Conditioning by Subthreshold Synaptic Input Changes the Characteristic Firing Pattern of CA3 Hippocampal Neurons

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Abstract

Conventionally, the action potential discharge dynamics of neurons has been measured using somatic injection of step currents. Different morphological types of neurons are considered to have characteristic step responses, and so the response has been used for phenotypic classification. However, neuronal excitability is subject to homeostasis at both network and single neuron level, suggesting that discharge patterns should also be subject to change. We indeed show here that the firing patterns of neurons in the CA3 field of the rat hippocampus in vitro change significantly after only a few minutes of low frequency subthreshold stimulation of the neuron's afferents. This effect could be reproduced by subthreshold somatic depolarizing pulses and was blocked by kinase inhibitors, indicating that discharge dynamics is modulated locally. Cluster analysis of the firing patterns before and after subthreshold conditioning revealed systematic transitions towards adapting and intrinsic burst behaviours, irrespective of the patterns initially exhibited by the cells. Using a conductance-based model, we demonstrate that the observed transitions can be mediated by recruitment of calcium and M-type potassium conductances. We conclude that CA3 neurons adapt their conductance profile to the statistics of ongoing activity in their embedding circuits, making their intrinsic firing pattern not a constant signature, but rather the reflection of their history of activity.

Significance Statement

Different anatomical types of neuron express a characteristic action potential discharge pattern in response to intra-somatic injections of step currents. Together with the cell's morphology and molecular markers, these patterns have been used to classify neuronal phenotypes. However, we show that in the case of hippocampal CA3 neurons, this discharge pattern is not as characteristic as generally assumed. Instead, they modify their fundamental processing according to the statistics of the sub-threshold signals that they receive from their embedding circuit. This result implies that CA3 neurons collectively adapt their network processing, and also that their discharge response patterns can not be used for phenotypic classification.

Introduction

It is widely accepted that the diversity of morphological, molecular, and electrophysiological properties exhibited by neurons of the neocortex and hippocampus reflects functionally distinct classes of cells (Ramon y Cajal, 1893; McCormick et al., 1985; Ren et al., 1992; DeFelipe, 1993; Kawaguchi and Kubota, 1997; Markram et al., 2004; Somogyi and Klausberger, 2005). Therefore, neurons have been classified electrophysiologically according to the pattern of their action potential discharge in response to applied intra-somatic step currents (Connors and Gutnick, 1990; Cauli et al., 2000; Markram et al., 2004; Butt et al., 2005; Dumitriu et al., 2007; Hemond et al., 2008; Tasic et al., 2016). These responses may be for example: adapting, accelerating, bursting, or fast spiking. With some exceptions (Steriade, 2004), the patterns are assumed to be a sufficiently stable property of a neuron to be used as a basis for phenotypic classification (Markram et al., 2004; Ascoli et al., 2008; Tricoire et al., 2011; Van Aerde and Feldmeyer, 2015).

However, there are substantial reasons to doubt that firing patterns are static properties of neurons. The discharge dynamics depends on the distribution and activations of the membrane conductances that it expresses (Hille, 2001; Markram et al., 2004). This distribution is subject, over the course of hours and days, to homeostatic control via up- or down-regulation of conductances in response to the neuron's own activity (Turrigiano et al., 1994; Turrigiano and Nelson, 2004; Marder and Goaillard, 2006). Furthermore, neurons have conserved molecular pathways that link network activity to the recruitment of genes and signaling factors implicated in neural excitability (Flavell and Greenberg, 2008; Cohen and Greenberg, 2008), and activity-dependent maturation is necessary for the emergence of the whole spectrum of electrical types (Moody and Bosma, 2005; García et al., 2011). These lines of evidence suggest that the firing pattern is not a static characteristic of the cell, but rather the consequence of adaptive mechanisms that adjust the behavior of the neuron in response to the patterns of activity in its embedding network. In fact, some studies show that specific features of the discharge response are actually modulated homeostatically upon activity or after learning. For example, the spike delay (Cudmore et al., 2010; Dehorter et al., 2015) or the accommodation rate (Thompson et al., 1996). On faster time scales, changes in intrinsic excitability have also been well characterized (Aizenman and Linden, 2000; Paz et al., 2009; Mahon and Charpier, 2012; Brager and Johnston, 2007). These shifts

are postulated to increase the probability of firing, integrating neurons into memory engrams (Zhang and Linden, 2003; Titley et al., 2017). However, the fact that key conductances with potential impact on the discharge pattern have also been found to be rapidly modulated by activity (Belmeguenai et al., 2010; Hyun et al., 2013; Brown and Randall, 2009) suggest neurons may be doing more than just increasing their overall excitability. Despite the evidence, it remains unexplored whether ongoing activity can impact not just specific features of the discharge or its average rate, but the whole spectrum of firings classically described (Ascoli et al., 2008).

We have addressed this issue by studying the effect that subthreshold activity has on the suprathreshold response of neurons using whole-cell recordings in the CA3 region of the rat hippocampus in organotypic cultures. The discharge patterns were characterized before and after a conditioning phase of periodic subthreshold synaptic stimulation lasting a few minutes. Pre-conditioned cells presented diverse discharge patterns (Ascoli et al., 2008). However, conditioning by subthreshold synaptic input elicited significant changes in the behavior of most of the neurons examined, requiring substantial re-classification of their type. This effect was reproduced when conditioning the cells via subthreshold intra-somatic current pulses and was blocked by adding protein kinase A (PKA) and protein kinase C (PKC) inhibitors to the recording pipette, suggesting that changes are mediated at the single cell level via phosphorylation. Using a conductance-based neuron model we found that the results can be explained by a recruitment of voltage dependent calcium and M-type potassium conductances. We conclude that CA3 neurons rapidly adapt their characteristic firing patterns in response to their circuit subthreshold input by possibly tuning key conductances.

Materials and Methods

All experiments were conducted in accordance with guidelines and regulations of the Cantonal Veterinary Office of Zurich; License Nr 81-2014.

Electrophysiological Recordings

Rat hippocampal organotypic cultures (Gähwiler, 1981) of average postnatal age 21 days were transferred to a recording chamber and mounted on an upright microscope (Axioskop FS1; Zeiss). The cultures were superfused with an external

solution ($pH7.4$) containing (in mM) 148.8 Na^+, 2.7 K^+, 149.2 Cl^-, 2.8 Ca^{2+}, 2.0 Mg^{2+}, 11.6 HCO_3^-, 0.4 $H_2PO_4^-$, 5.6 D-glucose, and 10 mg/l Phenol Red. All experiments were performed at 34°C. Whole-cell recordings of CA3 neurons were obtained with patch pipettes (4-7 $M\Omega$). Pipettes were filled (in mM) with 126 Kgluconate, 4 $NaCl$, 1 $MgSO_4$, 0.1 $BAPTA-free$, 0.05 $BAPTA-Ca^{2+}$, 15 glucose, 3 ATP, 5 $HEPES$ (pH was adjusted to 7.2 with KOH) 0.1 GTP, and 10.4 byocitin. IPSPs in the recorded cells were reduced by adding picrotoxin (1 mM) to the intracellular patch solution in order to elicit reliable depolarization in the cell.

The recording pipettes were manually positioned under microscope control. Recorded neurons were located mostly in the pyramidal cell layer. Electrophysiology and subsequent histology in a subset of the cells recorded suggest that the neurons described below include both pyramidal cells and smooth cells.

Current-voltage relationships were determined by step command potentials and had duration of 1 s to ensure steady-state responses. Data were recorded using an Axopatch 200B amplifier (Molecular Devices). Series resistance was monitored regularly, and was typically between 5 and 15 $M\Omega$. Cells were excluded from further analysis if this value changed by more than 20% during the recording. Junction potential and bridge was not corrected.

Mossy fibers were stimulated with a bipolar tungsten electrode. The intensity of the stimulus was adjusted to evoke subthreshold post-synaptic potential responses of 15 mV on average in the recorded neuron (minimal stimulation + 20% stimulation intensity).

Action potential discharges were evoked by injected current steps (-0.08 up to 1.8 nA; step increment 0.05 - 0.15 nA, depending on the input resistance of the recorded cell) each lasting 5 seconds. After this control, the neurons were conditioned by mossy fibers activation, consisting of a double pulse (0.1 ms duration pulses, interval 10 - 20 ms) at a frequency of 1 Hz, repeated 500 times. Thus, the conditioning period was approximately 8 minutes. Immediately after this conditioning, the firing pattern of the neuron was assessed again using the same step protocol. The step protocols were repeated 3 times with 5 min intervals to assess stability. In a subset of experiments, mossy fiber subthreshold responses were mimicked by injecting somatically and at a frequency of 1 Hz double step current pulses of 50ms of duration and 20ms of interstep interval. The amplitude of the pulse was adjusted in order to get a depolarization of 15

mV on average.

Histology

Hippocampal slice cultures were prepared for morphological assessment by fixing in freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at pH 7.4 overnight at 4 °C; washing three times in phosphate-buffered saline (PBS, 1.5 mM KH_2PO_4, 8.5 mM Na_2HPO_4, 137 mM $NaCl$, and 3 mM KCl, pH 7.4); and permeabilizing at room temperature in PBS that contained 10% heat-inactivated donkey serum, and 1% Triton X-100. Then they were incubated overnight at 4 °C with streptavidin conjugated with Alexa (546λ). The cultures were washed again three times in PBS, and then mounted in Fluorostab (Bio-Science Products AG, Emmenbrucke, Switzerland) and coverslipped. High-resolution images were obtained using laser scanning confocal microscopy (Leica TCS SP2, Leica Microsystems, Heidelberg, Germany).

Data analysis

Signals were digitized at 4 kHz for current clamp and 5 kHz for voltage clamp. These data were analyzed off-line using pCLAMP 10 (Molecular Devices) and MatlabR2011b (MathWorks). Analysis of the voltage traces was performed similar to Chen et al. (2015). The average resting membrane potential of each neuron was estimated as the mean membrane potential during the first 100 ms of current-injection protocol (before injection of the step-current pulses). Input resistance was obtained by measuring the voltage drop across the hyperpolarizing trace of the step-current pulses. APs were located using median filtering, and the threshold was inferred as the point at which the derivative of the voltage trace exceeded 5 mV/ms. AP amplitude was measured from threshold-to-peak and AP afterhyperpolarization (AHP) from the threshold-to through. Half-width was estimated as the full width at half-maximal amplitude.

