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Supplemental Information

Pauses in Cholinergic Interneuron Activity Are Driven by Excitatory Input and Delayed Rectification, with Dopamine Modulation

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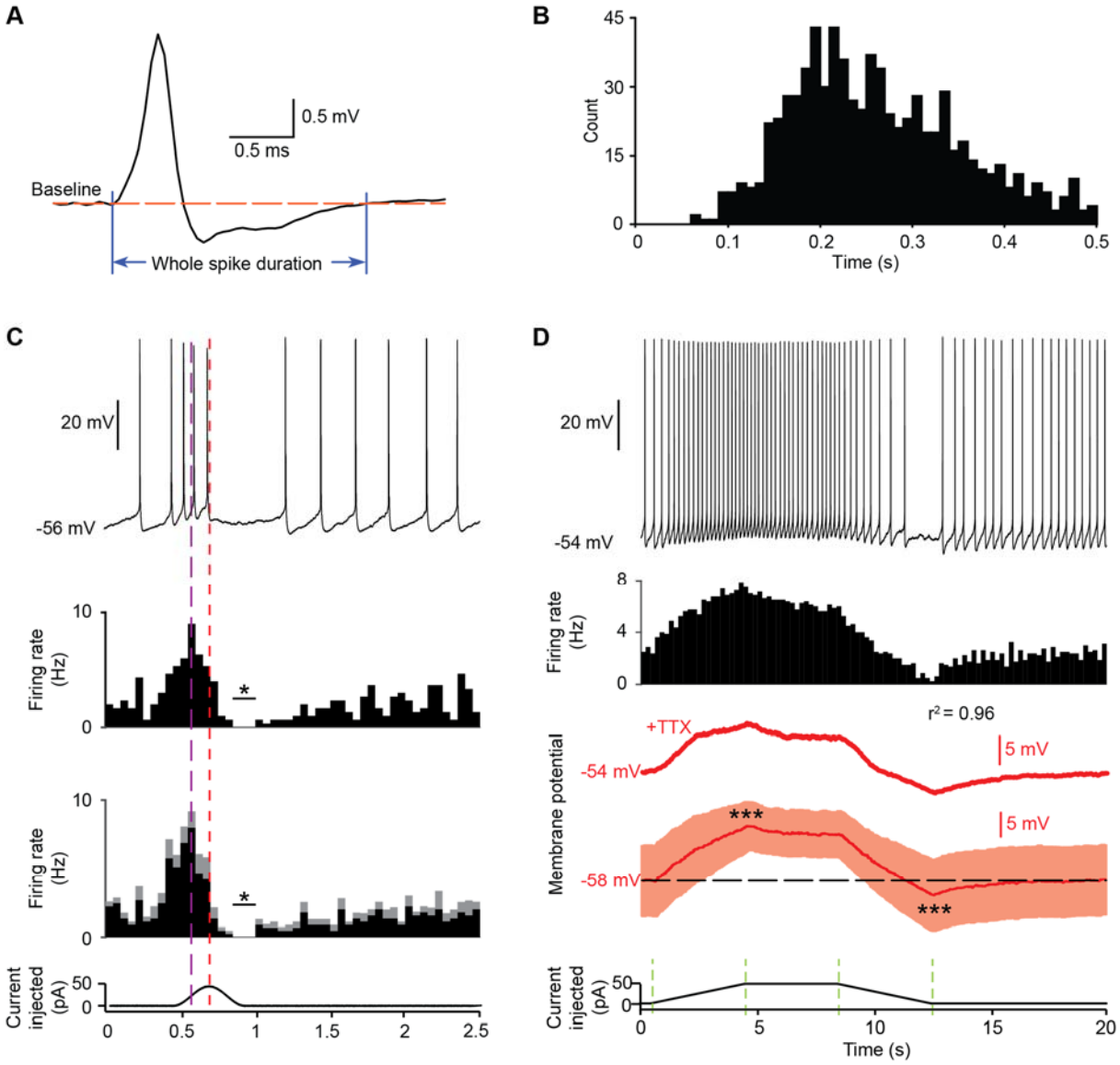

Figure S1
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Figure S1. **Rat ChI firing rate reflects changes to excitatory input. Related to Figures 1 & 2.**
(**A**) A typical characteristic waveform of a pChI recorded *in vivo*, with long whole spike duration. (**B**) Typical firing rate of a pChI *in vivo* is regular with a modal interspike interval (*x*-axis) of ~0.2 s. Neurons were classified as pChIs by tonic firing rate and waveform duration. (**C**) *Upper to lower,* Example sweep, histogram (10 sweeps) and mean histogram ± SEM (*grey shaded*) (*n*=5) of ChI response to sine-shape current. Highest firing rate (*purple dashed line*), input current maximum (*red dashed line*), pause in firing rate versus baseline **P*<0.05, t-test. (**D**) Responses to trapezoid current injections for depolarizing input. *Upper to lower*, Example sweep, histogram (10 sweeps), representative and mean (± SEM, *shaded*) membrane potential in TTX 1 µM (*red*), and current injection. Correlation, firing rate and membrane potential, r^2=0.96, 100 bins. ****P*<0.001, paired t-test for peaks versus plateaus (*n*=10).

