I_h-mediated depolarization enhances the temporal precision of neuronal integration

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Feed-forward inhibition mediated by ionotropic GABA_A receptors contributes to the temporal precision of neuronal signal integration. These receptors exert their inhibitory effect by shunting excitatory currents and by hyperpolarizing neurons. The relative roles of these mechanisms in neuronal computations are, however, incompletely understood. In this study, we show that by depolarizing the resting membrane potential relative to the reversal potential for GABA_A receptors, the hyperpolarization-activated mixed cation current (I_h) maintains a voltage gradient for fast synaptic inhibition in hippocampal pyramidal cells. Pharmacological or genetic ablation of I_h broadens the depolarizing phase of afferent synaptic waveforms by hyperpolarizing the resting membrane potential. This increases the integration time window for action potential generation. These results indicate that the hyperpolarizing component of GABA_A receptor-mediated inhibition has an important role in maintaining the temporal fidelity of coincidence detection and suggest a previously unrecognized mechanism by which I_h modulates information processing in the hippocampus.
Microelectrode and electroencephalography studies show that groups of neurons can fire synchronously with millisecond precision. To maintain temporal fidelity of information encoding, neurons act predominantly as coincidence detectors rather than neuronal integrators. The precision of coincidence detection in pyramidal cells depends critically on feed-forward inhibition. Such inhibition, mediated by GABA_A receptors, acts both by shunting excitatory currents (shunting inhibition) and by hyperpolarizing neurons (voltage inhibition). Shunting inhibition reduces the amplitude and duration of excitatory postsynaptic potentials (EPSPs) by increasing the membrane conductance. The hyperpolarizing action of inhibitory postsynaptic potentials (IPSPs), on the other hand, offsets the depolarization mediated by EPSPs and is long lasting, resulting in a biphasic EPSP–IPSP sequence in many neurons.

It has been suggested that the temporal precision of neuronal integration also depends on I_H, but this effect has been attributed to HCN-mediated shunting of excitatory inputs. I_H is a mixed cationic current with a reversal potential of ~ −30 mV present in neurons throughout the brain (for reviews see refs 7, 8). As I_H is present at resting membrane potential, it depolarizes neurons6. I_H has a marked effect on dendritic processing by directly shunting excitatory inputs7,8 and through interactions with other membrane conductances4,10. Consequently, blocking I_H facilitates the temporal summation of EPSPs and action potential firing during repetitive stimulation12,13,16. As I_H can also affect IPSP kinetics17,18, we asked what effect I_H blockade has on coincidence detection, when inhibition is left intact.

Here, we report that blocking I_H results in a significant broadening of the window for neuronal integration. This, however, is not due to the influence of I_H on PSP kinetics, but rather is secondary to the hyperpolarizing effect of I_H blockade. Indeed, I_H is required to maintain the hyperpolarizing action of synaptically released GABA, and so, blocking I_H broadens the excitatory phase of the EPSP–IPSP sequence evoked by afferent stimulation. These results show an essential role for I_H in determining the relative values of the resting membrane potential (V_R) and the reversal potential for GABA_A receptor-mediated currents (E_GABA_A), and also show that hyperpolarizing inhibition is necessary for temporally precise neuronal integration of synaptic inputs.

Results

Blocking I_H increases the integration time window. We assessed coincidence detection by recording from CA1 pyramidal cells using gramicidin perforated-patch in current-clamp mode. We stimulated two separate populations of Schaffer collaterals (Fig. 1a) representing weak and strong synaptic inputs (see Methods). The stimulus intensities were adjusted so that simultaneous activation of the two pathways resulted approximately in a 50% chance of the neuron spiking. We then measured the spike probability while systematically varying the interstimulus interval. As previously reported, the spike probability decreased as the interval increased (Fig. 1b,c). We used 10 μM ZD-7288 to block I_H. Again consistent with previous studies3,19, this resulted in a hyperpolarization, an increase in input resistance and complete disappearance of the characteristic depolarizing sag of the membrane potential following a hyperpolarizing step current injection (Supplementary Fig. S1). We then readjusted the stimulation intensities to match the spiking probability for simultaneous stimulation observed under control conditions. Blocking I_H resulted in a significant broadening of the...
time window for integration of the two input stimuli (Fig. 1d–f; \( n = 6 \); repeated measures analysis of variance (ANOVA): \( F(1,5) = 15.5, P = 0.011 \)). We further confirmed this effect using cell-attached recordings at near physiological temperature and with the same stimulation paradigm (Supplementary Fig. S2; \( n = 4 \); repeated measures ANOVA: \( F(1,3) = 37.3, P = 0.009 \) for the effect of ZD-7288).

