SUPPLEMENTAL INFORMATION

Intracellular Determinants of Hippocampal CA1 Place and Silent Cell Activity in a Novel Environment

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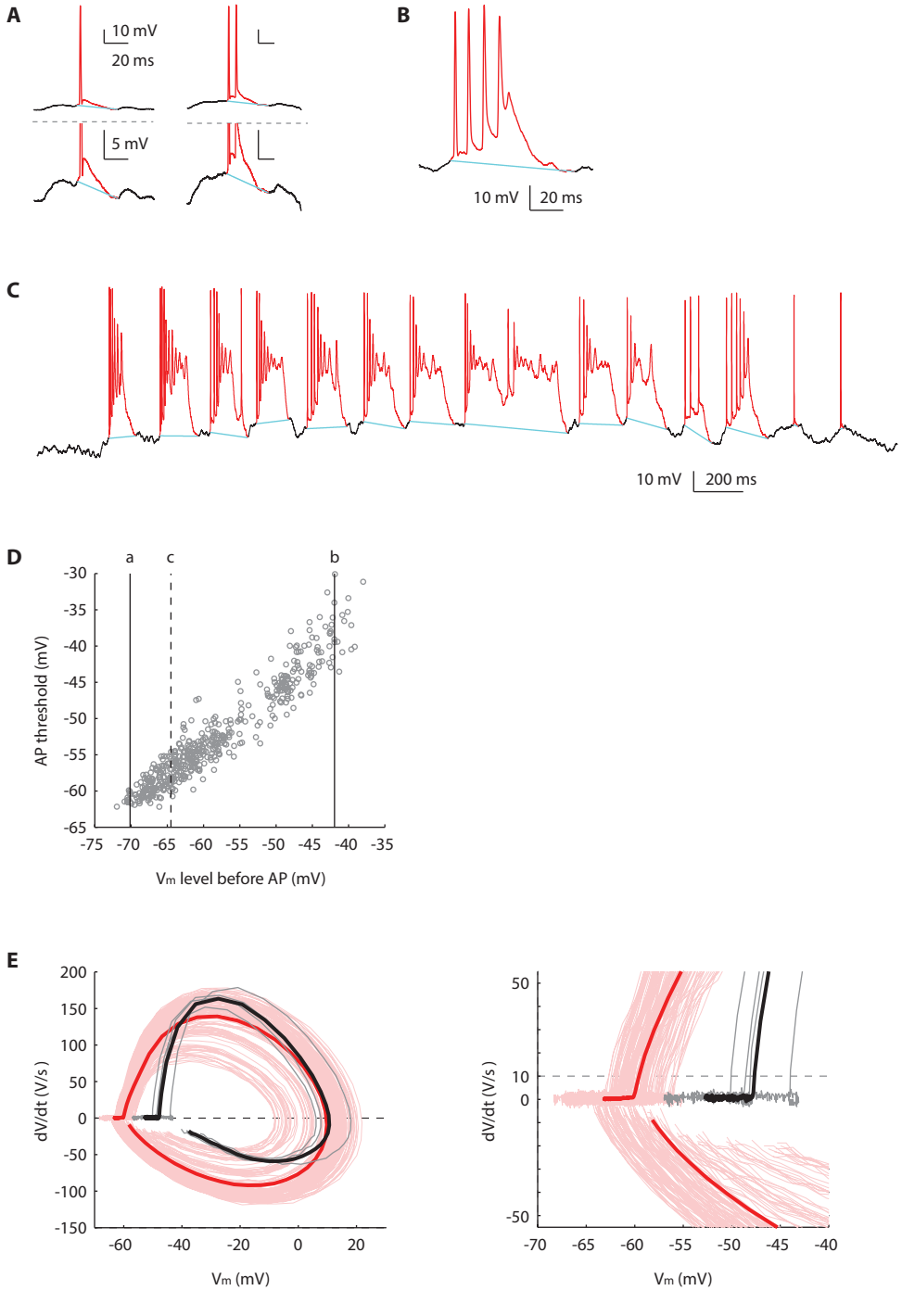
A
10 mV
20 ms
5 mV
B
10 mV
20 ms
C
10 mV
200 ms
D
a
c
b
AP threshold (mV)
-30
-35
-40
-45
-50
-55
-60
-65
-75 -70 -65 -60 -55 -50 -45 -40 -35
Vm level before AP (mV)
E
dV/dt (V/s)
200
150
100
50
0
-50
-100
-150
-60 -40 -20 0 20
Vm (mV)
dV/dt (V/s)
50
10
0
-50
-70 -65 -60 -55 -50 -45 -40
Vm (mV)

