Non-uniform distribution of dendritic nonlinearities differentially engages thalamostriatal and corticostriatal inputs onto cholinergic interneurons

Abbreviated title: Dendritic nonlinearities in striatal cholinergic interneurons

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Abstract

The tonic activity of striatal cholinergic interneurons (CINs) is modified differentially by their afferent inputs. Although their unitary synaptic currents are identical, cortical inputs onto distal dendrites only weakly entrain CINs, whereas proximal thalamic inputs trigger abrupt pauses in discharge in response to salient external stimuli. To test whether the dendritic expression of the active conductances that drive autonomous discharge contribute to the CINs' capacity to dissociate cortical from thalamic inputs, we used an optogenetics-based method to quantify dendritic excitability. We found that the persistent sodium (NaP) current gave rise to dendritic boosting and that the hyperpolarization-activated cyclic nucleotide-gated (HCN) current gave rise to a subhertz membrane resonance. This resonance may underlie our novel finding of an association between CIN pauses and internally-generated slow wave events in sleeping non-human primates. Moreover, our method indicated that dendritic NaP and HCN currents were preferentially expressed in proximal dendrites. We validated this non-uniform distribution with two-photon imaging of dendritic back-propagating action potentials, and by demonstrating boosting of thalamic, but not cortical, inputs by NaP currents. Thus, the localization of active dendritic conductances in CIN dendrites mirrors the spatial distribution of afferent terminals and may promote their differential responses to thalamic vs. cortical inputs.

36 Introduction

37 The striatal cholinergic interneuron (CIN) is a key modulator of the striatal
38 microcircuitry, impacting the neuronal excitability, synaptic transmission and synaptic
39 plasticity of spiny projection neurons (SPNs), as well as other striatal interneurons
40 (Abudukeyoumu et al., 2019; Assous, 2021; Goldberg et al., 2012). There are two processes
41 that drive the ongoing release of acetylcholine (ACh) by CINs. First, the two glutamatergic
42 inputs arising from cerebral cortex and intralaminar nuclei of the thalamus can drive CINs to
43 discharge (Bradfield et al., 2013; Ding et al., 2010; Doig et al., 2014; Kosillo et al., 2016;
44 Lapper and Bolam, 1992; Matsumoto et al., 2001; Sharott et al., 2012; Thomas et al., 2000).
45 While the unitary synaptic currents generated by these two inputs in CINs are identical
46 (Aceves Buendia et al., 2019), that thalamic inputs to CINs dominate in the sense that they
47 that give rise to larger excitatory post-synaptic potentials (EPSPs) in acute striatal slices
48 (Johansson and Silberberg, 2020) and can trigger abrupt pause responses, often flanked by
49 excitatory peaks, to external saliency-related cues (Apicella et al., 1991; Goldberg and
50 Reynolds, 2011; Graybiel et al., 1994; Kimura et al., 1984; Matsumoto et al., 2001; Morris et
51 al., 2004; Raz et al., 1996). In contrast, cortical inputs to CINs are weaker in that, in acute
52 striatal slices, they give rise to smaller EPSPs and cannot trigger the pause response (Ding et
53 al., 2010), although, these differences are less pronounced in intact animals (Doig et al.,
54 2014).

55 Second, even in the absence of afferent input, CINs exhibit multiple autonomously
56 generated discharge patterns – including regular and irregular pacemaking, as well as burst
57 firing (Bennett et al., 2000; Bennett and Wilson, 1999; Goldberg and Reynolds, 2011;
58 Goldberg and Wilson, 2010). These firing patterns are generated by an interplay between
59 various nonlinear ionic currents including voltage- and calcium-activated K^+ currents, as well
60 as two voltage-dependent pacemaker currents: the hyperpolarization-activated cyclic
61 nucleotide-gated (HCN) current; and the persistent Na^+ (NaP) current (Bennett et al., 2000;
62 Deng et al., 2007; Goldberg et al., 2009; Goldberg and Wilson, 2010, 2005; McGuirt et al.,
63 2021; Oswald et al., 2009; Song and Surmeier, 1996; Wilson, 2005; Wilson and Goldberg,
64 2006). The main purpose of these pacemaker currents presumably is to guarantee the ongoing
65 release of ACh onto the striatal microcircuitry by sustaining the autonomous discharge of
66 CINs. Nevertheless, these pacemaker (and other subthreshold) currents will also impact how
67 cortical and thalamic inputs are integrated by the CINs.

Attaining a mechanistic (e.g., dynamical systems) understanding of how the repertoire of CIN firing patterns is generated, requires a full characterization of the nonlinear properties of the pacemaker (and other) currents (e.g., by determining their voltage dependence and kinetics) – which is a daunting endeavor. In contrast, understanding how these currents impact synaptic inputs is a simpler task. Because individual synaptic inputs are small, the membrane nonlinearities can be *linearized* making the analysis of their impact simpler and more general - a treatment called the quasi-linear membrane approximation. This analysis dates back to Mauro (Koch, 1984; Mauro et al., 1970) and has shown that quasi-linear membranes can give rise to two qualitatively different transformations of inputs: *amplification* and *resonance* (Goldberg et al., 2007; Hutcheon and Yarom, 2000). Being a linear approximation, the quasi-linear approximation is amenable to Fourier analysis, which helps to better define these transformations as linear (time-invariant) filters on the input in frequency space.

Amplification arises from regenerative ionic currents – that provide positive feedback – including inward (depolarizing) currents activated by depolarization, such as the NaP current. Here the main effect in frequency space is amplification of the amplitude response (as compared to the response of a passive linear membrane). Resonance arises from restorative currents – that provide negative feedback – including inward currents activated by hyperpolarization, such the HCN current. In frequency space, the defining properties of resonance is a peak (at a non-zero frequency) in the amplitude response, and a zero-crossing of the phase delay (at a nearby frequency). On a practical level, the quasi-linear response properties of a membrane can be measured by providing a small-amplitude, sinusoidally-modulated voltage command to an intracellularly-recorded neuron and recording the resultant sinusoidal output current. Calculating the ratio of the voltage amplitude to the current response yields an estimate of the membrane’s impedance (which is, loosely speaking, an indication of the neuron’s “input resistance” to a sinusoidal input as a function of its frequency). Membrane impedances have been reported for various neuronal types in the brain (Hutcheon et al., 1996; Ulrich, 2002), including in the striatum (Beatty et al., 2015). Beatty and collaborators (2015) found that CINs exhibit a resonance in the vicinity of 1 Hz. Moreover, they found that the shape of the impedance function depended on the holding voltage, which is understandable given that the amplitude of the various subthreshold currents is voltage-dependent. Finally, with use of tetrodotoxin (TTX), a selective antagonist

of voltage-activated Na⁺ (Nav) channels, Beatty and collaborators (2015) demonstrated that NaP currents contributed to these filtering properties of the CIN membrane.