Blocking \( I_h \) prolongs excitation through hyperpolarization. Input summation in the above experiments depends on the time course of the EPSP–IPSP sequence\(^{1,19,20} \). The long membrane time constant of hippocampal principal cells permits EPSP summation over a large time window, but disynaptic feed-forward inhibition limits the temporal summation of the excitatory inputs by curtailing over a large time window, but disynaptic feed-forward inhibition altering membrane conductance\(^{21,22} \), interneuron recruitment\(^{23} \) and/or \( V_m^{2-11} \). To address the relative roles of these effects of \( I_h \), we evoked an EPSP–IPSP sequence in CA1 pyramidal cells by stimulating Schaffer collaterals, and then blocked \( I_h \) with and without correcting the membrane voltage (Fig. 2). Blocking \( I_h \) with ZD-7288 completely abolished the hyperpolarizing component of the EPSP–IPSP sequence and resulted in considerable broadening of the half-width of the depolarizing phase of the response to \( 251 \pm 27\% \) of control (\( n = 4 \); \( P = 0.003 \); Fig. 2a,b).

We first examined whether the effect of \( I_h \) block on the EPSP–IPSP sequence could be entirely accounted for by hyperpolarization of \( V_m \). Following the application of ZD-7288, \( V_m \) was returned to the baseline level by injecting a constant current into the recorded neuron and the EPSP–IPSP sequence was again recorded (Fig. 2b). Almost full recovery of the hyperpolarizing phase of the response was observed (\( 80 \pm 8\% \) of the baseline amplitude before ZD-7288 application; \( n = 4 \); \( P = 0.1 \)). In these experiments, there was only a relatively small increase in peak amplitude (\( 36 \pm 10\% \); \( P = 0.04 \)) and half-width (\( 39 \pm 9\% \); \( P = 0.02 \)) of the depolarization, consistent with some direct effect of \( I_h \) on the EPSPs; these effects were significantly smaller than those observed with the addition of ZD-7288 without current injection (that is, without correcting the neuronal hyperpolarization; \( P = 0.02 \) and \( P = 0.004 \) for amplitude and half-width of depolarizing phase of the EPSP–IPSP sequence, respectively (Fig. 2b)). The changes in the EPSP amplitude and half-width following ZD-7288 application and direct current injection (Fig. 2b) were similar to those observed when testing the effect of ZD-7288 on EPSPs when GABA\(_A\) receptors were also blocked with 100 \( \mu \)M picrotoxin (Fig. 2c, \( V_m \) was fixed at the control level with direct current injections). These results argue against a significant contribution of non-specific effects of ZD-7288 on presynaptic function\(^4\). In addition, because of the limited space clamp in our experimental conditions (see Methods), recovery of the hyperpolarizing component of the postsynaptic response by current injection indicates that the majority of the inhibitory synapses recruited by Schaffer collateral stimulation impinge either close to the soma or on proximal dendrites of pyramidal cells. This is in line with previous findings that feed-forward inhibition in hippocampal CA1 pyramidal neurons underlying temporally precise synaptic integration is predominantly perisomatic\(^1\).