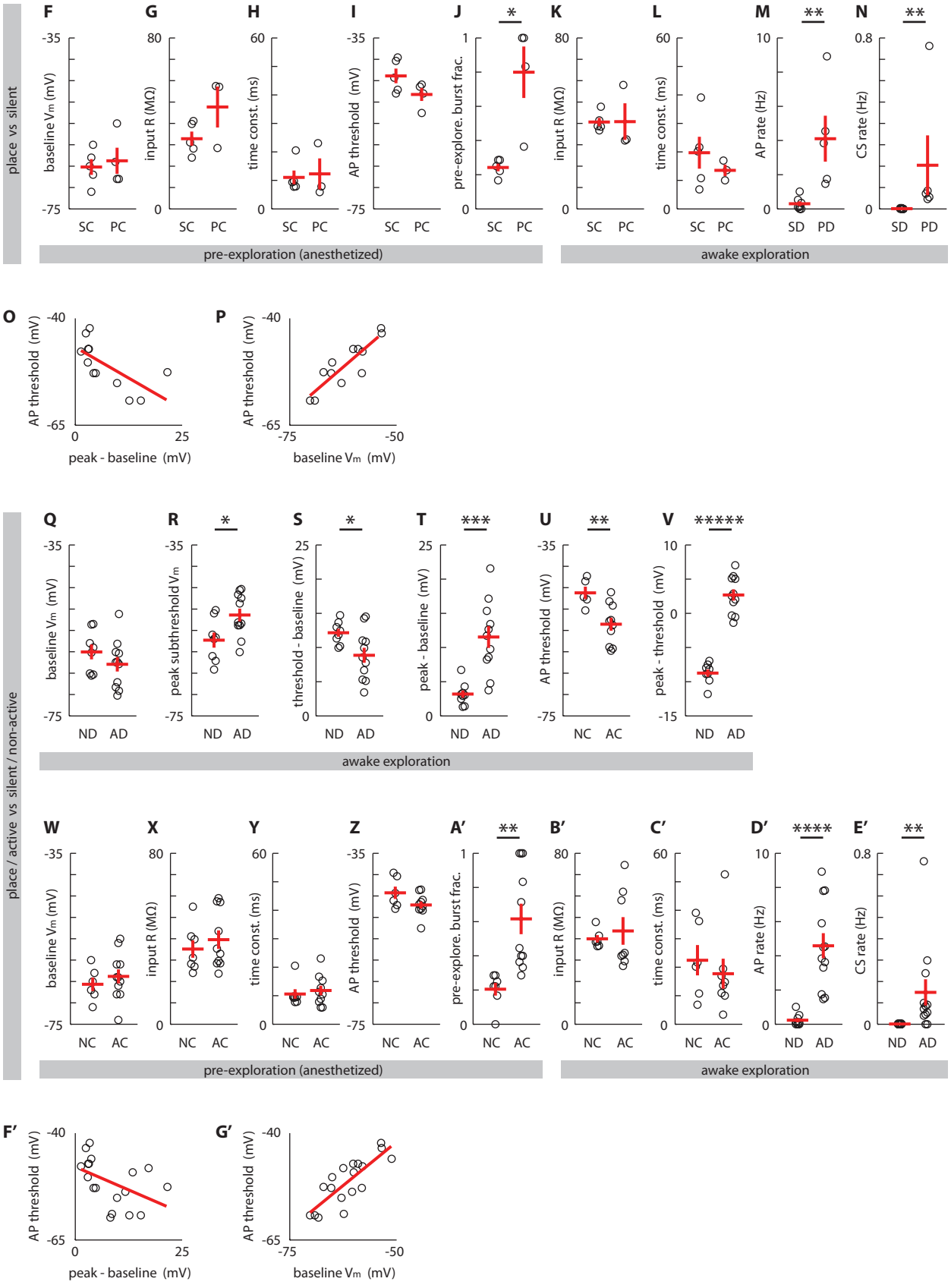
place vs silent
F
G
H
I
J
*
K
L
M
**
N
**
baseline Vm (mV)
input R (MΩ)
time const. (ms)
AP threshold (mV)
pre-explore. burst frac.
input R (MΩ)
time const. (ms)
AP rate (Hz)
CS rate (Hz)
SC PC
SD PD
pre-exploration (anesthetized)
awake exploration
O
P
AP threshold (mV)
peak - baseline (mV)
baseline Vm (mV)
place / active vs silent / non-active
Q
R
*
S
*
T
***
U
**
V
*****
baseline Vm (mV)
peak subthreshold Vm
threshold - baseline (mV)
peak - baseline (mV)
AP threshold (mV)
peak - threshold (mV)
ND AD
NC AC
awake exploration
W
X
Y
Z
A'
**
B'
C'
D'
****
E'
**
baseline Vm (mV)
input R (MΩ)
time const. (ms)
AP threshold (mV)
pre-explore. burst frac.
input R (MΩ)
time const. (ms)
AP rate (Hz)
CS rate (Hz)
NC AC
ND AD
pre-exploration (anesthetized)
awake exploration
F'
G'
AP threshold (mV)
peak - baseline (mV)
baseline Vm (mV)

