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.

Introduction

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

Second, even in the absence of afferent input, CINs exhibit multiple autonomously generated discharge patterns – including regular and irregular pacemaking, as well as burst firing (Bennett et al., 2000; Bennett and Wilson, 1999; Goldberg and Reynolds, 2011; Goldberg and Wilson, 2010). These firing patterns are generated by an interplay between various nonlinear ionic currents including voltage- and calcium-activated K^+ currents, as well as two voltage-dependent pacemaker currents: the hyperpolarization-activated cyclic nucleotide-gated (HCN) current; and the persistent Na^+ (NaP) current (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). The main purpose of these pacemaker currents presumably is to guarantee the ongoing release of ACh onto the striatal microcircuitry by sustaining the autonomous discharge of CINs. Nevertheless, these pacemaker (and other subthreshold) currents will also impact how 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 (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.

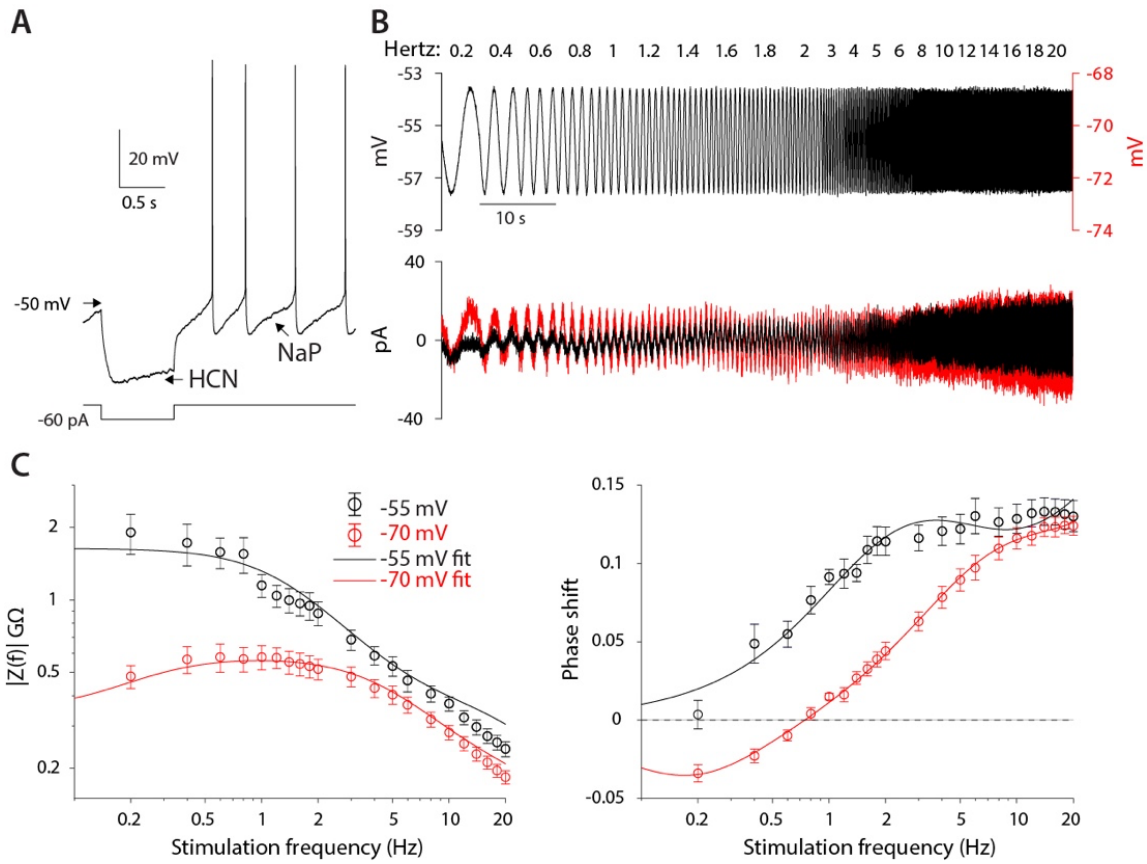

Figure 1: CIN membranes exhibit voltage-dependent quasi-linear properties. **A.** CINs exhibit a voltage sag due to HCN currents, and autonomous pacemaking due to NaP currents. **B.** Application of 2 mV sinusoidal voltage commands to the soma, of increasing frequencies, elicits a current response that 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

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

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

163 The previous experiments suggest that the amplifying effect at –55 mV occurs due to
164 prominence of the amplifying NaP current in that voltage range, whereas the resonance
165 visible at –70 mV is due to the HCN current that dominates that voltage range. This
166 conclusion was supported by the fact that application of 1 μM TTX ($n = 9$ neurons, $N = 4$
167 mice), that abolished autonomous spiking and slightly hyperpolarized the CIN (Fig. 2A),
168 reduced the impedance ($P = 9 \cdot 10^{-9}$) and exhibited a trend toward a shortened phase delay (P
169 $= 0.137$, ANCOVA, Fig. 2B). These changes are captured by the amplification parameter
170 being estimated as less negative ($\mu_n = -2.0$), which reduces ϕ_s (Eq. 4, in Materials and