We further tested the effect of \( I_h \) block on GABA\(_A\) receptor-mediated transmission, as \( I_h \) has been reported to be present in some interneurons\(^{23,25,26} \). We stimulated Schaffer collaterals and recorded in whole-cell voltage-clamp configuration with the membrane potential clamped to 0 mV (the reversal potential for glutamatergic currents) to isolate inhibitory postsynaptic current (IPSCs). Consistent with the results obtained in current clamp, ZD-7288 only reduced the IPSC amplitude by 17.5 \( \pm \) 3\% (\( n = 3 \)). The effect of \( I_h \) on the EPSP–IPSP sequence can therefore be largely attributed to the \( I_h \)-dependent depolarization of \( V_m \) and to the loss of the hyperpolarizing action of the IPSPs. Does shunting inhibition also modify the EPSP–IPSP sequence? To test this, we applied the GABA\(_A\) receptor antagonist picrotoxin with \( I_h \) blocked. This resulted in further prolongation of the EPSPs, indicating an additional role of shunting inhibition in regulating the shape of the EPSP–IPSP sequence (Fig. 2a).

One prediction from these results is that genetic ablation of \( I_h \) should similarly hyperpolarize neurons, change the driving force for chloride and prolong the depolarizing phase of the EPSP–IPSP sequence. We examined HCN1 knockout mice and compared them with wild-type littermate controls. Consistent with previous studies\(^{7,24,26} \), HCN1 knockouts lacked the \( I_h \)-mediated membrane potential sag following hyperpolarizing step current injection (Fig. 3a). The resting membrane potential of pyramidal cells was more hyperpolarized in the knockout mice (\( \approx 72.6 \pm 2.5 \) mV) compared with
wild-type littermate control animals (−61.9±1.6 mV; P = 0.0037; Fig. 3a–c); however, \(E_{\text{GABA}}\) was similar in both genotypes (Fig. 3b,d). As predicted, the hyperpolarizing phase of the EPSP–IPSP sequence was either absent or reduced in the knockout animals, and the width of the EPSPs was broadened to 193.4±18.4% of the wild-type value (P = 0.009; Fig. 3e).

\(I_h\) maintains \(V_R\) more depolarized than \(E_{\text{GABA}}\). The results thus far indicate that \(I_h\)-dependent neuronal depolarization is necessary to maintain a hyperpolarizing effect of \(GABA_A\) receptor currents. We directly tested this hypothesis using gramicidin perforated-patch current-clamp recordings, which minimize perturbation of the neuronal membrane potential to the baseline level. Using this protocol, we observed no change in the time window for coincidence detection (Fig. 5; \(n = 5\); repeated measures ANOVA: F (1,4) = 0.374, P = 0.57). This implies that it is primarily the depolarizing effect of \(I_h\) and loss of the hyperpolarizing effect of \(GABA_A\) receptor currents that maintains the narrow coincidence detection for input integration. A further
Hyperpolarizing inhibition underlies temporal precision. A possible confounder in the above experiments is that in order to maintain a 50% spiking probability, we had to adjust the stimulation intensity to compensate for the membrane hyperpolarization, potentially affecting interneuron recruitment. This would tend to increase the interneuron recruitment and therefore would be expected to narrow the integration time window (in contrast to the broadening that we observed). Nevertheless, to control for this and to address the question of whether loss of the hyperpolarization effect of GABA A receptor currents is sufficient to explain our results, we constructed a simple integrate-and-fire model of a neuron that receives two inputs. Each input consisted of an excitatory followed by an inhibitory conductance, and the parameters were adjusted to simulate the kinetics of the experimentally obtained EPSP–IPSP sequence waveform (see Methods; Fig. 6a). In agreement with the experimental findings, systematic variation of the delay between the two inputs revealed a narrow integration time window for spike generation (Fig. 6b).

Hyperpolarization of V m broadens the integration time window. Removing I h from the modelled cell, hyperpolarized the membrane from −70 to −80 mV and led to an increase in the width of the integration time window (Fig. 6c; the area under the spike probability curve was increased by 26.3 ± 2.3%; P < 0.01). This effect was robust and relatively insensitive to varying EPSP and IPSP kinetic parameters, delay times between excitatory and inhibitory synapse activation, as well as synapse locations on the dendrites (Supplementary Fig. S4). We also systematically changed the maximal probability of action potential generation to test whether this could affect our experimental results. The effect of membrane potential hyperpolarization was constant across a wide range of tested probability values (Supplementary Fig. S5).

