Roles of GABA\textsubscript{A} and GABA\textsubscript{B} receptors in regulating thalamic activity by the zona incerta: a computational study

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Park A, Hoffman K, Keller A. Roles of GABA\textsubscript{A} and GABA\textsubscript{B} receptors in regulating thalamic activity by the zona incerta: a computational study. J Neurophysiol 112: 2580–2596, 2014. First published August 20, 2014; doi:10.1152/jn.00282.2014.—The posterior thalamic nucleus (PO) is a higher order nucleus heavily implicated in the processing of somatosensory information. We have previously shown in rodent models that activity in PO is tightly regulated by inhibitory inputs from a GABAergic nucleus known as the zona incerta (ZI). The level of incertal inhibition varies under both physiological and pathological conditions, leading to concomitant changes in PO activity. These changes are causally linked to variety of phenomena from altered sensory perception to pathological pain. ZI regulation of PO is mediated by GABA\textsubscript{A} and GABA\textsubscript{B} receptors (GABA\textsubscript{AR} and GABA\textsubscript{BR}) that differ in their binding kinetics and their electrophysiological properties, suggesting that each may have distinct roles in incerto-thalamic regulation. We developed a computational model to test this hypothesis. We created a two-cell Hodgkin-Huxley model representing PO and ZI with kinetically realistic GABA\textsubscript{AR}- and GABA\textsubscript{BR}-mediated synapses. We simulated spontaneous and evoked firing in PO and observed how these activities were affected by inhibition mediated by each receptor type. Our model predicts that spontaneous PO activity is preferentially regulated by GABA\textsubscript{BR}-mediated mechanisms, while evoked activity is preferentially regulated by GABA\textsubscript{AR}. Our model also predicts that modulation of ZI firing rate and synaptic GABA concentrations is an effective means to regulate the incerto-thalamic circuit. The coupling of distinct functions to GABA\textsubscript{AR} and GABA\textsubscript{BR} presents an opportunity for the development of therapeutics, as particular aspects of incerto-thalamic development of therapeutics, as particular aspects of incerto-thalamic regulation can be targeted by manipulating the corresponding receptor class. Thus these findings may provide interventions for pathologies of sensory processing.

THE POSTERIOR THALAMIC NUCLEUS (PO) is a higher order somatosensory nucleus (Sherman and Guillery 2009) involved in processing both innocuous and noxious sensory information. Its analogs in primates, including humans, include the thalamic posterior complex (Jones 2007) and the anterior pulvinar (Cusick 2002). PO receives peripheral inputs through dense afferent projections from the spinalthalamic (STT) and spinal trigeminal tracts (Gauriau and Bernard 2004; Lund and Webster 1967; Peschanski et al. 1985) and also receives inputs from several brain regions, including the cortex (Sherman and Guillery 2009), thalamic reticular nucleus (TRN; Crabtree et al. 1996 Pinault et al. 1995; Pinault and Deschenes 1998; Yen et al. 1985), and zona incerta (ZI; Bartho et al. 2007; Power et al. 1999).

While responsive to both innocuous and noxious stimuli, Poggio and Mountcastle (1960) found that PO preferentially responds to noxious inputs (Casey 1966; Perl and Whitchlock 1961; Whitlock and Perl 1961). We have shown that chronic pain after spinal cord injury (SCI-Pain) is causally related to dramatic changes in PO activity: spontaneous firing of PO neurons increase 30-fold, and their responses to peripheral stimuli also increase significantly (Keller and Masri 2014; Masri et al. 2009). These findings strongly implicate PO as a site of maladaptive plasticity in SCI-Pain.

We also demonstrated that both spontaneous and evoked activities in PO are regulated by inhibitory inputs from the GABAergic nucleus ZI (Trageser et al. 2006; Trageser and Keller 2004). Indeed, in animals with SCI-Pain, the increased spontaneous and evoked activity in PO is causally related to the decreased tonic and feedforward inhibition exerted upon PO from ZI (Keller and Masri 2013; Masri et al. 2009).

Here, we explore the mechanisms by which ZI regulates PO activity and test the hypothesis that incerto-thalamic regulation is differentially exerted by GABA\textsubscript{A} and GABA\textsubscript{B} receptors (GABA\textsubscript{AR} and GABA\textsubscript{BR}, respectively). For this we developed a computational model of the incerto-thalamic circuit. The model allows us to explore the interplay among modulation of ZI firing rates, concentration of GABA in the synaptic cleft ([GABA]), and the respective roles of GABA\textsubscript{AR} and GABA\textsubscript{BR}. Our model predicts that GABA\textsubscript{BR}-mediated inhibition is the main mechanism for regulating the spontaneous firing of PO neurons, whereas evoked PO activity is preferentially regulated by GABA\textsubscript{AR}. Furthermore, our model predicts that modulation of ZI firing rate and [GABA] are effective means of regulating PO activity.

METHODS

We used single compartment models of each of the two neuronal types involved in the circuit of interest: PO and ZI neurons. We modified models of the generic thalamocortical neuron and the TRN neuron (Destexhe et al. 1994, 1996a, 1996b, 1998), with each neuron’s behavior defined as an interplay between both its intrinsic and synaptic currents. All intrinsic currents, with the exception of the t-type calcium current in PO, were defined using Hodgkin-Huxley formalisms (Hodgkin and Huxley 1952); the PO t-current was modeled using a constant field (Goldman-Hodgkin-Katz) formalism, as the ratio of intracellular to extracellular calcium concentrations is large (De Schutter and Smolen 1998; Destexhe and Huguenard 2000). All models were simulated using NEURON (Carnevale and Hines 2006).
**Model PO Neuron**

The model PO neuron was based on a model of a generic, single compartment thalamocortical neuron (Destexhe et al. 1998). It modeled the neuron as a single compartment cylinder with a surface area of 24.058 μm² (length = 100 μm and diameter = 76.58 μm) and used Hodgkin-Huxley type kinetics for the intrinsic currents, with the exception of the t-type calcium current, which used a constant field equation. Destexhe et al. (1998) established the membrane area and capacitance so that the simplified single compartment model behavior matched that of passive voltage-clamp recordings. The membrane potential was described by:

\[
C_m \frac{dV_{PO}}{dt} = I_{LPO} - I_h - I_{na,PO} - I_{KPO} - I_{pas,PO} - I_{GABAa} - I_{GABAb} - I_{AMPA}, \tag{1}
\]

where \(V_{PO}\) is the membrane voltage, \(C_m = 0.88 \mu F/cm^2\) is the specific membrane capacitance, \(I_{LPO}\) is the t-type calcium current, \(I_h\) is the hyperpolarization-mediated nonspecific cation current, \(I_{na,PO}\) and \(I_{KPO}\) are sodium and potassium currents underlying action potentials, and \(I_{pas,PO}\) is the leakage current. \(I_{GABAa}\) and \(I_{GABAb}\) are synaptic currents mediated by GABA_aR and GABA_bR, and \(I_{AMPA}\) is the AMPA receptor-mediated current.

The t-type calcium current is a transiently activated, low-threshold, voltage-dependent calcium current with characteristic kinetics (Crunelli et al. 1989). At rest, or at more depolarized potentials, the channel is inactivated, requiring hyperpolarization below the resting membrane potential to deinactivate and to open upon depolarization. The t-current, taken directly from the Destexhe et al. (1998) model, was defined by the equation:

\[
I_{LPO} = \frac{p_c m^2 h^2 F V (C_{ah} - C_{eh}) e^{-ZFV} \frac{RT}{RT} \sqrt{e^{2ZFV}}}{1 - e^{ZFV}},
\]

where \(p_c = 6 \times 10^{-5}\) cm/s is the maximum permeability of calcium ions, \(m\) and \(h\) are the activation and inactivation (gating) parameters, \(Z = 2\) is the valence of calcium ions, \(F\) is Faraday’s constant, \(V\) is the membrane voltage, \(R\) is the ideal gas constant, \(T\) is temperature in Kelvins, and \(C_{ah} = 240\) nM and \(C_{eh} = 2\) mM are intracellular and extracellular calcium concentrations, respectively. The chosen t-channel permeability accurately reflects the peak t-current amplitude although it slightly deviates from the experimental I-V curve (Destexhe et al. 1998). The activation and inactivation kinetics of all voltage-dependent currents were defined in the standard Hodgkin-Huxley style:

\[
m(t) = m_0 - \left[ (m_0 - m_n) (1 - e^{-\tau_m n}) \right],
\]

\[
h(t) = h_0 - \left[ (h_0 - h_n) (1 - e^{-\tau_h n}) \right],
\]

\[
n(t) = n_0 - \left[ (n_0 - n_n) (1 - e^{-\tau_n n}) \right],
\]

where \(m_h/n_h/n_n\) are the initial gating parameter states, \(m_h/n_h/n_n\) are the gating parameter states at infinite time, and \(\tau_m/\tau_h/\tau_n\) are the time constants dictating the rate at which the gating parameters reach their infinite-time states.

The voltage dependence of t-current activation and inactivation constants are described by the equations:

\[
m_{t,PO}(V) = \frac{1}{1 + e^{\frac{-V+56\text{ mV}}{62\text{ mV}}}}, \quad h_{t,PO}(V) = \frac{1}{1 + e^{\frac{-V+80\text{ mV}}{4\text{ mV}}}}, \quad \tau_{m,PO}(V) = 0.204\text{ ms} + \frac{0.333\text{ ms}}{e^{\frac{V+131\text{ mV}}{16.7\text{ mV}}} + e^{\frac{V+15.8\text{ mV}}{18.2\text{ mV}}}}.
\]

\[
\tau_{h,PO}(V) = 9.32\text{ ms} + 0.333\text{ ms} \cdot e^{\frac{V+21\text{ mV}}{10.5\text{ mV}}}, \quad \tau_{n,PO}(V) = 1.0\text{ ms}, \quad \tau_{m,PO}(V) = 0.333\text{ ms} \cdot e^{\frac{V+46.6\text{ mV}}{66.6\text{ mV}}}, \quad \tau_{h,PO}(V) = 9.32\text{ ms} + 0.333\text{ ms} \cdot e^{\frac{V+21\text{ mV}}{10.5\text{ mV}}}, \quad \tau_{n,PO}(V) = 1.0\text{ ms}.
\]

These kinetics were derived from experiments performed at 24°C (Huguenard and McCormick 1992; Huguenard and Prince 1992). For this and all other currents, \(Q_{10}\) values were used to adjust the temporal kinetics to those of the temperature of the simulation; e.g., because our simulations were run at 36°C and the t-current kinetics were experimentally determined at 24°C, the t-current kinetics were adjusted by using their \(Q_{10}\) values to reflect the kinetics the t-channels would express at 36°C. For the PO t-current, \(Q_{10}\) of 2.5 were used for both \(m\) and \(h\).

Although calcium enters through t-channels, we chose not to vary intracellular calcium concentrations in our model PO neuron because the model neuron contains no calcium-dependent processes. The only process that may be affected by calcium influx is the magnitude of the t-current itself, due to changes in calcium reversal potential. To ensure that PO neuron behavior is not significantly altered by our constant calcium concentration approximation, we compared sample simulations with, and without, mechanisms for t-current-dependent changes in calcium concentrations (similar to those implemented in ZI described later) and ascertained that variable calcium concentrations have no effect on PO activity (data not shown). Tscherter et al. (2011) further showed that changes in intracellular calcium concentration due to t-channel-mediated calcium influx have negligible effect on thalamic neuron activity.

