Network and neuronal membrane properties in hybrid networks reciprocally regulate selectivity to rapid thalamocortical inputs

Michael J. Pesavento\textsuperscript{1,2} and David J. Pinto\textsuperscript{1,2,3}

\textsuperscript{1}Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, New York; \textsuperscript{2}Department of Biomedical Engineering, University of Rochester School of Medicine, Rochester, New York; and \textsuperscript{3}Department of Neurology, University of Rochester School of Medicine, Rochester, New York

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Pesavento MJ, Pinto DJ. Network and neuronal membrane properties in hybrid networks reciprocally regulate selectivity to rapid thalamocortical inputs. \textit{J Neurophysiol} 108: 2452–2472, 2012. First published August 15, 2012; doi:10.1152/jn.00914.2011.—Rapidly changing environments require rapid processing from sensory inputs. Varying deflection velocities of a rodent’s primary facial vibrissa cause varying temporal neuronal activity profiles within the ventral posteromedial thalamic nucleus. Local neuron populations in a single somatosensory layer 4 barrel transform sparsely coded input into a spike count based on the input’s temporal profile. We investigate this transformation by creating a barrel-like hybrid network with whole cell recordings of in vitro neurons from a cortical slice preparation, embedding the biological neuron in the simulated network by presenting virtual synaptic conductances via a conductance clamp. Utilizing the hybrid network, we examine the reciprocal network properties (local excitatory and inhibitory synaptic convergence) and neuronal membrane properties (input resistance) by altering the barrel population response to diverse thalamic input. In the presence of local network input, neurons are more selective to thalamic input timing; this arises from strong feedforward inhibition. Strongly inhibitory (damping) network regimes are more selective to timing and less selective to the magnitude of input but require stronger initial input. Input selectivity relies heavily on the different membrane properties of excitatory and inhibitory neurons. When inhibitory and excitatory neurons had identical membrane properties, the sensitivity of in vitro neurons to temporal vs. magnitude features of input was substantially reduced. Increasing the mean leak conductance of the inhibitory cells decreased the network’s temporal sensitivity, whereas increasing excitatory leak conductance enhanced magnitude sensitivity. Local network synapses are essential in shaping thalamic input, and differing membrane properties of functional classes reciprocally modulate this effect.

thalamocortical transformation; barrel cortex; somatosensory cortex; neural circuit; feedforward inhibition; recurrent excitation

UNDERSTANDING THE RELATIONSHIP between single-neuron membrane properties and network processing is a necessary component of understanding neuronal circuit processing. Many studies have examined the response of cortical neurons to sensory stimuli within networks (in vivo whole animal preparations), whereas others have examined the activity of such neurons responding to injected current in relative isolation (in vitro slice preparations). Because of the difficulty in separating the roles of network connectivity and neuronal membrane properties in reshaping transient input, this relationship requires an innovative approach. We create a hybrid network, consisting of a biological neuron embedded in a simulated conductance-based computational network, to assist in the isolation of neuronal and network contributions to reshaping transient input. This technique gives unprecedented levels of control over network connectivity and neuronal membrane properties while permitting comparison of in vitro neuron responses across a multitude of conditions.

The rodent vibrissa system is uniquely positioned to provide insight into neuron- and network-level processing of sensory inputs. Discrete, dense clusters of neurons within layer 4 of rodent primary somatosensory cortex, known as “barrels” (Welker 1971; Welker and Woolsey 1974; Woolsey and Van der Loos 1970), receive the majority of their afferent input from analogous groups of neurons in the ventral posteromedial (VPM) nucleus in the thalamus (Jensen and Killackey 1987; Land et al. 1995; Simons and Carvell 1989). Within a layer 4 barrel, three electrophysiologically distinct neuronal subpopulations form a strongly interconnected synaptic network: excitatory regular-spiking (RS) neurons, inhibitory fast-spiking (FS) neurons, and inhibitory low-threshold-spiking (LTS) neurons (Beierlein et al. 2003; McCormick et al. 1985). Although several neuronal morphologies have been observed in each class (Kawaguchi 1993; Kawaguchi and Kubota 1997; Staiger et al. 2004; Sun et al. 2006), electrophysiological measures allow accurate classification by observed intrinsic membrane properties in response to square pulses of current injection (Gibson et al. 1999; McCormick et al. 1985; Pesavento et al. 2010). In this study, we focus on RS and FS neuron transient responses to thalamic input; LTS neurons largely do not receive direct thalamic input (Cruikshank et al. 2010; Gibson et al. 1999) and therefore are not discussed here.

The temporal features of sensory stimuli are conveyed through the thalamus to the sensory cortex via multiple sensory modalities (Buracas et al. 1998; DeWeese et al. 2003; Phillips et al. 1988; Reinagel and Reid 2000; Wehr and Zador 2003). A number of studies have examined the contribution of either cortical neurons or cortical networks in shaping the responses to rapid thalamic input. Layer 4 barrel neurons respond preferentially to fast, synchronous or near-synchronous thalamic input (Arabzadeh et al. 2005; Ito and Kato 2002; Pinto et al. 2000; Wilent and Contreras 2004), and similar results have been reported in both visual cortex (Alonso et al. 1996; Hirsch et al. 1998) and auditory cortex (Wehr and Zador 2003). This sensitivity may be due in part to the membrane properties of individual neurons (Azouz and Gray 2000; Chung and Ferster 1998; Pesavento et al. 2010; Wilent and Contreras 2005). Several studies also suggest that network interactions contrib-
ute to barrel response sensitivity (Arabzadeh et al. 2005; Pinto et al. 2000; Wilent and Contreras 2004). Our goal is to clarify these reciprocal contributions of neuronal and network properties in context of temporal selectivity in the barrel system.

Neurons within the barrel form an interconnected synaptic network, including direct thalamic input (Jensen and Killackey 1987), feedforward inhibition (Bruno and Sakmann 2006; Cruikshank et al. 2007; Gabernet et al. 2005; Lawrence and McBain 2003; Miller et al. 2001; Pouille and Scanziani 2001; Swadlow and Gusev 2002; Wehr and Zador 2003), and recurrent excitation (Adorján et al. 1999; Douglas et al. 1995; Miller et al. 2001). In barreIs, the overall effect of network interactions is inhibitory or damping due to the strength of feedforward inhibition (Pinto et al. 2003a; Swadlow et al. 2005). We further explore how each class of synaptic inputs (thalamic, local excitatory, and local inhibitory) contributes to modifying neuronal response properties. Previous modeling studies have explored the balance of local and thalamic inputs (Kyriazi and Simons 1993) and excitation and inhibition within the local network (Pinto et al. 2003a). The role of network feedback has also been examined via bath application of muscimol, effectively inhibiting all local synaptic connections (Liu et al. 2007). However, this likely alters the intrinsic membrane properties of the neurons, making an accurate comparison difficult. The hybrid network is well suited to the task of comparing the responses of a single neuron or neuronal population in the absence of local network input versus the presence of network input. An additional strength of the hybrid network over modeling studies is that we are examining the responses of biological neurons.

Previously, we characterized the activity of individual neurons in rat barrel cortex in response to simulated thalamocortical input (Pesavento et al. 2010). We found that neurons responding in the absence of local network activity cannot account for the response sensitivity of ensemble barrel responses observed in vivo (Pinto et al. 2000). In this study, we examine the combined effect of intrinsic neuronal and network properties using a unique combination of whole cell patch recordings and real-time computer simulations. Recording from individual barrel neurons in vitro, we use a conductance clamp to embed the neurons in a simulated barrel network. This arrangement allows us to change the intrinsic properties of the barrel neurons and examine the effects of those changes on the network’s response. Our hybrid network is activated by simulated thalamic synaptic inputs modeled after real thalamic responses to whisker deflections of different velocities (Pesavento et al. 2010; Pinto et al. 2000).

Using the hybrid network, we find that the presence of local synaptic input from the surrounding network explains much of the difference of response measures between in vivo neurons and in vitro neurons. We then examine what aspects of the network contribute to shaping response selectivity to thalamic input, and how these principles can apply to other sensory modalities. Damping networks (Pinto et al. 2003a) with strong feedforward inhibition enhance response selectivity to fast inputs while decreasing the sensitivity to input magnitude. Recurrent excitation may play an important role in nontransient input processing.

The intrinsic membrane properties of neurons, such as input resistance and firing rate adaptation, have been used to classify electrophysiological differences between subpopulations; however, it is also important to note that these classifications also distinguish between different functional classifications, such as excitatory and inhibitory synaptic projections. Excitatory RS neurons have higher input resistance than inhibitory FS neurons, which results in broader response selectivity in the inhibitory neurons and higher temporal selectivity in the excitatory neurons (Pesavento et al. 2010). This difference between neuronal subclasses may be an essential component of how local synaptic input shapes the population response to transient thalamic input.

Using both the hybrid network and off-line simulations, we find that differences in the intrinsic properties of RS vs. FS barrel neurons are crucial for enabling the network to enhance barrel sensitivity to input timing. In addition, changes to the input resistance (leak conductance) of either FS or RS neurons strongly alter the processing ability of the network, consistent with our previous model predictions.

Our hybrid network allows full control over synaptic connections and intrinsic membrane properties, enabling us to explore how they each affect response sensitivity to the number of active thalamic synapses as well as sensitivity to the timing of their input. The results presented here indicate a close interrelationship between neuron- and network-level processing in determining local circuit function. The differences of individual neuronal membrane properties result in selective responses to input in the absence of local network synapses. The addition of network synapses then additionally increases the selectivity of the neurons. The final step in this reciprocal interaction occurs when the membrane properties of individual neurons are changed, thus altering the effect of the network input on neuronal population output.

METHODS

We generated a computational model of a barrel network by synaptically connecting RS and FS neurons and have constructed thalamic spike volleys based on known responses to whisker deflections. Using the dynamic clamp, we present virtual synaptic conductances from the model neurons and thalamic inputs. Finally, we modify the intrinsic properties of the simulated neurons and observe the results. Many aspects of our simulated network are conceptually based on previous work (Kyriazi and Simons 1993). Model neurons and simulated networks are constructed probabilistically by varying parameters to generate responses that conform to experimental data at the level of both the barrel network and barrel neurons. Membrane conductance values and individual synaptic strengths are drawn from normal distributions centered on mean values such that simulated neural responses have the same mean and variance as real neurons. Network connections are assigned randomly, but with convergence values in accord with the known connectivity estimates of the barrel cortex network. This strategy ensures that each instance of our simulated and hybrid networks is unique and that our results are robust to heterogeneities (Skinner et al. 2005). At the same time, however, both the neurons and network of each simulation closely approximate real features of barrel circuitry to the extent of our present knowledge.

Preparation and Electrophysiology

All electrophysiological methods were performed as reported previously (Pesavento et al. 2010). Whole cell dynamic clamp recordings were taken from layer 4 RS neurons. Cortical slices 400 µm thick were obtained with a mechanical vibratome (World Precision Instruments,...
ments. Sarasota, FL) from Sprague-Dawley rats on postnatal days 13–24, using the near-cortical slicing angle described by Land and Kandler (2002). Slices were bathed in artificial cerebrospinal fluid (ACSF) containing (in mM) 126 Na, 3 KCl, 1.25 NaH2PO4, 2 MgSO4, 26 NaHCO3, 10 dextrose, and 2 CaCl2 and saturated with 95% O2-5% CO2 at a temperature of 35°C. Borosilicate glass micropipettes were pulled with a Flaming-Brown puller (P97; Sutter Instruments) to a tip resistance of 5–10 MΩ and access resistance of 18–30 MΩ, and they were filled with an internal solution consisting of (in mM) 135 K-gluconate, 4 KCl, 2 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, and 7 phosphocreatine-Tris (pH 7.25, 290 mosmol/l). Whole cell patch recordings were made in current-clamp mode using an Axon Instruments Multiclamp 700B amplifier. All protocols were reviewed and approved by the University of Rochester Committee on Animal Resources.