To determine whether the effect on the integration window in the simulations was due to the conductance or voltage effect of I h removal, we repolarized the neuronal soma back to −70 mV by introducing a depolarizing current. In agreement with experimental observations, this restored the width of the time window for integration, indicating a crucial role for the depolarizing action of I h (Fig. 6d).

We next asked whether the effect that we observed was due to the voltage change itself rather than the change in the polarity of GABA A receptor-mediated transmission. We therefore assessed the effects of membrane potential on the integration time window in the absence of GABAergic transmission. As expected, removing feed-forward inhibition broadened the integration time window by 106.8 ± 6.7%. However, the width of the window was minimally affected by changes in V m in the range observed in our experiments and simulations (Fig. 7a; an increase of 3.1 ± 0.4% with 10 mV hyperpolarization).

In the above simulations, the magnitude of the inhibitory synaptic conductance was fixed to match the simulated EPSP–IPSP waveform with that observed experimentally. We therefore tested the effect of different inhibitory synaptic conductances. In all instances, hyperpolarizing V m, so that the IPSPs became depolarizing, widened the integration time window. This effect initially increased with increases in the IPSP amplitude (Fig. 7b) up to a maximum and then decreased probably because of the increasing shunting versus voltage effect of inhibitory synapses.

Finally, we considered the possible confounding effect of variability in the size of the inhibitory conductance. At high stimulation intensity, the inhibitory conductance is relatively invariant. However, at lower stimulation intensities, inhibitory conductances vary with stimulation intensity (and magnitude of excitatory conductance). We therefore covaried the conductances of excitatory and inhibitory synapses (see Methods) to model this situation. Under these conditions, the window of integration was still increased following hyperpolarization of the neuron by 38.1 ± 11.4% compared with 20.2 ± 2.6%, with constant inhibitory synaptic conductances (Fig. 7c; P < 0.05).

Discussion

We have shown that I h is necessary to maintain a hyperpolarizing driving force for GABA A receptor-mediated transmission by depolarizing the resting membrane potential of pyramidal cells. Hyperpolarizing the resting membrane potential through pharmacological inhibition of I h or deletion of HCN1 results in depolarizing GABA A receptor-mediated potentials and broadening of the excitatory phase.
of the EPSP–IPSP sequence, leading to a prolonged time window for synaptic integration. These results also demonstrate an important and unexpected role of voltage inhibition.

$I_h$ channels are densely expressed in the dendritic arbour of CA1 pyramidal cells. A significant proportion of these channels is active...
at rest, reducing the membrane resistance and neuronal time constant. These effects of \( I_h \) have been shown to determine the time course of EPSPs onto CA1 pyramidal cells\(^\text{12}\). The previous studies have concentrated on the impact of \( I_h \) on the integration properties of excitatory inputs, with GABA\(_\text{A}\) receptor-mediated inhibition blocked. However, GABAergic inhibition has a profound effect on the width of the integration time window of excitatory inputs\(^\text{1}\). The relative roles of shunting and hyperpolarizing inhibition in maintaining precise coincidence detection have not been addressed. Although one study has considered the effect of \( I_h \) on the integration time window without GABA\(_\text{A}\) receptor blockade\(^\text{6}\), that work was performed in whole-cell mode, with the membrane potential of the neurons fixed and the reversal potential of GABA\(_\text{A}\) responses set to be depolarizing.

In hippocampal neurons, \( E_{\text{GABA(A)}} \) is negative to \( V_h \) by the end of the second postnatal week\(^\text{36}\), so that GABA\(_\text{A}\) receptor-mediated potentials predominantly hyperpolarize the postsynaptic membrane in adult tissue, for example, see ref. (31). In adult neurons, \( E_{\text{GABA(A)}} \) is mainly determined by the Cl\(^\text{−}\) cotransporter KCC2, which uses the transmembrane K\(^{+}\) gradient to maintain a low intracellular Cl\(^\text{−}\) concentration, thereby clamping the reversal potential for Cl\(^{−}\) (\( E_{\text{Cl}^{−}} \)) closer to that of K\(^{+}\) (\( E_{\text{K}^{+}} \))\(^\text{36,36a}\). \( E_{\text{GABA(A)}} \) is, however, more depolarized than \( E_{\text{Cl}^{−}} \) due to the permeability of the GABA\(_\text{A}\) receptors to HCO\(_3\)\(^−\) atoms\(^\text{37}\). Therefore, to maintain hyperpolarizing IPSPs, \( V_h \) also has to be depolarized relative to \( E_{\text{GABA(A)}} \). A number of conductances may contribute to this depolarization (such as persistent sodium currents and leak conductances); in CA1 pyramidal cells, \( I_h \) provides a significant depolarizing drive at resting membrane potential\(^\text{1}\). In our study, this effect of \( I_h \) was more evident with pharmacological block than with genetic ablation, perhaps because of compensatory changes in other conductances in the HCNI knockout mice.