Experimental Design and Statistical Analysis

Two conditions were designed for statistical comparison. The firing pattern of the cell was assessed in control conditions and after a period of conditioning (see first section of the Methods). Two different conditioning protocols were tested, synaptic and somatic. As a control, two similar experiments were performed: in one the cell was recorded

for 15 minutes without conditioning (and the firing pattern tested thereafter) and in a second set of experiments the firing pattern was analyzed at different holding potentials. For assessing the mechanism of the effect the synaptic conditioning experiment was repeated in the presence of kinase inhibitors. For any set of experiments, 1 cell per slice was recorded and 2 slices per rat (of either sex) were used for the preparation of the organotypic cultures. A minimum of 10 cells were used for each set of experiments. Data was tested for normality using a one-sample Kolmogorov-Smirnov test. A paired t-test was used for statistical comparisons between conditions with normally distributed data and a two-sided Wilcoxon signed rank test was used otherwise. The exact p-values of the tests and the sample number for each experiment are indicated on the figure legends in the results section. A clustering analysis was also performed for the synaptic conditioning group, and it is described on the following section. All the statistical analysis were performed using MatlabR2011b (MathWorks).

Cluster analysis of discharge traces

The firing patterns of the neurons were categorized by hierarchical clustering of their discharge patterns. The dataset consisted of all voltage traces recorded from neurons in response to step-wise current injections with different amplitudes, including recordings before and after conditioning. For any one neuron, the collection of responses to different current injections represents the signature of the electrical type. However, for inherent verification of our cluster procedure, we chose to treat each response independently. In this way successful clustering could be confirmed by its ability to assign responses from the same neuron into the same category.

The clustering measured similarity of a feature vector derived from the voltage traces. First the recorded voltage traces were converted into a time series of the instantaneous firing rates. The instantaneous firing rate at each spike was taken as 1/Inter-spike-Interval (ISI). Then the instantaneous rates where linearly interpolated across the spike times at 1 ms time intervals over 6 seconds (5 second current injection step, plus 1 second on and offset), and normalized by the maximum firing rate. Finally, a characteristic feature vector of a common length of 600 elements was obtained by down-sampling the interpolated rate traces by a factor of 10, in order to make them computationally tractable to the similarity measurement.

Similarity distances between pairs of traces were calculated using the Dynamic Time Warping (DTW) measure (Berndt and Clifford, 1994). DTW takes into account that two similar signals can be out of phase temporarily, and aligns them in a non-linear manner through dynamic programming (Keogh and Ratanamahatana, 2005). The algorithm takes two time series $Q = \langle q_1, q_2, \ldots, q_n \rangle$ and $C = \langle c_1, c_2, \ldots, c_m \rangle$ and computes the best match between the sequences by finding the path of indices that minimizes the total cumulative distance

$$\mathrm{DTW}(Q,C) = \min \sum_{k=1}^{K} w_k \tag{1}$$

where w_k is the cost of alignment associated with the kth element of a warping path W. A warping path starts at q_1 and c_1 respectively, and finds a monotonically increasing sequence of indices i^k and j^k, such that all elements q_i in Q and c_j in C are visited at least once, and for the final step of the path $i^{end} = n$ and $j^{end} = m$ holds. The optimal DTW distance is the cumulative distances $y(i,j)$, corresponding to the costs of the optimal warping path $\langle q_1, \ldots, q_i \rangle$ and $\langle c_1, \ldots, c_j \rangle$. This distance can be computed iteratively by dynamic programming:

$$y(i,j) = d(q_i, c_j) + \min\{y(i-1,j-1), y(i-1,j), y(i,j-1)\} \tag{2}$$

where $d(q_i, c_j)$ is the absolute difference between the elements of the sequence. The optimal warping path is obtained by backtracking from the final element $y(n,m)$, and finding which of the three options (increasing i only, increasing j only, or increasing i and j simultaneously) led to the optimal warping distance, until $i = 1, j = 1$ is reached. A warping window constraint of 10% of the vector size was chosen (Keogh and Ratanamahatana, 2005).

The pairwise DTW distances were used to perform hierarchical clustering by Ward's algorithm (Ward Jr, 1963). The number of classes increases with the level of the hierarchy. We choose to cut the tree at a level that provided sufficient structure to interpret the hierarchy in terms of recognized response types (Ascoli et al., 2008).

Every recording for a given cell was treated as an independent observation, and could in principle be assigned to any cluster. If the electrophysiological state of the cell is expressed in all of its responses, then we expect that all the

independent observations derived from that cell should be assigned to the same cluster. However, traces derived from current injections to the same cell in different conditions (pre- or post-stimulation) are expected to be assigned to different clusters if there is significant change in the underlying electrophysiological state.

In fact the independent traces did not cluster perfectly. Instead, the majority of independent observations derived from a given state clustered together and there were a few that fell into other clusters. Therefore, we chose to label the electrical type of each cell according to the cluster that contained the mode of the traces for one set of current injections. Cells for which no clear dominant cluster could be identified, e.g. because half of the traces fell into one cluster, and half of them into another, were labeled as unclassified. A cluster transition was recognized whenever the cell was assigned to different clusters before and after the stimulation protocol.

The analysis was performed using custom-written software in MatlabR2011b. The implementation of the DTW algorithm was obtained from Matlab Central (http://www.mathworks.com/matlabcentral/fileexchange/43156-dynamic-time-warping–dtw).

Neuron simulation model

A single cylindrical compartment, conductance-based neuronal model was used for all simulations. The length and diameter of the cylinder are set at equal dimensions to avoid spatial discretization problems in a single compartment (Cooley and Dodge, 1966; De Schutter and Bower, 1994). The passive properties associated with the model were obtained from Hemond et al. (2008). We set the length and diameter of our compartment to 50 μm. The active properties were modeled by including appropriate voltage and calcium gated ion channels whose density and kinetics were obtained from experimental recordings performed in CA3 neurons (Hemond et al., 2008). Throughout the manuscript, we refer to the channels as conductances. The simulations were performed using NEURON (Hines and Carnevale, 1997). We choose an integration step of 25 μs, which was approximately 1% of the shortest time constant in the model. The voltage- and time-dependent currents followed the Hodgkin and Huxley formalism (1952):

$$C \cdot \frac{\mathrm{d}V}{\mathrm{d}t} = -(I_{Na} + I_{Kdr} + I_{Kd} + I_{KA} + I_{Km} + I_{CaK} + I_{CaL} + I_{CaT} + I_{CaN} + I_{Leak}) \tag{3}$$

Each current I_x is described by the equation

$$I_{(v,t)} = \bar{g} \cdot m \cdot h \cdot (V_{(t)} - E) \tag{4}$$

where $\bar{g}$ is the maximal conductance, m and h are activation and inactivation terms, V is the membrane potential, and E the reversal potential of the channel. The reversal potentials for $Na+$ and $K+$ were E_{Na} = 50 mV and E_K = -85 mV, respectively. The equations describing the different channel kinetics (m,h) for every current were obtained from Hemond et al. (2008). Following this reference, the three calcium conductances (T, N and L) were incorporated into a single parameter g_{Ca}.

The set of maximal conductance values that are consistent with all our experimentally observed firing patterns are shown in the Table 5-1. The intracellular calcium dynamics were modeled (Hemond et al., 2008), as follows:

$$\frac{\mathrm{d}[Ca^{2+}]_i}{\mathrm{d}t} = \frac{\mathrm{I}_{Ca}}{2Fv} - \frac{[Ca^{2+}]_i - 0.0001}{\tau_{Ca}} \tag{5}$$

The first term of the above equation describes the change caused by Ca^{2+} influx into a compartment with volume v. F is the Faraday constant, I_{Ca} is the calcium current and τ_{Ca} is the time constant of Ca^{2+} diffusion.

The occasional decrease in spike amplitude seen in some of the experimental traces is probably due to sodium inactivation. We choose not to include this feature in the model, because it does not affect the overall dynamics of the spike discharge itself.

The model is available online at ModelDB (https://senselab.med.yale.edu/ModelDB/enterCode.cshtml?model=228599)

Results

Firing patterns of CA3 neurons change after subthreshold synaptic stimulation

Whole-cell patch clamp recordings of CA3 neurons were performed in rat hippocampal organotypic cultures. The intrinsic firing patterns of the neurons were recorded before and after conditioning by extracellular stimulation of the mossy fibers originating in the dentate gyrus. The conditioning stimuli consisted of paired pulses (0.1 ms duration pulses, interval 10 – 20 ms) applied at 1 Hz, and repeated 500 times for a total period of approximately 8 minutes. The amplitude of the pulses was adjusted for each recorded cell to elicit only subthreshold excitatory post-synaptic potentials (EPSPs). This mossy fiber stimulation protocol is a modification of that described by Brandalise and Gerber (2014) and Brandalise et al. (2016), which has been previously shown to elicit heterosynaptic subthreshold plasticity in CA3 pyramidal-pyramidal synapses. We tested whether the same type of subthreshold stimulation could also induce plasticity on the action potential discharge patterns of the CA3 cells. The firing patterns of neurons were assessed with a sequence of constant current injections. For convenience, we label these patterns according to the Petilla classification terminology (Ascoli et al., 2008). Interestingly, we observed that for most neurons the conditioning protocol elicited a change in the Petilla discharge pattern, independently of their type of firing on control conditions. For example, the pyramidal cell shown in Figure 1A had a non-adapting burst pattern before stimulation (gray traces) but after conditioning (blue traces), this response changed to intrinsic burst. The same transition was observed for the pyramidal cell on panel 1B, whose initial pattern was delayed accelerating. The bipolar cell on panel 1C switched from non-adapting continuous to adapting continuous firing. The most common transition was towards adapting and intrinsic burst patterns. This observation is supported by measuring the mean fraction of spikes in the first half versus the second half of the voltage traces for the population of recorded cells. The distribution of the spikes favors the first half (Figures 1D, 1E) (n=50), supporting our observation that the main pattern transitions are towards adapting and intrinsic burst behaviors after the conditioning. The changes in firing pattern were present in most cells immediately after the stimulation protocol, and were stable at least 15 minutes after cessation of stimulation. The mossy fiber conditioning was followed by a significant 36 MΩ (25%) decrease in input resistance (Rin), (from 144.8 ± 73.0MΩ to 108.4 ± 65.3MΩ, two-sided Wilcoxon signed rank test, p=1.1e-5), while there was no significant change

