Supplementary Information

To accompany "Asymmetric Temporal Integration of Layer 4 and Layer 2/3 Inputs in Visual Cortex" by Giao B. Hang and Yang Dan.

Measuring the reversal potential for excitation and inhibition:

To measure the reversal potential of inhibition, we blocked glutamate receptors with 50 μM APV and 15 μM CNQX. The left panel of supplemental Fig. S1 shows the I-V curve from one example experiment. The reversal potential (x-intercept) for the measured inhibitory current was -79.3 mV. The mean reversal potential measured in all experiments was -78.8 mV ± 8.5 mV (s.d., n = 11). The reversal potential for excitation (-7.5 ± 12.9 mV, s.d., n = 4) was measured in a similar fashion except that inhibition was blocked with 10 μM of bicuculline. For estimating the excitatory and inhibitory conductances in this study, we have used 0 and -80 mV for the reversal potentials for excitation and inhibition, respectively, very close to the experimentally measured values.

Note that the measured reversal potential for inhibition is far from the theoretical calculation of -45 mV (based on the Cl^- concentration in the internal solution). This could be due to incomplete dialysis of cell, such that the Cl^- concentration at the inhibitory synapses is quite different from that in the recording pipette. To test this possibility, we performed another set of experiments with a different internal solution that has been used in previous studies (Liu et al. 2007). The composition of this solution (in mM) was the following: 125 K-gluconate, 5 KCl, 10 Hepes, 4 MgATP, 0.4 NaGTP, 0.4 TrisPhosphocreatine, 2 QX-314, 1 EGTA. The calculated reversal potential for chloride was -85 mV. The right panel of supplemental Fig. S1 shows the I-V curve from one example experiment in the presence of CNQX and APV, and the mean reversal potential from all the experiments was -77.8 mV ± 3.0 mV (s.d., n=7). These results indicate that the reversal potential is insensitive to the chloride concentration in the patch electrode, supporting the hypothesis that the intracellular Cl^- concentration is not strongly influenced by the intra-pipette Cl^- concentration. Consistent with the more hyperpolarized reversal potential for inhibition of ~-80 mV, when cells were held at -70 mV, PSPs were never observed to increase in amplitude after wash in of bicuculline (supplemental Fig. S2).

Evoked responses before and after blockade of excitatory transmission:

In this study, we adjusted the stimulation strength such that the PSPs from the two pathways have comparable amplitudes. Under this condition, the excitatory inputs from the two pathways were similar, but inhibition was stronger in layer 2/3. This difference is likely to be due to differences in the functional circuits recruited. To further understand the basis of this difference, we blocked excitation with glutamate receptor antagonist CNQX (15μM) and APV (50 μM) and found a marked reduction in inhibition from stimulation in layer 4 but not in layer 2/3 (supplemental Fig. S3). This suggests that inhibition from layer 2/3 stimulation recruits primarily monosynaptic inhibition, while inhibition from layer 4 is likely to be indirect, requiring activation of the GABAergic neurons through excitatory input from layer 4.

References

Liu BH, Wu GK, Arbuckle R, Tao HW, and Zhang LI. Defining cortical frequency tuning with recurrent excitatory circuitry. *Nat Neurosci* 10: 1594-1600, 2007.

Williams SR, and Mitchell SJ. Direct measurement of somatic voltage clamp errors in central neurons. *Nat Neurosci* 11: 790-798, 2008.