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Supplemental Information

Dendrite-Specific Amplification of Weak Synaptic Input during Network Activity *In Vivo*

Leiron Ferrarese, Jean-Sébastien Jouhanneau, Michiel W.H. Remme, Jens Kremkow, Gergely Katona, Balázs Rózsa, Susanne Schreiber, and James F.A. Poulet

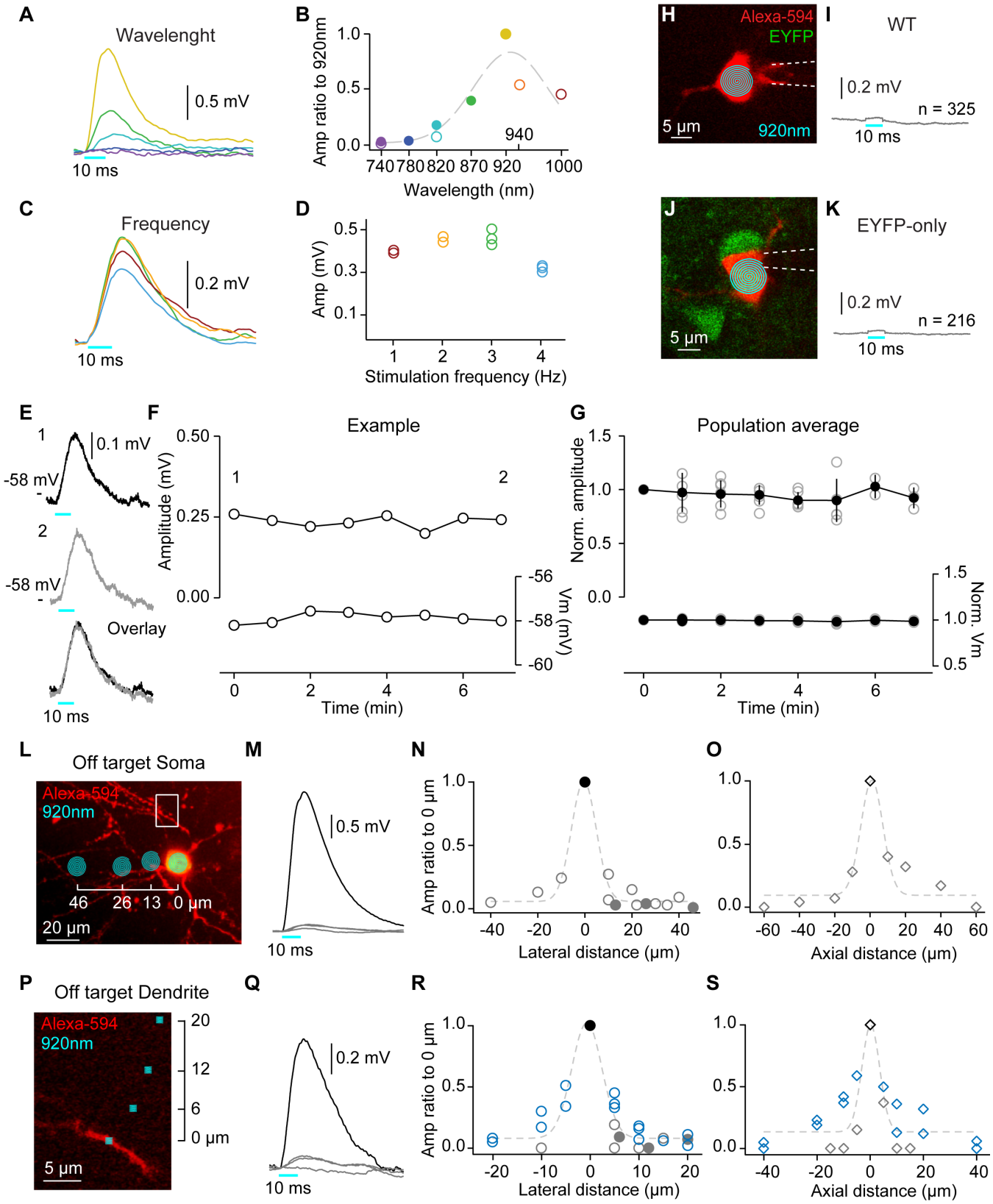
A
Wavelenght
0.5 mV
10 ms
B
Amp ratio to 920nm
1.0
0.5
0.0
940
740 780 820 870 920 1000
Wavelength (nm)
H
Alexa-594
EYFP
5 µm
920nm
I
WT
0.2 mV
n = 325
10 ms
C
Frequency
0.2 mV
10 ms
D
Amp (mV)
0.5
0.3
0.1
1 2 3 4
Stimulation frequency (Hz)
J
5 µm
K
EYFP-only
0.2 mV
n = 216
10 ms
E
1
0.1 mV
-58 mV
2
-58 mV
Overlay
10 ms
F
Example
Amplitude (mV)
0.50
0.25
0.00
1
2
Vm (mV)
-56
-58
-60
0 2 4 6
Time (min)
G
Population average
Norm. amplitude
1.5
1.0
0.5
0.0
Norm. Vm
1.5
1.0
0.5
0 2 4 6
Time (min)
L
Off target Soma
Alexa-594
920nm
46 26 13 0 µm
20 µm
M
0.5 mV
10 ms
N
Amp ratio to 0 µm
1.0
0.5
0.0
-40 -20 0 20 40
Lateral distance (µm)
O
1.0
0.5
0.0
-60 -40 -20 0 20 40 60
Axial distance (µm)
P
Off target Dendrite
Alexa-594
920nm
20
12
6
0 µm
5 µm
Q
0.2 mV
10 ms
R
Amp ratio to 0 µm
1.0
0.5
0.0
-20 -10 0 10 20
Lateral distance (µm)
S
1.0
0.5
0.0
-40 -20 0 20 40
Axial distance (µm)