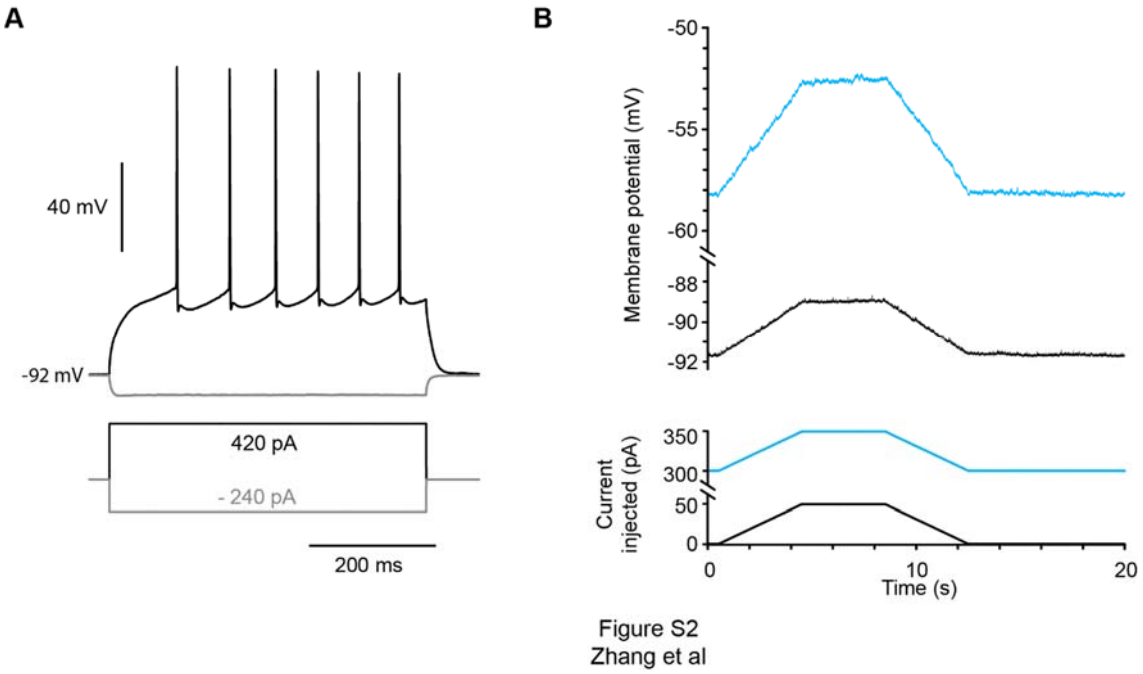

Figure S2. Response of SPNs *ex vivo* to current injection. Related to Figure 2.
(**A**) Characteristic SPN response to depolarizing and hyperpolarizing current injections in acute slices of mouse striatum, including hyperpolarized resting membrane potential and delayed spike activity in response to positive current injection. (**B**) Membrane potential in SPNs responds linearly to current injection when resting membrane potential was normal (-92 mV) or is raised by a holding current to a value (-58 mV, 300 pA holding current, *blue*) that is close to that of ChIs. Representative data, from *n*=5.