Barrels were clearly visible in the living slice. Only layer 4 barrel neurons were examined in this study. A small constant holding current (maximum ±100 pA) was applied to bring the neuron’s dialed resting potential to −65 mV to facilitate comparisons between cells. Previous studies have found that slight modulations of the membrane potential strongly influence the amplitude of postsynaptic potentials, as well as the timing of evoked action potentials (Petersen et al. 2003; Sachdev et al. 2004); here we attempted to minimize those differences as well as the timing of evoked action potentials (Petersen et al. 2003; Petersen et al. 2003). In previous studies, slight modulations of the membrane potential have been shown to influence the amplitude of postsynaptic potentials, as well as the timing of evoked action potentials (Petersen et al. 2003; Sachdev et al. 2004); here we attempted to minimize those differences as well as the timing of evoked action potentials (Petersen et al. 2003; Petersen et al. 2003).

We controlled current injection, data collection, and real-time (i.e., dynamic clamp) stimulation by using LabVIEW-RT software (National Instruments) written specifically for the task. A host computer system running custom LabVIEW software controlled stimulus parameters and file input/output. A slave computer system running custom software on the LabVIEW-RT operating system performed all real-time calculations for network simulation and conductances to apply to a single in vitro neuron via an analog-to-digital converter (National Instruments). Membrane potentials were recorded using an Axon Instruments amplifier, and response waveforms were sampled and digitized at 10 kHz.

We injected square current pulses into each neuron to classify the neuron and quantify several standard response measures. Pulses lasted 1,000 ms, ranged in amplitude between ±0.4 nA in 0.1-nA increments, and were presented at a rate of 0.5 Hz. Cells were classified as RS, FS, or LTS according to previously established criteria (Gibson et al. 2010). Currents and virtual conductances were applied to a single cell, eliciting at most one or two spikes. Stimulating a single neuron with single-compartment conductance-based models of RS and FS neurons (Pesavento et al. 2010). In previous studies, slight modulations of the membrane potential have been shown to influence the amplitude of postsynaptic potentials, as well as the timing of evoked action potentials (Petersen et al. 2003; Petersen et al. 2003); here we attempted to minimize those differences as well as the timing of evoked action potentials (Petersen et al. 2003; Petersen et al. 2003).

Simulated RS and FS Neurons

Single-compartment conductance-based models of RS and FS neurons were created using current-balance equations (Pesavento et al. 2010). For each model, we varied the values of each maximal conductance so that the intrinsic membrane properties of the model neurons were within the range of those measured from real barrel neurons recorded in vitro (Beierlein et al. 2003; Pesavento et al. 2010). Network simulations generated populations of model neurons by randomly selecting the maximum conductances and radius based on a Gaussian distribution with means listed and standard deviation of 5% of the mean. Membrane capacitance density was held constant at 1.0 μF/cm2 for all neuron classes. All membrane parameters are given in terms of both the maximum conductance and specific conductances; actual values vary about these means.

We modeled conductance-based RS and FS neurons using current-balance equations that describe membrane voltage:

$$C_m \frac{dV}{dt} = I_{\text{leak}}(V) + I_{Na}(V, h) + I_{K_a}(V, n) + I_{ATP}(V, w) + I_{\text{app}} + I_{\text{synX}}$$

$$C_m \frac{dV}{dt} = I_{\text{leak}}(V) + I_{Na}(V, h) + I_{K_a}(V, n) + I_{Ih}(V, w) + I_{\text{app}} + I_{\text{synX}}$$

$$I_{\text{son}} = g_{\text{ion}} e^{-b[V-E_\text{ion}]}/(V-V_f(t))$$

where $C_m$ is the specific membrane capacitance, $V_s$ is the membrane potential for a neuron of class $Y$ (RS or FS), $I_{\text{app}}$ are ionic currents, $I_{\text{app}}$ is the applied current such as a square pulse, and $I_{\text{synX}}$ is the simulated synaptic input from presynaptic neuronal population $X$ (E, excitatory; I, inhibitory; T, thalamic). For each ionic current, $g_{\text{ion}}$ is the maximal conductance, $a$ and $b$ are the proportion of channels that are activated and deinactivated, respectively, and $p$ and $q$ are integers, and $E_\text{ion}$ is the reversal potential for the given population of ion channels. Currents in the model RS neuron include fast sodium ($I_{Na}$), fast delayed rectifier potassium current ($I_{K_a}$), an afterhyperpolarization current ($I_{Ih}$), and a passive leak current ($I_{\text{son}}$). Currents in the model FS neuron include fast sodium ($I_{Na}$) and potassium currents ($I_{K_a}$), a slowly inactivating potassium current ($I_{Ih}$), and a passive leak current ($I_{\text{son}}$).

Model RS neurons. In simulated RS neurons, the soma is modeled as a sphere with radius = 0.0031 cm. The leak conductance is $g_{\text{leak}} = 0.057$ mS/cm2 (6.883 nS), with a reversal potential of $E_{\text{leak}} = -67$ mV.

$$I_{\text{leak}}(V) = g_{\text{leak}} (E_{\text{leak}} - V)$$

The fast sodium current $I_{Na}$ is defined as

$$I_{Na}(V, h) = g_{\text{Na}} m^3 h (E_{Na} - V)$$

$$m_{\text{h}}(V) = \frac{1}{1 + \exp \left(\frac{V - \theta_{m}}{\sigma_m}\right)}$$

$$h_{\text{h}}(V) = \frac{1}{1 + \exp \left(\frac{V - \theta_{h}}{\sigma_h}\right)}$$

$$\tau_{\text{h}}(V) = \frac{0.37 + 2.78}{1 + \exp \left(\frac{V - \theta_{h}}{\sigma_{h}}\right)}$$

where $g_{\text{Na}} = 42$ mS/cm2 (5.072 nS), $E_{Na} = 55$ mV, and the kinetic equation parameters are $\theta_m = -20$ mV, $\sigma_m = 9.5$ mV, $\theta_h = -40$ mV, $\sigma_h = -7$ mV, $\theta_{h'} = -40.5$ mV, and $\sigma_{h'} = -6$ mV. $\theta_m$ was shifted 10 mV to the right along the voltage axis to match the observed in vitro spike threshold (Golomb and Amitai 1997).

The delayed rectifier potassium current $I_{K_a}$ is defined as

$$I_{K_a}(V, n) = g_{K_a} n^4 (E_K - V)$$

$$n_{\text{d}}(V) = \frac{n_{\text{h}}(V) - n}{\tau_{\text{h}}(V)}$$

$$n_{\text{h}}(V) = \frac{1}{1 + \exp \left(\frac{V - \theta_{h}}{\sigma_h}\right)}$$

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\[ \tau_d(V) = 0.37 + 1.85 \frac{1}{1 + \exp \left( - \frac{V - \theta_d}{\sigma_d} \right)} \]

where \( g_K = 2.2 \text{ mS/cm}^2 \) (0.266 nS), \( E_K = -90 \text{ mV}, \theta_d = -20 \text{ mV}, \sigma_d = 9.5 \text{ mV}, \theta_n = -40.5 \text{ mV}, \) and \( \sigma_n = -6 \text{ mV} \). \( \theta_n \) is shifted 12 mV to the right along the voltage axis to match the observed in vitro spike threshold.

The afterhyperpolarization current \( I_{AHP} \) is a slow K\(^{+}\) current that is responsible for firing rate adaptation in RS neurons, similar to other slow K\(^{+}\) currents used in other models (Pinto et al. 2003b; Prescott and Sejnowski 2008). The form of the equation that we use is based on Pinto et al. (2003b) and Kopell et al. (2000):

\[
I_{AHP}(V, n) = g_{AHP}(E_K - V)
\]

\[
\frac{dw}{dt} = \frac{w_n(V) - w}{\tau_n(V)}
\]

\[
w_n(V) = \frac{1}{1 + \exp \left( - \frac{V - \theta_n}{\sigma_n} \right)}
\]

\[
\tau_n(V) = 800 \frac{3.3 \exp \left( - \frac{V - \theta_n}{\sigma_n} \right) + \exp \left( - \frac{V - \theta_n}{\sigma_n} \right)}{\exp \left( - \frac{V - \theta_n}{\sigma_n} \right)}
\]

where \( g_{AHP} = 0.08 \text{ mS/cm}^2 \) (0.00966 nS), \( E_K = -90 \text{ mV}, \theta_n = -25 \text{ mV}, \sigma_n = 10 \text{ mV} \), and \( \sigma_w = 20 \text{ mV} \). \( \theta_n \) is shifted 10 mV to the right along the voltage axis to match the observed in vitro spike threshold. The scaling parameter in the numerator of \( \tau_n(V) \) has also been changed to give a peak time constant of 22 ms at -37 mV, matching the observed time constant of in vitro RS neurons (Pesavento et al. 2010).

**Model FS neurons.** In simulated RS neurons, the soma is modeled as a sphere with radius = 0.0019 cm. The leak conductance is \( g_{\text{leak}} = 0.25 \text{ mS/cm}^2 \) (11.3 nS), with a reversal potential of \( E_{\text{leak}} = -67 \text{ mV} \).

\[ I_{\text{leak}}(V) = g_{\text{leak}} (E_{\text{leak}} - V). \]

The fast sodium current \( I_{\text{Na}} \) is defined as

\[
I_{\text{Na}}(V, h) = g_{\text{Na}} m_a^3 h (E_{\text{Na}} - V)
\]

\[
\frac{dh}{dt} = h_a(V) - h
\]

\[
m_a(V) = \frac{1}{1 + \exp \left( - \frac{V - \theta_m}{\sigma_m} \right)}
\]

\[
h_a(V) = \frac{1}{1 + \exp \left( - \frac{V - \theta_n}{\sigma_h} \right)}
\]

\[
\tau_s(V) = 0.5 + 14.0 \frac{1}{1 + \exp \left( - \frac{V - \theta_{\text{hit}}}{\sigma_{\text{hit}}} \right)}
\]

where \( g_{\text{Na}} = 50 \text{ mS/cm}^2 \) (2.2682 nS), \( E_{\text{Na}} = 55 \text{ mV}, \theta_m = -24 \text{ mV}, \sigma_m = 11.5 \text{ mV}, \theta_n = -58.3 \text{ mV}, \sigma_n = -6.7 \text{ mV}, \theta_b = -60 \text{ mV}, \) and \( \sigma_b = -12 \text{ mV} \). Our value of \( \theta_b \) gives simulation results that are in agreement with Erisir et al. (1999) and closely approximate observed in vitro responses of real FS neurons to simulated thalamic input.