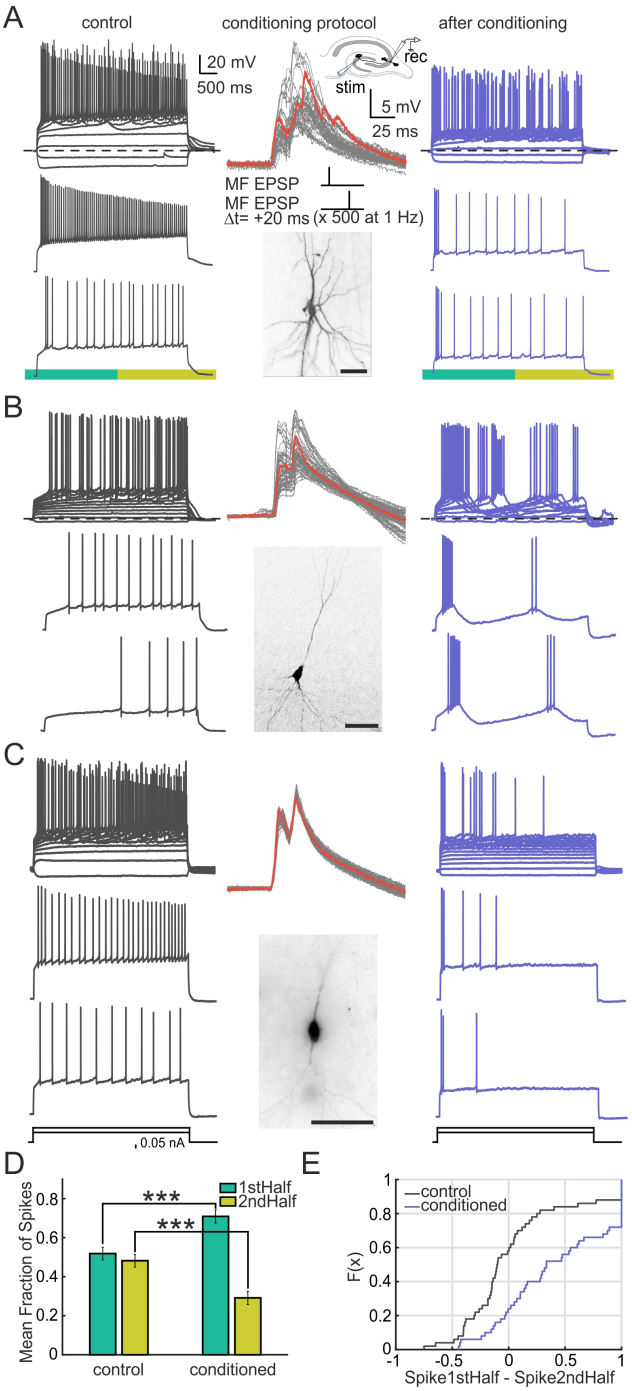

Figure 1. Firing pattern transitions occur in CA3 neurons after subthreshold paired-pulse stimulation of afferents. Three examples of neurons in the CA3 area presenting different morphologies and different firing patterns in control conditions. The discharge patterns were measured by injection of step currents of increasing amplitude. Control measurements (gray traces, left) were followed by stimulation of the mossy fibers. The upper trace shows all voltage traces elicited upon different levels of current injection on that cell. Two sample traces of this set are shown below. EPSPs (middle panel) were evoked in response to a stimulation with double current pulses, separated by 20 ms and repeated 500 times at 1 Hz. The series of repeated pulses are shown superimposed. A sample trace is highlighted in red. The inset shows the configuration of recording and stimulating electrodes (on the CA3 region of the hippocampus and on the dentate gyrus, respectively). Below, the morphology obtained by labeling the cells with biocytin is shown. After the conditioning, patterns were measured again (blue traces, right). A) Pyramidal cell switches from non-adapting burst to intrinsic burst firing. B) Pyramidal cell switches from delay accelerating to intrinsic burst continuous pattern. C) Bipolar cell switches from non-adapting continuous to adapting continuous firing (scale bars = 50μm). D) Mean Fraction of Spikes for the population in the first and second half of the voltage trace (green and yellow rectangle below the trace, respectively) for both control and conditioned cases. A significant redistribution on the fraction of spikes is observed after the conditioning, where the fraction of spikes on the first half is increased while it decreases in the second half (n=50, p=1.92e-6, two-sided Wilcoxon signed rank test). E) Empirical Cumulative Distribution Function for the data shown in D. Every individual cell, for both control and conditioned cases, is represented as the number of spikes for the first half of the trace minus the spikes for the second half (n=50)

in rheobase (from 0.36 ± 0.32 nA to 0.3 ± 0.6 nA, two-sided Wilcoxon signed rank test, p=0.59). There was also a significant 5 mV (7%) depolarization of the resting membrane potential (Vm) (-65.3 ± 5.0mV) with respect to resting level (-70.4 ± 5.7mV, two-sided Wilcoxon signed rank test, p=2.3e-5, n = 50). However, the firing pattern transitions could not be induced by simply clamping the membrane potential at different values (see Figure 2 D-F, n = 10), nor could they be induced by the step-currents used to measure the discharge patterns (see Figure 2 A-C, n = 15). No significant changes in Vm or Rin were found in unconditioned cells (Vm: -69.3 ± 2.0mV, -69.1 ± 1.9mV, paired t-test, p=0.64, Rin: 148.8 ± 56.1MΩ, 158.9 ± 55.6MΩ, paired t-test, p=0.063, n = 15). Intracellular dialysis could also be excluded as the cause of the pattern transitions, as firings did not change spontaneously over intracellular recording time (see Figure 2 D-F).

Firing pattern transitions occur also via somatic conditioning and are blocked by protein kinase A and C inhibitors

We attempted to resolve whether synaptic input was necessary to elicit the changes, or whether they could be induced by direct stimulation of the soma. To this end, we used intra-somatic injection of paired step current pulses whose parameters were chosen to elicit a similar somatic voltage response compared to that generated by the mossy fiber stimulation (Figure 3). This direct subthreshold somatic stimulus evoked changes in discharge pattern that were similar to those elicited by the indirect mossy stimulation. For example, the cell in Figure 3A displayed a delay accelerating firing pattern in control conditions and underwent a transition towards intrinsic burst pattern after somatic conditioning. The population data for direct stimulation showed a significant redistribution in the fraction of spikes in favor of the first half of the trace versus the second half after the conditioning (Figures 3B and C) (n=12). In these experiments we observed the same tendency of neurons to become adapting and intrinsic burst after conditioning as for mossy fiber stimulation. This result suggests that the mechanism underlying the changes in firing pattern is not localized to synapses, but rather acts at a more central, probably somatic or proximal dendritic level.

Next, we sought to identify what internal mechanism could be responsible for the firing pattern transitions. The firing pattern of the cell depends on the distribution of ion channels, or conductances, that the cell presents at its membrane