The h-current is the hyperpolarization-mediated nonspecific cation current. This current is important for oscillatory behaviors such as delta and theta oscillations and has important interactions with the t-channels; the h-current causes the membrane voltage to return to a more depolarized state after a hyperpolarizing event, allowing deinactivated t-channels to become active (McCormick and Pape 1990a). This current was omitted in the Destexhe et al. (1998) model upon which we based our current model. While our study does not involve intrinsic oscillations, we include this current because of evidence that h-currents may regulate neuronal excitability and responses due to their dampening effects on perturbations and their interactions with t-channels (reviewed in Biel et al. 2009). For this, we modified a previously described biophysical model of the h-current (Destexhe et al. 1996a), where the activity of the h-current to produce intrinsic oscillations is modeled. This model included a calcium dependence. However, it is not known if and how calcium affects h-current kinetics in thalamic neurons, and the calcium dependence of the original model attempted to match the effects of h-currents in sino-atrial cells of the heart, despite lack of evidence that the two h-currents have similar kinetics. Furthermore, the original model (Destexhe et al. 1996a) caused large and long-lasting membrane voltage fluctuations that are not consistent with intracellularly recorded activity (not shown). Thus we chose to ignore the calcium dependence, and defined the h-current equation by

\[
I_h = g_h m(V - E_h),
\]

where \(g_h = 2 \times 10^{-5}\) S/cm², and \(E_h = -43\) mV (Destexhe et al. 1996a). The activation kinetics, determined at 36°C with \(Q_{10} = 3\), were defined by

\[
m_{h,PO}(V) = \frac{1}{1 + e^{\frac{-V+75\text{ mV}}{5.5\text{ mV}}}}, \quad \tau_{m,h}(V) = e^{(-14.59\text{ mV} - 0.086\cdot V)} + e^{(-1.87\text{ mV} - 0.0701\cdot V)}.
\]

The sodium and potassium currents underlying action potentials were modeled using the Traub formalism (Traub and Miles 1991) with parameters taken from Destexhe et al. (1998). The ionic equations were

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Additionally, the model was isoelectronic, rendering it homogenous in unaffected by its surface area, as all intrinsic currents were defined per inputs onto PO and ZI. However, the intrinsic behaviors of ZI were and I

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where \( g_{\text{pas,PO}} = 0.01 \text{ S/cm}^2, E_{\text{pas,PO}} = 50 \text{ mV}, g_{\text{k,PO}} = 0.01 \text{ S/cm}^2, \) and \( E_{\text{k,PO}} = -100 \text{ mV}. \) These activation and inactivation kinetics are written in alpha and beta forms. While these can readily be converted to more commonly used steady-state and tau forms, alpha and beta forms are shown for fidelity to original derivation and how they are explicitly modeled. These kinetics, determined at 36°C with \( Q_{10} = 3 \) for both currents, were

\[
\alpha_{\text{m,na,PO}}(V) = 0.032 \left( \frac{13 \text{ mV} - (V - V_{\text{transab}})}{4 \text{ mV}} \right) e^{\frac{-(V - V_{\text{transab}})}{5 \text{ mV}}} - 1
\]

\[
\beta_{\text{m,na,PO}}(V) = 0.28 \left( \frac{40 \text{ mV} - (V - V_{\text{transab}})}{40 \text{ mV}} \right) e^{\frac{(V - V_{\text{transab}})}{5 \text{ mV}}} - 1
\]

\[
\alpha_{\text{h,na,PO}}(V) = 0.128 e^{\frac{17 \text{ mV} - (V - V_{\text{transab}})}{18 \text{ mV}}}
\]

\[
\beta_{\text{h,na,PO}}(V) = 4 \left[ e^{\frac{-40 \text{ mV} - (V - V_{\text{transab}})}{5 \text{ mV}}} + 1 \right]
\]

\[
\alpha_{\text{nh,k,PO}} = 0.32 \left( \frac{15 \text{ mV} - (V - V_{\text{transab}})}{5 \text{ mV}} \right) e^{\frac{(V - V_{\text{transab}})}{5 \text{ mV}}} - 1
\]

\[
\beta_{\text{nh,k,PO}} = 0.5 \left( \frac{10 \text{ mV} - (V - V_{\text{transab}})}{40 \text{ mV}} \right) e^{\frac{-10 \text{ mV} - (V - V_{\text{transab}})}{40 \text{ mV}}}
\]

where \( V_{\text{transab,PO}} = -52 \text{ mV}. \)

The leak current was defined by

\[ I_{\text{pas,PO}} = g_{\text{pas,PO}}(V - E_{\text{pas,PO}}), \]

where \( g_{\text{pas,PO}} = 3.79 \times 10^{-5} \text{ S/cm}^2 \) and \( E_{\text{pas,PO}} = -65 \text{ mV}. \) The conductance was taken from Destexhe et al. (1998) while the reversal potential was chosen to keep the membrane potential around \(-62 \text{ mV}. \) Although physiologically this resting potential is determined through an interplay of intrinsic currents and tonic levels of small synaptic events, our model does not incorporate such small, tonic synaptic events that contribute to the resting potential. Thus we use the passive leakage reversal potential to maintain the resting potential at a physiologically realistic value.

Model ZI Neuron

Intrinsic properties of ZI neurons are largely unknown; however, ZI is electrophysiologically (cf. Destexhe and Sejnowski 2003; Tragesser et al. 2006), functionally (Bartho et al. 2002; Lavallee et al. 2005; Pinault 2004; Sherman and Guillery 2009), and developmentally (Inamura et al. 2011) very similar to TRN. Thus the model ZI neuron was based on a TRN model (Destexhe et al. 1994). Like the PO model, the ZI model is also represented as a cylindrical, single compartment model. We set the ZI surface area to match that of PO (24 058 \mu m^2) to allow comparisons between the relative strength of inputs onto PO and ZI. However, the intrinsic behaviors of ZI were unaffected by its surface area, as all intrinsic currents were defined per surface area which compensated for any change in surface area. Additionally, the model was isoelectronic, rendering it homogenous in space. The membrane potential was defined by the equation

\[
C_m \frac{dV_{\text{ZI}}}{dt} = -I_{\text{Na, ZI}} - I_{\text{Ca, ZI}} - I_{\text{K, ZI}} - I_{\text{pas, ZI}} - I_{\text{AMPA}},
\]

where \( V_{\text{ZI}} \) is the membrane voltage; \( C_m = 1 \mu F/cm^2; I_{\text{Na, ZI}}; I_{\text{Ca, ZI}}; I_{\text{K, ZI}}; \) and \( I_{\text{pas, ZI}} \) are analogous to those of PO (1); \( I_{\text{AMPA}} \) is the AMPA receptor-mediated current, which is only active during investigations of evoked activity (see Regulation of Evoked Activity).

The t-type calcium current and kinetic equations, determined at 24°C, have the form

\[ I_{\text{t,ZI}} = g_{\text{t,ZI}} m^2 h (V - E_{\text{Ca}}) \]

\[ m_{\text{t,ZI}}(V) = \frac{1}{1 + e^{\frac{V - 48 \text{ mV}}{7.4 \text{ mV}}}} \]

\[ h_{\text{t,ZI}}(V) = \frac{1}{1 + e^{\frac{V - 80 \text{ mV}}{3 \text{ mV}}}} \]

\[ \tau_m = 3 \text{ ms} + e^{\frac{V + 27 \text{ mV}}{10 \text{ mV}}} + e^{\frac{-V - 98 \text{ mV}}{15 \text{ mV}}} \]

\[ \tau_h = 1 \text{ ms} + e^{\frac{V + 48 \text{ mV}}{4 \text{ mV}}} + e^{\frac{-V - 403 \text{ mV}}{50 \text{ mV}}} \]

where \( E_{\text{Ca}} \approx 120 \text{ mV} \) at rest and \( g_{\text{t,ZI}} \) varied from 0.2 to 1.2 S/cm² when quiescent (\( g_{\text{t,ZI}} \) is used to manipulate ZI firing rate; below). \( Q_{10} \) values were 5 and 3 for \( m \) and \( h \), respectively. These current kinetics are taken from the model of TRN neurons (Destexhe et al. 1994) and are distinct from the t-current kinetics used for the model PO neuron. Also, unlike the model PO neuron, the model ZI neuron had a mechanism that controlled intracellular calcium concentrations. This allowed us to include the afterhyperpolarization (AHP) current and calcium-dependent nonspecific cation (CAN) current, both of which have calcium-dependent kinetics. This also had the effect that \( E_{\text{Ca}} \) varied in time as calcium concentrations changed, causing changes to the t-current magnitude, although these changes were minor. The calcium kinetics were defined by the following equations

\[
d\left[ [Ca]_i \right] \over dt = \text{Channel + Pump + Decay}
\]

Channel = \( \frac{10,000 I_{\text{t,ZI}}}{2F \text{ depth}} \)

Pump = \(- k_i [Ca]_i / \tau_m \)

Decay = \( [Ca]_i - [Ca]_i / \tau_{\text{decay}} \)

where \( F \) is Faraday’s constant, depth = 1 \mu m is the depth of the shell of intracellular calcium that is affected by t-channel opening, \( k_i = 10^{-4} \text{ mM/ms} \) and \( k_d = 10^{-6} \text{ mM} \) are the time constants defining how rapidly an active calcium pump removes calcium from the intracellular shell, \( [Ca]_i \) is the intracellular calcium concentration, \( [Ca]_i = 2.4 \times 10^{-4} \text{ mM} \) is the steady-state intracellular calcium concentration, and \( \tau_{\text{decay}} = 10^{10} \text{ ms} \) is the time constant defining how rapidly passive decay of calcium from the shell occurs. \( [Ca]_i \) was a constant equal to 2 mM.

The AHP current is a slow potassium current assumed to be independent of voltage, and whose activation depends on intracellular calcium concentration (Bal and McCormick 1993). This current causes membrane AHP following calcium influx through t-channels (Bal and McCormick 1993). The AHP current was defined by the equations

\[ I_{\text{am},ZI} = g_{\text{am},ZI} m^3 h (V - E_{\text{am},ZI}) \]

\[ \frac{dm}{dt} = \left( m - m_0 \right) / \tau_m \]

\[ m_{\text{am},ZI}([Ca]_i) = \frac{\alpha [Ca]_i^2}{\alpha [Ca]_i^2 + \beta} \]
These parameters were systematically varied (Destexhe et al. 1996a; Wallenstein 1994). This is applied to the ZI model, where $g_{\text{pas},ZI}$ empirically (i.e., through a manual parameter search) to keep the resting membrane potential at $-64$ mV.

The above parameters recreated a cell that was quiescent at rest. While ZI is heterogeneous and contains both intrinsically firing and quiescent neurons, we have shown that incertal neurons that project to the PO are found exclusively in the ventral ZI (Trageser et al. 2006) and that essentially all ventral ZI neurons fire spontaneously, both in vitro and in vivo (Masri et al. 2009; Trageser et al. 2006). Thus the ZI neuron of interest warrants intrinsic firing. To recreate this intrinsic firing, we found that intrinsic firing could be induced by significantly increasing the conductances of depolarizing currents, such as the sodium current. However, the large conductances required for this approach was deemed physiologically unlikely.

\[
\tau_{\text{m,ahp}}([\text{Ca}]) = \frac{1}{\alpha [\text{Ca}]^2 + \beta}
\]

where $g_{\text{ahp}} = 0.01 \text{ S/cm}^2$; $E_{\text{k,zi}} = -95 \text{ mV}$; the rate constants $\alpha$ and $\beta$ equal 48 ms$^{-1}$ and 0.03 ms$^{-1}$, respectively; and $[\text{Ca}]$ is the intracellular calcium concentration.