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

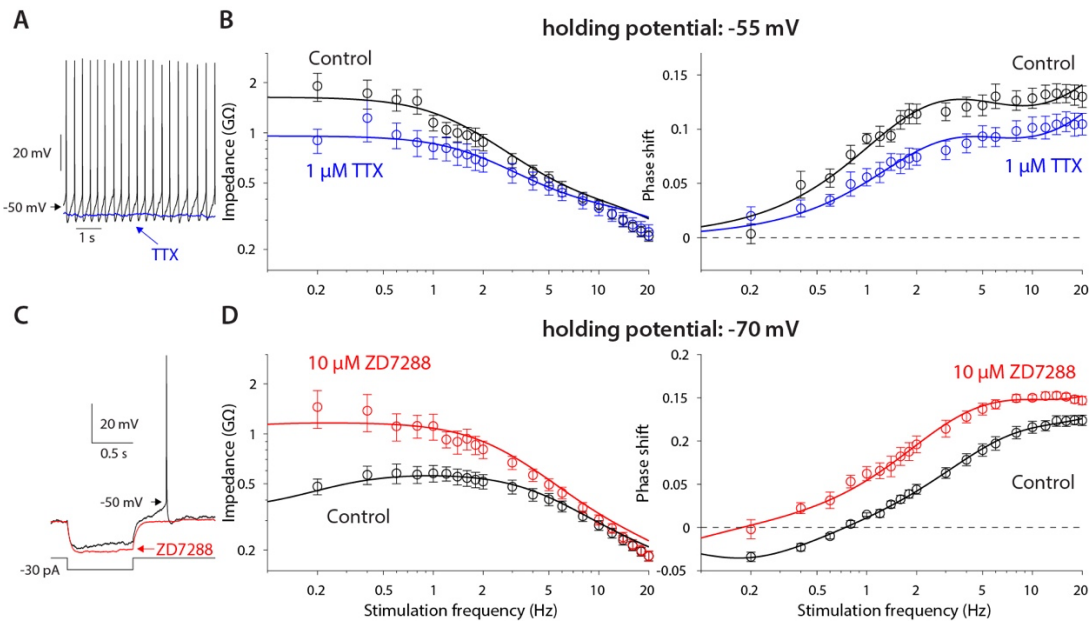

Figure 2: Amplification is caused by NaP currents, whereas the resonance is caused by HCN currents. A. TTX (blue) prevents autonomous spiking (black). **B.** TTX prevents amplification of the impedance (left), and reduces the phase shift (right). **C.** ZD7288 (red) abolishes the voltage sag (black). **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.

176 <u>Optogenetic interrogation of the spatial distribution of NaP and HCN currents in the CIN</u>
177 <u>dendritic arbor</u>

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

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

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

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

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

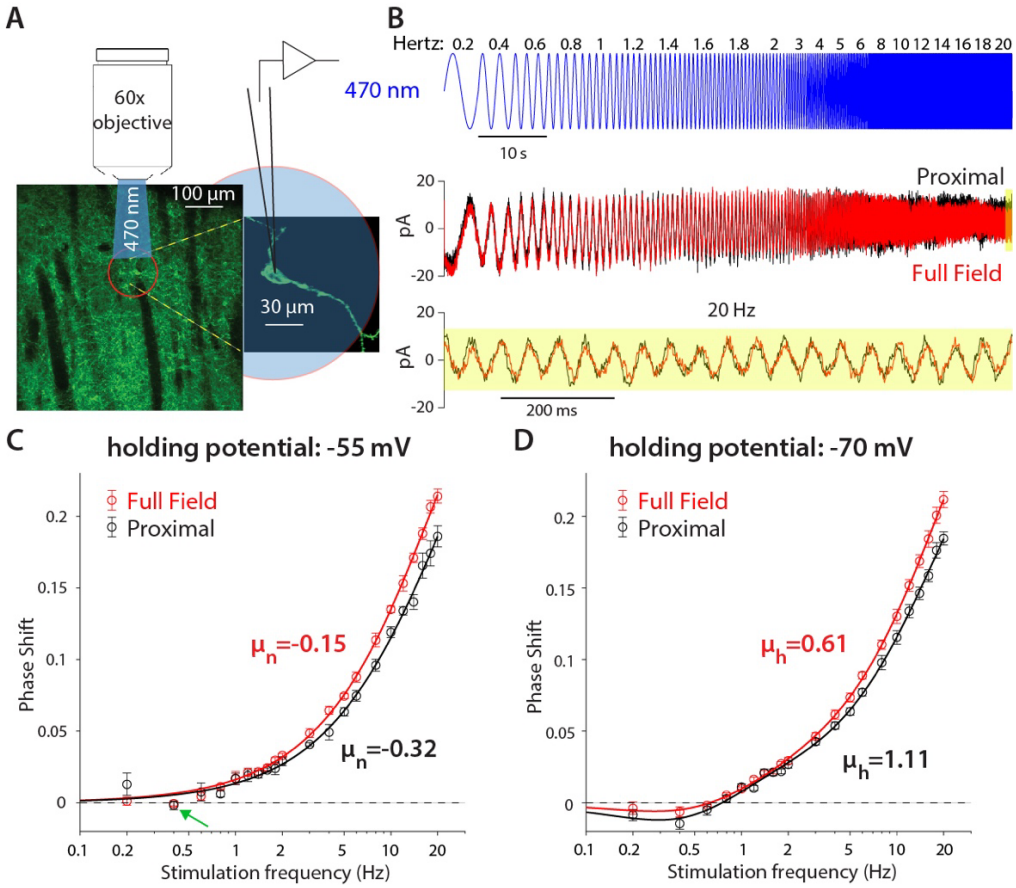

Figure 3: Optogenetic interrogation of the quasilinear properties of CIN dendrites indicates that dendritic nonlinearities are more prominent proximally. A. A CIN in a sagittal slice from ChAT-ChR2 mouse is patch-clamped in the whole-cell mode while either a small proximal region around the soma or the 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.

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

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

242 A closer look at the fits (at the –55 mV holding potential) reveals that the phase delay
243 at 0.4 Hz (for both the proximal and full field illumination), is not captured by this model.
244 This dip in phase delay (Fig. 3C, green arrow) at this low frequency is reminiscent of what a
245 restorative current is expected to do. In order to accentuate the effect of the restorative HCN
246 currents, we repeated the experiment at the –70 mV holding potential (Fig. 3D). In this case
247 ($n = 14$ neurons, $N = 11$ mice), the phase delays at the lower frequencies (especially at 0.4
248 Hz) were negative, which was reminiscent of the results from the electrical voltage
249 stimulation experiments (Fig. 1) at the –70 mV holding potential.