Figure S1. Subcellular two-photon stimulation of cortical L2/3 pyramidal neurons in vivo. Related to Figure 1.

(A) Mean membrane potential (V_m) response to 10 ms somatic spiral optogenetic stimulation (cyan bar) at different excitation wavelengths from an example neuron, colors correspond to closed circles in (B).
(B) The ratio of the measured optogenetic potential (OP) amplitude relative to the response at 920 nm from the same example neuron as in (A). Open circles show OPs from neurons not shown in (A).
(C) Same as (A) but for different frequencies of stimulation. Colors correspond to frequencies used in (D).
(D) Mean OPs amplitudes at frequencies from 1 to 4 Hz from an example neuron, open circle show mean from different trials in the same neuron.
(E) Top, black trace shows an example average OP to 3 Hz stimulation 0–30 s from the start of stimulation during V_{hyp}; middle, grey trace shows the OP average from the mean response during the last 30 s block of stimulation; bottom, overlay of the two responses.
(F) Top, open circles show the mean amplitude across 8 minutes of recording with each circle representing the average OP during a 30 s period; bottom, circles show the mean V_m for the same time periods. Marks 1 and 2 highlight the example average responses shown in (I).
(G) Top, grey open circles represent mean V_{hyp} amplitude OP from examples cells (n = 8), black filled circles show the population average across time with SD error bars; bottom, same as above but for the corresponding population average V_m. Amplitude and V_m are normalized to the mean OP during the first 30 s period.
(H) Somatic stimulation of a wild type cortical layer 2/3 pyramidal neuron and, (I) the corresponding averaged somatic response, show no OP in V_{hyp}.
(J, K) same as (H, I) but for a cortical neuron expressing EYFP without ChR2.
(L) In vivo image showing on and off target somatic stimulation spots (cyan) at the same axial depth but different lateral distances from the soma.
(M) The corresponding somatic mean example OP from the neuron shown in (I), with responses to on (black) and off (grey) target somatic stimuli.
(N) Plot of the OP amplitude normalized to the amplitude over the soma (0 μm, black filled circle), closed circles show examples shown in (M), open circles show examples from off target stimulation in 3 other neurons.
(O) same as (N) but keeping the same lateral position and varying the axial distance.
(P to S) same as (L to O) but for dendritic stimulation. In (R and S) light blue dots show higher stimulation power responses and grey show lower power responses.