Similarly, the CAN current, responsible for slow membrane after-depolarization after calcium influx, was also assumed to be voltage independent. The CAN current was defined by the same kinetics used for the AHP current but with slower parameters: $\alpha = 20 \text{ ms}^{-1}$ and $\beta = 0.002 \text{ ms}^{-1}$. The conductance and reversal potentials were also different with $g_{\text{can}} = 2.5 \times 10^{-4} \text{ S/cm}^2$; and $E_{\text{can}} = -20 \text{ mV}$. The kinetics for both of these calcium-dependent currents were determined at $22^\circ \text{C}$, and $Q_{10}$ of 3 are used for both (Bal and McCormick 1993; Destexhe et al. 1994).

The sodium, potassium, and leak currents were defined by the same mechanisms described for PO (2, 3, 4) with different parameters: $g_{\text{na},ZI} = 0.1 \text{ S/cm}^2$; $E_{\text{na},ZI} = 50 \text{ mV}$; $g_{\text{cli},ZI} = 0.01 \text{ S/cm}^2$; $E_{\text{cli},ZI} = -95 \text{ mV}$; $g_{\text{pas},ZI} = 5 \times 10^{-5} \text{ S/cm}^2$ at rest, $E_{\text{pas},ZI} = -70 \text{ mV}$, and $V_{\text{t,mem,hi}} = -55 \text{ mV}$. These parameters were obtained from Destexhe et al. (1994), except for $E_{\text{pas},ZI}$; we determined the parameter value of $E_{\text{pas},ZI}$ empirically (i.e., through a manual parameter search) to keep the resting membrane potential at $-64$ mV.

Synaptic Components

Kinetic models of GABAergic synaptic currents use chemical kinetics representations of the physical neurotransmitter-receptor interaction (Destexhe et al. 1996a). In the current investigation, [GABA] was set at 0 mM at baseline. Then, a brief pulse of GABA release was initiated if an action potential in the presynaptic cell (ZI) caused its membrane potential to exceed a threshold (set to 0 mV). This release event was defined as a square pulse of $[\text{GABA}] = 0.5 \text{ mM lasting 0.3 ms}$, creating postsynaptic potentials according to the following equations:

\[
I_{\text{GABA}_{\alpha}} = g_{\text{GABA}_{\alpha}}[\text{O}]_{\text{GABA}}(V - E_{\text{l}})
\]

\[
I_{\text{GABA}_{\beta}} = g_{\text{GABA}_{\beta}}(g_{\text{GABA}_{\beta}}[\text{G}] + K_{d}(V - E_{\text{l}})),
\]

where $g_{\text{GABA}_{\alpha}} = 0.02 \text{ S}$; $E_{\text{GABA}_{\beta}} = -70 \text{ mV}$; $g_{\text{GABA}_{\beta}} = 0.04 \mu\text{S}$; $K_{d}$ the postsynaptic potassium reversal potential is $-100 \text{ mV}$; $[\text{O}]$ is the channel open probability; $[\text{G}]$ is the G-protein concentration; and $K_{d}$ the dissociation constant for GABAB$_{\beta}$ kinetics, is 100 M. Unlike the intrinsic currents, synaptic conductances were not normalized to the surface area of the neuron. The GABAB$_{\beta}$ current Eq. 5 shows that the current follows a simple Hodgkin-Huxley formalism multiplied by the channel open probability. This channel open probability was determined by

\[
[C] + [\text{GABA}] = \frac{[\text{O}]}{[\text{GABA}]}.
\]

where $[\text{O}]$ and $[\text{C}]$ represent channel open and closed probabilities, respectively, $\alpha = 20 \text{ ms}^{-1} \text{mM}^{-1}$ and $\beta = 0.16 \text{ ms}^{-1}$.

The GABAB$_{\beta}$ current Eq. 6 was a similar formalism multiplied by the binding of four G-protein molecules. Following the metabotropic character of the GABAB$_{\beta}$ and its associated potassium channels, the kinetics describe the activation of G-protein by the GABA bound GABAB$_{\beta}$ and the opening of potassium channels by the binding of activated G-proteins. These were defined by

\[
\frac{d[R]}{dr} = K_{d}[\text{GABA}][1 - [R]] - K_{d}[R]
\]

\[
\frac{d[G]}{dr} = K_{d}[R] - K_{d}[G],
\]

where $[R]$ represents concentration of activated receptor, $K_{d}$ is 0.5 ms$^{-1}$mM$^{-1}$; $K_{d}$ is 0.0012 ms$^{-1}$, $K_{d}$ is 0.0012 ms$^{-1}$, $K_{d}$ is 0.012 ms$^{-1}$, and $K_{d}$ is 0.034 ms$^{-1}$.

AMPA-mediated excitation can be described as having two components: a pulse generator and a synapse. The pulse generator was created using NEURON’S NetStim function, which generates a train of pulses with interspike intervals following a negative exponential distribution (Poisson distribution of events) around a defined mean interspike interval. This pulse generator was then set to trigger excitatory postsynaptic potentials (EPSPs) to its target following the equation:

\[
I_{\text{AMPA}} = g_{\text{AMPA}} e^{-\frac{V - E_{\text{AMPA}}}{\tau_1} - e^{-\frac{V - E_{\text{AMPA}}}{\tau_2}}},
\]

where $g_{\text{AMPA}} = 0.016 \text{ S}$; $\tau_1 = 0.1 \text{ ms}$, $\tau_2 = 7 \text{ ms}$, and $E_{\text{AMPA}} = 0 \text{ mV}$. The reversal potential was taken from Destexhe et al. (1996a), while the time constants and conductance were determined empirically, so as to keep the time course of the EPSP approximately consistent with that of Destexhe et al. (1996a). $g_{\text{AMPA}}$ was chosen to be just suprathreshold for a single action potential initiation in PO. Thalamocortical neurons are unlikely to receive large EPSPs capable of reaching spike threshold and are more likely to fire as a result of...
summation of smaller synaptic events. Modeling such summation requires expanding the single-compartment models used here to include multicompartiment models of both ZI and PO, with multiple input generators, a project beyond the scope of the current one. Therefore, in our single-compartment models, larger amplitude EPSPs were used to model the summation of small EPSPs to trigger action potentials. Two independent instances of the pulse generator were used to simulate spontaneous PO activity and peripherally evoked activity in PO and ZI (external PO driver and STT, respectively). Three instances of the synapse mechanism were used: PO driver→PO, STT→PO, and STT→ZI synapses.

Regulation of Spontaneous PO Activity

To simulate regulation of spontaneous PO activity by ZI, a single PO model neuron was set up to receive excitatory inputs from the external PO driver and GABA_A- and GABA_B-mediated inhibition from the ZI, which was also a single neuron. The external PO driver was set up to provide excitatory inputs at an average rate of 2 Hz; this rate was chosen because prior work has shown PO spontaneous firing was set up to provide excitatory inputs at an average rate of 2 Hz; this rate is significantly lower rates were observed with intact ZI neurons (Lavalle et al. 2005; Masri et al. 2009). When other rates were tested, however, the overall firing rate was chosen because prior work has shown PO spontaneous firing is effective at suppressing PO evoked activity (see Figs. 5 and 6). For all investigations examining the regulation of spontaneous PO activity, ZI firing rates are entirely regular, determined only by the intrinsic firing. These rates are expressed in Hertz.

For each case, we also show how spontaneous ZI firing rate and [GABA] affect PO firing rate by systematically varying these parameters. We used heat maps to illustrate these effects. The [GABA] vs. ZI firing rate heat maps (see Figs. 3C and 4, G and J) were generated by varying [GABA] over the range of 0 to 0.5 mM with a step size of 0.05 mM and varying ZI firing rate from ~0 Hz to 14 Hz (varying \( g_{ZI} \) from 0.2 to 1.2 mS/cm² by 0.05 mS/cm²). Each of these simulations was run for 100 s, and the PO firing rate was calculated to find the value of each pixel.

For all investigations examining the regulation of spontaneous PO activity, ZI firing rates are entirely regular, determined only by the intrinsic firing. These rates are expressed in Hertz.

Regulation of Evoked PO Activity

To simulate peripherally evoked activity, a different external pulse generator was introduced to represent STT inputs. This pulse generator provided AMPA-mediated excitatory currents to both ZI and PO, modeling peripherally evoked nociceptive inputs to both nuclei. To observe only the effects of these evoked inputs, the external PO driver (that simulated spontaneous PO activity) was turned off. Under this condition, we examined the two GABA receptor types independently, and then examined their combined effect. The relative timing of STT→PO and STT→ZI excitation was modeled by introducing a variable time lag between the time when the model STT generated a pulse and when the excitation arrived at the target. Then, we searched and show the relative lag parameter space during which each receptor class is effective at suppressing PO evoked activity (see Figs. 5 and 6).

Consistent with physiological data (Tragesser et al. 2006), the model ZI neuron fires spontaneously at a fixed rate (expressed in Hz). For investigations examining the regulation of evoked activity, ZI firing is determined by interactions between its intrinsic firing rate and the external drive (STT). ZI fires regularly unless transiently perturbed by the STT-mediated drive. ZI responses to STT inputs are determined by the baseline intrinsic firing of ZI (see Fig. 5, D and E).

Simulation Parameters

All simulations were run at 36°C and with step size of 0.1 ms. Because of the pseudorandom nature of the Poisson pulse generator, each simulation in a series forming a single heat map used identical trains of presynaptic pulses from the PO driver and/or STT; i.e., the seed of the random number sequence in the simulation environment was set to the same number for all runs. To ascertain that patterns observed were not artifacts specific to the random sequence, the same set of simulations were run while using a randomly generated list of seeds such that each simulation within a set had a different random number sequence. These results with random seeds are not shown but reveal the same patterns as those without the random seeds (shown in present paper).

All heat maps displaying the results of the spontaneous PO activity share an identical scale for their pixel magnitude (see Figs. 3C and 4, G and J) so that comparisons across different conditions can easily be made. The maps are scaled by the largest value across the plots. Similarly, all heat maps displaying the results of the evoked activity also share an identical scale (see Figs. 5, C and F, and 6, A–C) with the one exception (see Fig. 5B).

RESULTS

PO Neuron

The model PO neuron expressing intrinsic currents described in METHODS did not exhibit intrinsic, spontaneous activity. In the absence of experimental or synaptic perturbations, PO was quiescent at a stable membrane potential. As expected, the resting membrane potential was influenced most by the leak current. The resting membrane potential was maintained, for example, at \(-69, -62, \) and \(-54 \text{ mV} \) when the leak current reversal potential (\( E_{\text{pas,PO}} \)) is set to \(-75, -65, \) and \(-55 \text{ mV} \), respectively. Similarly, changes to the maximum leak conductance (\( g_{\text{pas,PO}} \)) were reflected in the resting membrane potential: decreased conductance caused the membrane potential to be less sensitive to \( E_{\text{pas,PO}} \) and vice versa.