Use of somatic voltage perturbations, however, fails to discriminate the role played by the CINs' dendritic arbor *per se* in transforming synaptic inputs. The CINs' dendritic arbor can span well over half a millimeter from the soma (Wilson, 2004), with cortical inputs terminating on distal dendrites and thalamic inputs terminating perisomatically and on proximal dendrites (Doig et al., 2014; Lapper and Bolam, 1992; Thomas et al., 2000). Both dendritic morphology (as taught by cable theory) and dendritic nonlinearities will lead to distal cortical inputs being integrated differently from proximal thalamic input. Thus, the impact of membrane nonlinearities on the quasi-linear approximation will depend on *where* they are expressed throughout the dendritic arbor. To address this question, we recently developed an optogenetics-based experimental method (that relies on the use of quasi-linear cable theory and Fourier analysis) to determine the duration of the delays introduced by dendrites, and how these delays impact the rapidity and fidelity of a neuron's response to its input. We used our method to study GABAergic neurons of the substantia nigra pars reticulata (SNr) that expressed channelrhodopsin-2 (ChR2). We illuminated (with a 470 nm LED) either a small perisomatic region or the entire dendritic arbor. Comparison of the two illumination regimes enabled us to demonstrate that dendrites (that in the SNr can be >700 μm long) introduce a significant integration delay. The analysis also yielded that SNr dendrites behaved like passive linear filters, without evidence for amplification or resonances, or any dependence on holding voltage (Tiroshi and Goldberg, 2019).

In the present study, we apply our method to study nonlinearities of CIN dendrites. We demonstrate that HCN and NaP currents shape the quasi-linear response properties of CINs, and that their presence in the dendrites contributes additional phase delays. Furthermore, we show that our analysis can reveal information about dendritic location of membrane nonlinearities, and use the analysis to deduce that both HCN and NaP currents are expressed primarily proximally. The proximal distribution of NaP currents is supported by measuring: a) how far autonomously generated backpropagating action potentials (bAPs) actively invade the dendritic arbor; and b) the differential boosting by NaP currents of proximal thalamostriatal vs. distal corticostriatal EPSPs.

Figure 1: CIN membranes exhibit voltage-dependent quasi-linear properties. **A.** CINs exhibit a voltage sag due to HCN currents, and autonomous pacemaking due to NaP currents. **B.** Application of 2 mV sinusoidal voltage commands to the soma, of increasing frequencies, elicits a current response that is voltage dependent (black trace, -55 mV; red trace, -70 mV). **C.** Estimation of the impedance (left) and phase shift (right) show that at -55 mV, CINs exhibit an amplified impedance and that at -70 mV, CINs exhibit a resonance (non-monotonic impedance and negative phase delays). Solid lines are parameter fits for $(\alpha^2 + \beta^2)^{-1/2}$ up to a scale factor (left), and phase shift, ϕ_s (right, see Eq. 4).

Results

Ionic currents underlying amplification and resonance in cholinergic interneurons

The HCN and NaP currents depolarize CINs over largely non-overlapping voltage ranges. The HCN current is mostly active below -60 mV and is responsible for the voltage sag in response to a hyperpolarizing current pulse (Fig. 1A, “HCN”), whereas the NaP current takes over at -60 mV and is necessary and sufficient (Bennett et al., 2000) to drive CINs to action potential threshold (Fig. 1A, “NaP”). Therefore, because NaP is a regenerative current, while HCN is a restorative current, we would expect the current responses to an oscillating voltage command to depend strongly on whether the membrane voltage is clamped above or below -60 mV (Beatty et al., 2015). We therefore held mouse CINs in whole-cell voltage clamp ($n = 10$ neurons, $N = 4$ mice), first at -55 mV, and subjected them

to a voltage command that was composed of a continuous sequence of sinusoidal cycles with an amplitude of 2 mV and a frequency that increases discretely from 0.2 to 20 Hz. The current amplitude was very small at low frequencies and increased monotonically to the high frequency (Fig. 1B, black), which is suggestive of an impedance curve with amplified lower frequencies. Loosely speaking, this means that the CINs’ “input resistance” to a low frequency oscillatory input currents is boosted. As expected (Fig. 1C, black), the impedance curve, $|Z(f)|$, exhibited an amplifying structure, with the phase delay being strictly positive. In order to quantify the degree of amplification, we fit the model of the phase delay, ϕ_s , for an iso-potential cell with quasi-linear properties (see Materials and Methods). In this fit, there is a (negative) amplifying parameter, μ_n – that is derived from biophysical properties of amplifying current (e.g., the slope of the activation curve, reversal potential, etc.) (Goldberg et al., 2007) – which was estimated to be $\mu_n = -3.9$.

When neurons were held at -70 mV ($n = 10$ neurons, $N = 7$ mice), the current response was very different, it was much larger at subhertz frequencies as compared to the experiment at -55 mV, and then exhibited what looks like a slightly decreasing amplitude for frequencies near 1 Hz, followed by an amplitude increase at higher frequencies (Fig. 1B, red). Estimation of the amplitude response and phase delay (Fig. 1C, red), revealed significantly different curves (amplitude: $P = 6 \cdot 10^{-25}$, phase: $P = 1 \cdot 10^{-17}$, ANCOVA) with a clear resonance peak at approximately 1 Hz, and a zero crossing at a slightly lower frequency (Fig. 1C, red). Fitting the somatic phase delay yielded a reasonable fit only when both an amplifying and resonant component were included in the fit with the amplifying parameter ($\mu_n = -3.4$) being only slightly reduced relative to the -55 mV fit. In contrast, the additional (positive) resonance parameter, μ_h – which is derived from the biophysical properties of the restorative current – was estimated to be $\mu_h = 1.6$ (see Materials and Methods) based on the fit to the negative lobe in the phase delay.

The previous experiments suggest that the amplifying effect at -55 mV occurs due to prominence of the amplifying NaP current in that voltage range, whereas the resonance visible at -70 mV is due to the HCN current that dominates that voltage range. This conclusion was supported by the fact that application of $1 \mu\text{M}$ TTX ($n = 9$ neurons, $N = 4$ mice), that abolished autonomous spiking and slightly hyperpolarized the CIN (Fig. 2A), reduced the impedance ($P = 9 \cdot 10^{-9}$) and exhibited a trend toward a shortened phase delay ($P = 0.137$, ANCOVA, Fig. 2B). These changes are captured by the amplification parameter being estimated as less negative ($\mu_n = -2.0$), which reduces ϕ_s (Eq. 4, in Materials and

Methods). Similarly, application of 10 μ M ZD7288, the selective HCN antagonist ($n = 9$ neurons, $N = 7$ mice), which abolished the sag response (Fig. 2C), abolished the resonance peak in the impedance curve ($P = 4 \cdot 10^{-16}$) and significantly reduced the negative lobe in the phase delay ($P = 0.018$, ANCOVA, Fig. 2D), which was captured by the resonance parameter being reduced to $\mu_h = 0.5$.

Figure 2: Amplification is caused by NaP currents, whereas the resonance is caused by HCN currents. **A.** TTX (blue) prevents autonomous spiking (black). **B.** TTX prevents amplification of the impedance (left), and reduces the phase shift (right). **C.** ZD7288 (red) abolishes the voltage sag (black). **D.** ZD7288 abolishes the resonance peak in the impedance, and the negative phase shifts in the subhertz range. Solid lines are parameter fits as in Fig. 1.

Optogenetic interrogation of the spatial distribution of NaP and HCN currents in the CIN dendritic arbor

While the above experiments demonstrate the NaP and HCN currents are capable of transforming subthreshold voltage fluctuations, the question remains as to where within the CIN's somatodendritic compartments these currents perform their amplifying and restorative actions, respectively. One extreme scenario is that they are restricted to the axosomatic region (where they are needed for sustaining the autonomous firing patterns of the CINs). In that scenario, the dendrites could be entirely linear, passively transmitting the distal depolarizations to the soma. Only at the soma are the inputs then transformed by these currents. However, a more realistic scenario is that these currents are also expressed dendritically and exert their nonlinear influence on synaptic inputs more distally. But in this scenario, we would still want to know where along the dendrites the currents are expressed.

