

1 Title: Cholinergic Modulation Shifts the Response of CA1 Pyramidal Cells to Depolarizing Ramps via
2 TRPM4 Channels with Potential Implications for Place Field Firing

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4 Abbreviated Title: Cholinergic Modulation Shifts the Response of CA1 Pyramidal Cells

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25 **Abstract**

26 A synergistic combination of *in vitro* electrophysiology and multicompartmental modeling of rat CA1
27 pyramidal neurons identified TRPM4 channels as major drivers of cholinergic modulation of the firing rate
28 during a triangular current ramp, which emulates the bump in synaptic input received while traversing the
29 place field. In control, fewer spikes at lower frequencies are elicited on the down-ramp compared to the
30 up-ramp due to long-term inactivation of the Na_v channel. The cholinergic agonist carbachol (CCh)
31 removes or even reverses this spike rate adaptation, causing more spikes to be elicited on the down-
32 ramp than the up-ramp. CCh application during Schaffer collateral stimulation designed to simulate a
33 ramp produces similar shifts in the center of mass of firing to later in the ramp. The non-specific TRP
34 antagonist flufenamic acid and the TRPM4-specific blockers CBA and 9-phenanthrol, but not the TRPC-
35 specific antagonist SKF96365, reverse the effect of CCh; this implicates the Ca^{2+} -activated nonspecific
36 cation current, I_{CAN} , carried by TRPM4 channels. The cholinergic shift of the center of mass of firing is
37 prevented by strong intracellular Ca^{2+} buffering but not by antagonists for IP_3 and ryanodine receptors,
38 ruling out a role for known mechanisms of release from intracellular Ca^{2+} stores. Pharmacology
39 combined with modeling suggest that $[\text{Ca}^{2+}]$ in a nanodomain near the TRPM4 channel is elevated
40 through an unknown source that requires both muscarinic receptor activation and depolarization-induced
41 Ca^{2+} influx during the ramp. Activation of the regenerative inward TRPM4 current in the model
42 qualitatively replicates and provides putative underlying mechanisms for the experimental observations.

43 Introduction

44 Release of the neuromodulator acetylcholine is thought to be correlated with certain brain states or
45 functions (Pepeu and Giovannini, 2004). In the hippocampal formation, elevated acetylcholine is
46 associated with exploration (Bianchi et al., 2003), particular memory processes (Micheau and Marighetto,
47 2011), REM sleep (Hutchison and Rathore, 2015), and response to novelty (Acquas et al., 1996; Thiel et
48 al., 1998; Giovannini et al., 2001).

49 The major cholinergic input to the hippocampus is from the medial septum and diagonal band of Broca,
50 which innervate all hippocampal subfields (Dutar et al., 1995). As for the degree to which acetylcholine
51 release is spatially targeted, this is still under debate (Disney and Higley, 2020; Sarter and Lustig, 2020);
52 however it has been shown that CA1 receives cholinergic afferents in all layers, although at different
53 densities (Nyakas et al., 1987; Aznavour et al., 2002). Pyramidal neurons express G-protein-coupled
54 metabotropic muscarinic acetylcholine receptors (mAChRs) (Levey et al., 1995), more so than they do
55 ionotropic (nicotinic) receptors, which are primarily on interneurons in this region (Dani and Bertrand,
56 2007). M₁ mAChRs, in particular, are found on both dendritic shafts and spines of CA1 pyramidal
57 neurons (Yamasaki et al., 2010).

58 Elevated acetylcholine in the hippocampal formation is thought to favor memory encoding, and
59 modulates theta oscillations (Hasselmo, 2006), which in turn modulate place cell firing (Easton et al.,
60 2012). There is evidence that cholinergic activity regulates long-term synaptic plasticity (Huerta and
61 Lisman, 1995; Palacios-Filardo and Mellor, 2019; Fernández de Sevilla et al., 2021). Cholinergic
62 activation also influences intrinsic properties of neurons, which result in membrane potential
63 depolarization (Brown and Adams, 1980), increase in firing rate (Benardo and Prince, 1982), and
64 modulation of input/output relationships, for example switching from burst to regular firing (Azouz et al.,
65 1994).

66 In addition to suppressing several potassium currents (Brown and Adams, 1980; Hoffman and Johnston,
67 1998; Buchanan et al., 2010; Giessel and Sabatini, 2010), cholinergic stimulation has been linked to the
68 activation of a calcium-activated, non-specific cation current, I_{CAN} (Colino and Halliwell, 1993; Egorov et
69 al., 2002; Yoshida et al., 2012). I_{CAN} is attributed to current flowing through channels of the transient
70 receptor potential (TRP) family (Guinamard et al., 2010; Reboreda et al., 2011). The canonical TRP
71 channel, TRPC, has been implicated in cholinergic-mediated persistent firing ((Zhang et al., 2011; Arboit
72 et al., 2020), but see (Egorov et al., 2019)). There is indirect evidence, however, of involvement of
73 TRPM4 channels in the cholinergic activation of I_{CAN}. TRPM4 channel activation downstream of group I
74 metabotropic glutamate receptors in pre-Botzinger cells (Mironov, 2008), implicates the G_{q/11} pathway
75 shared by mGluR_{1/5} and mAChR M_{1/3/5}. Although TRPM4 channels are known to be expressed in the
76 soma and apical dendrites of CA1 pyramidal dendrites (Riquelme et al., 2021), their known functions in

77 these neurons are currently limited to a minor role in the afterdepolarization following action potential
78 trains (Lei et al., 2014) and involvement in NMDA-mediated LTP (Menigoz et al., 2016).

79 Previously, we found that when triangular shaped current ramps intended to emulate the depolarizing
80 input received by a place cell during a place field traversal (Epsztein et al., 2011) were injected at the
81 soma or dendrites of CA1 pyramidal neurons, they elicited fewer spikes on the down-ramp than on the
82 up-ramp (Upchurch et al., 2022). In the current study, we investigated the effect of cholinergic modulation
83 on this firing pattern *in vitro* (slice electrophysiology) and *in silico* (multi-compartmental NEURON model).
84 We found that, at comparable maximum firing rates, cholinergic activation shifts the center of mass of
85 firing to the latter half of the ramp, both for current injection ramps as well as for synaptically-driven
86 depolarizations. Both CCh and sustained depolarization are required to produce the rightward shift in the
87 center of mass of firing along the ramp. This shift is blocked by antagonists specific for TRPM4 channels
88 or by intracellular Ca^{2+} buffering, but not by IP_3 or ryanodine receptor antagonists. The TRPM4 channels
89 have a micromolar half-activation concentration of Ca^{2+} (Nilius et al., 2004), which implies that a
90 restricted Ca^{2+} nanodomain near the channel mouth is required to elicit substantial opening of TRPM4
91 channels. Thus, whatever the Ca^{2+} source that activates the TRPM4 channels, it is likely co-localized
92 with them.

93

94 **Results**

95 *Asymmetric firing on symmetric ramps is modulated by cholinergic activation*

96 We have previously used 2 and 10 second long triangular-shaped current ramps (Upchurch et al., 2022)
97 to emulate the depolarizing input received by a place cell during a place field traversal (Epsztein et al.,
98 2011); the amplitude of the ramps was adjusted for each cell to produce peak frequencies between 10
99 and 25 Hz, as observed in place cells *in vivo* (Hargreaves et al., 2007; Resnik et al., 2012; Bittner et al.,
100 2015). Regardless of whether these ramps are injected at the soma or in the dendrites of CA1 pyramidal
101 neurons, they elicit fewer spikes on the down-ramp than on the up-ramp, due to adaptation mediated by
102 long-term sodium channel inactivation (Fernandez and White, 2010; Venkatesan et al., 2014; Upchurch
103 et al., 2022). Place cell firing is modulated by experience such that the center of mass of the place field
104 shifts in a direction opposite the direction of motion as the environment becomes more familiar (Mehta et
105 al., 2000). This modulation is strongest when animals are first introduced to a novel environment (Roth et
106 al., 2012), suggesting involvement of a novelty signal. Since elevated acetylcholine is thought to be such
107 a signal (Acquas et al., 1996; Giovannini et al., 2001), we investigated the effect of cholinergic
108 neuromodulation on this firing rate adaptation (Figure 1).

109 Figure 1A1 and 1E1 show typical voltage responses to two-second-long symmetric ramps injected in the
110 soma or dendrites (approximately 200 μm from soma) under control conditions. This type of response

111 results in a positive value for the normalized difference between spikes fired on the up-ramp versus the
112 down-ramp (we call this an adaptation index, see Methods for definition). A positive index means that
113 neurons fire more on the up-ramp than the down-ramp, whereas a negative value indicates the converse,
114 and is therefore a good metric to determine shifts in the center of mass of the firing of CA1 pyramidal
115 neurons in different conditions. In some trials, under control conditions we applied a baseline
116 depolarization prior to the ramp, in order to capture the variability observed *in vivo* (Harvey et al., 2009;
117 Epsztein et al., 2011). Application of the cholinergic agonist carbachol (CCh, 2 μ M) caused a
118 depolarization of 2-6 mV. We compensated for this depolarization by injecting tonic hyperpolarizing
119 current to reestablish the original membrane potential (see also (Losonczy et al., 2008)), as indicated by
120 an offset from the 0 pA current level in the traces of the injected current ramps. The amplitude of
121 background fluctuations in the resting membrane potential increased from a few tenths of a mV in control
122 to 2-4 mV in CCh. Moreover, the threshold for action potential generation became more hyperpolarized.
123 For all these reasons, we were not able to consistently vary the membrane potential using baseline
124 depolarizations in the presence of CCh, because baseline depolarization alone frequently evoked
125 spiking. As a result of the increased excitability in CCh, smaller current ramps were sufficient to produce
126 the same peak frequencies as in control (note the difference in the injected ramp currents below the
127 voltage trace in Figures 1A1 vs. 1B1 and 1E1 vs. 1F1). Under these conditions, CCh caused a shift of
128 the center of mass of firing to later in the ramp (Figure 1B1 and 1F1), which resulted in a decreased
129 value of the adaptation index which became negative for most cells, as shown in the scatter plot of group
130 data (Figure 1D and 1H). The shift is evident in the plots of the frequency as a function of time (compare
131 Figure 1A2 vs. 1B2 and 1E2 vs. 1F2). As in (Mainen and Sejnowski, 1995), the raster plots of the spike
132 times were characterized by variability in the timing of action potentials among different trials in the same
133 neuron (Figures 1A3, 1B3 and 1E3, 1F3). However, the variability appears to be independent of the
134 amount of current injected, as demonstrated by trials that are grouped as a function of the injected peak
135 current amplitude. Moreover, the raster plots show how the center of mass of firing overall shifted from
136 early in the ramp in control conditions to later in the ramp in the presence of carbachol. This shift was
137 significant for short (2 s) ramps in the soma (control 0.33 ± 0.02 , CCh -0.25 ± 0.03 ; $t(20) = 16.741$; $p <$
138 0.0005 ; $n = 21$), long (10 s) ramps in the soma (control 0.24 ± 0.02 , CCh -0.33 ± 0.06 ; $t(18) = 9.854$; $p <$
139 0.0005 ; $n = 19$), as well as for short and long ramps in the dendrites (2 s ramps: control 0.49 ± 0.04 , CCh
140 -0.45 ± 0.07 ; $t(18) = 12.529$; $p < 0.0005$; $n = 19$; 10 s ramps: control 0.37 ± 0.02 , CCh -0.16 ± 0.06 ; $t(9) =$
141 10.582 ; $p < 0.0005$; $n = 10$).

142 Plots of instantaneous frequency versus current injection are shown in Figure 1C1, 1C2, 1G1 and 1G2.
143 These plots provide a way to assess firing rate adaptation or acceleration when the injected current is not
144 a square pulse (Venugopal et al., 2015). In control (Figure 1C1 and 1G1), frequencies were higher on the
145 up-ramp (filled circles) and decreased on the down-ramp (open circles) at similar values of injected
146 current. This hysteretic clockwise motion corresponds to spike rate adaptation, in which the firing rate

decreases at a constant value of injected current. In contrast, in the presence of CCh, the motion was variable; it could be essentially linear as shown in the somatic example in Figure 1C2 or even counter-clockwise as in the dendritic example in Figure 1G2. The frequency/current (f/I) plots in Figure 1 – supplement 1 show the clockwise hysteresis in control and different cases of reversal or removal of adaptation in the presence of CCh for somatic recordings, with panels A and B showing acceleration, and panels C and D showing linearization. A linear motion indicates no hysteresis, although often the neurons fire at lower current injections on the down-ramp (defined as sustained linear in (Venugopal et al., 2015)), whereas counter-clockwise motion corresponds to an acceleration of the firing rate that would be observed during injection of a constant current. Both of these variants correspond to an effective removal of the spike rate adaptation by cholinergic activation. These results also demonstrate that cholinergic activity shifts the center of mass of firing to later times during a symmetric ramp. We speculate that the level of cholinergic modulation could therefore be a source of rapid modification of the timing of place cell firing relative to position in the place field. Our summary results in Figure 1 show that carbachol similarly affects 2 s and 10 s ramps, and we present only the data for the 2 second ramps for subsequent figures.

Since the initial membrane potential and the amount of current injected could potentially affect the center of mass of firing, confounding the effects of cholinergic modulation, we plotted the adaptation index as a function of the initial membrane potential (Figure 1 – figure supplement 2A) and of injected current amplitude (Figure 1 - figure supplement 2B), in control conditions and in the presence of carbachol (2 μ M) for each trial for all recorded neurons, both for somatic and dendritic recordings. Likewise, since the input resistance of the neuron could potentially skew the center of mass of firing, we plotted the average adaptation index as a function of the steady-state input resistance in control conditions and in the presence of carbachol for each somatic and dendritic recording (Figure 1 - figure supplement 2C). In all cases, it is apparent that the main factor determining the adaptation index, and therefore whether the center of mass of firing occurs earlier or later in the ramp, is the presence or absence of carbachol rather than initial membrane potential, current injection amplitude, or input resistance.

The later onset of spiking in CCh in the experiments described above may be attributable to the smaller current ramps, but the persistence of spiking without adaptation is striking in comparison to control. The use of smaller ramps to achieve similar firing rates may be justified by analogy with the presynaptic action of ACh to reduce glutamate release (Hasselmo, 2006). Since the current injection protocols described above only account for the effects of cholinergic agonists on the postsynaptic neuron, we examined the effects of carbachol on synaptically-driven membrane potential ramps, where pre- and postsynaptic effects can be probed at the same time. To this end, we stimulated the Schaffer collateral fibers with bipolar electrodes and recorded the responses from the somata of CA1 pyramidal neurons (Figure 2). The electrical stimulation consisted of 30 stimuli for a total duration of ~2 s. The stimulation

pattern was adjusted according to a linear, symmetric ramp as in (Hsu et al., 2018), such that the frequency increased and decreased symmetrically, starting at 6.7 Hz and peaking in the middle of the ramp at 25 Hz, the target peak frequency in the current injection experiments. The intensity of stimulation was adjusted such that neurons would fire in response to 40-65% of the total inputs under control conditions, and was kept constant in control conditions and in the presence of carbachol. In both conditions, firing was more frequent in the middle of the ramp, because of the higher stimulation frequency. However, as in the current injection protocols in Figure 1, some variability was observed in the timing of the spikes across trials, as can be appreciated in the raster plots. In control conditions, the neurons tended to fire more in the first half of the ramp (Figure 2A), whereas in the presence of carbachol they tended to fire more on the second half of the ramp (Figure 2B). The adaptation index was calculated in a similar manner as in Figure 1, as the normalized difference between the number of spikes in the first and the second half of the ramp. On average, the adaptation index decreased from 0.18 ± 0.03 in control to -0.17 ± 0.04 in the presence of carbachol, $t(10) = 6.873$, $p < 0.0005$, $n = 11$ (Figure 2C). This shift was partly due to a decrease in the amplitude of the EPSPs (Figure 2D); the average amplitude of the first EPSP in control (8.83 ± 0.82 mV) was significantly larger than the average first EPSP in CCh (6.81 ± 0.52 mV; $t(10) = 4.127$; $p = 0.002$; $n = 11$). In addition, the decay of the EPSPs appeared to slow down later in the ramp in the presence of CCh (compare EPSPs indicated by diamonds in the two conditions), resulting in an increase in temporal summation and excitability. These results show that carbachol tends to shift the center of mass of firing to later in the ramp in the case of a synaptically-driven depolarization as well as in response to a ramp of injected current.

Carbachol is often used to more easily study cholinergic signaling in biological tissues, because it is not susceptible to hydrolysis by acetylcholinesterase, which is preserved and active in hippocampal slices (Avignone and Cherubini, 1999). To verify that the effects of the endogenous neuromodulator were comparable to those of its synthetic analog, we repeated the experiments in Figure 1 with different concentrations of acetylcholine. In this and all following sets of experiments, the amplitude of current injection was adjusted for each cell and condition to produce peak frequencies between 10 and 25 Hz as described above. Acetylcholine caused a depolarization of the V_m in a dose-dependent manner. As before, we compensated for this depolarization by injecting hyperpolarizing current to restore the initial V_m (Figure 3). Like carbachol, acetylcholine shifted the center of mass of firing to later in the ramp, albeit to a more moderate degree (Figure 3A-D). A one-way repeated measures ANOVA was carried out to determine if there was an effect on the adaptation index of three concentrations of ACh within the same neurons. The assumption of sphericity was violated, so a Greenhouse-Geisser correction was applied ($\epsilon = 0.574$). There was a significant effect of ACh on the adaptation index, plotted in Figure 3E (control 0.35 ± 0.04 ; 2 μ M ACh 0.14 ± 0.04 ; 10 μ M ACh 0.00 ± 0.06 ; 15 μ M ACh -0.02 ± 0.06 ; $F(1.722, 20.665) = 44.360$; $p < 0.0005$, $n = 13$). Bonferroni-adjusted post hoc tests demonstrated significant differences between the control condition and all concentrations of ACh (all comparisons $p < 0.0005$), and between

the lowest concentration and the subsequent higher concentrations (for 2 μ M compared to 10 μ M, $p = 0.001$; for 2 μ M compared to 15 μ M, $p = 0.01$). There was not a significant difference between 10 μ M and 15 μ M ACh. Due to likely degradation via acetylcholinesterase, we speculate that the actual concentration in the slices is lower than the applied one. CCh is not susceptible to degradation by acetylcholinesterase, and its concentration, which in our experiments is markedly lower than that used in many studies on cholinergic modulation of neuronal activity (Bland et al., 1988; Fisahn et al., 1998; Knauer et al., 2013), is therefore more consistent, which may explain the more pronounced shift in spiking in Figure 1 as compared to Figure 3. Nonetheless, our results with ACh suggest that there are concentration-dependent effects of the endogenous neuromodulator on firing rate adaptation. Fluctuations in ACh levels could therefore have implications for the firing of a place cell within its place field.

