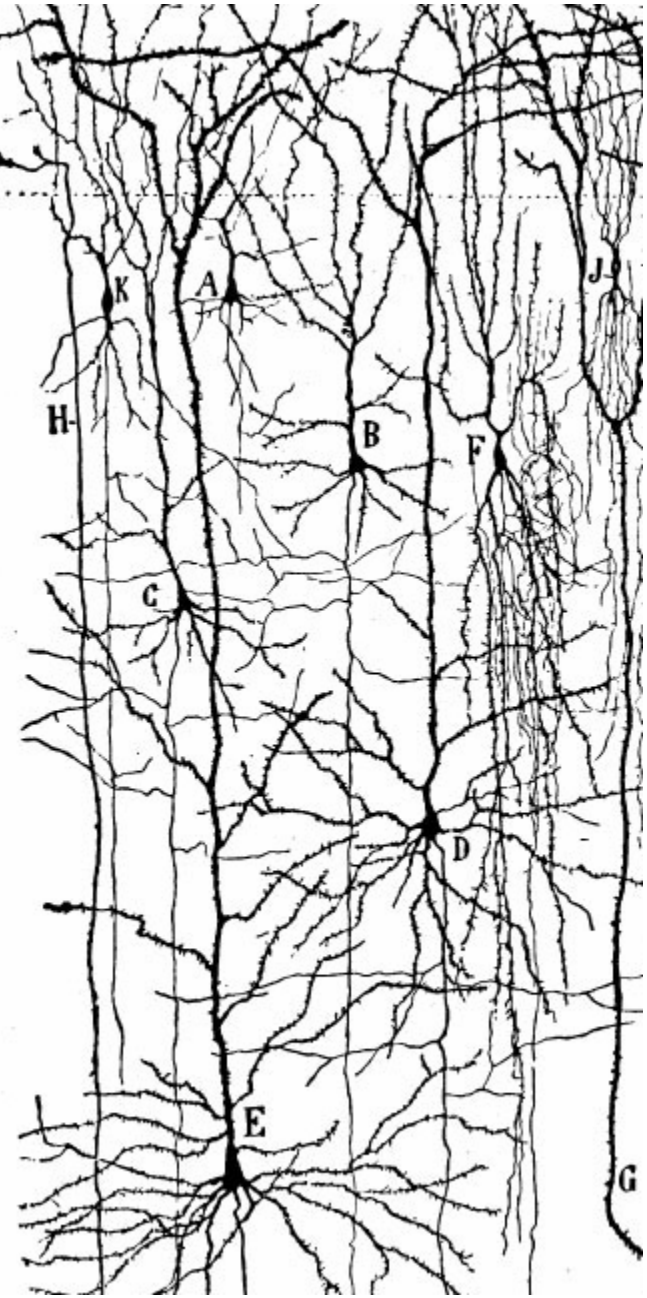


Sodium Channels and Membrane Excitability in Neocortical Pyramidal Neurons

Mike Gutnick

The Hebrew University of Jerusalem

Rehovot, Israel

KITP, Santa Barbara, October 12, 2010

ELECTROPHYSIOLOGY

Ilya Fleidervish

Andreas Neef

Nadav Astman

Lior Libman

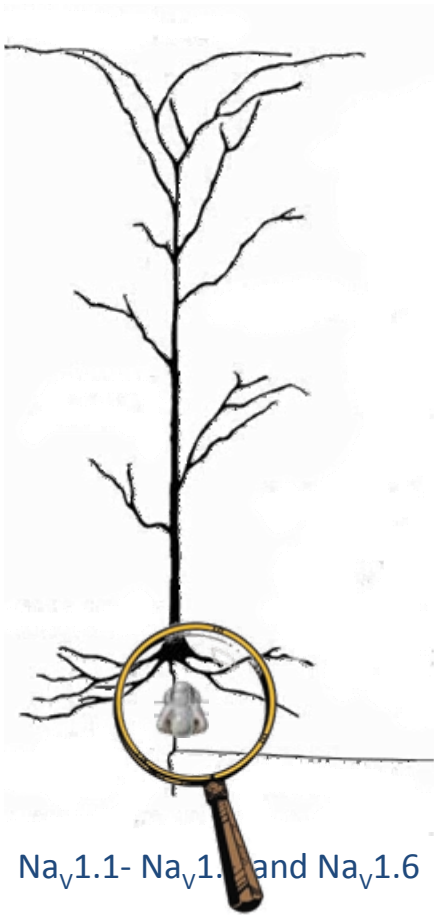
Efrat Katz

Na^+ IMAGING

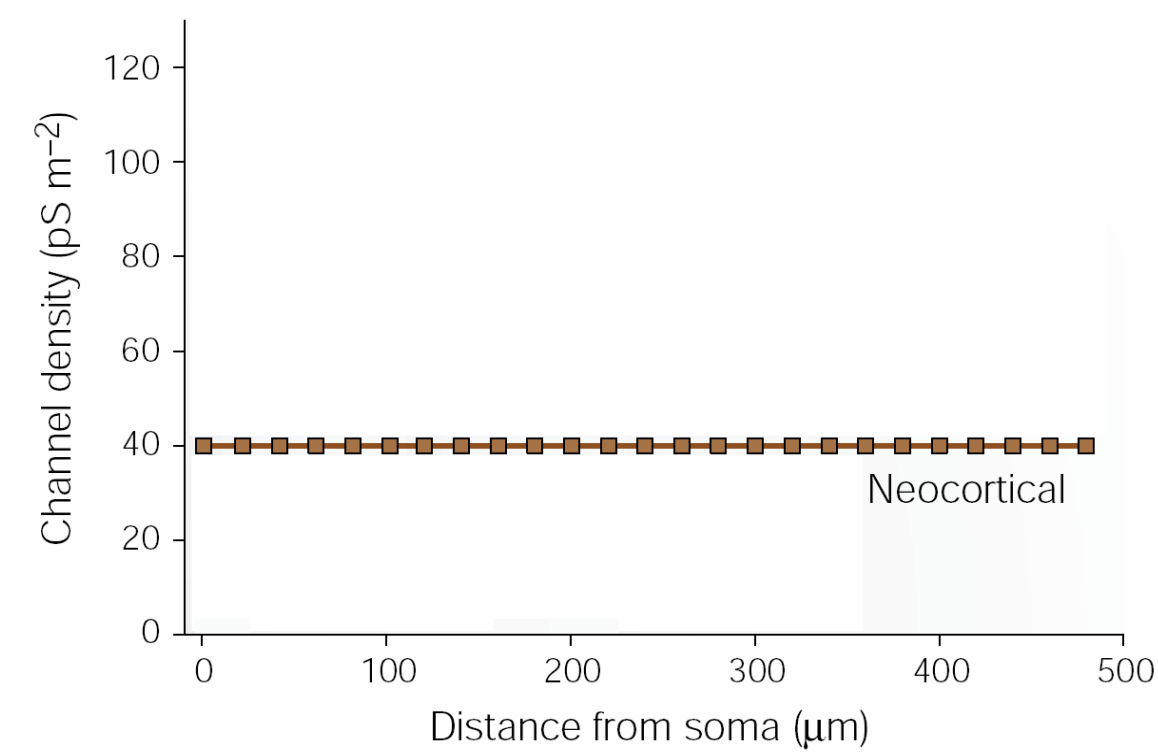
Ilya Fleidervish

Bill and Nehama Ross

COMPUTATION



$Na_V1.1$- $Na_V1.$ and $Na_V1.6$



Hodgkin-Huxley theory

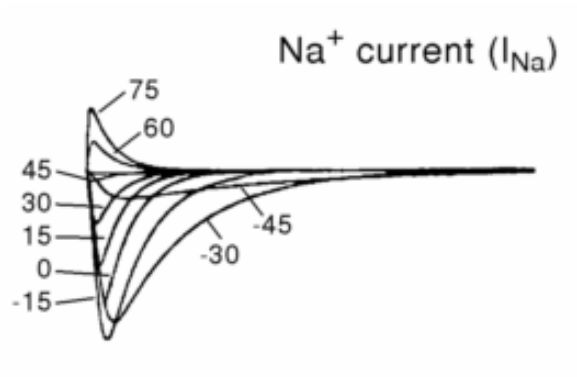
J. Physiol. (1952) 117, 500–544

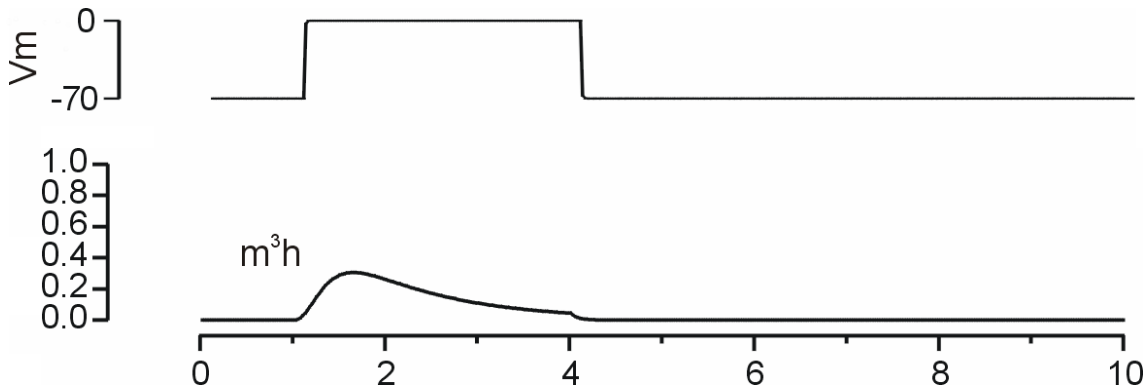
A QUANTITATIVE DESCRIPTION OF MEMBRANE CURRENT AND ITS APPLICATION TO CONDUCTION AND EXCITATION IN NERVE

By A. L. HODGKIN and A. F. HUXLEY

From the Physiological Laboratory, University of Cambridge

(*Received* 10 *March* 1952)





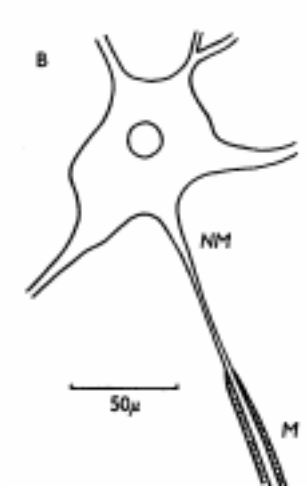
$g_{Na}(V,t) = m^3 h \bar{g}_{Na}$ where *m* is the Na channel activation variable, *h* is the Na channel inactivation variable and they follow exponential time course:

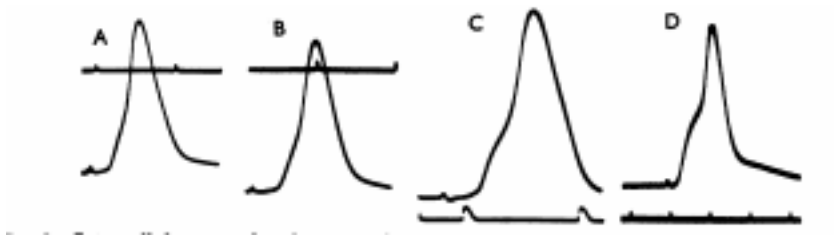
$$m(t) = m_\infty(1 - e^{-t/\tau_m}) \qquad h(t) = h_\infty(1 - e^{-t/\tau_h})$$

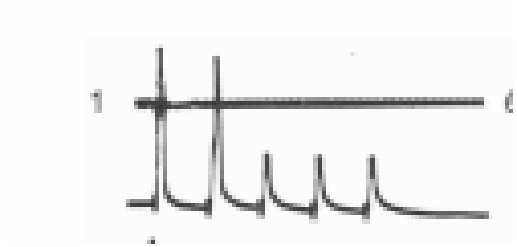
J. Physiol. (1953) 122, 429–461

INTRACELLULAR RECORDING FROM ANTIDROMICALLY ACTIVATED MOTONEURONES

By L. G. BROCK, J. S. COOMBS and J. C. ECCLES



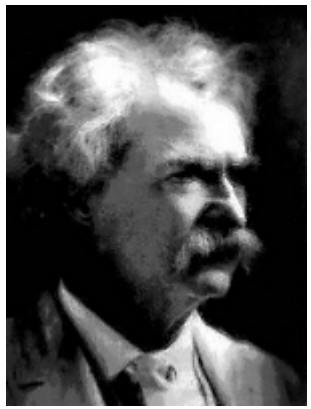




SUMMARY

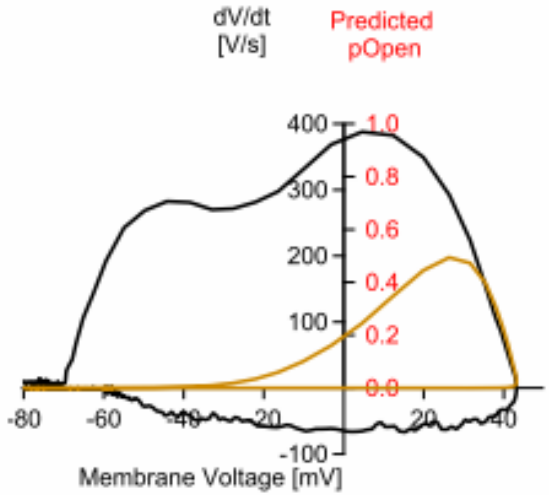
1. Intracellular recording from motoneurones in the lumbar region of the cat's spinal cord has provided evidence on most of the controversial issues concerning antidromic responses of motoneurones.

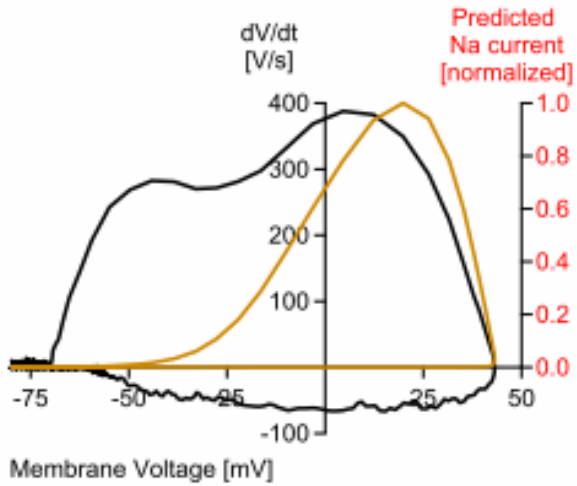
2. The geometry of the antidromic pathway indicates that low safety-factors for transmission would occur at the medullated–non-medullated junction and at the axon–soma junction, and on this basis detailed explanations are given for most of the experimental observations. With blockage at the latter site there is a simple *NM* (non-medullated) spike of about 30–40 mV, in contrast to the *SD* (soma–dendritic) spike of up to 100 mV on full antidromic invasion. With blockage at the former site there is a very small *M* (medullated) spike of about 1 mV.