To determine the precise location of these channels would require advanced *in situ* molecular and anatomical techniques and/or direct electrophysiological recordings from CIN dendrites in conjunction with advanced imaging techniques. While these experiments may, in principle, be done, we wondered whether the optogenetics-based technique that we recently developed to interrogate the role of dendrites in synaptic integration (Tiroshi and Goldberg, 2019) could help us address this question.

We previously showed that the impact of dendrites on synaptic integration can be quantified by studying the response of neurons that express ChR2 post-synaptically to (470 nm) illumination of their dendritic arbor. In particular, we compared between two spatial patterns of illumination of the somatodendritic arbor of SNr neurons: either a small perisomatic region (approximately 100 μ m in diameter) or the entire dendritic arbor. By using sinusoidally-modulated blue LED illumination at various temporal frequencies, we were able to calculate the phase delays produced by both spatial patterns, and found that illumination of the entire dendritic arbor introduced larger phase delays. In order to quantify the effect, we fit the data to a tractable theoretical model of a semi-infinite cable (Goldberg et al., 2007; Tiroshi and Goldberg, 2019). As mentioned above, in the case of the SNr neuron, we found that the dendrites were well-fit by a passive linear cable model, whose parameters (i.e., time and space constants) we could estimate. The conclusion that SNr dendrites were largely linear was further supported by the finding that these phase delays were voltage-independent (Tiroshi and Goldberg, 2019).

Because CINs exhibit prominent amplifying and resonating currents that are strongly voltage dependent (Figs. 1 & 2), we posited that by using the same optogenetic technique and semi-infinite cable model we would be able to quantify the contribution of CIN dendrites to post-synaptic integration. In particular, we hypothesized that by fitting a *quasi-linear* cable model we would be able to quantify to what degree CIN dendrites *per se* possess amplifying or resonating properties (See Materials and Methods). Finally, by comparing illumination of the proximal vs. the entire dendritic arbor, we could learn something about the localization of the nonlinearities along the dendritic arbor. To this end, we crossed mice that express Cre-recombinase under a ChAT promoter with the Ai32 mouse that expresses ChR2 and EYFP in a Cre-dependent manner (see Materials and Methods). The cholinergic neuropil and individual CINs could be clearly visualized in the dorsal striatum of these ChAT-ChR2 mice (Fig. 3A). Individual CINs were patched and recorded in voltage clamp, while illuminating

223 either the proximal region with a 60X water-immersion objective (Fig. 3A) or the entire slice
 224 with a 5X air objective, with a continuous sequence of sinusoidally modulated illumination
 225 waveforms at various frequencies (Fig. 3B, blue).

Figure 3: Optogenetic interrogation of the quasilinear properties of CIN dendrites indicates that dendritic nonlinearities are more prominent proximally. **A.** A CIN in a sagittal slice from ChAT-ChR2 mouse is patch-clamped in the whole-cell mode while either a small proximal region around the soma or the full-field are illuminated with a sinusoidally modulated 470 nm LED. **B.** The current response to the proximal (black) and full-field (red) illumination differ, with the phase of the full-field illumination right-shifted at the higher frequencies (20 Hz is highlighted in yellow). **C.** Phase shifts at -55 mV holding potential, calculated for proximal (black) and full-field illumination (red). A tendency towards negative phase shifts is present at 0.4 Hz (green arrow). **D.** Phase shifts at -70 mV holding potential, exhibit a negative phase shift. Fitting the quasi-linear model at -55 mV demonstrate the magnitude of the amplification parameter (μ_n) is smaller for the full field fit (Eqs. 1-3). Similarly, at -70 mV the resonance parameter (μ_h) is smaller for the full field fit. The elevation in these parameters' magnitudes when illuminating proximally relative to full-field suggest that the surface densities of NaP and HCN currents is higher proximally.

226 Comparison of the somatic current traces in response to proximal (Fig. 3B, black) vs.
 227 full-field (red) illumination demonstrated that the phase of the full-field-generated current is
 228 delayed relative to the proximally-generated current. This could be observed in the raw data
 229 both for the low and high (yellow inset) frequencies. The effect at the high frequency was
 230 very evident when plotting the phase delay curves both when the CINs were held at -55 mV

(Fig. 3C) and at -70 mV (Fig. 3D). Estimation of the phase delays revealed delays that were considerably larger than those observed with the electrical somatic stimulation (Fig. 1). Thus, to account for these large delays (Figs. 3C&D), we fit a model of the sum of a somatic delay, ϕ_s (Eq. 4, in Materials and Methods), and a dendritic delay, ϕ_d (Eq. 1, in Materials and Methods). For the curve calculated at the -55 mV holding potential ($n = 5$ neurons, $N = 4$ mice), we fit a model with only an amplifying current. As expected, the fits were significantly different ($P = 4 \cdot 10^{-3}$, ANCOVA) and extracted a larger effective radius (in units of space constants) of $r = 1.47$ for the full field stimulation as compared to $r = 0.79$ (See Materials and Methods). Additionally, the amplifying parameter was more negative ($\mu_n = -0.32$) for the proximal fit than for the full field fit ($\mu_n = -0.15$). At face value, this latter finding means that the amplifying nonlinearity appears denser when activating only the proximal arbor, than when activating the entire arbor. In other words, this finding suggests that the NaP currents are denser more proximally and probably drop off farther out in the CIN dendrites.

A closer look at the fits (at the -55 mV holding potential) reveals that the phase delay at 0.4 Hz (for both the proximal and full field illumination), is not captured by this model. This dip in phase delay (Fig. 3C, green arrow) at this low frequency is reminiscent of what a restorative current is expected to do. In order to accentuate the effect of the restorative HCN currents, we repeated the experiment at the -70 mV holding potential (Fig. 3D). In this case ($n = 14$ neurons, $N = 10$ mice), the phase delays at the lower frequencies (especially at 0.4 Hz) were negative, which was reminiscent of the results from the electrical voltage stimulation experiments (Fig. 1) at the -70 mV holding potential.

When fitting a full soma-plus-dendrite model (incorporating both amplifying and restorative parameters) the fits were significantly different ($P = 8 \cdot 10^{-4}$, ANCOVA) and the differences between the estimates of the effective radii were larger: $r = 1.4$ for the full-field vs. $r = 0.41$ for the proximal illumination. Here too the restorative parameter was estimated to be larger for the proximal fit ($\mu_h = 1.11$) relative to the full field fit ($\mu_h = 0.61$), suggesting that the HCN currents are denser more proximally. This conclusion is buttressed by the fact that the negative phase delays in the subhertz frequency range tended to be less negative for the full-field illumination ($P = 0.037$, Wilcoxon rank sum test on phase delays at 0.4 Hz). In the framework of our quasi-linear model, the only mathematically possible way to recreate this finding while increasing the effective radius r is by reducing the restorative parameter μ_h .

Otherwise, if μ_h were constant then illuminating a larger region of the cable – by increasing r – would necessarily recruit more restorative current, thereby making the negative delays more negative. Thus, the conclusion of our analysis is that CIN dendrites indeed express the amplifying and resonating nonlinearities, which can contribute to post-synaptic integration. Moreover, the analysis strongly suggests that these nonlinearities are concentrated proximally and likely taper off distally. In the following section, we provide independent evidence in support of this conclusion vis-à-vis NaP currents.

