Supplemental Material

Discussion

It has long been suggested that CICR contributes significantly to somatic and proximal dendritic Ca^{2+} signals in TC neurons during tonic firing. Previously, Budde et al. (2000) described a major contribution of CICR through activation of ryanodine receptors (RyRs) in TC neurons. The results of our study disagree to some extent with these previous findings. Under our conditions we found that bAP evoked $\Delta[Ca^{2+}]$ summated linearly in a manner consistent with Ca^{2+} entry through HVA Ca^{2+} channels and intracellular application of ryanodine had no effect upon bAP or LTS evoked $\Delta[Ca^{2+}]$. We believe the discrepancies between these studies may be related to numerous important factors. Firstly, these earlier experiments were largely performed in acutely isolated cells lacking dendrites whilst imaging experiments performed on neurons *in situ* in brain slices were restricted to only somatic and very proximal dendritic compartments. Secondly, several of the experiments in the earlier study were performed using relatively long (200 ms) and large (-50 mV to +5 mV) depolarising steps in voltage clamped neurons. In fact, in their Fig. 5 significant effects of CICR are only observed when voltage steps are longer than 500 ms in duration. Such voltage steps are likely to induce far larger Ca^{2+} influx than would be obtained in response to bAPs at physiological rates. Indeed, in our Fig. 2 we show that 60 mV steps in voltage clamped neurons induce significantly larger Ca^{2+} influx than a 50 Hz AP train of the same duration. Moreover, in experiments where $\Delta[Ca^{2+}]$ were evoked by AP trains very prolonged (7 s) steady-state current injections (100-350 pA) were used. Once again these less physiological stimuli may have the effect of directly gating HVA Ca^{2+} channels in the electrotonically compact TC neurons and thus promote larger Ca^{2+} build-up. In our experiments using brief (2 ms) steps to evoke bAPs $[Ca^{2+}]_{plat}$ were achieved within several hundred milliseconds and remained stable over several seconds (Fig. S2) reflecting balanced Ca^{2+} influx and extrusion as described by others (Helmchen et al., 1996; Scheuss et al., 2006). Thirdly, the results reported by these authors were obtained from experiments conducted at 21-23 °C rather than at physiological temperatures such as those used in our study (35 °C). This is significant as it has been well documented by others that mechanisms governing the rate of Ca^{2+} extrusion including Na^{+}/Ca^{2+} exchange, uptake into intracellular stores by SERCAs and extrusion by PMCAs are highly temperature-dependent processes.

As a result, performing experiments at lower temperatures could result in significantly larger Ca^{2+} accumulation during long or repeated stimuli than would occur at physiological temperatures. This is partly reflected in the very slow τ_{decay} (several seconds) of $\Delta[Ca^{2+}]$ observed in these experiments compared with those that we report (< 200 ms). Taken together we believe that these factors could explain the differences observed between our study and the earlier report. The conditions used by Budde et al. may favour the accumulation of larger Ca^{2+} concentrations sufficient to activate CICR via RyRs. However, under more physiological conditions (i.e. 35 °C fully functional extrusion mechanisms, more realistic stimulus protocols, less added Ca^{2+} buffer) we believe that normal TC neuron activity does not result in Ca^{2+} levels sufficient to activate CICR, at least in dendrites.

References

Scheuss V, Yasuda R, Sobczyk A, Svoboda K (2006) Nonlinear [Ca^{2+}] signalling in dendrites and spines caused by activity-dependent depression of Ca^{2+} extrusion. *J. Neurosci.* 26(31): 8183-8194.

Helmchen F, Imoto K, Sakmann B (1996) Ca^{2+} buffering and action potential-evoked Ca^{2+} signalling in dendrites of pyramidal neurons. *Biophys. J.* 70: 1069-1081.

Budde T, Sieg F, Braunewell K-H, Gundelfinger E, Pape H-C (2000) Ca^{2+}-induced Ca^{2+} release supports the relay mode of activity in thalamocortical cells. *Neuron* 26: 483-492.