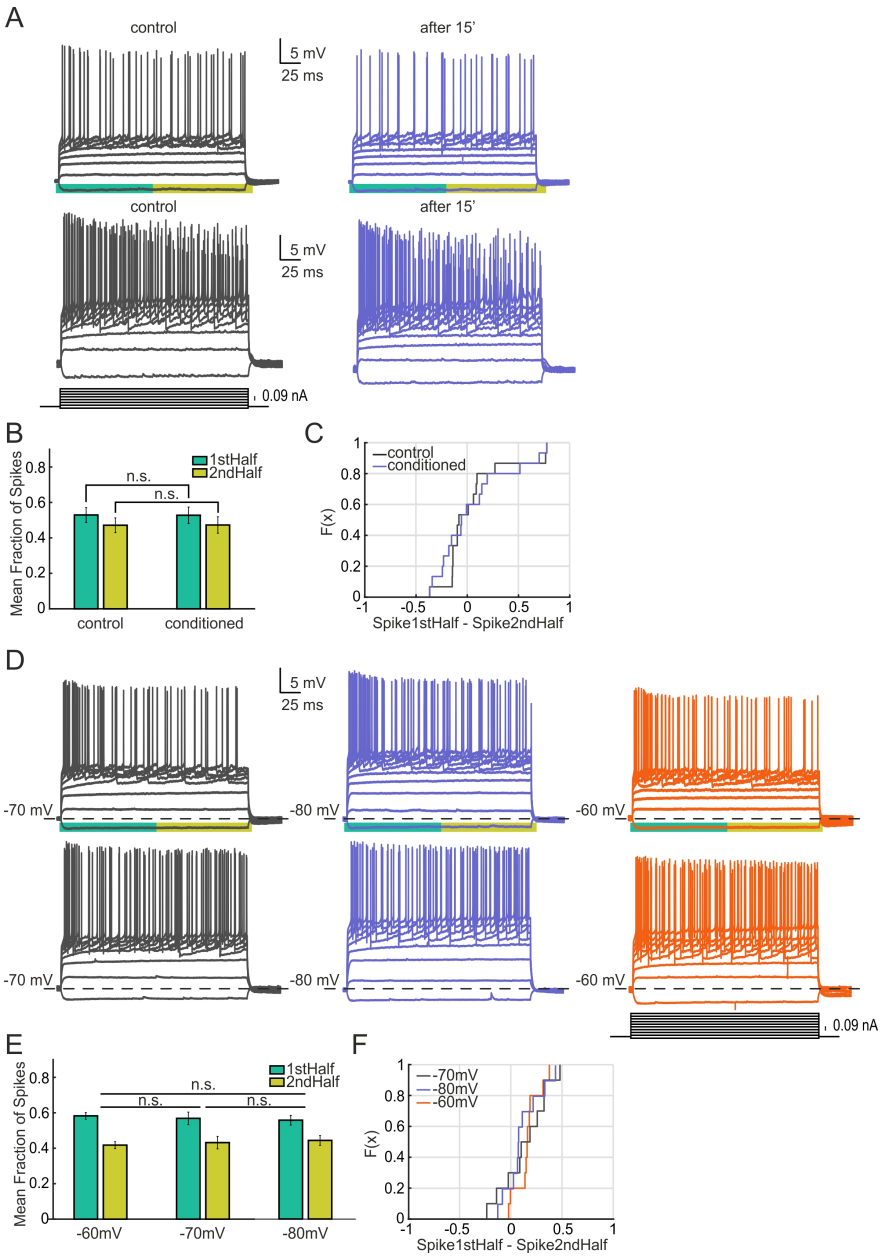

Figure 2. CA3 firing patterns are stable over time and also to changes in resting membrane potential. Firing pattern transitions are not elicited by step current injection alone. A) Examples of two cells whose firing pattern have been measured by step-wise current injection (protocol showed in the inset). The cells do not show changes in firing pattern after 15 min of recording. B) Mean Fraction of Spikes for the population in the first and second half of the voltage trace for both control and conditioned cases. No significant redistribution on the fraction of spikes is observed (n = 15, p=0.583, two-sided Wilcoxon signed rank test). C) Empirical Cumulative Distribution Function for the data shown in B. Every individual case is represented as the number of spikes for the first half of the trace minus the spikes for the second half. D) Firing pattern transitions are not elicited by sustained shifts in membrane potential. Examples of two cells that have been hold at different membrane potentials through steady current injection (-70, -80 and -60 approximately). After changing the holding potential of the recorded neuron the firing patter was measured by step-wise current injection (protocol showed in the inset). No transitions of firing pattern were observed at any of the different holding potentials. E) Mean Fraction of Spikes for the population in the first and second half of the voltage trace for every condition. No significant redistribution on the fraction of spikes is observed (Vm 60 vs 70, p=0.652; Vm 60 vs 80, p=0.084; Vm 70 vs 80, p=0.695) (n = 10, two-sided Wilcoxon signed rank test)). F) Empirical Cumulative Distribution Function for the data shown in E. Every individual case is represented as the number of spikes for the first half of the trace minus the spikes for the second half.

(Hille, 2001). A possible mechanism would act by changing this distribution. Due to the time scale of the response (on the order of minutes) we ruled out protein synthesis of new channels on the membrane. An alternative could be channel phosphorylation, a mechanism known to affect the maximal conductance of the channel on a relatively short timescale (Davis et al., 2001). We reproduced the conditioning protocol in the presence of the PKA and PKC inhibitors H-89 and Go 6983 in the intracellular recording pipette. Figure 3D shows a cell whose firing pattern in control conditions was delay accelerating is shown. After mossy fiber conditioning in the presence of the inhibitors the cell retained this initial pattern. 84% of cells recorded with phosphorylation inhibitors showed no visible modulation of the Petilla label pattern (11 out of the 13 cells). Panels 3E and F show the population response for these cells. Unlike Figure 1D, no significant redistribution of the spikes was found on the presence of the inhibitors (n=13). These results suggest that phosphorylation is implicated in the mechanism of firing pattern transition.

Cluster analysis of experimental traces: quantification of identity changes in neurons

We observed that the conditioning induced firing pattern changes from more regular patterns towards early bursting and adapting patterns. We sought to quantify these changes using hierarchical clustering methods (Druckmann et al., 2013; Tricoire et al., 2011; Hosp et al., 2014) to establish more objectively which discharge type to associate with every response, and to quantify the frequencies of transitions between them.

Previous studies have used clustering methods to quantify the similarity between vectors of features extracted from the voltage traces, such as action potential (AP) amplitude, firing rate, or accommodation index (Druckmann et al., 2013; Tricoire et al., 2011; Hosp et al., 2014). However, those metrics are not suitable for of our dataset, because several features commonly used in those methods are unaffected by the conditioning. For example, AP amplitude, width and afterhyperpolarization (AHP) showed no difference before and after the stimulation (AP amplitude: 78.63 ± 14.95mV, 75.60 ± 9.77mV, paired t-test, p=0.11, AP half width: 1.11 ± 0.26ms, 1.10 ± 0.24ms, paired t-test, p=0.74, AHP: 13.62 ± 3.76mV, 12.66 ± 4.15mV, paired t-test, p=0.12, n = 50). Consequently, we chose to use Dynamic Time Warping (DTW), because it operates directly on the action potential sequence rather than relying on a pre-defined set of features (see Methods for a detailed explanation). Feature vectors of the instantaneous firing rate of the voltage traces were

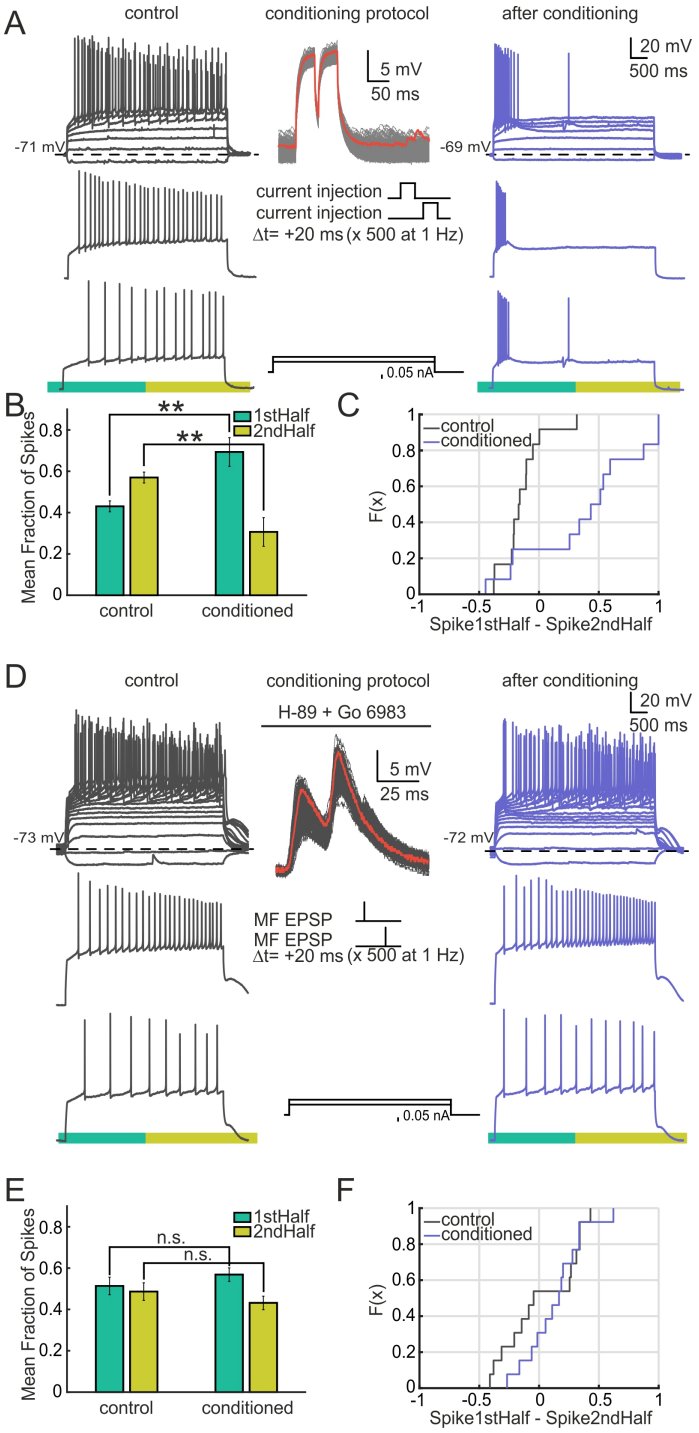

Figure 3. CA3 firing pattern transitions occur upon somatic conditioning and are blocked by kinase inhibitors. A) Example of an intrasomatic conditioned cell that switched from delay accelerating (gray traces) to intrinsic burst firing (blue traces). The conditioning protocol is shown in the middle column. EPSPs were evoked by injection of paired current steps, of 50 ms in duration and separated by 20 ms. The double steps were repeated 500 times at 1 Hz. The series of repeated pulses are shown superimposed. A sample trace is shown in red. B) Mean Fraction of Spikes for the population in the first and second half of the voltage trace for both control and conditioned cases. A significant redistribution on the fraction of spikes occurs after the conditioning. The fraction of spikes on the first half is increased while it decreases in the second half (n=12, p=0.0024, two-sided Wilcoxon signed rank test). C) Empirical Cumulative Distribution Function for the data shown in B. Every individual cell is represented as the number of spikes for the first half of the trace minus the spikes for the second half (n=12). D) Example of a mossy fiber conditioned cell (as described in Figure 1) under the presence of H-89 and Go 6983 (PKA and PKC inhibitors) on the intracellular pipette. The cell expresses a delay accelerating pattern in control conditions and remains under such pattern after the conditioning protocol is applied. E) Mean Fraction of Spikes for the population in the first and second half of the voltage trace for both control and conditioned cases. The redistribution of the fraction of spikes was not significant after the conditioning (n=13, p=0.266, two-sided Wilcoxon signed rank test). F) Empirical Cumulative Distribution Function for the data shown in D. Every individual cell is represented as the number of spikes for the first half of the trace minus the spikes for the second half (n=13).