The delayed rectifier K\(^{+}\) current \( I_{\text{Kdr}} \) is based on Kv3.1/2 channels found in FS neurons and is responsible for both their narrow action potential width (Beierlein et al. 2003; Chow et al. 1999) and their high firing frequency (Erisir et al. 1999; Lien and Jonas 2003). All parameters are identical to those used in Erisir et al. (1999):

\[
I_{\text{Kdr}}(V, n) = g_{\text{Kdr}} n^2 (E_K - V)
\]

\[
\frac{dn}{dt} = \frac{n_a(V) - n}{\tau_n(V)}
\]

\[
n_a(V) = \frac{1}{1 + \exp \left( - \frac{V - \theta_n}{\sigma_n} \right)}
\]

\[
\tau_n(V) = \left[ \frac{0.087 + \frac{11.4}{1 + \exp \left( \frac{V + 14.6}{8.6} \right)}}{1 + \exp \left( - \frac{V - 1.3}{18.7} \right)} \right]
\]

where \( g_{\text{Kdr}} = 150 \text{ mS/cm}^2 \) (6.8047 nS), \( E_K = -90 \text{ mV}, \theta_n = -12.4 \text{ mV}, \) and \( \sigma_n = 6.8 \text{ mV} \).

The \( I_{\text{D}} \) current is a voltage-dependent K\(^{+}\) current with fast activation and slow inactivation (Coetee et al. 1999; Storm 1988; Toledo-Rodriguez et al. 2004) and is dendrotoxin-sensitive. Slowly inactivating Kv1.1 channels have been found in FS cells (Goldberg et al. 2008) and serve to regulate the firing rate of FS neurons in response to near-threshold depolarizations. This channel is distinct from the Kv1.3 channel used by Erisir et al. (1999), which was based on human T-lymphocytes. Parameters were chosen so that our simulated FS neuron responded to square current pulses similar to real FS neurons recorded in vitro:

\[
I_{\text{D}}(V, a, b) = g_{\text{D}} a^3 b (E_K - V)
\]

\[
\frac{da}{dt} = \frac{a_a(V) - a}{\tau_a}
\]

\[
\frac{db}{dt} = \frac{b_a(V) - b}{\tau_b}
\]

\[a_a(V) = \frac{1}{1 + \exp \left( - \frac{V - \theta_a}{\sigma_a} \right)}\]

\[b_a(V) = \frac{1}{1 + \exp \left( - \frac{V - \theta_b}{\sigma_b} \right)}\]

where \( g_{\text{D}} = 0.15 \text{ mS/cm}^2 \) (6.805 nS), \( E_K = -90 \text{ mV}, \theta_a = -50 \text{ mV}, \sigma_a = 20 \text{ mV}, \tau_a = 2 \text{ ms}, \theta_b = -70 \text{ mV}, \sigma_b = 60 \text{ mV}, \) and \( \tau_b = 150 \text{ ms} \). The maximum conductance \( g_{\text{D}} \) was selected such that the model FS neuron displays classic type-2 dynamics, as we and others have observed in vitro (unpublished data; Golomb et al. 2007).

### Changing Neuronal Leak Conductance

In several experiments, we systematically varied the leak conductance density of the simulated RS and FS neuronal subpopulations (\( g_{\text{leak}1} \) and \( g_{\text{leak}2} \), respectively). Simulated RS neurons had a baseline mean leak conductance density (\( g_{\text{leak}1} \)) of 0.057 mS/cm\(^2\) and was varied from 0.025 to 0.12 mS/cm\(^2\) (3.02–14.49 nS). Simulated FS neurons had a baseline mean leak conductance density (\( g_{\text{leak}2} \)) of 0.25 mS/cm\(^2\) and was varied from 0.05 to 0.45 mS/cm\(^2\) (2.27–20.41 nS). Changing the neuronal leak conductance exhibits an inverse relationship with input resistance and the membrane time constant, as expected from the current-balance equations (Pesavento et al. 2010).
Simulated Inhibitory RS Neurons

A major goal of our study was to explore how differences in the intrinsic properties of RS vs. FS neurons influence network processing. To examine this, we made the intrinsic membrane properties of simulated RS and FS neurons identical. That is, simulated FS neurons were given the same membrane capacitance, radius, conductances, and channel kinetics as simulated RS neurons. The role in the network of the modified FS neurons was left unchanged, e.g., synaptic convergence, kinetics, and maximum synaptic conductances were kept the same as before (Fig. 1A, right). Henceforth, we refer to the modified FS neurons as inhibitory RS (RSi) neurons.

Simulated Synapses

The arrival of a spike onto either real or simulated neurons evokes a synaptic conductance. Simulated synaptic conductances were governed by the following equation:

\[
\alpha(t) = \begin{cases} 
0 & t < 0 \\
\frac{1}{\tau_1} - \frac{1}{\tau_2} & t \geq 0 
\end{cases}
\]

where thalamic (T) and excitatory (E) synapses were based on AMPA synapses, with time constants \(\tau_1 = 0.0935 \text{ ms}\) and \(\tau_2 = 1.4286 \text{ ms}\), which are related to the rise and fall times of a unitary excitatory postsynaptic current (EPSC), respectively (Ermentrout 1998; Hausser and Roth 1997; Kleppe and Robinson 1999). Inhibitory (I) synapses

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**Fig. 1.** Hybrid networks and simulating whisker-evoked volleys of thalamic input in vitro. A: conductance-based computational models of excitatory (E) and inhibitory (I) barrel neurons receive simulated synaptic conductances from thalamic inputs. Synaptic conductances are summed and passed to an in vitro (R) neuron via a conductance clamp. The in vitro neuron receives synaptic conductances from either thalamic inputs alone (left) or thalamic inputs as well as synaptic conductances from the simulated barrel neurons (middle). Synaptic conductances are also applied from the real neuron back to the simulated network. The hybrid network allows easy alteration of membrane parameters across a neuronal subclass, such as giving all inhibitory cells the same membrane properties as regular-spiking (RS) neurons (right). RSi, inhibitory RS neurons.

B: population spike time histograms from in vivo neurons (n = 63) in response to caudal whisker deflections at 3 different velocities (fast, 2,300°/s; medium, 1,666°/s; slow, 800°/s), with 630 responses per histogram. [Adapted from Pinto et al. 2000.]

C: probability density functions (PDF) used to generate thalamic input spike times, quantified by the median time of the skewed distribution. D: spike density histograms with 600 spikes per distribution, showing similar characteristics to in vivo thalamic responses. E: an example of the evoked excitatory postsynaptic potential (EPSP) of an RS neuron in vitro in response to a single simulated synaptic input. F: average synaptic conductance envelopes presented to an in vitro neuron with 15 thalamic input spikes (at threshold \(T\)) from 25 repetitions of fast, medium, and slow thalamic input distributions. G: average total \(G_{\text{syn}}\) and component synaptic conductance envelopes presented to an in vitro neuron with 26 thalamic input spikes (at threshold \(T\)). Strong inhibition (\(g_I\), red) follows the thalamic inputs \(g_{\text{thal}}\) (blue), with weak recurrent excitation \(g_E\) (green) occurring just after the inhibition. H: estimated synaptic reversal potentials \(E_{\text{syn}}\) for thalamic input alone (left) and thalamic + network input (right). With thalamic input alone, only excitatory synapses are activated, giving rise to a constant reversal potential \(E_{\text{AMPA}}\). In the presence of network connections, the timing of inhibitory input sharply hyperpolarizes the reversal potential, decreasing the driving force. F, fast; M, medium; S, slow.
were based on GABA_A synapses, with time constants \( \tau_1 = 0.1930 \) ms and \( \tau_2 = 5.5555 \) ms for a unitary inhibitory postsynaptic current (IPSC) (Destexhe et al. 1996).

Synaptic currents were generated by summing the scaled conductances from all neurons from subpopulation \( X \) and multiplying it by the driving force for the postsynaptic neuron \( i \):

\[
I_{\text{syn}}(t) = \sum_{j} g_{\text{syn}} \sum_{k} E_{\text{syn}} \sum_{l} \alpha(t - t_k)
\]

where \( g_{\text{syn}} \) is the maximum synaptic channel conductance from presynaptic neuron \( j \) of type \( X \) (T, E, or I as defined above) to postsynaptic neuron \( i \), and the driving force is given by the difference of the synaptic reversal potential \( E_{\text{syn}} \) (\( E_{\text{GABA}} = 0 \) mV, \( E_{\text{AMPA}} = 0 \) mV, \( E_{\text{GABA}} = -80 \) mV) and the membrane potential \( V(t) \) of postsynaptic neuron \( i \). The membrane potential of an in vitro neuron was obtained in real time via conductance clamp. The function \( \alpha(t) \) was summed at each time step over each spike time \( t_k \), which is the time of spike number \( k \) from neuron \( j \) onto neuron \( i \).

Values for the maximum synaptic conductance \( g_{\text{syn}} \) were selected to provide a good fit to experimental data examining unitary postsynaptic potentials (PSPs) onto barrel neurons via paired recordings or minimal current stimulation between thalamic, RS, and FS neurons (Beierlein et al. 2003; Bruno and Sakmann 2006). Importantly, these recordings were made at or near the soma after synaptic PSPs had been shaped by dendritic spatial integration and/or active dendritic processing. Thus our simulations partially capture the effects of dendritic processing on unitary responses. The same synaptic dynamics are used in both simulated and hybrid networks. Figure 1E presents an example voltage trace obtained from an RS neuron generated in response to the current evoked by a single simulated input spike.

To introduce heterogeneity across synapses, we varied each maximum synaptic conductance by using a normal distribution with mean \( \mu \) and standard deviation of 0.2 \( \times \) \( \mu \) or 20% of the mean maximum conductance, resulting in PSPs with amplitude variance similar to those observed in vitro (Beierlein et al. 2003). We believe that this is a reasonable first-order approximation for the observed amplitude distribution, which is positively skewed. Because of the transient nature of the thalamic inputs and cortical responses, we are not including synaptic depression or facilitation in our model.

Maximum synaptic conductance values were selected from Gaussian distributions with means \( \pm SD \) as follows (in \( nS \)): thalamic to RS (TE) = 0.0036 \( \pm 0.0007 \), thalamic to FS (TI) = 0.0038 \( \pm 0.0006 \), RS to RS (EE) = 0.0019 \( \pm 0.00038 \), RS to FS (EI) = 0.002 \( \pm 0.0004 \), FS to RS (IE) = 0.0037 \( \pm 0.00074 \), and FS to FS (II) = 0.002 \( \pm 0.0004 \). With the canonical neuronal membrane properties given above, these values result in PSP amplitudes (in \( mV \)) of TE = 2.4, TI = 4.1, EE = 1.1, EI = 2.2, IE = 1.1, and II = 0.94.

It is known that FS neurons in barrel cortex receive stronger thalamic inputs than do RS neurons (Beierlein et al. 2003; Cruikshank et al. 2007). In contrast to our previous study (Pesavento et al. 2010), we scaled thalamic EPSPs onto model FS neurons to an amplitude of 4 mV. The increased EPSP resulted in a thalamic input magnitude threshold (number of spikes) that is 60% of what is observed in RS neurons (9 vs. 16, respectively). For all simulated thalamic inputs in this study, we held the ratio of thalamic input magnitude to inhibitory neurons (TI) to be 60% of the thalamic inputs on excitatory neurons (TE); thus, as we varied TE, TI was 0.6 \( \times \) TE. FS neurons typically have a higher probability of thalamic convergence than RS neurons (Bruno and Simons 2002). However, in this study we are not modeling the nonlinear effects of synaptic integration on dendritic arbors, only the PSPs observed at the soma.

Changes in the leak conductance for a given neuron also result in a change to the size and shape of PSPs arising from synaptic conductances. To isolate the effect of intrinsic properties from those of the network, each change in leak conductance was accompanied by a change in the maximum conductance for all arriving synapses so that PSPs retained their original size and approximate shape.

