THE UNIVERSITY OF CHICAGO

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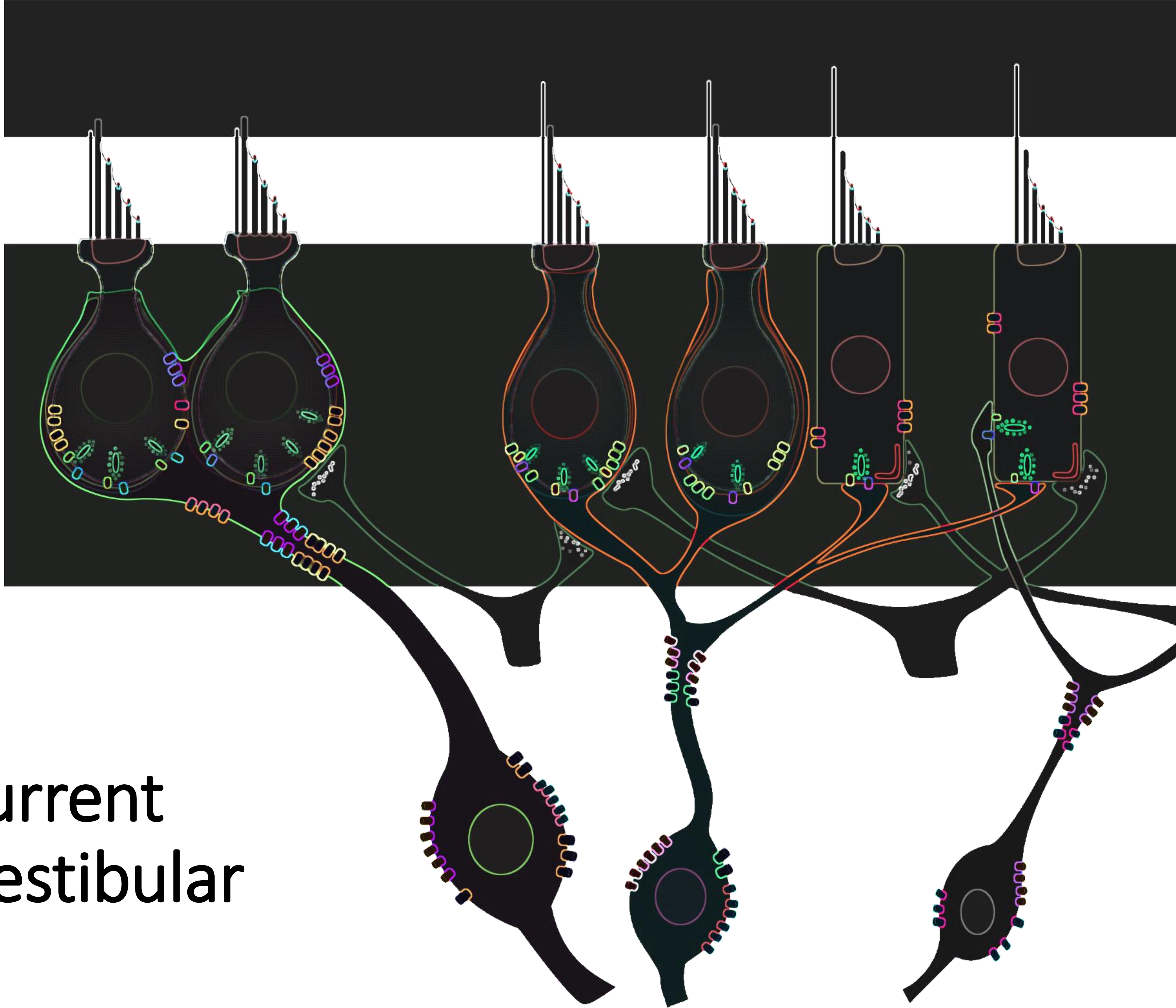
Eatock Lab