229

230 *Cholinergic-associated shift in the timing of firing during the ramp is mediated by TRPM4 channels*

An increase in firing on the down-ramp compared to the up-ramp induced by cholinergic activation could result from 1) a decrease in an outward current and/or 2) an increase in an inward current. The cascade of events that follow cholinergic activation in these neurons includes the suppression of a number of outward currents (Halliwell and Adams, 1982; Hoffman and Johnston, 1998; Buchanan et al., 2010; Giessel and Sabatini, 2010), some of which are also involved in firing rate adaptation (Stocker et al., 1999; Gu et al., 2005; Pedarzani et al., 2005; Otto et al., 2006; Chen et al., 2014). Although the SK channel clearly contributes to adaptation when the dendrites are stimulated at high frequency (>50 Hz) (Combe et al., 2018), we have previously examined the contribution of small-conductance calcium-activated potassium (SK) current, M-type potassium current, and A-type potassium current with respect to the asymmetric firing in response to symmetric current ramps (Upchurch et al., 2022). We found that, in the relatively low range of firing frequencies evoked by this type of stimulation, the adaptation induced by these K^+ currents (including SK) does not play a role in shifting action potentials to earlier times during the ramp. However, cholinergic activation has also been shown to activate a non-specific inward cationic current, I_{CAN} , that is thought to be carried by certain TRP channels (Guinamard et al., 2010; Reboreda et al., 2011). Flufenamic acid (FFA) has been shown to block a variety of TRP channels in hippocampal neurons (Partridge and Valenzuela, 2000) and elsewhere (Guinamard et al., 2013). After observing the typical response to CCh, responses to symmetric ramps were recorded while FFA (100 μ M) was applied along with CCh (Figure 4A and 4B). FFA shifted most action potentials back to the up-ramp as shown in the examples in Figure 4A1-4A3 and 4B1-4B3, effectively reversing the shift triggered by CCh. The differences in adaptation index across the three conditions were found to be significantly different by repeated measures ANOVA in both somatic recordings (control 0.35 ± 0.05 , CCh -0.36 ± 0.09 , CCh+FFA 0.25 ± 0.06 ; $F(2,16) = 65.835$; $p < 0.0005$; $n = 9$; Figure 4A4) and dendritic recordings (control $0.52 \pm$

0.02, CCh -0.54 ± 0.11 , CCh+FFA 0.20 ± 0.08 ; $F(2,16) = 60.680$; $p < 0.0005$; $n = 9$; Figure 4B4). Bonferroni-adjusted pairwise comparisons indicated that for somatic recordings, the differences were significantly different between control and CCh ($p < 0.0005$), and between CCh and CCh+FFA ($p < 0.0005$), but not between control and CCh+FFA ($p = 0.43$). For dendritic recordings, the differences were significantly different between control and CCh ($p < 0.0005$), CCh and CCh+FFA ($p < 0.0005$), and control and CCh+FFA ($p = 0.02$). In most cases, the adaptation index reverted to a positive value with the application of FFA from a negative value in CCh alone, and in all cases was closer to the control value with FFA than with CCh alone. The failure of the adaptation index to return to control values in FFA may reflect an incomplete block of TRP channels or an additional effect of CCh. Overall, these data suggest that the shift in the center of mass of firing to later along the ramp that is associated with cholinergic activity is due, in large part, to activation of TRP channels.

The TRPC subfamily has been implicated as being responsible for I_{CAN} in some neurons and is thought to be activated by elements downstream of muscarinic acetylcholine receptor activation (Putney, 2005), which makes it an attractive candidate, mechanistically, for this cholinergic-mediated shift in action potential firing along the depolarizing ramp. Cholinergic stimulation of $G_{q/11}$ -coupled mAChRs results in phospholipase C hydrolysis of membrane constituent phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 stimulates IP_3 receptor (IP_3R) associated Ca^{2+} release from the endoplasmic reticulum, regulating TRPC channel activity via calmodulin (Zhu, 2005), while DAG activates TRPC channels directly (Hofmann et al., 1999; Mederos Y Schnitzler et al., 2018).

In a set of experiments similar to those above, the TRPC-specific antagonist SKF96365 (50 μM) was added after CCh was applied (Figure 4C and 4D). The differences in adaptation index across the three conditions were found to be significantly different by repeated measures ANOVA in somatic recordings (control 0.43 ± 0.07 , CCh -0.36 ± 0.09 , CCh+SKF -0.24 ± 0.10 ; $F(2,14) = 42.453$; $p < 0.0005$; $n = 8$; Figure 4C4) and dendritic recordings (control 0.47 ± 0.11 , CCh -0.24 ± 0.07 , CCh+SKF -0.23 ± 0.06 ; $F(2,14) = 118.541$; $p < 0.0005$; $n = 8$; Figure 4D4). However, Bonferroni-adjusted pairwise comparisons demonstrated that the significant differences lie both between control and CCh (soma $p < 0.0005$; dendrite $p < 0.0005$) and control and CCh+SKF (soma $p = 0.001$; dendrite $p < 0.0005$), not between CCh and CCh+SKF (soma $p = 0.68$; dendrite $p = 1.00$). Thus, SKF96365 did not significantly shift the adaptation index in the same manner as did FFA, suggesting that the TRP channels involved in the CCh-mediated forward shift are not of the TRPC subtype.

TRPM4 channels have likewise been implicated in downstream effects associated with G_q -coupled signaling (Launay et al., 2002; Dutta Banik et al., 2018). TRPM4 channels are activated by Ca^{2+} binding to the cytoplasmic side combined with depolarization (Nilius et al., 2003; Autzen et al., 2018), but these channels are impermeable to Ca^{2+} . Instead, TRPM4 channels facilitate a rise in $[Ca^{2+}]_i$ indirectly, by

288 depolarizing the membrane potential (Launay et al., 2002) and therefore activating voltage-gated Ca^{2+}
289 channels (Li et al., 2021). Cationic current through these channels and the resulting $[\text{Ca}^{2+}]_i$ elevation has
290 been implicated in bursting, persistent firing, and oscillatory activity in some neurons (Lin et al., 2017;
291 O'Malley et al., 2020; Li et al., 2021). In order to investigate the involvement of TRPM4 channels in the
292 cholinergic-mediated shift in adaptation index, the TRPM4 channel antagonist CBA (50 μM) was added
293 after recording the response to CCh (Figure 5A and 5B). Repeated measures ANOVA showed significant
294 differences in the adaptation indices in somatic recordings (control 0.29 ± 0.05 , CCh -0.37 ± 0.10 ,
295 CCh+CBA 0.17 ± 0.07 ; $F(2,16) = 66.257$; $p < 0.0005$; $n = 9$; Figure 5A4) and dendritic recordings (control
296 0.39 ± 0.07 , CCh -0.41 ± 0.10 , CCh+CBA 0.08 ± 0.05 ; $F(2,16) = 34.848$; $p < 0.0005$; $n = 9$; Figure 5B4).
297 Bonferroni-corrected pairwise comparisons indicated that for somatic recordings, the differences were
298 significant between control and CCh ($p < 0.0005$), and between CCh and CCh+CBA ($p < 0.0005$), but not
299 between control and CCh+CBA ($p = 0.16$). For dendritic recordings, Bonferroni-corrected pairwise
300 comparisons showed that the differences were significant between control and CCh ($p < 0.0005$),
301 between CCh and CCh+CBA ($p = 0.002$), and between control and CCh+CBA ($p = 0.02$). In most cells,
302 the adaptation index became negative in CCh and reverted to a positive value with the addition of CBA,
303 and in all cases the adaptation index was higher in CCh+CBA than in CCh alone.

304 In order to corroborate that the effect of CBA is due to block of TRPM4 channels, we investigated the
305 effect of a second TRPM4 channel antagonist, 9-phenanthrol, added to CCh in somatic recordings.
306 Figure 5C illustrates the shift of most of the spiking activity towards the up-ramp, moving the adaptation
307 index in the positive direction, when 9-phenanthrol (100 μM) was applied on top of CCh. The differences
308 in adaptation index between groups are significant, as demonstrated by repeated measures ANOVA
309 (control 0.40 ± 0.03 , CCh -0.14 ± 0.06 , CCh+9Ph 0.19 ± 0.06 ; $F(2,14) = 57.640$; $p < 0.0005$; $n = 8$; Figure
310 5C4). Bonferroni-corrected pairwise comparisons indicated that the differences were significant between
311 control and CCh ($p < 0.0005$), between CCh and CCh+9Ph ($p = 0.001$), and between control and
312 CCh+9Ph ($p = 0.03$).

313 Taken together, these data with the non-selective TRP channel blocker FFA, the TRPC channel blocker
314 SKF96365, and two different TRPM4 channel antagonists suggest that the shift in adaptation index that
315 we see with CCh is largely mediated through activation of TRPM4, rather than TRPC, channels.

316

317 *Does Ca^{2+} release from internal sources activate TRPM4 channels?*

318 We next tried to determine the source of Ca^{2+} required to activate TRPM4 channels. We first focused on
319 IP_3 receptors (IP_3Rs), which are activated through mAChRs and G_q as described above, using the
320 antagonist Xestospongin C (Gafni et al., 1997) in the intracellular solution (Figure 6A). Since the
321 antagonists rapidly dialyzed into the cytosol, in this figure control data reflect the effect of the antagonists

322 before the addition of CCh. We initially used Xestospongin C at 1 μ M, which was previously found to be
 323 effective in inhibiting TRPM4 channel activation via mGluRs in neurons of the preBötzing nucleus
 324 (Pace et al., 2007). Since this concentration was not effective in preventing the cholinergic shift in the
 325 center of mass of firing in our experiments, in six out of nine experiments we increased the concentration
 326 to 2 μ M. Neither concentration of intracellular Xestospongin C appeared to change the firing features in
 327 control conditions. The shift induced by carbachol did not appear to depend on the concentration of
 328 Xestospongin C (Figure 6A3), therefore we averaged the data in these two conditions. The adaptation
 329 index still decreased significantly ($t(8) = 10.574$, $p < 0.0005$; $n = 9$, as per a paired t-test) from $0.31 \pm$
 330 0.02 in control conditions to -0.38 ± 0.07 in the presence of CCh (2 μ M). This result argues strongly
 331 against the hypothesis that the effects of the activation of muscarinic receptors on the shift of the center
 332 of mass of firing are mediated by activation of IP₃Rs. Ryanodine receptors (RyRs) are also expressed on
 333 the endoplasmic reticulum in CA1 pyramidal neurons, and appear to contribute selectively to spine Ca²⁺
 334 elevations during LTP at the CA3-CA1 synapses (Raymond and Redman, 2006). We therefore tested the
 335 hypothesis that the increase of [Ca²⁺]_i in a nanodomain leading to TRPM4 channel activation could be
 336 due to Ca²⁺-induced Ca²⁺ release (CICR) through RyRs following Ca²⁺ influx through voltage-dependent
 337 Ca²⁺ channels. In these experiments, we included ryanodine at 40 μ M, a concentration known to inhibit
 338 RyRs (Ehrlich et al., 1994; Isokawa and Alger, 2006), in the intracellular solution. Notably, one out of nine
 339 of the neurons recorded from in these conditions had a slightly negative adaptation index already under
 340 control conditions (Figure 6B3) when RyR were inhibited. Moreover, three more neurons appeared to fire
 341 irregularly, with some doublets, under control conditions (an example is shown in Figure 6C), a feature
 342 that was not observed in other experimental conditions. This change could be due to the close
 343 juxtaposition of RyRs in the endoplasmic reticulum and various K⁺ channels on the membrane of CA1
 344 neurons (Vierra et al., 2019; Tedoldi et al., 2020; Sahu and Turner, 2021). Upon addition of CCh in the
 345 bath, the adaptation index became significantly more negative in all nine neurons recorded with
 346 intracellular ryanodine, as per a paired t-test (control 0.28 ± 0.05 , CCh -0.46 ± 0.07 ; $t(8) = 11.99$; $p <$
 347 0.0005 ; $n = 9$), suggesting that RyRs also do not play a role in the cholinergic shift of the center of mass
 348 of firing.

349

350 *Multicompartmental modeling of cholinergic modulation of asymmetric firing responses*

351 We next turned to our detailed multicompartmental model, in order to help identify the underlying
 352 mechanisms that contribute to the cholinergic modulation that we observe experimentally *in vitro*. The
 353 model was implemented in a reconstructed morphology (Megías et al., 2001) with 144 compartments,
 354 each of which can be represented with an equivalent circuit with heterogeneous channel conductance
 355 densities and kinetics as described in the Methods. Previously (Upchurch et al., 2022), this model was
 356 used to capture the firing rate adaptation observed under control conditions as shown in Figure 7A1 for

357 short triangular ramps of injected current. The model performs a material balance on Ca^{2+} ions to provide
358 estimated free Ca^{2+} concentrations in concentric shells beneath the membrane, as shown in the top
359 panels of Figure 7. The control simulation for a 2 second somatic ramp in Figure 7A1 captures the
360 experimentally observed decrease in spike number and frequency on the down-ramp as compared to the
361 up-ramp (adaptation index = 0.28), as well as a slight decrease in spike amplitude, previously shown to
362 be due to a decrease in available Na_v channels (Upchurch et al., 2022). The plot of instantaneous
363 frequency versus level of injected current at the right captures the clockwise pattern observed in Figure
364 1C1 and G1 corresponding to firing rate adaptation. Figure 7A2 shows the $[\text{Ca}^{2+}]$ in the outer shell of the
365 soma in nM, Figure 7A3 replots this same concentration in μM (the nanodomain in this case is
366 contiguous with the outer shell so it is not actually a nanodomain), and Figure 7A4 shows that this
367 concentration is insufficient to activate TRPM4 channels. In order to model the effect of CCh, we added a
368 nanodomain for the spatially restricted Ca^{2+} concentration which we hypothesize is sensed by the Ca^{2+}
369 binding site on TRPM4 channels; also note that, as in the experiments, the amount of injected current is
370 lower for the simulated CCh. We showed in Figure 6 that TRPM4 channel activation is not affected by
371 antagonists of IP_3Rs and RyRs , excluding a contribution of Ca^{2+} release from the ER. Although the
372 source of the Ca^{2+} increase that activates TRPM4 channels is currently unknown, it likely depends in
373 some way upon the activation of voltage-gated Ca^{2+} channels during the ramp. We therefore made the
374 Ca^{2+} influx in the nanodomain proportional to the total instantaneous Ca^{2+} current across the membrane
375 in the model, but only during simulated activation of the muscarinic receptor. Adding the Ca^{2+} influx from
376 an unknown source to a nanodomain in order to simulate bath application of CCh (Figure 7B1) replicates
377 the experimentally observed shift in adaptation index, with more spikes occurring on the down-ramp as
378 compared to the up-ramp (adaptation index = -0.29). The plot of instantaneous frequency versus injected
379 current captures the more extreme observed effect of CCh as illustrated in Figure 1G2; the motion is
380 counter-clockwise corresponding to the acceleration of the firing rate. Figure 7B2 shows the Ca^{2+}
381 concentration in the outer shell of the soma in nM, Figure 7B3 shows the Ca^{2+} concentration in μM in the
382 hypothesized nanodomain, and Figure 7B4 shows that this concentration is sufficient to activate TRPM4
383 channels.

384 Figure 8 shows why spike rate adaptation is not observed in the simulated presence of CCh. Figure 8A
385 repeats the voltage and current traces from the previous figure, except that the simulated CCh traces
386 (magenta) are superimposed over the control traces (black). Our model includes a four state Markov
387 model of the Na_v channel (see Methods). Occupancy in the open state between action potentials is the
388 substrate for the persistent Na_v current during the interspike interval. Figure 8B shows that the
389 occupancy in the open state of the Markov model of the Na_v channel in the soma is not greatly different
390 between the two conditions, except for excursions produced by spiking, and Figure 8D shows the same
391 result for the somatic Na_v current. Our previous work (Upchurch et al., 2022) attributed spike rate
392 adaptation to reduced persistent Na_v current due to increased long-term inactivation. Figure 8C shows

393 that although long-term inactivation of the Na_v channel (occupancy in the I2 state) starts later in
394 simulated CCh, it eventually progresses to higher levels than in control because of the increased number
395 of spikes. Nonetheless, no spike rate adaption was observed in simulated CCh in Figure 7B1 because
396 the TRPM4 current is about 4-fold larger than the persistent Na_v current (compare Figures 8D and 8E).
397 Figure 8F shows that the additional TRPM4 current more than compensates for the smaller level of
398 injected ramp current; as a consequence, the net inward current is comparable on the up-ramp and
399 larger in magnitude during the down-ramp for the simulated CCh case than control. This causes spiking
400 to persist longer despite higher levels of Na_v channel inactivation.

401 When activated by Ca^{2+} , TRPM4 channels cause a depolarization and additional spiking that further
402 increases Ca^{2+} influx resulting in a positive feedback loop, with the regenerative inward TRPM4 current
403 contributing to action potential initiation. Figure 9 elaborates on the mechanisms by which Ca^{2+} and
404 voltage differentially contribute to TRPM4 channel activation in removing or reversing spike frequency
405 adaptation. It repeats the traces in control (black) and simulated CCh (magenta) from Figure 7 on an
406 expanded scale and adds two conditions as described below. The insets to the right in Figure 9A show f/I
407 curves that capture the qualitative features of the f/I curves shown in Figure 1C1, C2, G1 and G2.
408 Qualitatively, in control (black traces), there are more spikes on the up-ramp than on the down-ramp, and
409 the instantaneous frequency is lower on the down-ramp for similar values of injected current during the
410 up- ramp. The contribution of the increase in Ca^{2+} concentration in the nanodomain was examined by
411 turning off the voltage-dependence of the TRPM4 channel (light blue traces). In this case, the voltage
412 component of TRPM4 channel activation was set to its steady state value at the resting V_m (see
413 Methods).

414 Simply adding the nanodomain accomplishes the shift in center of mass (compare blue trace in Figure
415 9A to control trace in black) and removes the adaptation, with a very slight tendency to accelerate the
416 firing. However, without the contribution of the voltage-dependence of TRPM4 channels, the resulting
417 Ca^{2+} influx is lower than in the original simulation of the effects of CCh (compare the blue traces to the
418 magenta traces in Figure 9B and C), resulting in less TRPM4 channel activation (compare the blue trace
419 to the magenta trace in Figure 9D) and therefore fewer total spikes. To determine the contribution of the
420 positive feedback loop by which Ca^{2+} entry recruits more Ca^{2+} entry via additional spiking and
421 depolarization, we recorded the Ca^{2+} traces in each nanodomain (one for each segment of every section
422 in NEURON, see Methods) of the original CCh simulation (magenta trace in Figure 9C). We then used
423 this Ca^{2+} trace as the $[\text{Ca}^{2+}]$ in the nanodomain during simulations with the voltage dependence removed
424 as described above. The purple trace in Figure 9A shows that the positive feedback loop added two
425 spikes (16 in the purple trace versus 14 in the blue trace) by slightly increasing the frequency during the
426 spike train and allowing spiking to continue longer. The f/I plot shows a little more acceleration of the
427 firing rate. The purple trace in Figure 9B shows that $[\text{Ca}^{2+}]$ in the outermost shell is slightly different than

both the blue and magenta traces, reflecting the different numbers of spikes. Note that the purple and magenta traces in Figure 9C are identical because the magenta trace for $[Ca^{2+}]$ in the nanodomain (original CCh simulation) was input to the nanodomain in the simulations shown in purple in the other panels. The additional TRPM4 channel activation (compare purple to blue trace in Figure 9D) results from the increased $[Ca^{2+}]$ in the nanodomain. Adding the full voltage-dependence to the TRPM4 channel slightly raises the overall level of TRPM4 channel activation. The voltage-dependence also allows for fast excursions in channel activation that could contribute to action potential initiation (compare magenta trace in Figure 9D to the blue and purple ones). The voltage-dependence contributes one additional spike (17 in the magenta trace versus 16 in the purple trace in Figure 9A) and accentuates spike rate acceleration due to the positive feedback loop, as shown in the f/I curves.