Simulated Thalamic Input Volleys

We used the dynamic clamp to generate conductance waveforms that simulate thalamic synaptic input volleys evoked by fast, medium, and slow whisker deflections as described previously (see Fig. 2 in Pesavento et al. 2010). We constructed simulated input spike volleys (Fig. 1D) by modeling real thalamic spike time distributions evoked by fast-, medium-, and slow-velocity caudal whisker deflections, as reported previously (Pesavento et al. 2010; Pinto et al. 2000) (Fig. 1B). Simulated spike times were selected using a family of log-logistic functions known as Fisk distributions (Fisk 1961) (Fig. 1C).

For each stimulus presentation, a pool of 200 thalamic spike times was generated, approximating the number of thalamic neurons that project to a single barrel (Bruno and Sakmann 2006; Land et al. 1995; Varga et al. 2002). Thalamic inputs to an individual neuron, simulated or in vitro, were applied as a fixed number of spike times (input magnitude) drawn from the pool of 200 spikes. This allows for the possibility that a given thalamic spike may arrive at more than one cortical neuron via thalamocortical divergence. In a simulation with intracortical connections present, 35 excitatory neurons receiving a mean input of 26 thalamic spikes and 15 inhibitory neurons receiving 16 thalamic spikes, or 1,150 total thalamic input spikes, are required for the simulation. Given that there are 200 spike times available, each thalamic spike will be propagated to about 6 neurons on average, allowing limited synchrony of thalamocortical synapses onto separate neurons.

We quantify thalamic input volleys in terms of timing and magnitude. Input timing is defined as the time required to generate 50% of the total spike count: 2, 5, and 8 ms for fast (F), medium (M), and slow (S) volleys, respectively (Fig. 1C). This can also be understood as the median time for the cumulative distribution function (CDF). Input magnitude is defined as the total number of spikes presented to a single neuron due to a single stimulus. Input magnitude was varied in increments of 3 spikes centered around a threshold value (\( T \)) that evoked a single output spike on 50% of all trials using the medium timing distribution. This is consistent with experimental data suggesting that barrel neurons fire sparsely in response to whisker deflections (Brecht and Sakmann 2002). An increment of 3 spikes roughly corresponds to the 15% change in thalamic input magnitude observed in vivo with whisker deflections of different magnitudes (Pesavento et al. 2010; Pinto et al. 2000). For example, if a RS neuron generates a spike one-half of the time with presentation of 26 spikes, \( T = 26 \) and the input magnitudes used would be 23, 26, 29, 32, and 35 spikes over each of the input timing distributions. The threshold value \( T \) varies with each in vitro neuron (Pesavento et al. 2010); therefore, we estimate this value before running the full data collection. Input threshold is expressed in the normalized units around \( T \) throughout all figures.

We assessed the responses of each neuron to input volleys from the 3 temporal distributions and 5 different magnitudes, for a total of 15 volleys. Each volley was presented 25 times at a rate of 2 Hz with both the timing and magnitude randomized between trials. A new set of specific thalamic spike times was constructed each time a stimulus was presented, as described above.

Simulated Neuronal Network

Network simulations consisted of 35 RS neurons and 15 FS neurons, thereby maintaining a ratio of 70:30 excitatory-to-inhibitory neurons in a network with 50 neurons total. The barrel circuit was modeled as a random sparse network. Connections between subpopulations (RS and FS) were determined probabilistically based on estimates from pairwise recordings (Beierlein et al. 2003; Cruikshank et al. 2007; Gibson et al. 1999; Sun et al. 2006). Convergence from one subpopulation to another was defined as the mean probability that one cell will connect to another. Feedforward inhibition (IE) had a default convergence probability of 0.7 (a given inhibitory neuron had
RESULTS. Synaptic conductances were constructed as described above. Probabilities determined experimentally using paired recordings. Con- 
timing, net damping effect; c.f. Fig. 3) and conform to connection 
values were found to give barrel-like responses (e.g., sensitive to input 
timing, net damping effect: c.f. Fig. 3) and conform to connection 
probabilities determined experimentally using paired recordings. Con- 
vergence values were also varied systematically as described in the 
RESULTS. Synaptic conductances were constructed as described above.

Hybrid Network

Hybrid networks were constructed by using the dynamic clamp to 
mediate real-time interactions between real and simulated barrel 
nurons. In effect, we removed one of the simulated RS neurons from 
a 50-neuron network simulation and inserted a biological RS neuron 
from the in vitro slice recording in its place (Fig. 1A).

Spike times from the real RS neuron were taken to be at the peak 
of recorded action potentials and were passed to the simulated neurons 
to generate simulated synaptic conductances as described below. 
Spikes in simulated RS or FS neurons that were connected to the real 
neuron evoked synaptic conductances scaled to match excitatory 
(EPS) or inhibitory postsynaptic potential (IPSP) amplitudes and 
variability as described below and were applied to the in vitro RS 
nuron using the dynamic clamp with an update rate of 10 kHz.

Individual Neuron Response Measures

For each network condition, we assessed the responses of each 
nuron to input volleys across the 3 timing distributions (F, M, and S) 
each having 5 different magnitudes, for a total of 15 volleys. We 
quantified responses of individual neurons in terms of response 
threshold, latency, and variability. Response threshold was defined as 
the minimum number of input spikes required to evoke an output 
spike within 50 ms of stimulus onset on 50% of the trials. We obtained 
the response threshold by fitting a linear regression over spike gener- 
ation probabilities between 10% and 90% in response to the medium 
stimulus condition (F, M, and S) or thalamic input as well as synaptic input from the 
individual synaptic conductances from thalamic, excitatory, and 
inhibitory synapses from the simulated network: 

\[ g_{E} = g_{EAMPA} + g_{EAMPA} + g_{I} \]

We also consider the effective synaptic reversal 
potential: 

\[ E_{\text{syn}} = (g_{\text{Thal}}E_{\text{AMPA}} + g_{E}E_{\text{AMPA}} + g_{I}) \]

The sensitivity of population responses to input timing and mag- 
nitude was quantified as the absolute values of the two corresponding 
slopes of the regression plane fit to the excitatory activity (spikes/ 
stimulus) over the input timing (F = 2 ms, M = 5 ms, S = 8 ms) and 
input magnitudes T, T+3, and T+6. T−3 is included in figures for a 
subthreshold comparison and is not included in the regression analy- 

All in vivo data are taken from previously published results (Pinto 
et al. 2000).

Randomization of Network Properties

For each stimulus presentation, simulated neurons and networks 
were constructed probabilistically by varying parameters to generate 
responses that conform to experimental data at the level of both the 
barrel network and barrel neurons. Membrane conductance values and 
individual synaptic strengths are drawn from normal distributions 
centered on mean values such that simulated individual neural re- 

Software and Statistics

Our network simulation software was written using C++ and 
compiled into a dynamically shared library that was used in offline 
network simulations in MATLAB as well as the real-time hybrid 
network simulations within the dynamic clamp using LabVIEW. 
Importantly, simulated and hybrid networks as well as the dynamic 
clamp interface used the same library to simulate neurons, networks, 
and conductances. The dynamic clamp was implemented on a custom- 
built personal computer with an Intel Core 2 Duo E6850 that was 
stable overclocked to 3.4 GHz. This allowed us to simulate up to 200 
nurons (up to 5 differential equations each) in real time with a 
10-kHz sample rate. However, the stability of real-time interactions 
decreased with more network connections and/or spiking activity. 
Thus we used networks of 50 neurons so that stability could be 
maintained for all network conditions we examined. Numerical inte-

RESULTS

Hybrid Network Enhances Differences Between Input 
Conductance Timing Distributions

Utilizing the dynamic clamp to apply simulated synaptic 
conductances to layer 4 RS neurons in vitro, we first examine the 
summed conductance in response to thalamic input (Fig. 
1F) or thalamic input as well as synaptic input from the 
simulated barrel network (Fig. 1G). We define the total synap- 
tic conductance onto a single RS neuron as the sum of each 
individual synaptic conductance from thalamic, excitatory, and 
inhibitory synapses from the simulated network: 

\[ G_{\text{syn}} = (g_{\text{Thal}} + g_{E} + g_{I}) \]

Note that both quantities vary over time and depend on activity in the hybrid network.
When the neuron is acting without the hybrid network (Fig. 1F), the total synaptic conductance is due solely to thalamic input. Both the rate of rise and the peak magnitude depend on the timing of the input volley. The cumulative sum of synaptic conductance (i.e., the area under each curve) is consistent across all input timing distributions. Because of the lack of inhibition, the synaptic reversal potential is constant, resulting in a constant depolarizing synaptic drive to the subthreshold membrane potential (Fig. 1H, left).

In the presence of the hybrid network, the total synaptic conductance is shaped by the combination of thalamic input as well as local excitatory and inhibitory synapses. Importantly, neurons in the barrel-like hybrid network require more thalamic input to reach threshold compared with neurons acting without the network. Because the simulation parameters were set so that the cell spiked, the total synaptic conductance is larger, resulting in a bigger difference in both the rate of rise and the peak conductance magnitude between fast and slow inputs (Fig. 1F).

The presence of late inhibition further increases the synaptic conductance, as well as rapidly hyperpolarizing the synaptic reversal potential just after the thalamic conductance reaches its maximum (Fig. 1H, right). Excitatory synapses from recurrent excitation have little effect on the late component of the net conductance waveform but serve to slightly depolarize the late component (>5 ms for fast inputs, >10 for slow inputs) of the synaptic reversal potential. Interestingly, since fast stimuli evoke stronger inhibition (Pesavento et al. 2010), the changes in driving force occur earlier for fast inputs (Fig. 1H). This effectively shortens the duration in which rapid thalamic input can generate an action potential in excitatory neurons, which in turn requires more thalamic input to reliably generate a response. Thus fast inputs will generate responses that occur more reliably and with shorter latency than slow inputs in the presence of network connections.

Individual Neuron Responses to Hybrid Network Input

We examined the responses of 39 RS barrel neurons recorded in vitro in response to simulated thalamic input, with and without the presence of simulated barrel network connections via the hybrid network. Figure 2 shows example traces and measures from an example in vitro RS neuron in response to three timing distributions of thalamic input at the threshold input magnitude $T$. In the presence of the hybrid network, spikes occur earlier and with less variability than with thalamic input alone (Fig. 2A). The same neuron requires more thalamic input to reach threshold $T$ in the presence of network connections versus the absence (24 vs. 15 input spikes, respectively). When embedded in the network, the input threshold is less sensitive to changes of input magnitude and more sensitive to changes of input timing (Fig. 2B). Moreover, both the spike latency (Fig. 2C) and spike variability (Fig. 2D) decrease in the presence of network connections.