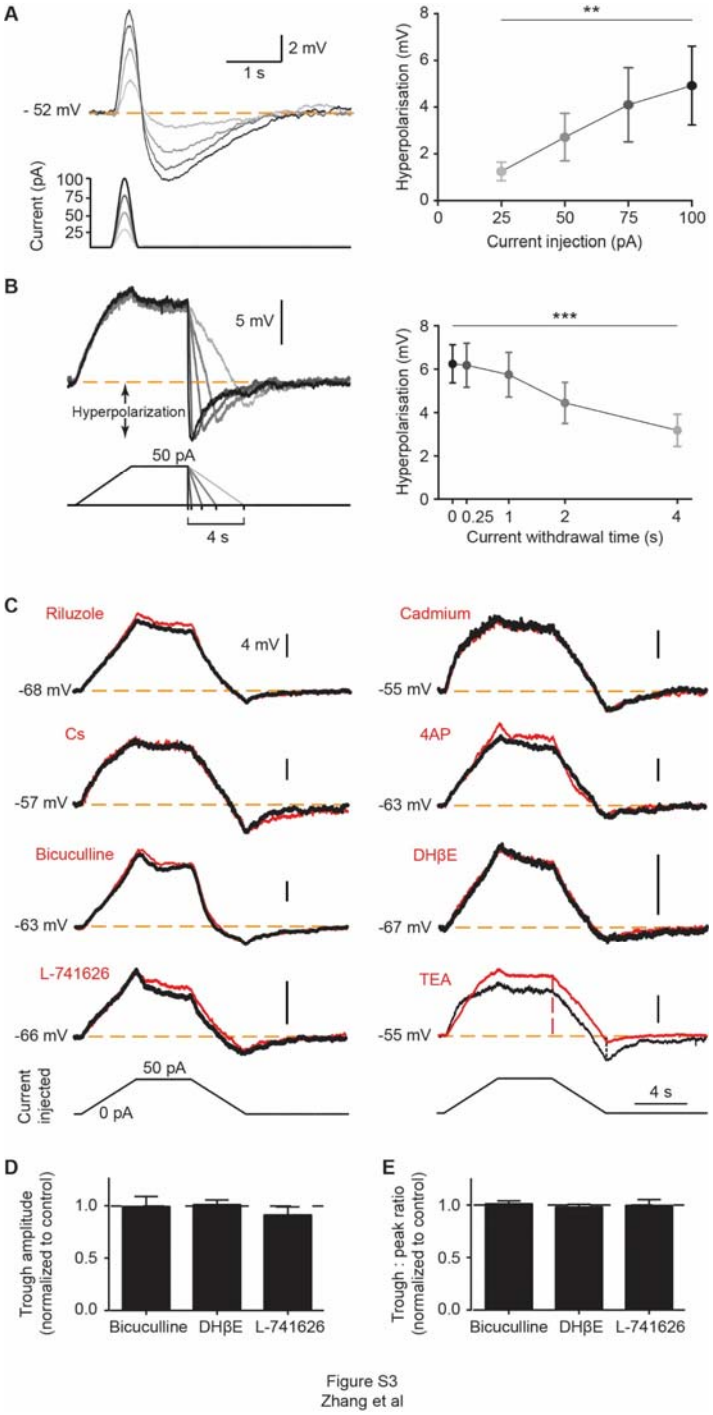

Figure S3. Hyperpolarization in ChIs scales with amplitude and rate of withdrawal of excitatory input, is TEA-sensitive, and does not require $GABA_A$, nACh or D_2-receptors. Relates to Figures 2 and 3.

(**A**) Typical responses (*left*) of ChI membrane potential (*upper*) to four different amplitudes of sine-shape current injections (*lower*) in ChIs recorded in striatal slices. Hyperpolarization (± SEM) in ChIs following input withdrawal scales with depolarization (*right*, *n*=5, ***P*<0.01, Pearson's correlation). (**B**) Typical responses (*left*) of ChI membrane potential (*upper*) to withdrawal from depolarizing current of 50 pA to 0 pA at different rates. Hyperpolarization trough amplitude (± SEM) varies inversely with duration of current withdrawal, (*right, n*=5, ****P*<0.001, One-way ANOVA). (**C**) Typical recordings of before (*black*) and during (*red*) either riluzole, cadmium, CsCl, 4-AP, bicuculline, DHβE, L-741626 or TEA. Resting membrane potentials were kept at pre-drug values using current injections when necessary. Vertical scale bar, 4 mV. (**D**) Mean ± SEM of amplitude of hyperpolarization trough (*black dashed*) from RMP (*orange dashed*) or (**E**) mean ± SEM of ratio of hyperpolarization trough to peak (*black* versus *red dashed lines* in *panel C*), normalized to control condition for bicuculline 10 µM, DHβE 1 µM, L-741626 1 µM.