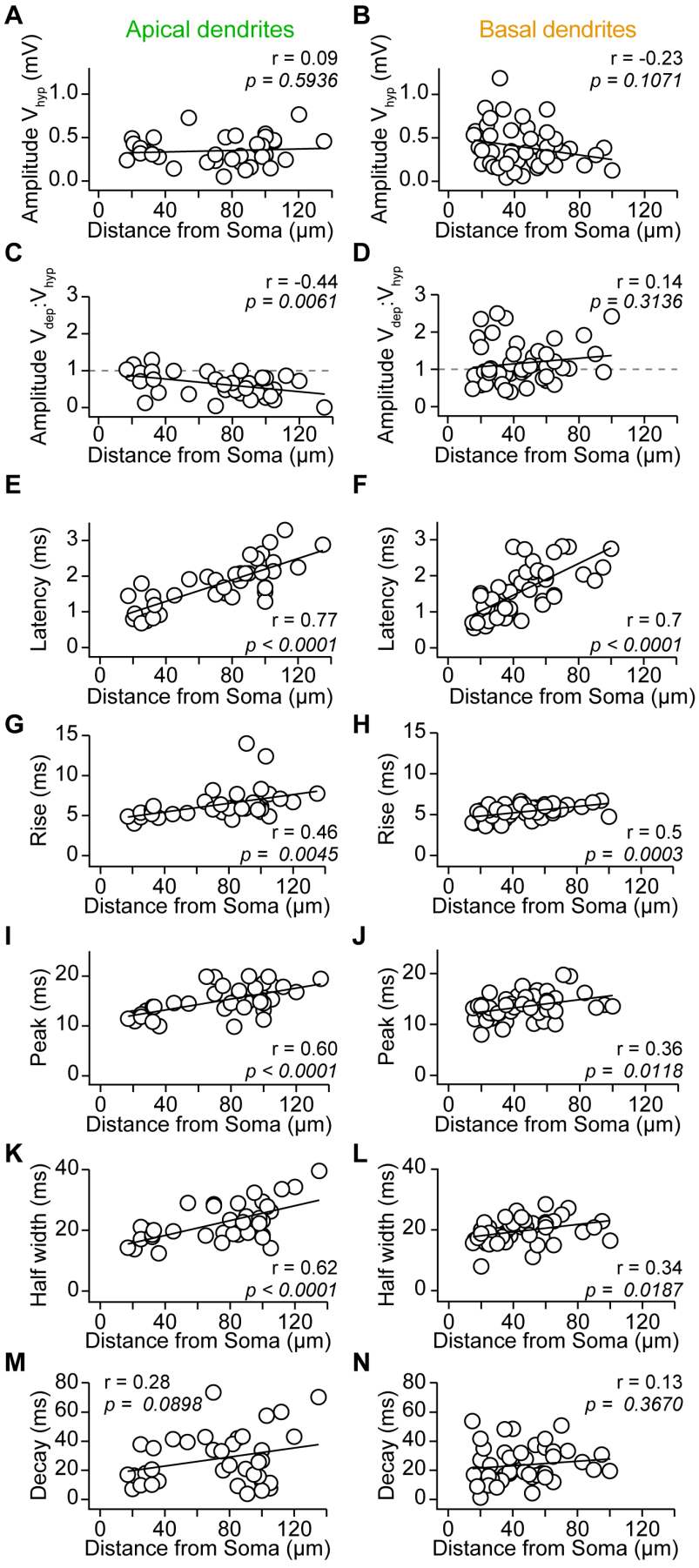
A
Apical dendrites
B
Basal dendrites
Amplitude V_hyp (mV)
r = 0.09
p = 0.5936
r = -0.23
p = 0.1071
C
D
Amplitude V_dep:V_hyp
r = -0.44
p = 0.0061
r = 0.14
p = 0.3136
E
F
Latency (ms)
r = 0.77
p < 0.0001
r = 0.7
p < 0.0001
G
H
Rise (ms)
r = 0.46
p = 0.0045
r = 0.5
p = 0.0003
I
J
Peak (ms)
r = 0.60
p < 0.0001
r = 0.36
p = 0.0118
K
L
Half width (ms)
r = 0.62
p < 0.0001
r = 0.34
p = 0.0187
M
N
Decay (ms)
r = 0.28
p = 0.0898
r = 0.13
p = 0.3670
Distance from Soma (µm)

Figure S2. OP amplitude and kinetics as a function of the distance from soma of dendritic optogenetic stimulation. Related to Figures 2 and 3.

(A) V_{hyp} OP amplitude to apical dendrite stimulation as a function of the distance from the soma for entire dataset, open circles represent the mean response from one dendrite, black line shows linear fit.

(B) Same as (A) but for basal dendrites.

(C) A significant negative correlation between the ratio of V_{dep} : V_{hyp} OP amplitude to apical dendrite stimulation and the stimulation site distance from the soma.

