Supplementary Information

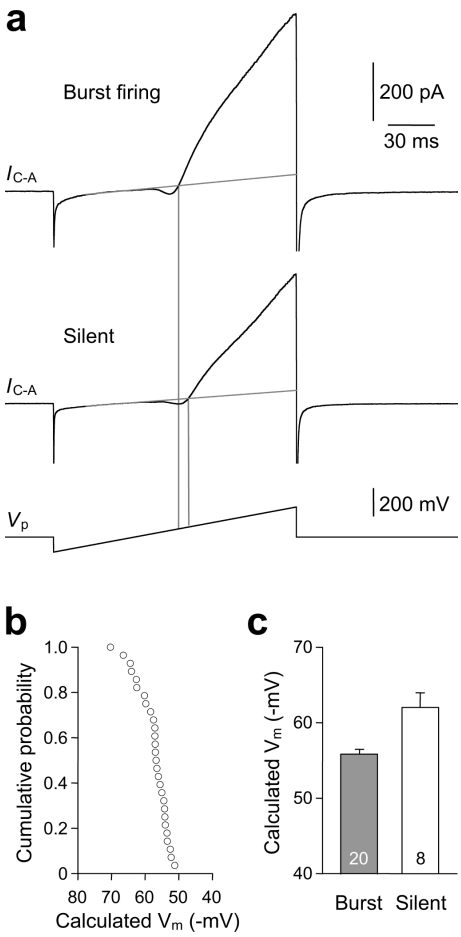
Errors in the measurement of voltage activated ion channels in cell attached patch clamp recordings

Stephen R. Williams[1,2] and Christian Wozny[2]

[1] Queensland Brain Institute, The University of Queensland, St. Lucia, Queensland, 4072, Australia.

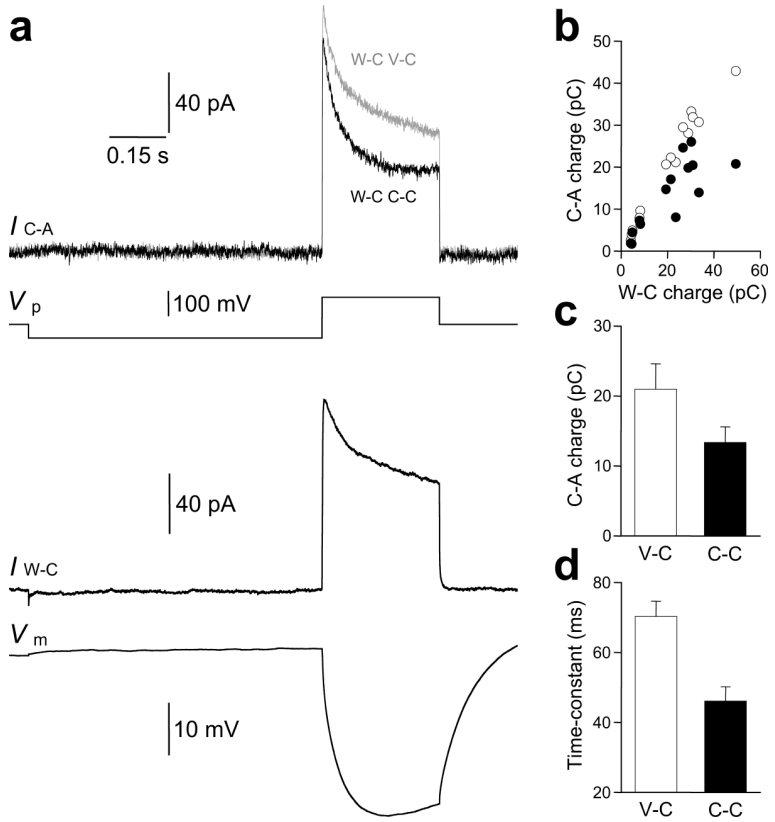
[2] Medical Research Council Laboratory of Molecular Biology, Cambridge, CB2 0QH, United Kingdom.

Supplementary Figure S1. Non-invasive measurement of the resting membrane potential of Thalamocortical neurons.