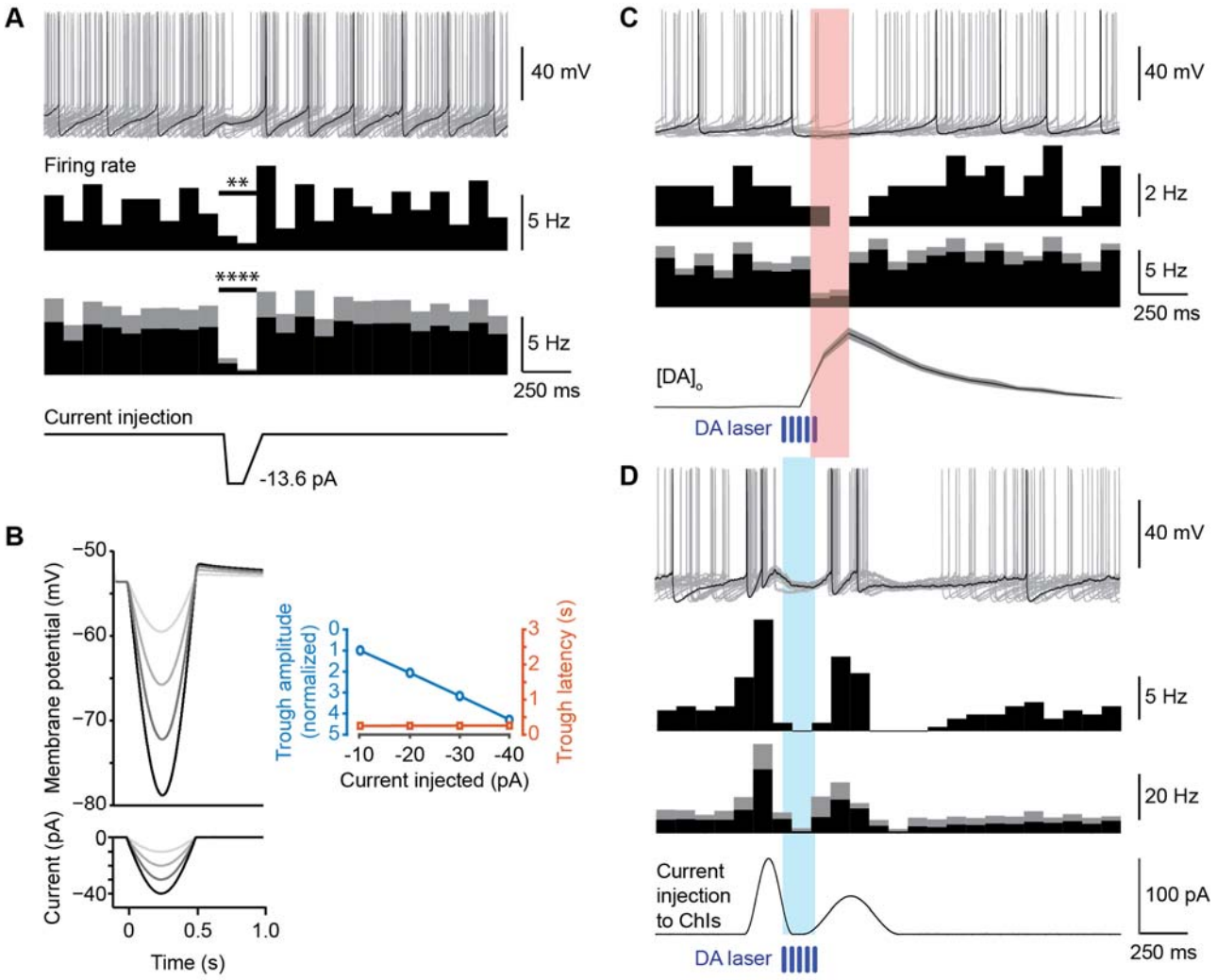

Figure S4
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Figure S4. ChIs can pause in response to changing input current or burst activity in dopamine neurons, but when concurrent, dopamine effects are too slow. Relates to Figure 2 and 4.

(**A**) Firing rate in mouse ChI in slices showing (*upper to lower): e*xample activity (20 sweeps), example firing rate histogram, mean firing rate histogram ± SEM (*n*=5), in response to -13.6 pA current injection (equivalent to the I_{Kr} current evoked by a 50 pA sine wave, see Methods). ***P*<0.01, *****P*< 0.0001, t-test. (**B**) Response of ChI membrane potential (*upper*) to a series of sine-shape hyperpolarizing current injections (*middle*) in the computational model shown in Fig. 4. *Right*, the trough amplitude (*blue*) but not latency (*orange*) scales with amplitude of current injected. (**C**) Firing rate in mouse ChI recorded in slices showing (*upper to lower*): example activity (15 sweeps), example firing rate histogram, mean firing rate histogram ± SEM (*n*=5), as well as extracellular dopamine concentration ($[DA]_o$) (mean ± SEM) detected with voltammetry (*n*=14), in response to optogenetic activation of dopamine axons with blue light pulses (*DA laser*). Dopamine-induced pause, *red shade.* (**D**) Firing rate in mouse ChI recorded in slices showing (*upper to lower*): example activity (20 sweeps), example firing rate histogram, mean firing rate histogram ± SEM (*n*=5) of ChI response to current injection, while concurrently dopamine axons were light-activated *(DA laser,* 5 pulses) to coincide with the pause induced by withdrawal of excitation of ChIs (*blue shade*). The dopamine-induced pause seen in C occurs too late to coincide with the ChI pause in D.