Figure 4: Autonomous action potentials actively back-propagate in CIN dendrites up to 70 μm from the soma. **A.** Autonomous discharge of a CIN that was patched and filled with fluo-4 and Alexa Fluor 568 for 2PLSM imaging (image). **B.** Line scans at various distances from the soma exhibit Ca^{2+} oscillations caused by bAPs. **C.** Calculating the spike-triggered average of these oscillations and fitting an alpha-function gives an estimate of the amplitude of these oscillations (in % $\Delta F/F_0$). **D.** The scatter plot of these amplitudes as a function of distance from the soma (11 CINs from 7 mice are pooled) exhibits a large degree of variability. However, a 35 μm moving average (black line) exhibits that the Ca^{2+} transients begin to decay approximately 70 μm from the soma, indicating that bAPs are supported by Nav channels up to that point (the initial increase up to 20 μm is due to the increase in the surface-to-volume ratio in the large proximal dendrites).

Distance of dendritic bAP invasion indicates location of amplifying Nav channels

The persistent and fast-inactivating Nav currents flow through the same Nav channels (Alzheimer et al., 1993). Therefore, determining to what distance from the soma dendritic bAPs invade provides a measure of the Nav channels density – and therefore where the NaP

current is present – in the dendritic arbor. To this end, we used 2PLSM Ca^{2+} imaging to measure the size of the Ca^{2+} transients elicited by bAPs in autonomously firing CINs at various distances from the soma (Fig. 4A). We conducted line scans ($n = 11$ neurons, $N = 7$ mice) to measure the Ca^{2+} signals at various distances from the soma (Fig. 4B). Next, we estimated the size of the spike triggered average (STA) of the $\Delta F/F_0$ Ca^{2+} signal, by averaging around spontaneous APs measured at the soma (Fig. 4C). The amplitude of the STA was estimated by fitting it with an alpha-function (see Materials and Methods). A scatter plot of STA amplitude vs. distance from soma demonstrates a large degree of variability (Fig. 4D). Nevertheless, applying a 35 μm moving average to the scatter plot reveals a trapezoidal dependence of Ca^{2+} transient sizes (Fig. 4D, black). The initial dip in the size of the transients at short distances from the soma result from the large size of the proximal dendrites (Fig. 4D), which reduces the size of the Ca^{2+} transient due to the low surface-to-volume ratio of the thick proximal dendrites. Neglecting that effect, we found that the size of the bAP driven Ca^{2+} transients remains constant up to approximately 70 μm from the soma, and then drops off. This spatial dependence of the Ca^{2+} transient amplitude, suggests that the bAP maintains a constant amplitude due to the presence of Nav channels that sustain their regenerative nature out to some 70 μm from the soma. Farther out the Ca^{2+} transients decrease presumably due to a drop off in Nav channel expression, which leads to a lower amplitude bAPs, and hence less Ca^{2+} via voltage-activated Ca^{2+} channels. Thus, this measurement strengthens the conclusion that NaP currents are present in CIN dendrites – primarily in proximal dendrites (up to approximately 70 μm from the soma).

NaP currents boost proximal thalamic inputs but not distal cortical inputs to CINs

The above data indicate that Nav channels are preferentially expressed in proximal dendrites. Therefore, their ability to boost small voltage perturbations, including synaptic inputs, should be restricted to proximal dendrites. Because thalamic inputs terminate proximally, whereas cortical inputs terminate distally (Doig et al., 2014; Lapper and Bolam, 1992; Thomas et al., 2000), we would expect only the former to be boosted by Nav current. To test this, we transcranially inoculated the PfN of Vglut2-ires-Cre mice with adeno-associated viruses (AAVs) harboring Cre-dependent ChR2 and EYFP genes (Fig. 5A) (Aceves Buendia et al., 2019; Rehani et al., 2019). Two weeks later, we tested the sensitivity of optogenetically-evoked EPSPs to ranolazine (30 μM), a selective antagonist of the NaP current, in CINs patched in acute striatal slices. The mean amplitude of the EPSP was

reduced by a median of 16% ($n = 11$ neurons, $N = 4$ mice, $P = 0.04$, Wilcoxon signed-rank test, Fig. 5B). In order to test whether the more distal cortical inputs are modulated by NaP, we used the Thy1-ChR2 mice (Aceves Buendia et al., 2019; Arenkiel et al., 2007) that expressed ChR2 in corticostriatal fibers (Fig. 5C). The EPSPs elicited by cortical inputs to CINs in these mice were unaffected by ranolazine ($n = 11$ neurons, $N = 6$ mice, $P = 0.74$, Wilcoxon signed-rank test, Fig. 5D). Thus, the post-synaptic modulation of glutamatergic inputs to CINs by NaP currents corresponds to the spatial distribution of these currents as derived from our analysis of the quasi-linear properties of CINs and 2PLSM imaging of bAPs.

Figure 5: Thalamo – but not cortical EPSPs – onto CINs are boosted by NaP currents in healthy mice. **A.** The parafascicular nucleus (Pfn) of Vglut2-Cre mice was inoculated with AAVs harboring Cre-dependent ChR2, so that 470 nm LED illumination of striatal slices activated Pfn terminals while CINs were patched in current clamp mode. **B.** Optogenetically evoked thalamic EPSPs in CINs (held between -50 mV and -60 mV) were reduced by $30 \mu\text{M}$ ranolazine. **C.** CINs were patched in current clamp in Thy1-ChR2 mice, so that 470 nm LED illumination of striatal slices activated nominally cortical terminals. **D.** Optogenetically evoked cortical EPSPs were unaffected by ranolazine.

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Figure 6: TONs, but not SPNs, exhibit a pause-like response to slow-wave events occurring during natural non-REM sleep in non-human primates (NHPs). **A.** Top: simultaneous recording of LFP and a TAN in an NHP during N2 and N3 stages of sleep. Bottom: simultaneous recording of LFP and an SPN in an NHP during N2 and N3 stages of sleep. **B.** Top: TAN firing rate conditioned on the occurrence of an LFP slow wave event (28,603 triggers). Middle: Average striatal LFP signal triggered on the occurrence of slow wave events (see Materials and Methods). Bottom: SPNs firing rate conditioned on the occurrence of slow wave event (6,065 triggers). Gray box indicates the 99% confidence intervals.

316 Tonically active neurons in non-human primates exhibit a pause-like response to slow wave
 317 oscillations during sleep

318 Our data, alongside previous studies (Beatty et al., 2015), demonstrate that
 319 somatodendritic HCN currents give rise to resonances in the subhertz range in CIN surface
 320 membranes. In contrast, SPNs exhibit no resonance (Beatty et al., 2015). This suggests CINs
 321 in intact animals may exhibit increased sensitivity to oscillatory inputs in that frequency
 322 range, whereas SPNs should not. Delta waves in the electroencephalogram (EEG) and in
 323 cortical and sub-cortical local field potentials (LFP) are prominent during non-REM sleep
 324 across species (Brown et al., 2012; Liu and Dan, 2019), including in non-human primates
 325 (NHPs) (Mizrahi-Kliger et al., 2018). Because the LFP is widely thought to represent

