepted class of models, cortical neurons are assumed to receive a large number of uncorrelated small excitatory and inhibitory synaptic inputs that, on average, balance each other[1–3]. However, several recent *in vivo* intracellular recording studies[4–6] have reported that membrane potential dynamics consisting of quiescent periods are interrupted by short intervals of high-amplitude depolarizations, presumably produced by synchronous firing of many presynaptic neurons, which is clearly inconsistent with the random walk models of membrane potential mentioned above. It remains unclear whether excitation and inhibition are correlated, mainly because it is practically impossible to simultaneously measure them both in a single cell. A particular membrane potential value can be produced by different combinations of excitatory and inhibitory inputs (just as $a + b = 7$ holds true for different values of a and b). Hence, a single-cell recording can only provide the average relation between these inputs, calculated from the average response to repeated stimulation, recorded at different holding potentials[7–9]. In this study, we resolve the questions regarding temporal and magnitude correlations between excitatory and inhibitory signals during ongoing and sensory-evoked activities by means of a new experimental technique that allows the probing of the instantaneous relation between excitation and inhibition in the local cortical circuit.

Nearby cortical neurons receive highly similar synaptic inputs, as demonstrated by the high correlation of their membrane potentials[10–12]. To determine the correlation between excitation and inhibition, we simultaneously recorded pairs of closely located neurons (47 pairs, distance <500 μm) in the barrel cortex of lightly anesthetized rats in current-clamp mode. In each pair, one cell was recorded near the reversal potential of inhibition ($\sim$−75 mV in our preparation, **Supplementary Figs. 1–3** and **Results** online), which required the injection

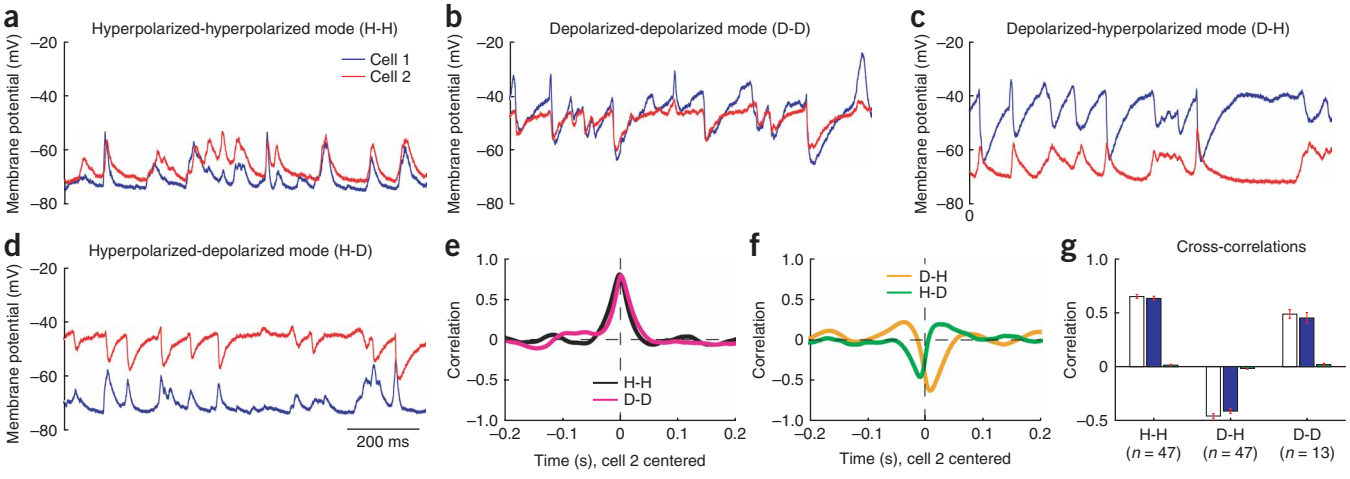

Figure 1 Excitatory and inhibitory synaptic potentials of nearby neurons are synchronized during spontaneous activity. (**a**–**d**) Simultaneous recordings from two cortical cells at four different combinations of current injection (H-H, D-D, D-H and H-D). D, depolarized; H, hyperpolarized. (**e**,**f**) Corresponding cross-correlations (each from an average cross-correlation of 20 1-s epochs). (**g**) Cross-correlation in the population: correlation at zero time lag (blue bars), peak correlation (white bars), and cross-correlation among shuffled traces (green bars). Note that the D-D mode was tested only in a subset of pairs. Error bars represent the s.e.m.