250 When fitting a full soma-plus-dendrite model (incorporating both amplifying and
251 restorative parameters) the fits were significantly different ($P = 8 \cdot 10^{-4}$, ANCOVA) and the
252 differences between the estimates of the effective radii were larger: $r = 1.4$ for the full-field
253 *vs.* $r = 0.41$ for the proximal illumination. Here too the restorative parameter was estimated
254 to be larger for the proximal fit ($\mu_h = 1.11$) relative to the full field fit ($\mu_h = 0.61$), suggesting
255 that the HCN currents are denser more proximally. This conclusion is buttressed by the fact
256 that the negative phase delays in the subhertz frequency range tended to be less negative for
257 the full-field illumination ($P = 0.037$, Wilcoxon rank sum test on phase delays at 0.4 Hz). In
258 the framework of our quasi-linear model, the only mathematically possible way to recreate
259 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.

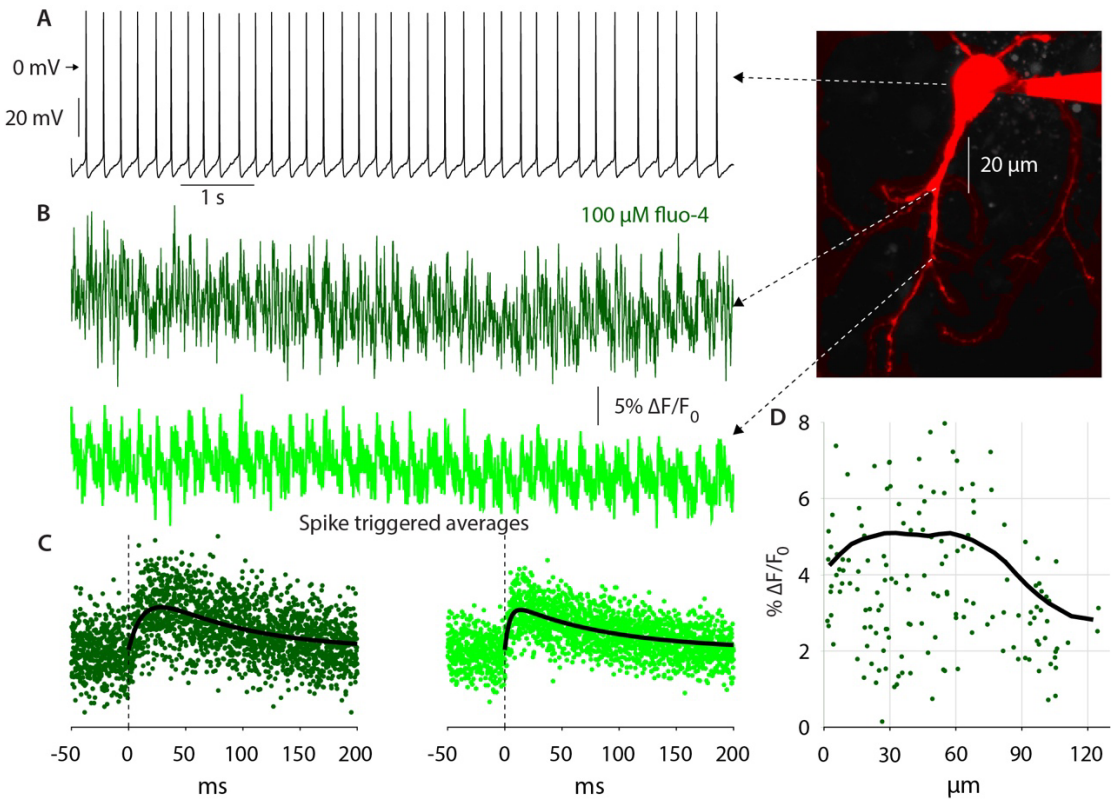

Figure 4: Autonomous action potentials actively back-propagate in CIN dendrites up to 70 µm from the soma. A. Autonomous discharge of a CIN that was patched and filled with fluo-4 and Alexa Fluor 568 for 2PLSM imaging (image). **B.** Line scans at various distances from the soma exhibit Ca^{2+} oscillations 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 % $\Delta F/F_o$). **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).

<u>Distance of dendritic bAP invasion indicates location of amplifying Nav channels</u>

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

271 current is present – in the dendritic arbor. To this end, we used 2PLSM Ca^{2+} imaging to
272 measure the size of the Ca^{2+} transients elicited by bAPs in autonomously firing CINs at
273 various distances from the soma (Fig. 4A). We conducted line scans (*n = 11* neurons, *N = 7*
274 mice) to measure the Ca^{2+} signals at various distances from the soma (Fig. 4B). Next, we
275 estimated the size of the spike triggered average (STA) of the $\Delta F/F_0$ Ca^{2+} signal, by
276 averaging around spontaneous APs measured at the soma (Fig. 4C). The amplitude of the
277 STA was estimated by fitting it with an alpha-function (see Materials and Methods). A scatter
278 plot of STA amplitude *vs.* distance from soma demonstrates a large degree of variability (Fig.
279 4D). Nevertheless, applying a 35 µm moving average to the scatter plot reveals a trapezoidal
280 dependence of Ca^{2+} transient sizes (Fig. 4D, black). The initial dip in the size of the transients
281 at short distances from the soma result from the large size of the proximal dendrites (Fig.
282 4D), which reduces the size of the Ca^{2+} transient due to the low surface-to-volume ratio of the
283 thick proximal dendrites. Neglecting that effect, we found that the size of the bAP driven
284 Ca^{2+} transients remains constant up to approximately 70 µm from the soma, and then drops
285 off. This spatial dependence of the Ca^{2+} transient amplitude, suggests that the bAP maintains
286 a constant amplitude due to the presence of Nav channels that sustain their regenerative
287 nature out to some 70 µm from the soma. Farther out the Ca^{2+} transients decrease presumably
288 due to a drop off in Nav channel expression, which leads to a lower amplitude bAPs, and
289 hence less Ca^{2+} via voltage-activated Ca^{2+} channels. Thus, this measurement strengthens the
290 conclusion that NaP currents are present in CIN dendrites – primarily in proximal dendrites
291 (up to approximately 70 µm from the soma).