January 25th, 2021

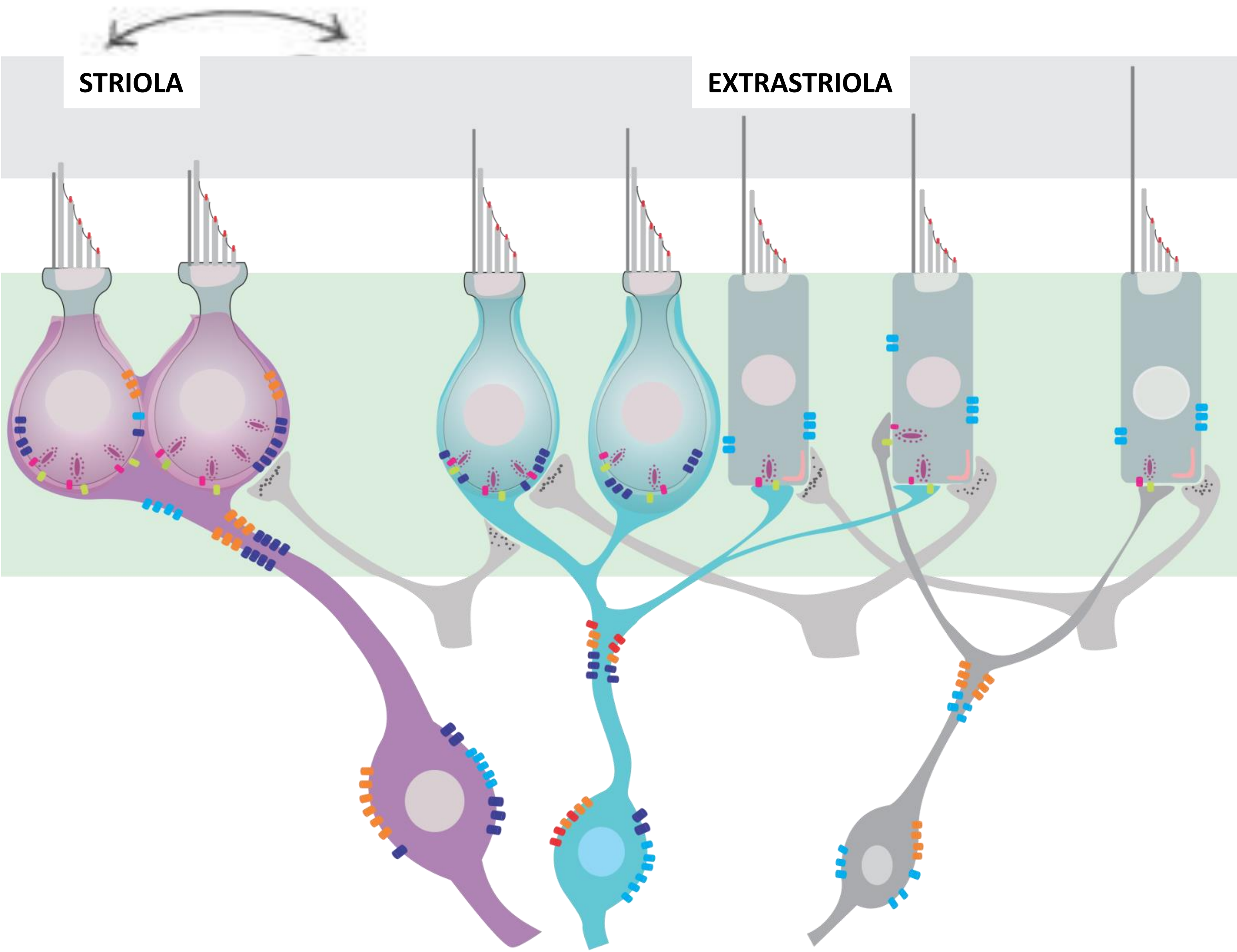


How does sodium current diversity influence vestibular afferent firing?

The peripheral vestibular organs are the primary balance receptors

How do vestibular ganglion neurons (VGN) encode info about head motion?

Otolith motion
↓
Transduction
↓
Receptor potential
↓
Synaptic transmission
↓
Spike initiation
↓
Encoding
↓
Brain

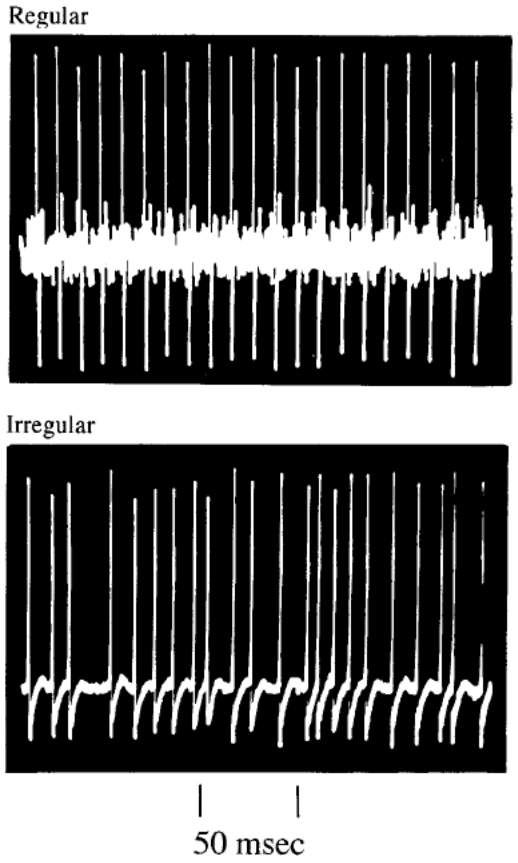


1st Right: "Vestibular Senses", Above: hear-it.org. 2nd Right: sketch of the mammalian inner ear [adapted from I Brödel in Hardy (1934) with permission from John Wiley and Sons],

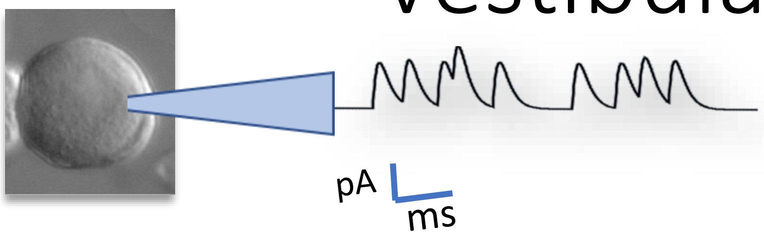
Spike timing in Vestibular Ganglion Neurons