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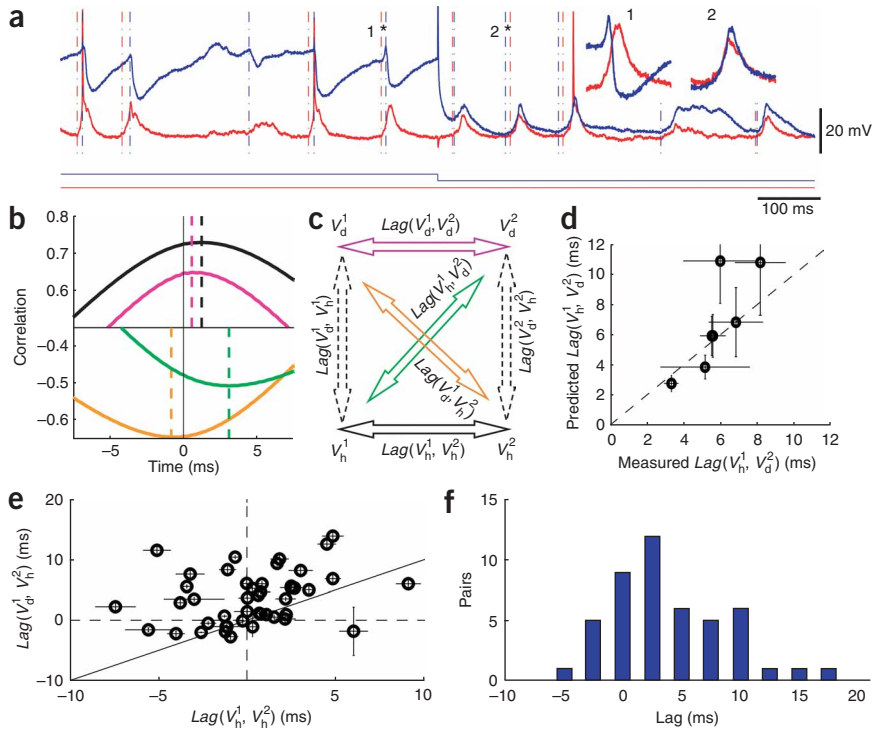

Figure 2 Inhibition lags behind excitation during spontaneous activity. (**a**) Simultaneous recordings from two cortical cells. One cell (red) was continuously recorded in hyperpolarized mode and the other cell (blue) was switched between depolarized and hyperpolarized modes (current depicted below traces). Dashed lines mark the onsets of synaptic events (detection algorithm described in **Supplementary Methods** online). Insets show examples of two events (marked by asterisks). (**b**,**c**) V_m average cross-correlograms, cell 2 centered (corresponding to the combinations in **Fig. 1e**,**f**, but for a different pair). Peak times (dashed lines) were used to estimate the lags presented in the diagram (**c**). (**d**) No significant difference ($P = 0.3$, $n = 7$) was found between the measured $Lag(V_h^1, V_d^2)$ and its prediction by the expression $Lag(V_h^1, V_h^2) - Lag(V_d^1, V_h^2) + Lag(V_d^1, V_d^2)$. Error bars represent 95% confidence intervals. (**e**,**f**) For each of the pairs ($n = 47$), V_h^2 was used as a reference to measure the lag of V_d^1 relative to V_h^1. The distribution of the lags computed by subtracting the abscissa from the ordinal in **e** is presented in **f**. The error bars (**e**, barely visible for most pairs) represent the s.e.m.

of zero or small negative current (hyperpolarized mode), while at the same time a positive current was injected into the other cell to reveal the inhibitory potentials (depolarized mode; to prevent firing, we added QX-314 to the pipette). Positive excursions of the membrane potential in the hyperpolarized mode reflect mostly excitatory synaptic currents, whereas the negative excursions in the depolarized mode reflect mostly inhibitory synaptic currents[9,12]. This dual recording technique is the only currently available method that provides an adequate single-trial picture of the magnitude and timing of both excitatory and inhibitory inputs and that is suitable for elucidating the excitatory-inhibitory dynamics during ongoing activity and evoked responses.

Synchrony in spontaneous activity was noticeable in the appearance times, shapes and amplitudes of the spontaneous large synaptic potentials, or 'bumps'[5] (**Fig. 1a**), indicating that this pair of cells received highly correlated excitatory inputs. The two cells also received synchronized inhibitory inputs, as was revealed when the cells were depolarized to about –40 mV (**Fig. 1b**). To observe the instantaneous excitatory-inhibitory dynamics in the local network, we depolarized one cell to reveal inhibitory potentials, while the second cell was recorded near its resting level (**Fig. 1c** and vice versa in **Fig. 1d**). Note that almost every negative bump in the depolarized neuron was accompanied by a positive bump in the hyperpolarized neuron. These recordings strongly suggest that the excitatory and inhibitory inputs of the cells were highly synchronized during spontaneous activity (**Fig. 1e**,**f**). A similar behavior was observed in all of the analyzed pairs (**Fig. 1g**) and in other cortical areas (parietal association cortex, data not shown). It is important to emphasize that the spontaneous activity observed here does not show Up-Down dynamics[9,12] but is rather similar to the dynamics in the awake animal (**Supplementary Figs. 4–6** and **Results** online).