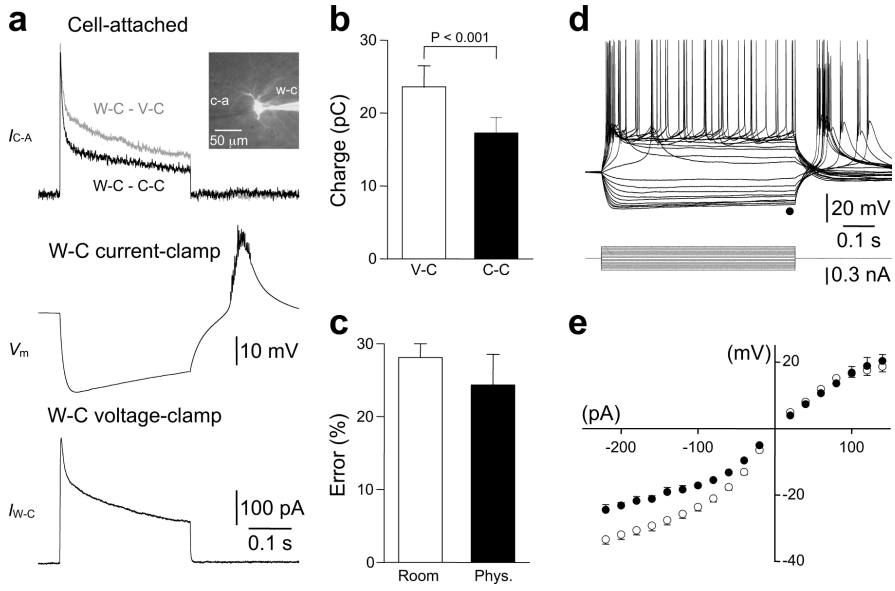
(**a**) Representative traces of ensemble potassium channel activity (I_{C-A}) evoked by voltage ramps in cell-attached patches made from the soma of Thalamocortical neurons. The linear, leak, portion, of the cell-attached current recording was fit by linear regression (near horizontal lines). The time points at which these lines intersect with the potassium channel activity is assumed to be the resting membrane potential (vertical drop lines), which can be read-out from the pipette command voltage (V_P); a method based on a previously described technique[34]. Example traces are shown for Thalamocortical neurons that either showed action potential burst firing following the generation of the ensemble potassium current (upper trace) or did not (lower trace). (**b**) Cumulative probability distribution of the resting membrane potential of Thalamocortical neurons calculated using the procedure shown in (a) (n = 28). (**c**) The resting membrane potential of Thalamocortical neurons which exhibited cell-attached current evoked action potential burst firing (filled bar) was significantly ($P < 0.001$) depolarised with respect to those that did not (open bar). Pooled data are shown as mean ± SEM, together with the number of replicates, statistical significance was tested with an unpaired Students T-test.

Supplementary Figure S2. Cell-attached measurement errors in midbrain dopaminergic neurons.