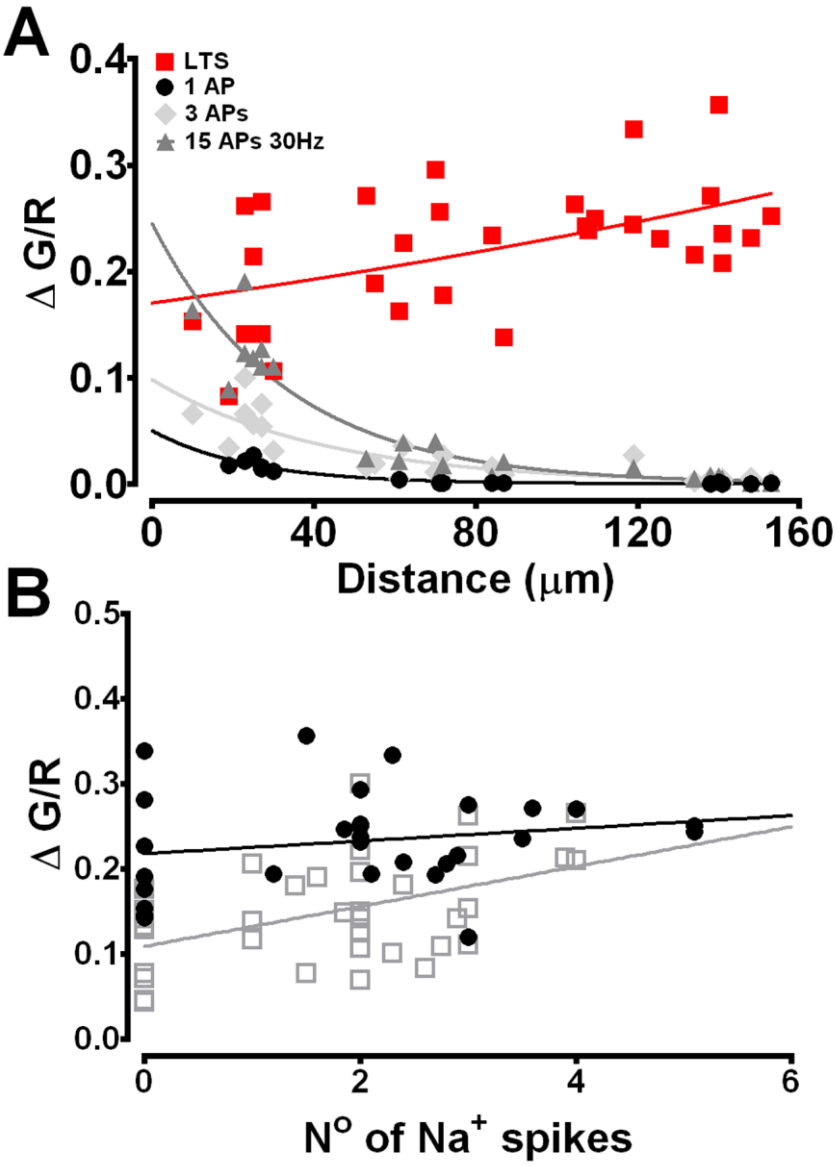

Fig. S1. Distance dependent distribution of evoked Δ[Ca^{2+}] in TC neuron dendrites. (A) Plot showing the Δ[Ca^{2+}] evoked by LTS, single bAPs, 3 bAPs 200Hz and 15 bAPs at 30 Hz for the individual TC neurons summarised in Fig. 1D. Lines represent mono-exponential fits to the data sets. (B) The number of APs per LTS burst correlates with the amplitude of dendritic Ca^{2+} transients in proximal but not distal dendrites. Linear regression fits reveal significant slope deviation from zero for data from proximal (P = 0.0036, n = 36) but not distal (P = 0.3089, n = 29) locations. Data pooled from several groups of experiments.