We compiled similar data from 39 in vitro RS neurons and 35 simulated RS neurons both while isolated and while embedded in a hybrid network (Table 1). In the presence of network connections, the mean threshold and threshold sensitivity to input timing are significantly higher than with thalamic input alone. The unexpectedly lower threshold sensitivity to timing in the simulated neuron can be explained by the lack of dendrites in the computational model. The sharp rise of sensitivity in the real neuron compared with the simulated neuron is likely due to dendritic shunting, in which a slow current applied at the soma has a greater propensity to be shunted by the dendrites than a fast input would. Our simulated neurons do not have dendrites, so they would not experience this shunting effect. Latency, variability, and the sensitivities of each to input timing and input magnitude are reduced in the presence of network connections. All changes of response measures are similar between in vitro and simulated RS neurons.
Comparison of real and simulated single-neuron response measures

Table 1. Comparison of real and simulated single-neuron response measures

<table>
<thead>
<tr>
<th></th>
<th>In Vitro</th>
<th>Simulated</th>
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<tbody>
<tr>
<td></td>
<td>Net OFF</td>
<td>Net ON</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold, no. of spikes</td>
<td>16.7 ± 0.8</td>
<td>29.0 ± 1.7</td>
</tr>
<tr>
<td>Latency, ms</td>
<td>9.8 ± 0.4</td>
<td>6.8 ± 0.15</td>
</tr>
<tr>
<td>Variability, ms</td>
<td>1.95 ± 0.17</td>
<td>1.11 ± 0.08</td>
</tr>
<tr>
<td>Sensitivity to input timing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold, no. of spikes</td>
<td>0.41 ± 0.02</td>
<td>3.11 ± 0.33</td>
</tr>
<tr>
<td>Latency, ms</td>
<td>1.27 ± 0.10</td>
<td>1.00 ± 0.03</td>
</tr>
<tr>
<td>Variability, ms</td>
<td>0.28 ± 0.04</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>Sensitivity to input magnitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, ms</td>
<td>0.56 ± 0.15</td>
<td>0.02 ± 0.012</td>
</tr>
<tr>
<td>Variability, ms</td>
<td>0.24 ± 0.11</td>
<td>0.03 ± 0.011</td>
</tr>
</tbody>
</table>

Values are means ± SE of single-neuron response threshold, latency, and variability from in vitro (n = 39) and simulated regular-spiking (RS) neurons (n = 35) as a function of network connectivity (net OFF vs. net ON). Within each neuronal type, all measures are significantly different between network OFF and ON (P < 0.01, pairwise t-test).

Taken together, our results suggest that neurons embedded in barrel circuits require more thalamic input to reach threshold but fire earlier and with less variability than neurons acting alone. In all of our measures, with the exception of the previously noted threshold sensitivity to input timing, the effect of network connections on the responses of both real and simulated neurons of the hybrid network was the same (Table 1).

Population Response of Connected vs. Individual Excitatory Barrel Neurons

One of the primary goals of this study was to examine how the presence of local excitatory and inhibitory input alters the population response to transient thalamic input. We combined the responses from the same 39 in vitro RS neurons to the different simulated thalamic inputs in the presence or absence of hybrid network connections. The additional transient synaptic conductances significantly altered the ensemble response to different input conditions.

Population spike density histograms, showing the pooled spike times from 39 RS neurons in vitro, indicate the temporal distributions of responses to simulated thalamic input. Responses for each neuron are aligned to input threshold (T) (Fig. 3, A and B). When acting within the hybrid network (thalamic + network input), in vitro population responses are less temporally distributed but are more sensitive to input timing (compare rows) and less sensitive to input magnitude (compare columns) than when acting individually (thalamic input only). Hybrid network responses are similar to responses observed in vivo (Pinto et al. 2000), specifically in the difference of response magnitude across input timing (Fig. 3C). However, the in vivo responses have a broader temporal distribution than those observed with the in vitro hybrid network responses. Despite the temporal difference, it is likely that the downstream neurons integrate the responses from layer 4 RS neurons, indicating the importance of spike count across the population.

As described in METHODS, we quantify RS neuron population responses as spikes per stimulus for the different simulated synaptic input conditions (Fig. 3, D–F). In the absence of network connections, excitatory RS neurons are sensitive to both input magnitude (the distance between lines for responses across magnitudes) and input timing (the slopes of the lines) (Fig. 3D), consistent with our previous findings for individual neurons (Pesavento et al. 2010). In the presence of network connections, RS excitatory responses are less sensitive to input magnitude but more sensitive to input timing (Fig. 3E).

A comparison of the hybrid network population responses and the in vivo responses shows many similarities, in particular high sensitivity to input timing and low sensitivity to input magnitude. In our data, the in vivo neurons typically generate up to two spikes per stimulus, compared with the in vivo one spike per stimulus. This may be a result of the lack of spatial interactions from applying simulated conductances directly to the soma in vitro, as opposed to the dendritic arbor, as happens naturally in vivo. In particular, the soma would be refractory after the initial spike, whereas charge from the dendritic inputs would continue to facilitate a second spike within the in vivo network. This would also explain the wider temporal profile with the in vivo spike density histogram (Fig. 3C). It is also important to note that some studies have found more sparse spiking responses to whisker deflections in vivo (Brecht and Sakmann 2002; de Kock et al. 2007). Many of those observed differences of in vivo spike count can be attributed to differences in whisker stimulation.

Population sensitivity measures elucidate how changes of input conditions, such as timing and magnitude, affect the excitatory population response. The presence of the hybrid network significantly increases the population sensitivity to different input timing distributions (P < 0.001) (Fig. 3G) and significantly decreases the sensitivity to input magnitude (P < 0.001) (Fig. 3H). Both of these measures are comparable to the sensitivity measures calculated from excitatory neuron responses in vivo. In addition, we also found that neurons embedded in networks require more thalamic input to reach threshold (Fig. 3I). This is consistent with the known damping properties of barrel circuitry (Pinto et al. 2003a). Taken together, these data suggest the sufficiency of network interactions in shaping the local network response to thalamic input.

Effect of Connectivity on Population Response Sensitivity

Next, we examined the effect of synaptic convergence on population response sensitivity. We combined the responses of 10 in vitro RS neurons while varying the convergence probabilities of feedforward inhibition and recurrent excitation in the hybrid network. Our data suggest that the increased sensitivity...
to timing in ensemble responses depends mainly on feedforward inhibition (IE); recurrent excitation with relatively low convergence probabilities has little effect on sensitivity to timing (Fig. 4). However, decreased sensitivity to magnitude depends on the balance of both recurrent excitation and feedforward inhibition. Because of the time required to obtain data from multiple network connectivity patterns while holding a cell in vitro, we restricted the hybrid networks to no local connectivity (thalamic input only) and four combinations of feedforward inhibition and recurrent excitation: normal IE (0.7), weak IE (0.3), normal EE (0.15), and strong EE (0.5).

When IE is decreased from “normal” to “weak,” the population responses are less sensitive to the timing of thalamic inputs and more sensitive to input magnitude (Fig. 4, B and C). Increasing the strength of recurrent excitation in both the normal and weak IE networks shows little effect on the sensitivity to timing but significant effect on the sensitivity to input magnitude (Fig. 4, D and E). We quantified response sensitivity as the slope of the line of best fit through the data shown in D–F. Sensitivity to input timing is significantly higher in the presence of local hybrid network input (t-test, ***P < 0.001), whereas the in vivo sensitivity is similar to the responses we observe with the hybrid network. H: sensitivity to input magnitude, defined as the magnitude slope of the linear plane fit over the data in D–F. Sensitivity to input magnitude is significantly lower with local hybrid network input (t-test, ***P < 0.001). I: average population input threshold in the absence and presence of local network input. The threshold is significantly higher with the hybrid network (t-test, ***P < 0.001). Error bars are ±SE.

**Network Connectivity in Simulated Networks**

Although the hybrid network allows us to examine the effect of different network configurations on the response properties of real barrel neurons, the number of networks we can explore...
is limited by how long we can hold each cell. Therefore, to more thoroughly explore the effect of network connectivity, we examined purely simulated networks over a broad range of values for both feedforward inhibition convergence (IE) and recurrent excitation convergence (EE) values.

Figure 5, A and B, compares the population responses of simulated RS neurons acting alone (thalamic input) vs. synaptically connected (thalamic + network input). As with the in vitro neurons (c.f. Fig. 3, D and E), network connections result in population responses that are more sensitive to thalamic input timing and less sensitive to input magnitude.

To understand the specific effects of different convergence probabilities, we change the convergence probability of one class of connections while holding the others constant within the simulated network. While the recurrent excitation is held constant at barrel-like levels (see METHODS), the sensitivity to thalamic input timing increases as the convergence probability of inhibitory to excitatory synapses (IE) increases (Fig. 5C, black). The simulated RS neuron population shows slightly lower sensitivity than the in vitro population responses (Fig. 5C, red). Increasing EE also results in a decrease of sensitivity to thalamic input magnitude (Fig. 5E). While feedforward inhibition is held constant, increasing the strength of recurrent excitation has little effect on the population sensitivity to input timing (Fig. 5D); again, there is a noticeable decrease in sensitivity to timing in the simulated population response compared with the in vitro population. Increasing recurrent excitation significantly increases the sensitivity to input magnitude (Fig. 5F). We show the sensitivity to input timing and input magnitude over the full range of convergence probabilities (Fig. 5, G and H). The sensitivity to timing is almost bowl shaped, with a high sensitivity for high EE and IE convergence probabilities, with the IE convergence dominating the overall shape of the surface (Fig. 5G). The sensitivity to input magnitude is predictably high for high EE and low IE and falls off as EE decreases or IE increases (Fig. 5H).

**Network Damping**

Previous studies have examined the role of strong intracortical inhibition in rendering a net suppressive or “damping” effect on layer 4 barrel responses to thalamic input (Gabernet et al. 2005; Pinto et al. 2003a; Wilent and Contreras 2004). Moreover, theoretical models have shown that damping circuits are selectively responsive to fast inputs (Pinto et al. 2003a). The methods used in the present study allow a more detailed investigation of the role of network damping on thalamocortical response sensitivity.

We define network gain as the mean excitatory population response in the presence of network connections divided by the mean excitatory population response without network connections. Values <1 indicate that the overall effect of connectivity is inhibitory, or damping, whereas values >1 indicate that the overall effect is excitatory or amplifying.

Consistent with previously published results using reduced models (Pinto et al. 2003a), we find that damping networks exhibit responses that are more sensitive to timing and less sensitive to magnitude than networks that are amplifying. We show the response sensitivities of a representative damping network and amplifying network. In the presence of network connections, the damping network is significantly more sensitive to input timing than the amplifying network (Fig. 6A). The damping network also exhibits a significantly reduced sensitivity to input magnitude, whereas the amplifying network...
the network off. A network sensitivity gain equal to 1 indicates that the presence of network connections has no effect on response sensitivity to input conditions. We examined the effect of network gain on network sensitivity gain by changing the IE and EE convergence probabilities (IE = 0.1 to 1.0; EE = 0.05 to 0.75) and thus systematically varying network gain. Simulated thalamic inputs were presented as before, and we recorded the network sensitivity gain of the subsequent responses. Thus we can compare the functional effect of the network (damping vs. amplifying) against the effect of the network in altering the sensitivity to input conditions.

We found that nearly all damping networks are more sensitive to the timing of transient synaptic inputs, whereas amplifying networks are more sensitive to the number of inputs received (Fig. 6C). Networks with low network gain (damping) exhibit high sensitivity to timing and low sensitivity to magnitude, regardless of the specific network configuration giving rise to the network gain. Conversely, amplifying networks exhibit high sensitivity to input timing and magnitude.