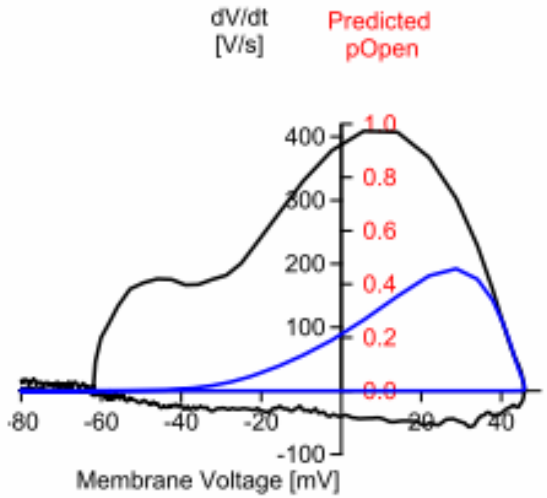


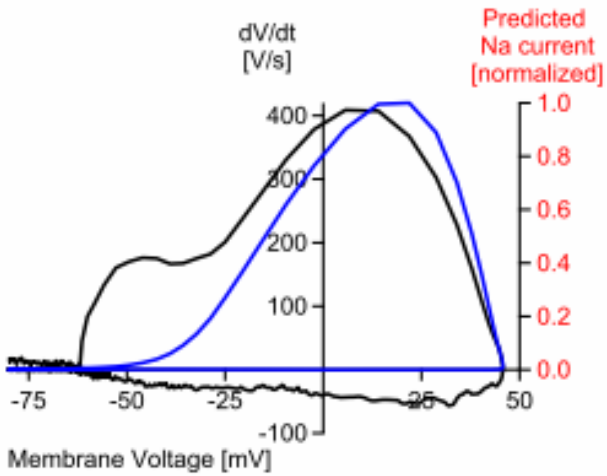
“The researches of many commentators have already thrown much darkness on this subject, and it is probable that if they continue we shall soon know nothing at all about it.”

Mark Twain


dV/dt
[V/s]
Predicted
pOpen
400
300
200
100
1.0
0.8
0.6
0.4
0.2
0.0
-80
-60
-40
-20
20
40
-100
Membrane Voltage [mV]


dV/dt
[V/s]
Predicted
Na current
[normalized]
400
300
200
100
1.0
0.8
0.6
0.4
0.2
0.0
-75
-50
-25
25
50
-100
Membrane Voltage [mV]


dV/dt
[V/s]
Predicted
pOpen
400
300
200
100
1.0
0.8
0.6
0.4
0.2
0.0
-80
-60
-40
-20
20
40
-100
Membrane Voltage [mV]


dV/dt
[V/s]
Predicted
Na current
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400
300
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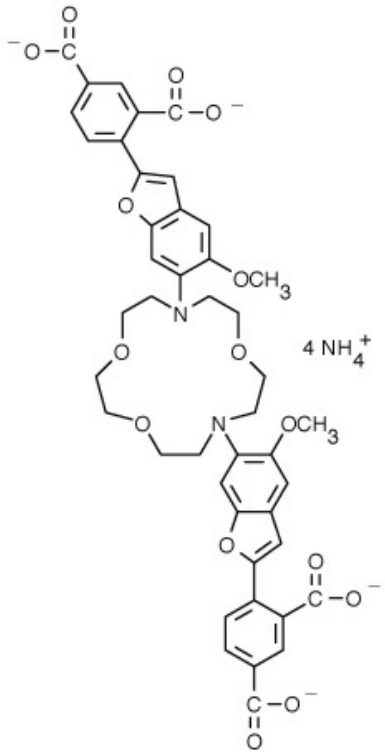
100
1.0
0.8
0.6
0.4
0.2
0.0
-75
-50
-25
25
50
-100
Membrane Voltage [mV]

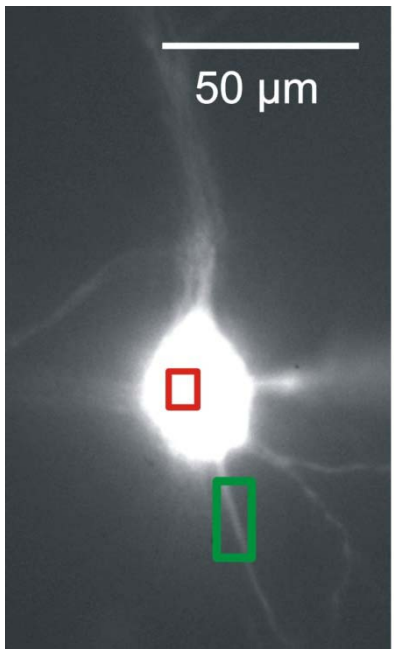
Properties of the Na^+ indicator SBFI



- selective for changes in $[Na^+]_i$
- low affinity (K_D ~ 26 mM), linear
- non buffering
- accurately follows time course of $[Na^+]_i$

Minta and Tsien, 1989

Methods



Patch clamp recordings

- ***whole cell recordings***
- ***cell-attached Na^+ channel recording***

High-speed optical recording

- ***RedShirt Imaging (1 kframes/s)***

3D reconstruction of live cell

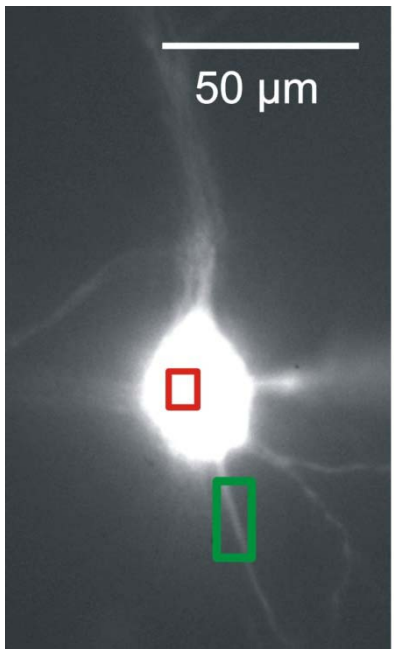
- ***Two-photon microscope (Zeiss LSM510 -- 710)***

Simulations

- ***Neuron***

Interpretation of Na^+ signals

Changes in $[Na^+]_i$ in a given neuronal compartment reflect:



Transmembrane Na^+ influx

- ***local Na^+ channel density***
- ***Na^+ channel properties (molecular type, kinetics)***
- ***shape of the action potential***

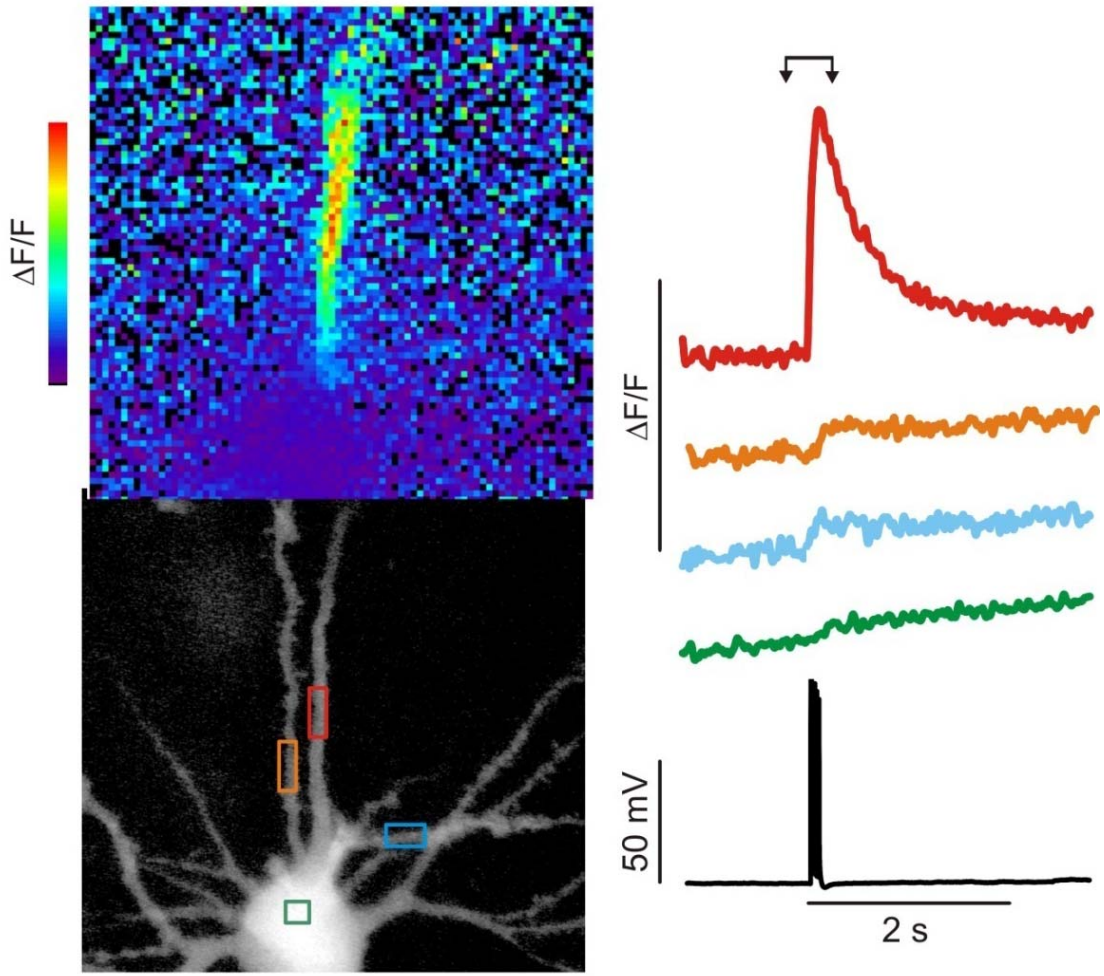
Build-up of Na^+ concentration

- ***neuronal morphology (e.g. surface-to-volume ratio)***

Na^+ diffusion

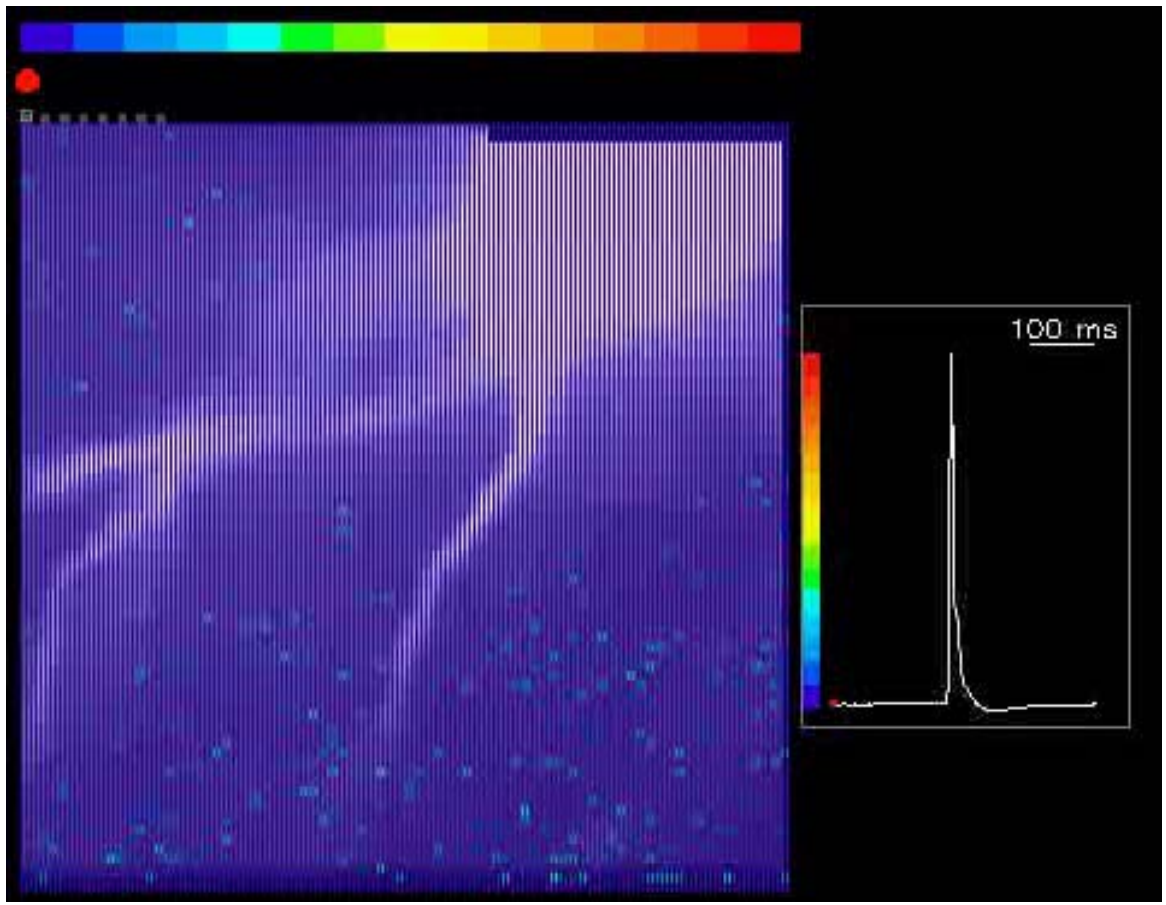
Active extrusion by Na^+/K^+ pump

The largest AP-evoked $[Na^+]_i$ increases are in the AIS

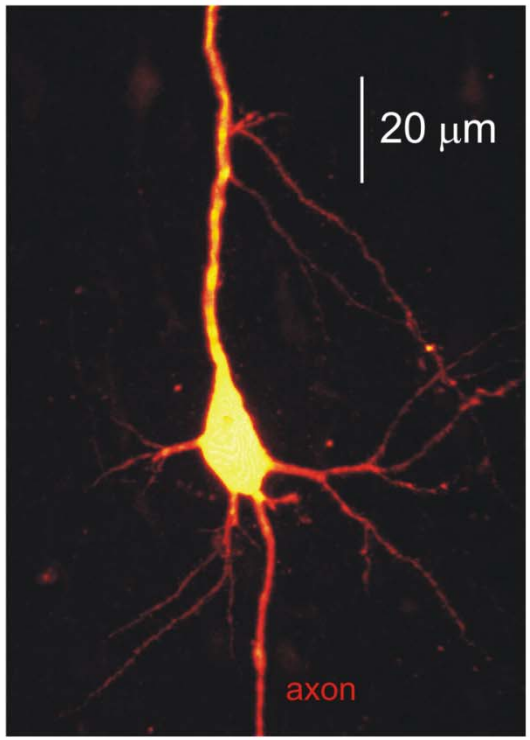


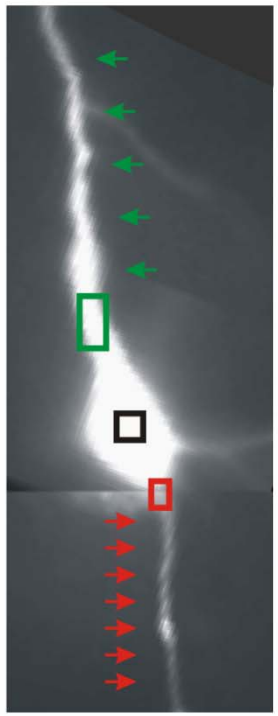
The largest AP-evoked $[Na^+]_i$ increases are in the AIS