292 <u>NaP currents boost proximal thalamic inputs but not distal cortical inputs to CINs</u>

293 The above data indicate that Nav channels are preferentially expressed in proximal
294 dendrites. Therefore, their ability to boost small voltage perturbations, including synaptic
295 inputs, should be restricted to proximal dendrites. Because thalamic inputs terminate
296 proximally, whereas cortical inputs terminate distally (Lapper and Bolam, 1992; Thomas et
297 al., 2000), we would expect only the former to be boosted by Nav current. To test this, we
298 transcranially inoculated the PfN of Vglut2-ires-Cre mice with adeno-associated viruses
299 (AAVs) harboring Cre-dependent ChR2 and EYFP genes (Fig. 5A) (Aceves Buendia et al.,
300 2019; Rehani et al., 2019). Two weeks later, we tested the sensitivity of optogenetically-
301 evoked EPSPs to ranolazine (30 µM), a selective antagonist of the NaP current, in CINs
302 patched in acute striatal slices. The mean amplitude of the EPSP was reduced by a median of

303 16% (*n = 11* neurons, *N = 4* mice, *P = 0.04*, Wilcoxon signed-rank test, Fig. 5B). In order to
304 test whether the more distal cortical inputs are modulated by NaP, we used the Thy1-ChR2
305 mice (Aceves Buendia et al., 2019; Arenkiel et al., 2007) that expressed ChR2 in
306 corticostriatal fibers (Fig. 5C). The EPSPs elicited by cortical inputs to CINs in these mice
307 were unaffected by ranolazine (*n = 11* neurons, *N = 6* mice, *P = 0.74*, Wilcoxon signed-rank
308 test, Fig. 5D). Thus, the post-synaptic modulation of glutamatergic inputs to CINs by NaP
309 currents corresponds to the spatial distribution of these currents as derived from our analysis
310 of the quasi-linear properties of CINs and 2PLSM imaging of bAPs.

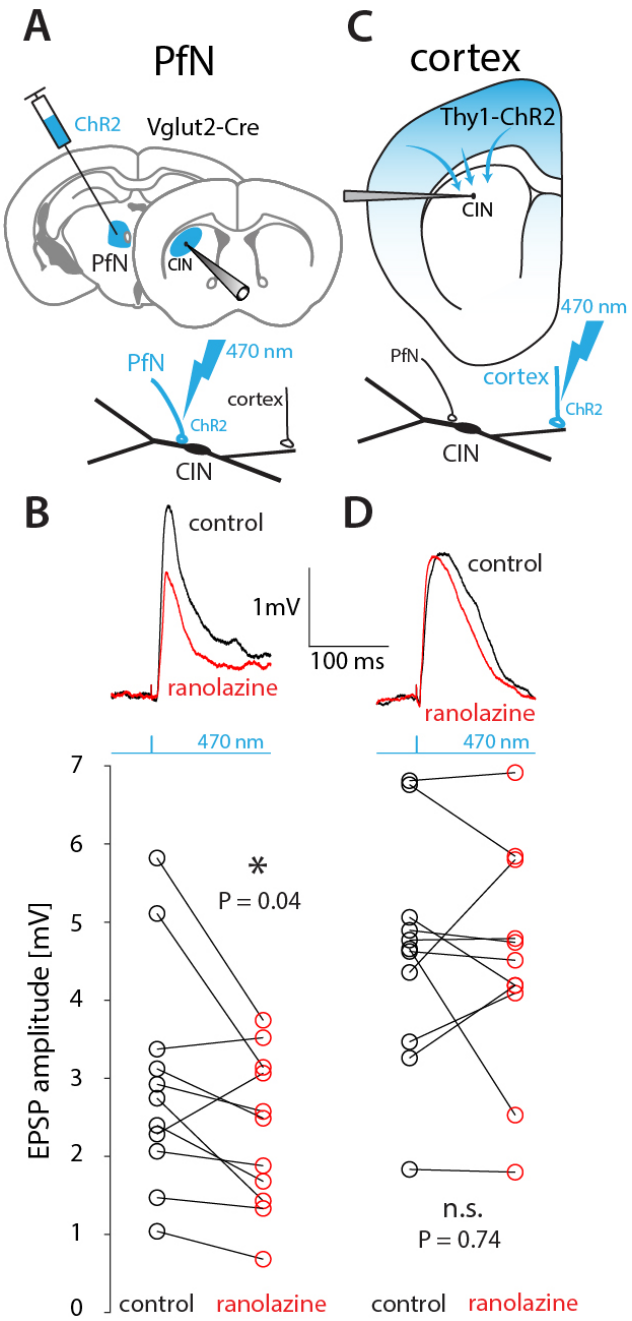

Figure 5: Thalamic – but not cortical EPSPs – onto CINs are boosted by NaP currents in healthy mice. A. The parafascicular nucleus (PfN) of Vglut2-Cre mice was inoculated with AAVs harboring Cre-dependent ChR2, so that 470 nm LED illumination of striatal slices activated PfN terminals while CINs were patched in current clamp mode. **B.** Optogenetically evoked thalamic EPSPs in CINs (held between –50 mV and –60 mV) were reduced by 30 µM 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.