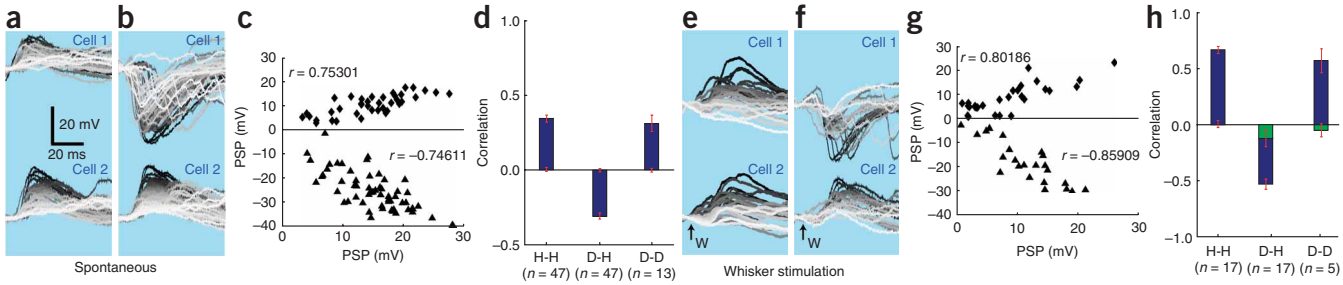

Figure 3 The magnitude of excitatory and inhibitory potentials in paired recordings is positively correlated. (**a**) Automatically detected synaptic events (randomly selected from 30 s of recording) in the second cell (Cell 2) of a pair are shown at the bottom, sorted by their amplitude (indicated by the color intensity). The corresponding events in Cell 1 are shown above with the same color. Here, both neurons were in the hyperpolarized mode. (**b**) As in **a**, when the first cell is in the depolarized mode. Baseline membrane potential was ~–77 mV for the second cell and ~–70 (in **a**) and ~–28 mV (in **b**) for the first cell. (**c**) Amplitudes of the events presented in **a** and **b**. A significant correlation ($P < 0.0001$) between the amplitudes is clearly visible in each case. (**d**) The mean of amplitude correlations of spontaneous events that appear at the same time in both cells ($n = 47$ pairs). The amplitude correlations were significant ($P < 0.05$) for 39 out of 47 pairs in the H-H and D-H modes, and for 10 out of 13 pairs in the D-D mode. The green bar (invisible) presents the correlation when the amplitudes in Cell 2 of each pair were randomly shuffled. (**e**–**g**) Data is presented as in **a**–**c** for events evoked by whiskers' stimulation (in the same pair). (**h**) Average amplitude correlations for sensory-evoked postsynaptic potentials in $n = 17$ pairs. The green bars represent the correlation after the response traces in one of the cells were randomly shuffled. Errors bars (in **d** and **h**) represent the s.e.m.

The cross-correlations of the membrane potentials attained their peak values at nonzero time lags (**Fig. 1e**,**f**). On average across all pairs ($n = 47$), the lag between the membrane potential of a depolarized neuron (V_d^1) and the membrane potential of a hyperpolarized neuron (V_h^2) was 3.5 ± 4.3 ms. This highly significant lag ($P < 0.001$) was not a result of biased measurements, as evidenced by the nearly zero and nonsignificant ($P > 0.6$) lag between V_h^1 and V_h^2, and between V_d^1 and V_d^2, when averaged across all pairs ($n = 47$, -0.2 ± 4.0 ms and $n = 13$, -0.6 ± 5.6 ms, respectively). Therefore, although excitation and inhibition were measured in two different cells, one can conclude that, on average, inhibition lags behind excitation by several milliseconds during spontaneous activity.