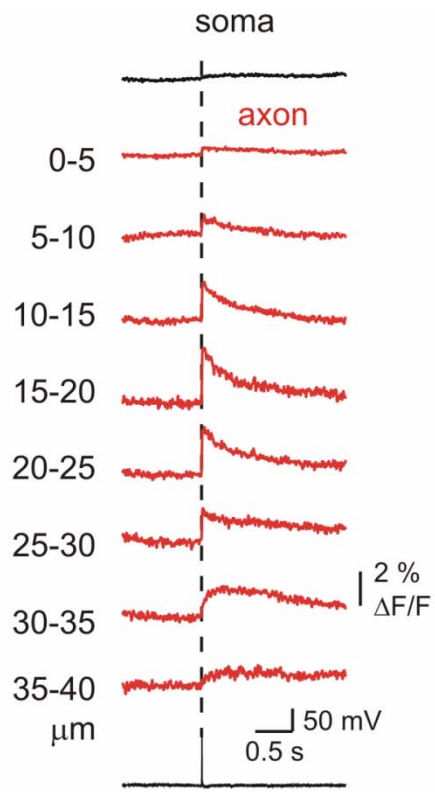
Single
Action Potential

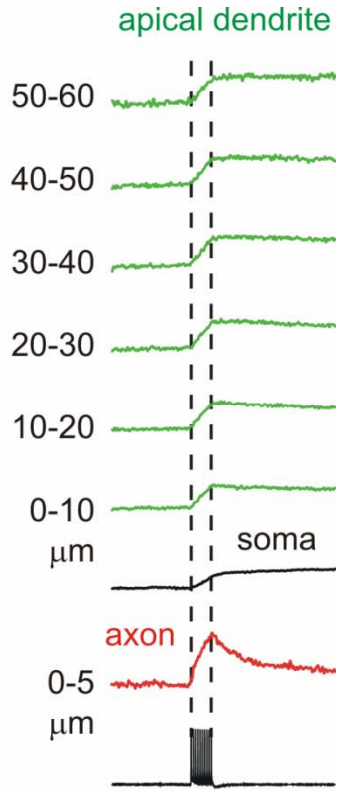


The shapes of the Na^+ signals differ in different neuronal compartments

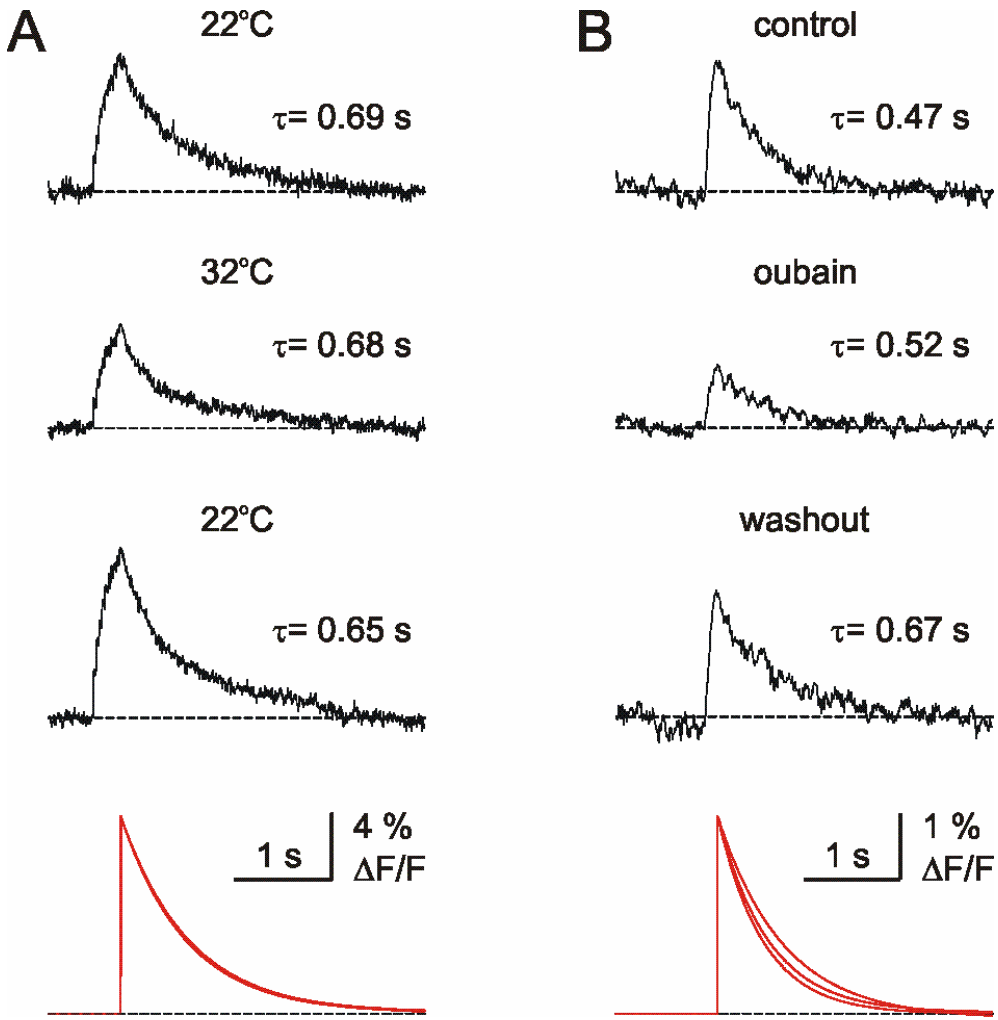




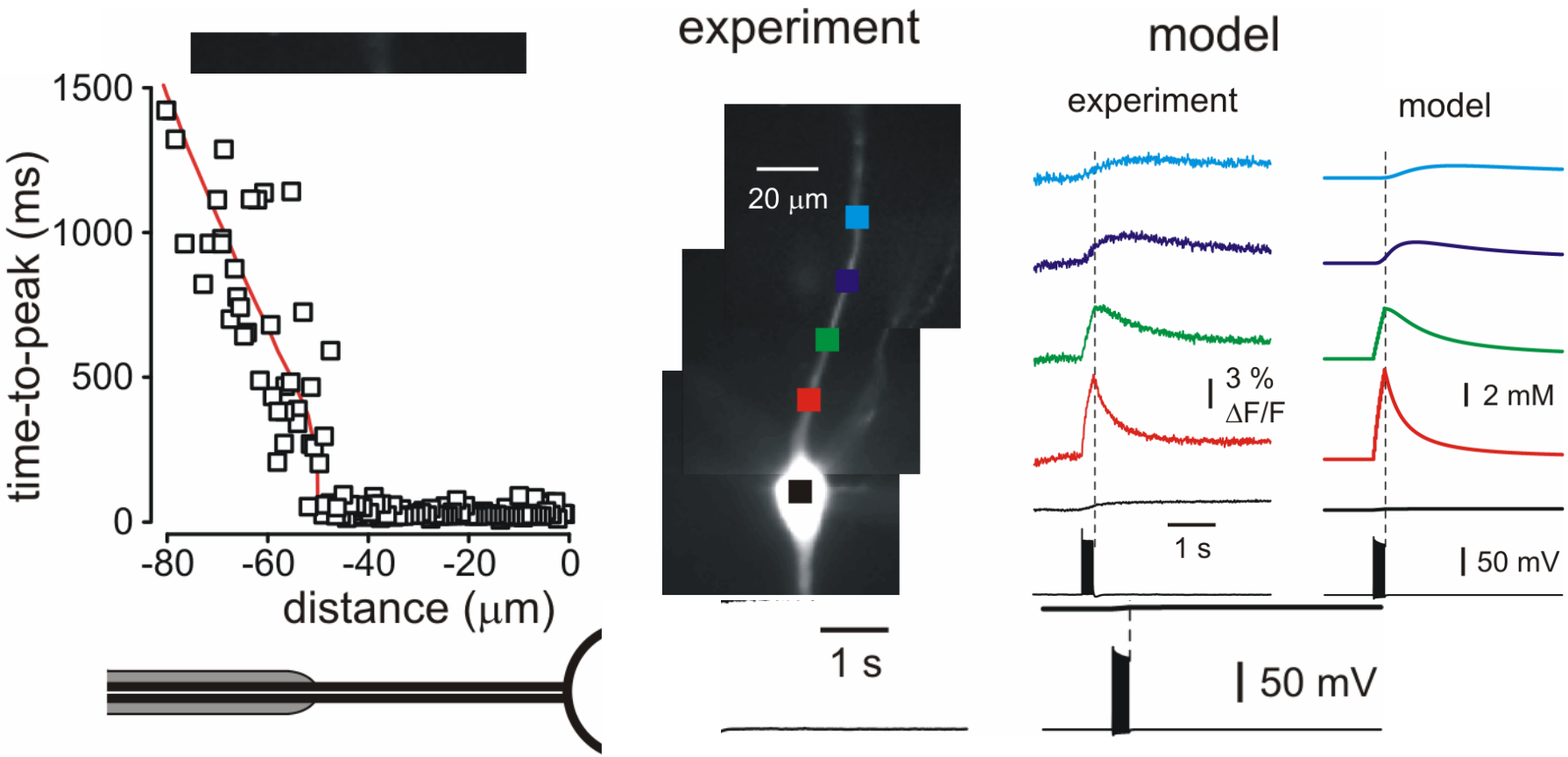




Inhibition of the Na^+/K^+ pump has little effect on the time course of Na^+ transients



The time course of the axonal Na^+ transient reflects localized Na^+ influx into AIS followed by diffusion to the soma and to the first myelinated internode



nature neuroscience

Action potential generation requires a high sodium channel density in the axon initial segment

Maarten H P Kole[1], Susanne U Ilschner[1], Björn M Kampa[1,4], Stephen R Williams[1,2], Peter C Ruben[1,3] & Greg J Stuart[1]

The axon initial segment (AIS) is a specialized region in neurons where action potentials are initiated. It is commonly assumed that this process requires a high density of voltage-gated sodium (Na^+) channels. Paradoxically, the results of patch-clamp studies suggest that the Na^+ channel density at the AIS is similar to that at the soma and proximal dendrites. Here we provide data obtained by antibody staining, whole-cell voltage-clamp and Na^+ imaging, together with modeling, which indicate that the Na^+ channel density at the AIS of cortical pyramidal neurons is ~50 times that in the proximal dendrites. Anchoring of Na^+ channels to the cytoskeleton can explain this discrepancy, as disruption of the actin cytoskeleton increased the Na^+ current measured in patches from the AIS. Computational models required a high Na^+ channel density (~2,500 pS μm^{-2}) at the AIS to account for observations on action potential generation and backpropagation. In conclusion, action potential generation requires a high Na^+ channel density at the AIS, which is maintained by tight anchoring to the actin cytoskeleton.

Action potentials are the primary means of fast communication between neurons. Work dating back to the mid-1950s, using sharp

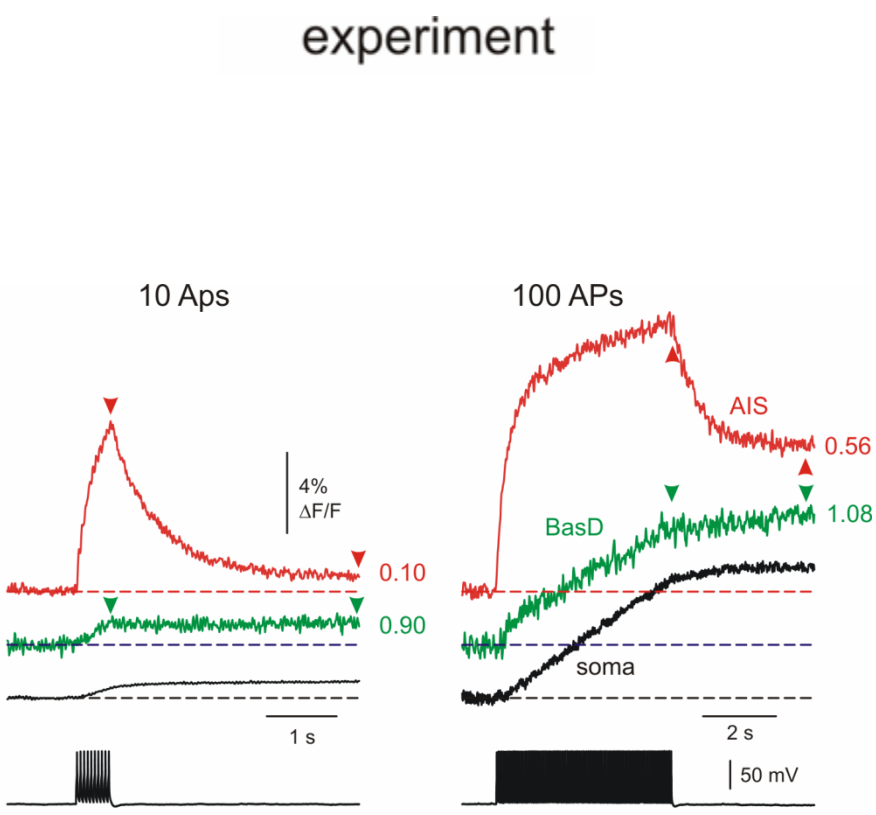
Here we describe studies of the distribution and properties of Na^+ channels in the AIS of cortical layer 5 pyramidal neurons.

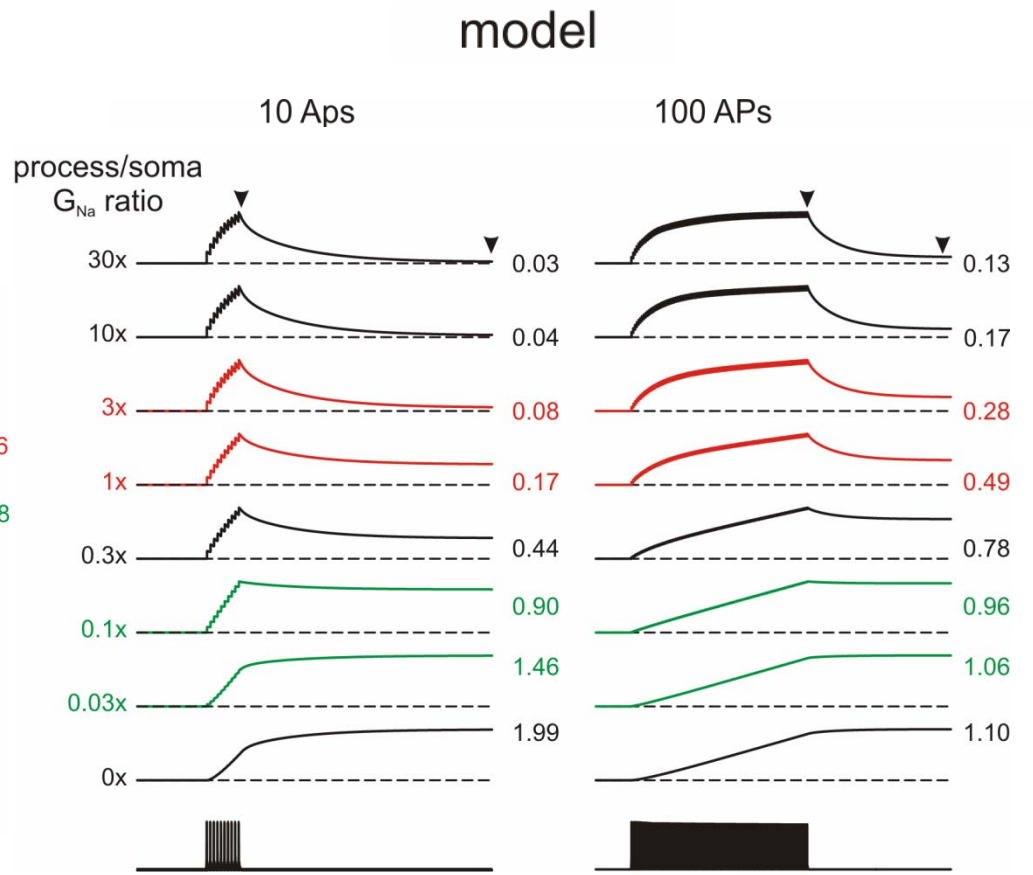
MUST CHANNEL DENSITY REALLY BE SO MUCH GREATER IN AIS THAN IN SOMA?