We have shown that \( I_h \) sets the polarity of GABAergic events by making \( V_h \) more depolarized than \( E_{\text{GABA(A)}} \) and most of the effects of \( I_h \) on the EPSP–IPSP sequence can be rescued by repolarizing \( V_h \) to its baseline value, arguing for a major role of GABAergic voltage inhibition in regulating the integration properties of synaptic inputs of CA1 pyramidal cells. As observed in our study and in other studies, for example, see ref. (33), GABA\(_\text{A}\) receptor activation can still shunt EPSPs even when \( E_{\text{GABA(A)}} \) is more depolarized than \( V_h \). However, the relative importance of shunting and hyperpolarizing effects of inhibition for neuronal computations and precise timing of action potential generation has received much less attention. Some experimental and theoretical studies have suggested that the hyperpolarizing effects of GABAergic neurotransmission are critical for network synchronization, for example, see ref. (34), whereas others emphasize the role of shunting inhibition in oscillatory behaviour of interconnected neurons\(^\text{38}\). The ability of \( I_h \) to switch the inhibitory mode of GABAergic signals from hyperpolarizing to shunting or depolarizing adds further complexity in defining the computational properties of hippocampal neurons\(^\text{39}\).

Further, it might be expected that the large, early shunting effect of GABAergic inhibition has the predominant part in curtailing the EPSPs. Our results, however, underscore the contribution of GABA\(_\text{A}\) receptor-mediated hyperpolarization in determining the temporal precision of coincidence detection by hippocampal pyramidal neurons. How does changing the GABA\(_\text{A}\) receptor-mediated response from hyperpolarizing to an increased integration time? Although shunting is maintained regardless of the polarization of GABA\(_\text{A}\) receptor-mediated responses (see Fig. 2a) attenuating the EPSPs generated in response to the second stimulation, this attenuation would be much stronger when the IPSP produces hyperpolarization. More importantly, because the voltage effect of an IPSP outlasts its shunting effect\(^\text{30}\), it would have an impact on the excitatory potentials over an extended period. In the situation when the hyperpolarizing phase of the GABAergic response is abolished (and even more so if the polarity of the GABA\(_\text{A}\) response is changed), this would facilitate EPSP summation. Therefore, the hyperpolarization that results from inhibition of \( I_h \) results in a considerable increase in the half-width of the time window for coincidence detection. Inhibition of other membrane conductances through hyperpolarization cannot account for this, as voltage changes of similar magnitudes, when \( I_h \) is blocked, result in minimal change in the membrane resistance or time constant\(^\text{1}\). The critical role of hyperpolarization for coincidence detection has extensive implications, as anything that alters the polarity of GABA\(_\text{A}\) receptor-mediated transmission will affect the time window for input integration. This would include either a shift in \( E_{\text{GABA(A)}} \) (for example, due to loss or inhibition of KCC2) or changes in resting membrane potential mediated through, for example, changes in membrane potassium conductances.