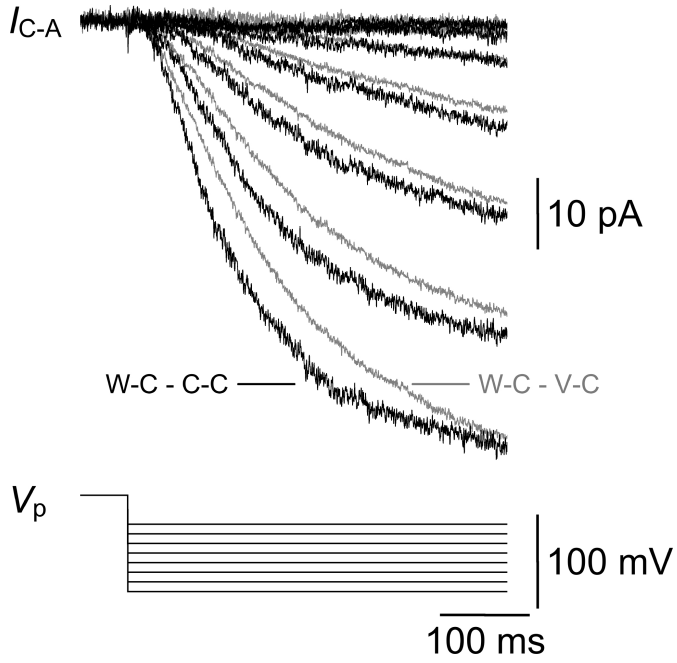
(**a**) Overlain leak-subtracted ensemble potassium channel activity recorded in cell-attached patches (I_{C-A}) from the soma of a visually identified dopaminergic neuron of the substantia nigra, during simultaneous whole-cell (W-C) voltage-clamp (V-C, gray trace) or current-clamp (C-C) conditions. The voltage command step delivered to the cell-attached pipette is shown (V_p). In whole-cell voltage-clamp mode the cell-attached current was accompanied by a prominent outward current (I_{W-C}), while in current-clamp mode, a large amplitude hyperpolarizing voltage response was apparent (V_m, lower trace). (**b**) Relationship between the charge of ensemble potassium channel activity simultaneously recorded at the cell-attached and whole-cell level, under voltage-clamp (open symbols). The charge of cell-attached currents in the same cell-attached patches was decreased when recordings were made in the whole-cell current-clamp mode (filled symbols). (**c-d**) Pooled data showing the reduction of the charge (c) and acceleration of the decay kinetics (d) of ensemble potassium channel activity in cell-attached patches determined under whole-cell voltage-clamp (V-C) and current-clamp (C-C). In both cases a significant difference was revealed ($n = 14$, $P < 0.001$, paired Students T-test). Pooled data represents mean ± SEM. The maximum and minimum 3rd quadrant apparent input resistance of dopaminergic neurons were: maximum: 197 ± 25 MΩ; minimum: 70 ± 24 MΩ ($n = 5$).

Supplementary Figure S3. The influence of recording temperature on ensemble potassium channel evoked voltage errors in Thalamocortical neurons.



(**a**) Leak subtracted ensemble potassium channel activity ($I_{C\text{-}A}$) recorded from a cell-attached patch during simultaneous whole-cell voltage-clamp (W-C V-C, gray) and current-clamp (W-C C-C) recording from the soma of a Thalamocortical neuron maintained in a brain-slice at near physiological temperature (36 ℃). Cell-attached currents were accompanied by the generation of a whole-cell recorded outward current ($I_{W\text{-}C}$) or a hyperpolarizing voltage response (V_m) under voltage- or current-clamp conditions, respectively. The inset shows an overlain image of the recorded Thalamocortical neuron under differential interference and florescence microscopy; note the bright appearance of the neuron and the whole-cell electrode, which was filled with Alexa Fluor 568. (**b**) Pooled data show the significant difference in the charge underlying ensemble potassium channel activity in cell-attached patches when simultaneously recorded under whole-cell voltage- and current-clamp conditions at near physiological temperatures (35-37 ℃, n = 13). (**c**) Summary of the measurement error of the charge of ensemble potassium channel activity in cell-attached patches for recordings made at room (23 -25 ℃, n = 32) and near physiological temperature (35-37 ℃, n = 13). Measurement error was calculated as the difference in charge under simultaneous whole-cell current- and voltage-clamp recording conditions. (**d**) A family of voltage responses evoked by a series of current steps recorded from a Thalamocortical neuron at near physiological temperature. (**e**) Pooled current-voltage relationship for Thalamocortical neurons recorded at room (23-25 ℃, open symbols, n = 7) and near physiological temperature (35-37 ℃, closed symbols, n = 5). Pooled data are represented as mean ± SEM, significance was tested for with a paired Students T-test.

Supplementary Figure S4. Trans-membrane voltage changes influences the activation kinetics of HCN1 channels.



Under whole-cell current-clamp conditions (W-C – C-C, black traces) the activation kinetics of HCN1 channels recorded from a cell-attached patch (I_{C-A}) are accelerated, an effect shown by the overlay of a scaled version of traces recorded under whole-cell voltage-clamp (W-C – V-C gray traces). The lower traces show the pipette command voltage (V_p).