Thalamocortical neurons in our model were set up to exhibit no intrinsic activity, as thalamocortical neurons are quiescent in vitro (Steriade 2001b). Thalamocortical neurons in vivo, however, exhibit spontaneous activity as a result of synaptic inputs, particularly in response to corticothalamic barrages (Contreras et al. 1996; Steriade 2001a). To model this spontaneous activity, an external driver was created to provide excitatory inputs to PO, as outlined in METHODS. Briefly, the external driver is a pulse generator whose interpulse interval is governed by a Poisson distribution. When a pulse occurs, it triggers a synaptic event that passes depolarizing current into PO in an alpha function-like time course (see METHODS). Synaptic weight of the external driver was set at 0.016 μS, a value that is just suprathreshold for action potential generation. This value was chosen to model the effects of summation of small amplitude EPSPs to a suprathreshold level. Figure 1, A and B, shows sample traces of PO activity and the corresponding external driver pulses (timestamps below traces) at different driving firing rates (2 and 10 Hz). The excitatory inputs reliably evoked action potentials in the PO neuron, unless the interpulse interval was too short (examples marked with stars in Fig. 1B). For example, two stimuli needed to be \( \sim 50 \text{ ms} \) or more apart to each trigger an action potential due to the latency to action potential from stimulus arrival (\( \sim 17 \text{ ms} \); this latency decreased with increasing strength of excitation, not shown), duration of action potential (\( \sim 0.5 \text{ ms} \) full width at half-maximum), refractory period, and afterhyperpolarization (\( \sim 30 \text{ ms} \)), although these interactions between stimuli rapidly become complex as more stimuli occur within a short time interval.
D i c t a n c e (ZI. We implemented this intrinsic activity based on findings by firing in the ZI neurons to replicate the biological behavior of neuron displays no intrinsic activity, we implemented intrinsic inputs (Trageser et al. 2006). Therefore, unlike in the original and repetitive intrinsic firing that is independent of synaptic resistances.

While synaptic conductances are not defined per area, they are defined by their surface area throughout the investigation. While synaptic conductances are not relevant in the current amplitude of the injected current (not shown). We do not report expected, the firing rate of the train varied directly with the magnitude of the injected current (not shown). We do not report input resistances because they are not relevant in the current context. Because our intrinsic currents are defined per surface area, input resistance can be manipulated by altering the size of the neuron. However, for consistency, we did not alter the input resistance. The model also recapitulated other well-established characteristics of PO neurons: rebound spike bursts after release from hyperpolarization, and trains of single action potentials in response to depolarization (Jahnsen and Llinàs 1984). In Fig. 1C, the neuron is exhibiting a rebound burst in response to release from a hyperpolarizing current injection (−0.2 nA for 500 ms). A sag following current injection due to an h-current characteristic of thalamic neurons (McCormick and Pape 1990a) was also observed. Figure 1D shows the neuron responding with a train of single action potentials in response to a depolarizing current injection (+0.2 nA for 500 ms). As expected, the firing rate of the train varied directly with the amplitude of the injected current (not shown). We do not report input resistances because they are not relevant in the current context. Because our intrinsic currents are defined per surface area, input resistance can be manipulated by altering the size of the neuron. However, for consistency, we did not alter the input resistance. While synaptic conductances are not defined per area, they are defined by their postsynaptic potentials, which accounts for changes to input resistances.

ZI Neuron

Unlike their counterparts in PO, ZI neurons display regular and repetitive intrinsic firing that is independent of synaptic inputs (Trageser et al. 2006). Therefore, unlike in the original TRN model of Destexhe et al. (1994), in which the TRN neuron displays no intrinsic activity, we implemented intrinsic firing in the ZI neurons to replicate the biological behavior of ZI. We implemented this intrinsic activity based on findings by Hughes et al. (1999, 2002) that changes in passive leak conductance (g_{pas,ZI}) and t-current conductance (g_{t,ZI}) drive intrinsic firing in thalamic cells. Figure 2A demonstrates intrinsic firing resulting from a reduction in g_{pas,ZI}; The neuron is quiescent (left trace) when g_{pas,ZI} = 5 \times 10^{-6} \text{S/cm}^2 while it is intrinsically firing (right trace) when g_{pas,ZI} = 5 \times 10^{-5} \text{S/cm}^2. Figure 2B shows three traces of ZI intrinsically firing at different firing rates (4, 7, and 15 Hz) when g_{t,ZI} was set at different levels (g_{t,ZI} = 0.000342, 0.000590, and 0.00125 S/cm², respectively).

While altering t-current conductance affected intrinsic firing rate, large increases in g_{t,ZI} resulted in nonphysiological firing patterns, characterized by extremely hyperpolarized membrane potentials and irregular spike profiles. The physiological firing patterns (Fig. 2, A and B) were characterized by membrane potentials of −71 mV at their most hyperpolarized points and the regular and stereotypical profile of their spikes. In comparison, the nonphysiological firing pattern (Fig. 2C; g_{t,ZI} = 0.01 \text{S/cm}^2) was characterized by a membrane potential of −93 mV at its most hyperpolarized point and an unusual profile of its spikes, such as the subthreshold depolarization that occurs just before action potential triggering (arrow) and nonsmooth repolarization following the action potential (arrowhead).

Fig. 1. Characteristics of model posterior thalamic nucleus (PO) neuron. A: trace shows PO membrane voltage, and timestamps show pulse generation in the external PO driver. The PO driver is firing at an average of 2 Hz, and PO responds with action potentials with high fidelity. B: PO driver is firing at an average of 10 Hz. Stars show examples of instances when the external pulse fails to trigger an action potential in PO. C: PO displays rebound burst by being released from hyperpolarizing current. Driving current is −0.2 nA for 500 ms. D: PO displays tonic firing under depolarizing current clamp input. Driving current is 0.2 nA for 500 ms.

Fig. 2. Characteristics of model zona incerta (ZI) neuron. A: inducing intrinsic firing in ZI. Left trace: quiescent ZI neuron (g_{pas} = 5e-5 \text{S/cm}^2). Right trace: intrinsically firing ZI neuron induced by partial obstruction of leak current (g_{pas} = 5e-6 \text{S/cm}^2). B: t-current conductance is utilized to control the intrinsic firing rate of ZI. g_{t} = 0.000342, 0.000590, 0.00125 \text{S/cm}^2 to induce 4 Hz (top), 7 Hz (middle), and 15 Hz (bottom) firing rates. C: breakdown of physiological intrinsic firing behavior in ZI. Unrealistic firing characteristics are observed when parameters used to determine ZI intrinsic activity are significantly changed. Inset shows magnified view of a single action potential (dotted box). Arrows and arrowhead point to nonphysiological waveform characteristics. D: ZI activity phase plot. The plot shows zones of quiescence (dark blue; firing rate = 0), physiological firing (firing rates shown as colors), and nonphysiological firing (beyond the last datapoint) as a function of leak and t-current conductances. E: ZI exhibits rebound firing behavior in response to release from hyperpolarizing currents. Injected currents are −0.02, −0.03, and −0.04 nA.
To examine how the leak current and t-current interacted to result in these different types of intrinsic activity (quiescence, physiological firing, and nonphysiological firing), a plot of ZI firing rate (denoted by color) as a function of these two parameters is shown in Fig. 2D. The deep blue region at the bottom of the plot represents ZI is quiescence (firing rate = 0 Hz). From quiescence, the firing rate steadily increases as t-current conductance increases (moving up vertically in the plot) for any value of \( g_{\text{pas,ZI}} \) until plot is discontinued at the top of the color map. This discontinuation marks the transition from physiological to nonphysiological firing. Figure 2D shows that while manipulation of either \( g_{\text{pas}} \) or \( g_{t,ZI} \) alone could modulate firing rate, the firing rate was much more sensitive to changes in \( g_{t,ZI} \) over changes in \( g_{\text{pas,ZI}} \). However, lower firing rates (<10 Hz), which occur physiologically (Masri et al. 2009; Trageser et al. 2006), occurred only at lower values of \( g_{\text{pas,ZI}} \); at higher values, the firing rate transitioned directly from a quiescent state to a high frequency (~20 Hz) state. For these reasons, \( g_{\text{pas,ZI}} = 5 \times 10^{-6} \text{S/cm}^2 \) was used while \( g_{t,ZI} \) was varied within the physiologically firing range (~2.2 × 10^{-4} to ~5.5 × 10^{-3} \text{S/cm}^2) to regulate ZI firing rate for the remainder of this investigation.

An additional characteristic of ZI is rebound spiking behavior, similar to that seen in PO. Figure 2E shows the model neuron exhibiting rebound spikes upon release from hyperpolarizing current injections of ~0.1, ~0.08, or ~0.06 nA. Note, however, that ZI rebounds with single action potentials rather than high-frequency bursts, consistent with physiological data (Trageser et al. 2006).

**Regulation of Spontaneous Activity**

Our model consisted of a single ZI neuron providing both GABA\(_A\)-R- and GABA\(_B\)-R-mediated inhibitory inputs to a single PO neuron that also received excitatory input from the external PO driver. Because previous work showed that PO activity is closely linked with states of arousal and with sensory and pain processing (Masri et al. 2009; Trageser et al. 2006), the main metric of interest was PO firing rate. As illustrated above, PO spontaneous activity was entirely dependent on external excitation in the absence of inhibition. However, when ZI inhibition is active, PO activity is significantly affected by these GABAergic inputs.

**GABA\(_A\)-R-mediated inhibition of spontaneous activity.** To model the inhibitory action of ZI we introduced GABA\(_A\)-R-mediated synaptic mechanisms to act on PO, as described in METHODS. Briefly, a square pulse of GABA (0.5 mM for 0.3 ms) is released in response to an action potential in ZI. The pulse of GABA interacts with and activates the receptors in PO, according to the chemical kinetics defined in METHODS. Following this formalism, in response to an isolated ZI action potential, the GABA\(_A\)-R-mediated synaptic current caused an inhibitory postsynaptic current (IPSC) across the PO membrane, characterized by a time constant of 6.3 ms and a peak of 0.14 nA (Fig. 3A, top trace). Consistent with this short time course, the GABA\(_A\)-R-mediated current showed no temporal summation to a tonic train of ZI action potentials, unless ZI fired at a rate greater than ~20 Hz (not shown). Such fast time course and limited temporal summation of GABA\(_A\)-R-mediated current are consistent with physiological observations (Otis and Mody 1992; Zhang et al. 1997). The model neuron’s input resistance remained fixed throughout the investigation. Thus the time constant was not affected by any changes to input resistance.

Because of the high affinity of GABA\(_A\)-R for GABA, the GABA\(_A\)-R-mediated currents are relatively insensitive to changes in [GABA] in the synaptic cleft (Karim et al. 2013). Indeed, Fig. 3A shows that as [GABA] (0.50 mM by default) is decreased to 0.35 mM (70% of original) and 0.175 mM (35% of original), GABA\(_A\)-R-mediated current peak magnitudes (0.139 nA at default [GABA]) only decreased to 0.122 nA (~88% of original) and 0.081 nA (~58% of original).