We used three approaches to estimate Na^+ charge transfer (channel density) in soma, apical and basal dendrites, and in the axon

1

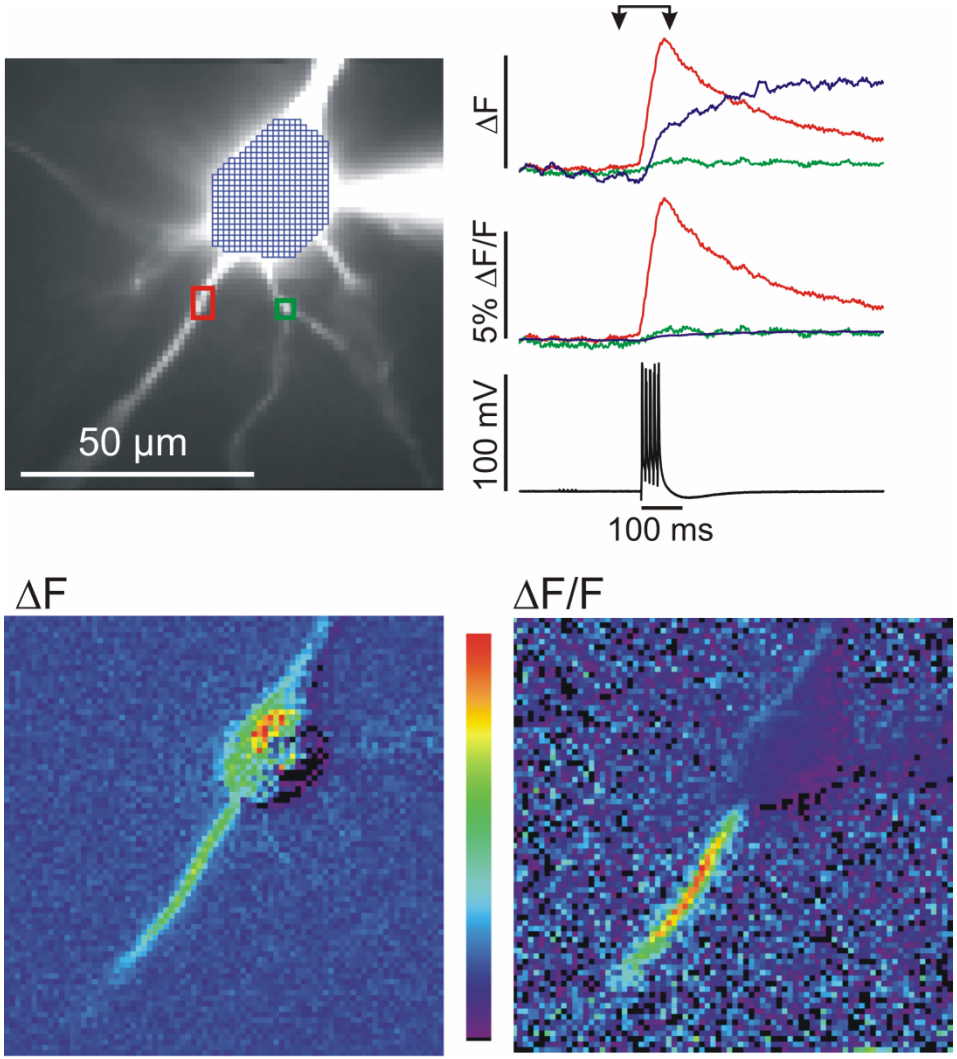
Shapes of Na^+ transients indicate a difference in Na^+ channel density





2

ΔF value, which is proportional to Na^+ charge transfer, is about 2 times higher in the AIS than in the soma. ΔF in the basal dendrites is very much lower than in the soma.



$\Delta F/F \sim \Delta Na^+_i$

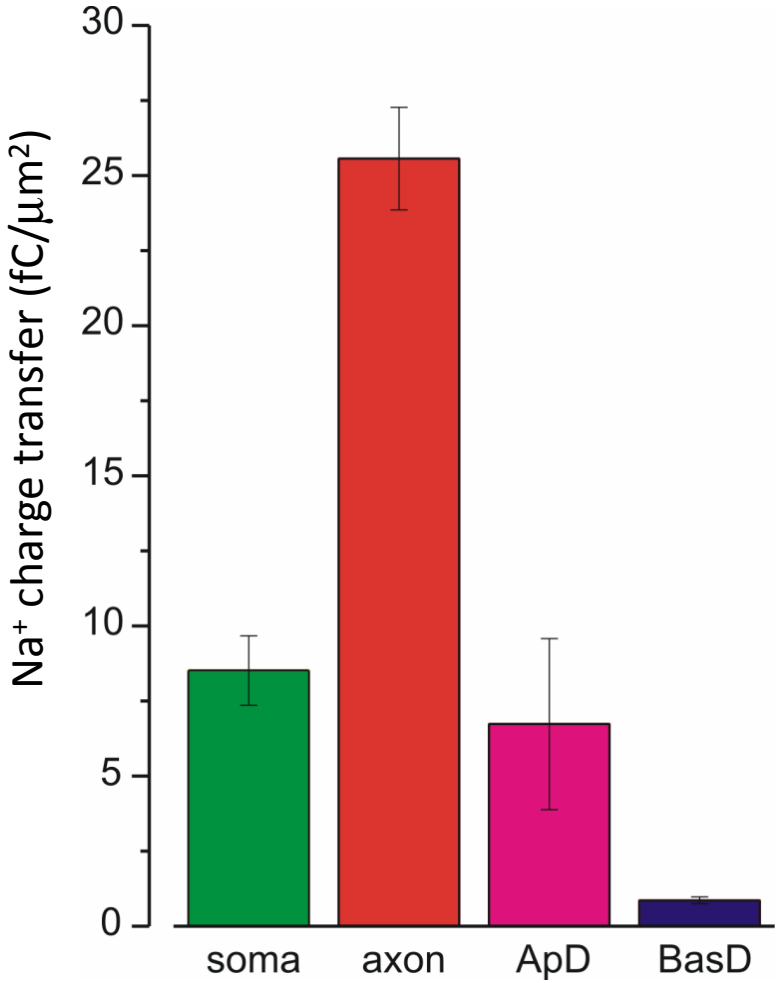
since F ~ volume,

$\Delta F \sim Na^+$ flux

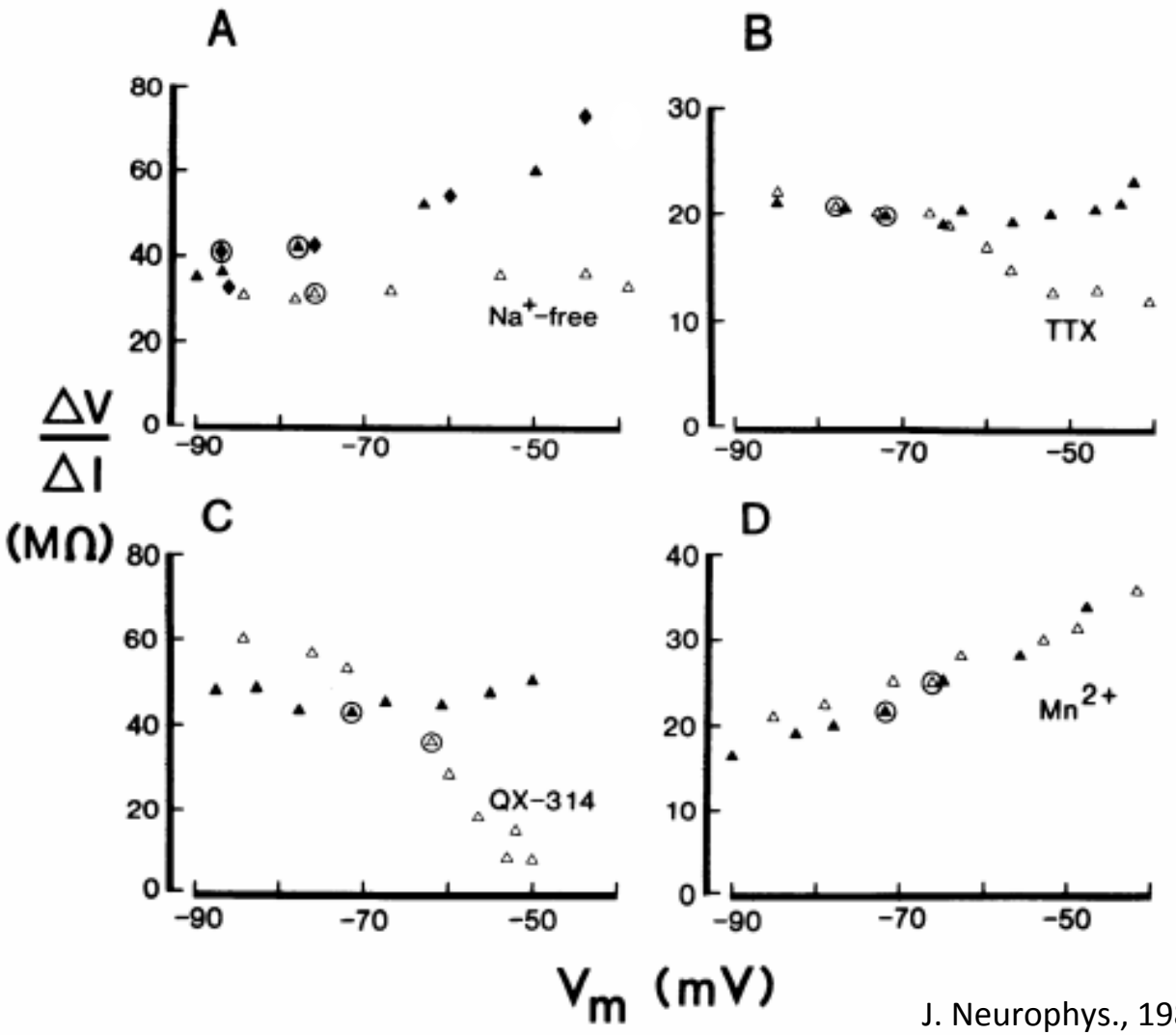
3 Direct evaluation of AP-mediated Na^+ charge transfer from the amplitude of Na^+ transients and morphological data

$$Q_{Na+} = k \bullet \Delta F/F \bullet \text{Faraday constant} \bullet \text{volume/surface area}$$

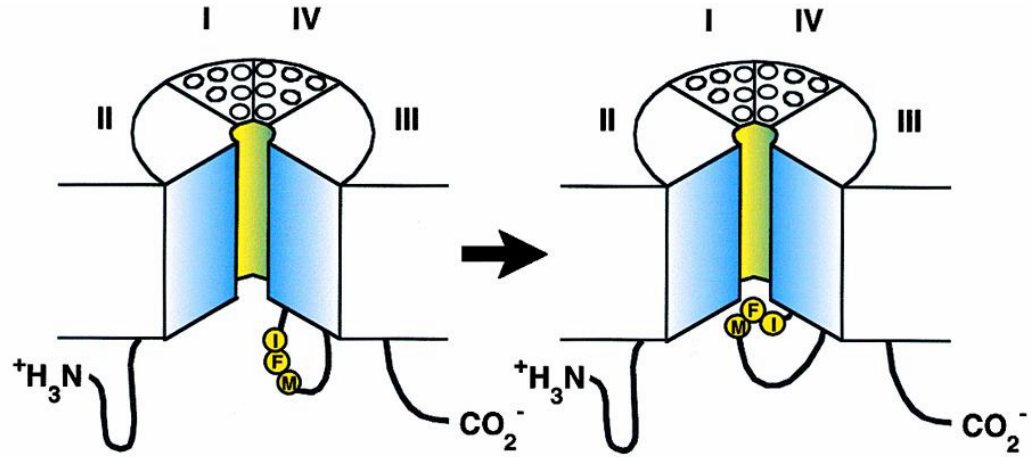
Na^+ channel mediated charge transfer in the proximal axon is ~3 times larger than in the soma, and ~30 times larger than in the basal dendrites



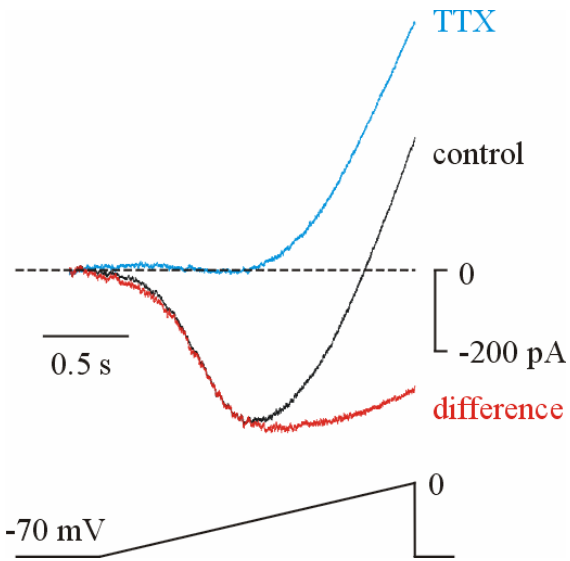
A voltage-gated, non-inactivating Na^+ current contributes to the apparent input resistance



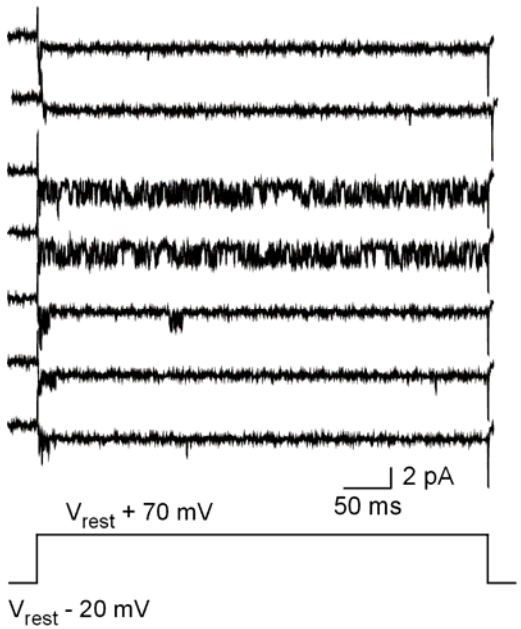
J. Neurophys., 1982



whole cell



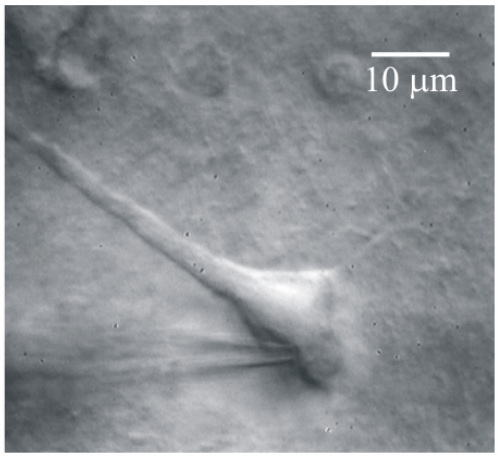
cell-attached



FUNCTIONAL CONSEQUENCES OF I_{NaP}

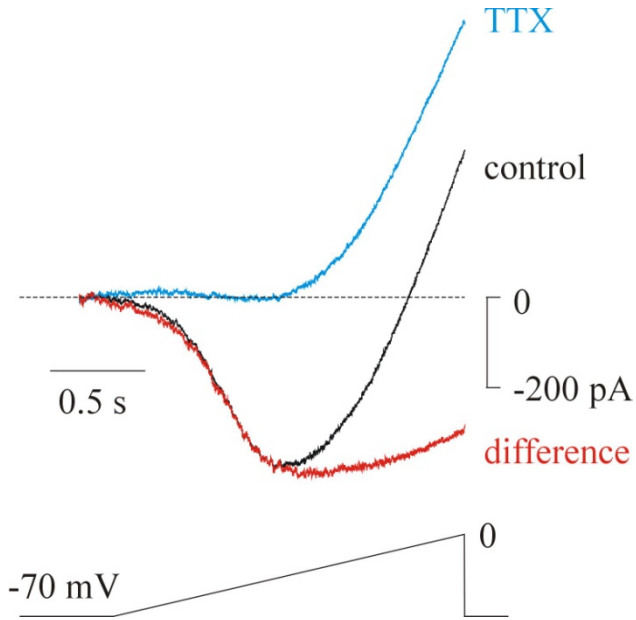
- “Boosting” of synaptic excitation and inhibition
- Voltage-dependent rhythmic activity
- Determination of spike threshold