subthreshold cellular activity, affected by afferent and recurrent synaptic inputs (Buzsáki et al., 2012), we hypothesized that the tonically active neurons (TANs) in the NHP striatum – that are comprised primarily of CINs (Aosaki et al., 1995; Kawaguchi, 1993; Wilson et al., 1990) – will exhibit stronger entrainment to slow-wave LFP events during sleep, while SPNs under the same conditions will not. Indeed, triggering the spike trains of TANs and SPNs (from $N = 2$ NHPs) on slow wave events that occur during sleep stages N2 and N3 (Mizrahi-Kliger et al., 2018; Riedner et al., 2007), which represent relatively deep non-REM sleep, demonstrates that the firing rates of TANs ($n = 122$) is modulated by these slow-wave events, whereas the firing rate of SPNs ($n = 83$) is not. Importantly, the biphasic response exhibited by TANs to slow-wave events bears a striking resemblance to the “classical” TAN response to external cues (Aosaki et al., 1995; Apicella et al., 1991; Joshua et al., 2008; Kimura et al., 1984). Because these responses in awake primates require an intact thalamic projection (Bradfield et al., 2013; Goldberg and Reynolds, 2011; Matsumoto et al., 2001; Schulz and Reynolds, 2013; Smith et al., 2004; Yamanaka et al., 2018) (and there is no reason to assume that this is altered in sleep), they provide support to the hypothesis that the CIN membrane resonance contributes to a thalamically-driven biphasic CIN response during slow-wave sleep. Importantly, because the primates are asleep in a sound-proof room, this is, to the best of our knowledge, the first report of striatal TANs responding to an internally-generated brain event, and not an external saliency-related cue.

Discussion

Quasi-linear dendritic properties of CINs and the non-uniform distribution of their active conductances

In the current study, we applied a cable-theoretic and optogenetics-based formalism – that we developed previously to study dendritic properties of SNr neurons (Tiroshi and Goldberg, 2019) – to study the dendritic properties of CINs. Unlike SNr neurons that were found to act like passive linear cables exhibiting no voltage dependent properties, we find that CIN dendrites exhibit both amplification and resonances in a voltage dependent manner: amplification is more prominent at depolarized subthreshold potentials (approximately -55 mV), whereas resonances are more prominent at more hyperpolarized potentials (approximately -70 mV). Moreover, we found that our method is able to provide information about where in the dendritic arbor these quasi-linear properties are localized. We extracted boosting (μ_n) and resonance (μ_h) parameters for proximal vs. full-field illumination, and

found that the magnitude of both parameters is smaller when the entire dendritic arbor is illuminated. In other words, activating more of the distal dendritic membrane dilutes the amplifying and resonating effects, meaning that the additional membrane illuminated in the full-field condition contributed less to amplification and resonances. The simplest interpretation of this finding is that the active conductances that give rise to amplification and resonance are more highly expressed proximally and apparently taper off farther out in the dendritic arbor. We identified the relevant currents by studying the quasi-linear properties of CIN somata with voltage perturbations. We found that amplification is TTX sensitive and therefore arises from Nav channels, that give rise to the NaP current. Similarly, we found that resonances require HCN channels.

We used two indirect methods to validate the preferentially proximal localization of NaP currents. First, we implemented a widely-used approach of estimating the distance of dendritic invasion of bAPs by imaging the Ca^{2+} influx that accompanies them (Carter and Sabatini, 2004; Day et al., 2008; Goldberg et al., 2009; Kerr, 2004; Rehani et al., 2019; Tanimura et al., 2016). We found – not unlike in SPN neurons – that bAPs maintain their amplitude out to 70 μm from the soma and then begin to decay, suggesting that Nav currents that support bAP propagation taper off from that point onwards. Future work can verify this by directly testing where locally puffed TTX attenuates the bAP (perhaps even with the use of voltage sensitive probes). Second, we demonstrated that only thalamic – but not cortical – EPSPs exhibit sensitivity to the selective NaP blocker. Because the thalamic terminals are located more proximally (Lapper and Bolam, 1992; Thomas et al., 2000), this provides further evidence that NaP currents are more prominent locally. The preferential localization of HCN currents proximally in CIN dendrites requires additional validation, particularly because other neurons exhibit an opposite pattern (Berger et al., 2001). However, we were less interested in validating the proximal localization of HCN currents, because unlike NaP currents, activation of HCNs is conditioned on CINs being actively (after-)hyperpolarized, during which bAPs are unlikely to occur. We did however test a context in which HCN currents, and the resonance they underlie, are likely to affect CIN collective dynamics. We found that TANs respond biphasically to internally-generated slow wave activity during sleep. This preferential entrainment is consistent with the fact that CIN membranes exhibit a resonance in the subhertz range as found by us and others (Beatty et al., 2015). The elevated impedance (or frequency dependent access resistance) in this range means that inputs fluctuating in this range will more efficiently depolarize the CINs and therefore more likely to trigger additional action potentials than inputs fluctuating at a frequency that is far from

the resonance frequency. Accordingly, SPNs that do not exhibit resonances (Beatty et al., 2015) are not entrained by the slow wave oscillation. Our findings in sleeping NHPs are at odds with a rodent study by Sharott and collaborators (Sharott et al., 2012) that found only a weak association between cortical slow-wave activity and the discharge of TANs in anesthetized mice. Moreover, they reported a stronger association of SPNs with the slow wave activity. These differences may be attributable to species differences and/or differences between natural sleep and anesthesia.

Advantages and limitations of the quasi-linear formalism and the optogenetics-based approach

As mentioned in the introduction, the quasi-linear approach provides a tool to characterize membrane excitability in general functional terms, without requiring the complete biophysical characterization of membrane currents. Unfortunately, the method is not invertible: even after estimating all the quasilinear parameters one cannot deduce the full biophysical characterization of the underlying channels even if one has a precise mathematical model of these channels. Moreover, even though we extracted the values of μ_n and μ_h , we do not know how to translate them into a quantitative measure of channel density, or have a proper explanation as to why the somatic values of these parameters (estimated with voltage perturbations) are larger than the dendritic ones. Still, the ability to compare parameters for various spatial illumination patterns enables us to reach qualitative conclusions regarding relative channel density. Additionally, our attribution of μ_n to NaP and μ_h to HCN is only provisional. CINs possess other channels that have a major influence on their firing patterns such as A-type, inward rectifying, and various Ca^{2+} -activated K^+ channels, to mention a few (Bennett et al., 2000; Deng et al., 2007; Goldberg et al., 2009; Goldberg and Wilson, 2010, 2005; McGuirt et al., 2021; Oswald et al., 2009; Song and Surmeier, 1996; Wilson, 2005; Wilson and Goldberg, 2006), that were not included in our analysis. Future work, should elaborate how these and other channels contribute to the quasi-linear properties of CINs and their dendrites.

Using optogenetics provides another level of practicality. The seminal studies that characterized the filtering properties of dendrites or pyramidal neurons (Berger et al., 2001; Goldberg et al., 2007; Hutcheon et al., 1996; Ulrich, 2002) required dual soma-dendrite patching which is not practical for all neuronal types whose dendrites taper off rapidly. Expressing opsins in the membrane being activated, as we did, means that a single somatic

patch electrode can suffice to conduct the quasi-linear characterization. We currently use this approach to illuminate large regions of the dendritic arbor simultaneously, which means we can only derive large-scale dendritic properties. In the future, localized laser stimulation of dendritic regions (visualized with the help of a fluorescent marked in the patch pipette), or even two-photon laser activation of opsin (provided one can find stimulation parameters that do not harm the dendrites) could be used as an alternative approach. Another point to consider is that the temporal kinetics of the opsin also introduces additional linear filtering of the input (Higgs and Wilson, 2017; Tiroshi and Goldberg, 2019). However, by demonstrating differential responses to various spatial patterns we circumvent this concern. On the backdrop of optogenetics being utilized almost exclusively to study circuit mapping (Häusser, 2021; Kim et al., 2017) by expressing opsins presynaptically, we believe our study joins other studies (Higgs and Wilson, 2017; Tiroshi and Goldberg, 2019) to underscore the value of using opsins expressed postsynaptically to study neuronal – even dendritic – excitability.