Evidence for tight coupling between TRPM4 channels and the calcium source that activates them

In order to test experimentally the model prediction that the highly localized Ca^{2+} elevation in a nanodomain next to the TRPM4 channels gives rise to the CCh-mediated shift in the center of mass of firing, the calcium chelator BAPTA was included in the recording pipette, and somatic recordings obtained before and during addition of CCh. In Figure 10, control data reflect the effect of BAPTA before the addition of CCh, rather than control data such as in previous figures, because the dialysis occurred quickly. As in previous sets of experiments, the amount of current injected was chosen in order to achieve a target firing frequency of 10-25 Hz. With intracellular dialysis of BAPTA, the current needed to reach these frequencies was consistently higher in these experiments (compare scale bars of injected current to those in previous figures). In addition, intracellular dialysis with BAPTA tended to shift the firing earlier in the ramp, with fewer spikes on the down-ramp, therefore making the adaptation index closer to 1. In Figure 10A, 10 mM BAPTA was included in the internal solution. As previously noted, the addition of CCh in the bath caused a depolarization that was compensated for by the injecting hyperpolarizing current. Moreover, despite the presence of BAPTA, the adaptation index became significantly smaller in the presence of CCh, as per a paired t-test (control 0.82 ± 0.05 , CCh -0.12 ± 0.11 ; $t(9) = 8.594$; $p < 0.0005$; $n = 10$; Figure 10A3); therefore, 10 mM BAPTA did not prevent the cholinergic-mediated shift. It has been shown that a higher concentration of BAPTA may be required to prevent TRPM4 channel activation in neurons of the preBötzinger nucleus (Pace et al., 2007; Picardo et al., 2019), therefore, in Figure 10B, BAPTA in the internal solution was increased to 30 mM. With a free $[Ca^{2+}]_i$ of 100 nM (see Methods), CCh never produced negative adaptation indices that were seen in previous figures, although the V_m did depolarize and was restored by the injection of tonic hyperpolarizing current. CCh still had a significant effect on the adaptation index, as per a paired t-test (control 0.62 ± 0.05 , CCh 0.37 ± 0.06 ; $t(7) = 5.069$; $p = 0.001$; $n = 8$; Figure 10B3). However, when the free $[Ca^{2+}]_i$ was adjusted to 10 nM in the presence of intracellular 30 mM BAPTA (Figure 10C), the adaptation index did not change significantly when CCh was applied in the bath (control 0.73 ± 0.03 , CCh 0.76 ± 0.03 ; $t(11) = 0.776$; $p = 0.45$; $n = 12$;

Figure 10C3). In addition, the depolarization normally observed in the presence of CCh did not occur in these conditions, and the amount of injected current was comparable to that injected in control. These data demonstrate that a concentration of BAPTA higher than the typical value (10 mM) used to disrupt Ca^{2+} -dependent mechanisms (Harney et al., 2006) is required to prevent TRPM4 channel activation, and that free $[\text{Ca}^{2+}]_i$ of <100 nM is necessary to disrupt TRPM4 channel activation. Together, these data suggest a close physical relationship between the source of Ca^{2+} and TRPM4 channels.

470

471 Discussion

472 Summary of major results

Whereas place cell firing can only be observed *in vivo*, our bidirectional approach of modeling place cell firing *in vitro* and *in silico* can be precisely targeted to uncover molecular mechanisms responsible for modulation of firing patterns that lead to place cell firing *in vivo*. Manipulations that are difficult or impossible even in the *in vitro* preparation can be carried out *in silico* using our detailed multicompartmental model, with the added benefit that the model is transparent. All underlying state variables (ion concentrations, channel open fractions) are accessible so that mechanisms can be positively identified, facilitating design of experiments to test the putative mechanisms.

In this study we show that: 1) muscarinic receptor activation shifts the center of mass of firing from earlier to later during a depolarizing ramp, through 2) activation of I_{CAN} mediated by TRP channels, most likely of the TRPM4 subtype; 3) the shift is not affected by the inhibition of either IP_3Rs or RyRs , arguing against a role of Ca^{2+} released from the endoplasmic reticulum in this process; 4) multicompartmental modeling suggests a close physical relationship between the source of Ca^{2+} increase and the TRPM4 channels; 5) the model prediction is supported by recordings showing that a very high concentration of BAPTA (30 mM), combined with buffering $[\text{Ca}^{2+}]_i$ at 10 nM are required to prevent the cholinergic-mediated rightward shift.

More broadly, our results suggest that some rapid changes to place cell firing could be triggered via neuromodulation of intrinsic excitability, in addition to and distinct from longer lasting experience-dependent changes that are brought about by synaptic and structural plasticity. Specifically, we speculate that increasing levels of ACh could lead to forward shifts in place cell firing, whereas decreasing levels of ACh could contribute to backwards shifts.

493

494 Mystery current identified

495 Muscarinic activation of a Ca^{2+} -dependent nonselective cation current in rat CA1 pyramidal neurons has
496 been known for nearly three decades (Colino and Halliwell, 1993; Fraser and MacVicar, 1996; Dasari et
497 al., 2017). A previous study (Yamada-Hanff and Bean, 2013) found that in mouse CA1 neurons as well,
498 muscarinic stimulation not only inhibits background potassium currents but also activates a nonselective
499 cation current. They found that the latter effect was dominant and in fact converted CA1 pyramidal
500 neurons into pacemakers with the application of 5-25 μM acetylcholine, 5-25 μM carbachol, or 5-10 μM
501 oxotremorine-M. They suggested TRPC channels might mediate this current but were unable to
502 positively identify the source of the nonselective cation current. Based on our results, this unidentified
503 channel could be the TRPM4 channel. We show that TRPM4 channels primarily mediate a nonspecific
504 Ca^{2+} -activated cation current evoked by cholinergic modulation in CA1 pyramidal neurons. Our model
505 shows that the activation of TRPM4 channels contribute an additional inward current that activates and
506 deactivates slowly upon depolarization. In the case of synaptically-driven depolarizations (Figure 2), this
507 inward current, with its slow kinetics (see Figures 8 and 9) is likely to cause an increased decay time of
508 the EPSPs (see Figure 2). This aspect has likely implications for synaptic integration by these neurons,
509 as shown by the observation of an impairment of LTP in TRPM4 knockout mice (Menigoz et al., 2016).

510 One of our most surprising findings was that TRPC channels did not seem to play a role in cholinergic
511 modulation of the firing pattern along a triangular ramp. TRPC channels have been implicated in
512 cholinergic signaling via direct activation by DAG, one of the two bioactive molecules generated by PLC
513 hydrolysis of PIP_2 (the other being IP_3). Cholinergic-associated persistent firing, whereby neurons
514 continue to fire after a depolarizing stimulus has ceased, has been attributed to activation of TRPC
515 channels (Zhang et al., 2011; Arboit et al., 2020). However, the presence of cholinergic-induced
516 persistent firing in TRPC knockout mice has called this into question (Egorov et al., 2019). In a recent
517 study, TRPM4 channels were found to be involved in persistent firing generated upon Ca^{2+} influx via T-
518 type channels in thalamic neurons (O'Malley et al., 2020). Delayed bursting in a subtype of cerebellar
519 granular cells was also shown to be mediated by TRPM4 channels (Masoli et al., 2020). Our study
520 therefore provides further evidence regarding the role of these channels in the control of neuronal firing,
521 possibly including persistent firing.

522

523 *Nanodomain resulting from colocalization of cholinergic-induced increases in $[\text{Ca}^{2+}]_i$ and TRPM4*
524 *channels*

525 In HEK cells transfected with cDNA encoding TRPM4 channels, and in cardiac myocytes, activation of
526 TRPM4 channels is secondary to IP_3 receptor activation (Launay et al., 2002; Gonzales et al., 2010;
527 Gonzales and Earley, 2012), suggesting that TRPM4 channels in myocytes are activated by Ca^{2+} release
528 from intracellular stores. Furthermore, in situ proximity ligation assay was used to demonstrate co-
529 localization of TRPM4 channels and IP_3Rs in human detrusor smooth muscle cells (Provence et al.,

2017). In addition, in neurons of the preBötzing nucleus TRPM4 channels are recruited by metabotropic glutamate receptors to generate robust inspiratory drive potentials through IP₃R activation (Pace et al., 2007). Surprisingly, we found that Ca²⁺ release from the ER was not involved in the cholinergic shift of the center of mass of firing, since neither the IP₃R antagonist Xestospongine C (1-2 μM) nor the RyR antagonist ryanodine (40 μM) prevented the effects of carbachol. Therefore, although the mechanisms that produce the micromolar [Ca²⁺] increases needed for TRPM4 channel activation are unknown, our results point to these channels acting as coincidence detector, requiring both muscarinic receptor activation and depolarization-induced Ca²⁺ influx during the ramp to be activated

Our modeling results imply that, upon activation of muscarinic receptors, TRPM4 channels have privileged access to a nanodomain in order to achieve the micromolar concentrations of Ca²⁺ necessary for their activation (Nilius et al., 2004), since microdomains (like the multicompartamental model's outer shell) only achieve submicromolar concentrations (Fakler and Adelman, 2008). The affinity of TRPM4 channels for Ca²⁺ is heavily dependent on the availability of PIP₂ (Nilius et al., 2006) and binding of ATP and calmodulin or PKC-dependent phosphorylation (Nilius et al., 2005), which all function to increase the sensitivity of TRPM4 channels for Ca²⁺. If under our conditions, the dependence of TRPM4 channels on [Ca²⁺] has a much lower EC₅₀ and/or is much steeper than in the model we implemented (Nilius et al., 2004), perhaps the nanomolar [Ca²⁺] in the outer shell could be sufficient to activate TRPM4 channels. However, the experimental result that 30 mM BAPTA was required to block the TRPM4 channels' access to Ca²⁺ argues for a spatially restricted nanodomain. In a previous study, we found that intracellular BAPTA at a concentration 5 mM can mimic SK block (Combe et al., 2018), presumably by interfering with the calcium activation of those channels. The requirement of using 30 mM BAPTA while buffering free [Ca²⁺]_i to 10 nM in order to prevent TRPM4 channel activation suggests that TRPM4 channels are activated by a more spatially restricted Ca²⁺ domain than SK channels.

553

554 *Convergence of pharmacological blockers*

One caveat for our experimental results is that TRP channel blockers, like most drugs, have been reported to have some non-specific effects. In cardiac cells, both CBA and 9-phenanthrol have been shown to decrease the I_{to1} potassium current, attributed to Kv4.3 (Veress et al., 2018; Dienes et al., 2021) and a late sodium current (Hou et al., 2018; Dienes et al., 2021). Kv4.3 is likely more important in interneurons than in pyramidal cells in the CA1 area (Martina et al., 1998), and neither of the two currents are affected by FFA at the concentration we used here (Guinamard et al., 2013). The other reported non-specific effects do not overlap between the three blockers, flufenamic acid, CBA and 9-phenanthrol (Simard et al., 2012; Guinamard et al., 2013; Veress et al., 2018; Dienes et al., 2021). The convergent effects of these three distinct drugs implicate TRPM4 channels as the most probable effector of the CCh-mediated modulation of the center of mass of firing. A similar pharmacological approach was

565 used to implicate TRPM4 channel activation in subthreshold oscillations underlying pacemaker firing of
566 chemosensory neurons in the retrotrapezoid nucleus (Li et al., 2021).

567

568 *Diverse cholinergic effects on CA1 pyramidal neurons*

569 In CA1 pyramidal neurons, ACh binding to muscarinic receptors results in neuromodulation of neuronal
570 excitability and presynaptic release probability. In our study, focused mainly on intrinsic properties, the
571 predominant effect of ACh and CCh was to activate TRPM4 channels; however, the effect of ACh on
572 CA1 pyramidal cells *in vivo* is multipronged. Muscarinic receptors are known to suppress several K⁺
573 currents in CA1 pyramidal neurons (Halliwell and Adams, 1982; Hoffman and Johnston, 1998; Buchanan
574 et al., 2010; Giessel and Sabatini, 2010). The effects of ACh on SK, M-type currents and I_{CAN} are
575 mediated by M₁ (G_q-coupled) receptors (Dasari and Gulledge, 2011). Synaptic depression of Schaffer
576 collateral inputs to CA1 is mediated by M₄ (G_i-coupled) receptors (Dasari and Gulledge, 2011) located on
577 presynaptic glutamatergic terminals, and synaptic depression of temporoammonic pathway glutamatergic
578 inputs to CA1 is mediated by M₃ (G_q-coupled) receptors (Palacios-Filardo et al., 2021). When we used a
579 synaptically-driven depolarization (Figure 2), decreased release from Schaffer collateral was indicated by
580 the smaller amplitude EPSPs in the presence of CCh. In these experiments, as in the current injection
581 protocols, the center of mass of firing occurred earlier in the ramp in control and shifted to later in the
582 ramp in the presence of CCh, suggesting that a combination of pre- and postsynaptic effects of
583 cholinergic agonists can contribute to the shift. Moreover, the cholinergic down-regulation of these
584 afferents justifies the use of smaller depolarizing current injection ramps in our recordings in the
585 presence of carbachol, because less current injection is required to produce the same output frequencies
586 due to enhanced intrinsic excitability in carbachol.

587 In a model of place field formation (Savelli and Knierim, 2010), if a neuron randomly fires within a place
588 field (Bittner et al., 2015), its inputs from the currently active grid cells are strengthened and the input
589 from those grid cells onto silent cells are weakened. Previously, ACh was thought to act in a manner
590 consistent with volume transmission (Dannenberg et al., 2017). Recently, this view has been challenged
591 by the finding that all hippocampal cholinergic terminals form synapses (Takács et al., 2018), and
592 vesicles dock only at synapses. Since cholinergic neuromodulation of TRPM4 channels greatly increases
593 excitability in CA1 pyramidal cells, selective synaptic ACh inputs may underlie the formation of place
594 fields in a novel environment. Moreover, (Takács et al., 2018) found that the ACh terminals co-released
595 GABA in separate vesicles, and that ACh acting on M₂ (G_i-coupled) autoreceptors on cholinergic
596 terminals inhibited the release of both ACh and GABA. Future work is required to disentangle the distinct
597 effects of ACh acting at the various muscarinic receptors (M₁-M₅).

598

600 Acetylcholine acting at muscarinic receptors has been hypothesized to be a global novelty signal in the
601 dentate gyrus (Gómez-Ocádiz et al., 2022), dorsal hippocampus (Huff et al., 2022), and the
602 parahippocampal gyrus (Frank and Kafkas, 2021). A novel unconditioned stimulus caused a large
603 increase in ACh extracellular levels in the hippocampus, but little locomotion (Acquas et al., 1996). On
604 the other hand, hippocampal ACh release in an animal placed in a novel environment has two
605 components, one related to novelty, that disappears in a familiar environment (Thiel et al., 1998), and
606 one related to motor activity, associated with increased exploration (Giovannini et al., 2001). Indeed, a
607 recent fiber photometric study showed that part of the population activity in medial septal cholinergic
608 neurons is correlated with the logarithm of running speed (Kopsick et al., 2022). We are primarily
609 interested in the component of the ACh signal related to novelty because of previous studies
610 demonstrating modulation of place cell firing that was more pronounced during the first day in a novel
611 environment (Roth et al., 2012).

612

613 *In vivo* recordings in rats demonstrated that place fields expand and the center of mass shifts backward
614 with respect to the direction of travel on continuous tracks over several laps during each recording
615 session (Mehta et al., 2000). This phenomenon was predicted by modeling studies that implemented
616 Hebbian plasticity in the form of NMDA-mediated LTP (Abbott and Blum, 1996; Blum and Abbott, 1996),
617 whereby spatially tuned input gradually strengthens over repeated traversals of a place field, causing a
618 CA1 place cell to fire earlier in the place field. NMDA blockers were subsequently shown to occlude the
619 backwards shift of the place field (Ekstrom et al., 2001) and an NMDA agonist partially restored it in aged
620 rats (Burke et al., 2008). Calcium imaging in mice running on a treadmill in a virtual environment showed
621 an abrupt backward shift in place cell firing (relative to where the first episode of place field firing
622 occurred) during the first exposure to a novel environment (Priestley et al., 2022), as well as much
623 smaller backward shifts in place fields in novel environments that leveled off as the animals became
624 more familiar with the environment during subsequent exposures (Dong et al., 2021). These backwards
625 shifts, which were much larger than those observed previously and attributed to Hebbian plasticity, were
626 ascribed to behavioral time scale plasticity, which is also dependent on NMDA receptor activation, and
627 strengthens glutamatergic inputs active during an asymmetric seconds-long window skewed towards
628 synapses activated before a burst triggered by a dendritic plateau (Bittner et al., 2017).

629 Despite the ability of elegant theories of synaptic plasticity to account for backward shifts in the center of
630 mass of the place field, forward shifts have also been observed. Forward shifting of the center of mass of
631 place field firing occurred, shifting toward prospective goal locations in the continuous T-maze alternation
632 task as a reward was approached (Lee et al., 2006). Thus, task-specific demands may allow for the
633 modulation of firing fields by neuromodulators such as ACh and dopamine. In a study that did not involve

reward, but only exploration of a novel virtual linear maze (Dong et al., 2021), the center of mass of CA1 place fields in laps 2-4 were forward shifted compared to the first lap, for cells that already had a place field on the first lap (a lap required approximately 25 seconds). Laps 5 and beyond were backward shifted compared to the immediately preceding lap, so averaging over all runs, the place field was shifted backwards. Perhaps the neuromodulatory environment, including cholinergic tone, was different during the first four laps. The data in that study indicated that backward shifting in CA1 in a novel environment is due to a combination of place field expansion, skewness and translation of the point at which peak firing occurs. They suggested that since skewness and width dynamics are inconsistent across studies, multiple mechanisms may be involved in place field shifting.

643

644 *Ideas and Speculations: Implications of our results for place fields in intact rodents*

Our results suggest that increasing levels of ACh could lead to forward shifts in place field center of mass whereas decreasing levels of ACh could contribute to backwards shifts. Since cholinergic activity in CA1 is elevated when animals encounter novel environments, our results with CCh could correspond to an initial encounter with a novel environment, where firing of an existing place cell is shifted “forward” or later during the depolarizing ramp that simulates spatially-tuned input. Our control conditions, then, would be analogous to lower cholinergic tone, occurring as an animal becomes familiar with an environment, and the associated “backward” shift earlier in the depolarizing ramp. The rapid modulation of ion channels by removal of cholinergic tone could therefore provide a possible mechanism for the rapid shifting of place cell firing in a novel versus familiar environment, perhaps complementary to Hebbian plasticity that develops over time.

If our hypothesis is correct, optogenetic silencing of medial septum cholinergic afferents to CA1 during runs through a place field in a novel environment should partially occlude backwards shifts in place field center on subsequent runs that are due to diminished ACh in a familiar environment. Optogenetic stimulation of the same afferents during runs in a familiar environment should induce forward shifts in place field centers. Behaviorally, it could be informative to design experiments to test the effects of these manipulations on the animal’s subjective sense of position, for example, demonstrated by overshoot or undershoot locations where the animal has been trained to pause.