We have therefore shown the critical role of GABA\(_\text{A}\) receptor-mediated hyperpolarization in information processing and an unexpected mechanism by which \( I_h \) can modulate coincidence detection. As \( I_h \) is regulated by developmental\(^\text{\text{40}}\), physiological\(^\text{\text{40}}\) and pathological processes\(^\text{\text{41,42}}\), such processes may consequently affect the properties of GABA\(_\text{A}\) receptor-mediated signalling and the temporal fidelity of coincidence detection. This may contribute to cognitive and psychiatric consequences of epilepsy by increasing the probability of aberrant associations. Moreover, the magnitude of somatic \( I_h \) is dependent upon excitatory synaptic activity, and this has been proposed to be a homeostatic mechanism regulating neuronal excitability\(^\text{\text{43}}\). We further hypothesize that such a mechanism may have an additional homeostatic role by narrowing the time window for coincidence detection with increases in neuronal activity and conversely broadening the time window for integration when synaptic activity decreases. The role of \( I_h \) in restricting temporal input integration may also contribute to IBS, explaining the enhanced learning behaviour observed in HCNI knockout mice\(^\text{\text{27}}\), which may occur at the expense of loss of temporal discrimination of inputs.

**Methods**

**Electrophysiology.** We used transverse hippocampal slices (350 μm) from 3–6-week-old male Sprague–Dawley rats, or HCNI knockout mice and their wild-type littermate controls. The HCNI knockout animals were re-derived from that described previously, and maintained on a 129SVEV background\(^\text{\text{24,25}}\).

Animals were killed according to schedule 1 of the UK Animals (Scientific Procedures) Act 1986. The brains were rapidly removed, dissected and cut with Leica VT1000S vibratome in ice-cold solution containing (in mM) sucrose (70), NaCl (80), KCl (2.5), MgCl\(_2\) (7), CaCl\(_2\) (0.5), NaHCO\(_3\) (25), NaH\(_2\)PO\(_4\) (1.25) and glucose (22), and were equilibrated with 95% O\(_2\)/5% CO\(_2\) (pH 7.4). The slices were allowed to recover in an interface chamber (>1 h) at room temperature before being transferred to the recording chamber. Storage and perfusion solutions contained (in mM) NaCl (119), KCl (2.5), MgSO\(_4\) (1.3), CaCl\(_2\) (2.5), NaHCO\(_3\) (26.2), NaH\(_2\)PO\(_4\) (1) and glucose (22), and were gassed with 95% O\(_2\)/5% CO\(_2\). All recordings, except for those in Figures 1, 5 and Supplementary Figure S3, were carried out at 30 °C. Current-clamp recordings (Supplementary Fig. S1) were performed using pipettes (5–5.5 MΩ) filled with an intracellular solution containing (in mM) K-gluconate (145), NaCl (8), KOH-HEPES (10), EGTA (0.2), Mg-ATP (2) and Na\(_2\)GTP (0.3); pH 7.2; 290 mOsm. Granamicidin (50 μg ml\(^{-1}\)) was added for perforated-patch recordings\(^\text{\text{44}}\), and patch pipettes (8–12 MΩ) were front-filled with granamicidin-free solution; either QX-314 Br (5 mM) was added or [Cl\(^{−}\)] was increased to 26 mM (\( E_{\text{Cl}^{−}} \) = −41 mV) to monitor patch integrity. Series resistance was monitored throughout. Data acquisition began when the series resistance was < 150 MΩ (15–20 min after obtaining cell-attached configuration). To isolate GABA\(_\text{A}\) receptor-mediated IPSPs, NMDA, AMPA and GABA\(_\text{B}\) receptors were blocked with AP5 (50 μM), NBQX (20 μM) and CGP52432 (3 μM), respectively. Constant current stimuli were delivered to Schaffer collaterals through bipolar stainless steel electrodes placed in stratum radiatum. Monopolar IPSPs were evoked by positioning the stimulating electrode close to the recording site. EPSP–IPSP sequences were evoked by positioning the electrodes in stratum radiatum at least 300 μm away from the recording site; CGP52432 (5 μM) was added to the perfusate. Although application of CGP52432 reduced the duration of the depolarizing phase by 16.3 ± 3.3% (Supplementary Fig. S6a–c), it did not affect the depolarizing component.