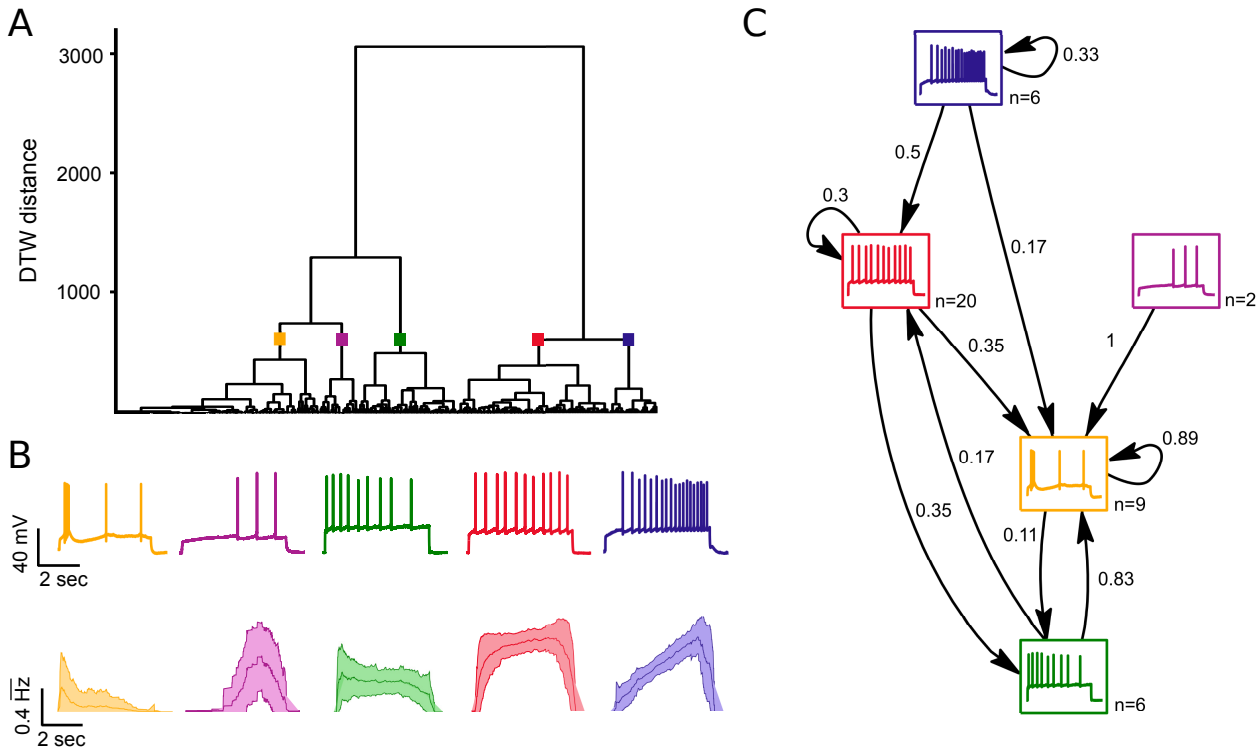

Figure 4. Hierarchical clustering of experimental discharge traces. A) Dendrogram of clustered traces. The data included in the cluster corresponds to the mossy fiber conditioned cells of Figure 1. Two main families can be identified: one containing adapting and bursting traces, together with delayed spiking patterns (left branch); and another branch containing regular and accelerating traces (right branch) (n=50). B) Representative traces from each cluster. Below, average instantaneous firing rate over all traces belonging to the same cluster. Middle lines indicate the mean; light outer lines indicate standard deviations. The instantaneous firing rate (in Hz) is normalized to 1. C) Transitions observed between firing patterns before and after conditioning. Each cell is assigned to a single cluster (represented as a box) for both the control and conditioned cases. Arrows indicate transitions between types whenever a cell changed cluster. Self-loops indicate that the firing pattern was retained after conditioning. Numbers indicate percentages of observed transitions, and the number of cells in each category under control conditions is displayed next to each pattern type. Cells tend to transition towards adapting and bursting patterns following conditioning (n = 43). Seven cells were assigned as unclassified.

compared pairwise using the DTW algorithm. As an internal control, vectors coming from the same set of step current injections of a cell were treated independently. The results of the cluster analysis of discharge patterns are shown in Figure 4. We set the threshold of the clustering tree at a level that separates the traces into 5 distinct families. The threshold was chosen large enough to yield sufficient structure to interpret the hierarchy in terms of recognized response types (Ascoli et al., 2008). Representative traces of each family are shown in Figure 4B. The average of the firing rate vectors of every cluster is depicted beneath each representative trace.

The clustering algorithm captures well the typical time courses of the firing patterns. The right branch of the cluster tree contains accelerating and non-adapting firing patterns, while the other contains adapting and intrinsic bursting

patterns together with a smaller group of traces that have delayed spiking profiles (Figure 4A). The consistency of the algorithm was confirmed by its successful clustering of independent feature vectors derived from the same set of current injections (same cell under the same conditions) into a single cluster. Indeed, in 86% of cases (43 of the 50 cells) the algorithm successfully allocated the majority of vectors from the same set of current injections into single clusters. Vectors from the 7 remaining cells were not consistently classified. For 50% of the cells all of their voltage traces fell into the same cluster, and for 90% of the cells at least 50% did. The allocation of some responses from the same cell into more than a single cluster does however follow a biological logic. For example, for cells classified as accelerating, some of their voltage traces could reasonably fall into the non-adapting cluster because acceleration may vanish at high current injections. A similar reasonable misclassification is possible for adapting traces. In this case low current injections may be classified as non-adapting because the currents are not high enough to elicit adaptation. In particular, many of the traces belonging to the delayed spiking cluster are derived from cells whose traces at low current injections were assigned to the accelerating cluster, or belonged to non-adapting cells with spiking delay. The transitions between cluster types induced by the stimulation protocol are shown in Figure 4C. This figure considers only those cells in which responses both before and after conditioning could be clearly assigned to a cluster. In total, 68% of the cells (n = 50) changed their original cluster as a result of subthreshold conditioning. This quantitative result supports the qualitative observation that cells tend to transition towards more adapting and intrinsic burst profiles. 70% of cells initially belonging to the non-adapting cluster exhibited such changes in response (14 cells), with 35% moving into the intrinsic burst category, and 35% exhibiting adapting spike patterns. 5 of the 6 cells from the adapting cluster (83%) switched to the intrinsic burst type. Most of the cells for which the firing pattern did not change were already in the most common target states of transitions. For example, 89% of the intrinsic bursting cells did not change cluster. This provides further evidence for a predominantly unidirectional change of firing patterns in response to conditioning. The 7 cells that could not be consistently classified under control conditions were all correctly classified after the stimulation. They showed the same transition tendencies: 5 moved into the intrinsic bursting cluster, the other 2 became adapting.

A conductance based model explains the transitions between firing patterns

The consistent transition towards adapting and intrinsic bursting behaviors suggests a common underlying mechanism for most cell types. Our results showing that phosphorylation inhibition blocks firing pattern change after conditioning (Figure 3) support the hypothesis that the prime candidate for this mechanism is a change in the profile of active conductances contributing to action potential discharge dynamics. We explored this possibility using simulations of action potential discharge in a conductance-based single compartment neuron model containing 9 voltage and calcium gated ion channels (see Methods). The densities and kinetics of these channels were derived from experimental measurements of CA3 pyramidal neurons (Hemond et al., 2008). We tuned only their maximum conductances to reproduce the discharge patterns observed in our experiments. The allowed ranges of maximum conductances were restricted to those reported in the literature (Hemond et al., 2008).

In order to explain the experimental transitions, we compared the performance of the clustering procedure on the model and the experimental data. In a first step, the maximal conductance densities of the model were tuned to match the various experimentally observed firing patterns. This tuning was performed manually, and the match to the traces was qualitative. The absolute values for the conductances required to match the main experimental categories (Figure 4: adapting, intrinsic burst, delay spiking, accelerating and non-adapting) are reported in Table 5-2. We also were able to reproduce the experimental traces in the morphologically realistic model described by Hemond et al. (2008) (see Table 5-1 for the conductance values). Although the maximal conductance values had to be adjusted to satisfy the different impedance of the more detailed morphology, the same key conductances are responsible for each category of firings both the single compartment and in the realistic CA3 model.

In a second step, a database of representative ranges of maximal conductances that could plausibly explain the discharge patterns observed experimentally was generated using the single compartment model. To do this, the maximal conductances of the different channels were swept through ranges that would likely encompass the experimentally observed patterns (see Table 5-2 for the exact ranges). In this way a total of 861 conductance profiles were generated. We obtained the discharge response to different levels of current injection for each conductance profile, giving a total of 5166 voltage traces with their associated maximal conductance profiles. Every single experimental trace (coming