Previous studies suggest that the degree of incertal inhibition of spontaneous PO activity is modulated by changes in both ZI firing rate (Masri et al. 2009) and the amount of GABA released (Keller 2011; Keller and Masri 2014). However, the relative insensitivity of GABA\(_A\)-R to changes in [GABA] suggests that GABA\(_A\)-R-mediated inhibition is unlikely to be significantly affected by changes in [GABA]. Likewise, limited temporal summation and the short time course of GABA\(_A\)-R currents suggest that changes in ZI firing rates are unlikely to significantly modulate incertal inhibition. Indeed, our simulation shows that GABA\(_A\)-R-mediated mechanisms are insufficient to significantly inhibit PO activity. In Fig. 3B, the first 10 s of the trace depict PO firing spontaneously at ~2 Hz in the absence of inhibition from ZI. In the next 10 s, we set ZI to fire regularly at 4 Hz, as evidenced by the resulting inhibitory postsynaptic potential (IPSPs) in PO. However, ZI activity did not suppress the spontaneous activity in PO. These traces show that GABA\(_A\)-R-mediated IPSPs are mostly out-of-phase, with in-phase inhibition occurring only by chance. Thus due to the phasic nature of both the excitation and inhibition, GABA\(_A\)-R-mediated mechanisms do not effectively inhibit PO activity.

![Image](http://jn.physiology.org/Downloadedfrom)
when ZI is firing at 4 Hz and releasing 0.5 mM pulses of GABA, which are physiologically relevant parameters (Destexhe et al. 1996a; Destexhe and Sejnowski 1995; Trager et al. 2006).

For a more systematic examination of ZI firing rates and [GABA], PO firing rate was recorded over a series of simulations while varying these two parameters. The results are presented in Fig. 3C in the form of a heat map. Every pixel in the heat map represents the results of a single simulation in which the two parameters being examined were set to a specific value designated by their abscissa and ordinate while all else were held the same. The scale in this figure is made to be consistent with those of Fig. 4, G and J, to allow comparisons across the different conditions. The first noticeable characteristic is that the PO firing rate is constant across the bottom row and down the leftmost column (~2 Hz). These represent cases in which no inhibition is available to PO; across the bottom row, [GABA] is zero, while down the leftmost column, ZI firing rate is zero. As ZI firing rate increases (across the abscissa; iso-[GABA]), PO firing rate decreases gradually. This illustrates that GABA<sub>R</sub>-mediated inhibition is approximately proportional to ZI firing rate. At the same time, minimal differences in PO firing rate across different [GABA] (vertically on the ordinate; iso-ZI firing rate) illustrate that GABA<sub>R</sub>-mediated inhibition is mostly insensitive to changes in [GABA]. Overall, this inhibition is weak, reaching a minimum PO firing rate (maximum inhibition) of ~1 Hz, even at a high ZI firing rate of 14 Hz. Thus our model predicts that GABA<sub>R</sub>-mediated mechanisms may have a limited role in the suppression of spontaneous PO activity.

GABA<sub>R</sub>-mediated inhibition of spontaneous activity. In contrast to GABA<sub>R</sub>-mediated current, GABA<sub>B</sub>-mediated current was not activated in response to single action potentials from ZI (Fig. 4A cf. Fig. 3A; note difference in scales); rather, it required a series or cluster of presynaptic action potentials (Kim et al. 1997). This is due to fundamental kinetic differences between the receptor types: GABA<sub>R</sub> are ionotropic while GABA<sub>B</sub> are metabotropic (Chebib and Johnston 1999). Thus GABA<sub>R</sub> receptors, when activated by GABA, activate G-proteins, several of which are required to then activate ion channels mediating the current. The metabotropic nature of GABA<sub>B</sub> requires prolonged activation of receptors to accumulate sufficient concentrations of activated G-proteins required to activate the ion channels. Furthermore, the G-protein

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**Fig. 4. GABA<sub>R</sub>-mediated incerto-thalamic inhibition of spontaneous PO activity.** A: GABA<sub>R</sub>-mediated current in PO in response to a single ZI action potential. Note the scale; GABA<sub>R</sub> is barely activated by isolated events. B: Concentrations of activated G-protein (in µM) at 3 different ZI firing rates: 0.5 Hz (bottom), 2 Hz (middle), 4 Hz (top). C: GABA<sub>R</sub>-mediated currents at the same ZI firing rates. GABA<sub>R</sub>-mediated currents activate slowly and require significant temporal summation to induce significant levels of inhibition. D: GABA<sub>R</sub>-mediated currents in response to different levels of [GABA] released. The 3 traces represent different [GABA] released: 0.5 mM (top), 0.35 mM (middle), and 0.175 mM (bottom). The GABA<sub>R</sub> current has a long time course and is highly sensitive to changes in [GABA]. E: GABA<sub>R</sub>-mediated current in response to irregular ZI activity (timestamps). The GABA<sub>R</sub>-mediated current is sensitive to the instantaneous firing rate of ZI. F: Effect of GABA<sub>R</sub>-mediated current on PO spontaneous activity. PO is firing at 2 Hz for the first 10 s in the absence of inhibition. The next 10 s are in the presence of GABABR-mediated inhibition driven by 4-Hz ZI activity. PO spontaneous activity is significantly attenuated by the inhibition. G: Spontaneous PO firing rate under GABA<sub>R</sub>-mediated inhibition as a function of ZI firing rate and [GABA]. GABA<sub>R</sub>-mediated inhibition is extremely effective at suppressing PO spontaneous activity under specific [GABA] and ZI firing rate conditions. Note the sharp delineation of the zone of suppression. H: PO displays burst firing when [GABA] and ZI firing rate are very high under GABA<sub>R</sub>-mediated influences. Strong inhibition by GABA<sub>R</sub> causes prolonged hyperpolarization of PO, leading to burst firing. I: Effect of both GABA<sub>R</sub> and GABA<sub>B</sub>-mediated currents on PO spontaneous activity. Inhibition of PO spontaneous activity by both receptor types is qualitatively identical to that solely by GABA<sub>R</sub>. J: PO firing rate under both GABA<sub>R</sub>- and GABA<sub>B</sub>-mediated inhibition as a function of ZI firing rate and [GABA]. The heat map recapitulates all salient features of the heat maps of individual receptor types.
concentrations take time to accumulate and to deactivate. As a result, GABA<sub>B</sub>R-mediated current has a long time course, and a long integration time window. Figure 4B illustrates the concentration of activated G-protein as a function of different ZI firing rates: at 0.5 Hz (bottom trace), there was negligible accumulation of activated G-proteins, while as ZI firing rate increases to 2 Hz (middle trace) and 10 Hz (top trace), accumulation increased significantly. Thus, as expected, the magnitude of the GABA<sub>B</sub>R-mediated current also increased as ZI firing rate increases (Fig. 4C), and when ZI was firing regularly, the current magnitude was entirely dependent on the firing rate of ZI activity.

An IPSC evoked in response to a train of ZI action potentials, firing at 1.25 Hz, is illustrated in Fig. 4D, top trace. This shows a peak amplitude of 1.2 pA and a time constant of 235 ms, demonstrating that GABA<sub>B</sub>R-mediated current is generally smaller but longer lasting compared with GABA<sub>A</sub>R-mediated current. Such characteristics are consistent with physiological studies of GABAergic IPSCs (Davies et al. 1990).

When ZI activity was irregular, i.e., interspike intervals were nonuniform, GABA<sub>B</sub>R-mediated current magnitude became sensitive to the instantaneous ZI firing rate. Figure 4E shows that the current fluctuates significantly over time and that the amplitude at any time is strongly dependent on the instantaneous frequency of ZI activity immediately preceding that time point. This indicates that activation of GABA<sub>B</sub>R-mediated current does not depend on the regularity of ZI firing. This also demonstrates that GABA<sub>B</sub>R-mediated current has supralinear summation, which may contribute to the sensitivity of the magnitude of this current to ZI firing rate. Although this irregular firing effect may be physiologically relevant, it was not pursued further in the current investigation.

Another important characteristic of GABA<sub>B</sub>R-mediated current is its [GABA] dependence. Figure 4D shows that, unlike GABA<sub>A</sub>R-mediated current (cf. Fig. 3B), it rapidly attenuates with decreasing [GABA]: as [GABA] (default, 0.50 mM) decreased to 0.35 and 0.175 mM, the peak magnitudes of GABA<sub>B</sub>R-mediated current (1.22 pA at default [GABA]) decreased to 1.22 pA (26% of original) and 0.0224 pA (2% of original) when ZI is firing at 1.25 Hz. In comparison, GABA<sub>A</sub>R-mediated current was reduced to 88 and 58% of the original amplitude (see above).

To examine whether GABA<sub>B</sub>R-mediated current plays a significant role in regulating spontaneous PO activity, simulations analogous to those performed for GABA<sub>A</sub>R-mediated current were performed. Figure 4F shows 10 s of PO firing at 2 Hz with no inhibition, followed by 10 s with 4 Hz of ZI activity; when GABA<sub>B</sub>R-mediated current was activated by ZI inputs, PO firing was effectively reduced. A systematic examination of how different [GABA] and ZI firing rates affected the GABA<sub>B</sub>R-mediated regulation of PO is depicted in Fig. 4G. The bottom row and the leftmost column are characterized by maximal spontaneous activity (2-Hz firing in absence of any inhibition). However, unlike with GABA<sub>A</sub>R-mediated inhibition, which exhibited a gradual strengthening of inhibition with increasing ZI firing rate, GABA<sub>B</sub>R-mediated inhibition showed no such gradient. Instead, there was a sharply delineated zone of effective suppression (PO firing rates <0.5 Hz) as both ZI firing rate and [GABA] increased. Such sharp boundaries indicate that very small changes in [GABA] or ZI firing rate, past a certain threshold, can cause a dramatic difference in PO activity.

As both ZI firing rate and [GABA] reached their maxima (14 Hz and 0.50 mM), PO firing rate began to increase to values even higher than those in the absence of inhibition. While counterintuitive, this phenomenon was due to significant hyperpolarization (less than −75 mV; Fig. 4H) caused by GABA<sub>B</sub>R current summation. This hyperpolarization caused significant t-channel deactivation, such that the channels were primed for activation by excitatory synaptic inputs (t-channel inactivation constant when ZI firing at 4 Hz = 0.0187; when firing at 14 Hz = 0.372; note that inactivation = 0 reflects all gates being closed while inactivation = 1 reflects all gates being open). These responses caused burst firing, which explain the paradoxical increases in PO firing rate to values higher than those without any inhibition.

Finally, we incorporated both GABA<sub>A</sub>R and GABA<sub>B</sub>R-mediated currents into the circuit and repeated the simulations. Figure 4I shows a trace of PO activity with and without inhibition from ZI. As in Fig. 4F, the thalamic membrane voltage trace shows spontaneous firing for the first 10 s; when incerto-thalamic inhibition activates 10 s later, the inhibition effectively suppresses PO spontaneous activity. These results show that coexpression of GABA<sub>A</sub>R- and GABA<sub>B</sub>R-mediated mechanisms effectively inhibits PO activity. Significantly, PO behavior, in the presence of both GABA receptor types, was qualitatively similar to that when only GABA<sub>B</sub>R-mediated current was present (cf. Fig. 4F), indicating that GABA<sub>B</sub>R-mediated inhibition is the dominant mechanism of action in the regulation of PO spontaneous activity by ZI.

Figure 4J illustrates the systematic investigation of the effects of [GABA] and ZI firing rate with both receptor types present. Compared with the analogous figures with each of the GABA mechanisms independently (Figs. 3C and 4G), Fig. 4J essentially depicts the sum of the most salient features of the two prior figures; the distinct zones of effective suppression and of paradoxically increased firing rate from GABA<sub>B</sub>R-mediated inhibition are present, as well as the minor, ZI firing rate-dependent suppression from GABA<sub>A</sub>R-mediated inhibition. Thus the combination of individual GABAergic inhibitions fully explains the overall behavior observed in the simulations, with both receptor types present. These results suggest that GABA<sub>B</sub>R-mediated inhibition likely predominates the incertal inhibition of PO spontaneous activity while GABA<sub>A</sub>R-mediated inhibition has negligible or minor contributions only when ZI firing rates are significantly high.