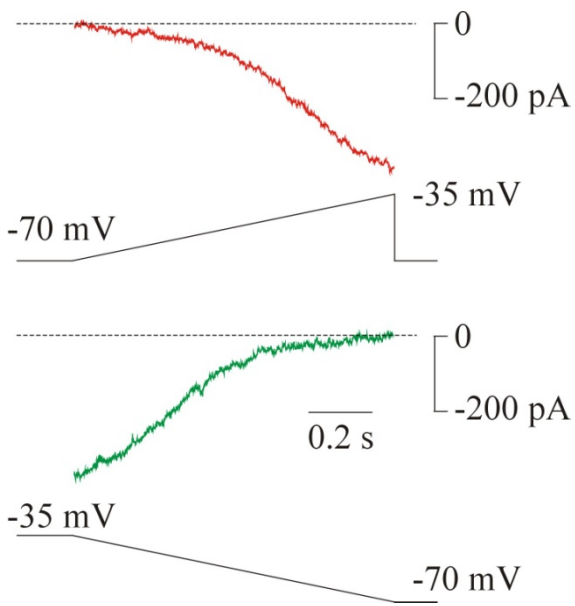
I_{NaP} in whole cell recordings

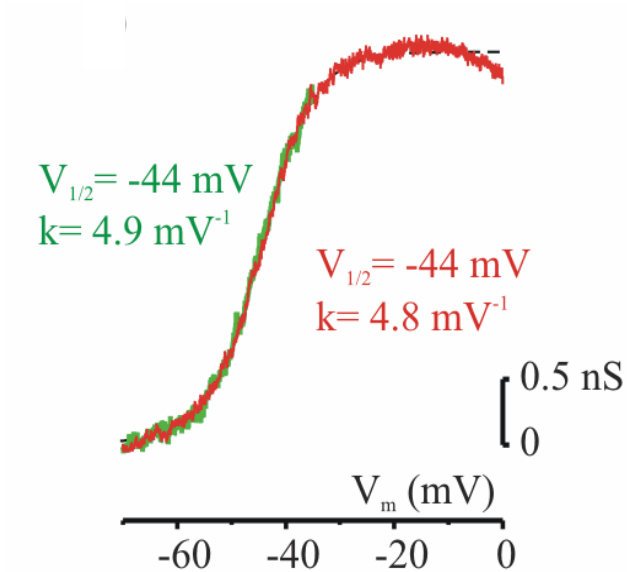


Cs^+ in electrode

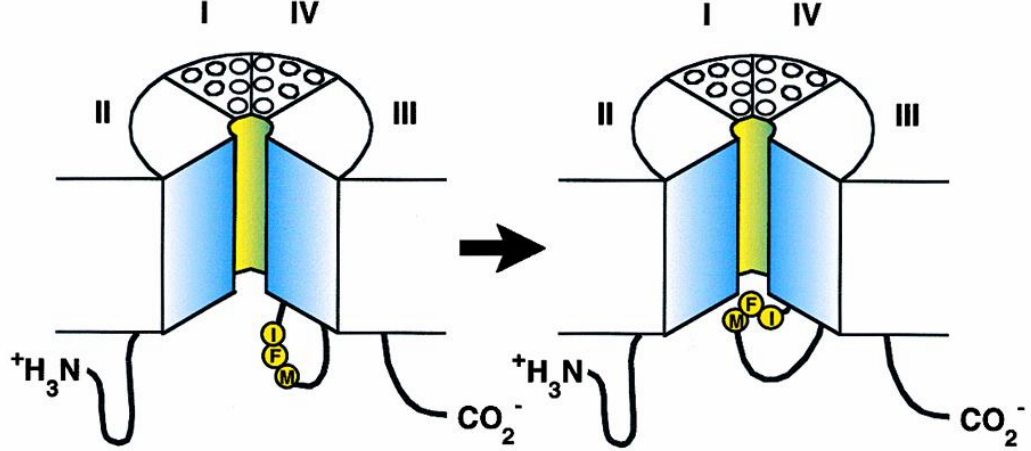
Cd^{2+} and synaptic blockers in the bath







Astman et al, J. Neurosci. 2006

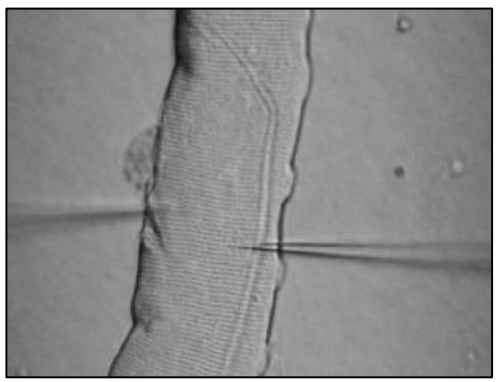


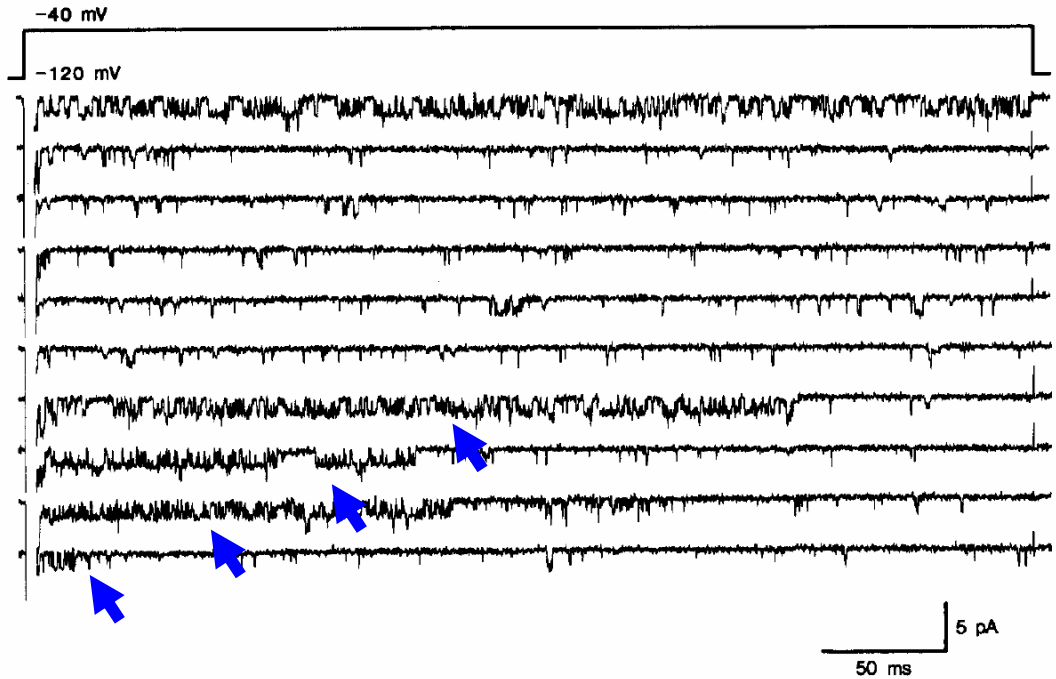
Na^+ CHANNELS THAT FAIL TO INACTIVATE

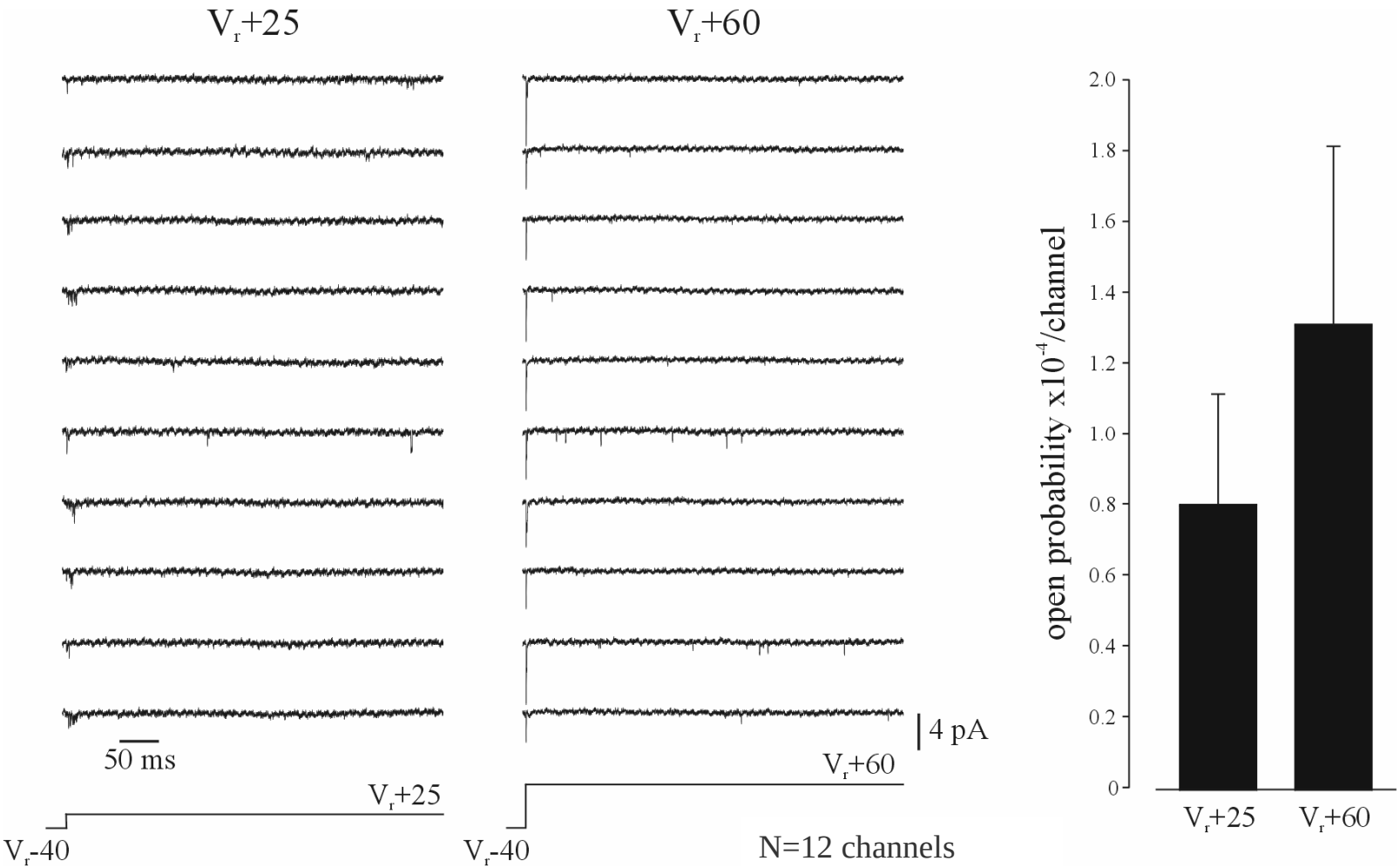
Two Modes of Gating During Late Na^+ Channel Currents in Frog Sartorius Muscle

JOSEPH B. PATLAK and MAURICIO ORTIZ

J. GEN. PHYSIOL. Volume 87 February 1986 305–326

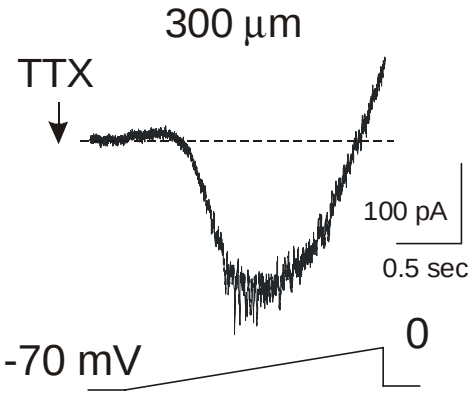




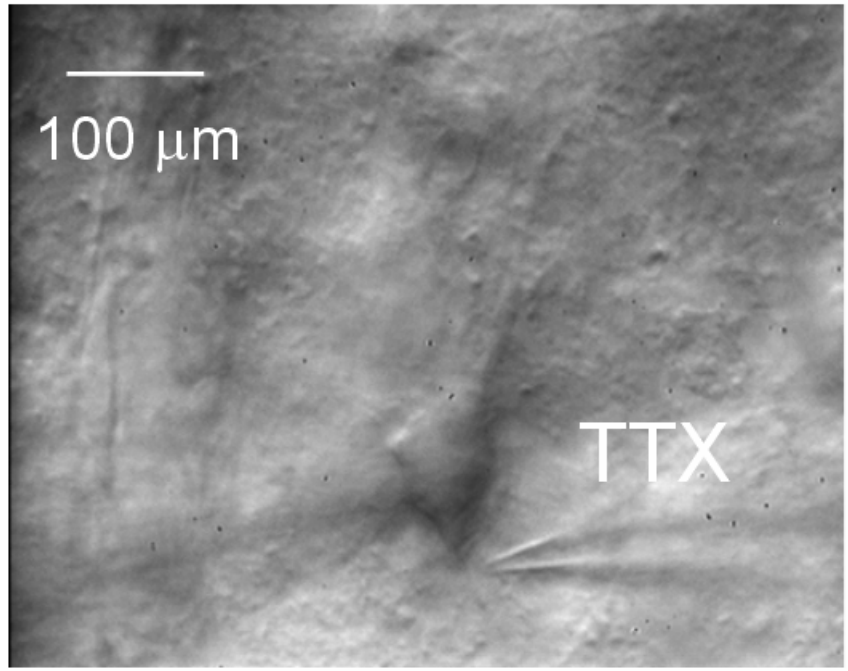


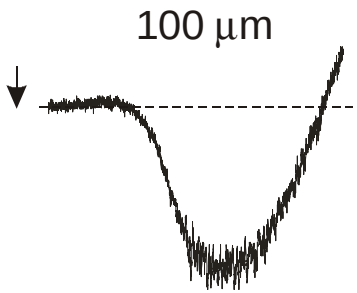
if I_{NaP} is 1% (10^{-2}) of transient I_{Na}, in a patch that contains 10 channels, the late Na channel should be persitently open in one out of every ten depolarizations

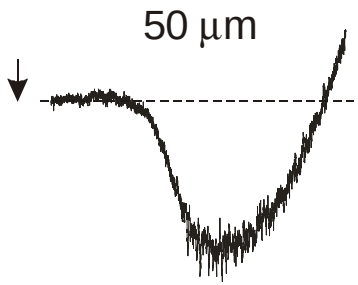
Analysis: at soma, probability of late openings is ~10^{-4}