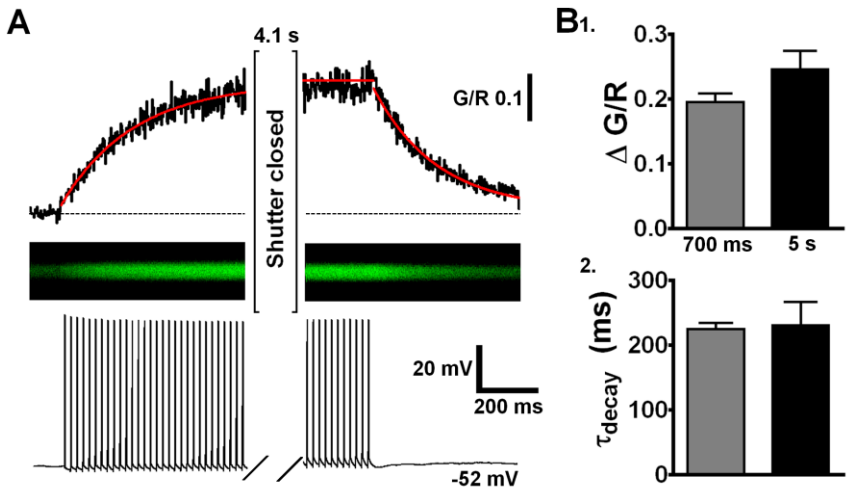

Fig. S2. Equilibrium between dendritic Ca^{2+} influx and extrusion is maintained during prolonged tonic firing. (A) Continuous sustained 5 second AP trains (50Hz) did not produce significantly (P>0.05) higher plateau Ca^{2+} accumulations than those achieved during our standard 700ms trains. Fitting a single exponential function to the rising phase of the Ca^{2+} signal reveals that after 5 seconds of activity $[Ca^{2+}]_{plat}$ is no larger than the level predicted by the linear sum of single AP transients. (B) Histograms summarising the similarity between (1) $[Ca^{2+}]_{plat}$ and (2) τ_{decay} after 700ms (n=18) and 5s (n=3) AP trains. These findings suggest that even during sustained periods of AP firing at typical TC neuron firing frequencies bAPs are unable to raise the mean dendritic Ca^{2+} concentration to sufficient levels to evoke CICR from intracellular stores. The lack of change in Ca^{2+} extrusion rate after longer firing periods also reaffirms the apparent absence of non-linear Ca^{2+} extrusion mechanisms in TC neuron dendrites during 'physiological' activity.

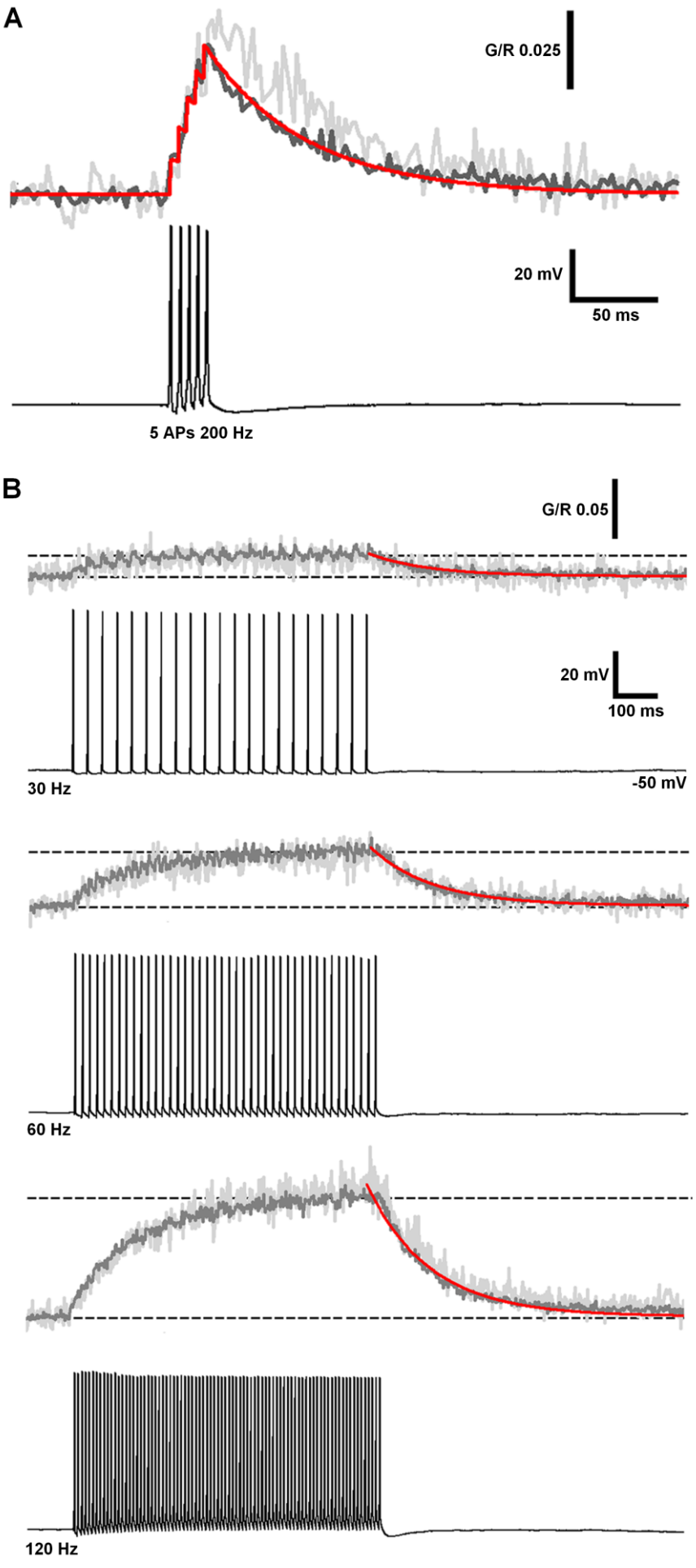
A
G/R 0.025
20 mV
50 ms
5 APs 200 Hz
B
G/R 0.05
20 mV
100 ms
30 Hz
-50 mV
60 Hz
120 Hz