**Feed-Forward Inhibition**

Thus far we explored the regulation of spontaneous PO activity, and our model predicted that such regulation of spontaneous activity is mediated by GABA<sub>B</sub>R. However, PO and ZI receive peripheral inputs, including nociceptive inputs relayed through the STT (Craig 2004; Jones 2007; Shammah-Lagnado et al. 1985). To explore the role of the incerto-thalamic pathway in regulating this peripherally evoked activity, we included an additional external driver, the STT, to model the peripheral inputs to both ZI and PO. The STT used the same mechanism as the PO driver; it generates pulses according to a Poisson distribution that act as presynaptic triggers for evoking AMPA receptor-mediated EPSP at both.
PO and ZI. The physiological responses of PO and ZI to this feed-forward input likely depend on several factors, including the relative strengths of STT inputs to PO and ZI and the relative timing of arrival of these inputs. Because these parameters are largely unknown, we use a range of values to explore the parameter space.

GABA<sub>R</sub>-mediated feed-forward inhibition. We first examined feed-forward inhibition mediated by GABA<sub>R</sub> alone. STT input strength was set to evoke EPSPs large enough to elicit action potentials in both PO and ZI and in the absence of ZI→PO inhibition (0.016 μS). In the presence of ZI→PO inhibition, STT evoked an EPSP in the ZI neuron, causing a transient feed-forward IPSP in PO. When STT inputs simultaneously engaged both ZI and PO, the feed-forward hyperpolarization suppressed the action potential in PO (Fig. 5A, orange traces). However, the effectiveness of this feed-forward inhibition depended on the relative timing of arrival of these EPSPs and IPSPs. To examine this temporal dependence, we introduced a variable temporal delay between the EPSPs generated in ZI and in PO in response to STT input, while keeping the strength of the inputs constant.

Figure 5A shows a series of traces representing the behavior of the PO neuron in response to the STT→PO EPSP arriving at different times relative to the STT→ZI EPSP. The first trace in the series shows a simulation when STT excites PO 10 ms before it excites ZI. It is shown in blue to represent the evocation of an action potential in PO. Each subsequent trace shows simulations in which STT excites PO 2 ms later than in the previous simulation, while keeping the time of ZI excitation the same; thus the second trace shows excitation of PO 8 ms before that of ZI. In the third trace (PO excited 6 ms before ZI), the excitation no longer evoked an action potential due to the feed-forward inhibition. The simulations in which the STT failed to evoke an action potential are represented by orange traces. This effective feed-forward inhibition continued until the second to last trace (PO excited 32 ms after ZI) when the excitation once again evoked an action potential in PO. Thus if STT→PO excitation occurred anywhere between 6 ms before and 30 ms after the STT→ZI excitation, feed-forward inhibition suppressed STT-evoked action potentials in PO. However, if STT→PO excitation occurred outside this time window, feed-forward inhibition was ineffective.

These findings suggest that GABA<sub>R</sub>-mediated feed-forward inhibition effectively suppresses PO EPSPs that arrive within a ~40-ms window. Physiologically, the delays in EPSP generation between PO and ZI are likely to be on the order of several milliseconds; indeed, Lavalle et al. (2005) have shown that, in PO, peripheral stimulation evokes direct EPSPs and incerto-thalamic feed-forward IPSPs that tend to occur within 15 ms of each other. We have simulated latency differences on the order of several tens of milliseconds and shown the time window during which inhibition is effective. This time window encompasses the physiologically likely differences in delay; thus GABA<sub>R</sub>-mediated feed-forward inhibition is likely effective at suppressing STT inputs to PO.

Next, we explored how changing the strength of STT→PO excitation affects this feed-forward mechanism. We generated a heat map that depicts how PO firing rate changes by varying the relative strengths of STT→ZI and GABA<sub>R</sub>-mediated ZI→PO synapses, respectively. The abscissa represents relative timing of the EPSPs; t = 0 represents coincident arrival, positive values represent conditions in which STT inputs arrive first in PO, and negative values represent conditions in which ZI is activated first. These relative latencies ranged from +75 ms (EPSP arrives at PO 75 ms earlier than an EPSP arrives at ZI) to −75 ms in 5 ms steps. The strength of STT→PO was varied from 0.016 to 0.025 μS on the ordinate in step sizes of 0.001 μS. The STT generated pulses at an average of 2 Hz following a Poisson distribution.

This analysis revealed that there is a sharply delineated time window in which feed-forward inhibition from ZI effectively suppressed PO firing. The window was widest when STT→PO strength was weak (0.016 μS; Fig. 5B, bottom row), a condition depicted also in Fig. 5A. This window narrowed as the strength of STT→PO input increased. Beyond a threshold of 0.024 μS, GABA<sub>R</sub>-mediated feed-forward inhibition could not suppress STT→PO excitation. These findings suggest that GABA<sub>R</sub>-mediated inhibition can effectively suppress PO responses to STT inputs, provided that STT activates PO and ZI within a relatively narrow time window, and that STT→PO excitation is not strong enough to overcome the feed-forward inhibition.

In the analysis above, [GABA] and ZI intrinsic firing rates were kept constant. Recall, however, that these two parameters modulate the incerto-thalamic circuit (Keller 2011; Keller and Masri 2014; Masri et al. 2009; see also Figs. 3C and 4, G and J). We, therefore, explored how changes in [GABA] and ZI firing rate affect this feed-forward inhibition. We did this by repeating the simulation and analyses as in Fig. 5B while varying [GABA] and ZI intrinsic firing rate. [GABA] was varied from 0.1 to 0.5 mM at 0.1 mM steps, and ZI firing rate was varied from 0 to 15 Hz at 3-Hz steps. Doing this resulted in plots (Figs. 5, C and F, and 6, A–C) made of a series of subplots, each of which is analogous to Fig. 5B.

In Fig. 5C, examining the subplots vertically across the ordinate suggests that GABA<sub>R</sub>-mediated feed-forward inhibition is effective even as [GABA] is significantly decreased; the window of suppression was only slightly narrower over a significant drop in [GABA]. For example, when ZI firing rate = 0 Hz (leftmost column of subplots) and STT→PO strength = 0.016 μS (bottom row of each subplot), the time window of effective suppression of PO firing was not affected significantly by large changes in [GABA] (0.1 to 0.5 mM). However, the peak of the suppressed zone decreased as [GABA] decreased.

Examining the subplots horizontally across the abscissa showed that as ZI firing frequency increased, the zone of suppression became less well delineated, representing increased variability in the latency between the STT→ZI EPSP arrival and the ZI action potential. This was likely due to temporal interactions between the intrinsically evoked spikes in ZI and STT-evoked EPSPs in these neurons, resulting in increased variability in the latencies of evoked spikes in ZI. Figure 5D, top trace, illustrates this phenomenon, showing ZI intrinsically firing at 3 Hz and excited by STT at times indicated (T1, T2). The STT input at T1 arrives soon after an intrinsically driven action potential, when the membrane voltage is relatively hyperpolarized (~75 mV), increasing the latency of the evoked spike (12.6 ms from the time of excita-
tion arrival to the peak of action potential). In contrast, STT input at T2 arrives long after the last intrinsically driven action potential, when the membrane potential is more depolarized (approximately $-59 \text{ mV}$), resulting in a shorter latency spike ($4.8 \text{ ms}$ to peak). Additionally, the intrinsically driven ZI action potential may occur just before the arrival of the STT input, effectively inhibiting the STT→PO excitation even when the feed-forward inhibition is not time locked. Thus increased ZI background firing reduced the delineation of the zone of suppression and enhanced the inhibition outside the time-locked region.

Increased ZI intrinsic firing rate resulted also in the appearance and drift of a second zone of PO suppression (Fig. 5C), reflecting the generation of multiple spikes in response to STT inputs. Increased ZI intrinsic firing rates were associated with enhanced activation of voltage dependent conductances, including those mediated by t-channels (see above). As a result, STT-evoked EPSPs activated a larger magnitude of t-current, enhancing the depolarization in the postsynaptic cell. This is also illustrated in Fig. 5D, which shows that as ZI intrinsic firing increases from 3 to 6 Hz (cf. first and second traces), STT inputs (at T1 and T2) result in two ZI evoked spikes. As ZI intrinsic firing further increases to 12 Hz, spike doublets occur at shorter interspike intervals (peak-to-peak interspike interval of the doublet at T1 is $31.0 \text{ ms}$ when intrinsic firing rate is 6 Hz while the interval decreases to $18.4 \text{ ms}$ when firing rate is 12 Hz).

The strength of STT→ZI input also significantly affected the dynamics of ZI inhibition of PO. This was evaluated by increasing the strength of the STT→ZI input from 0.016 to 0.032 uS (Fig. 5E). Increasing the input strength resulted in an increase in the number of action potentials evoked by a single STT event at all ZI firing rates; an STT input event evoked two action potentials when ZI firing rate was 3 Hz and evoked three action potentials when the firing rate was 6 or 12 Hz. As seen

Fig. 5. GABA$_A$R-mediated incerto-thalamic inhibition of evoked PO activity. A: effect of latency differences of spinothalamic (STT) inputs onto PO and ZI. Each trace represents a separate simulation in which the relative timing of STT excitation onto PO vs. ZI is varied. The red dotted line marks the time at which STT excites ZI: time of PO excitation varies. The first trace represents STT exciting PO 10 ms before it excites ZI. Every subsequent trace represents STT exciting PO 2 ms later than the previous trace. Feed-forward inhibition is noticeable after the excitation of ZI. In blue traces, PO excitation is temporally distant enough from excitation of ZI (and thus feedforward inhibition) that STT successfully triggers PO action potentials. In orange traces, feedforward inhibition prevents the PO excitatory postsynaptic potentials (EPSPs) from reaching threshold. B: effect of STT→PO excitation strength and relative timing of PO and ZI excitation on GABA$_A$R-mediated feedforward inhibition. Color of each pixel represents the averaged PO firing rate over a 100-s simulation. STT→PO excitation strength (ordinate) and relative excitation times of PO and ZI (abscissa) are varied. Inhibition is most effective when the PO and ZI excitations are time locked. C: GABA$_A$R-mediated feed-forward inhibition as a function of ZI firing rate and [GABA]. Color of each pixel represents the averaged PO firing rate. The pixels across a subplot reflect different simulations in which STT→PO strength and relative excitation times of PO and ZI are varied (analogous to Fig. 5B). The subplots reflect different sets of simulations in which [GABA] and ZI intrinsic firing rates are varied. Strength of inhibition increases with increases in [GABA] and ZI firing rate. D: traces show intrinsic and STT evoked activity of a ZI neuron. STT inputs arrive at T1 and T2. Comparing T1 and T2 illustrates the variability in the time-to-peak. This variability arises from the fluctuations of membrane voltage underlying the intrinsic activity. Comparing the responses across traces illustrates the increasing number of action potentials evoked by the same stimulus due to the increased t-current conductance in higher intrinsic firing rate ZI neurons. STT→ZI excitation strength = 0.016 uS. E: STT→ZI excitation strength = 0.032 uS. Compared with those traces in D, these traces respond with larger numbers of action potentials and with shorter latencies due to the increased strength of STT→ZI excitation strength. F: same setup as C, except with increased STT→ZI excitation strength from 0.016 uS (C) to 0.032 uS (F). Stronger excitation of ZI results in enhanced inhibition of PO.
suppression was mediated by an increased number of action potentials, as discussed above; stronger EPSPs were able to induce more than one action potentials in ZI. Thus even when there was no intrinsic ZI firing (Fig. 5F, leftmost column), the EPSP was able to induce two action potentials in very close succession, creating a large zone of suppression that is temporally bimodal. As intrinsic firing rate increased, the two modes moved closer together, representing the decrease in interspike interval, and a third peak appeared and also drifted toward time zero.