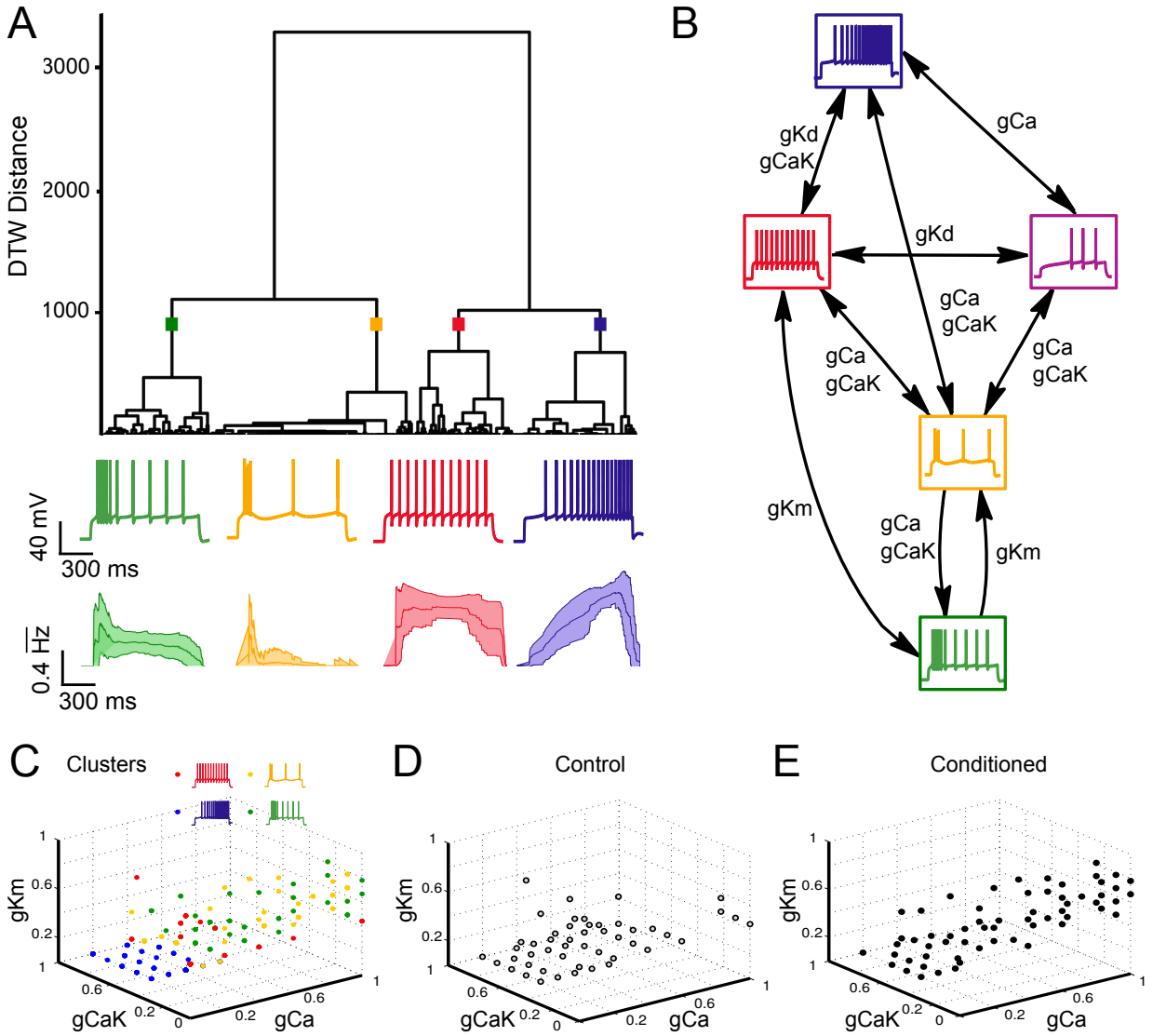

Figure 5. Hierarchical clustering of the model-generated discharge traces mapped to the experimental traces. Every experimental trace was matched to a model trace using the DTW algorithm as a search tool on a model database of traces. Hierarchical clustering was then applied to the model traces. A) The clustering algorithm distinguishes four main families, which correspond to adapting, intrinsic burst, non-adapting and accelerating patterns. Below the dendrogram, a representative model trace of every cluster is depicted. The single compartment model could reproduce the sample experimental traces of Figure 4. The exact maximal conductance values used to produce every model pattern and the amount of current injection are shown in the Table 5-1. Underneath, average instantaneous firing rate within each cluster with its standard deviation (n = 50) B) Conductance road map showing the key conductances responsible for a transition in firing pattern on the model generated traces. The main conductances implicated are *gCa*, *gCaK*, *gKd* and *gKm*. C) Distribution of the maximal conductance vectors of the model traces clustered in (A) in 3D space. Axes correspond to: calcium (*gCa*); calcium-dependent potassium (*gCaK*); and potassium (*gKm*) maximum conductances. The dots are color coded according to their cluster assignment. D) Distribution of the maximal conductance vectors in 3D space of the model traces matched to cells in control conditions E) Distribution of the conductance vectors of traces matched to cells after conditioning. Conditioned cells present a higher content of *gCa* and *gKm*.

from both, control and conditioned cases) was matched against the collection of traces in the model database using the DTW algorithm. The best fit was then selected, allowing us to obtain an estimate of the maximal conductance profile likely to be present in the experimental neuron. These estimates also define the subset of model traces that best represent their experimental counterparts. This subset was then fed to the same hierarchical clustering procedure that was previously performed for the experimental data (Figure 4). The result of hierarchical clustering of the model traces is shown in Figure 5A. There are four main families, corresponding to adapting, intrinsic bursting, accelerating and non-accommodating behavior. Thus, the classification of the model traces is very similar to the experimental one. We noted however the absence of the small class of delayed-spiking patterns (second cluster of Figure 4), which in the case of the model were allocated mostly to the accelerating cluster. The transition diagram of Figure 5B represent the crucial conductances determining the transitions between discharge patterns, obtained during the first step of manual tuning. These are gKm, $gCaK$, gCa and gKd. In this manner, for the delayed discharge pattern, the presence of gKd is required for a delayed onset of the spiking, and the slow inactivation of gKd is important for generating the accelerating discharge pattern. In the case of the adapting and intrinsic burst patterns, the inclusion of gKm and gCa (given the presence of basal levels of $gCaK$) is necessary for the slowing down of the action potentials after the initial discharge. In panel 5C each point indicates the location of an experimental discharge response matched to the model in conductance space. The color of a point shows its cluster assignment. There is a systematic segregation of the data, indicating how the discharge classes of Figure 5A conform to localized regions of conductance space. This correspondence of firing patterns and biophysical parameters offers an interpretation of the causes of transitions between firing behaviors induced by mossy fiber stimulation (Figure 4C). The shift towards adapting and intrinsic bursting behavior after the conditioning corresponds to an increase in calcium related, and gKm conductances (Figures 5D,E).

1 Discussion

The different firing patterns upon step current injection that neurons present are a feature for neuronal classification (Markram et al., 2004; Ascoli et al., 2008), and yet it is increasingly evident that ongoing activity may play a crucial role on their specification (Moody and Bosma, 2005; García et al., 2011; Dehorter et al., 2015). We have shown in here

that CA3 neurons can switch between the main established suprathreshold discharge categories (Ascoli et al., 2008) after only a few minutes of subthreshold conditioning to their somata either by activation of their synapses, or by direct intrasomatic current injection. The effect of conditioning was abolished in the presence of PKA and PKC inhibitors, indicating that phosphorylation over the few minutes of conditioning is necessary for the changes in firing pattern. Hierarchical cluster analysis showed that the transitions observed are more likely towards adapting and intrinsic burst responses. Using a conductance-based neuron model, we found that this shift can be explained by recruitment of calcium and M-type potassium conductances, being phosphorylation of channels a possibility for this recruitment. These results indicate that the intrinsic firing pattern of CA3 neurons on the time scale of seconds can be modified by the statistics of ongoing subthreshold activity on a longer time scale. Our results also imply that the firing pattern of neurons in this hippocampal region is not constant and so does not provide a reliable descriptor of a neural phenotype.

1.1 A novel plasticity mechanism shapes the intrinsic firing pattern of CA3 neurons

Neurons are plastic elements that can adjust their excitability by tuning membrane conductances in response to network activity (Turrigiano et al., 1994; Desai et al., 1999; Fan et al., 2005). This phenomena has been well characterized in the context of homeostatic plasticity (Turrigiano and Nelson, 2004). However, the time scale of those mechanisms typically extends over hours, and involves processes of gene expression (Lee et al., 2005), whereas in our experiments the changes were observed after only a few minutes of conditioning. Furthermore, we show that the effect is abolished by blocking phosphorylation, which also points towards a faster plasticity mechanism. Indeed, rapid up- or down-regulation of ion channel maximal conductances via phosphorylation or vesicle modulation due to calcium signaling has been demonstrated frequently (Flavell and Greenberg, 2008; Davis et al., 2001; Zhang and Linden, 2003) and it has also been shown that the channels possess a complex of scaffold proteins containing protein kinases that could selectively regulate their conductance through phosphorylation (Davis et al., 2001). More recent studies indicate that changes in intrinsic excitability occur on faster time scales (Aizenman and Linden, 2000; Paz et al., 2009; Mahon and Charpier, 2012; Brager and Johnston, 2007; Hyun et al., 2013). The variables considered are generally the firing rate or threshold of the cell, because it is thought that their modulation cooperates with synaptic hebbian learning (Titley et al., 2017) . The neurons in our study significantly adapt their spiking dynamics, adding an extra

dimension to the previous reports.

Most of the work on intrinsic plasticity require that the cell fire during the conditioning, whereas we observe that the firing patterns are modulated even although the conditioning is by subthreshold input. Thus the post-synaptic spiking of the cell is not necessary to induce change in discharge dynamics. Golowasch et al. (1999) were the first to demonstrate that subthreshold current pulses could modulate the currents of STA ganglion neurons, and so their discharge. However, this change required hours of stimulation. Brown and Randall (2009) and Sánchez-Aguilera et al. (2014) reported that subthreshold pulses or constant subthreshold depolarization increases the first ISI or reduces the overall excitability of the cell a few minutes after conditioning. Brown and Randall (2009) reported also that transient subthreshold depolarizing pulses are more effective for the induction. Interestingly, we observe that the first ISI is reduced after conditioning, rather than increased. Finally, other studies have shown that intrinsic cell properties can be affected by different neuromodulators (Brager and Johnston, 2007; Graves et al., 2012; Fujisawa et al., 2005). However, the changes observed here were induced after direct subthreshold somatic conditioning, ruling out a synaptic cause.

1.2 Candidate conductances for the firing pattern transitions

Our model suggests that the likely conductances supporting transitions through the firing pattern space of CA3 cells are gKd, gKm and gCa coupled with $gCaK$. Alternative conductances could potentially elicit a similar dynamical response. For example, the effect on the spike delay mediated by a slow inactivating current, such as gKd can also be elicited by a slow non-inactivating current such as $gNap$. However, the candidates we propose have previously been reported to shape the spiking response of hippocampal cells via activity dependent mechanisms. For example, $gCaT$ is strongly associated with the switch to bursting mode upon status epilepticus (Kim et al., 2001; Su et al., 2002) while gKd is modulated by network activity and influence the delay firing of the cell (Cudmore et al., 2010; Hyun et al., 2013). Modulation of the M-type current upon activity has also been shown in CA1 (Wu et al., 2008) and in CA3 (Brown and Randall, 2009). An increase of a linear conductance can also translate into more adaptation (Prescott et al., 2006), although this is associated with a change in rheobase, which we did not see in our experiments.