Fig. S3. Ca^{2+} accumulation during trains with low exogenous Ca^{2+} buffering. (A) Trains of 5 spikes at 200 Hz were used to estimate the amplitude and τ_{decay} constant for single bAP transients (signal to noise limitations prevented direct measurement of these parameters in Fluo 4ff experiments). We assumed linear summation of transients and unchanged τ_{decay} for our estimates. The red line shows a simulation of linearly summating $\Delta[Ca^{2+}]$ based upon our estimate of the mean single bAP amplitude and τ_{decay} (n = 8). This closely matches the actual measured average $\Delta[Ca^{2+}]$ evoked by a burst of 5 spikes (dark grey) thus validating our assumptions. (B) Typical $\Delta[Ca^{2+}]$ evoked in an individual TC neuron filled with Fluo 4ff (500 μM) proximal dendrite (light grey) by 700ms AP trains at 30, 60 and 120 Hz. Overlays (dark grey) show the average $[Ca^{2+}]_{plat}$ pooled from 13 different TC neurons. Decay phases are fitted with mono-exponential functions (red lines) to yield τ_{decay}. Dashed lines show baseline and $[Ca^{2+}]_{plat}$ levels.

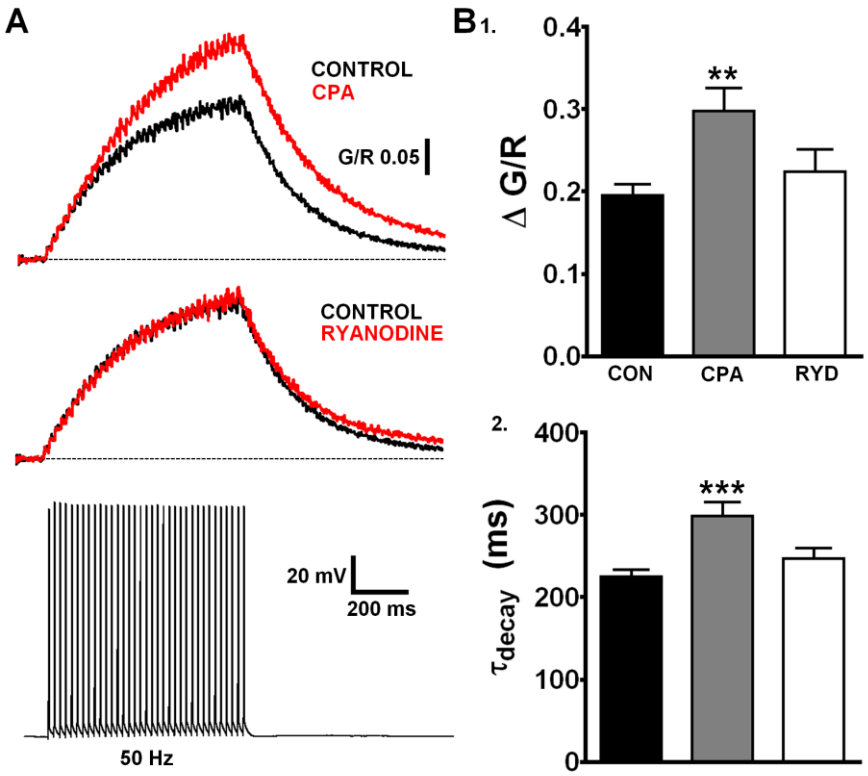

Fig. S4. Net Ca^{2+} uptake into ER stores by SERCA during LTS and bAP evoked dendritic $\Delta[Ca^{2+}]$. Amplitude and τ_{decay} of $[Ca^{2+}]_{plat}$ evoked by a 700 ms train at 50 Hz are not affected by ryanodine but are significantly increased by CPA.

Video S1. **Quasi-synchronous dendritic $\Delta[Ca^{2+}]_{LTS}$ in TC neurons.** A region of interest covering an area of the proximal dendritic (~42 x 24 µm, 63 x 36 pixels) field of a TC neuron filled with Fluo 5F was scanned at a rate of 20 frames per second. To enhance green fluorescence signals the laser was tuned to λ = 1000 for this experiment. A series of 8 LTSs were evoked manually by injecting 50ms (110 pA) current pulses through the patch pipette (indicated by asterisk). Quasi-synchronous Ca^{2+} influx can be observed across all dendrites within the field of view in response to LTSs.