Thus our model predicts that GABABR-mediated inhibition is capable of producing high-fidelity feed-forward inhibition as long as the evoked excitation and inhibition are closely time locked. However, we predict that GABABR-mediated inhibition is incapable of suppressing nontime-locked inputs.

GABABR-mediated feed forward inhibition. We demonstrated above that GABABR-mediated inhibition likely plays a key role in the regulation of spontaneous PO firing. Here, we examine the role of GABABR in regulating STT-evoked responses in PO by including only GABABR-mediated feed-forward inhibition in the model and omitting the GABAAR. GABABR-mediated feed-forward inhibition was significantly less effective, compared with GABAAR inhibition (cf., Figs. 6A and 5C). GABABR-mediated feed-forward inhibition lacked a distinct time-locked component: PO firing rates varied little across delays between STT inputs to ZI and PO (abscissa of each subplot in Fig. 6A). Furthermore, inhibition of PO firing occurred only at relatively high [GABA] and weak STT→PO inputs. Inhibition was more effective when STT input arrived at ZI before PO (negative values on abscissa of subplots); this was expected because of the long time course of GABABR-mediated inhibition; as shown in Fig. 4D, GABABR-mediated current requires several tens of milliseconds to reach its maximum magnitude. Thus when the STT input arrives at ZI before PO, GABABR-mediated current is given the time to build up to a stronger amplitude. However, this effect was relatively minor compared with the strong and well time-locked GABAAR-mediated inhibition (Fig. 5, C and F).

The effects of GABABR-mediated inhibition depended on the intrinsic firing rate of ZI. In Fig. 5, C and F, leftmost column, with no intrinsic ZI firing, there was minimal feed-forward inhibition because the sporadic STT evoked responses could not elicit sufficient temporal summation to exert a noticeable effect. As intrinsic ZI firing rate increased, moving across the abscissa, feed-forward GABABR-mediated inhibition exerted a stronger effect. As ZI intrinsic firing and [GABA] increased further, GABABR-mediated hyperpolarization became strong enough to cause paradoxical increases in PO firing rate by causing burst firing; this was analogous to the findings in Fig. 4, G and H, where high ZI firing rate and [GABA] caused burst firing mediated increases in PO firing rate.

Increasing the strength of STT inputs to ZI (to 0.032 µS) resulted only in minor effects on the dynamics of feed-forward inhibition. As expected, the increase in STT→ZI input strength led to a small enhancement of inhibition when the STT input to ZI arrived earlier than that of PO. This was due to the increased STT input strength onto ZI, evoking multiple ZI action potentials (Fig. 5E), and the closely timed action potentials causing supralinear summation of GABABR-mediated current (Fig. 4E). Other aspects of these feed-forward dynamics remained

in Fig. 5D, the variability in interspike intervals and latencies were dependent on the temporal interactions between the evoked and the intrinsic activities.

These effects were systematically analyzed by creating a new heat map with the increased STT→ZI strength (Fig. 5F). Compared with the results shown in Fig. 5C, the zone of suppression was both wider and taller when STT→ZI excitation strength is increased. This enhancement in feed-forward

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**Fig. 6.** GABAAR-mediated incerto-thalamic inhibition of evoked PO activity. A: identical setup as in Fig. 5C but with GABAAR inserted instead of GABAAR. GABAAR-mediated feedforward inhibition is less effective at regulating evoked activity. Increased ZI firing rate and [GABA] led to paradoxical increases in activity mediated by rebound firing. B: identical setup as in Fig. 5F but with GABAAR. C: identical setup as in Figs. 5C and 6A but with both GABA receptor types included.
firing rate and [GABA] both reach high levels, the GABABR—which is highly sensitive to changes in [GABA]. Then, as ZI confining some neurons responses to certain stimuli, while controlling the magnitude of evoked responses, as well as for GABABR antagonists increased spontaneous activity of a population of neurons in the supraoptic nucleus but also that the inhibition mediated hyperpolarization and burst firing dominates. Thus as was the case in the suppression of spontaneous PO activity, a combination of the effects of the two individual GABA receptor types fully explains the effects observed when both receptor types are present.

DISCUSSION

Here, we developed the first computational model of the incerto-thalamic circuit based on Hodgkin-Huxley formalisms. Our model predicted that spontaneous and evoked activities of PO neurons are differentially affected by the two GABA receptor types and that modulation of both ZI firing rate and [GABA] can alter the nature of incerto-thalamic inhibition, although in different ways. These results provide important insights both to the general role of the incerto-thalamic pathway in sensory processing, as well as to underlying mechanisms of central pain.

Differential Role of GABA_{A/B}R

The literature is sparse on the respective roles of GABA_{A}R and GABA_{B}R-mediated mechanisms in regulating spontaneous and evoked neuronal activity. Wu et al. (2011) have shown that noradrenergic cells of the spinal cord are under tonic inhibitory control mediated by GABA_{B}R in basal conditions and that GABA_{B}R-mediated inhibition is capable of effectively eliminating spontaneous firing in these neurons. Ibrahim et al. (1998) have shown that application of both GABA_{A}R and GABA_{B}R antagonists increased spontaneous activity of a population of neurons in the supraoptic nucleus but also that the degree of their inhibitory effect varied between populations. Thus it is difficult to determine whether GABA receptor types play consistent roles across different brain regions or cell types with respect to spontaneous activity.

Similarly, there is no consensus on the receptors roles in evoked activity. Lee et al. (1994) have shown that GABA_{A}R-mediated current inhibits evoked activity in the ventral posteromedial thalamic nucleus, evoked from the center of the whisker receptive field, while GABA_{B}R-mediated current inhibits evoked activity from the periphery of the receptive field. Vahe-Hinz and Hicks (2003) reported that, in the same nucleus, GABA_{A}R-mediated inhibition was responsible for controlling the magnitude of evoked responses, as well as for confining some neurons responses to certain stimuli, while suggesting that GABA_{B}R play little or no role.

In the thalamocortical system, GABA receptors, particularly GABA_{B}R, have been implicated in brain rhythms, including dictating the frequencies of oscillation, modulating their power, and, in certain cases, generating pathological rhythms (reviewed in Kohl and Paulsen 2010). These investigations, however, focus on behaviors arising mainly from networks due to significant internuclear interactions, namely involving thalamus, TRN, and cortex. Furthermore, high-frequency burst firing is an essential feature to strongly activate GABA_{B}R; however, in our system, ZI does not exhibit such bursts and instead activates GABA_{A}R through lower frequency tonic firing. Thus such rhythms are not observed in our model.

Here, we use our model to predict that PO spontaneous activity is preferentially regulated by GABA_{A}R-mediated current while GABA_{B}R-mediated current had minimal effect on PO spontaneous activity. The differential effects of the two receptor types on spontaneous and evoked activity are related to the kinetics of the currents they mediate. The short time course of GABA_{A}R-mediated current (~20 ms) means that these currents can only provide effective inhibition if excitation happens to occur at the same time or at least within that short time window. As a result, during spontaneous PO firing, the excitatory current occurs without any time relation to that of ZI, the effective suppression of PO activity only occurs when the excitation and inhibition occur in-phase by chance. To enhance the chances of such in-phase firing, ZI neurons would have to fire at high rates (>50 Hz). By contrast, the slow time course and effective temporal summation of GABABR-mediated current results in effective suppression of spontaneous PO activity (Fig. 4D).

The rapid onset and high conductance of GABA_{A}R-mediated current, however, effectively suppressed evoked activity in PO, as long as the evoked inputs excited both PO and ZI in a closely time-locked fashion.

Physiological Implications

The incerto-thalamic circuit has key roles in both physiological and pathophysiological processes. The ZI sends dense GABAergic projections to PO (Bartho et al. 2007; Power et al. 1999) and exerts potent feed-forward and tonic inhibition of PO neurons (Lavallee et al. 2005; Trageser et al. 2006; Trageres and Keller 2004). This incerto-thalamic pathway is regulated by the cholinergic reticular activating system that is responsible for regulating arousal and sleep-wake transitions: we have shown that cholinergic inputs, acting on m2 receptors expressed on ZI terminals in PO (Bartho et al. 2002), regulate GABA release from these terminals (Masri et al. 2006). Cholinergic activity also modulates the firing rate of ZI neurons (Masri et al. 2006; Trageser et al. 2006). The cholinergic regulation of the incerto-thalamic pathway provides a substrate for physiological modulation of ZI firing and of GABA release, explored in the present study.

We have also demonstrated that maladaptive plasticity of the incerto-thalamic pathway is causally related to chronic pain that develops after SCI-Pain (reviewed in Keller and Masri 2014). In brief, in rats with SCI-Pain, spontaneous firing of PO neurons increases 30-fold, and their responses to peripheral stimuli are also significantly increased (Masri et al. 2009). These increases are causally related to a concomitant, threelfold decrease in spontaneous firing of ZI neurons and 50% reduction in their responses to tactile stimuli (Masri et al. 2009). In addition, SCI-Pain is associated with a 30% decrease in the frequency but not the amplitude of miniature IPSCs recorded from PO neurons, supporting the conclusion that SCI results in
suppression of inhibitory control of these thalamic nuclei by ZI (Keller 2011; Keller and Masri 2014).

These changes in incerto-thalamic activity during SCI-Pain are accurately reflected in our model. Assuming that physiological [GABA] is 0.5 mM, as was determined to accurately fit experimental data (Destexhe et al. 1996a), maximal inhibition of spontaneous PO activity was achieved in our model when ZI firing rate was 2.8 Hz (Fig. 4J; PO firing rate = 0.17 Hz). From these parameters that exert maximal inhibition, decreasing [GABA] by 30% to 0.35 mM increases PO spontaneous firing rate almost 3-fold to 0.49 Hz. Decreasing ZI firing rate two- to fourfold to 1.4 and 0.7 Hz increases PO spontaneous firing rate to 0.25 and 1.55 Hz, respectively (the resolution of Fig. 4J does not show 0.9 Hz, which is the 3-fold decrease). When [GABA] and ZI firing rate are decreased together, mimicking the parameters of SCI-Pain, PO spontaneous firing rate increases almost 10-fold to 1.5 Hz (when ZI firing at 1.4 Hz), and 1.63 Hz (when ZI firing at 0.7 Hz). Thus we show that small changes in ZI function, which occur in SCI-Pain, result in a substantial increase in PO spontaneous firing rate, consistent with our previous experimental findings.