Somatic amplification and resonance in CINs

We preceded our optogenetic characterization of dendritic nonlinearities with the electrical characterization of quasilinear somatic properties using a sinusoidal voltage command applied through the patch pipette. As previously reported by Beatty and collaborators (Beatty et al., 2015), CIN somata exhibit a membrane resonance in the subhertz range, that depends on the holding potential. However, while Beatty and collaborators found that an amplified resonance is present in the more depolarized (approximately -55 mV) range, we found that the resonance is only pronounced in the more hyperpolarized (approximately -70 mV) range, where it depends on the HCN current. It is possible that the resonances observed by Beatty and collaborators (2015) at -55 mV arose from ineffective clamping of dendrites with the slightly higher resistance electrodes used in that study. In support of this proposition, we also found evidence for the resonance arising from unclamped (dendritic) membranes. When the CINs were clamped at -55 mV, while somatic voltage perturbations did not produce a resonance, optogenetic stimulation (both proximal and full field) at 0.4 Hz produced a more negative phase than at the neighboring 0.2 Hz and 0.6 Hz stimulation (Fig. 3C, green arrow). This downward deflection in phase is reminiscent of the full flown negative subhertz region that occurred with optogenetic stimulation when the CINs were clamped at -70 mV. Thus, it is likely that resonance originated in both studies from dendritic HCN currents. Because in the study of Beatty and collaborators (Beatty et al., 2015)

TTX lowered the impedance, without removing the subhertz resonance peak, we conclude that the effect of TTX in both studies is to remove the NaP-dependent boosting, without directly affecting resonance.

Dendritic contribution to capacity of CINs to differentiate between their excitatory inputs

In NHPs, TANs that are comprised primarily of CINs (Aosaki et al., 1995; Goldberg and Wilson, 2010; Kawaguchi, 1993; Wilson et al., 1990), encode – through a brief pause in their tonic firing – external stimuli that are salient (and often unexpected, even conveying a stop or behavioral shift signal (Aoki et al., 2018; Thorn and Graybiel, 2010)) or stimuli that are associated with reward (Apicella et al., 1991; Goldberg and Reynolds, 2011; Kimura et al., 1984). The pause response requires an intact projection from the parafascicular nucleus (Pfn) of the thalamus (Bradfield et al., 2013; Goldberg and Reynolds, 2011; Matsumoto et al., 2001; Schulz et al., 2011; Schulz and Reynolds, 2013; Smith et al., 2004; Yamanaka et al., 2018), indicating that TANs are attuned to thalamic input. While TANs do not respond to ongoing movement (Aosaki et al., 1994; Apicella et al., 1991; Kimura et al., 1984; Raz et al., 1996), and hence seem less attuned to sensorimotor cortical input (Sharott et al., 2012), CIN activity in awake behaving mice is strongly modulated by self-initiated movements (Gritton et al., 2019; Howe et al., 2019; Yarom and Cohen, 2011). Nevertheless, slice physiology studies in rodents (Aceves Buendia et al., 2019; Assous, 2021; Assous et al., 2017; Ding et al., 2010; Johansson and Silberberg, 2020; Kosillo et al., 2016; Threlfell et al., 2012) clearly show that thalamic inputs to CINs are stronger in the sense that they give rise to larger EPSPs (Johansson and Silberberg, 2020) and can trigger a pause-like responses (Ding et al., 2010), whereas cortical inputs to CINs are weaker in that they give rise, in acute slices, to smaller EPSPs and cannot trigger the pause-like response – although this distinction is less pronounced in anesthetized rodents (Doig et al., 2014). Thus, it is clear that CINs differentiate between these two excitatory inputs and respond differently to them. A major contributor to the CINs' capacity to dissociate thalamic and cortical input is the differential distribution of their respective terminals on the dendritic arbor: thalamic input terminate perisomatically and on proximal dendrites, whereas cortical input terminates on distal dendrites (Doig et al., 2014; Lapper and Bolam, 1992; Thomas et al., 2000). Our finding that the higher expression of NaP currents in proximal dendrites preferentially boosts thalamic over cortical inputs, suggests that active conductances are expressed in CIN dendrites in a manner that

corresponds and reinforces the effect of the spatial separation between the terminals of the two inputs.

Adaptive changes in dendritic excitability in movement disorders

The capacity of the CIN dendritic excitability to mirror the distribution of afferent terminals is also an adaptive process. We previously reported that CIN dendritic excitability is elevated in the Q175 mouse model of Huntington's disease (HD) (Tanimura et al., 2016). In contrast to control (Thy1-ChR2) mice in which cortical EPSPs in CINs are insensitive to ranolazine (Fig. 5), cortical excitatory postsynaptic currents (EPSCs) in CINs from Q175 mice (crossed with Thy1-ChR2 mice) are strongly reduced by ranolazine. The acquired dependence of distal cortical inputs on NaP currents in Q175 mice results from an upregulation of the NaP current, which was also evidenced by bAPs invading farther out into the CINs' dendritic arbor (Tanimura et al., 2016). This elevated excitability in the Q175 mouse is probably a homeostatic response aimed at elevating the postsynaptic sensitivity to the remaining synaptic contents after the loss of afferent cortical and thalamic inputs observed in HD mouse models (Deng et al., 2013). Because thalamostriatal inputs are also altered in models of Parkinson's disease (PD) (Aceves Buendia et al., 2019; Parker et al., 2016; Tanimura et al., 2019), future work should determine whether the excitability of CIN dendrites is altered in these models, as well. Importantly, dendrites are positioned at the interface between synaptic inputs and the intrinsic properties of CIN. While changes in synaptic transmission and intrinsic excitability of CINs have received attention in models of PD, HD and other movement disorders (Abudukeyoumu et al., 2019; Aceves Buendia et al., 2019; Choi et al., 2020; Ding et al., 2006, 2011; Eskow Jaunarajs et al., 2015; Mallet et al., 2019; Paz et al., 2021; Pisani et al., 2007; Plotkin and Goldberg, 2019; Poppi et al., 2021; Tanimura et al., 2019; Tubert and Murer, 2021), studying alterations in dendritic excitability in these models is a complementary approach that remains a largely uncharted territory.

The membrane channels that are expressed in CINs (e.g., Nav, HCN, Kv4, etc.) are targets for neuromodulation under normal, healthy circumstances (Deng et al., 2007; Helseth et al., 2021; Maurice, 2004; Song and Surmeier, 1996). Therefore, it is likely that the dendritic excitability of CINs can be modulated under physiological conditions, as well, as a way to adjust the tuning of CINs to their afferent inputs. Conversely, if boosting and resonances are present primarily proximally, the selective tuning of CINs to subhertz slow-wave oscillations is probably stronger for thalamic inputs (that terminate proximally) than for

cortical inputs. Future experiments can address this issue by testing how silencing the PfN affects the observed entrainment of TANs to slow-waves (Fig. 6).