The relationship between response sensitivity and network function (damping vs. amplifying) appears to be robust across a broad range of network convergence values. Observations of

Fig. 5. Simulated barrel network response sensitivity to changes in synaptic convergence. A and B: Population responses of 35 simulated RS neurons in response to simulated thalamic input alone (A) and connected in the local network (B). Population responses are comparable to those observed in real RS neurons (cf. Fig. 3, D and E). C–F: response sensitivity in both simulated (black) and hybrid (red) excitatory populations over different feedforward inhibition (IE) and recurrent excitation (EE) convergence probabilities. EE is fixed at 0.15 for C and E as IE changes; IE is fixed at 0.7 for D and F as EE changes. C: sensitivity to input timing increases as IE convergence increases over a broad range, matching the change of sensitivity observed in the hybrid networks. D: increasing EE convergence has no effect on either simulated or hybrid network response sensitivity to input timing. E: Increasing IE convergence results in decreasing sensitivity to input magnitude, closely matching response sensitivity from the hybrid network. F: Increasing EE convergence increases sensitivity to magnitude weakly over low values and strongly for values >0.5. G: Surface plot of the sensitivity to input timing for different values of IE and EE convergence values. Response sensitivity to input timing primarily depends on feedforward inhibition, so long as recurrent excitation is not too strong. H: Surface plot of the sensitivity to input magnitude over different values of IE and EE. As IE decreases and EE increases, sensitivity to input magnitude increases dramatically. Further analysis of strong EE networks suggest that these are not damping networks (Pinto et al. 2003a) and lead to multiple spikes and epileptiform activity. Error bars are ±SE.

shows a significant increase of sensitivity to input magnitude (Fig. 6B).

To describe the effect of the network on the response sensitivity to input timing or magnitude, we define “network sensitivity gain” as the ratio of sensitivity to input timing or magnitude with the network on divided by the sensitivity with
response sensitivity to both input timing and magnitude can serve as a way to determine the effect of network connections experimentally. For example, high values of sensitivity to both timing and magnitude may indicate an amplifying network, whereas high sensitivity to timing and low sensitivity to magnitude indicates that the network is predominantly inhibitory, or damping. Importantly, these results give a valuable insight for understanding the functional role of network connectivity, described as sensitivity to input timing, input magnitude, or a combination thereof, that is independent of specific synaptic convergence values.

**Neuronal and Network Responses With Inhibitory RS Neurons**

How much of the effect of the network relies on the difference of intrinsic membrane properties between RS and FS neurons? We create a network of 35 simulated RS and 15 simulated FS neurons (RS-FS network) and present it with simulated thalamic inputs in the presence or absence of local synaptic connections. Previous results have found that in the absence of synaptic inputs from the local network, RS and FS neurons exhibit different input thresholds, latencies, and variability, resulting from their having different intrinsic membrane properties (Pesavento et al. 2010). In a different set of simulations, we replace all FS neurons in the model with RS neurons while retaining their inhibitory synapses, yielding inhibitory RS (RSi) neurons, i.e., a RS-RSi network. Thus all neurons in the network have identical intrinsic membrane properties but different network-level effects. Here, we show that differences in the intrinsic properties of FS vs. RS neurons are crucial for enhancing the network’s sensitivity to input timing. This is illustrated with example networks in Fig. 7.

The presence of fast inhibition increases the thalamic input threshold; the increased number of input spikes results in reduced temporal variability across both populations. In a simulated RS-FS network, FS neurons spike ~5 ms before RS neuron responses in both the absence and presence of network connections (Fig. 7A). In the RS-RSi network, inhibitory neurons respond concurrently with the excitatory neurons in both the absence and presence of network connections; this remains consistent for all thalamic input timing distributions (Fig. 7F). RSi neurons exhibit a slight increase of spike probability later in time, suggesting that these late responses are enhanced by disynaptic feedforward excitation from the RS neurons (Fig. 7F, right). Thus the input threshold of excitatory RS neurons is slightly higher with than without network input. Because of the transient nature of the stimuli used, these later interactions have no effect on initial RS neuron responses.

Again, we utilize the total synaptic conductance envelopes to visualize the timing of each class of input received, as well as estimated synaptic reversal potential as before (c.f. Fig. 1, G and H). In the RS-FS network, thalamic synapses ($g_{\text{thal}}$) generate the initial rapid increase of conductance, with inhibition ($g_i$) peaking within 5 ms for fast inputs (Fig. 7B). Strong and rapid inhibition immediately but incompletely hyperpolarizes the effective synaptic reversal potential, $E_{\text{syn}}$ (Fig. 7C). Recurrent excitatory conductances slow membrane hyperpolarization, but more preferentially to fast vs. slow inputs (Fig. 7D, arrow). In the RS-RSi network, thalamic inputs are solely responsible for the initial conductance, with inhibition peaking ~10 ms after stimulus initiation for fast thalamic inputs (Fig. 7G), much later than inhibition in the RS-FS network (cf. Fig. 7B). Compared with the RS-FS network, $E_{\text{syn}}$ of an excitatory neuron in the RS-RSi network remains depolarized for longer and is rapidly hyperpolarized to the GABA reversal potential (~80 mV) for all three thalamic input timings (Fig. 7H).

The inhibitory neurons in the network exhibit different synaptic conductances than the excitatory neurons, with the difference of synaptic convergence readily obvious. Inhibitory FS neurons in our barrel-like network receive feedforward excitatory input (EI) with 0.5 convergence probability and recurrent inhibitory input with 0.2 convergence probability, as described in Methods. Strong thalamic input is enhanced in duration by feedforward excitation (Fig. 7G). It is clear that the excitatory input from RS neurons is highly dependent on the timing of thalamic input, with high amplitude and duration excitatory conductance in response to fast thalamic inputs and very little response to slow inputs. Because FS neurons respond early, the recurrent inhibition occurs before the start of feedforward excitation for all thalamic input timing. The estimated synaptic reversal potential for FS neurons exhibits a initially depolarizing drive, followed by hyperpolarization from recurrent inhibition (Fig. 7E). The third wave of synaptic conductance is from the slow excitatory neurons, keeping the driving force depolarized. The relative amount of time depolarized varies between different thalamic input timing distributions, more so than with RS neurons (c.f., Fig. 7D). From these results, it is apparent that RS and FS neurons receive different inputs in context of the connected network. The second half of this study aims to identify the role of membrane properties in shaping connected population responses to thalamic input.

To examine the effects of identical intrinsic membrane properties in modulating population responses, we utilize the hybrid network and embed in vitro RS neurons into both the RS-FS and RS-RSi networks. Figure 8 presents data from 10 in vitro RS neurons, showing their pooled responses to both network conditions. As observed previously (c.f. Fig. 3), population responses of in vitro neurons in the presence of hybrid network virtual synapses are more sensitive to input timing and less sensitive to input magnitude than with the connections off (Fig. 8, A and B). The calculated values of sensitivity to input timing and input magnitude are shown in Fig. 8, E and F (FS), respectively. In contrast, networks with RSi neurons in place of FS neurons do not show notable change between networks with or without connections (Fig. 8, C and D). The sensitivity to input timing in RSi networks increases slightly when connectivity is added (Fig. 8E, RSi, not significant), but this change is significantly lower than that observed in FS networks (Fig. 8E). RSi networks exhibit a slight decrease in sensitivity to input magnitude (Fig. 8F, RSi). This decrease in sensitivity to input magnitude is not significantly different from that in FS networks, suggesting the importance of the properties of RS neurons in network processing.

It is clear that by removing the differential effects of FS neuron single-cell properties, the processing of the network is significantly altered. However, the changes were not consistent across each measure, suggesting different roles of the membrane properties between RS and FS neurons. Next, we examine how changes in input resistance and membrane time con-
Effect of Neuronal Leak Conductance on Network-Level Processing

RS and FS neurons differ in a number of membrane properties. Our previous work suggested that the difference of input resistance is particularly important to account for differences in their responses to thalamic input (Pesavento et al. 2010). Here, we examine how changes in input resistance in both populations affect network-level processing. Note that in our computational models, changes of input resistance are induced by varying the maximum leak conductance. All other membrane parameters are held constant while maximum synaptic conductances ($g_{syn}$) are scaled to maintain the same PSP amplitude as in the canonical models.

We examine the responses of 11 RS barrel neurons embedded in hybrid barrel networks while systematically varying the leak conductance of both the excitatory RS and inhibitory FS neurons in the simulated network (Fig. 9). Our results demonstrate that the effect of network interactions depends strongly on the leak conductance of inhibitory neurons and only marginally on the leak conductance of excitatory neurons. As discussed previously, network connections under normal conditions enhance response sensitivity to input timing and decrease sensitivity to magnitude (compare Fig. 9, A and B).
Timing, substantially lower than responses in the absence of network connections (Fig. 9E). This combination of membrane conductances negates the influence of the network, decreasing the population sensitivity to input timing. In addition, high values of $g_{\text{leakE}}$ with low $g_{\text{leakI}}$ enhance RS neuron sensitivity to timing (Fig. 9E, far right bar). Sensitivity to magnitude is less affected by the differences of leak conductance between the neuronal subpopulations (Fig. 9F). Decreasing $g_{\text{leakE}}$ slightly increases the sensitivity to input magnitude, regardless of $g_{\text{leakI}}$ (Fig. 9F, light gray bars). Note that these effects occur after the synaptic conductances are adjusted to maintain the same PSP amplitude as used in the null condition; the effects observed are not a direct result of synapse efficacy. These results indicate that the network’s sensitivity to input timing depends in part on the fact the input resistance of inhibitory neurons is lower than that of excitatory neurons.

Fig. 9. Effect of leak conductance on hybrid network population responses. A–D: averaged excitatory population responses from 11 in vitro RS neurons embedded in hybrid networks in the absence (A) or presence of network connectivity (B–D). Leak conductance values are as follows (in mS/cm²): excitatory ($g_{\text{leakE}}$), normal = 0.057, low = 0.025, high = 0.11; inhibitory ($g_{\text{leakI}}$), normal = 0.25, low = 0.1, high = 0.4. B: under barrel-like $g_{\text{leak}}$, for both excitatory and inhibitory populations, the network exhibits sensitivity to input timing (slope of lines) and reduced sensitivity to input magnitude (spacing of lines). C: $g_{\text{leakE}}$ is reduced in all simulated RS neurons, resulting in little apparent change of network’s sensitivity to input timing depends in part on the fact the input resistance of inhibitory neurons is lower than that of excitatory neurons.

Decreasing the leak conductance in the excitatory RS neurons ($g_{\text{leakE}}$) has little effect on the role of the network (compare Fig. 9, B and C). However, if we also increase the leak conductance in the inhibitory neurons ($g_{\text{leakI}}$), the enhancement of response sensitivity due to network connections is lost (compare Fig. 9, C and D).

We compare the sensitivity to timing and sensitivity to magnitude of in vitro RS neurons across a subset of networked population leak conductance conditions and the baseline condition without network connections. The number of conditions examined was limited by how long we could reliably hold each neuron using whole cell patch. Nonetheless, the $g_{\text{leakE}}$ and $g_{\text{leakI}}$ conditions we examined exhibit complex interactions in their effects on population sensitivity to input timing. Whereas decreasing $g_{\text{leakE}}$ alone has little effect on the sensitivity to input timing, the addition of high $g_{\text{leakI}}$ decreases response sensitivity to input timing, substantially lower than responses in the absence of network connections (Fig. 9E). This combination of membrane conductances negates the influence of the network, decreasing the population sensitivity to input timing. In addition, high values of $g_{\text{leakE}}$ with low $g_{\text{leakI}}$ enhance RS neuron sensitivity to timing (Fig. 9E, far right bar). Sensitivity to magnitude is less affected by the differences of leak conductance between the neuronal subpopulations (Fig. 9F). Decreasing $g_{\text{leakE}}$ slightly increases the sensitivity to input magnitude, regardless of $g_{\text{leakI}}$ (Fig. 9F, light gray bars). Note that these effects occur after the synaptic conductances are adjusted to maintain the same PSP amplitude as used in the null condition; the effects observed are not a direct result of synapse efficacy. These results indicate that the network’s sensitivity to input timing depends in part on the fact the input resistance of inhibitory neurons is lower than that of excitatory neurons.