There is a growing realization that there may be multiple mechanisms for place field shifting in addition to the well-known mechanisms for backwards shifts dependent on Hebbian plasticity (Blum and Abbott, 1996; Ekstrom et al., 2001; Burke et al., 2008; Roth et al., 2012; Mehta et al., 2000) or, more recently, behavioral timescale synaptic plasticity (Bittner et al., 2017; Priestley et al., 2022). We propose that neuromodulation of intrinsic properties by ACh, and potentially other neuromodulators like dopamine,

667 provide an additional level of modulatory control in parallel with synaptic plasticity mechanisms,
 668 contributing to rapid place field shifts.

669

670 **Materials and Methods**

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain, strain background	Male Sprague Dawley rats	Envigo (Inovit)	Hsd:Sprague Dawley® SD®	7-12 weeks old when sacrificed for experiments
chemical compound, drug	NBQX	Alomone Labs	cat# N-186	AMPA receptor antagonist
chemical compound, drug	DL-APV	HelloBio	cat# HB0252	NMDA receptor antagonist
chemical compound, drug	Gabazine	Alomone Labs	cat# G-216	GABA _A receptor antagonist
chemical compound, drug	CGP55845	Abcam	cat# ab120337	GABA _B receptor antagonist
chemical compound, drug	Carbachol	Tocris	cat# 2810	Acetylcholine receptor agonist
chemical compound, drug	Acetylcholine,	Sigma-Aldrich	cat# A6625	Ligand for acetylcholine receptors
chemical compound, drug	flufenamic acid	Sigma-Aldrich	cat# F9005	TRP channel antagonist
chemical compound, drug	SKF96365	Alomone Labs	cat# S-175	TRPC channel antagonist

chemical compound, drug	CBA	Tocris	cat# 6724	TRPM4 channel antagonist
chemical compound, drug	9-phenanthrol	Tocris	cat# 4999	TRPM4 channel antagonist
chemical compound, drug	Xestospongine C	Abcam	cat# ab120914	IP ₃ R antagonist
chemical compound, drug	low molecular weight heparin	Sigma-Aldrich	cat# H3149	IP ₃ R antagonist
chemical compound, drug	D-myo-IP ₃	Cayman Chemical	cat# 60960	IP ₃ R agonist
chemical compound, drug	Adenophostin A	MilliporeSigma	cat# 115500	IP ₃ R agonist
chemical compound, drug	Ryanodine	HelloBio	cat# HB1320	RyR antagonist at [μM]
chemical compound, drug	Ruthenium red	Tocris	cat# 1439	RyR antagonist
chemical compound, drug	Dantrolene	Tocris	cat# 0507	RyR antagonist
chemical compound, drug	caffeine	Tocris	cat# 2793	RyR agonist
chemical compound, drug	Thapsigargin	Alomone Labs	cat# T-650	SERCA inhibitor
chemical compound, drug	BAPTA tetra-potassium salt (K4-BAPTA)	Sigma-Aldrich	cat# A9801	fast Ca ²⁺ chelator
Software, algorithm	MAXCHELATOR, WEBMAXC EXTENDED	https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcE.htm		Online free metal calculator
Software,	SPSS	IBM	RRID:SCR_00	Statistics

algorithm			2865	software
Software, algorithm	NEURON	Hines and Carnevale, 1997; Carnevale and Hines, 2006	doi:10.1162/neuro.1997.9.6.1179; doi.org/10.1017/CBO9780511541612	Computational modelling and simulation software
other	Laerd Statistics	https://statistics.laerd.com/		Online tutorial and software guide

671

672

673 *Experimental methods*

674 *Slice preparation.* All the procedures described below were conducted according to protocols developed
675 by following guidelines on the responsible use of laboratory animals in research from the National
676 Institutes of Health and approved by the Louisiana State University Health Sciences Center-New Orleans
677 Institutional Animal Care and Use Committee (IACUC, internal protocol numbers 3583 and 3851).
678 Animals were housed in pairs, whenever possible; in the event of single housing, Bed-r'nest pucks
679 (Andersons Lab Bedding) were provided for enrichment purposes.

680 For these experiments, 400 μ m-thick slices were prepared from 7 to 11-week-old male Sprague Dawley
681 rats as previously described (Combe et al., 2018). Briefly, rats were deeply anesthetized via
682 intraperitoneal injection of ketamine and xylazine (90 and 10 mg/kg, respectively), until the
683 disappearance of the toe-pinch and palpebral reflexes. After trans-cardiac perfusion with ice-cold
684 solution, decapitation, and rapid removal of the brains, transverse hippocampal slices were cut using a
685 vibratome. Slices were then transferred to a chamber filled with an oxygenated artificial cerebro-spinal
686 fluid (ACSF) containing, in mM: NaCl 125, NaHCO₃ 25, KCl 2.5, NaH₂PO₄ 1.25, MgCl₂ 1, CaCl₂ 2,
687 dextrose 25, ascorbate 1, sodium pyruvate 3.

688 *Patch clamp electrophysiology.* After recovery, individual slices were transferred to a submerged
689 recording chamber, and superfused with ACSF at about 2 ml/min; the solution was warmed through an
690 inline heater and measured to keep it at a temperature of 34-36°C in the chamber.

691 CA1 pyramidal cells were identified via differential interference contrast-infrared video microscopy.
692 Whole-cell current clamp recordings were made using Dagan BVC 700A amplifiers in the active "bridge"
693 mode. Recording pipettes were filled with an internal solution that contained, in mM: potassium
694 methanesulphonate 125, KCl 20, HEPES 10, EGTA 0.5, NaCl 4, Mg₂ATP 4, Tris₂GTP 0.3,

695 phosphocreatine 14. The electrode resistance was 1-3 MΩ for somatic recordings and 3-5 MΩ for
696 dendritic recordings. Series resistance was monitored throughout the recordings and was usually less
697 than 20 MΩ; recordings were discarded when series resistance reached 25 MΩ or 30 MΩ, for somatic
698 and dendritic recordings, respectively. Cells with resting membrane potentials depolarized beyond -60
699 mV at break-in were discarded.

700 To investigate the contribution of intracellular Ca^{2+} signaling to TRPM4 channel activation and the
701 cholinergic shift in the center of mass of firing, in some sets of experiments, the internal patch clamp
702 solution was modified as follows. In some experiments, EGTA was replaced by higher concentrations (10
703 or 30 mM) of the faster Ca^{2+} chelator BAPTA tetra-potassium salt. When 30 mM BAPTA was included,
704 0.69 mM or 5.7 mM Ca^{2+} was added to the internal solution to give 10 or 100 nM free $[\text{Ca}^{2+}]$,
705 respectively, at 33°C, pH 7.3 and an overall ionic concentration of 300 mM, according to the online
706 program MAXCHELATOR, WEBMAXC EXTENDED version
707 ([https://somapp.ucdmc.ucdavis.edu/pharmacology/](https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcE.htm)

708 [bers/maxchelator/webmaxc/webmaxcE.htm](https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcE.htm)). In other experiments, Xestospongine C (1-2 μM) or
709 ryanodine (40 μM) were added to the internal patch clamp solution to block IP_3Rs and RyRs ,
710 respectively. Xestospongine C and ryanodine were dissolved in DMSO; in this case the [DMSO] in the
711 final solution was $\leq 0.2\%$ due to the limited solubility of these compounds. As in (Pace et al., 2007),
712 Xestospongine C was added to the standard intracellular solution immediately prior to use and discarded
713 after 2 hours. In all these cases, intracellular dialysis appeared to take effect quickly, so the control data
714 refer to the effect of the modified internal solution. Nonetheless, dialysis was allowed for at least 20
715 minutes in these experiments to make sure that the drug could diffuse into distal compartments.

716 NBQX (10 μM), DL-APV (50 μM), Gabazine (12.5 μM) and CGP55845 (1 μM) were applied in the
717 external solution in all experiments, except those in which the Schaffer Collateral fibers were electrically
718 stimulated (Figure 2), to block glutamatergic and GABAergic neurotransmission, respectively, in order to
719 isolate the contribution of the intrinsic ion channels to the ramp responses. Carbachol (2 μM),
720 acetylcholine (2, 10, or 15 μM), flufenamic acid (100 μM), SKF96365 (50 μM), CBA (50 μM), and 9-
721 phenanthrol (100 μM) were added to the external solution as needed, from stock solutions made with
722 water, DMSO, or ethanol; the concentration of ethanol or DMSO in the final solution was $\leq 0.1\%$.
723 Carbachol, 9-phenanthrol, and CBA were purchased from Tocris, CGP55845 and Xestospongine C from
724 Abcam (Cambridge, MA), DL-APV and ryanodine from HelloBio (Princeton, NJ), NBQX, Gabazine, and
725 SKF96365 from Alomone Labs (Jerusalem, Israel), and acetylcholine, flufenamic acid, and K4-BAPTA
726 from Sigma (St. Louis, MO).

727 In order to approximate the depolarizing input that place cells receive as an animal traverses the place
728 field, ramp-shaped depolarizing current injections were applied via the recording electrode to CA1
729 pyramidal neurons at either the soma or the apical dendrite (150-250 μm from soma). Two second ramps
730 (1 s up, 1 s down) and ten second ramps (5 s up, 5 s down) were applied to simulate different running

731 speeds. Current amplitude was adjusted to evoke peak frequencies between 10 and 25 Hz, comparable
732 to place cell firing as recorded *in vivo* (Hargreaves et al., 2007; Resnik et al., 2012; Bittner et al., 2015).

733 Electrical stimulation was achieved by delivering constant current pulses through a tungsten bipolar
734 electrode placed in the stratum radiatum of area CA1 to stimulate the Schaffer collateral fibers from CA3
735 pyramidal neurons. The instantaneous input frequency of stimulation was adjusted according to a linear,
736 symmetric ramp (see also (Hsu et al., 2018)). The total duration of the ramp was ~2 s and the peak
737 frequency at the center of the ramp was 25 Hz, our target for the maximum peak frequency in the ramp
738 current injections (see above). The intensity of stimulation was adjusted such that neurons would fire in
739 response to 50-70% of the total inputs and kept constant in control conditions and in the presence of
740 carbachol. For each neuron and condition, we averaged the trials where the neurons did not fire in
741 response to the first pulse in the synaptic ramp and the amplitude of the EPSPs was measured to
742 estimate the degree of cholinergic modulation of presynaptic release. Antagonists of synaptic transmitter
743 receptors were omitted in these experiments.

744

745 *Experimental design and statistical analyses*

746 Experimental data were recorded and analyzed with Igor Pro software (WaveMetrics). Left or rightward
747 shifts in firing along the current ramp were quantified as an adaptation index, as previously described
748 (Upchurch et al., 2022):

$$\frac{\#APs\ on\ upramp - \#APs\ on\ downramp}{\#APs\ on\ upramp + \#APs\ on\ downramp}$$

749 Positive values indicate firing predominantly on the up-ramp, while negative values indicate firing mostly
750 on the down-ramp. For synaptically driven membrane potential ramps, the adaptation index was
751 calculated in a similar manner, where the up-ramp consisted of the first 15 (out of 30) stimuli. An
752 adaptation index of zero indicates symmetry in the number of action potentials on the up-ramp and the
753 down-ramp. Plots of instantaneous frequency versus time (f/t) or current (f/I) use the time or current
754 value at the midpoint of the interspike interval (ISI), respectively. Current amplitude is relative to tonic
755 baseline current. Raster plots in Figures 1 and 2 show the timing of action potential firing for several trials
756 in control conditions and in the presence of carbachol, where time = 0 indicates the beginning of the
757 ramp. The tick marks represent the timing of each action potential (when the V_m crossed -40 mV).

758 An n = 6-12 is required for a typical patch clamp electrophysiology experiment that has a difference in
759 means of 1.3-2X and a standard deviation of 0.2-0.3 for p = 0.05 and a power of 0.9 (Cohen, 1977).
760 Each experimental group in this study is made up of n ≥ 8 recording sessions; the number of cells
761 recorded for each experiment is indicated in the Results section. Recordings were from one cell per slice;
762 typically, a maximum of three recordings were obtained for each animal. Biological replicates (recording

763 from different cells) were $n \geq 8$ for each experiment and (recordings from different animals) $n \geq 6$ for each
764 experiment.

765 Statistical analyses were performed in SPSS (IBM, RRID:SCR_002865), following the tutorials and
766 software guide from Laerd Statistics (2015, <https://statistics.laerd.com/>). Comparisons were made in the
767 same cell before and during pharmacological treatment; the summary plots for these data include
768 individual points and means $\pm$ standard error of the mean. Parametric analyses (paired samples t-test
769 and repeated-measures ANOVA) were used to compare treatments in the same neurons, since data
770 were normally distributed as determined by the Shapiro-Wilk test for normality. Significant differences
771 demonstrated by repeated measures ANOVA were followed up by post-hoc analysis using Bonferroni-
772 corrected pair-wise comparisons. Differences were considered to be statistically significant when $p <$
773 0.05. Outliers, as assessed by boxplots, were not removed from analysis. To check the effect of the
774 outliers, analyses were also performed after removing outliers, and with non-parametric tests keeping the
775 outliers. The significance did not change, and for consistency, the results of parametric tests are reported
776 with all data included.

777 *Data Availability*

778 Data generated and analyzed during the study are included in the manuscript and supporting files.
779 Source data are provided for all experimental figures.

780 *Computational Modeling*

781 A multicompartmental CA1 pyramidal neuron model from our laboratory was used as a starting point
782 (Upchurch et al., 2022). This model was based on the model in the study by (Poirazi et al., 2003a), with
783 subsequent changes made in studies by (Shah et al., 2008), (Bianchi et al., 2012), and (Combe et al.,
784 2018). The model (Figure 11A) was created from a reconstructed morphology (Megías et al., 2001) with
785 144 compartments and implemented in the NEURON simulation software package (Hines and
786 Carnevale, 1997; Carnevale and Hines, 2006). The following currents (Figure 11B) were modeled using
787 Ohmic drive and conductances in parallel with a membrane capacitance (C_M): a Na^+ current (I_{Na}), L-type
788 (I_{CaL}), R-type (I_{CaR}), and T-type (I_{CaT}) Ca^{2+} currents, two delayed rectifiers (I_{Kv1} and I_{Kv2}), an A-type K^+
789 current (I_A), an inward rectifying potassium current (I_{KIR}), a nonspecific Ca^{2+} -activated current (I_{CAN}), a
790 hyperpolarization-activated mixed cationic h-current (I_h), and a leak current (I_L). All currents were as in
791 the previous model except (I_{CAN}), which was added to the model. In the soma and apical dendrites, we
792 kept the Markov model (Figure 11C) of the Na_v channel that we modified from (Balbi et al., 2017). This
793 Markov model was tuned to replicate the distance-dependent long-term inactivation of $\text{Na}_v1.6$ found in
794 the literature (Mickus et al., 1999) that causes spike frequency adaptation and the difference in half
795 activation between the soma and dendrites supported by (Gasparini and Magee, 2002). The sevenfold
796 gradient in the h-conductance supported by (Magee, 1998), sixfold gradient in A-type potassium

channels supported by (Hoffman et al., 1997) and lower sodium conductance in the dendrites versus the soma (75% of somatic value) supported by (Gasparini and Migliore, 2015) were also retained. A spine factor, to account for the additional membrane area due a higher density of spines (Megías et al., 2001) was varied as described in (Upchurch et al., 2022), and a sigmoidal decrease in membrane and axial resistance was implemented to account for the decrease in input resistance along the apical dendrites (Magee, 1998; Poirazi et al., 2003b).

The calcium handling for the model (see top panels in Figure 7A1 and B1) was modified from (Ashhad and Narayanan, 2013). The model performs a Ca^{2+} material balance on four shells in each compartment, and incorporates radial and longitudinal diffusion ($D_{Ca} \nabla^2 [\text{Ca}^{2+}]$), stationary and mobile Ca^{2+} buffers (R_{Buf}), calcium leak through endoplasmic reticulum leak channels (J_{leak}), Ca^{2+} uptake from a sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pump (J_{SERCA}), IP_3 -mediated Ca^{2+} release (J_{IP3R}), a plasma membrane extrusion pump (J_{Pump}) and Ca^{2+} from voltage gated channels on the membrane (J_{VGCC}). The latter two terms appear only in the outer shell. Details on the implementation of diffusion, the buffers, calcium leak and the pumps are described in (Ashhad and Narayanan, 2013). The differential equation for cytosolic calcium is described below.

$$\frac{\partial [\text{Ca}^{2+}]}{\partial t} = D_{Ca} \nabla^2 [\text{Ca}^{2+}] + A J_{IP3R} + B (J_{leak} - J_{SERCA}) + R_{Buf} + J_{VGCC} - J_{Pump}$$

812

We used an equation for Ca^{2+} dynamics in the nanodomain that is agnostic as to the source of the Ca^{2+} contributing to the nanodomain:

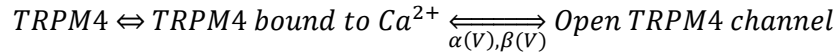
$$\frac{d[\text{Ca}^{2+}]_{ND}}{dt} = X * I_{Ca} + \frac{[\text{Ca}^{2+}]_{outer\ shell} - [\text{Ca}^{2+}]_{ND}}{400ms}$$

815

where X is a scale factor representing the effect of a small volume for Ca^{2+} accumulation in the restricted nanodomain. The model is agnostic regarding the source of the Ca^{2+} entry into the nanodomain, which was arbitrarily set to be proportional to the total Ca^{2+} current I_{Ca} . In the absence of simulated application of CCh, $X = 0$ so that the TRPM4 channel senses the bulk Ca^{2+} concentration in the outer shell. To simulate application of CCh, in contrast with our previous modeling efforts, based on a central role for IP_3 (see Figure 11 – Figure supplement 1), $[\text{IP}_3]$ and therefore J_{IP3R} was not increased from baseline levels; instead, influx to the nanodomain from an unknown source was simulated by setting $X = 500$. The relatively long time constant reflected the spatially restricted nature of the nanodomain, with some postulated impediment to diffusion between the nanodomain and the bulk cytosol. In one simulation (purple traces in Figure 9), the $[\text{Ca}^{2+}]_{ND}$ for every segment in every section was recorded as recorded in

the original CCh simulation (magenta traces) and played back using NEURON's vector play method in the model lacking the voltage-dependence of the TRPM4 channels in order to eliminate differences in $[Ca^{2+}]_{ND}$ between models and focus solely on the contribution of the voltage-dependence unrelated to additional Ca^{2+} influx.

The TRPM4 model was modified from (Nilius et al., 2004) and assumes that the required binding of Ca^{2+} to the channel precedes voltage-dependent channel activation. The model has three different states, an unbound state, a Ca^{2+} bound closed state, and an open state.



Ca^{2+} binding is assumed to be much faster than the voltage dependent gating and is assumed to instantaneously reach the steady state binding determined by the dissociation constant K_d (87 μM). The fraction of open channels m relaxes to the steady state activation m_{∞} with a time constant, τ , with first order kinetics:

$$\alpha' = \frac{\alpha}{1 + \frac{K_d}{[Ca^{2+}]_{ND}}}, m_{\infty} = \frac{\alpha'}{\alpha' + \beta} \quad \tau = \frac{1}{\alpha' + \beta}$$

where α is the forward rate of Ca^{2+} bound TRPM4 channel opening, $\alpha(V) = 0.0057 \exp(0.0060 V)$, α' is the forward rate scaled by the fraction of Ca^{2+} bound channels, β is the backward rate of TRPM4 channel closing, $\beta(V) = 0.033 \exp(-0.019 V)$ and $[Ca^{2+}]_{ND}$ is the Ca^{2+} concentration in the nanodomain. When removing the voltage-dependence of TRPM4 channels, we set V in the equations above to the steady-state value at -60 mV. We injected enough current into the model to hold the membrane potential at -60 mV long enough for all transients to equilibrate before injecting a two second temporally symmetric current ramp in the soma. The amplitude of the ramp was adjusted to reach similar peak frequencies as in the experiments, 10 to 25 Hz.