In summary, we have used a new optogenetics-based approach, complemented by other electrophysiological and imaging approaches, to demonstrate that the spatial localization of active dendritic conductances that endow CIN dendrites with quasi-linear filtering properties corresponds to the spatial distribution of their two main afferent excitatory inputs. This matching up of presynaptic terminals with post-synaptic excitability, probably contributes to the capacity of CINs to respond differentially to cortical and thalamic inputs. Sensorimotor information of cortical origin seems to be integrated continuously in a moment-by-moment fashion. In contrast, thalamic inputs lead to abrupt pauses in TAN firing, even in response to internally-generated brain-states (e.g., during slow-wave activity). The fact that dendritic arbors adapt to the spatiotemporal patterns of afferent inputs is likely an important principle of neural computation that deserves further attention, particularly in the framework of autonomously active neurons such as CINs, SNr neurons and other basal ganglia pacemakers.

Materials and Methods

Ethics statement: All experimental protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and with the Hebrew University guidelines for the use and care of laboratory animals in research. The experiments adhered to, received prior written approval and were supervised by the Institutional Animal Care and Use Committee of the Faculty of Medicine.

Mice: Homozygous Ai32 (RCL-ChR2(H134R)/EYFP) mice (The Jackson laboratory [Jax] stock: 012569) that express floxed ChR2 and an EYFP fusion protein under the CAG promoter were crossed with homozygous ChAT-IRES-Cre (Δ neo) mice that express Cre recombinase under the choline acetyltransferase promoter (Jax stock: 031661). The ChAT-ChR2 offspring (4-8 weeks old/both sexes) were used for the majority of experiments. To investigate corticostriatal transmission, we used homozygous transgenic Thy1-ChR2 mice (B6.Cg-Tg (Thy1-COP4/EYFP) 18Gfng/1, Jax stock: 007612), that express ChR2 under the Thy1 promoter in cortical neurons (Arenkiel et al., 2007). To investigate thalamostriatal transmission, we used Vglut2-ires-Cre mice (Jax stock: 016963).

Non-Human Primates (NHPs): Data were obtained from two healthy, young adult, female
vivet monkeys. The monkeys were habituated to sleeping in a primate chair, positioned in a
dark, double-walled sound-attenuating room. The primate chair restrained the monkeys' hand
and body movements but otherwise allowed them to be in a position similar to their natural
(sitting) sleeping posture. Detailed sleep habituation, surgery and sleep staging were reported
previously (Mizrahi-Kliger et al., 2018). For extracellular recordings, the monkeys' heads
were immobilized with a head holder and eight glass-coated tungsten microelectrodes were
advanced the dorsal striatum. Electrical signals were amplified with a gain of 20, filtered
using a 0.075 Hz (2 pole) to 10 kHz (3 pole) Butterworth filter and sampled at 44 kHz by a
16-bit analog/digital converter. Spiking activity was sorted online using a template matching
algorithm. The striatum was identified based on its stereotaxic coordinates according to MRI
imaging and primate atlas data (Martin and Bowden, 2000). Tonically active neurons (TANs)
and spiny projection neurons (SPNs) were identified using real-time assessment of their
electrophysiological features. Spiking and local field potentials (LFP) were recorded only for
identified recording sites with stable recording quality (i.e., where single-neuron spiking
yielded an average isolation score ≥ 0.85).

We followed established procedures for slow wave detection in the LFP (Riedner et al.,
2007). Briefly, the LFP signal was filtered at the 0.5-4 Hz range and putative slow wave events
whose duration was 0.25-2 seconds were kept for further analysis. Next, the slow wave peaks
were sorted according to their amplitude. Artifacts were removed by discarding all events
whose amplitude exceeded 5 standard deviations above the mean. Finally, conditional firing
rate analysis was only performed on 30% of the slow wave events with the highest amplitude.

Stereotaxic viral inoculation in Vglut2-ires-Cre mice: Mice were deeply anesthetized with
isoflurane in a non-rebreathing system (2.5% induction, 1-1.5% maintenance) and placed in a
stereotaxic frame (Kopf Instruments, Tujunga, CA). Temperature was maintained at 35°C
with a heating pad, artificial tears were applied to prevent corneal drying, and animals were
hydrated with a bolus of injectable saline (10 ml/kg) mixed with analgesic (2.5 mg/kg
Meloxicam). Stereotaxic injections into caudal intralaminar nuclei of thalamus were
performed under aseptic conditions. Adeno-associated viruses (AAV) serotype 9 carrying
double-floxed fusion genes for hChR2 (E123A) and EYFP under an EF1a promoter
(University of Pennsylvania Vector Core, Addgene #35507) were used to transfect Pfn
neurons. Injection coordinates were from Bregma: lateral, 0.65 mm; posterior, 2.3 mm; and
3.35 mm depth from surface of brain (Rehani et al., 2019). A small hole was bored into the

skull with a micro drill bit and a glass pipette was slowly inserted at the PfN coordinates. To minimize backflow, solution was slowly injected; a total volume of 280 nl ($>2.5 \times 10^{12}$ GC/ml) of the AAV constructs was injected over a period of approximately 1.5 min and the pipette was left in place for 5 min before slowly retracting it. Slice physiology experiments were conducted 2-3 weeks after surgery.

Slice preparation: Mice were deeply anesthetized with ketamine (200 mg/kg)–xylazine (23.32 mg/kg) and perfused transcardially with ice-cold-modified artificial cerebrospinal fluid (ACSF) bubbled with 95% O₂–5% CO₂, and containing (in mM) 2.5 KCl, 26 NaHCO₃, 1.25 Na₂HPO₄, 0.5 CaCl₂, 10 MgSO₄, 0.4 ascorbic acid, 10 glucose and 210 sucrose. The brain was removed, and 275 μ m thick sagittal slices containing the striatum were cut in ice-cold-modified ACSF. Slices were then submerged in ACSF, bubbled with 95% O₂–5% CO₂, containing (in mM) 2.5 KCl, 126 NaCl, 26 NaHCO₃, 1.25 Na₂HPO₄, 2 CaCl₂, 2 MgSO₄ and 10 glucose, and stored at room temperature for at least 1 h prior to recording.

Electrophysiological recording: The slices were transferred to the recording chamber mounted on a Zeiss Axioskop fixed-stage microscope and perfused with oxygenated ACSF at 31°C. During the optogenetic stimulation experiments, in order to guarantee that the effects we measured were generated post-synaptically, the ACSF solution contained (in μ M) 10 DNQX to block AMPA receptors, 50 D-APV to block NMDA receptors, 10 gabazine (SR95531) to block GABA_A receptors, and 2 CGP55845 to block GABA_B receptors. An Olympus 60X, 0.9 NA water-immersion objective with a 26.5 mm field number (FN) was used to examine the slice using standard infrared differential interference contrast video microscopy. Patch pipette resistance was typically 4-5 M Ω when filled with recording solutions. The junction potential estimated at 7-8 mV was not corrected. The intracellular solution contained (in mM) 135.5 KCH₃SO₃, 5 KCl, 2.5 NaCl, 5 Na-phosphocreatine, 10 HEPES, 0.2 EGTA, 0.21 Na₂GTP, and 2 Mg_{1.5}ATP, pH 7.3 with KOH (280–290 mOsm/kg). In the Ca²⁺ imaging experiments (see below) the internal solution contained (in mM) 135 K-gluconate, 5 KCl, 2.5 NaCl, 5 Na-phosphocreatine, 10 HEPES, 0.1 fluo-4 (Molecular Probes), 0.1 Alexa Fluor 568 (for morphological visualization, Molecular Probes), 0.21 Na₂GTP, and 2 Mg_{1.5}ATP, pH 7.3 with KOH (280–290 mOsm/kg). Electrophysiological recordings were obtained with a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Signals were filtered at 10 kHz online, digitized at 10 or 20 kHz and logged onto a personal computer with the Signal 6 software (Cambridge Electronic Design, Cambridge,

UK). All reagents, drugs and toxins were purchased from either Merck/Sigma-Aldrich (Darmstadt, Germany), Tocris Biosciences (Bristol, UK) or HelloBio (Bristol, UK).