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Network Sensitivity Gain

Although the hybrid network allowed us to examine the effect of different networks on the response properties of real barrel neurons, the number of networks we were able to explore was limited by how long we could hold each cell. Therefore, to examine the effect of neuronal leak conductances more thoroughly, we used simulated networks having a broad range of values for leak conductance in excitatory ($g_{\text{leak}E}$) and inhibitory ($g_{\text{leak}I}$) neurons. Our simulated results indicate that both $g_{\text{leak}E}$ and $g_{\text{leak}I}$ alter the effect of the network on response sensitivity (Fig. 10). Increasing values of $g_{\text{leak}I}$ decrease the effect of the network on sensitivity to input timing; predictably, changing $g_{\text{leak}I}$ has no effect on excitatory population responses in the absence of network connections (Fig. 10A). Increasing values of $g_{\text{leak}E}$ increase the sensitivity to input timing in both the presence and absence of the network (Fig. 10B).

To better quantify how intrinsic properties influence the role of the network, we define a measure of network sensitivity gain as the ratio of the response sensitivity (to timing or magnitude) when the network is present divided by the response sensitivity when the network is absent. A network sensitivity gain of 1 indicates that the presence of the network has no effect on the response sensitivity, a gain <1 indicates that network reduces response sensitivity, and a gain >1 indicates that the network increases response sensitivity. Network sensitivity gain to input timing depends strongly on the leak conductance of inhibitory neurons but only weakly on the leak conductance of excitatory neurons (Fig. 10, C and D). By measuring the regression line and testing whether the slope is significantly different from zero, we found that increasing $g_{\text{leak}I}$ significantly reduces the network sensitivity gain to input timing in simulated RS neurons ($P < 0.0001$) (Fig. 10C). Increasing $g_{\text{leak}E}$ weakly reduces network sensitivity gain ($P < 0.01$) (Fig. 10D). By contrast, the network sensitivity gain to input magnitude is modified by changes of $g_{\text{leak}E}$ but not $g_{\text{leak}I}$. Increasing $g_{\text{leak}I}$ has no significant effect on network sensitivity gain to input magnitude ($P > 0.05$) (Fig. 10E). However, increasing $g_{\text{leak}E}$ serves to significantly decrease the network sensitivity gain to magnitude ($P < 0.0001$) (Fig. 10F). These results suggest separate roles of excitatory and inhibitory subpopulation excitability in shaping the response selectivity to thalamic input, as modulated by leak conductance.

Why is the leak conductance of inhibitory neurons a critical parameter in altering the excitatory network sensitivity gain to input timing? To answer this, we examined model RS and FS neuron single-cell response measures to distinguish changes in threshold, latency, or variability across changes of $g_{\text{leak}I}$ across the simulated barrel network (Fig. 11). Changing $g_{\text{leak}I}$ has no effect on RS neurons in the absence of network connections; therefore, we only show the data for RS neurons in the presence of network connections (Fig. 11, top row). High-leak-conductance FS neurons exhibit an increase in spike threshold in both the absence (net off) and presence (net on) of network connections (Fig. 11D). Across increasing $g_{\text{leak}I}$, surprisingly, we did not observe changes of spike latency (Fig. 11E), but spike variability showed substantial changes with both network off and network on. The FS variability graphs have been separated for visual clarity. With the network off, increasing $g_{\text{leak}I}$ results in a decrease of FS neuron spike variability, particularly in response to slow inputs (Fig. 11G). In contrast, the presence of network connections results in an increase of spike variability as $g_{\text{leak}I}$ increases (Fig. 11F). This may occur as a result of feedback inhibition from early FS neuronal responses increasing the variability of membrane conductance and thus broadening the distribution of spike times without altering the mean latency.

Inhibition from high-leak-conductance FS neurons effectively decreases the spike threshold in RS neurons, decreasing the threshold sensitivity to timing (the distance between the different input timing lines) primarily through a substantial decrease of threshold for slow inputs (Fig. 11A). Notably, this decrease occurs concomitantly with an increase of FS response variability. RS response latency and variability remain unchanged for increasing values of $g_{\text{leak}I}$ (Fig. 11, B and C). It is clear that changing $g_{\text{leak}I}$ on FS neurons exhibits complex actions on single-cell and population responses of RS neurons.

![Fig. 10](http://jn.physiology.org/DownloadedFrom/10.1152/jn.00914.2011-77311/jn_10_77311_01_fig10.jpg)

Fig. 10. Effect of leak conductance on simulated network response sensitivity. A and B: sensitivity to input timing of 35 simulated RS neurons over changes of $g_{\text{leak}I}$ (A) or $g_{\text{leak}E}$ (B) in the absence (dashed lines, open symbols) or presence (solid lines, filled symbols) of network connections. Note that in B, changes of $g_{\text{leak}E}$ have a direct effect on sensitivity to input timing in both the presence and absence of network connections. To isolate the effect of the network, we normalize the measure to that with the network off, giving us the network sensitivity gain (Fig. 10). Increasing values of $g_{\text{leak}I}$ decrease the effect of the network on sensitivity to input timing; predictably, changing $g_{\text{leak}I}$ has no effect on excitatory population responses in the absence of network connections (Fig. 10A). Increasing values of $g_{\text{leak}E}$ increase the sensitivity to input timing in both the presence and absence of the network (Fig. 10B).

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RS and FS neurons were measured across fast (black), medium (red), and slow (blue) input timing distributions for increasing values of selectivity of population responses in the in vivo animal. The interplay between required thalamic input magnitude and the timing of thalamic and local network input in generating a single action potential is complex, overlying synaptic and cellular mechanisms that directly and indirectly modulate response probability. To overcome the effects of feedforward inhibition, more thalamic input is required to generate the same probability of output spike generation. In turn, this enhances the differences of responses to fast, medium, and slow thalamic inputs at the level of single neurons; specifically, the rate of total conductance rise of fast inputs is faster and earlier than that of slow inputs with identical input magnitude (Fig. 1, F and G). Feedforward inhibition additionally sharpens these differences by rapidly hyperpolarizing the synaptic reversal potential and correspondingly decreasing the synaptic driving force (Fig. 1H). Recurrent excitatory input begins just after the inhibitory input, serving to slow the rapid hyperpolarization of synaptic reversal potential. These dynamics rely heavily on the

DISCUSSION

Using a conductance clamp, we embedded in vitro RS neurons within a simulated barrel-like network. This hybrid network gives unique opportunity to control the timing and magnitude of synaptic inputs seen by the neuron’s soma. This allows the experimenter a unique chance to explore how the presence of simulated network input alters the response properties of neuronal subpopulations to different input, neuronal, and network conditions. By creating a biologically plausible heterogeneous network simulation and applying its synaptic output to in vitro neurons, we demonstrate the effectiveness of barrel-like conditions at enhancing selectivity to input timing. We defined the sensitivity to input timing as the change of population response magnitude (spikes per stimulus) as the median of the thalamic input latency increases. We defined the sensitivity to input magnitude as the change of population response magnitude as the number of active thalamic synapses increases. These metrics quantify how the network excitatory output transforms transient input. We then examine the effects of local network connectivity, dependence on the differences between neuronal subclass membrane properties, and how leak conductance can modulate the effects of network connectivity.

Studies that have examined the anatomical and electrophysiological characteristics of barrel neurons (McCormick et al. 1985; Pesavento et al. 2010) as well as their synapses and connectivity (Beierlein et al. 2003; Gibson et al. 1999) allow us to constrain neuronal and network simulations with known biological values and variability, adding to the robustness of our findings. Importantly, the synaptic parameters (e.g., PSP amplitude) were not tuned to give population responses that accurately represent those observed in vivo; nonetheless, our results closely approximate known response measures from anesthetized animals (cf. Fig. 3, G and H; Pinto et al. 2000). By matching observed single-cell responses (Pesavento et al. 2010) and adding network connectivity, we were able to take significant steps forward in understanding the mechanisms that underlie the selectivity of population responses in the in vivo animal.

Barrel-Like Connectivity Enhances Temporal Selectivity

Embedding in vitro RS neurons in the barrel-like hybrid network enhances the response selectivity to fast vs. slow inputs. When responding to transient thalamic input in context of the network, neurons have a higher probability of generating an action potential in response to fast temporally correlated inputs than when the neuron receives only thalamic input (Fig. 2B; Fig. 3, D and E). Strong feedforward inhibition is sufficient to enhance the sensitivity to input timing (Fig. 4F) while simultaneously decreasing the sensitivity to the number input magnitude (Fig. 4G). Governed by synaptic convergence between neuronal subtypes, the balance of excitation and inhibition can modify the processing modality of the cortical circuit. However, different network connectivity patterns give rise to identical functional effects (e.g., damping, sensitive to input timing) independent of the specific synaptic connectivity (Fig. 6C). Thus the synaptic convergence probabilities between neuronal subtypes can determine the temporal selectivity and magnitude selectivity within the thalamocortical transformation.

The interplay between required thalamic input magnitude and the timing of thalamic and local network input in generating a single action potential is complex, overlying synaptic and cellular mechanisms that directly and indirectly modulate response probability. To overcome the effects of feedforward inhibition, more thalamic input is required to generate the same probability of output spike generation. In turn, this enhances the differences of responses to fast, medium, and slow thalamic inputs at the level of single neurons; specifically, the rate of total conductance rise of fast inputs is faster and earlier than that of slow inputs with identical input magnitude (Fig. 1, F and G). Feedforward inhibition additionally sharpens these differences by rapidly hyperpolarizing the synaptic reversal potential and correspondingly decreasing the synaptic driving force (Fig. 1H). Recurrent excitatory input begins just after the inhibitory input, serving to slow the rapid hyperpolarization of synaptic reversal potential. These dynamics rely heavily on the
relative timing of synaptic input from FS, RS, and thalamic neurons. FS neurons respond earlier than RS neurons (Pesavento et al. 2010), and consequently, feedforward inhibition almost always precedes recurrent excitation.

Across all convergence values examined, we find that response sensitivity to input timing depends primarily on feedforward inhibition; recurrent excitation has little effect so long as the network has a net inhibitory effect. As EE increases beyond this threshold, the balance of excitation and inhibition is shifted to a positive feedback regime. The sensitivity of responses to both input magnitude and input timing both increase dramatically and/or the network exhibits unstable epileptiform activity in response to transient thalamic inputs (c.f. Fig. 5H). The sensitivity to input magnitude sharply increases as the strength of recurrent excitation increases and feedforward inhibition decreases, amplifying the strong excitatory thalamic inputs. With no fast inhibition, IE, the network responds easily to very few input spikes with little temporal correlation.

Note that the relative strengths of feedforward inhibition and excitation can shift the processing modality of the network. The connectivity within the local network can make a population of excitatory neurons sensitive to 1) input timing alone, 2) input magnitude alone, 3) both, or 4) neither. The local network accomplishes these processing modalities by altering the convergence probabilities within local excitatory and inhibitory populations.

The hybrid network also bridges the gap in understanding the structure and function of the network. As observed in single neurons, where a broad range of covarying maximum channel conductances give rise to similar functional outputs (Prinz et al. 2004), a broad range of covarying network parameters can also give rise to the same functional effect of the network. Here, we focused on the convergence probabilities of feedforward inhibition and recurrent excitation and found that multiple combinations of these parameters result in similar selectivity to input conditions (Kyriazi and Simons 1993). In particular, damping networks are more sensitive to input timing and less sensitive to input magnitude, regardless of the specific synaptic convergence (Fig. 6C). Other parameters that could be manipulated are total neuron number, ratio of excitatory and inhibitory neurons, and excitatory/inhibitory PSP amplitude. Although these manipulations may alter the fine-scale results, the general principles elucidated here will hold true.