The persistent current during the interspike intervals determines the spike frequency. In order to focus on the rate determining currents in Figures 8 and 9, we removed points from the traces during spiking by eliminating points at which the absolute value of the first temporal derivative of the current trace exceeded 0.005 $\mu A/ms \cdot cm^2$ for the net current, 0.15 $\mu A/ms \cdot cm^2$ for the TRPM4 current, and 0.2 $\mu A/ms \cdot cm^2$ for the NaV current. Points outside the range of -0.3 to 0.09 $\mu A/cm^2$ in the net current trace, greater than 0 $\mu A/cm^2$ in the TRPM4 trace and less than -10 μA in the Nav current were also removed. Points were removed from the occupancy in O trace if the absolute value of the first temporal derivative of the O state exceeded 0.00002/ms or when occupancy exceeded 0.0002.

854

855 *Software accessibility*

856 Model code is freely available and can be downloaded from ModelDB at:
857 <http://modeldb.yale.edu/267599>,
858 access code: cholinergicshift.
859

860

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1169

1170 **Figure legends**

1171 Figure 1. Carbachol shifts the center of mass of firing in response to depolarizing current ramps.

1172 A. Somatic recording, control. A1. Voltage trace for a two second triangular current ramp (represented
 1173 below) injected in the soma. A2. Instantaneous frequency for example in A1 as it changes along the
 1174 ramp. A3. Raster plot of different trials recorded from the same example neuron in A1. The trials are
 1175 ordered by the amplitude of the peak injected current, from lowest (bottom) to highest (top); trials with the
 1176 same amount of current injection are clustered together. B. Somatic recording, carbachol. B1, B2, and
 1177 B3. Same as A1, A2, and A3, but during superfusion with 2 μ M CCh. C1. Instantaneous frequency for
 1178 example in A1, as it changes with injected current. Filled circles represent firing on the up-ramp; open
 1179 circles, firing on the down-ramp. C2. Instantaneous frequency for example in B1. A, B, and C are
 1180 recordings from the same cell. D. Summary data of the adaptation index for somatic recordings, before
 1181 and during CCh, for two second ramps ($n = 21$) and ten second ramps ($n = 19$). Open circles connected
 1182 by gray lines represent indices for individual cells, before and during CCh. Black squares with error bars
 1183 represent group averages $\pm$ SEM. In control, all adaptation indices are positive, indicating more action
 1184 potentials on the up-ramp. In CCh, most adaptation indices are negative, indicating more action
 1185 potentials on the down-ramp. E. Dendritic recording, control. E1. Voltage trace for a two second
 1186 triangular current ramp (represented below) injected in the dendrite, approximately 200 μ m from the
 1187 soma. E2. Instantaneous frequency for E1 as it changes along the ramp. E3. Raster plot of different trials
 1188 recorded from same example neuron in E1. F. Dendritic recording, carbachol. F1, F2, and F3. Same as
 1189 E1, E2, and E3, but during superfusion with 2 μ M CCh. G1. Instantaneous frequency for example in E1,
 1190 as it changes with injected current. G2. Instantaneous frequency for example in F1. E, F, and G are from
 1191 the same cell. H. Summary data of the adaptation index for dendritic ramps, before and during CCh, for
 1192 two second ramps ($n = 19$) and ten second ramps ($n = 10$). *** $p < 0.0005$. Red dotted lines in the left
 1193 panels mark the middle of the current injection ramp. Source data in "Figure 1 - Source data". See also
 1194 Figure 1 – supplement 1 and Figure 1 – supplement 2.

1195

1196 Figure 2. Cholinergic modulation of the response to electrical stimulation of Schaffer collaterals, recorded
 1197 at the soma. A Top, synaptic response in control conditions to an input frequency that increases and
 1198 decreases symmetrically, from 6.7 to 25 Hz, as denoted by the pulses under the voltage trace. Bottom,

1199 Raster plot of multiple trials repeated at the same stimulation intensity. B same as A during superfusion
1200 of 2 μ M CCh. A and B are from the same neuron, and at the same stimulation intensity. Diamonds in A
1201 and B point to a longer EPSP decay time in the presence of CCh. C. Summary data of the adaptation
1202 index for synaptic ramps, before and during CCh. D. Summary data of the average EPSP amplitude
1203 measured during trials where the cells did not fire in response to the first stimulus pulse, before and
1204 during CCh. In both cases, open circles connected by gray lines represent indices for individual cells;
1205 black squares with error bars represent group averages $\pm$ SEM *** $p < 0.0005$, ** $p = 0.002$. Red dotted
1206 lines in the panels on the left mark the middle of the synaptic stimulation. Source data in "Figure 2 -
1207 Source data".
1208

1209 Figure 3. Modulation of response to symmetric ramps by acetylcholine is concentration dependent.
1210 A. Voltage trace recorded in the soma for a two second triangular current ramp injected in the soma in
1211 control. Current traces and instantaneous frequency plots are just below the voltage traces. B-D. Same
1212 as A, during superfusion with 2 μ M ACh (B), 10 μ M ACh (C), and 15 μ M ACh (D). A through D are from
1213 the same cell. E. Summary data of the adaptation index for control and the three concentrations of ACh,
1214 applied in order of increasing concentration ($n = 13$). Open circles connected by gray lines represent
1215 indices for individual cells over all four conditions. Black squares with error bars represent group
1216 averages $\pm$ SEM. *** $p < 0.0005$, ** $p = 0.001$, * $p = 0.01$. Red dotted lines in the panels on the left mark
1217 the middle of the current injection ramp. Source data in "Figure 3 - Source data".
1218

1219 Figure 4. Cholinergic-associated shift in the timing of firing on ramp is mediated by TRP channels, but
1220 notably not TRPC channels. A. Somatic recordings for a two second triangular current ramp injected in
1221 the soma in control (A1), CCh (2 μ M, A2), and CCh + FFA (100 μ M, A3) applied subsequently to the
1222 same cell. Current traces and instantaneous frequency plots are just below the voltage traces. Summary
1223 data (A4) of the adaptation index for control, CCh, and CCh + FFA ($n = 9$). B. Same as in A, but for
1224 dendritic injection and recordings. Summary data (B4) of the adaptation index for control, CCh, and CCh
1225 + FFA ($n = 9$). Open circles connected by gray lines represent indices for individual cells, over all three
1226 treatments. Black squares with error bars represent group averages $\pm$ SEM. C. Somatic recordings for a
1227 two second triangular current ramp injected in the soma in control (C1), CCh (2 μ M, C2), and CCh +
1228 SKF96365 (50 μ M, C3) applied subsequently to the same cell. Current traces and instantaneous
1229 frequency plots are just below voltage traces. Red dotted line marks the middle of the ramp. Summary
1230 data (C4) of the adaptation index for control, CCh, and CCh + SKF96365 ($n = 8$). D. Same as in C, but
1231 for dendritic injection and recordings. Summary data (D4) of the adaptation index for control, CCh, and
1232 CCh + SKF96365 ($n = 8$). *** $p < 0.0005$, ** $p = 0.001$, * $p = 0.02$, n.s. not significant. Source data in
1233 "Figure 4 - Source data".
1234

Figure 5. Cholinergic-mediated shift in the center of mass of firing is mediated by TRPM4 channels.

A. Somatic recordings for a two second triangular current ramp injected in the soma in control (A1), CCh (2 μ M, A2), and CCh + CBA (50 μ M, A3) applied subsequently to the same cell. Current traces and instantaneous frequency plots are just below voltage traces. Red dotted line marks the middle of the ramp. Summary data (A4) of the adaptation index for control, CCh, and CCh + CBA (n = 9). Open circles connected by gray lines represent indices for individual cells, over all three treatments. Black squares with error bars represent group averages $\pm$ SEM. B. Same as in A, but for dendritic injection and recordings. Summary data (B4) of the adaptation index for control, CCh, and CCh + CBA (n = 9). C. Same as A, but for control (C1), CCh (2 μ M, C2), and CCh + 9-phenanthrol (100 μ M, C3) applied subsequently to the same cell. Summary data (C4) of the adaptation index for control, CCh, and CCh + 9-phenanthrol (n = 8). *** p < 0.0005, ** p = 0.002, * p = 0.02, ## p = 0.001, # p = 0.03, n.s. not significant. Source data in "Figure 5 - Source data".

Figure 6. Pharmacological block of IP₃R or RyRs has no effect on CCh-mediated shift in firing along ramp.

A. Somatic recordings during intracellular dialysis with Xestospongin C (2 μ M), for a two second triangular current ramp injected in the soma before (A1), and during superfusion with CCh (2 μ M, A2). Current traces and instantaneous frequency plots are just below voltage traces. Summary data (A3) of the adaptation index for Xestospongin C (1-2 μ M) and CCh (n = 9). Circles connected by gray lines represent ratios for individual cells, before and during CCh. Filled circles represent cells dialyzed with 1 μ M Xestospongin C and open circles represent cells dialyzed with 2 μ M Xestospongin C. Black squares with error bars represent group averages $\pm$ SEM. B. Same as A, but intracellular dialysis is with ryanodine (40 μ M). Summary data (B3) of the adaptation index for ryanodine and CCh (n = 9). Individual cells represented by open circles. *** p < 0.0005. In both sets of experiments, control data reflect the effect of either IP₃R or RyR antagonist before the addition of CCh, because the dialysis started to be apparent quickly after break-in. Red dotted lines in the left panels mark the middle of the ramp. C1 and C2. Same as B1 and B2; C3 is an expanded version of the inset in C1. Source data in "Figure 6 - Source data".

Figure 7. A. Simulated control response to triangular current ramp. A1. Top. Simplified model calcium handling schematic. For simplicity, only one annulus (shell) is shown, and the rest are represented as the core. Ca²⁺ enters via voltage-gated Ca²⁺ channels (Ca_v) and is removed by the plasma membrane Ca²⁺ ATPase (PMCA) pump. Inside the cell, buffering and the endoplasmic reticulum (ER) are also modeled. The TRPM4 channel is not permeable to Ca²⁺ and senses [Ca²⁺] in a nanodomain (ND). In the control simulation, the nanodomain is contiguous with the cytosol. Bottom trace shows the voltage responses, plotted in the somatic compartment, to a triangular current ramp applied in the soma (shown below). A2.

1271 Ca^{2+} concentration (in nM) in the outermost shell of the somatic compartment. A3. The nanodomain Ca^{2+}
1272 concentration (same as the that of the outer shell in A2 in this case) is negligible when plotted in μM
1273 scale. A4. TRPM4 activation is negligible in control. B. Simulated response to triangular current ramp
1274 during simulated application of CCh. B1. As in A1 except for ligand (magenta dot) binding to the
1275 metabotropic acetylcholine receptor (mAChR), triggering Ca^{2+} influx into a nanodomain linked to the
1276 TRPM4 channel. The voltage trace shows that the center of mass of firing is shifted to later times. B2.
1277 Ca^{2+} concentration (in nM) in the outermost shell of the somatic compartment. B3. In contrast to control,
1278 the addition of a nanodomain with privileged Ca^{2+} access allows micromolar concentrations to be
1279 reached and sensed by TRPM4 channels. B4. In contrast to control, TRPM4 is now activated during the
1280 ramp. Vertical dashed red line shows the peak of the current ramp.

1281
1282 Figure 8. The model explains how simulated TRPM4 channel activation can reverse the adaptation
1283 mediated by long-term inactivation of $\text{Na}_v1.6$ channels. A. Voltage traces from 7A1 and B1 overlaid;
1284 control (in black), CCh (in magenta). The triangular current injection ramps are shown below. B.
1285 Occupancy of $\text{Na}_v1.6$ channel in the open state (O) in the somatic compartment, with values during
1286 spikes removed. C. Occupancy of $\text{Na}_v1.6$ channel in the long-term inactivated state (I2). D. Persistent
1287 Na_v current (spiking values removed) E. TRPM4 current. F. Net ionic current. All values are shown for
1288 somatic compartment.

1289
1290 Figure 9. The model explains how activation of TRPM4 channels affects the firing pattern in response to
1291 triangular ramps that evoke similar firing frequencies. A. Simulated voltage traces (top) during a two
1292 second triangular current ramp (just below voltage traces). Insets show the reciprocal of the interspike
1293 interval (ISI) plotted as the instantaneous frequency at the ISI midpoint. Vertical dashed red line shows
1294 the peak of the current ramp. The slower frequencies at similar values of injected current and fewer ISIs
1295 on the down-ramp (open circles) versus the up-ramp (closed circles) in control show hysteresis due to
1296 adaptation mediated by long-term inactivation of the sodium channel. TRPM4 channel activation causes
1297 the center of mass of firing to be shifted to the right in all other traces compared to control because a
1298 nanodomain for TRPM4 activation with special access to Ca^{2+} was added in simulated CCh. The
1299 hysteresis is in the opposite direction because the activation of TRPM4 channels by Ca^{2+} accumulation
1300 obscures and overwhelms adaptation. In the light blue traces, the voltage-dependence of TRPM4
1301 activation was not included, so there is less Ca^{2+} accumulation in the outer shell in B and in the
1302 nanodomain in C, resulting in less activation of TRPM4 channels in D. In order to isolate the effect of
1303 voltage-dependence from that of Ca^{2+} dependence, the Ca^{2+} waveforms in all nanodomains were
1304 recorded during the full CCh simulation shown in magenta, and played back into each nanodomain Ca^{2+}
1305 during the simulations shown in purple.

1306

Figure 10. A high concentration of BAPTA is required to interfere with CCh-mediated shift in firing along ramp.

A. Somatic recordings during intracellular dialysis with 10 mM BAPTA, for a two second triangular current ramp injected in the soma before (A1), and during superfusion with CCh (2 μ M, A2). Current traces and instantaneous frequency plots are just below voltage traces. Red dotted line marks the middle of the ramp. Summary data (A3) of the adaptation index for control and CCh (n = 10). Open circles connected by gray lines represent ratios for individual cells, before and during CCh. Black squares with error bars represent group averages $\pm$ SEM. B. Same as A, but intracellular dialysis is with 30 mM BAPTA and 100 nM free $[Ca^{2+}]$. Summary data (B3) of the adaptation index for control and CCh (n = 8). C. Same as A, but intracellular dialysis is with 30 mM BAPTA and 10 nM free $[Ca^{2+}]$. Summary data (C3) of the adaptation index for control and CCh (n = 12). *** p < 0.0005, ** p = 0.001, n.s. not significant. Source data in "Figure 10 - Source data".

Figure 11. Model Schematics.

A. Reconstructed morphology ported to NEURON simulation program, with somatic and dendritic current injection sites labeled. B. Equivalent circuit diagram, with the reversal potentials for each channel indicated by E_x . The following currents were modeled using Ohmic drive and conductances in parallel with a membrane capacitance (C_M): a Na^+ current (I_{Na}), L-type (I_{CaL}), R-type (I_{CaR}), and T-type (I_{CaT}) Ca^{2+} currents, two delayed rectifiers (I_{KV1} and I_{KV2}), an A-type K^+ current (I_A), an inward rectifying K^+ current (I_{KIR}), a nonspecific Ca^{2+} -activated current (I_{CAN}), a hyperpolarization-activated mixed cationic h-current (I_h), and a leak current (I_L). C. Markov Model of the NaV channel with an open state (O), a closed state (C), a short-term inactivated state (I1) and a long-term inactivated state (I2). See also Figure 11 – supplement 1.

Figure 1 – supplement 1. Plots of instantaneous frequency versus current injection showing different effects of carbachol on firing rate adaptation. A and B. Two examples of somatic recordings where the firing rate adaptation in control is reversed in the presence of CCh, such that the clockwise hysteresis becomes counter-clockwise. C and D. Two examples of somatic recordings where the firing rate adaptation in control is removed in the presence of CCh, such that the hysteresis disappears, and the motion becomes linear. Filled circles represent the firing frequencies on the up-ramp and open circles represent firing frequencies on the down-ramp.

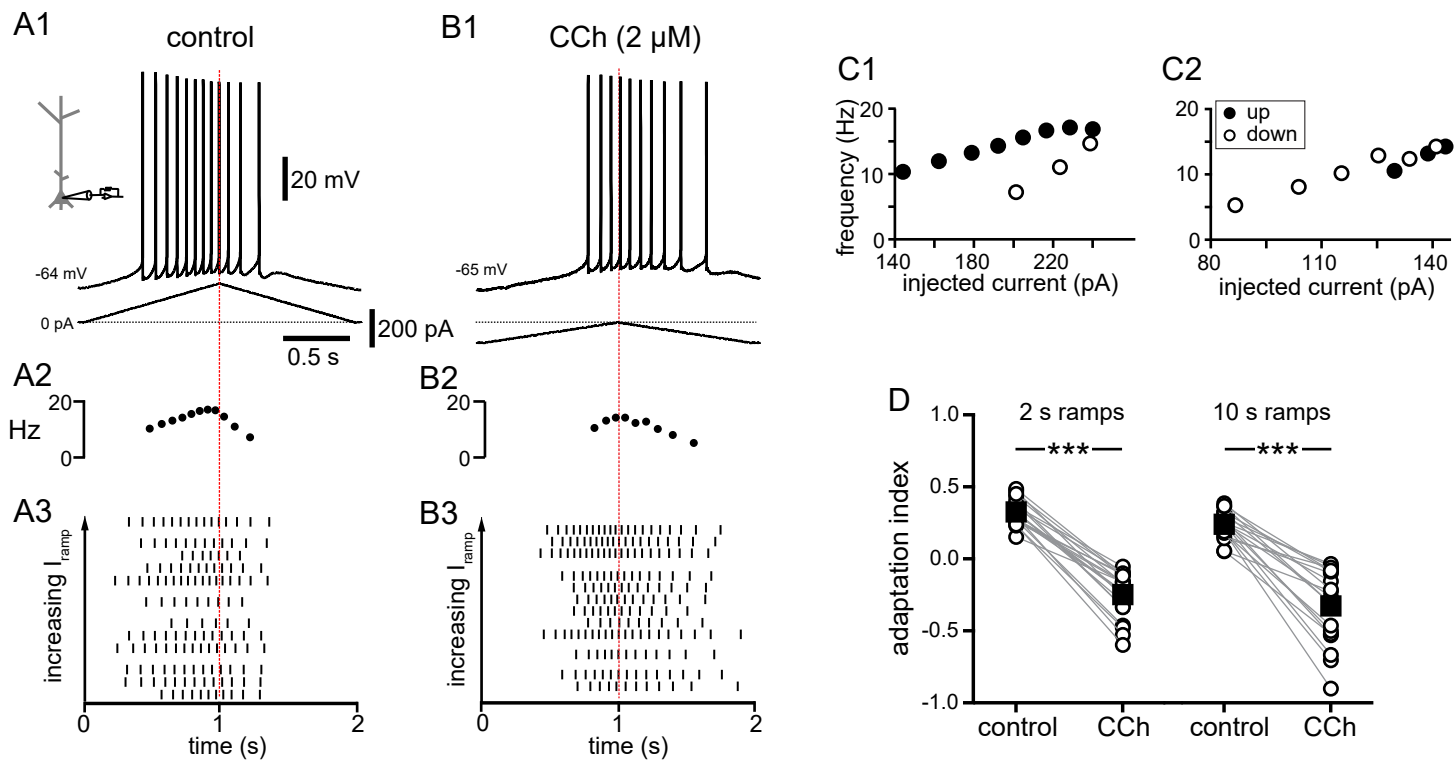
Figure 1 – supplement 2. A. Adaptation index as a function of membrane potential. Index values for each trial for all neurons are plotted against the membrane potential measured just before the current injection

1341 ramp for that trial, both for somatic recordings (n = 132 in control conditions, filled circles; n = 144 in the
1342 presence of CCh 2 μ M, open circles) and for dendritic recordings (n = 135 in control conditions, filled
1343 circles; n = 87 during CCh, open circles). B. Adaptation index as a function of the peak injected current.
1344 Index values for each trial for all recorded neurons are shown plotted against the peak amplitude of the
1345 triangular-shaped current injection for that trial, for both somatic and dendritic recordings (values for n
1346 and symbols are the same as in A). C. Adaptation index as a function of input resistance. Average
1347 adaptation index values per cell for both somatic recordings (n = 17) and dendritic recordings (n = 12)
1348 are plotted against the average steady state input resistance measured during control conditions (filled
1349 circles) and during perfusion with CCh (open circles). Source data in "Figure 1 supplement 2 - Source
1350 data".