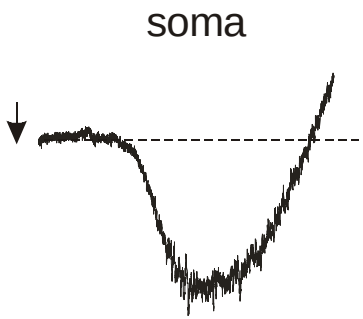

300 µm
TTX
100 pA
0.5 sec
0
-70 mV

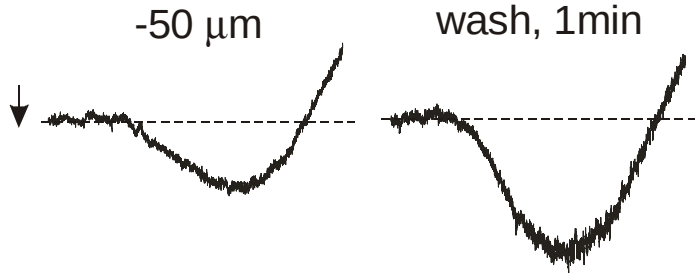
-50 µm


100 µm
TTX

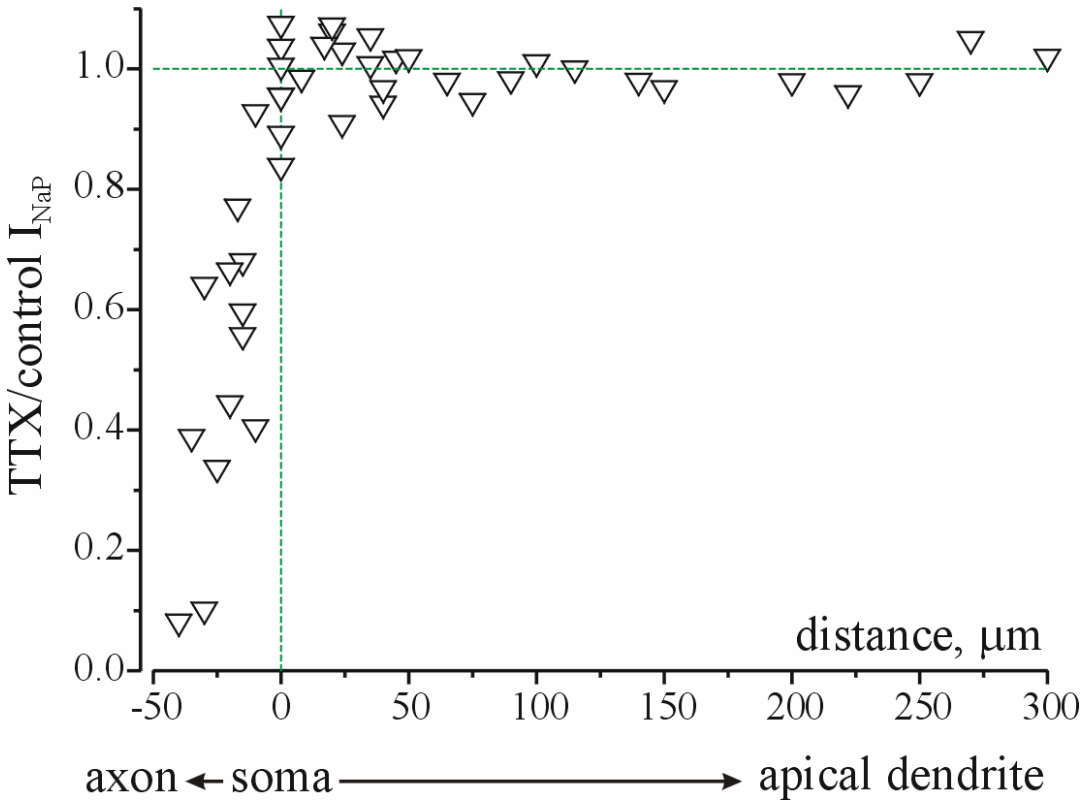

100 µm


50 µm

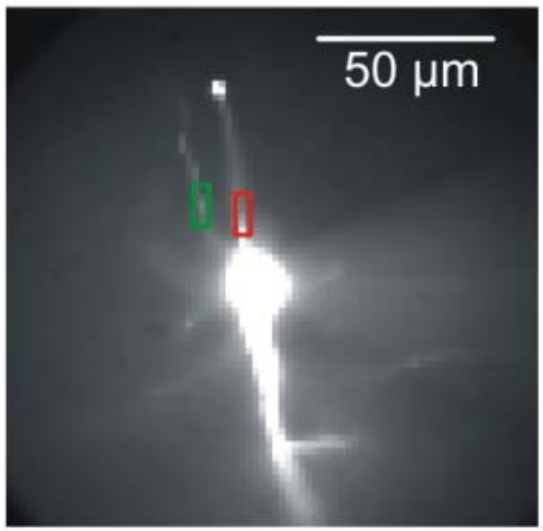

soma

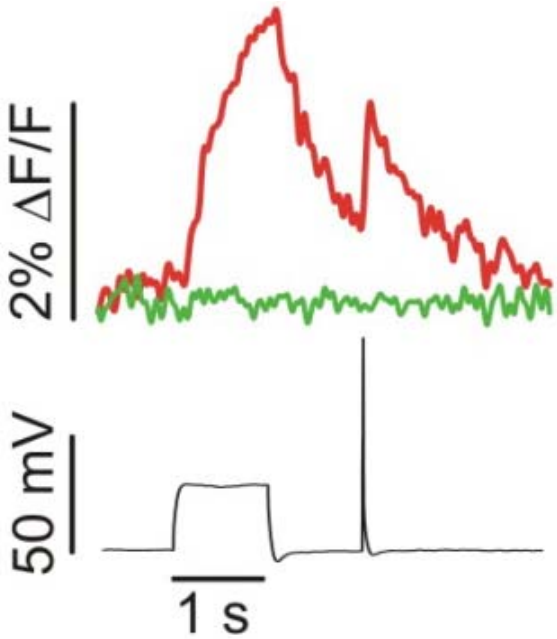

-50 µm
wash, 1min

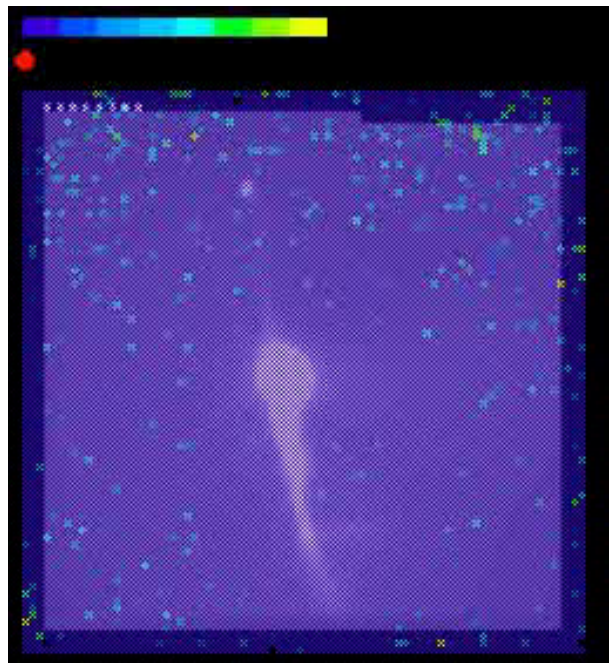
Effect of local application of TTX(10^{-7}) on whole-cell I_{NaP}

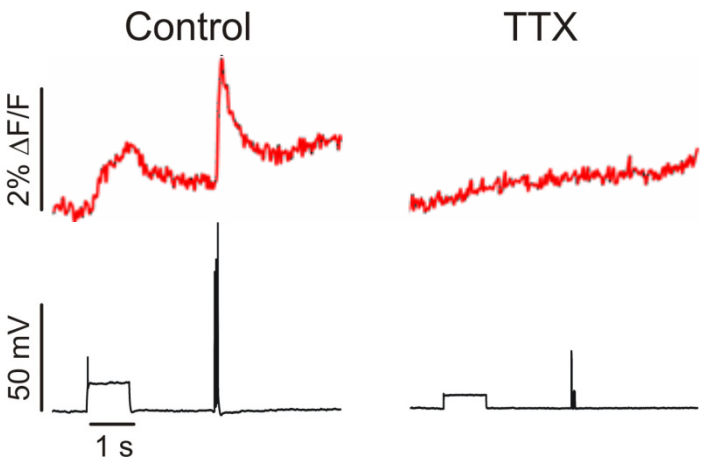


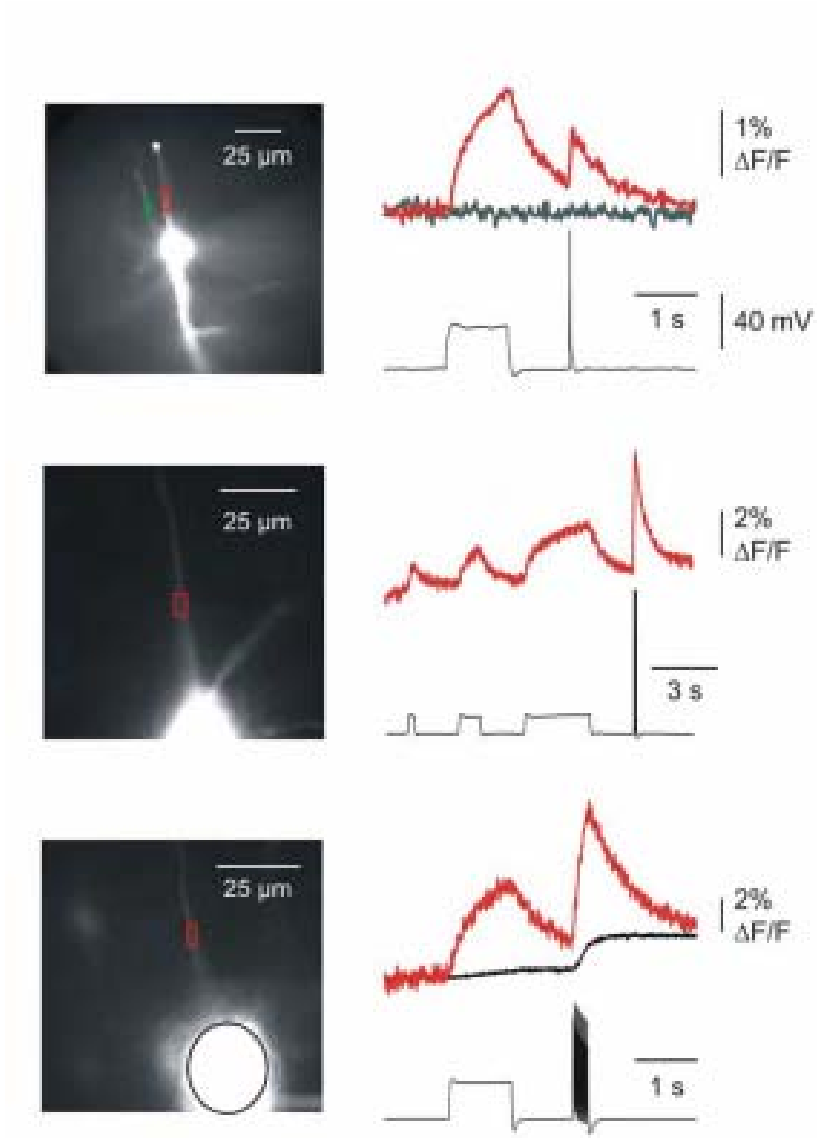
Persistent Na^+ conductance is predominately axonal