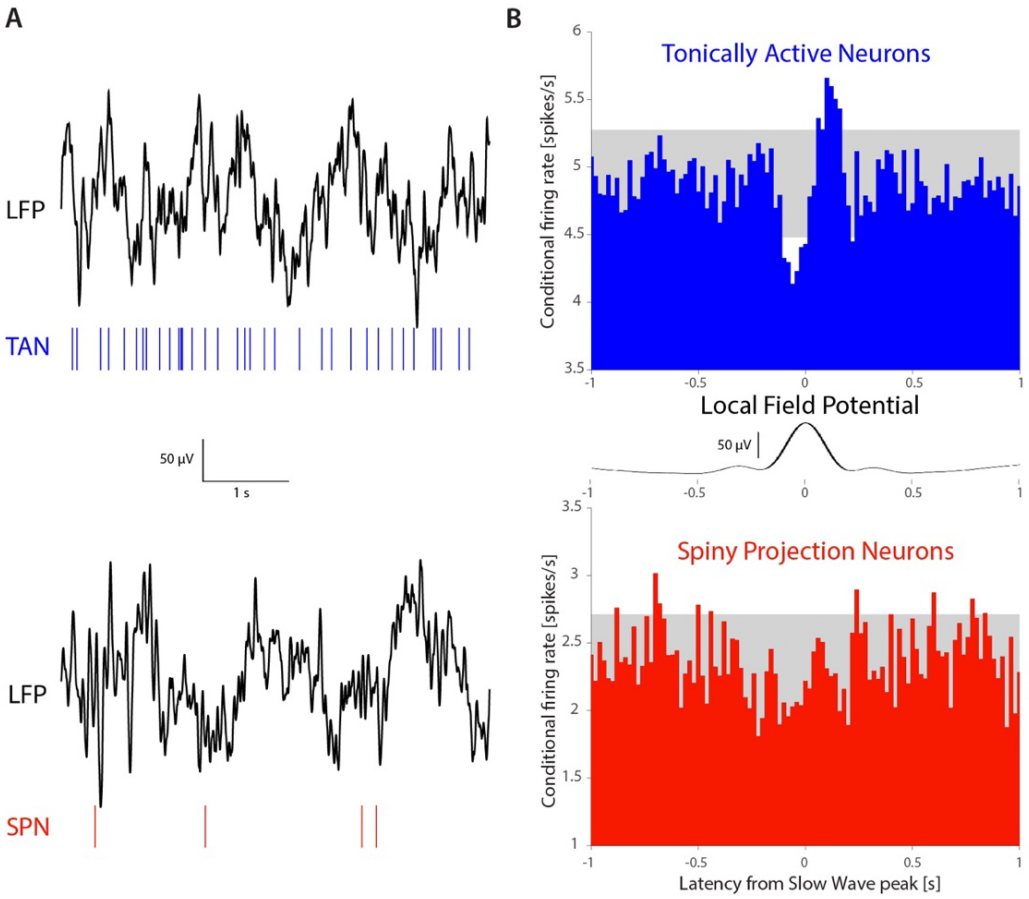

Figure 6: TANs, but not SPNs, exhibit a pause-like response to slow-wave events occurring during natural non-REM sleep in non-human primates (NHPs). A. Top: simultaneous recording of LFP and a TAN in an NHP during N2 and N3 stages of sleep. Bottom: simultaneous recording of LFP and an SPN in an NHP during 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.

<u>Tonically active neurons in non-human primates exhibit a pause-like response to slow wave oscillations during sleep</u>

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

354 found that the magnitude of both parameters is smaller when the entire dendritic arbor is
355 illuminated. In other words, activating more of the distal dendritic membrane dilutes the
356 amplifying and resonating effects, meaning that the additional membrane illuminated in the
357 full-field condition contributed less to amplification and resonances. The simplest
358 interpretation of this finding is that the active conductances that give rise to amplification and
359 resonance are more highly expressed proximally and apparently taper off farther out in the
360 dendritic arbor. We identified the relevant currents by studying the quasi-linear properties of
361 CIN somata with voltage perturbations. We found that amplification is TTX sensitive and
362 therefore arises from Nav channels, that give rise to the NaP current. Similarly, we found that
363 resonances require HCN channels.
364 We used two indirect methods to validate the preferentially proximal localization of
365 NaP currents. First, we implemented a widely-used approach of estimating the distance of
366 dendritic invasion of bAPs by imaging the Ca^{2+} influx that accompanies them (Carter and
367 Sabatini, 2004; Day et al., 2008; Goldberg et al., 2009; Kerr, 2004; Rehani et al., 2019;
368 Tanimura et al., 2016). We found – not unlike in SPN neurons – that bAPs maintain their
369 amplitude out to 70 µm from the soma and then begin to decay, suggesting that Nav currents
370 that support bAP propagation taper off from that point onwards. Future work can verify this
371 by directly testing where locally puffed TTX attenuates the bAP (perhaps even with the use
372 of voltage sensitive probes). Second, we demonstrated that only thalamic – but not cortical –
373 EPSPs exhibit sensitivity to the selective NaP blocker. Because the thalamic terminals are
374 located more proximally (Lapper and Bolam, 1992; Thomas et al., 2000), this provides
375 further evidence that NaP currents are more prominent locally. The preferential localization
376 of HCN currents proximally in CIN dendrites requires additional validation, particularly
377 because other neurons exhibit an opposite pattern (Berger et al., 2001). However, we were
378 less interested in validating the proximal localization of HCN currents, because unlike NaP
379 currents, activation of HCNs is conditioned on CINs being actively (after-)hyperpolarized,
380 during which bAPs are unlikely to occur. We did however test a context in which HCN
381 currents, and the resonance they underlie, are likely to affect CIN collective dynamics. We
382 found that TANs respond biphasically to internally-generated slow wave activity during
383 sleep. This preferential entrainment is consistent with the fact that CIN membranes exhibit a
384 resonance in the subhertz range as found by us and others (Beatty et al., 2015). The elevated
385 impedance (or frequency dependent access resistance) in this range means that inputs
386 fluctuating in this range will more efficiently depolarize the CINs and therefore more likely
387 to trigger additional action potentials than inputs fluctuating at a frequency that is far from

388 the resonance frequency. Accordingly, SPNs that do not exhibit resonances (Beatty et al.,
389 2015) are not entrained by the slow wave oscillation. Our findings in sleeping NHPs are at
390 odds with a rodent study by Sharott and collaborators (Sharott et al., 2012) that found only a
391 weak association between cortical slow-wave activity and the discharge of TANs in
392 anesthetized mice. Moreover, they reported a stronger association of SPNs with the slow
393 wave activity. These differences may be attributable to species differences and/or differences
394 between natural sleep and anesthesia.