1351

1352 Figure 11 – supplement 1. Old model in which the source of Ca^{2+} with privileged access to the
1353 nanodomain required to activate TRPM4 channels is IP_3R . This model captures the responses seen
1354 with cholinergic activation in the experiments. A. Simulated somatic recordings in control (low IP_3). A1.
1355 Voltage traces and current ramp. A2. Nanomolar Ca^{2+} concentration in outer shell beneath the cell
1356 membrane. A3. The nanodomain Ca^{2+} concentration is not elevated with low levels of IP_3 that simulate
1357 control. A4. There is negligible TRPM4 activation. B. Simulated somatic recordings in CCh (high IP_3). B1.
1358 Voltage traces and current ramp. B2. Nanomolar Ca^{2+} concentration in outer shell beneath the cell
1359 membrane is elevated due to increased spiking on the down-ramp. B3. The nanodomain Ca^{2+}
1360 concentration reached micromolar concentrations with high levels of IP_3 that simulate application of CCh.
1361 B4. TRPM4 is robustly activated. Black dashed line marks the end of the injected current ramp,
1362 highlighting that the return to baseline of Ca^{2+} and TRPM4 current lags behind injected current ramp. C.
1363 Simulated somatic recordings in CCh (high IP_3) with no nanodomain. C1. Voltage traces and current
1364 ramp. Note that more ramp current is required to achieve the same firing rates as were achieved in B1.
1365 C2. Nanomolar Ca^{2+} concentration in outer shell beneath the cell membrane. C3. No nanodomain. C4.
1366 There is negligible TRPM4 activation. In A2 and C2, the red arrows point to when the $[\text{Ca}^{2+}]$ in the outer
1367 shell starts decreasing while the neuron keeps firing, as opposed to B2.

somatic injection



dendritic injection

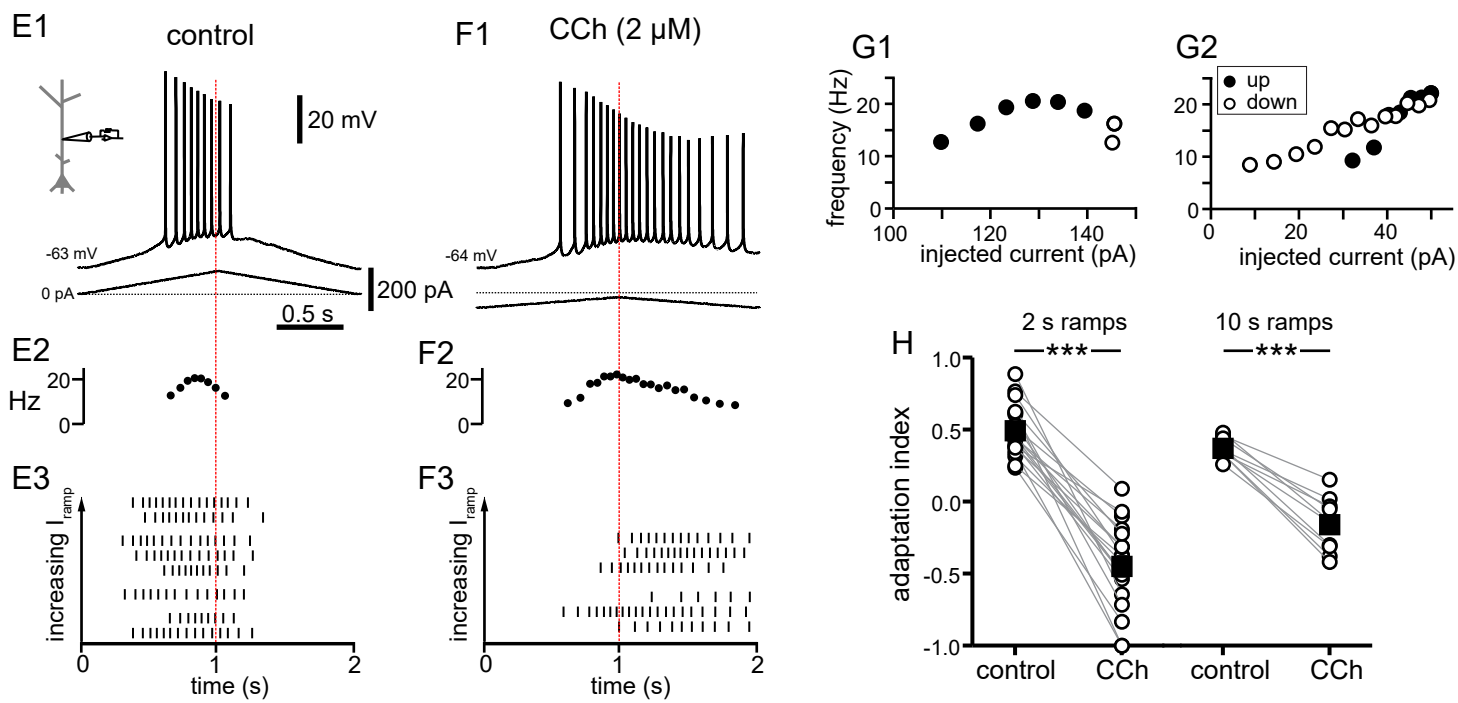
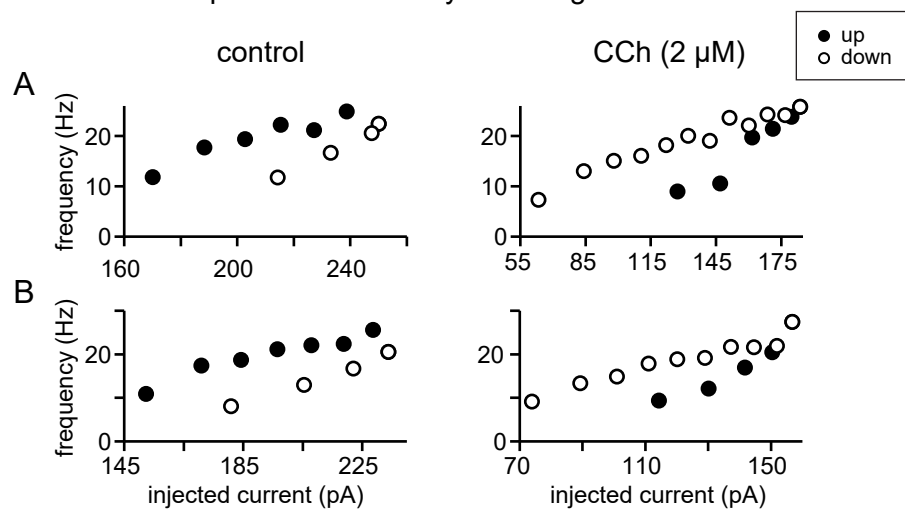


Figure 1

adaptation reversed by cholinergic modulation



adaptation removed by cholinergic modulation

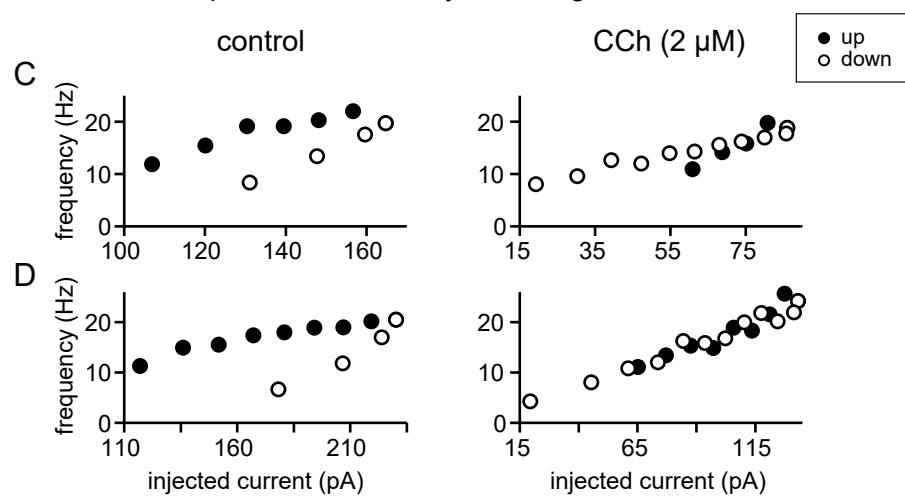
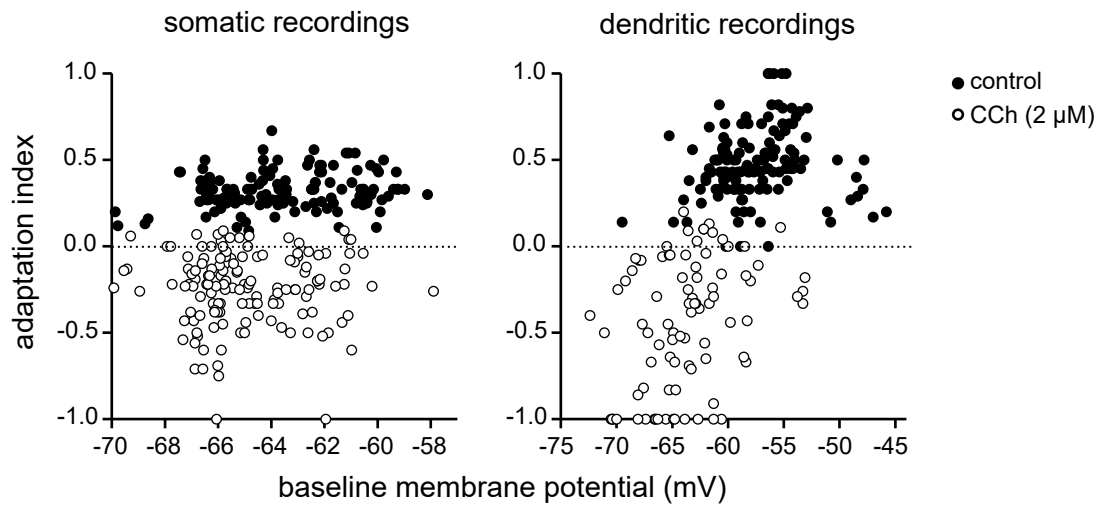
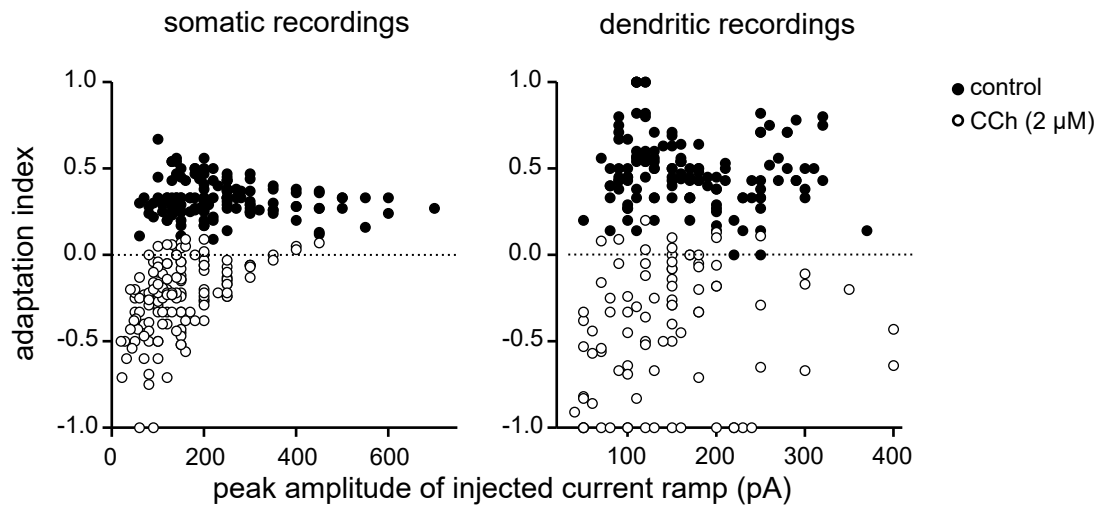