The relative timing between synaptic potentials in the two cells was fairly consistent during the recording (see below) and could be observed even in short spontaneous epochs (**Fig. 2a**). In the first half of the presented interval, positive current was injected into the test cell (blue), and then both cells were recorded at the hyperpolarized mode. During the first half of the recording, the inhibitory bumps in the test neuron lagged behind the excitatory bumps in the reference (red) neuron, whereas the excitatory bumps started simultaneously or even slightly before the corresponding events in the reference cell following hyperpolarization. This indicates that the inhibitory inputs to the first cell lagged behind its excitatory inputs. The average lag of inhibitory potentials behind the excitatory potentials in a single neuron, $Lag(V_d^1, V_h^1)$, was quantitatively estimated using the hyperpolarized membrane potential of the simultaneously recorded neuron, V_h^2, as a reference signal, namely $Lag(V_d^1, V_h^1) = Lag(V_d^1, V_h^2) - Lag(V_h^1, V_h^2)$, where $Lag(V_d^1, V_h^2)$ and $Lag(V_h^1, V_h^2)$ were measured from the peak cross-correlations. The accuracy of this method was additionally verified in pairs where all four combinations were recorded by comparing $Lag(V_h^1, V_d^2)$ with predictions based on similar measurements of the three other lags (**Fig. 2b–d**). No significant difference was found between the predicted and measured values for 6 out of the 7 pairs ($P = 0.05$; **Fig. 2d**). By using the above method and subtracting the abscissa values from the ordinate values (**Fig. 2e**), we found that the mean lag between inhibitory and excitatory potentials was 3.8 ± 4.9 ms ($n = 47$; **Fig. 2f**). The small error bars in **Figure 2e** and the ability to predict one of the four lags from the three complementary lags indicate that the lags between the activities in the two cells did not change substantially during the course of the recording session. Our conclusion that spontaneous inhibitory inputs lag by several milliseconds behind the excitatory inputs of the same cell is further supported by an additional, independent method, based on detection of event onsets (**Supplementary Fig. 7** and **Results** online).

The shape and amplitude of the synaptic events were highly variable when recorded at the neuron's resting potential (for example, as in **Fig. 1a**). A priori, the contribution of the excitatory and inhibitory synaptic inputs to the amplitude variability of these events could be anywhere between the following two diametrically opposing possibilities. It might be the case that large bumps occur when the inhibitory activity, which can damp the excitation, is weak. Alternatively, both types of synaptic inputs might reflect the overall level of activity in the local network and go hand in hand. Here we used our experimental approach to resolve this issue (**Fig. 3**). We found that the amplitudes of spontaneous events were significantly correlated, both for the depolarizing potentials when the two cells were held near their resting potential (**Fig. 3a**,**c**,**d**) and when one cell was depolarized to reveal inhibitory potentials (**Fig. 3b–d**). Hence, the latter alternative is correct; that is, for spontaneous activity, the larger the excitatory drive in the local circuit, the larger the inhibitory one (similar conclusions were drawn using yet another analysis, see **Supplementary Fig. 8** and **Results** online). The relatively low values of the correlation and the lack of significant correlation between the amplitudes of the bumps in a few pairs (**Fig. 3d**, population data) might be explained by the dependence of the correlation on the intrinsic properties of the two neurons, differences in their exact membrane potentials and the exact pattern of the synaptic inputs of the cells.

We used the same approach to examine the trial-to-trial correlation between excitation and inhibition in the highly variable cortical responses[13] to fast multi-whisker deflections. The amplitudes of sensory-evoked bumps in simultaneously recorded neurons were substantially correlated, rather similarly to the spontaneous activity (**Fig. 3e–h**). The coordinated activity of excitation and inhibition across two neurons in spontaneous and evoked activities strongly suggests that variations in excitation and inhibition reflect mostly changes in the local network activity rather than 'private' variability of the inputs of individual cells[4,14].

A likely role for cortical inhibition is to prevent runaway excitation by damping the recurrent excitatory activity[15]. The instantaneous coupling between excitation and inhibition that we have found in spontaneous and sensory-evoked activities supports this hypothesis. Such tight coupling is also consistent with the hypothesis that inhibition controls the integration time window of excitation, enabling neurons to operate as coincidence detectors[7,8]. The sources for the trial-to-trial variability in the response of the network to sensory stimulation are unclear. We speculate, however, that a large part of this variability results from thalamic variability and interactions of thalamic inputs with cortical ongoing activity[11]. Whether tight coupling between excitation and inhibition exists in awake animals is presently unknown. The similarity of subthreshold cortical activity in lightly anesthetized and awake rodents (**Supplementary Fig. 6**) suggests that this might be the case. In summary, our findings provide the first direct indication that the instantaneous balance between excitation and inhibition is continuously controlled with a millisecond precision during both spontaneous activity and the response to sensory stimulation.

Note: Supplementary information is available on the Nature Neuroscience website.

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