Figure S1 (related to Figure 4)

Subthreshold V_m trace

(A)-(C) Determination of subthreshold V_m trace used to compute mean subthreshold field. The trace was created by removing APs, bursts, and their associated ADPs ((A) and (B)), as well as the entirety of the large, slow depolarizations that sometimes follow APs (C), then linearly interpolating (blue) across the resulting gaps (see Experimental Procedures).

AP threshold

(D) Threshold for individual APs as a function of immediately preceding subthreshold V_m level for the place cell in Figure 2. Vertical lines (a) and (b) mark V_m below which 2.5% and 97.5% of preceding subthreshold levels lie, and line (c) marks the V_m value 20% of the way from (a) to (b). The AP threshold for this cell was set as the mean threshold of APs between vertical lines (a) and (c), i.e. those APs triggered from less-depolarized V_m levels. Only APs meeting certain criteria were considered and are plotted here (Experimental Procedures).

(E) Phase plots of the first derivative of V_m with respect to time (dV/dt) versus V_m for individual APs. Trajectories start 10 ms before and end 1.2 ms after the peak V_m of each AP. Phase plots for the APs used to determine the threshold for the place cell in Figure 2 (i.e. the APs between vertical lines (a) and (c) in (D)) (pink: individual APs, red: mean) and the silent cell in Figure 3 (gray: individual APs, black: mean). Close-up (right) showing "take-off" of V_m for each AP and intersection of trajectory with 10 V/s value used to determine individual AP thresholds.

Place and silent cell properties

(F)-(P) Additional properties of place cells (PC, n=4), silent cells (SC, n=5), directions with a place field (PD, n=5), and silent directions (SD, n=7) for cells and directions in Figure 4. For the pre-exploration period when the animal was anesthetized, we used values obtained immediately after achieving the whole-cell configuration. For the awake exploration period, we used values averaged over the entire awake recording session. Values and significance for (F) initial anesthetized baseline V_m (place: -63.8±3.1 mV versus silent: –65.2±1.9 mV; p=0.70), (G) initial anesthetized input resistance (R_N) (47.7±9.6 versus 32.9±3.3 MΩ; p=0.26), (H) initial anesthetized membrane time constant (τ_m) (12.3±5.4 versus 11.1±2.4 ms; p=0.85), (I) initial anesthetized AP threshold (-48.2±1.5 versus -43.9±1.7 mV; p=0.10), (J) initial anesthetized fraction of APs in bursts in response to depolarizing current step (0.80±0.15 versus 0.24±0.02, p=0.033), (K) awake R_N (40.8±8.6 versus 40.6±2.0 MΩ; p=0.98), (L) awake τ_m (13.5±2.0 versus 19.7±5.6 ms; p=0.34), (M) awake mean AP rate (4.1±1.3 versus 0.30±0.14 Hz; p=0.0025, Mann-Whitney), (N) awake mean CS rate (0.20±0.14 versus 0.0011±0.0007 Hz; p=0.0025, Mann-Whitney). For one place cell, the initial anesthetized R_N and τ_m could not be measured, and, for a different place cell, the awake R_N and τ_m could not be measured. (O) Scatterplot of AP threshold versus "peak – baseline" for all place field and silent directions together (ρ = -0.67; p=0.018; regression line: AP threshold = -0.58 × "peak – baseline" – 46.8 mV). (P) Scatterplot of AP threshold versus baseline V_m for all place field and silent directions together (ρ = 0.88; p=0.00013; regression line: AP threshold = 0.86 × baseline + 2.4 mV).