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

397 As mentioned in the introduction, the quasi-linear approach provides a tool to
398 characterize membrane excitability in general functional terms, without requiring the
399 complete biophysical characterization of membrane currents. Unfortunately, the method is
400 not invertible: even after estimating all the quasilinear parameters one cannot deduce the full
401 biophysical characterization of the underlying channels even if one has a precise
402 mathematical model of these channels. Moreover, even though we extracted the values of μ_n
403 and μ_h, we do not know how to translate them into a quantitative measure of channel density,
404 or have a proper explanation as to why the somatic values of these parameters (estimated
405 with voltage perturbations) are larger than the dendritic ones. Still, the ability to compare
406 parameters for various spatial illumination patterns enables us to reach qualitative
407 conclusions regarding relative channel density. Additionally, our attribution of μ_n to NaP and
408 μ_h to HCN is only provisional. CINs possess other channels that have a major influence on
409 their firing patterns such as A-type, inward rectifying, and various Ca^{2+}-activated K^+
410 channels, to mention a few (Bennett et al., 2000; Deng et al., 2007; Goldberg et al., 2009;
411 Goldberg and Wilson, 2010, 2005; McGuirt et al., 2021; Oswald et al., 2009; Song and
412 Surmeier, 1996; Wilson, 2005; Wilson and Goldberg, 2006), that were not included in our
413 analysis. Future work, should elaborate how these and other channels contribute to the quasi-
414 linear properties of CINs and their dendrites.
415 Using optogenetics provides another level of practicality. The seminal studies that
416 characterized the filtering properties of dendrites or pyramidal neurons (Berger et al., 2001;
417 Goldberg et al., 2007; Hutcheon et al., 1996; Ulrich, 2002) required dual soma-dendrite
418 patching which is not practical for all neuronal types whose dendrites taper off rapidly.
419 Expressing opsins in the membrane being activated, as we did, means that a single somatic

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

433 Somatic amplification and resonance in CINs

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

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

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

456 In NHPs, TANs that are comprised primarily of CINs (Aosaki et al., 1995; Goldberg
457 and Wilson, 2010; Kawaguchi, 1993; Wilson et al., 1990), encode – through a brief pause in
458 their tonic firing – external stimuli that are salient (and often unexpected, even conveying a
459 stop or behavioral shift signal (Aoki et al., 2018; Thorn and Graybiel, 2010)) or stimuli that
460 are associated with reward (Apicella et al., 1991; Goldberg and Reynolds, 2011; Kimura et
461 al., 1984). The pause response requires an intact projection from the parafascicular nucleus
462 (PfN) of the thalamus (Bradfield et al., 2013; Goldberg and Reynolds, 2011; Matsumoto et
463 al., 2001; Schulz et al., 2011; Schulz and Reynolds, 2013; Smith et al., 2004; Yamanaka et
464 al., 2018), indicating that TANs are attuned to thalamic input. While TANs do not respond to
465 ongoing movement (Aosaki et al., 1994; Apicella et al., 1991; Kimura et al., 1984; Raz et al.,
466 1996), and hence seem less attuned to sensorimotor cortical input (Sharott et al., 2012), CIN
467 activity in awake behaving mice is strongly modulated by self-initiated movements (Gritton
468 et al., 2019; Howe et al., 2019; Yarom and Cohen, 2011). Nevertheless, slice physiology
469 studies in rodents (Aceves Buendia et al., 2019; Assous, 2021; Assous et al., 2017; Ding et
470 al., 2010; Johansson and Silberberg, 2020; Kosillo et al., 2016; Threlfell et al., 2012) clearly
471 show that thalamic inputs to CINs are stronger in the sense that they give rise to larger EPSPs
472 (Johansson and Silberberg, 2020) and can trigger a pause-like responses (Ding et al., 2010),
473 whereas cortical inputs to CINs are weaker in that they give rise to smaller EPSPs and cannot
474 trigger the pause-like response. Thus, it is clear that CINs differentiate between these two
475 excitatory inputs and respond differently to them. A major contributor to the CINs' capacity
476 to dissociate thalamic and cortical input is the differential distribution of their respective
477 terminals on the dendritic arbor: thalamic input terminate perisomatically and on proximal
478 dendrites, whereas cortical input terminates on distal dendrites (Lapper and Bolam, 1992;
479 Thomas et al., 2000). Our finding that the higher expression of NaP currents in proximal
480 dendrites preferentially boosts thalamic over cortical inputs, suggests that active
481 conductances are expressed in CIN dendrites in a manner that corresponds and reinforces the
482 effect of the spatial separation between the terminals of the two inputs.