(D) Ratio of V_{dep} : V_{hyp} OP amplitude to basal dendrite stimulation is not correlated to the stimulation site distance from the soma.

(E) Same as (A) but for latency.

(F) Same as (E) but for basal dendrites.

(G) Same as (A) but for rise time.

(H) Same as (G) but for basal dendrites.

(I) Same as (A) but for peak time.

(J) Same as (I) but for basal dendrites.

(K) Same as (A) but for half width.

(L) Same as (K) but for basal dendrites.

(M) Same as (A) but for decay time.

(N) Same as (M) but for basal dendrites.

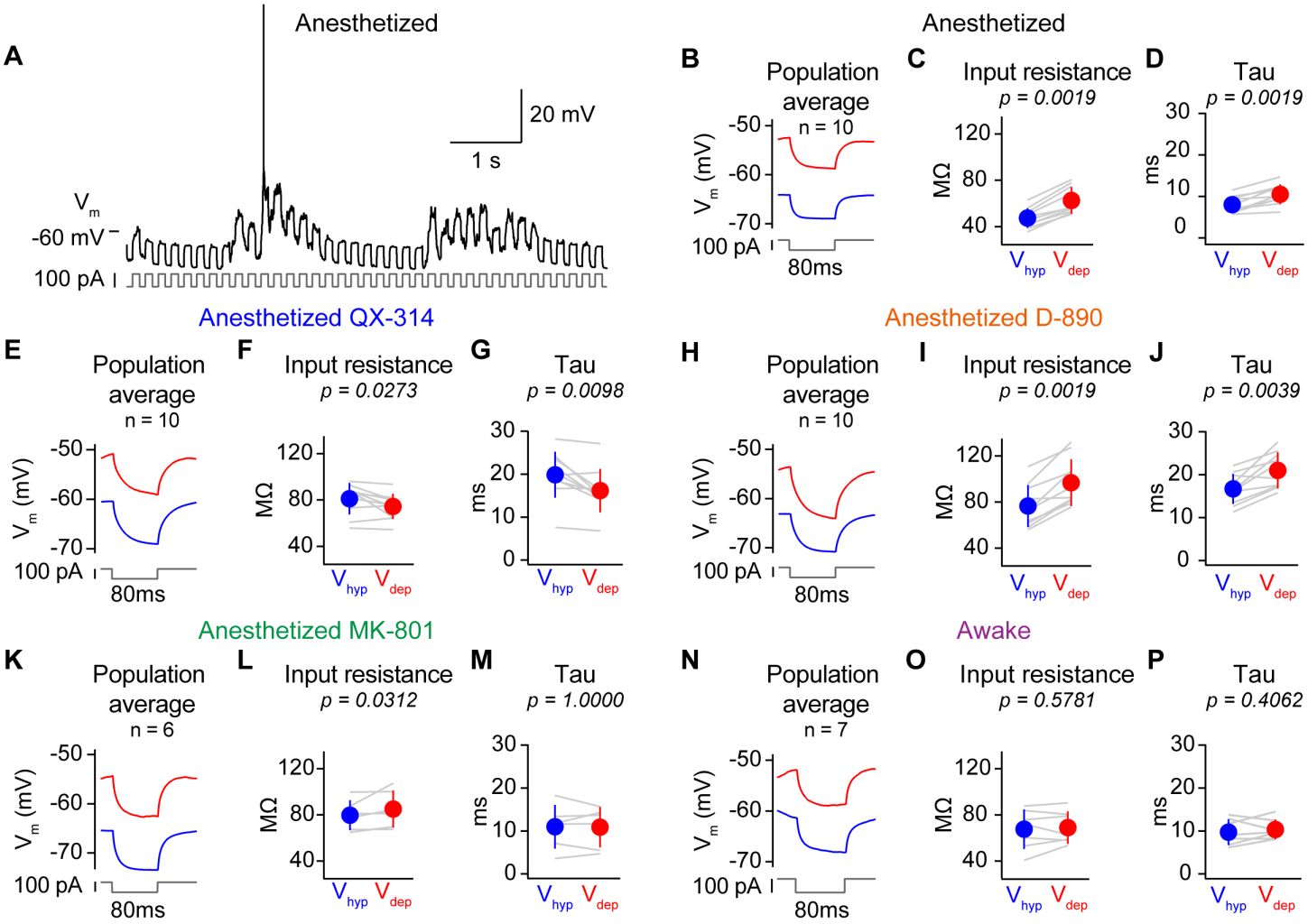

Figure S3. Input resistance measurements during V_{hyp} and V_{dep}. Related to Figures 1 and 6.