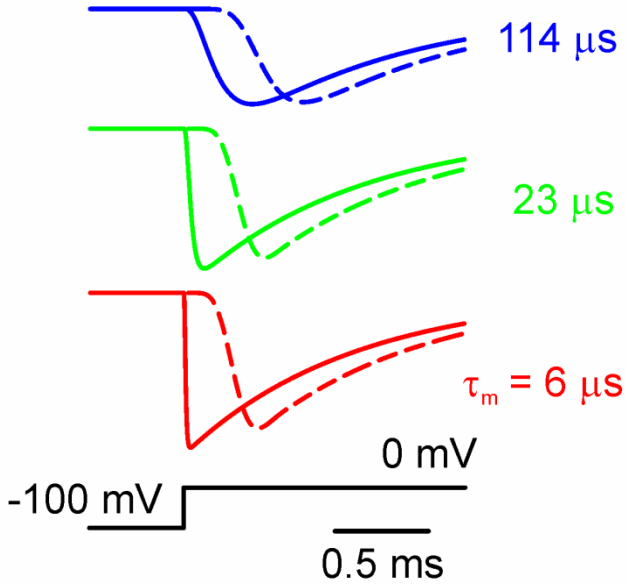


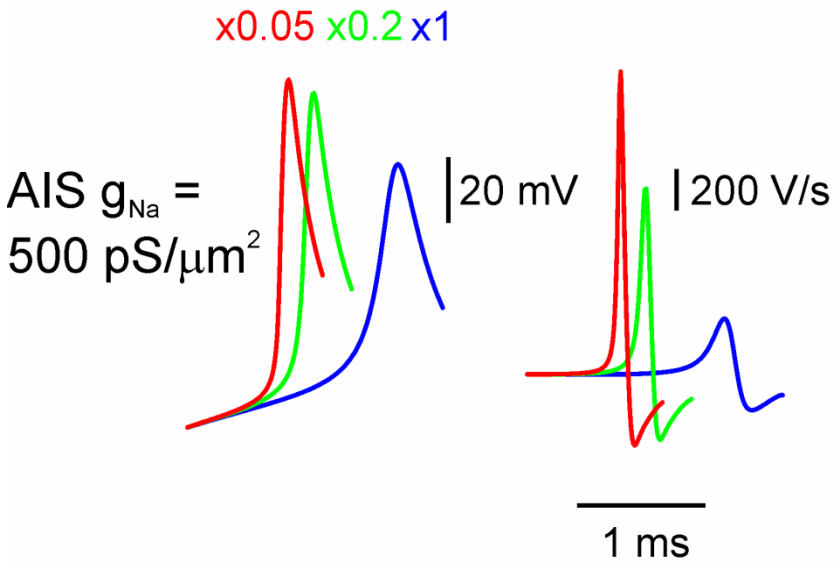


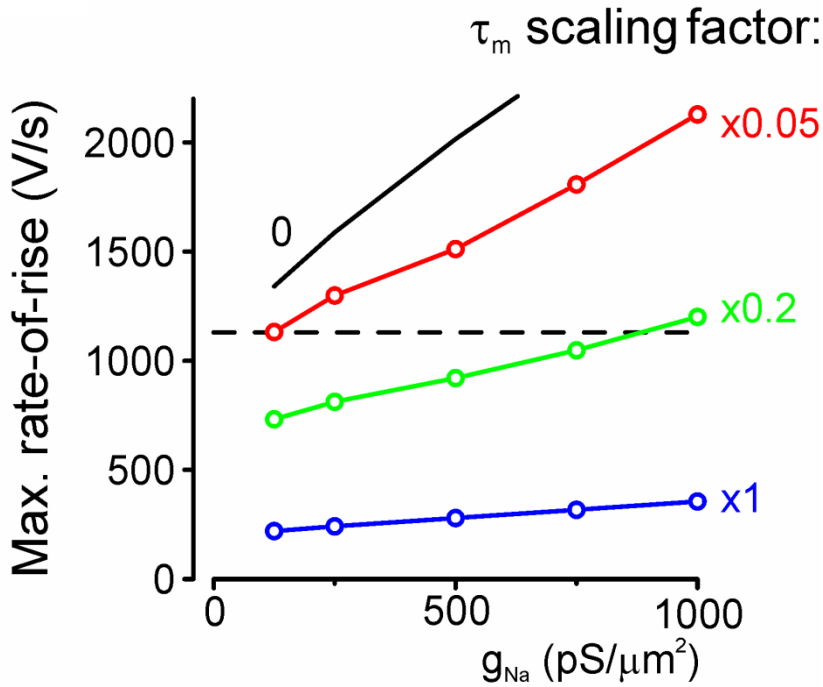

Control
TTX
2% ΔF/F
50 mV
1 s


25 µm
1%
ΔF/F
1 s
40 mV
25 µm
2%
ΔF/F
3 s
25 µm
2%
ΔF/F
1 s

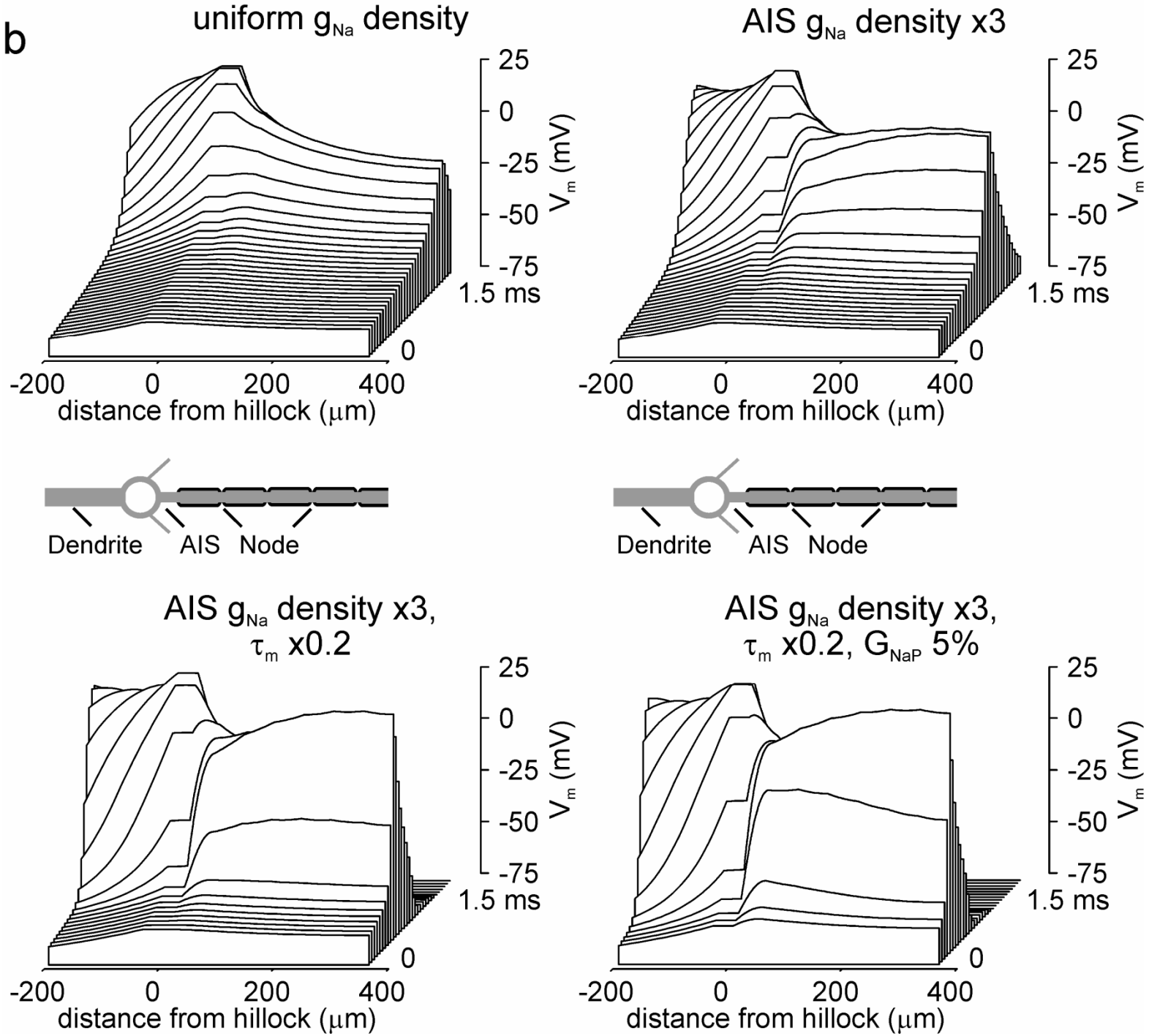
In model, when Na^+ channel kinetics are realistically fast, axonal APs have a very rapid rate-of-rise, despite the relatively low AIS Na^+ channel density







High persistent Na^+ conductance, shifted voltage dependence and fast tau m (not high channel density) are responsible for preferential AIS spike initiation

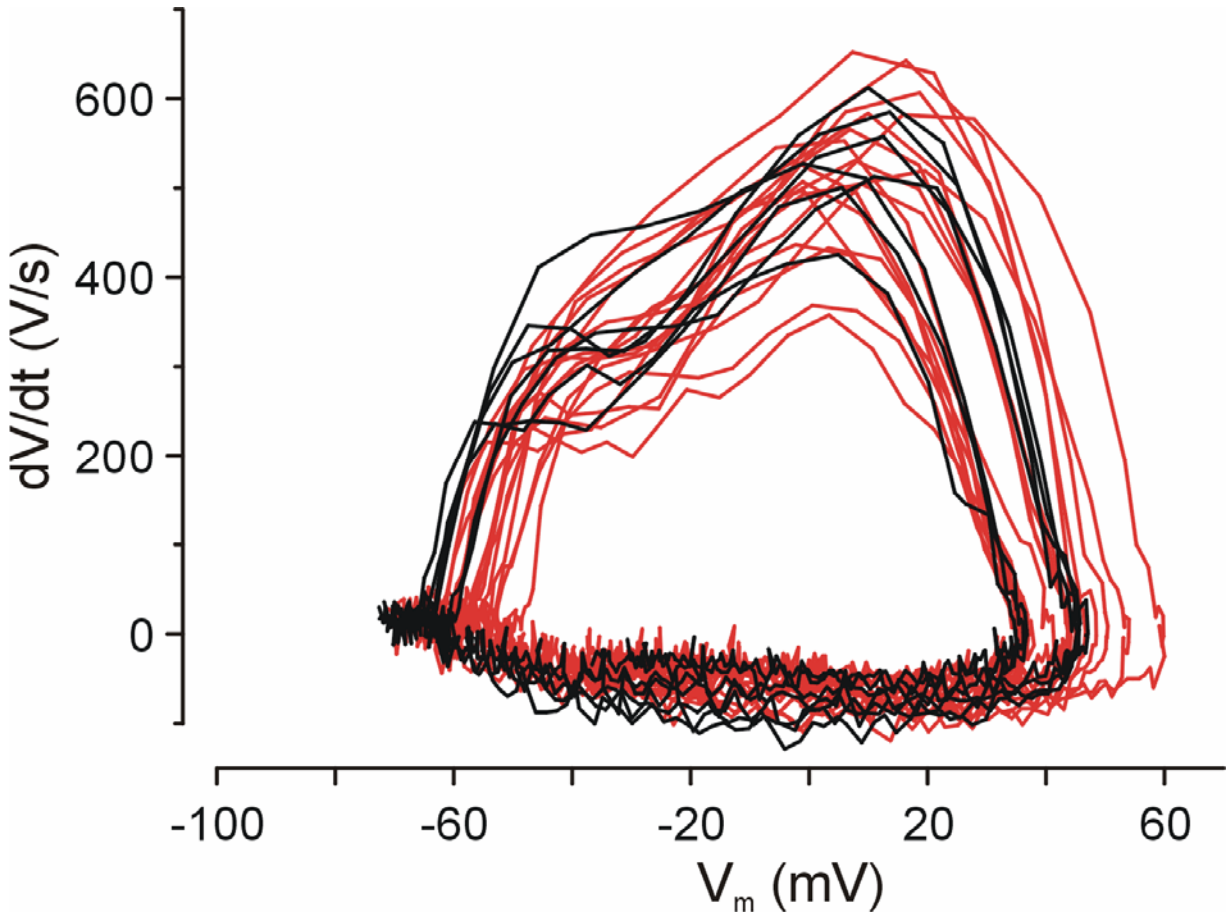

b
uniform g_{Na} density
AIS g_{Na} density x3
25
0
-25
-50
-75
V_m (mV)
1.5 ms
0
-200
0
200
400
distance from hillock (μm)
Dendrite
AIS
Node
AIS g_{Na} density x3,
τ_m x0.2
AIS g_{Na} density x3,
τ_m x0.2, G_{NaP} 5%

Conclusions

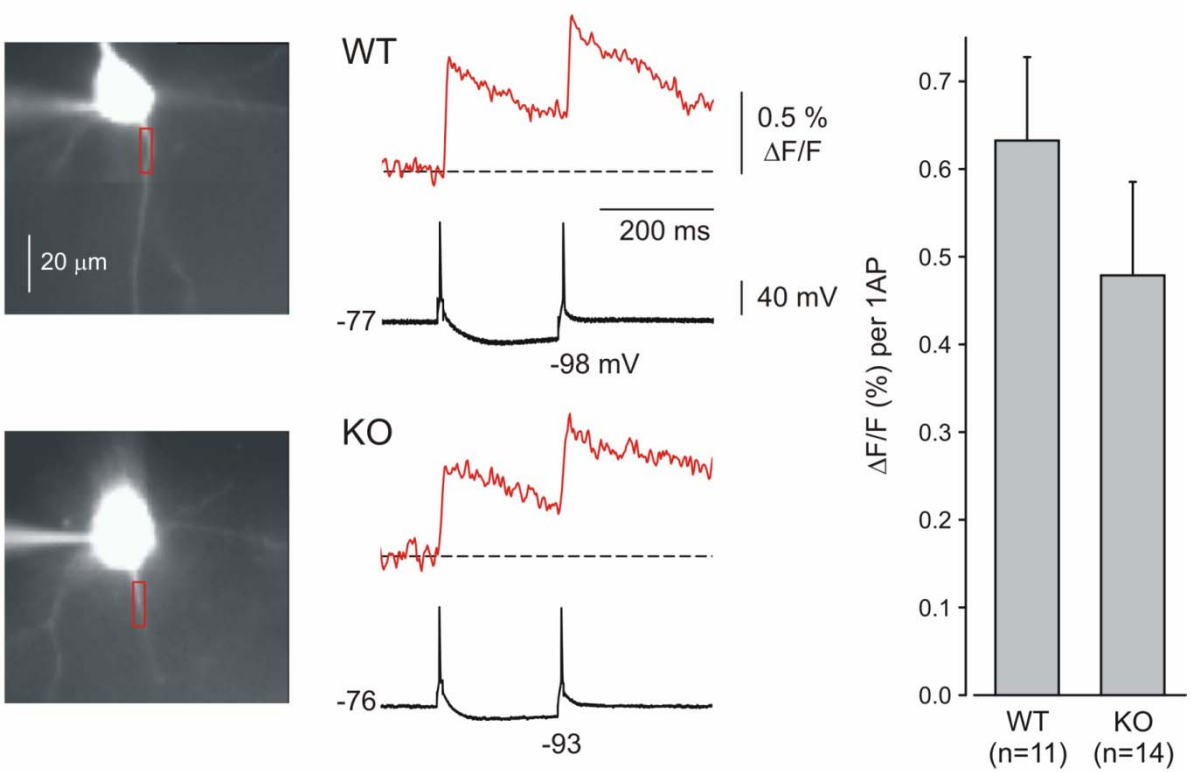
- Diffusion is the main regulator of $[Na^+]_i$ changes for short time intervals
- Na^+ channel mediated charge transfer in the soma is ~1/3 the value in the proximal axon and ~8 times larger than in the basal dendrites
- Na^+ conductance in the node is of the same order of magnitude as in the AIS
- Subthreshold depolarization only elicits I_{NaP} in axon of Layer 5 pyramidal cells
- We think that this large axonal I_{NaP} is due to higher propensity of the underlying Na^+ channels to enter the non-inactivating gating mode
- The unique properties of the axonal Na^+ channels explain preferential axonal initiation of action potentials in Layer 5 pyramidal cells

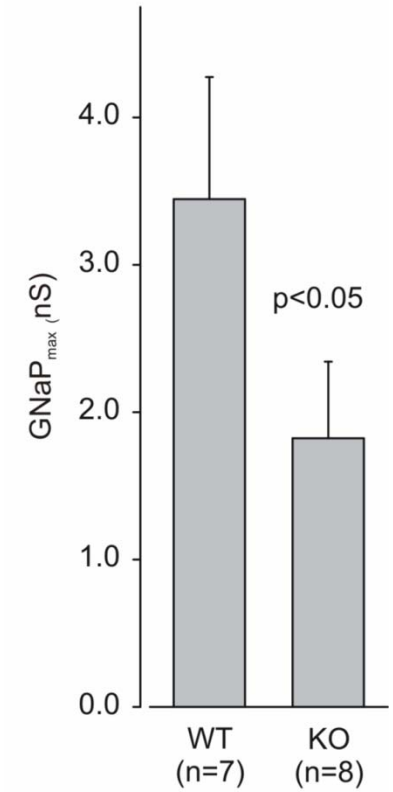
Do the unique properties of the Na current in the AIS reflect the difference in molecular subtype of Na channel?

In NaV1.6 KO mice, spikes are still biphasic and threshold is shifted.



$[Na^+]_i$ increases in the AIS associated with single APs was not different in KO and WT animals.





G_{NaP} was significantly smaller in KO animals.

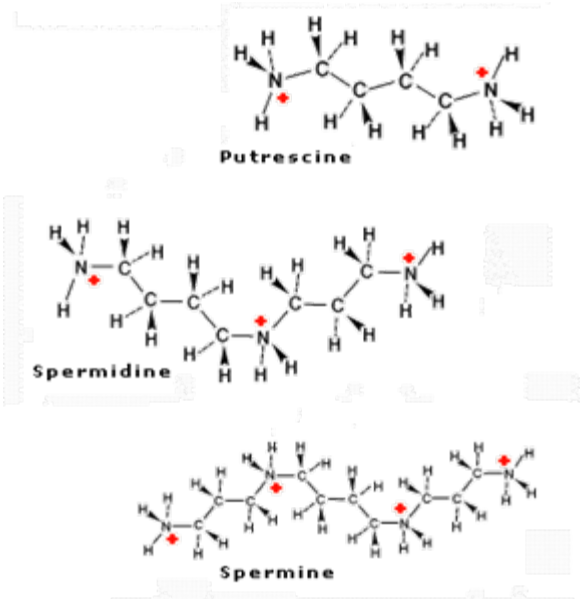
If it isn't the channel subtype, what is responsible for the unique characteristics of the AIS sodium channels?