Two Schaffer collateral pathways were stimulated for the coincidence-detection protocol (Figs 1 and 5, Supplementary Fig. S3). Experiments were performed in the presence of 30 μM AP5 (to avoid spike timing-dependent plasticity) and 3 μM CGP52432. AP5 had a minimal impact on the excitatory phase of the EPSP–IPSP sequence, decreasing the half-width by 14.4% (more than an order of magnitude smaller than the impact of changing the polarity of GABA\(_\text{A}\) responses; Supplementary}
Fig. S6d–f). To ensure recording stability, these experiments were performed at room temperature (23–25°C); this increased the time course of the synaptic responses (Supplementary Fig. S7), but the shape of the EPSP–IPSP waveform was maintained. Although modelling indicated that this does not qualitatively change the experimental findings (Supplementary Fig. S4a), we confirmed this with cell-attached recordings at 32°C (Supplementary Fig. S2). These experiments were performed using patch pipettes (8–12 mΩ) filled with ACSF in voltage-clamp with the voltage set so that no current was injected under baseline conditions. As afferent input strength to the hippocampus can vary considerably, we stimulated a weak and a strong input; the amplitude of the response in one pathway was adjusted to be approximately twice that of the other pathway. In the cell-attached experiments, the weaker pathway was stimulated at approximately half-threshold intensity, whereas the 'strong' pathway stimulation was set close to the firing threshold. Stimulation intensities were adjusted to obtain ~50% spike probability when both pathways were activated simultaneously. The two pathways were stimulated with an interstimulus delay varying from ~12 to ~12 ms in 3 ms steps (thus the order of inputs was reversed over the range investigated). To avoid any confounding effects of asymmetric distribution of spike probabilities, the data are presented as the averaged values from corresponding points on either side of the maximum.

In some experiments, constant current injection was used to repolarize the somatic membrane potential. Under our experimental conditions, there is a space clamp error in distal dendrites. However, as we observed experimentally that somatic current injection restored the hyperpolarization phase of the EPSP–IPSP waveform, we conclude that feed-forward inhibition in our study was predominantly perisomatic (Supplementary Fig. S8).

For experiments on the effect of Ih on the magnitude of IPSCs, the intracellular solution contained (in mM) Cs-methanesulfonate (120), NaCl (8), HEPES (10), EGTA (0.2), MgCl$_2$ (0.2), Mg-ATP (2), Na$_3$GTP (0.3) and QX-314 (0.9–1.1 μS; Poisson-distributed conductances of uniformly distributed variable magnitude (baseline conditions) or −80 mV (hyperpolarized conditions). The mean number of action potentials per bin (N) and s.e. (SE) were calculated. An example of the spike probability curve was used to quantify changes in the coincidence-detection time window. The s.e. of the ratio of surfaces S1/S2 was defined as:

\[
\varepsilon = \frac{\bin \cdot \sum e_i}{\bin \cdot \sum e_i}
\]

where N is the number of bins, bin is the bin size and $\rho_{\text{max}}$ is the maximum probability of the distribution.

The s.e. of the ratio of surfaces S1/S2 was defined as:

\[
\varepsilon_{\text{se}} = \frac{\bin \cdot \sum e_i}{\bin \cdot \sum e_i}
\]

The maximal probability of action potential generation (0.5) was set by scaling synaptic conductances with the parameter Stim: Stim×Gmax and Stim×Gmin. The strengths of inhibitory synapses were set to $G_{\text{inh}} = 450$ nS and $G_{\text{inh}} = 225$ nS for left and right synapse, respectively, for the majority of simulations. For Figure 7b, the strength of inhibitory connections varied as indicated. For simulations presented in Figure 7c in addition to excitatory conductances, we also scaled inhibitory conductances: Stim×Gmax and Stim×Gmin.

Simulations with maximal probabilities from 0.2 to 0.85 were performed (Supplementary Fig. S5). In these experiments, the actual maximal spike probability was determined post hoc.

**Statistics.** Two-tailed Student's t-test (paired or independent) and repeated measures ANOVA were used for statistical analysis. P<0.05 for significant differences. Data are presented as mean ± s.e.m.

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Author contributions
I.P. and A.S. designed and performed experiments, analysed data and wrote the paper; L.S. performed modelling; D.M.K. and M.C.W. designed experiments, directed the project and wrote the paper.

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