(A) Example whole-cell recording showing negative current injection pulses (-100 pA, 80 ms, dark grey trace) used to measure somatic input resistance.

(B) Population average membrane potential (V_m) response to hyperpolarizing current injection pulses (grey) during V_{hyp} (blue) and V_{dep} (red).

(C) Input resistance increases as neurons go from V_{hyp} to V_{dep} in wild type neurons (see methods). Grey lines show data from individual cells, filled circles with error bars show the mean ± SD.

(D) Tau increases as neurons go from V_{hyp} to V_{dep} in wild type neurons.

(E to G) Same as (B to D) but with 1 mM QX-314 in the intracellular solution.

(H to J) Same as (B to D) but with 200 µM D890 in the intracellular solution.

(K to M) Same as (B to D) but with 1 mM MK-801 in the intracellular solution.

(N to P) Same as (B to D) but from awake resting mice during slow network activity split by prestimulus V_m.

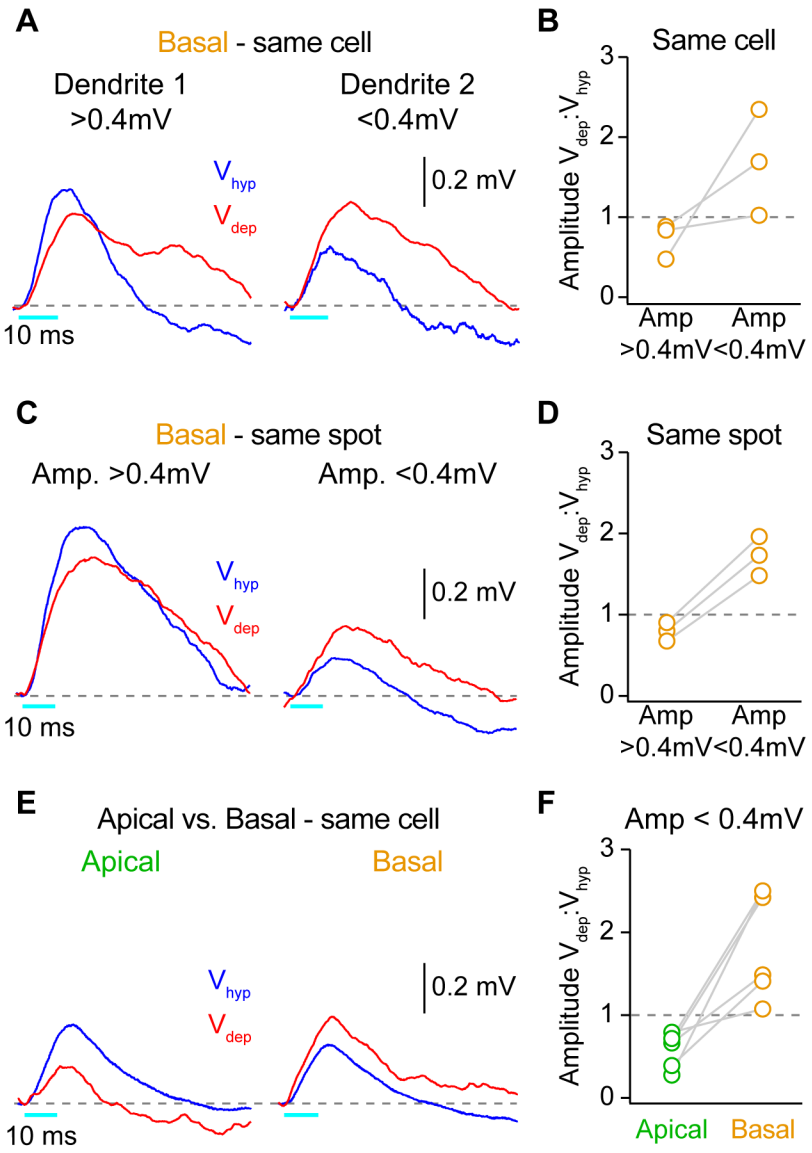

Figure S4. OP modulation to different dendritic stimulation locations and amplitudes on the same cell. Related to Figure 2.