Figure 1 Supplement 1

A adaptation index as a function of initial membrane potential



B adaptation index as a function of current injection amplitude



C adaptation index as a function of input resistance

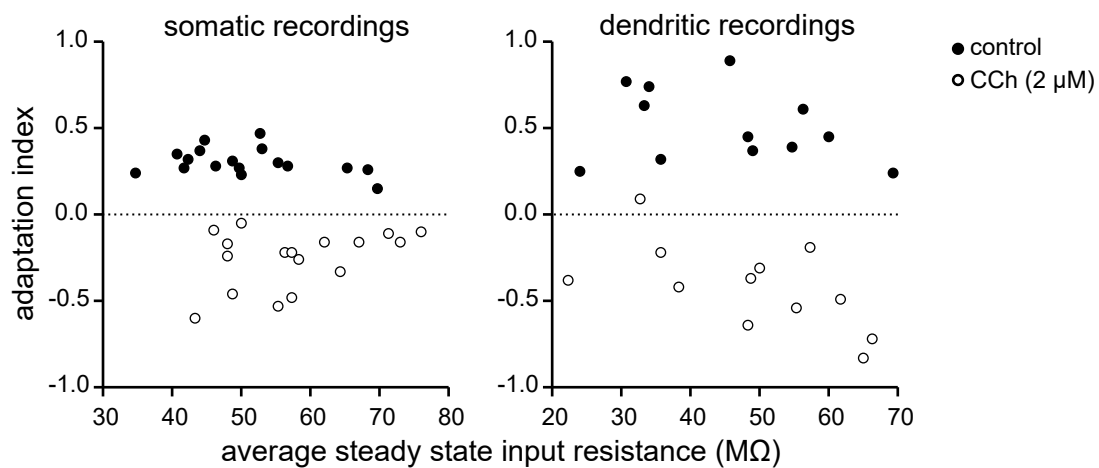


Figure 1 Supplement 2

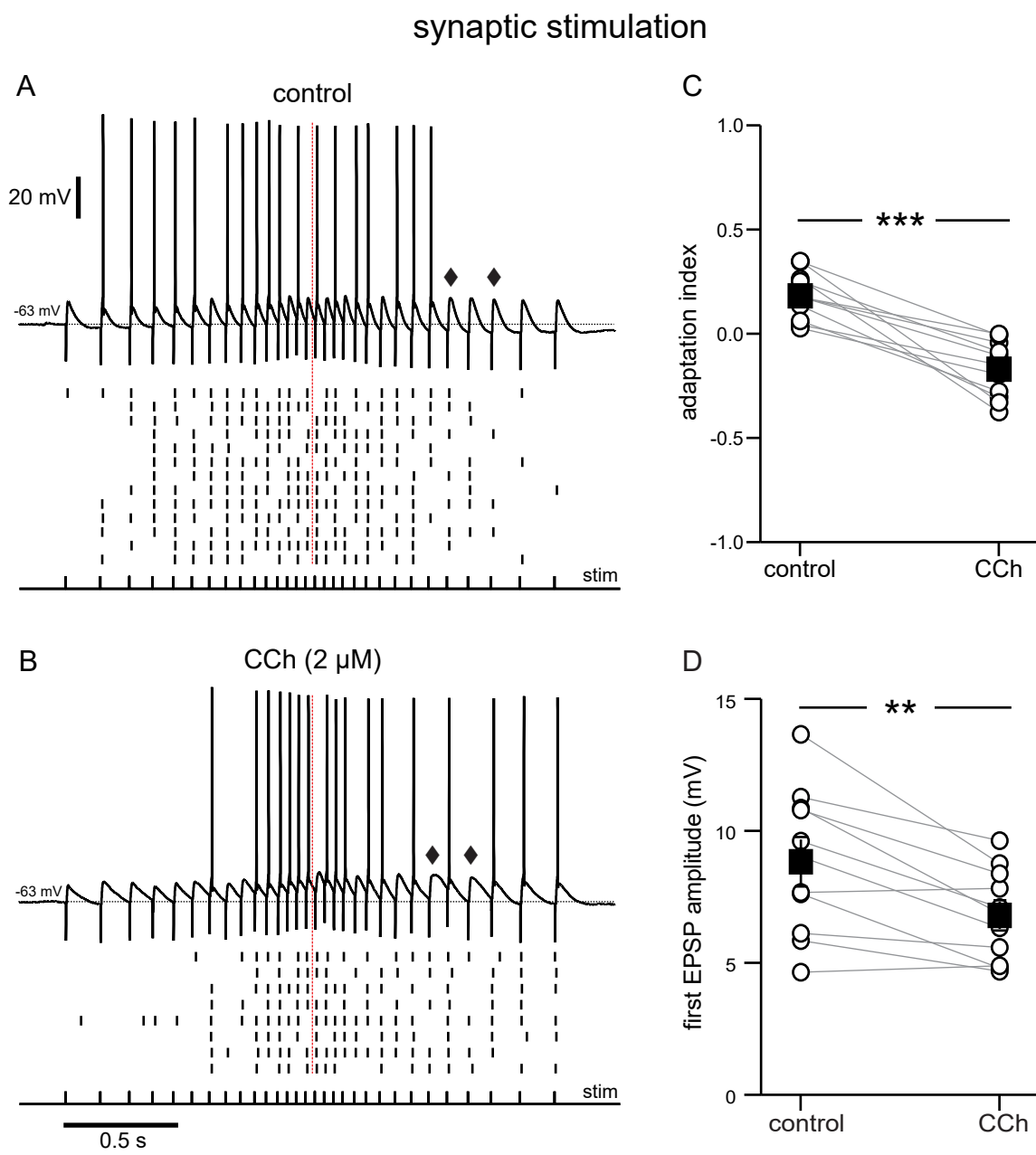


Figure 2

somatic injection

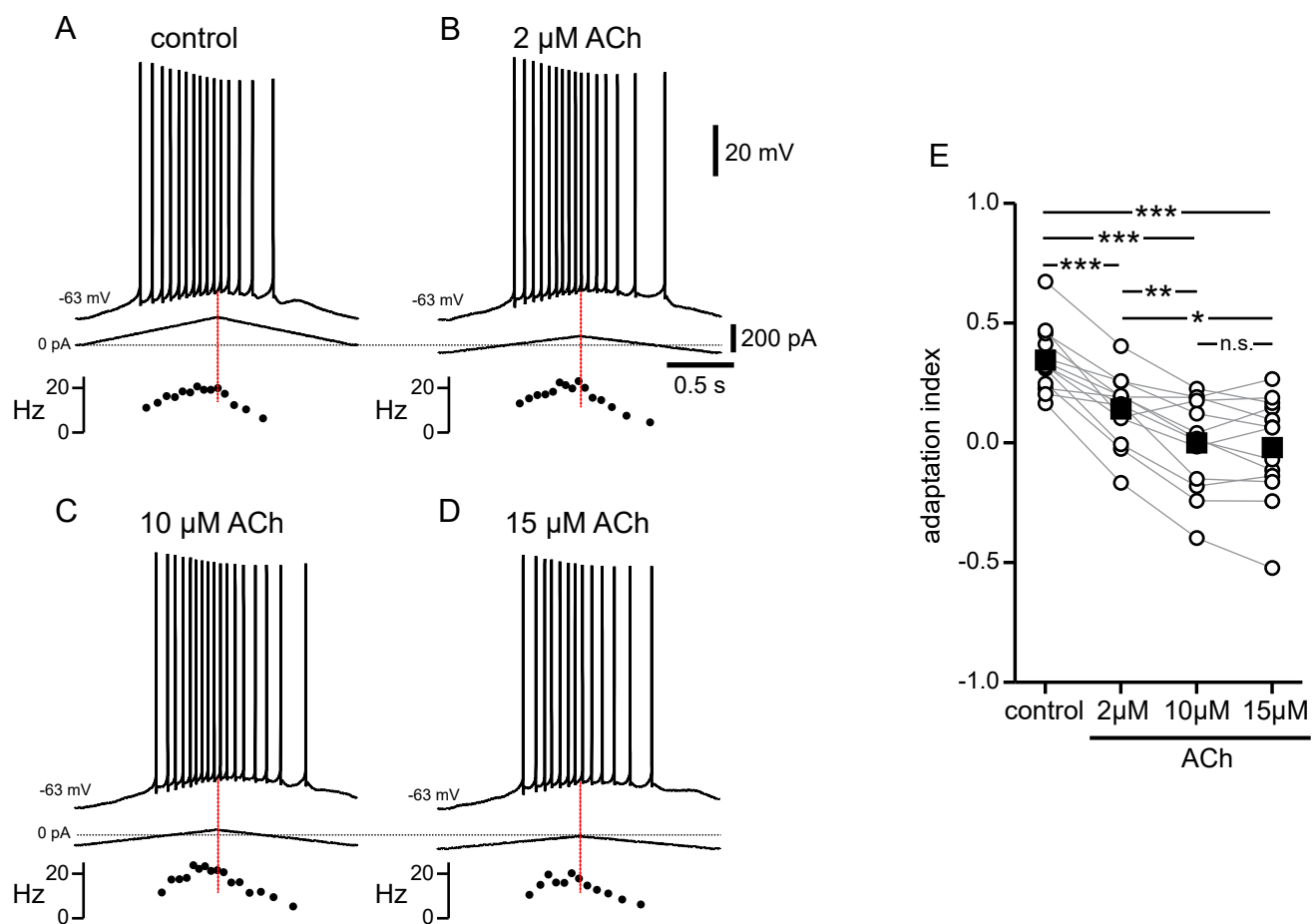


Figure 3

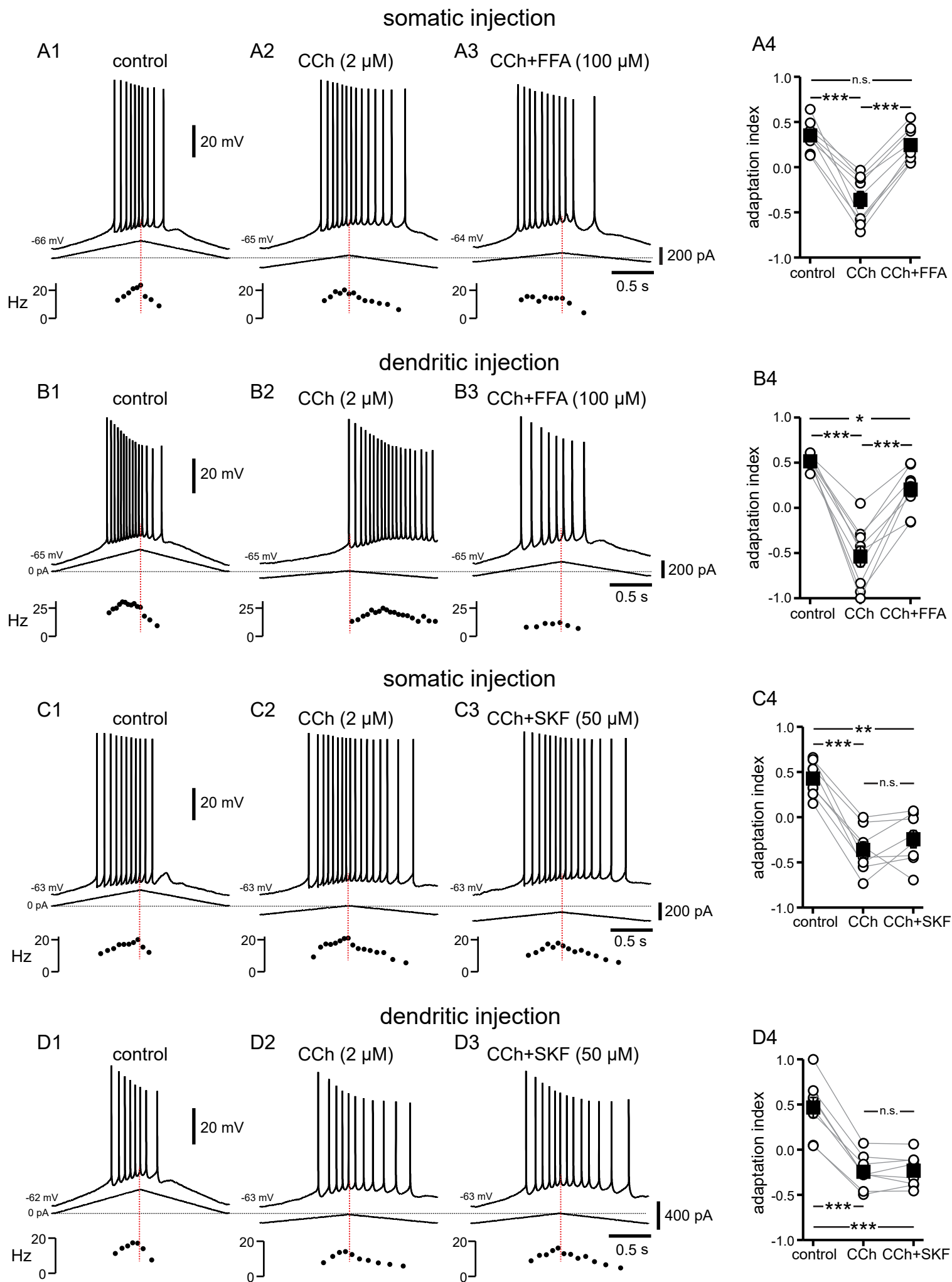
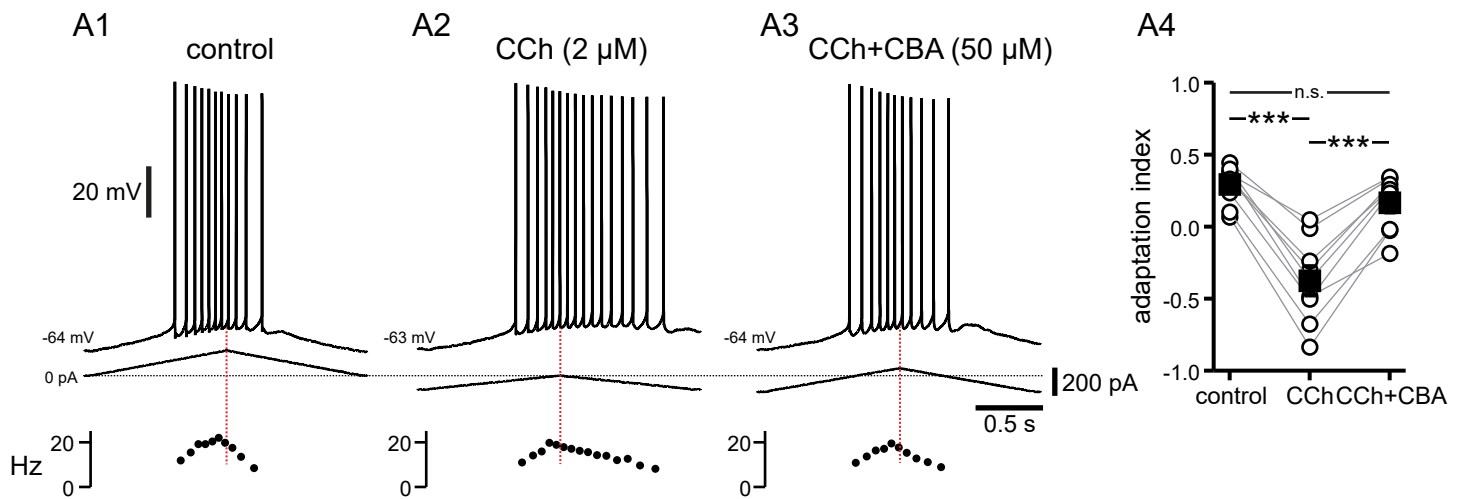
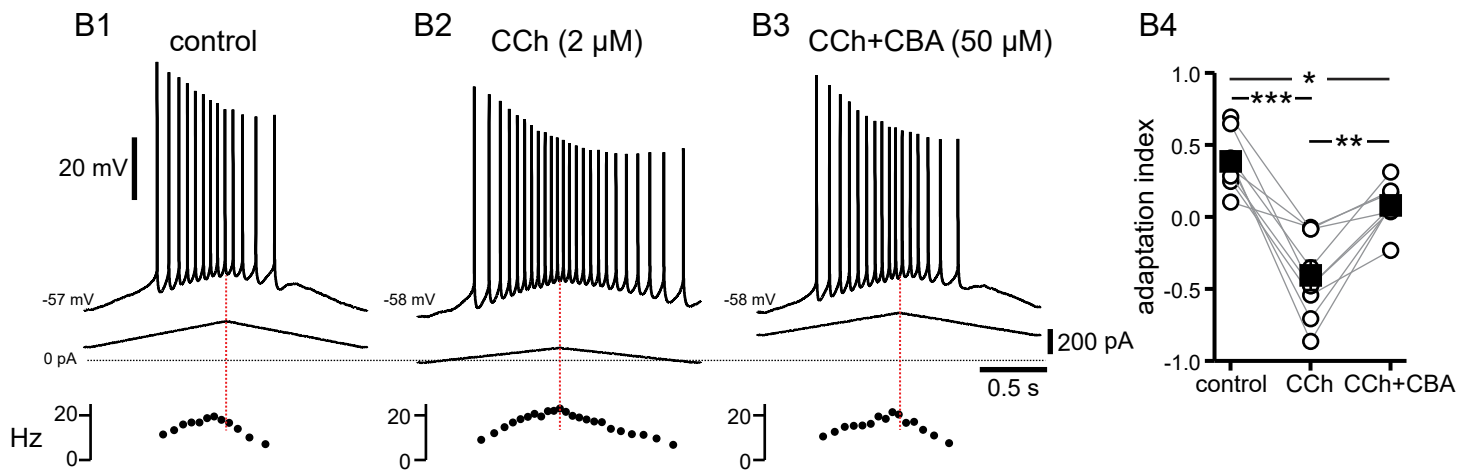