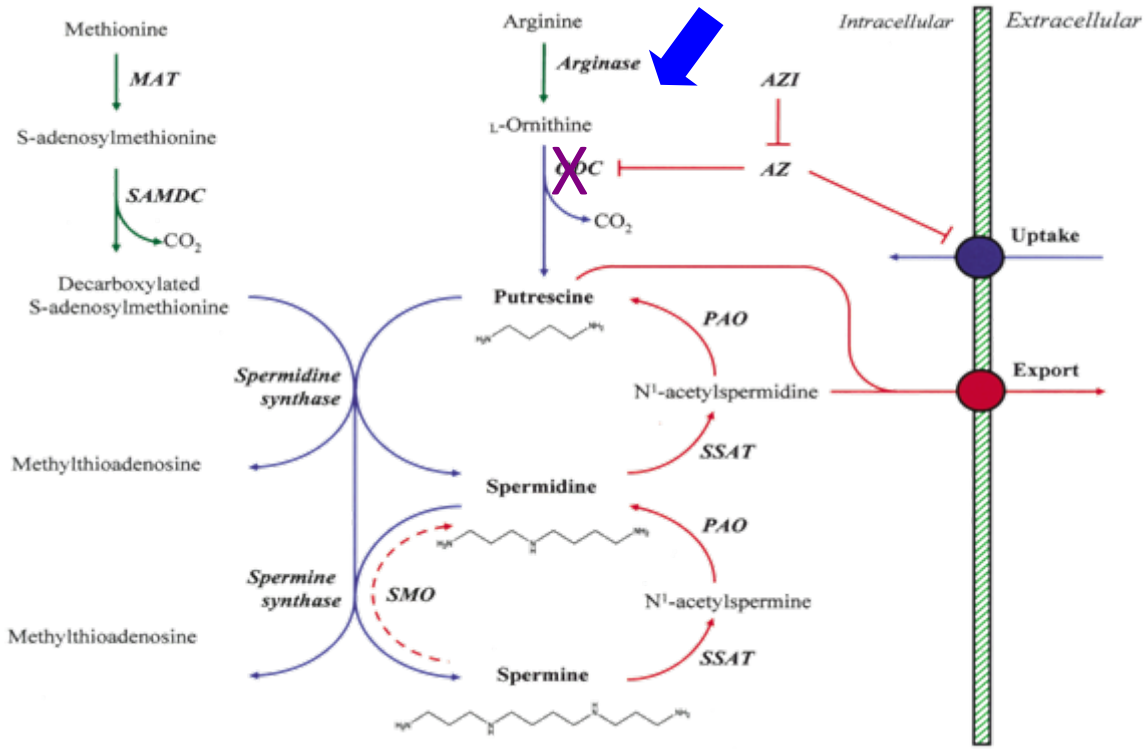
Why did evolution bother to change the AIS sodium channel subtype during the course of cortical maturation?

Differences between Na^+ channel properties *in situ* vs. dispersed cells led us to postulate the existence of *a soluble factor*, extrinsic to the Na^+ channel protein, that prevents late channel openings

We focus our attention on polyamines, because they are present in all eukaryotic cells they can be released from the cells they are known to affect gating of a variety of ion channels

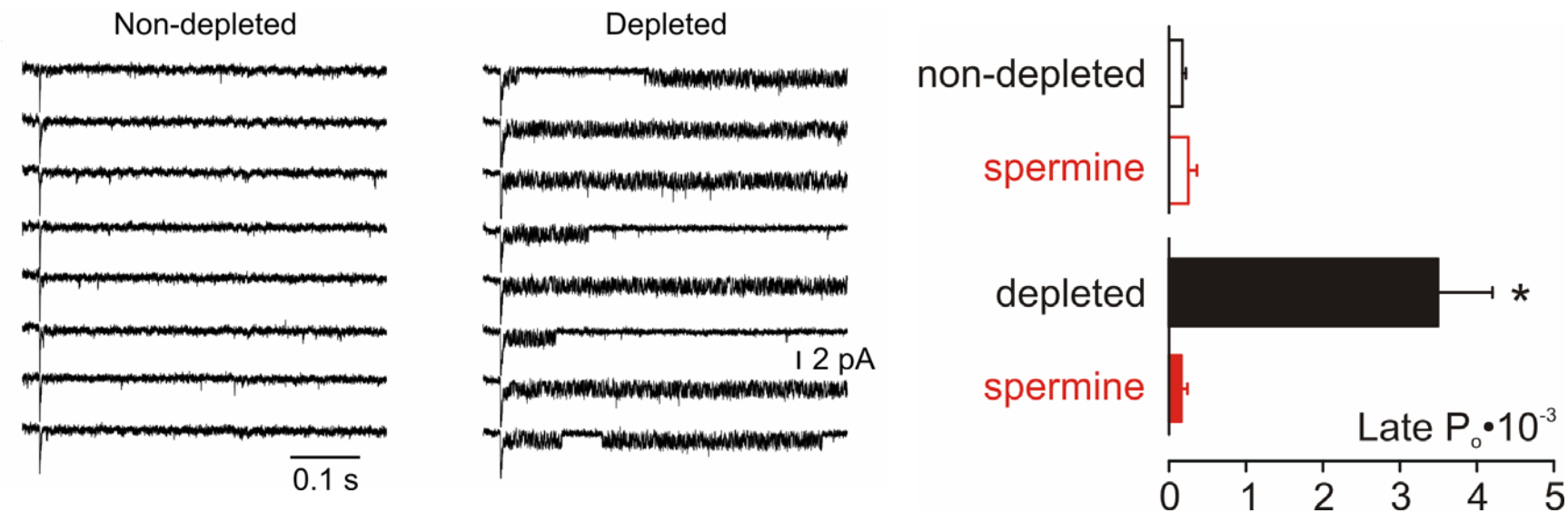
Polyamine metabolism





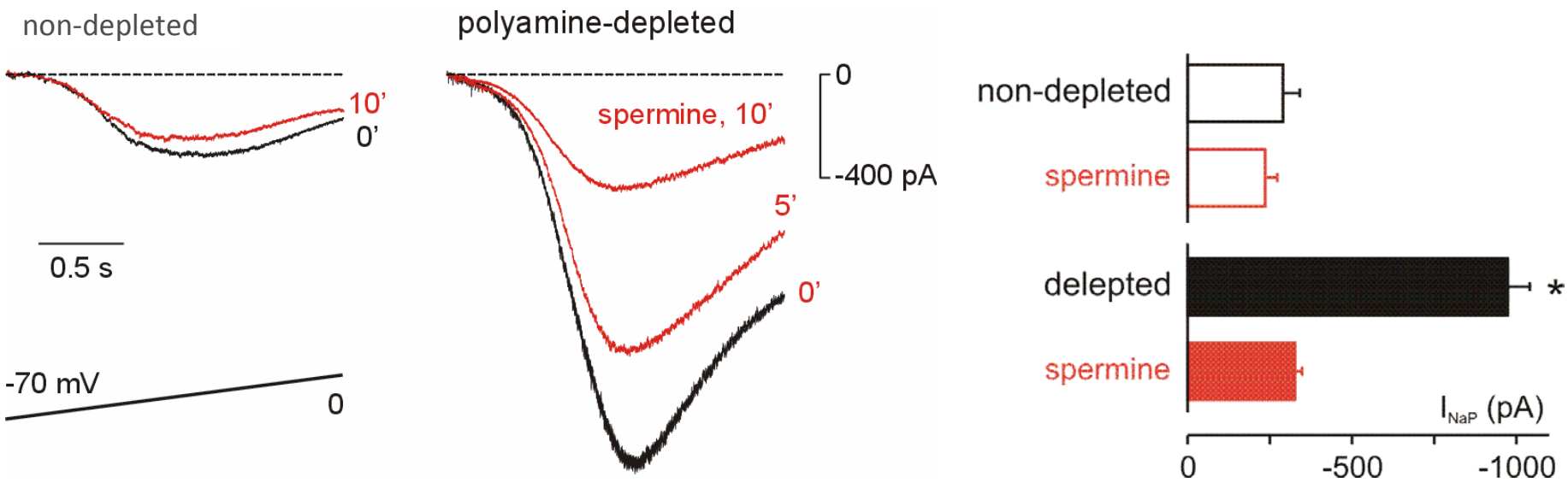
modified from Wallace et al., Biochem J *376*:1-14, 2003

In polyamine-depleted slices, the “modal gating” episodes were as frequent as in isolated neurons



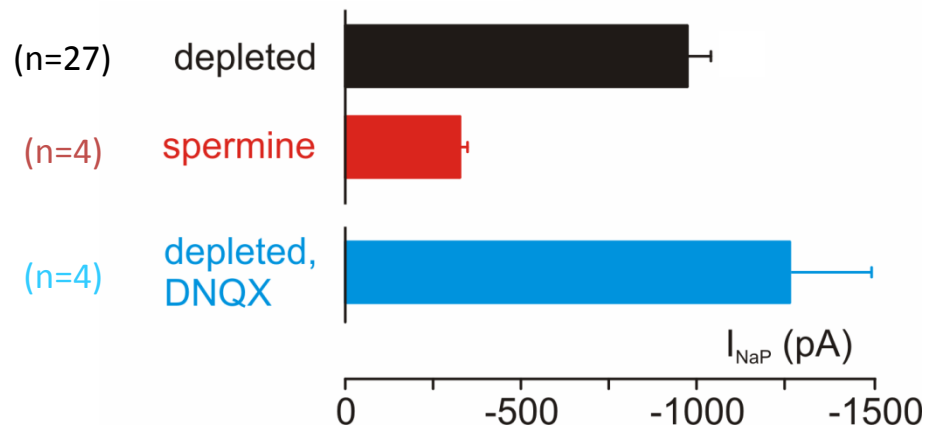
Fleidervish et al, PNAS, 2008

In polyamine-depleted slices, I_{NaP} amplitudes were 5-10 times larger than in control



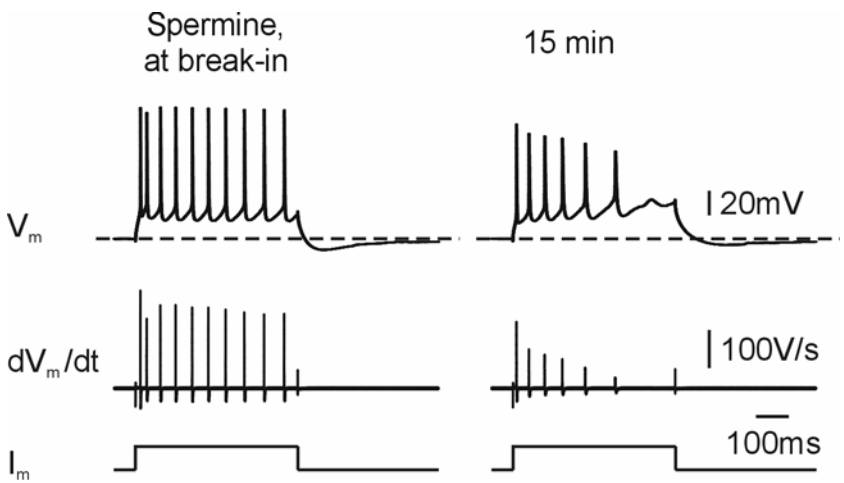
Exogenous spermine blocked I_{NaP} component which was elicited by polyamine depletion

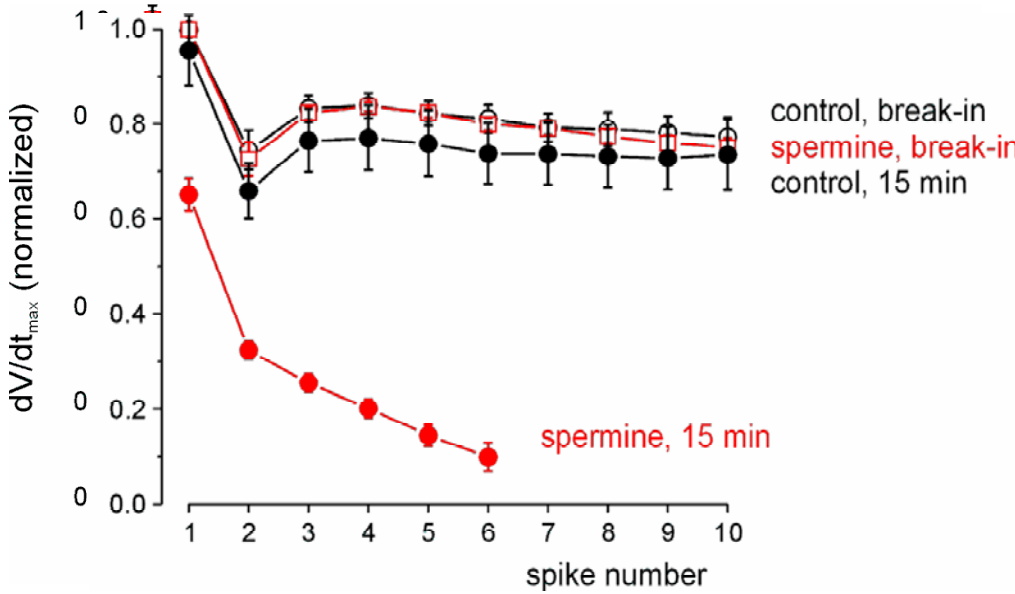
Effect of polyamine depletion on I_{NaP} is not related to modification of synaptic input…



…since it was not affected by bath-applied AMPAR blocker, DNQX

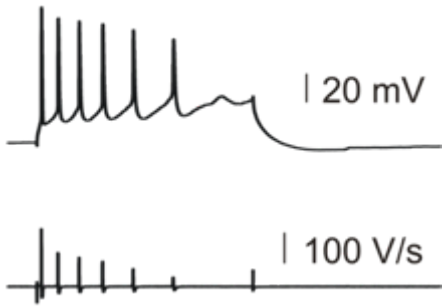
Exogenous spermine causes an activity-dependent Na^+ channel block



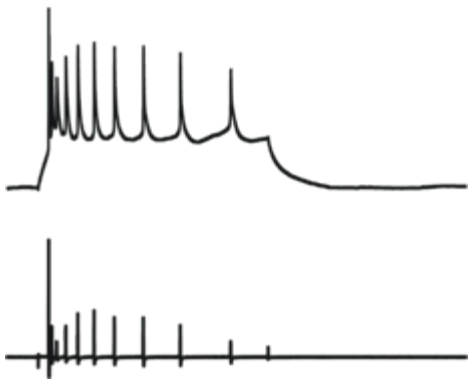


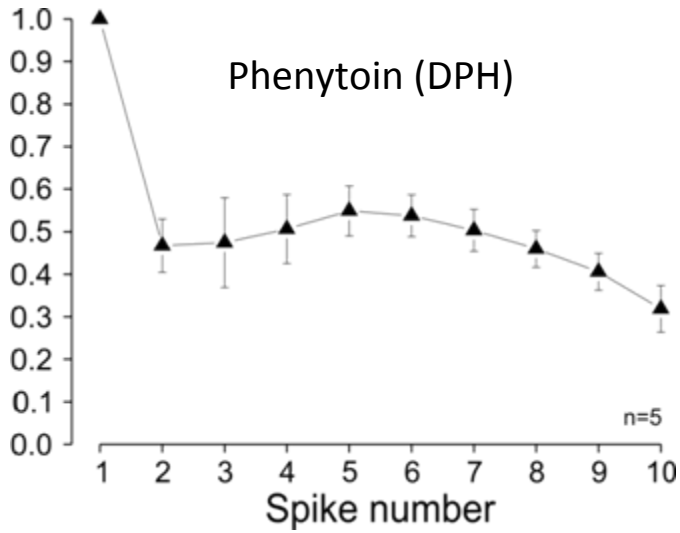
Polyamines block Na^+ channels in a manner reminiscent of the action of local anesthetic and anti-epileptic drugs

spermine



Phenytoin (DPH)





Activity-dependent block explains the preferential action of polyamines on I_{NaP} and the underlying repetitive late channel openings, which essentially represent heightened activity of the channel

CONCLUSIONS

1. Layer 5 pyramidal neurons possess a prominent, TTX-sensitive persistent Na current which contributes to synaptic integration and strongly influences threshold.

2. The persistent current and the transient current reflect “modal gating”of the same sodium channels

3. Somatic channels do not generated persistent current because they are bolcked by endogenous intracellular polyamines

4. The polyamine block is activity-dependent

5. Modulation of polyamine levels may be an important factor in regulating neuronal excitability, and hence, circuit function.