(A) Population average (n = 3 cells) OP_{bas} from two different basal dendrites in the same cells showing (left) the larger amplitude (> 0.4mV in V_{hyp}) response reducing and (right) the smaller amplitude (< 0.4mV in V_{hyp}) response increasing as the neurons go from V_{hyp} (blue) to V_{dep} (red).

(B) Data from (A) showing the ratio between OP_{bas} amplitude in V_{dep} : V_{hyp} to stimulation of two different basal dendrites in the same cells. Grey lines show data from individual cells.

(C) Population average (n = 3 cells) of two different amplitudes of OP_{bas} to stimulation at the same basal dendrite stimulation site in the same cell showing (left) the larger

amplitude response (> 0.4mV in V_{hyp}) showing a reduction and (right) the smaller amplitude response (< 0.4mV in V_{hyp}) showing an increase as neurons go from V_{hyp} (blue) to V_{dep} (red).
(D) Data from (C) showing the ratio between OP_{bas} amplitude in V_{dep} : V_{hyp} to stimulation of the same basal dendrites in the same cells with different amplitudes. Grey lines show data from individual cells.
(E) Population average of (left) OP_{bas} and (right) OP_{ap} on the same cell (n = 5 cells) showing a reduced response to weak apical inputs but an increased response to weak basal stimulation as neurons go from V_{hyp} (blue) to V_{dep} (red).
(F) Data from (E) showing the ratio between OP amplitude in V_{dep} : V_{hyp} to stimulation of basal and apical dendrites in the same cells. Grey lines show data from individual cells.