Figure 4

somatic injection



dendritic injection



somatic injection

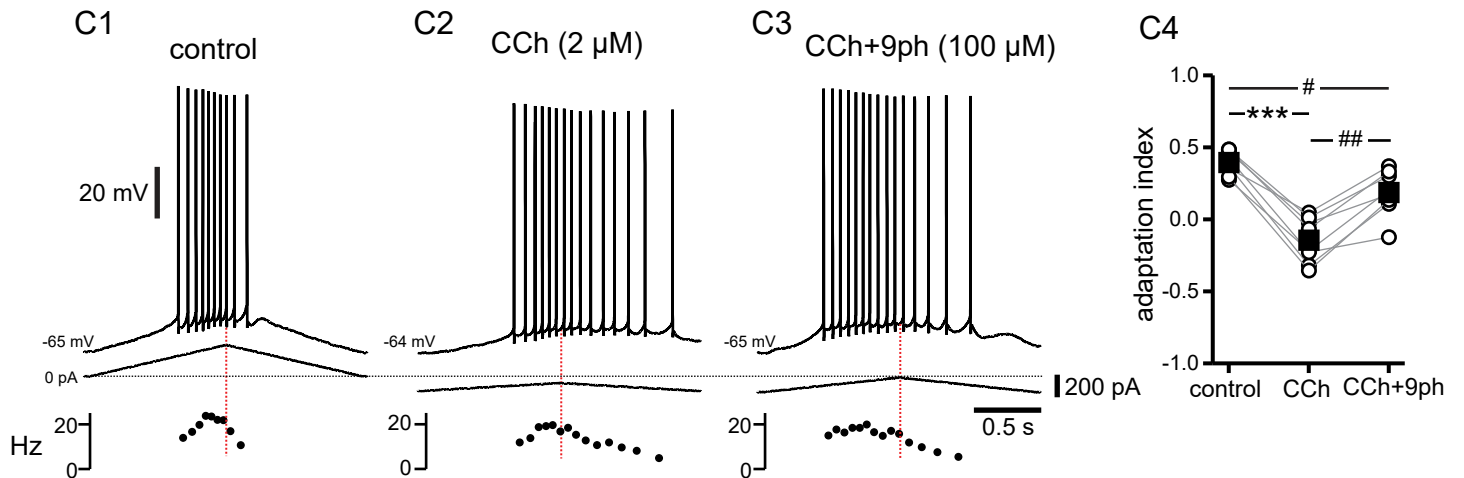


Figure 5

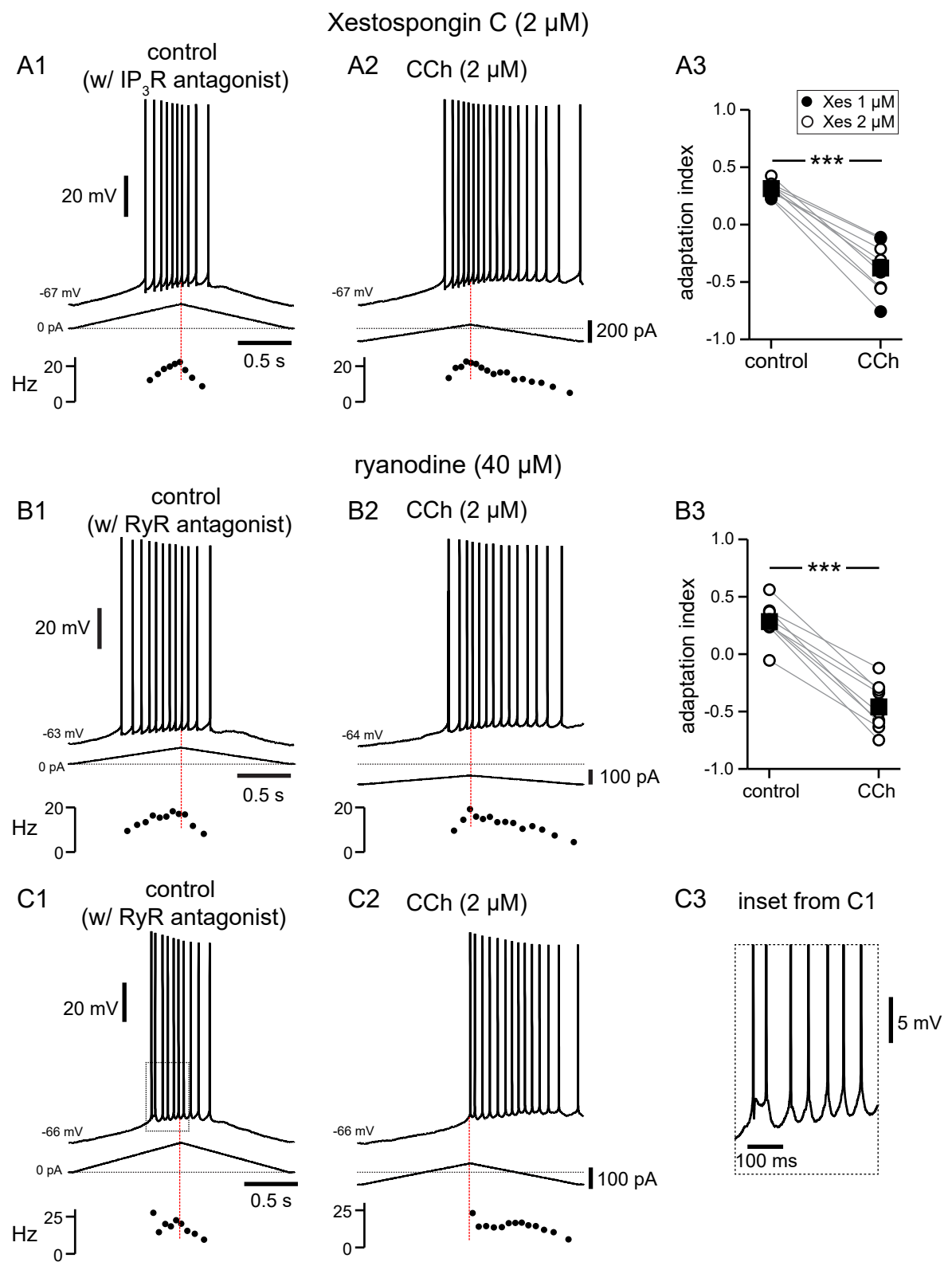


Figure 6

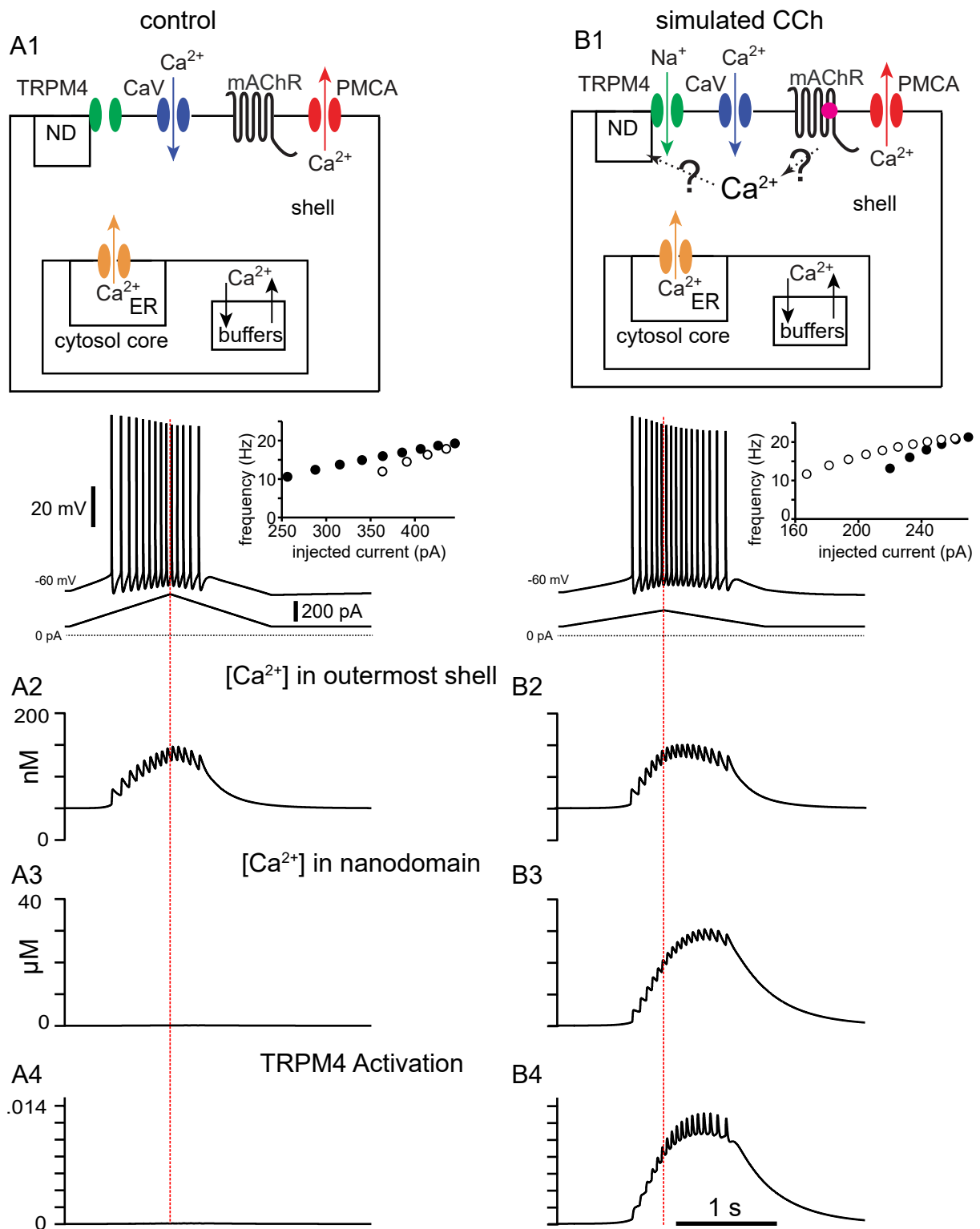


Figure 7

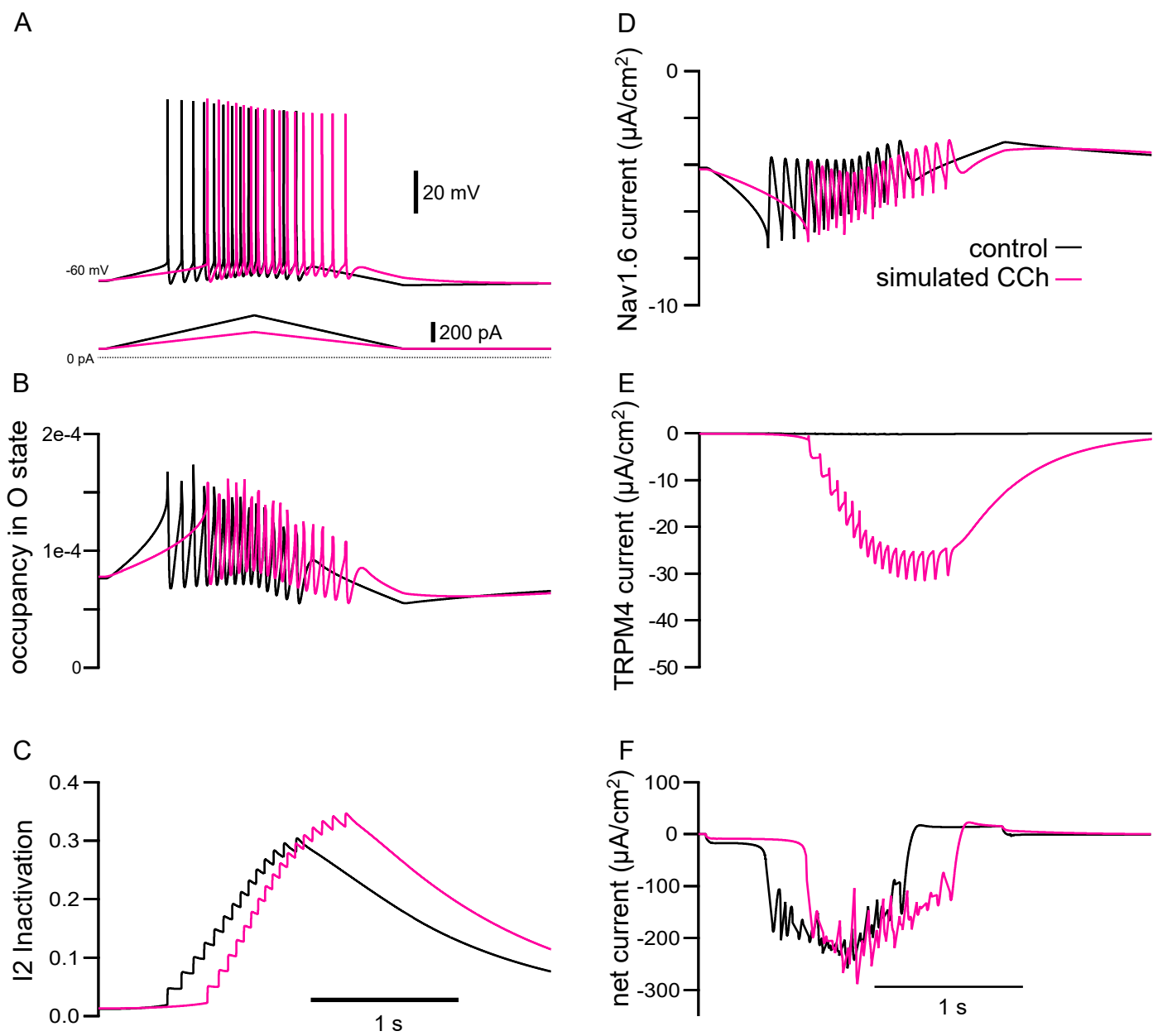


Figure 8

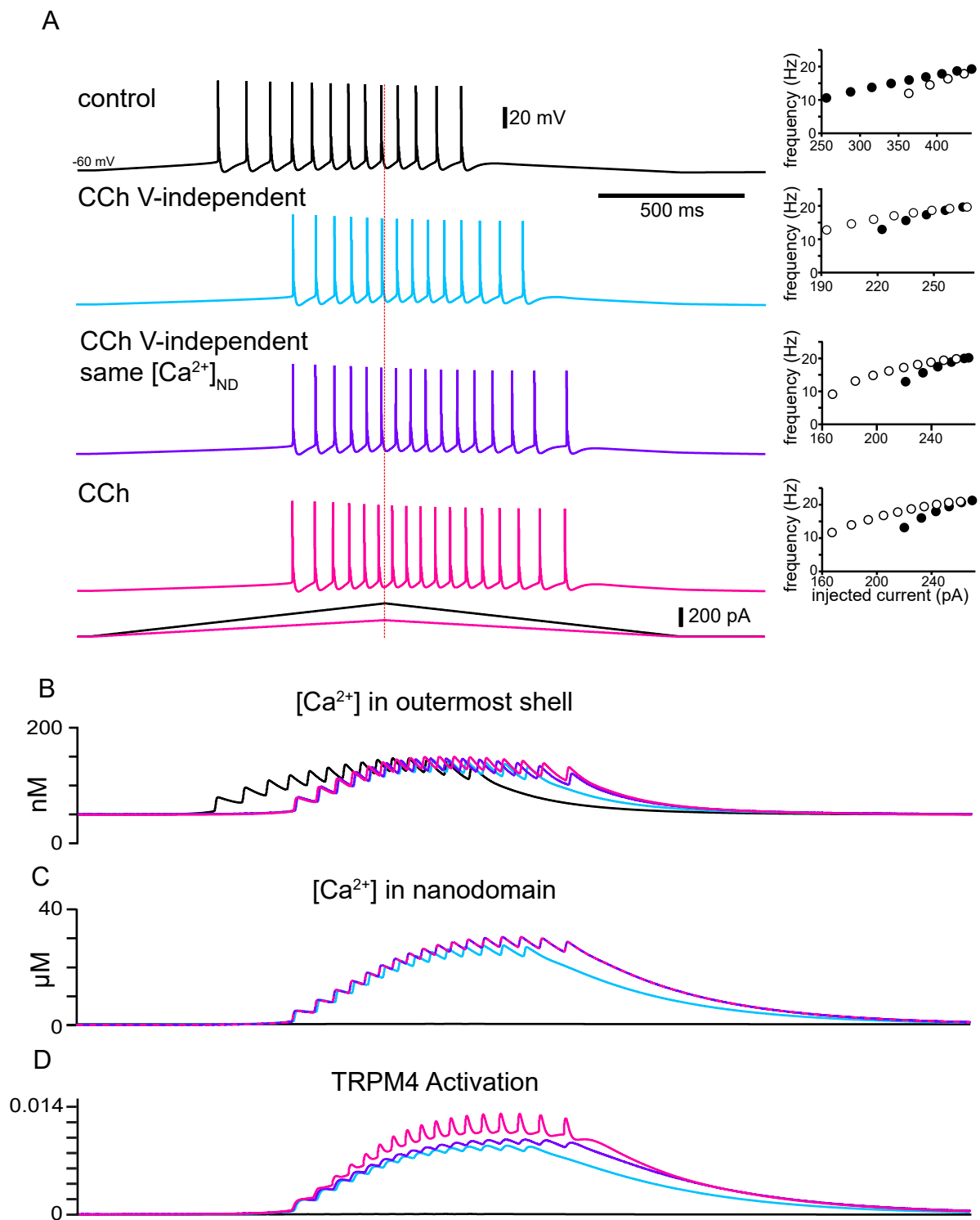


Figure 9

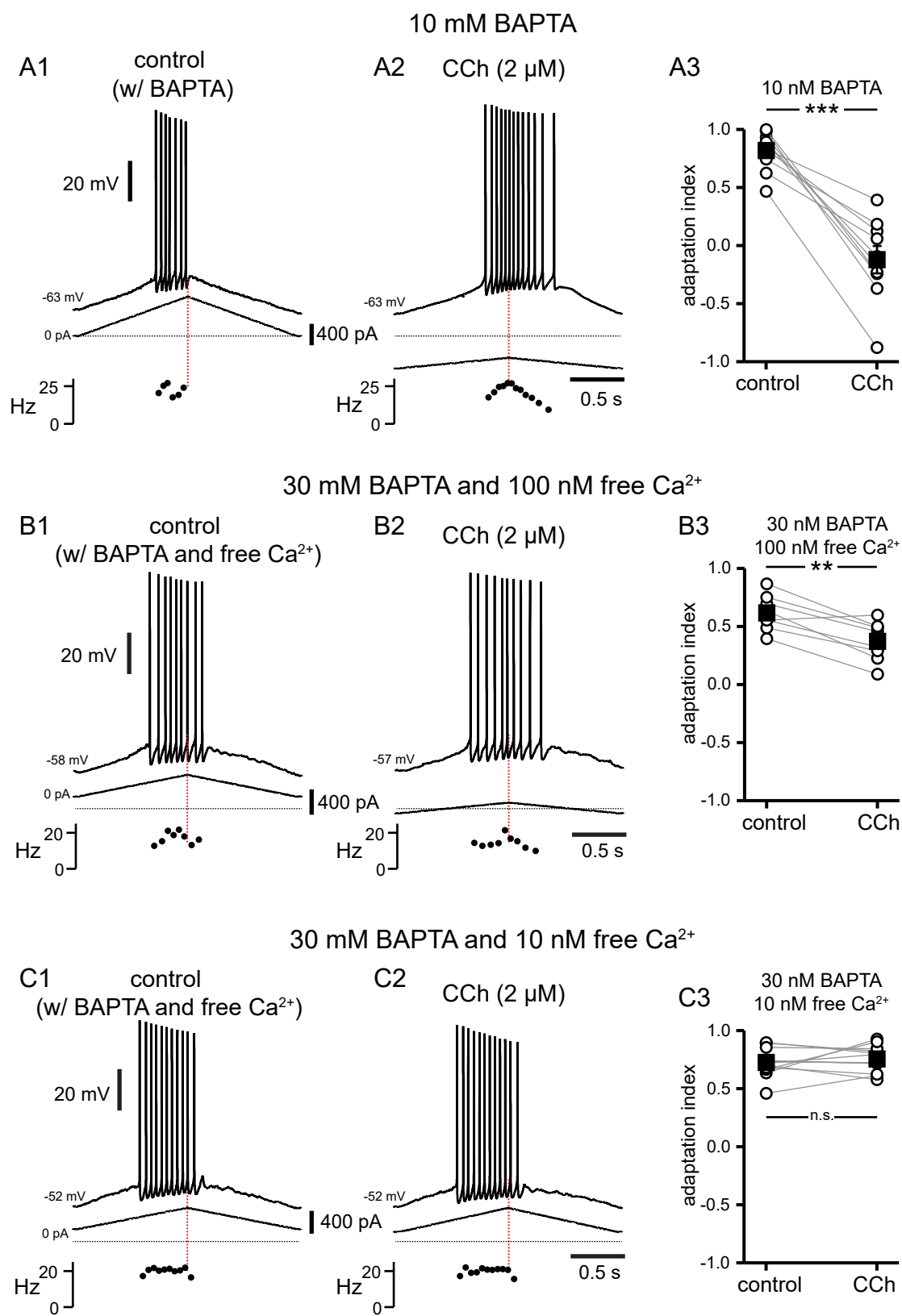


Figure 10

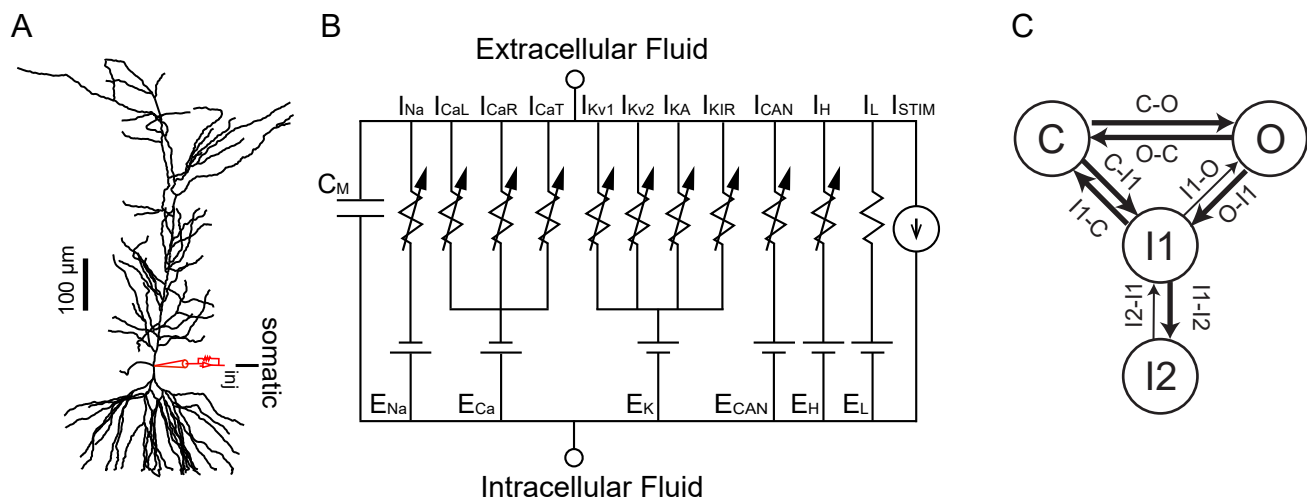


Figure 11

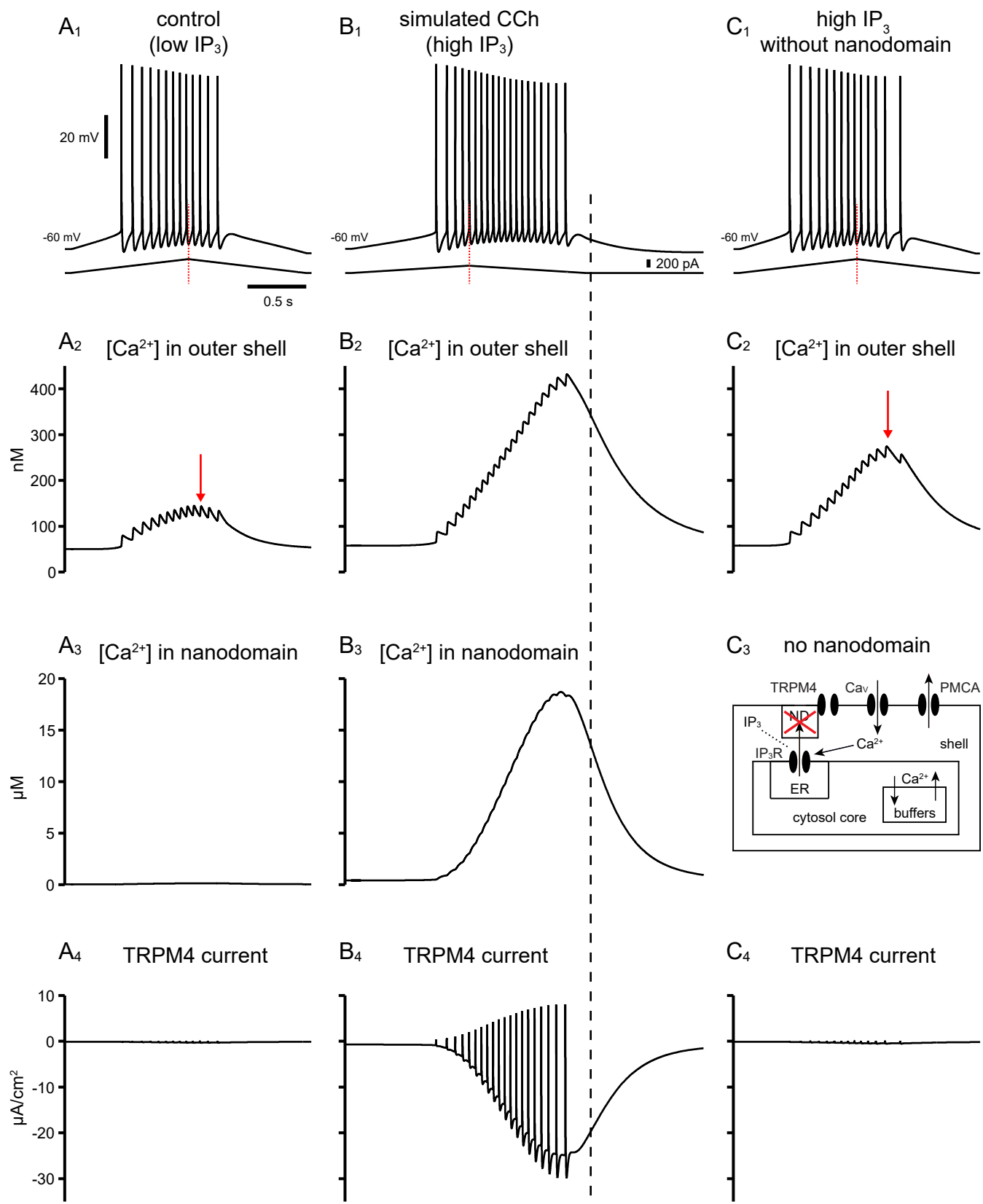


Figure 11 Supplement 1