The subthreshold conditioning elicited stereotypic transitions towards more adapting or intrinsic burst patterns. However, it was not equally likely for all cell types to undergo these transitions. For example, accelerating cells moved towards regular patterns with higher probability than other patterns (Figure 4C). An explanation for this could be that an accelerating cell tuning its conductances and moving towards adapting or intrinsic bursting necessarily become regular during the process. An alternative possibility is that there may be some cell types that obey different rules of conductance change, or that the different channel densities in control conditions favor different transitions. One of the typical transitions that we observe is the switch towards bursting behaviors. We emphasize that this is not the only transition induced, but rather that special attention should be given to this burst mechanism. It is known that some cells present this dual behavior. For example, relay cells on the thalamus become bursty upon hyperpolarization because of T-type conductance inactivation (Sherman, 2001). In our case, after conditioning, the cells depolarized 5 mV in average, so we rule out this hyperpolarization mechanism.

1.3 Functional implications of firing pattern modulation

Similar firing patterns are found in multiple species within the animal phyla (McCormick et al., 1985; Turrigiano et al., 1994; Yao and Wu, 2001), suggesting that they have a fundamental role in network computation. This raises questions about the computational advantages conferred by the transitions between patterns that we have observed. Shin et al. (1999) proposed that neurons that can dynamically adapt their output firing in response to their input statistics would have important advantages. By adjusting its threshold and dynamic range upon activity, a neuron could respond to stimuli over a broad range of amplitudes and frequencies without compromising sensitivity, and maximize the mutual information between its input and output discharge (Stemmler and Koch, 1999). Spike frequency accommodation has the characteristics of a high-pass filter (Benda and Herz, 2003). Since our conditioning stimuli occurred at constant frequencies, cells may have recruited a specific set of conductances that shift their integration properties to gain sensitivity in the new frequency range. Differences in filtering properties of brain stem neurons have also been shown to facilitate the extraction of spatial information from natural sounds (Remme et al., 2014) and most of the conductances that we identify in this study have reported to be frequency resonance candidates (Hutcheon and Yarom, 2000; Hu et al., 2002; Schreiber et al., 2004). These resonance properties of cells may also have important

functional implications for neural activity and brain rhythms (Llinás, 1988; Buzsáki and Draguhn, 2004). When adjusting their discharge to more adapting patterns neurons may be changing mode from integrator to a coincidence detector (Prescott et al., 2008), helping not only to detect synchrony but also to transmit it to the network (Cudmore et al., 2010). Additionally, this fast plasticity of the firings may also be important for specific memory acquisition on the hippocampus (Kumaran et al., 2016; Benna and Fusi, 2016). CA3 is thought to be the generator of sharp wave ripples (SPW-R), a state where neurons cooperatively switch to presumably transfer memories to cortex (Buzsáki, 2015; Kumaran et al., 2016). The stimulation protocol -resembling a SPW-R- may have push CA3 neurons to move to a different network state, similarly to Fujisawa et al. (2005), by possibly sensing the inputs and changing their spiking properties based on intrinsic plasticity rules (Srikanth and Narayanan, 2015).

Further studies will be needed in order to unravel the role that such firing pattern transitions may have for computations in neural circuits. A first step towards this goal must be to explore more generally how the form and frequency spectrum of somatic input signals on the long time scale affect the distinct firing patterns that neurons exhibit on the short scale. It appears that after decades of study and cataloging these patterns, the mystery is not whether neurons conform to classes or to a continuum but rather, what internal rules, and computational advantages are, that require neurons to converge onto these different discharge patterns.

Bibliography

Aizenman CD, Linden DJ (2000) Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. *Nature neuroscience* 3:109–111.

Ascoli GA, Alonso-Nanclares L, Anderson SA, Barrionuevo G, Benavides-Piccione R, Burkhalter A, Buzsáki G, Cauli B, DeFelipe J, Fairén A et al. (2008) Petilla terminology: nomenclature of features of gabaergic interneurons of the cerebral cortex. *Nature Reviews Neuroscience* 9:557–568.

Belmeguenai A, Hosy E, Bengtsson F, Pedroarena CM, Piochon C, Teuling E, He Q, Ohtsuki G, De Jeu MT, Elgersma Y et al. (2010) Intrinsic plasticity complements long-term potentiation in parallel fiber input gain control in cerebellar purkinje cells. *Journal of Neuroscience* 30:13630–13643.

Benda J, Herz AV (2003) A universal model for spike-frequency adaptation. *Neural computation* 15:2523–2564.

Benna MK, Fusi S (2016) Computational principles of synaptic memory consolidation. *Nature neuroscience* .

Berndt DJ, Clifford J (1994) Using dynamic time warping to find patterns in time series. In *KDD workshop*, Vol. 10, pp. 359–370. Seattle, WA.

Brager DH, Johnston D (2007) Plasticity of intrinsic excitability during long-term depression is mediated through mglur-dependent changes in ih in hippocampal ca1 pyramidal neurons. *Journal of Neuroscience* 27:13926–13937.

Brandalise F, Carta S, Helmchen F, Lisman J, Gerber U (2016) Dendritic nmda spikes are necessary for timing-dependent associative ltp in ca3 pyramidal cells. *Nature Communications* 7:13480.

Brandalise F, Gerber U (2014) Mossy fiber-evoked subthreshold responses induce timing-dependent plasticity at hippocampal ca3 recurrent synapses. *Proceedings of the National Academy of Sciences* 111:4303–4308.

Brown JT, Randall AD (2009) Activity-dependent depression of the spike after-depolarization generates long-lasting intrinsic plasticity in hippocampal ca3 pyramidal neurons. *The Journal of physiology* 587:1265–1281.

Butt SJ, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G (2005) The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron* 48:591–604.

Buzsáki G (2015) Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus* 25:1073–1188.

Buzsáki G, Draguhn A (2004) Neuronal oscillations in cortical networks. *Science* 304:1926–1929.

Cauli B, Porter JT, Tsuzuki K, Lambolez B, Rossier J, Quenet B, Audinat E (2000) Classification of fusiform neocortical interneurons based on unsupervised clustering. *Proceedings of the National Academy of Sciences* 97:6144–6149.

Cohen S, Greenberg ME (2008) Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. *Annual review of cell and developmental biology* 24:183–209.

Connors BW, Gutnick MJ (1990) Intrinsic firing patterns of diverse neocortical neurons. *Trends in neurosciences* 13:99–104.

Cooley J, Dodge F (1966) Digital computer solutions for excitation and propagation of the nerve impulse. *Biophysical journal* 6:583–599.

Cudmore RH, Fronzaroli-Molinieres L, Giraud P, Debanne D (2010) Spike-time precision and network synchrony are controlled by the homeostatic regulation of the d-type potassium current. *Journal of Neuroscience* 30:12885–12895.

Davis MJ, Wu X, Nurkiewicz TR, Kawasaki J, Gui P, Hill MA, Wilson E (2001) Regulation of ion channels by protein tyrosine phosphorylation. *American Journal of Physiology-Heart and Circulatory Physiology* 281:H1835–H1862.

De Schutter E, Bower JM (1994) An active membrane model of the cerebellar purkinje cell. i. simulation of current clamps in slice. *Journal of neurophysiology* 71:375–400.

DeFelipe J (1993) Neocortical neuronal diversity: chemical heterogeneity revealed by colocalization studies of classic neurotransmitters, neuropeptides, calcium-binding proteins, and cell surface molecules. *Cerebral Cortex* 3:273–289.

Dehorter N, Ciceri G, Bartolini G, Lim L, del Pino I, Marín O (2015) Tuning of fast-spiking interneuron properties by an activity-dependent transcriptional switch. *Science* 349:1216–1220.

Desai NS, Rutherford LC, Turrigiano GG (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nature neuroscience* 2:515–520.

Druckmann S, Hill S, Schürmann F, Markram H, Segev I (2013) A hierarchical structure of cortical interneuron electrical diversity revealed by automated statistical analysis. *Cerebral Cortex* 23:2994–3006.

Dumitriu D, Cossart R, Huang J, Yuste R (2007) Correlation between axonal morphologies and synaptic input kinetics of interneurons from mouse visual cortex. *Cerebral cortex* 17:81–91.

Fan Y, Fricker D, Brager DH, Chen X, Lu HC, Chitwood RA, Johnston D (2005) Activity-dependent decrease of excitability in rat hippocampal neurons through increases in ih. *Nature neuroscience* 8:1542–1551.

Flavell SW, Greenberg ME (2008) Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. *Annual review of neuroscience* 31:563.

Fujisawa S, Matsuki N, Ikegaya Y (2005) Single neurons can induce phase transitions of cortical recurrent networks with multiple internal states. *Cerebral Cortex* 16:639–654.

Gähwiler B (1981) Organotypic monolayer cultures of nervous tissue. *Journal of neuroscience methods* 4:329–342.

García NVDM, Karayannis T, Fishell G (2011) Neuronal activity is required for the development of specific cortical interneuron subtypes. *Nature* 472:351–355.

Golowasch J, Abbott L, Marder E (1999) Activity-dependent regulation of potassium currents in an identified neuron of the stomatogastric ganglion of the crab cancer borealis. *Journal of Neuroscience* 19:RC33–1.

Graves AR, Moore SJ, Bloss EB, Mensh BD, Kath WL, Spruston N (2012) Hippocampal pyramidal neurons comprise two distinct cell types that are countermodulated by metabotropic receptors. *Neuron* 76:776–789.

Hemond P, Epstein D, Boley A, Migliore M, Ascoli GA, Jaffe DB (2008) Distinct classes of pyramidal cells exhibit mutually exclusive firing patterns in hippocampal area ca3b. *Hippocampus* 18:411–424.

Hille B (2001) *Ion channels of excitable membranes*, Vol. 507 Sinauer Sunderland, MA.

Hines ML, Carnevale NT (1997) The neuron simulation environment. *Neural computation* 9:1179–1209.

Hosp JA, Strüber M, Yanagawa Y, Obata K, Vida I, Jonas P, Bartos M (2014) Morpho-physiological criteria divide dentate gyrus interneurons into classes. *Hippocampus* 24:189–203.

Hu H, Vervaeke K, Storm JF (2002) Two forms of electrical resonance at theta frequencies, generated by m-current, h-current and persistent na+ current in rat hippocampal pyramidal cells. *The Journal of physiology* 545:783–805.

Hutcheon B, Yarom Y (2000) Resonance, oscillation and the intrinsic frequency preferences of neurons. *Trends in neurosciences* 23:216–222.