(F)-(N) Mean ± SEM (red). Horizontal jitter added to individual values for visibility. *,** correspond to p < 0.05, 0.005 differences.

Active and nonactive cell properties

(Q)-(G') Properties of active cells (AC, n=11), nonactive cells (NC, n=7), active directions (AD, n=12), and nonactive directions (ND, n=9). These groups consisted of place and silent cells and directions from

Figures 4 and S1F-S1P combined with 9 additional cells in which animal sampled the maze ≤1 time (Experimental Procedures).

(Q)-(V) Same properties of active and nonactive cells and directions as those displayed for place and silent cells and directions in Figures 4B-4G. Values and significance for (Q) awake baseline V_m (active: -62.9±1.7 mV versus nonactive: -60.0±1.8 mV; p=0.25), (R) peak subthreshold V_m (-51.4±1.5 versus -57.3±1.8 mV; p=0.023), (S) "threshold – baseline" (8.9±1.1 versus 12.2±0.6 mV; p=0.019), (T) "peak – baseline" (11.5±1.6 versus 3.2±0.5 mV; p=0.00032), (U) awake AP threshold (-53.6±1.5 versus -46.2±1.5 mV; p=0.0049), (V) "peak – threshold" (2.7±0.8 versus -8.7±0.5 mV; $p=3.3\times10^{-9}$). Threshold could not be measured for 2 of the nonactive cells because one fired no APs during exploration, and the other had an unknown V_m offset for technical reasons. The unknown offset also prevented measurement of baseline and peak for that nonactive cell / direction. Measurements could not be made for 1 active cell / direction because the holding current was not recorded thus preventing any potential bridge correction.

(W)-(G') Same properties of active and nonactive cells and directions as those displayed for place and silent cells and directions in Figures S1F-S1P. Values and significance for (W) initial anesthetized baseline V_m (active: -63.8±1.7 mV versus nonactive: –65.7±1.6 mV; p=0.43), (X) initial anesthetized R_N (39.6±4.3 versus 35.2±4.1 MΩ; p=0.47), (Y) initial anesthetized membrane τ_m (11.8±1.7 versus 10.6±1.7 ms; p=0.62), (Z) initial anesthetized AP threshold (-47.1±0.8 versus -44.3±1.4 mV; p=0.12), (A') initial anesthetized fraction of APs in bursts in response to depolarizing current step (0.62±0.09 versus 0.20±0.04, p=0.00092), (B') awake R_N (43.6±6.5 versus 39.9±1.7 MΩ; p=0.60), (C') awake τ_m (17.7±5.3 versus 22.5±5.3 ms; p=0.54), (D') awake mean AP rate (4.6±0.7 versus 0.24±0.11 Hz; $p=6.8\times10^{-6}$, Mann-Whitney) (though note that the AP rate was used to split the data into active and nonactive groups in the first place), (E') awake mean CS rate (0.15±0.06 versus 0.00089±0.00059 Hz; p=0.00068, Mann-Whitney). For one active cell, the initial anesthetized R_N and τ_m could not be measured, and, for a different active cell, the initial anesthetized threshold could not be measured. For one nonactive cell, the initial anesthetized baseline and threshold could not be measured. For 3 active cells and one nonactive cell, the awake R_N and τ_m could not be measured. (F') Scatterplot of AP threshold versus "peak – baseline" for all active and nonactive directions together ($\rho = -0.47$; p=0.050; regression line: AP threshold = -0.43 × "peak – baseline" – 47.9 mV). (G') Scatterplot of AP threshold versus baseline V_m for all active and nonactive directions together ($\rho = 0.81$; p=0.000039; regression line: AP threshold = 0.82 × baseline – 1.3 mV).

(Q)-(E') Mean ± SEM (red). Horizontal jitter added to individual values for visibility. *,**,***,****,***** correspond to $p < 0.05$, 0.005, 0.0005, 0.00005, 5×10^{-9} differences.