483

484 Adaptive changes in dendritic excitability in movement disorders

485 The capacity of the CIN dendritic excitability to mirror the distribution of afferent
486 terminals is also an adaptive process. We previously reported that CIN dendritic excitability
487 is elevated in the Q175 mouse model of Huntington's disease (HD) (Tanimura et al., 2016).
488 In contrast to control (Thy1-ChR2) mice in which cortical EPSPs in CINs are insensitive to
489 ranolazine (Fig. 5), cortical excitatory postsynaptic currents (EPSCs) in CINs from Q175
490 mice (crossed with Thy1-ChR2 mice) are strongly reduced by ranolazine. The acquired
491 dependence of distal cortical inputs on NaP currents in Q175 mice results from an
492 upregulation of the NaP current, which was also evidenced by bAPs invading farther out into
493 the CINs' dendritic arbor (Tanimura et al., 2016). This elevated excitability in the Q175
494 mouse is probably a homeostatic response aimed at elevating the postsynaptic sensitivity to
495 the remaining synaptic contents after the loss of afferent cortical and thalamic inputs
496 observed in HD mouse models (Deng et al., 2013). Because thalamostriatal inputs are also
497 altered in models of Parkinson's disease (PD) (Aceves Buendia et al., 2019; Parker et al.,
498 2016; Tanimura et al., 2019), future work should determine whether the excitability of CIN
499 dendrites is altered in these models, as well. Importantly, dendrites are positioned at the
500 interface between synaptic inputs and the intrinsic properties of CIN. While changes in
501 synaptic transmission and intrinsic excitability of CINs have received attention in models of
502 PD, HD and other movement disorders (Abudukeyoumu et al., 2019; Aceves Buendia et al.,
503 2019; Choi et al., 2020; Ding et al., 2006, 2011; Eskow Jaunarajs et al., 2015; Mallet et al.,
504 2019; Paz et al., 2021; Pisani et al., 2007; Plotkin and Goldberg, 2019; Poppi et al., 2021;
505 Tanimura et al., 2019; Tubert and Murer, 2021), studying alterations in dendritic excitability
506 in these models is a complementary approach that remains a largely unchartered territory.
507 The membrane channels that are expressed in CINs (e.g., Nav, HCN, Kv4, etc.) are
508 targets for neuromodulation under normal, healthy circumstances (Deng et al., 2007; Helseth
509 et al., 2021; Maurice, 2004; Song and Surmeier, 1996). Therefore, it is likely that the
510 dendritic excitability of CINs can be modulated under physiological conditions, as well, as a
511 way to adjust the tuning of CINs to their afferent inputs. Conversely, if boosting and
512 resonances are present primarily proximally, the selective tuning of CINs to subhertz slow-
513 wave oscillations is probably stronger for thalamic inputs (that terminate proximally) than for
514 cortical inputs. Future experiments can address this issue by testing how silencing the PfN
515 affects the observed entrainment of TANs to slow-waves (Fig. 6).

516 In summary, we have used a new optogenetics-based approach, complemented by
517 other electrophysiological and imaging approaches, to demonstrate that the spatial
518 localization of active dendritic conductances that endow CIN dendrites with quasi-linear
519 filtering properties corresponds to the spatial distribution of their two main afferent excitatory
520 inputs. This matching up of presynaptic terminals with post-synaptic excitability, probably
521 contributes to the capacity of CINs to respond differentially to cortical and thalamic inputs.
522 Sensorimotor information of cortical origin seems to be integrated continuously in a moment-
523 by-moment fashion. In contrast, thalamic inputs lead to abrupt pauses in TAN firing, even in
524 response to internally-generated brain-states (e.g., during slow-wave activity). The fact that
525 dendritic arbors adapt to the spatiotemporal patterns of afferent inputs is likely an important
526 principle of neural computation that deserves further attention, particularly in the framework
527 of autonomously active neurons such at CINs, SNr neurons and other basal ganglia
528 pacemakers.
529

530 **Materials and Methods**

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

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

545 Non-Human Primates (NHPs): Data were obtained from two healthy, young adult, female
546 vervet monkeys. The monkeys were habituated to sleeping in a primate chair, positioned in a
547 dark, double-walled sound-attenuating room. The primate chair restrained the monkeys' hand

548 and body movements but otherwise allowed them to be in a position similar to their natural
549 (sitting) sleeping posture. Detailed sleep habituation, surgery and sleep staging were reported
550 previously (Mizrahi-Kliger et al., 2018). For extracellular recordings, the monkeys' heads
551 were immobilized with a head holder and eight glass-coated tungsten microelectrodes were
552 advanced the dorsal striatum. Electrical signals were amplified with a gain of 20, filtered
553 using a 0.075 Hz (2 pole) to 10 kHz (3 pole) Butterworth filter and sampled at 44 kHz by a
554 16-bit analog/digital converter. Spiking activity was sorted online using a template matching
555 algorithm. The striatum was identified based on its stereotaxic coordinates according to MRI
556 imaging and primate atlas data (Martin and Bowden, 2000). Tonically active neurons (TANs)
557 and spiny projection neurons (SPNs) were identified using real-time assessment of their
558 electrophysiological features. Spiking and local field potentials (LFP) were recorded only for
559 identified recording sites with stable recording quality (i.e., where single-neuron spiking
560 yielded an average isolation score ≥0.85).
561 We followed established procedures for slow wave detection in the LFP (Riedner et al.,
562 2007). Briefly, the LFP signal was filtered at the 0.5-4 Hz range and putative slow wave events
563 whose duration was 0.25-2 seconds were kept for further analysis. Next, the slow wave peaks
564 were sorted according to their amplitude. Artifacts were removed by discarding all events
565 whose amplitude exceeded 5 standard deviations above the mean. Finally, conditional firing
566 rate analysis was only performed on 30% of the slow wave events with the highest amplitude.