Hyun JH, Eom K, Lee KH, Ho WK, Lee SH (2013) Activity-dependent downregulation of d-type k+ channel subunit kv1. 2 in rat hippocampal ca3 pyramidal neurons. *The Journal of physiology* 591:5525–5540.

Kawaguchi Y, Kubota Y (1997) Gabaergic cell subtypes and their synaptic connections in rat frontal cortex. *Cerebral cortex* 7:476–486.

Keogh E, Ratanamahatana CA (2005) Exact indexing of dynamic time warping. *Knowledge and information systems* 7:358–386.

Kim D, Song I, Keum S, Lee T, Jeong MJ, Kim SS, McEnery MW, Shin HS (2001) Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking α 1g t-type ca 2+ channels. *Neuron* 31:35–45.

Kumaran D, Hassabis D, McClelland JL (2016) What learning systems do intelligent agents need? complementary learning systems theory updated. *Trends in cognitive sciences* 20:512–534.

Lee PR, COHEN JE, BECKER KG, FIELDS RD (2005) Gene expression in the conversion of early-phase to late-phase long-term potentiation. *Annals of the New York Academy of Sciences* 1048:259–271.

Llinás RR (1988) The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. *Science* 242:1654–1664.

Mahon S, Charpier S (2012) Bidirectional plasticity of intrinsic excitability controls sensory inputs efficiency in layer 5 barrel cortex neurons in vivo. *Journal of Neuroscience* 32:11377–11389.

Marder E, Goaillard JM (2006) Variability, compensation and homeostasis in neuron and network function. *Nature Reviews Neuroscience* 7:563–574.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience* 5:793–807.

McCormick DA, Connors BW, Lighthall JW, Prince DA (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *Journal of neurophysiology* 54:782–806.

Moody WJ, Bosma MM (2005) Ion channel development, spontaneous activity, and activity-dependent development in nerve and muscle cells. *Physiological reviews* 85:883–941.

Paz JT, Mahon S, Tiret P, Genet S, Delord B, Charpier S (2009) Multiple forms of activity-dependent intrinsic plasticity in layer v cortical neurones in vivo. *The Journal of physiology* 587:3189–3205.

Prescott SA, Ratté S, De Koninck Y, Sejnowski TJ (2006) Nonlinear interaction between shunting and adaptation controls a switch between integration and coincidence detection in pyramidal neurons. *Journal of Neuroscience* 26:9084–9097.

Prescott SA, Ratté S, De Koninck Y, Sejnowski TJ (2008) Pyramidal neurons switch from integrators in vitro to resonators under in vivo-like conditions. *Journal of neurophysiology* 100:3030–3042.

Ramon y Cajal D (1893) Nuevo concepto de la histologia de los centros nerviosos .

Remme MW, Donato R, Mikiel-Hunter J, Ballestero JA, Foster S, Rinzel J, McAlpine D (2014) Subthreshold resonance properties contribute to the efficient coding of auditory spatial cues. *Proceedings of the National Academy of Sciences* 111:E2339–E2348.

Ren J, Aika Y, Heizmann C, Kosaka T (1992) Quantitative analysis of neurons and glial cells in the rat somatosensory cortex, with special reference to gabaergic neurons and parvalbumin-containing neurons. *Experimental brain research* 92:1–14.

Sánchez-Aguilera A, Sánchez-Alonso J, Vicente-Torres M, Colino A (2014) A novel short-term plasticity of intrinsic excitability in the hippocampal ca1 pyramidal cells. *The Journal of physiology* 592:2845–2864.

Schreiber S, Fellous JM, Tiesinga P, Sejnowski TJ (2004) Influence of ionic conductances on spike timing reliability of cortical neurons for suprathreshold rhythmic inputs. *Journal of neurophysiology* 91:194–205.

Sherman SM (2001) Tonic and burst firing: dual modes of thalamocortical relay. *Trends in neurosciences* 24:122–126.

Shin J, Koch C, Douglas R (1999) Adaptive neural coding dependent on the time-varying statistics of the somatic input current. *Neural computation* 11:1893–1913.

Somogyi P, Klausberger T (2005) Defined types of cortical interneurone structure space and spike timing in the hippocampus. *The Journal of physiology* 562:9–26.

Srikanth S, Narayanan R (2015) Variability in state-dependent plasticity of intrinsic properties during cell-autonomous self-regulation of calcium homeostasis in hippocampal model neurons. *eNeuro* 2:ENEURO–0053.

Stemmler M, Koch C (1999) How voltage-dependent conductances can adapt to maximize the information encoded by neuronal firing rate. *Nature neuroscience* 2:521–527.

Steriade M (2004) Neocortical cell classes are flexible entities. *Nature reviews neuroscience* 5:121–134.

Su H, Sochivko D, Becker A, Chen J, Jiang Y, Yaari Y, Beck H (2002) Upregulation of a t-type ca2+ channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *The Journal of neuroscience* 22:3645–3655.

Tasic B, Menon V, Nguyen TN, Kim TK, Jarsky T, Yao Z, Levi B, Gray LT, Sorensen SA, Dolbeare T et al. (2016) Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nature neuroscience* 19:335–346.

Thompson L, Moyer J, Disterhoft J (1996) Transient changes in excitability of rabbit ca3 neurons with a time course appropriate to support memory consolidation. *Journal of Neurophysiology* 76:1836–1849.

Titley HK, Brunel N, Hansel C (2017) Toward a neurocentric view of learning. *Neuron* 95:19–32.

Tricoire L, Pelkey KA, Erkkila BE, Jeffries BW, Yuan X, McBain CJ (2011) A blueprint for the spatiotemporal origins of mouse hippocampal interneuron diversity. *The Journal of Neuroscience* 31:10948–10970.

Turrigiano G, Abbott L, Marder E (1994) Activity-dependent changes in the intrinsic properties of cultured neurons. *Science-AAAS-Weekly Paper Edition-including Guide to Scientific Information* 264:974–976.

Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. *Nature Reviews Neuroscience* 5:97–107.

Van Aerde KI, Feldmeyer D (2015) Morphological and physiological characterization of pyramidal neuron subtypes in rat medial prefrontal cortex. *Cerebral cortex* 25:788–805.

Ward Jr JH (1963) Hierarchical grouping to optimize an objective function. *Journal of the American statistical association* 58:236–244.

Wu WW, Chan CS, Surmeier DJ, Disterhoft JF (2008) Coupling of l-type ca2+ channels to kv7/kcnq channels creates a novel, activity-dependent, homeostatic intrinsic plasticity. *Journal of neurophysiology* 100:1897–1908.

Yao WD, Wu CF (2001) Distinct roles of camkii and pka in regulation of firing patterns and k+ currents in drosophila neurons. *Journal of neurophysiology* 85:1384–1394.

Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nature Reviews Neuroscience* 4:885–900.

Extended information

	Intrinsic Burst		Adapting		Accelerating		Non-Adapting		Delayed	
Maximal Conductances	Cylinder	Realistic CA3	Cylinder	Realistic CA3	Cylinder	Realistic CA3	Cylinder	Realistic CA3	Cylinder	Realistic CA3
g_{Na}	0.04	0.022	0.04	0.022	0.04	0.022	0.04	0.022	0.04	0.022
g_{Kdr}	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.01
g_{Ka}	0.07	0.02	0.04	0.02	0.04	0.02	0.12	0.02	0.08	0.02
g_{CaT}	0.001	0.00002	0.00	0.00001	0.0003	0.00001	0.00	0.00	0.0001	0.00
g_{CaN}	0.001	0.00002	0.00	0.00001	0.0003	0.00001	0.00	0.00	0.0001	0.00
g_{CaL}	0.001	0.00002	0.00	0.00001	0.0003	0.00001	0.00	0.00	0.0001	0.00
g_{CaK}	0.0001	0.00002	0.00	0.00	0.0005	0.00001	0.00	0.00	0.0006	0.00
g_{Km}	0.0006	0.021	0.00052	0.017	0.00	0.00	0.000003	0.00	0.000003	0.00
g_{Kd}	0.00045	0.002	0.00025	0.00	0.0008	0.005	0.00	0.00	0.00035	0.002
I	1	1.83	0.62	1.37	1.95	1.1	0.53	0.58	0.55	0.583

Table 5-1. Model maximal conductance values for the experimental fits. List of the absolute conductance values used to reproduce the traces of main Fig 5 (Intrinsic Burst, Adapting, Accelerating, Non-Adapting and Delayed) in the single compartment NEURON model. The same class of firing pattern traces could be also reproduced (second column for each trace) using the realistic CA3 pyramidal neuron model from Hemond et al. (2008). The conductances were tuned manually in order to fit qualitatively the overall dynamics of the voltage experimental traces. The bottom row lists the value of the current (in nA) used to generate every voltage trace. For more detailed description of the model see Methods.

Conductances Ranges	1	2	3
g_{Kdr}	0.015 : 0.05 : 0.04		
g_{Ka}	0.01 : 0.01 : 0.09		
g_{Ca}	0	0.2 : 0.1 : 0.6	0.8 : 0.1 : 1
g_{CaK}	0 : 0.1 : 0.9		
g_{Km}	0	0.4 : 0.1 : 1	
g_{Kd}	0 : 0.1 : 0.3	0.4 : 0.05 : 0.8	0.9 : 0.1 : 1
I	0.45 : 0.05 : 0.65	0.75 : 0.05 : 1	1.75 : 0.05 : 1.95
Total conductance vectors	861		
Total traces	5166		

Table 5-2. Range of maximal conductance values used to generate the model database of voltage traces. A model database of voltage traces, which includes all the observed experimental firing patterns, was generated by varying 6 maximal conductances ($gKdr$, gKa , gCa , $gCaK$, gKm and gKd) over a given range. Each row in the table lists the ranges of conductance values employed in every channel. The different ranges of conductances (columns) were produced in order to account for the different firing patterns reproduced in the model. Different ranges of current were also needed to reveal the different firing types. A total of 861 conductance vectors were generated by combining the different conductances. The firing pattern of every conductance vector was produced at several levels of step-current injection, obtaining a total of 5166 voltage traces. Note that $gCaT$, $gCaN$ and $gCaL$ are englobed under the single parameter gCa.