The fast, reliable, and broadly tuned responses of FS neurons (Pesavento et al. 2010) are an essential component of transforming the temporal signature of thalamic input into a spike count (Pinto et al. 2000, 2003a). Confirming earlier results, FS neurons respond earlier than RS neurons (Pesavento et al. 2010), giving rise to strong inhibition occurring 5–10 ms after initiation of thalamic input (Fig. 1G). This requires more excitatory thalamic inputs to be able to generate a response within this narrow window of opportunity. Fast thalamic inputs have more spikes within the early portion of the response, so they are able to withstand the rapid and strong inhibition from FS neurons. Conversely, slow inputs are more temporally distributed, and although feedforward inhibition will still lag behind the majority of excitatory thalamic synapses, a proportionally much greater number of input spikes are required to generate a response of the same probability as with the fast inputs (Fig. 2B). Thus strong temporal correlation of thalamic input will result in a higher probability of response from the excitatory neuronal population. The results shown in this report confirm and extend the results from previous studies (Arabzadeh et al. 2003; Wilent and Contreras 2004) by explicitly showing the contributions of feedforward inhibition, recurrent excitation, and the timing of thalamic input in the shaping of cortical responses.

Neuronal Membrane Properties Modulate Effect of Network Connectivity

The layer 4 barrel network responds preferentially to fast correlated input from thalamus, exhibiting reduced sensitivity to input magnitude (Fig. 3; Pinto et al. 2000). This is in contrast to observations of individual neurons acting outside the context of a connected network (Pesavento et al. 2010). Here, by altering the intrinsic membrane properties of neuronal subpopulations, we have been able to elucidate a crucial role of neuronal properties in shaping the population responses of excitatory barrel neurons to thalamic input.

Few studies have examined the reciprocal effect of how the membrane properties of participating neurons affect the response properties of the network. Our results suggest that network connections serve to modify response properties already inherent in individual neurons. The presence of network connections serves to shape the input received by a single neuron; that neuron will then respond in a manner dictated by its intrinsic membrane properties (Pesavento et al. 2010). By altering the mean leak conductance of a neuronal subpopulation, we alter how it responds to excitatory and inhibitory synaptic input. To capture this idea, we introduced the concept of network sensitivity gain, which elucidates how the intrinsic properties of individual neurons shape the effect of the local network in processing transient stimuli.

The difference of RS and FS intrinsic membrane properties within the barrel network is sufficient to establish the network’s preference for input timing vs. input magnitude. When excitatory and inhibitory neurons have identical membrane properties (RS-RSi network), network connections have little effect on network function. Such networks exhibit sensitivity to input timing and magnitude similar to that in neurons without network connections (Fig. 8, B and C).

A key difference between RS and FS neuron intrinsic membrane properties is input resistance (Pesavento et al. 2010). The hybrid network allowed us to manipulate the leak conductance in simulated neurons, thus directly altering the membrane input resistance and time constant of all neurons within a subpopulation. Simulated FS neurons with high leak conductance were associated with a decrease of sensitivity to input timing of in vitro RS neurons embedded in the hybrid network. Interestingly, increasing the difference of leak conductance between RS and FS values (via low $\delta_{\text{leak}}^E$ and high $\delta_{\text{leak}}^I$) resulted in RS neuron sensitivities to input timing below values observed without network input (Fig. 9E). Confirming this result, simulations showed that the input resistance of inhibitory neurons, as controlled by leak conductance, modulates network sensitivity to input timing within the excitatory neuronal population (Fig. 10C). Thus changing the relative leak conductances between the excitatory and inhibitory populations bestows the ability to substantially shift sensitivity to input timing. In combination with the role of excitatory leak conductance in shaping sensitivity to input magnitude (Fig. 10F), it is apparent that input resistance within a given neuronal subpopulation plays a direct part in modulating the role of the network’s response to transient input.
The leak conductance of the inhibitory FS neuronal subpopulation plays a significant role in shaping the network responses; however, the dynamics underlying this mechanism are subtle. To clarify these interactions, we examined the effect of leak conductance on single-cell response properties. We originally expected that lower leak conductance (higher input resistance, longer membrane time constant) would result in an increase of inhibitory spike latency, yielding responses similar to those observed with the RSI neurons. However, we did not observe a significant change of inhibitory latency (Fig. 11E). Unexpectedly, networks with high inhibitory leak conductance (low input resistance) in the presence of network connections exhibited increased spike time variability (Fig. 11F). This is in contrast to observations where high leak conductance in the absence of network connections resulted in a decrease of spike variability (Fig. 11G).

Inhibitory FS neurons typically respond rapidly, reliably, and with little preference to the timing of thalamic inputs (Pesavento et al. 2010), resulting in narrow spike timing distributions (cf. Fig. 7A), which are then propagated to excitatory RS neurons via feedforward inhibition. It is clear that networks containing inhibitory neurons with high leak conductance have reduced threshold sensitivity to timing, which in turn results in reduced network sensitivity to input timing. This stems from the increased spike time variability in high-leak-conductance FS neurons and the corresponding equalization of input threshold across different input times. With higher temporal variability of synaptic feedforward inhibition, fewer active thalamic synapses are required to generate an action potential. This reduces the difference of RS neuron input thresholds between fast, medium, and slow inputs (Fig. 11A), effectively decreasing the threshold sensitivity to input timing within single neurons (Pesavento et al. 2010). This, in turn, tends to equalize population spike probability across input timing distributions (c.f. Fig. 9D) and decreases the role of the network in facilitating temporal selectivity. Thus increased variability of inhibitory neurons resulting from increased leak conductance will reduce the network selectivity to high temporal correlation present in thalamic inputs (Fig. 10C).

In our simulated RS and FS cortical neurons, the leak conductance parameter directly alters the input resistance and membrane time constant. A multitude of mechanisms can persistently change the input resistance in specific neuronal subpopulations. Balanced excitatory and inhibitory synaptic input increases membrane permeability (Destexhe and Paré 1999; Paré et al. 1998) and can alter the efficacy of excitatory inputs (Mainen and Sejnowski 1995; Prescott and De Koninck 2003). Anesthesia can affect neuronal excitability and input resistance through a multitude of mechanisms (Ishizawa 2007; Kendig et al. 1991; MacIver and Roth 1987). Typically, general anesthetics reduce excitability by opening $K^+$ channels (Ishizawa 2007), thus reducing membrane input resistance. The norepinephrine system, via the locus coeruleus, can modulate neuronal excitability by modifying the input resistance of neuronal subpopulations and thus the transformation of information within a local network (Bergles et al. 1996; Constantinople and Bruno 2011). Many other potential mechanisms may directly alter a neuron’s membrane resistance, including persistent sodium channels, ion channel trafficking, and the effects of other neuromodulators, including acetylcholine, dopamine, and serotonin.

It must be noted that we have not exhaustively examined differences between RS and FS neurons. In this study, we focused on how differences in the leak conductance between RS and FS neurons is important, but RS and FS neurons have many other differences that are likely to also be important. For example, RS and FS neurons have different radii for the soma, spike properties, and firing rate adaptation. Although we are presenting our synaptic conductances as they are observed at the soma, RS and FS neurons are likely to integrate synaptic input differently within their dendritic arbors.

**Utility and Restrictions of the Hybrid Network**

The use of a hybrid network allows a unique comparison of biological neuronal responses to predictions from simulations. However, there are several notable differences between our simulated barrel-like network and an in vivo network. For instance, the spatial distribution of synapses along the dendritic processes can explain some of the differences in our results compared with those observed in vivo. Rather than depolarizing the soma all at once, as in current-clamp stimulation within whole cell recordings, dendritic processing would result in thalamic input arriving at the soma in a form that is more spatially diffuse, allowing time for a multiple spike response within the soma (Williams and Stuart 2003). Because of the nature of the transient input from the thalamus, our network does not include LTS neurons, which largely do not receive direct thalamic input (Gibson et al. Beierlein et al. 2003); short-term synaptic depression or facilitation (Beierlein et al. 2003; Chance et al. 1998; Cruikshank et al. 2007; Sun et al. 2006), NMDA receptor dynamics (Hull et al. 2009), or gap junction coupling between inhibitory interneurons (Gibson et al. 1999; Mancilla et al. 2007). Most of these component responses occur over longer time scales than we are examining here. The presence of gap junctions would serve to facilitate synchrony within the FS population, but would do so in response to ongoing activity and would have minimal contribution to shaping the timing of feedforward inhibition for the responses we observe.

Background synaptic activity is not present in the simulated network; that is, we do not induce spontaneous action potentials and thus activation of synaptic conductances. This is largely not present within the in vitro neurons, as previously discussed in Methods. The network used would most closely approximate that observed with an anesthetized animal. In such a preparation, there is a very low level of background activity within the barrel circuit (Brecht and Sakmann 2002). The presence of balanced background excitatory and inhibitory conductances serves to reduce the input resistance of each neuron (Destexhe and Paré 1999; Destexhe et al. 2001), which will have similar effects to what we have shown in this study.

Other caveats with the use of dynamic clamp to present simulated synaptic input have been discussed elsewhere (Pesavento et al. 2010). For example, we are not simulating short-term synaptic depression or facilitation, and the current applied on the soma may act differently than synaptic current distributed across the dendritic arbor. Despite these caveats, we feel that our method for simulating thalamic and cortical network input provides a realistic probe for comparing the responses of neurons when acting alone versus when they are functioning as part of the barrel circuit.

Although it may not be surprising that neuronal population responses depend on both neuronal and network properties, few studies have examined the reciprocal regulation of re-
response selectivity as we have done. Many studies focus on either single-neuron response properties or network-level interactions. Our results, however, suggest that neither approach is sufficient to fully understand circuit function. Network connections serve to modify response properties already apparent in individual neurons. Moreover, networks are more plastic than individual neurons, enabling the system to adapt its responses to different conditions. On the other hand, the effect of the network also depends on the membrane properties of participating neurons; when all of the neurons are the same, the network effects are significantly reduced. Moreover, when the properties of one subclass are altered within the network, as with leak conductance in FS neurons, such a change can modulate the timing and coherence of inhibition, thus altering the effect of the local network. The properties of neuronal subpopulations thus modulate the role of local network synapses, which in turn shape the output of the individual neurons.

Ultimately, the effect of the network breaks down to how it shapes the input to individual neurons via the timing and strength of excitatory and inhibitory synapses. Neurons, in turn, respond to the total input from both thalamus and the local network in a manner consistent with their own properties. The novel approach used here allows us to explain how the effect of feedforward inhibition depends on the properties of single neurons, namely, that RS neuron response latencies are more sensitive to the timing of input compared with the responses of inhibitory FS neurons (Pesavento et al. 2010). The intrinsic membrane properties of neuronal subpopulations are doing two things: shaping the input they receive from the network via local synaptic convergence properties, and determining how the cell responds to that input via membrane properties. Although the underlying mechanisms are reciprocally intertwined, this constitutes a simple mechanism by which a neuron filters and responds to input, providing multiple degrees of fine control over the processing abilities of cortical networks.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.J.P. and D.J.P. conception and design of research; M.J.P. performed experiments; M.J.P. analyzed data; M.J.P. interpreted results of experiments; M.J.P. and D.J.P. conceived and designed research; M.J.P. performed experiments; M.J.P. interpreted results of experiments.

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