567 Stereotaxic viral inoculation in Vglut2-ires-Cre mice: Mice were deeply anesthetized with
568 isoflurane in a non-rebreathing system (2.5% induction, 1-1.5% maintenance) and placed in a
569 stereotaxic frame (Kopf Instruments, Tujunga, CA). Temperature was maintained at 35˚C
570 with a heating pad, artificial tears were applied to prevent corneal drying, and animals were
571 hydrated with a bolus of injectable saline (10 ml/kg) mixed with analgesic (2.5 mg/kg
572 Meloxicam). Stereotaxic injections into caudal intralaminar nuclei of thalamus were
573 performed under aseptic conditions. Adeno-associated viruses (AAV) serotype 9 carrying
574 double-floxed fusion genes for hChR2 (E123A) and EYFP under an EF1a promoter
575 (University of Pennsylvania Vector Core, Addgene #35507) were used to transfect PfN
576 neurons. Injection coordinates were from Bregma: lateral, 0.65 mm; posterior, 2.3 mm; and
577 3.35 mm depth from surface of brain (Rehani et al., 2019). A small hole was bored into the
578 skull with a micro drill bit and a glass pipette was slowly inserted at the PfN coordinates. To
579 minimize backflow, solution was slowly injected; a total volume of 280 nl (>2.5x1012
580 GC/ml) of the AAV constructs was injected over a period of approximately 1.5 min and the

581 pipette was left in place for 5 min before slowly retracting it. Slice physiology experiments
582 were conducted 2-3 weeks after surgery.

583 Slice preparation: Mice were deeply anesthetized with ketamine (200 mg/kg)–xylazine (23.32
584 mg/kg) and perfused transcardially with ice-cold-modified artificial cerebrospinal fluid
585 (ACSF) bubbled with 95% O_2–5% CO_2, and containing (in mM) 2.5 KCl, 26 $NaHCO_3$, 1.25
586 Na2HPO4, 0.5 $CaCl_2$, 10 $MgSO_4$, 0.4 ascorbic acid, 10 glucose and 210 sucrose. The brain
587 was removed, and 275 µm thick sagittal slices containing the striatum were cut in ice-cold-
588 modified ACSF. Slices were then submerged in ACSF, bubbled with 95% O_2–5% CO_2,
589 containing (in mM) 2.5 KCl, 126 NaCl, 26 $NaHCO_3$, 1.25 Na_2HPO_4, 2 $CaCl_2$, 2 $MgSO_4$ and
590 10 glucose, and stored at room temperature for at least 1 h prior to recording.

591 Electrophysiological recording: The slices were transferred to the recording chamber
592 mounted on a Zeiss Axioskop fixed-stage microscope and perfused with oxygenated ACSF at
593 31ºC. During the optogenetic stimulation experiments, in order to guarantee that the effects
594 we measured were generated post-synaptically, the ACSF solution contained (in µM) 10
595 DNQX to block AMPA receptors, 50 D-APV to block NMDA receptors, 10 gabazine
596 (SR95531) to block $GABA_A$ receptors, and 2 CGP55845 to block $GABA_B$ receptors. An
597 Olympus 60X, 0.9 NA water-immersion objective with a 26.5 mm field number (FN) was
598 used to examine the slice using standard infrared differential interference contrast video
599 microscopy. Patch pipette resistance was typically 4-5 MΩ when filled with recording
600 solutions. The junction potential estimated at 7-8 mV was not corrected. The intracellular
601 solution contained (in mM) 135.5 KCH_3SO_3, 5 KCl, 2.5 NaCl, 5 Na-phosphocreatine, 10
602 HEPES, 0.2 EGTA, 0.21 Na_2GTP, and 2 $Mg_{1.5}ATP$, pH 7.3 with KOH (280–290 mOsm/kg).
603 In the Ca^{2+} imaging experiments (see below) the internal solution contained (in mM) 135 K-
604 gluconate, 5 KCl, 2.5 NaCl, 5 Na-phosphocreatine, 10 HEPES, 0.1 fluo-4 (Molecular
605 Probes), 0.1 Alexa Fluor 568 (for morphological visualization, Molecular Probes), 0.21
606 Na_2GTP, and 2 $Mg_{1.5}ATP$, pH 7.3 with KOH (280–290 mOsm/kg). Electrophysiological
607 recordings were obtained with a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale,
608 CA). Signals were filtered at 10 kHz online, digitized at 10 or 20 kHz and logged onto a
609 personal computer with the Signal 6 software (Cambridge Electronic Design, Cambridge,
610 UK). All reagents, drugs and toxins were purchased from either Merck/Sigma-Aldrich
611 (Darmstadt, Germany), Tocris Biosciences (Bristol, UK) or HelloBio (Bristol, UK).

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

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

636 Two-photon laser scanning microscopy (2PLSM): The two-photon excitation source was a
637 Chameleon Vision II tunable Ti:Sapphire pulsed laser (Coherent, Santa Clara, CA, USA)
638 tuned to 820 nm. The images were collected with the Femto2D system (Femtonics, Budapest,
639 Hungary) which includes two 3 mm galvo-scanners, one gated GaAsP and one multi-alkaline
640 non-descanned photomultiplier tube for imaging fluo-4 and Alexa Fluor, respectively. Z-
641 stacks of optical sections (spaced 2 μm apart) were collected using 0.2 μm pixels and 15 μs
642 dwell times. Optical and electrophysiological data were obtained using the software package
643 MES (Femtonics), which also integrates the control of all hardware units in the microscope.

644 The software automates and synchronizes the imaging signals and electrophysiological
645 protocols. Data was extracted from the MES package to personal computers using custom-
646 made code in MATLAB. We recorded spontaneously occurring bAPs with line scans at
647 various distances measured radially from the tip of the soma. Spike triggered averages of the
648 calcium measurements ($\Delta F/F_0$) were estimated and an alpha-function was fit to them. The
649 value of the peak of the fitted alpha-function was used as a measure of the size of the
650 spontaneous bAP at that location.

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

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

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

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

665

666 where

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

667

668 α and β are functions of frequency and are determined by the linearization of the dendritic
669 nonlinearities as explained in Goldberg et al. (2007) and Remme and Rinzel (2011), with a
670 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.

<u>Statistics</u>: 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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