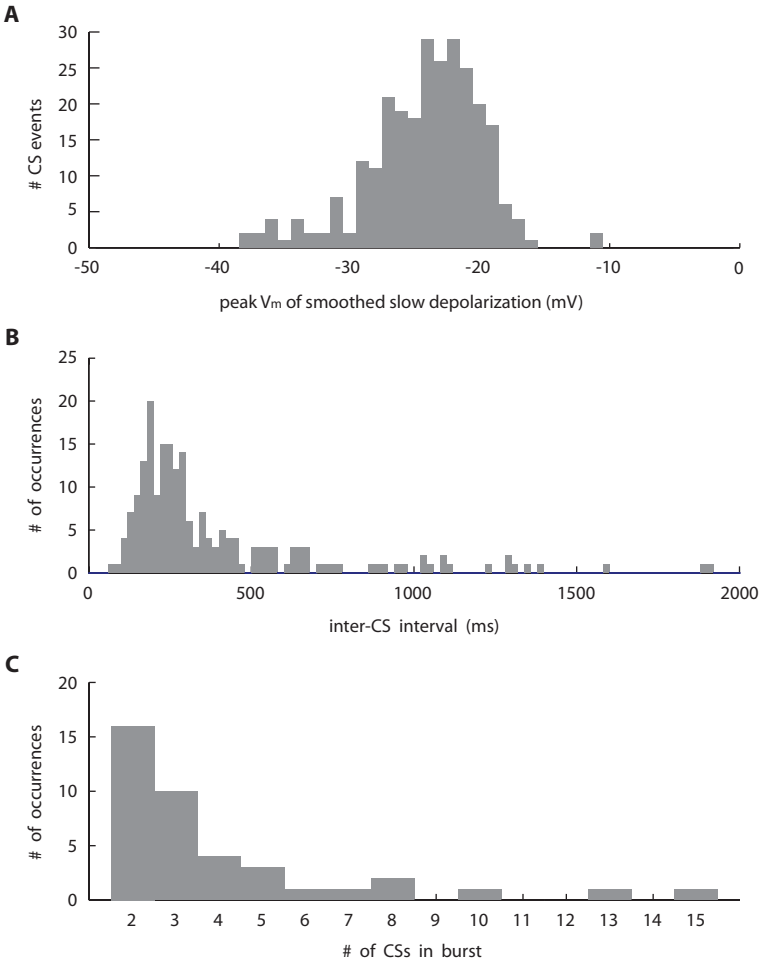

Figure S2 (related to Figure 6)

(A) Distribution of V_m values reached by the slow, large depolarization of CSs across all place and silent cells. Mean ± SD = -24.2±4.4 mV.

(B)-(C) Bursts of successive CSs. Both plots include all CSs from all place and silent cells.

(B) Inter-CS interval histogram peaks between 160-300 ms, corresponding to a frequency of ~4-5 Hz.

(C) Histogram of lengths of bursts of CSs in which successive CSs have inter-CS interval ≤300 ms.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Determination of Input Resistance and Membrane Time Constant

Immediately upon breaking into the neuron and achieving the whole-cell recording configuration, while the animal was anesthetized, we injected a series of 300-ms-long hyperpolarizing current steps of -0.6 to -0.1 nA. During awake exploration, we injected a 500-ms-long hyperpolarizing current step of -0.3 or -0.06 nA every 1 minute (marked by *'s in Figures 2E and 3E). To calculate R_N and τ_m, we eliminated any V_m responses to the current steps that were masked by large spontaneous fluctuations, averaged the remaining responses, then applied a previously described procedure (Crochet and Petersen, 2006) to the average response. Briefly, we automatically fit a single exponential to the V_m response starting ~5 ms (to minimize the effect of the series resistance (R_S) plus pipette capacitance) and ending ~100 ms (to minimize the effect of the activation of I_h current) after the onset of the current step. The difference between the pre-step baseline V_m and the intersection of this fitted curve with the step onset time corresponded to the voltage drop across R_S due to the current step and was subtracted off. This revealed the cell's average asymptotic V_m response to the current step, which, divided by the average magnitude of the current, yielded R_N. The time constant of the fitted curve yielded τ_m.

SUPPLEMENTAL REFERENCES

Crochet, S., and Petersen, C.C. (2006). Correlating whisker behavior with membrane potential in barrel cortex of awake mice. Nat. Neurosci. *9*, 608-610.