It is more difficult to quantify the effects of decreasing these parameters on evoked responses because of the large number of unknown variables, such as temporal relationships and strengths of peripheral inputs into PO and ZI. Our results do show that decreasing intrinsic ZI firing rate and [GABA] from 3 Hz and 0.5 mM (Fig. 6C, top row, second column) to 0 Hz and 0.3 mM (third row, first column) reveals both shorter temporal windows of effective suppression, and decreased ability to effectively suppress stronger STT inputs. At the same time, neither the decrease in ZI firing rate nor [GABA] affects the degree of inhibition when the STT→PO and STT→ZI latencies differences are small and when STT→PO excitation strengths are weak (near 0 ms of abscessa, and low values of ordinate in each subplot of Fig. 6C; effective inhibition remains even in bottom left subplot when [GABA] = 0.1 mM, and ZI intrinsic firing rate = 0 Hz). These results suggest that a subpopulation of evoked responses, ones with large latency differences and/or high excitation strength inputs, will be disinhibited by decreases in [GABA] and ZI firing rate, while another subpopulation with small latency differences and/or low excitation strength inputs will be unaffected, resulting in an overall moderate decrease in evoked responses.

Respective Roles of GABA and ZI Firing Rate

Our findings suggest that manipulation of [GABA] and of ZI firing rate have different consequences on PO activity. A moderate decrease in either [GABA] or ZI firing rate was sufficient to significantly increase spontaneous PO firing rate. However, a concurrent decrease in these two parameters (likely underlying biological changes described above) produced the same degree of inhibition, with a smaller decrease in parameter values. Figure 4G illustrates the sharp delineation between a state of full inhibition (high [GABA], moderate ZI firing rate; PO firing rate ~0 Hz) to a state of minimal inhibition (low [GABA], low ZI firing rate; PO firing rate ~2 Hz). This reflects the ability of ZI inhibition to rapidly and effectively turn inhibition on and off, with minimal changes in [GABA] and ZI firing rate.

Changes in [GABA] and ZI firing rate had different effects on the regulation of evoked activity. Reductions in [GABA] decreased the integration time window of effective suppression and made inhibition of stronger STT→PO inputs less effective. Decreases in ZI firing rate caused a noticeable change in the strength, duration, and properties (such as the number and size of peaks of inhibition; Fig. 5C). Thus it appears that changes in [GABA] are more effective in regulating the strength of inhibition, while ZI firing rate mainly shapes the temporal properties of evoked responses (e.g., the shape of peristimulus time histograms) in PO. These two parameters may be differentially manipulated to achieve further specificity in incerto-thalamic regulation. Whether these two phenomena do function independently or in concert is not yet known.

These different roles of [GABA] and ZI firing rate may also relate to our finding that SCI has a greater effect on PO spontaneous firing compared with PO evoked responses (see above). Physiological decreases in both parameters effectively abolished inhibition of spontaneous activity (Fig. 4J) but had a smaller effect on inhibition of evoked activity, especially when the latency differences between STT→PO and STT→ZI were large (Fig. 6C). We noted above that the magnitude of GABA<sub>B</sub>R-mediated current is significantly diminished in response to decreases in [GABA], while that of GABA<sub>A</sub>R-mediated current is relatively insensitive to such changes. It follows that in SCI-pain, spontaneous activity regulated by [GABA]-sensitive GABA<sub>B</sub>R is significantly more affected than evoked activity regulated by [GABA]-insensitive GABA<sub>A</sub>R.

Regulating ZI Firing Rate

Our findings suggest that the magnitude of the t-current may regulate both intrinsic and evoked activities of ZI neurons, consistent with the established role of t-currents in different types of intrinsic firing patterns (Williams et al. 1997). Indeed, it is common for computational models to employ passive or t-channel conductances to induce and tune intrinsic activity. Thus partial suppression of passive conductance is suggested to underlie intrinsic activity, whereas modulation of t-channel conductance is implicated in rate and mode of firing, both in neurons and cardiac pacemaker cells (Destexhe et al. 1996b; Ono and Iijima 2010; Wallenstein 1994).

While we intended to use the t-current only to control the intrinsic firing rate of ZI, we found that the magnitude of t-current also significantly affected evoked ZI responses: when we increased the intrinsic firing rate of the ZI neuron by increasing the t-current magnitude, the number of evoked action potentials in the ZI neuron in response to STT input also increased (Fig. 5, C and D). The t-current often acts to magnify depolarizing inputs due to its voltage-dependent characteristics (Crandall et al. 2010; Magee et al. 1995; Sun et al. 2012). Therefore, increasing the conductance of t-channels leads to a stronger response to a depolarizing input. For the same reason, the firing rate or the t-current conductance also affects the latency at which the ZI neuron responds to STT input (Fig. 5, C and D).

We previously showed that both spontaneous and evoked ZI firing rates are diminished in central pain (Masri et al. 2009). While distinct mechanisms may underlie these changes in spontaneous and evoked activities, we predict, by law of
parsimony, that there is a change in the t-current magnitude of ZI neurons in central pain that accounts for both of these changes. This is a prediction we aim to test experimentally.

**Divergence from Previous Models**

The starting point for the development of our model presented here was a computational model of the reticulo-thalamic circuit developed by Destexhe et al. (1996a) and related models (Destexhe et al. 1994, 1996b, 1998). While a number of components were borrowed from this previous model as detailed in the methods, our model differs from it in various aspects. The goal of our investigation was to dissect the synaptic mechanisms underlying the effective inhibition of a thalamic nucleus by ZI and to characterize the changes in incertal disinhibition due to changes that occur on the synaptic level. Destexhe et al. sought to capture oscillatory behaviors characteristic of reticulo-thalamic circuits. To this end, Destexhe et al. used reciprocally connected interactions and investigated a large-scale network of neurons. Our model, instead, focused more on the details of the interactions of two neurons, particularly, the unilateral effects of ZI activity on PO. Thus our model of the ZI neuron was significantly different from the model of TRN neurons included in previous models; our ZI neuron expressed intrinsically firing behavior, and we included biophysically relevant parameters to manipulate its intrinsic firing rate and the synaptic strength of its inhibitory outputs, parameters not examined in previous models. Our ZI neuron model also did not exhibit high-frequency bursts and instead produced weak, single spike rebounds. The original TRN neurons, on the other hand, exhibited robust bursting behavior. Finally, our investigation examines the activity of PO both with and without peripheral evoked activity. This was an important aspect of our investigation, as ZI and PO both receive direct peripheral somatosensory inputs. On the other hand, Destexhe et al. does not address effects of peripheral stimulation of the thalamus.

**Limitations and Methodological Considerations**

Although our model captures the essential features of the incerto-thalamic circuit, by necessity, we set aside some arguably important aspects that may contribute to the behavior of this circuit. We based our ZI model on a previous model of TRN, which may have minor differences. However, the many similarities between these two neuronal types justify this. Both exhibit similar electrophysiological properties, including tonic and burst firing (Destexhe and Sejnowski 2003; Trageser et al. 2006) and high spontaneous firing rates (Lavallee et al. 2005; Pinault 2004), and both provide inhibitory, GABAergic inputs to the thalamus (Bartho et al. 2002). Additionally, Inamura et al. (2011) show that the two cell types originate from the same precursors and develop similarly.

In addition to ZI, PO is regulated by GABAergic input from TRN (Sherman and Guillery 2009) and from the anterior pretectal nucleus (APT; Murray et al. 2010). In the present model, we did not include inputs from these other nuclei as we were interested in the specific mechanisms of control of PO that have been attributed to actions of ZI, such as gating by states of arousal (Masri et al. 2006), or by induction of chronic pain (Masri et al. 2009). We focused on ZI because of findings that ZI exerts significantly stronger inhibition upon PO, compared with inhibition from TRN or APT, and on findings showing significant increases in PO activity when ZI activity is decreased (Bartho et al. 2002; Bokor et al. 2005; Trageser and Keller 2004). Thus converging evidence suggests that PO is regulated primarily by ZI. We recognize that TRN may produce high-frequency spike bursts that likely have a significant effect on PO. Similarly, PO can also produce high-frequency spike bursts that can cause strong feedback inhibition from TRN, leading to sustained burst firing through oscillatory TRN-PO interactions. However, we are mostly interested in the interactions that occur during aroused states, and this internuclear oscillatory behavior is observed in nonaroused states, such as slow-wave sleep. The potential roles of TRN and APT will be considered in future studies.

We also ignore the potential role of presynaptic GABABR, because it is not known whether they play a role in the incerto-thalamic pathway. Putative presynaptic GABA receptors on ZI terminals in PO may regulate [GABA] in the synaptic cleft, a variable well controlled in our simulations. Presynaptic GABA receptors might also regulate glutamate release from SST terminals in PO; however, it is not known whether these presynaptic receptors exist. In the somatosensory, ventrobasal thalamus, Kulik et al. (2002) found no anatomical evidence for presynaptic GABA<sub>R</sub>. In contrast, Emri et al. (1996) report physiological data consistent with presynaptic, GABAergic regulation of sensory afferents to ventrobasal thalamus.

We also do not consider the possibility of extrasynaptic GABA receptors and that their localization, not their molecular kinetics, may underlie their unique behaviors, such as their requirements for high frequency presynaptic activity (Beenakker and Huguenard 2010; Fritschi et al. 1999). However, the GABA receptor kinetics we used were fit to electrophysiological data and behaves consistently with experimental behaviors of GABA receptors in all aspects (Destexhe et al. 1996a).

The PO neuron does not exhibit intrinsic delta oscillations, as may be expected from a thalamocortical neuron expressing h- and t-currents. However, McCormick and Pape (1990b) have shown that neuromodulators play a significant role in regulating the magnitude of the h-current and that they facilitate the transition between rhythmic states (such as the delta oscillation) of inattentive and sleep states to information transfer states during arousal. Indeed, a modeled thalamocortical neuron by Destexhe et al. (1996a) exhibited intrinsic delta oscillations only when the h-current conductance was significantly reduced from the “relay-state” (cf. Destexhe et al. 1996a, Fig. 3).

Our single cell models do not capture the complexity of the spatial distribution of synapses and dendritic processing. While these morphological details are undoubtedly important, such details are mostly unknown in the incerto-thalamic circuit and cannot yet be accurately modeled. Instead, we used single-compartment models and simplified the excitatory inputs onto PO as large amplitude EPSPs to represent the summation of smaller EPSPs as experienced by the soma. This negates the need for dendrites for excitatory currents. For inhibitory currents, anatomical evidence suggests that inhibitory inputs to PO from ZI are on proximal dendrites (Bartho et al. 2007; Power et al. 1999); Thus it is reasonable to assume that the soma also experiences such currents with high fidelity. Thus
the simplification of neurons into single-compartment models still provides valuable insight into the mechanisms of the incerto-thalamic circuit. We propose to use the current model as a starting point in creating expanded models, as well as designing parallel biological experiments. Through iterations of such modeling projects and experimental approaches, more light may be shed on the underlying mechanisms of incerto-thalamic regulation and may lead to critical findings for alleviating pathologies resulting from their disinhibition.

Despite these simplifying assumptions, our model revealed several key predictions regarding the underlying mechanics of incerto-thalamic regulation. Our model predicted that the two different GABA receptor types may mediate different aspects of the circuit, with GABAA,R preferentially affecting evoked activity and GABAB,R preferentially regulating spontaneous activity. It further predicted that these effects may explain several key aspects of physiological and pathophysiological processes such as cholinergic regulation of arousal and central pain. Thus the insights and predictions gathered from this investigation will help guide future work both in better understanding of sensory physiology and developing therapeutics for chronic pain.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
A.P., K.H., and A.K. conception and design of research; A.P. performed experiments; A.P. analyzed data; A.P., K.H., and A.K. interpreted results of experiments; A.P. prepared figures; A.P. drafted manuscript; K.H. and A.K. edited and revised manuscript; K.H. and A.K. approved final version of manuscript.

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