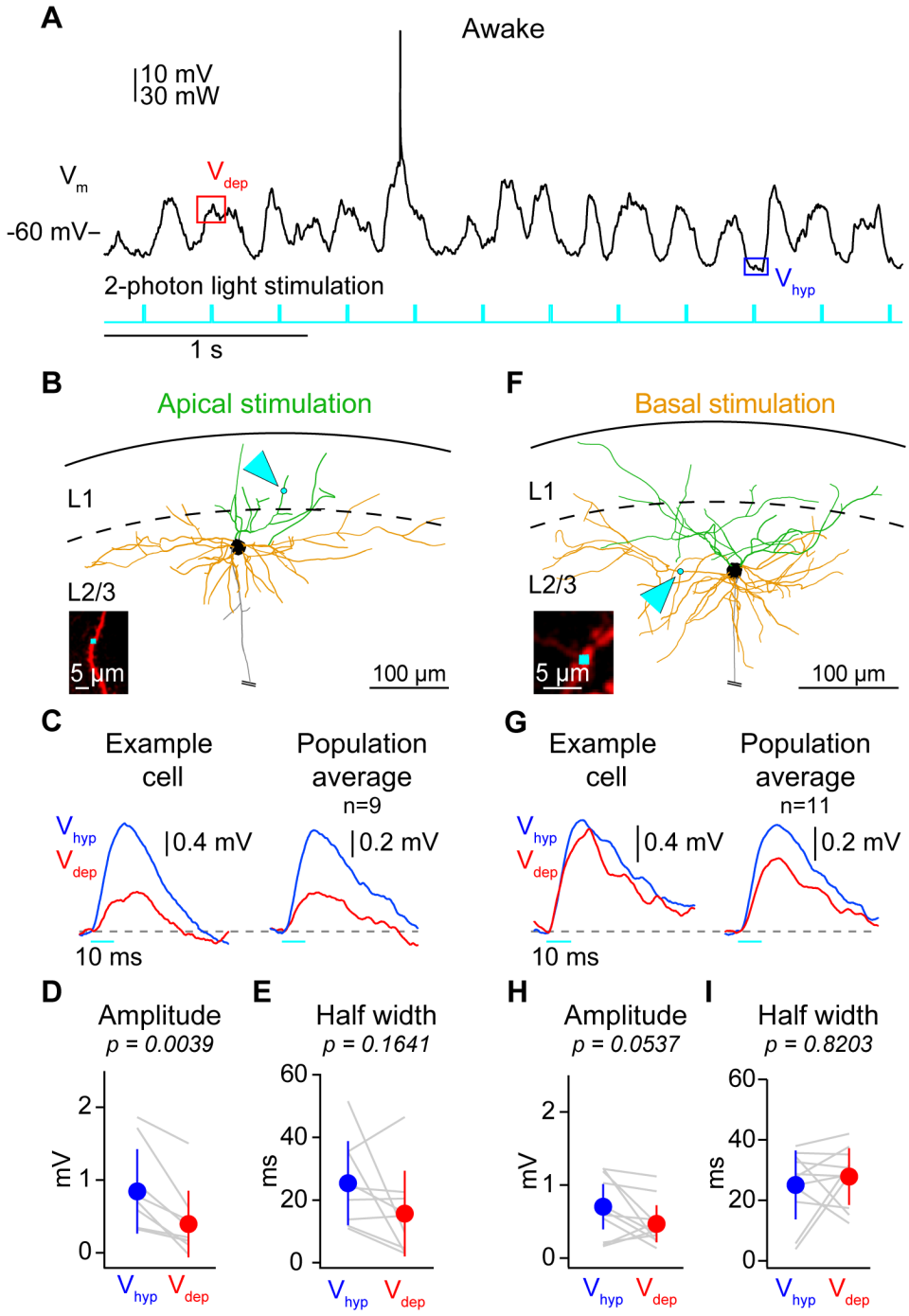

Figure S5. Basal and apical dendrite optogenetic stimulation during slow cortical activity in awake, resting mice. Related to Figures 2 and 3.

(A) Example somatic whole-cell recording of a layer 2/3 cortical neuron in an awake resting mouse during slow membrane potential (V_m) fluctuations with hyperpolarized (V_{hyp}) and depolarized (V_{dep}) epochs. Cyan trace shows optogenetic stimulation times.

(B) Reconstruction of example neuron showing apical dendrites in green and basal dendrites in orange with the apical dendrite two-photon stimulation spot highlighted by

cyan arrowhead. Inset shows in vivo image of Alexa-594 filled dendrite in red and optogenetic stimulation site in cyan.
(C) Averaged OPs to apical stimulation from an example neuron (left) and the population average (right) with V_{hyp} (blue) and V_{dep} (red) responses overlaid.
(D) OP_{bas} V_{dep} amplitude is significantly lower than during V_{hyp}. Grey lines show data from individual cells, filled circles with error bars show the mean ± SD.
(E) OP_{bas} half-width during V_{hyp} and V_{dep}.
(F) Reconstruction of example cell as in (B) but for basal dendrite stimulation.
(G) same as (D) but for basal dendrite stimulation.
(H) Amplitude of basal dendrite evoked OP in V_{hyp} and V_{dep} is not significantly different.
(I) OP_{ap} half-width during V_{hyp} and V_{dep}.

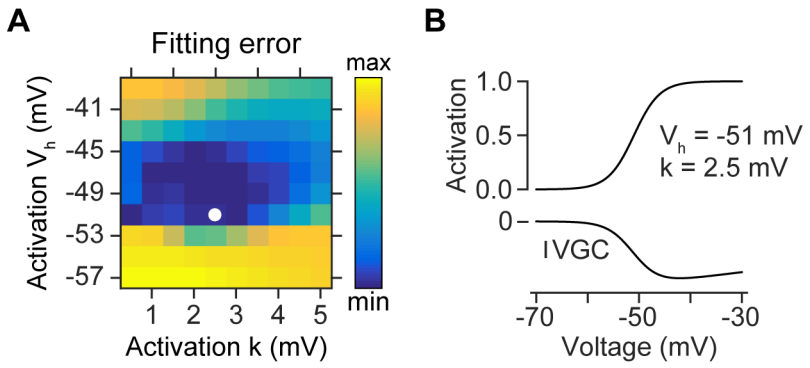

Figure S6. Putative voltage-gated current activation parameters. Related to Figure 7.

(A) Fitting error of model results and experimental data color coded as a function of the two parameters that characterize the voltage-dependence of the putative voltage-gated current activation: the half-activation voltage V_h and the reciprocal activation slope k. For each parameter combination, five further parameters (not shown, see Methods) are set such that the smallest fitting error is produced. White circle marks the activation function parameters used in Figure 7B, C: V_h = −51 mV and k = 2.5 mV; with the five further parameters being g_{leak} = 0.4 mS/cm^2, $\bar{g}_{VGC}$ = 0.15 mS/cm^2, x_b = 70 µm, x_a = 300 µm, and τ_n = 1 ms.

(B) Voltage-gated current activation curve (top) and current-voltage relationship (bottom; arbitrary scale).