Voltage-perturbation experiments: CINs were held at either -55 mV or -70 mV and were given an 83 second-long voltage command structured as a concatenated sequence of sinusoids from a discrete set of frequencies ranging from 0.2 to 20 Hz with an amplitude of 2 mV (3 or 5 seconds per frequency, such that each frequency was represented by an integer multiple of its fundamental period). Phase shifts between the voltage sinusoidal and the somatic current response were determined by the location of the peak in the cross-correlation function (CCF) of the two traces (whose units are mV·pA), for each stimulation frequency and for each illumination condition. The impedance at each frequency, $|Z(f)|$, was calculated from the maximal amplitude of the CCF as $|Z(f)| = (2 \text{ mV})^2 / \max(\text{CCF})$ (so that its units are $\text{G}\Omega$).

Optogenetic stimulation: Optogenetic stimulation was performed with blue-light (470 nm) LED illumination via the objective (Mightex, Toronto, ON, Canada). We used two spatial illumination regimes: a) *proximal illumination* wherein an opaque disk with a central pinhole was placed in the back focal plane of the 60X water-immersion objective such that a ~ 130 μm diameter region around the soma was illuminated (Tiroshi and Goldberg, 2019), thereby targeting the soma and proximal dendrites; and b) *full-field illumination* of the entire slice with a 5X air objective which excites the soma and the entire dendritic field. In all experiments, LED light intensity was chosen such that stimulation generated comparable current responses for both regimes. We used the same sequence of sinusoids described above, only this time the voltage driving the LED was modulated (the minimal voltage was the LED's voltage threshold, 40 mV). The phase delays were again calculated according to the latency of the peak of the CCF between the LED voltage command and somatic current. Note, that the phases were corrected by 0.5 (or by π in radians) due to the fact that the ChR2 inward current is in antiphase with the LED's voltage command.

Two-photon laser scanning microscopy (2PLSM): The two-photon excitation source was a Chameleon Vision II tunable Ti:Sapphire pulsed laser (Coherent, Santa Clara, CA, USA) tuned to 820 nm. The images were collected with the Femto2D system (Femtonics, Budapest, Hungary) which includes two 3 mm galvo-scanners, one gated GaAsP and one multi-alkaline non-descanned photomultiplier tube for imaging fluo-4 and Alexa Fluor, respectively. Z-stacks of optical sections (spaced 2 μm apart) were collected using 0.2 μm pixels and 15 μs

dwelt times. Optical and electrophysiological data were obtained using the software package MES (Femtonics), which also integrates the control of all hardware units in the microscope. The software automates and synchronizes the imaging signals and electrophysiological protocols. Data was extracted from the MES package to personal computers using custom-made code in MATLAB. We recorded spontaneously occurring bAPs with line scans at various distances measured radially from the tip of the soma. Spike triggered averages of the calcium measurements ($\Delta F/F_0$) were estimated and an alpha-function was fit to them. The value of the peak of the fitted alpha-function was used as a measure of the size of the spontaneous bAP at that location.

Drugs and Reagents: TTX was used to block voltage-activated Na^+ currents. Ranolazine was used to block NaP. ZD7288 was used to block the HCN current. Gabazine (SR-95531) and CGP 55845 were used to block GABA_A and GABA_B receptors, respectively. All reagents, drugs and toxins were purchased from either Merck/Sigma-Aldrich (Darmstadt, Germany), Tocris Biosciences (Bristol, UK) or HelloBio (Bristol, UK).

Data analysis and statistics: Data were analyzed and curve fitting was performed using custom code in Matlab (MathWorks, Natick, MA, USA).

Parameter fitting to the phase delay: In our previous study (Tiroshi and Goldberg, 2019), we modeled the dendritic arbor as a semi-infinite cable with a homogeneous quasi-linear membrane (i.e., the current density of each nonlinearity is constant along the dendrite). When a segment of length r (measured in units of the dendrite's space constant) from the soma is activated with a sinusoidal current injection (to model the 470 nm illumination of the ChR2-laden dendrites, which activates the inward current carried by the ChR2), the dendritic phase delay is given by

$$\phi_d = \frac{1}{2\pi} \left(\tan^{-1} \frac{q}{p} - \tan^{-1} \frac{\sin qr}{e^{pr} - \cos qr} \right) \quad (\text{Eq. 1})$$

where

$$p = \sqrt{\frac{\sqrt{\alpha^2 + \beta^2} + \alpha}{2}} \quad q = \sqrt{\frac{\sqrt{\alpha^2 + \beta^2} - \alpha}{2}} \quad (\text{Eq. 2})$$

α and β are functions of frequency and are determined by the linearization of the dendritic nonlinearities as explained in Goldberg et al. (2007) and Remme and Rinzel (2011), with a negative amplifying parameter, μ_n , and a positive resonance parameter, μ_h .

$$\alpha(f) = \gamma_R + \frac{\mu_n}{1 + (2\pi f \tau_n)^2} + \frac{\mu_h}{1 + (2\pi f \tau_h)^2} \quad (\text{Eq. 3a})$$

$$\beta(f) = 2\pi f \left[\tau - \frac{\mu_n \tau_n}{1 + (2\pi f \tau_n)^2} - \frac{\mu_h \tau_h}{1 + (2\pi f \tau_h)^2} \right] \quad (\text{Eq. 3b})$$

Additional parameters include total dendritic conductance (relative to leak), γ_R , the membrane time constant τ , and the time constants representing the kinetics of the nonlinear dendritic conductances, as explained in Goldberg et al. (2007) and Remme and Rinzel (2011). In some cases we only used the amplifying parameter in the fit (e.g., Fig. 1C).

Using the same formalism it is easy to show that for the (isopotential) soma with a quasi-linear membrane the phase delay is given by

$$\phi_s = \frac{1}{2\pi} \tan^{-1} \frac{\beta}{\alpha} \quad (\text{Eq. 4})$$

corresponding to an amplitude that is equal to $(\alpha^2 + \beta^2)^{-1/2}$ up to a scaling factor. In the main text, we point out that when μ_n becomes less negative ϕ_s is reduced. This is because when μ_n becomes less negative α is increased (Eq. 3a) and β is decreased (Eq. 3b). Thus, when we fit only somatic delays we use ϕ_s , but when we fit the dendritic delays we use the sum of $\phi_s + \phi_d$, because the dendritic perturbation as recorded by the intracellular electrode is filtered both by the dendritic and the somatic (quasi-linear) membranes.

Statistics: Null hypotheses were rejected if the P-value was below 0.05. The nonparametric two-tailed Wilcoxon signed-rank test was used for matched samples and the Wilcoxon rank-sum test was used for independent samples. The parametric ANCOVA test was used to test significant changes in the amplitude and phase curves as a function of the natural logarithm of the frequencies (an operation which spreads out this independent parameter more uniformly). Null hypotheses were rejected if the P-value was below 0.05.

For the TAN and SPN locking to slow wave peak analysis, confidence intervals (at P-value of 0.01) were calculated based on the distribution of conditional firing rates 1 s before and after the slow wave peak.

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Competing interests

The authors declare no competing interests.

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