Goldberg and Fernández, 1971a

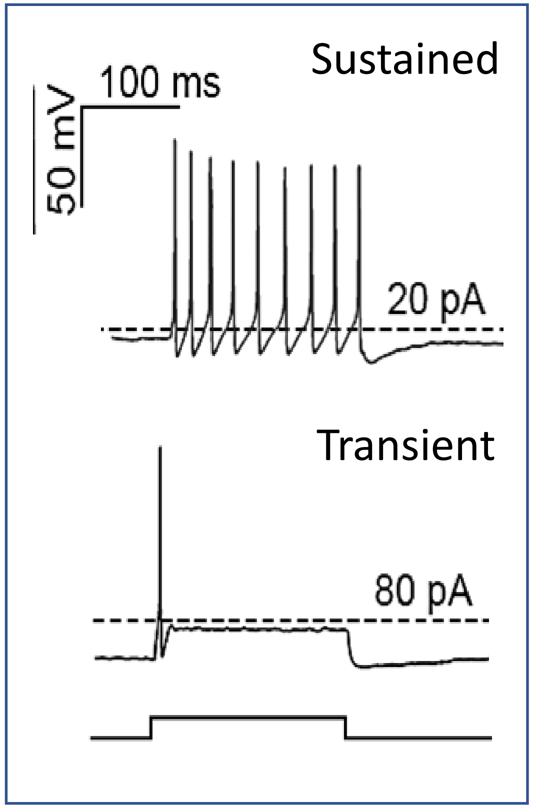
Spike timing *in vivo*

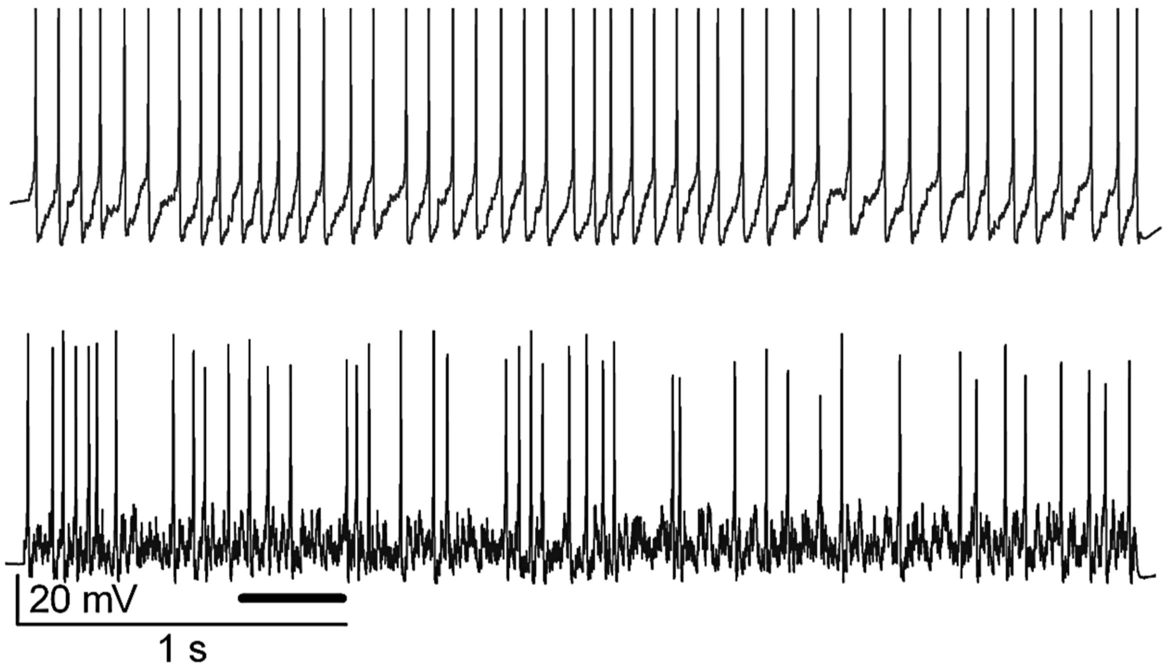


Kalluri et al., 2010



Firing patterns *in vitro*





Two parallel channels of information

- **Irregular** (transient) afferents and **Regular** (sustained) afferents
- Two encoding strategies; precise spike timing and firing rate respectively (Jamali et al., 2016; Cullen 2019).

Vestibular ganglion neurons are a model for studying the role of voltage-gated currents on spike timing (and indirectly encoding)

- **Low voltage activated potassium currents (I_{KL})** have been shown to be key in driving irregularity (Kalluri et al., 2010; Hight and Kalluri, 2016).
- Na_V currents in VGN were historically thought to be homogenous.

Sodium current diversity can arise from:

- Channel forming (α) subunits that carry current

- Curr

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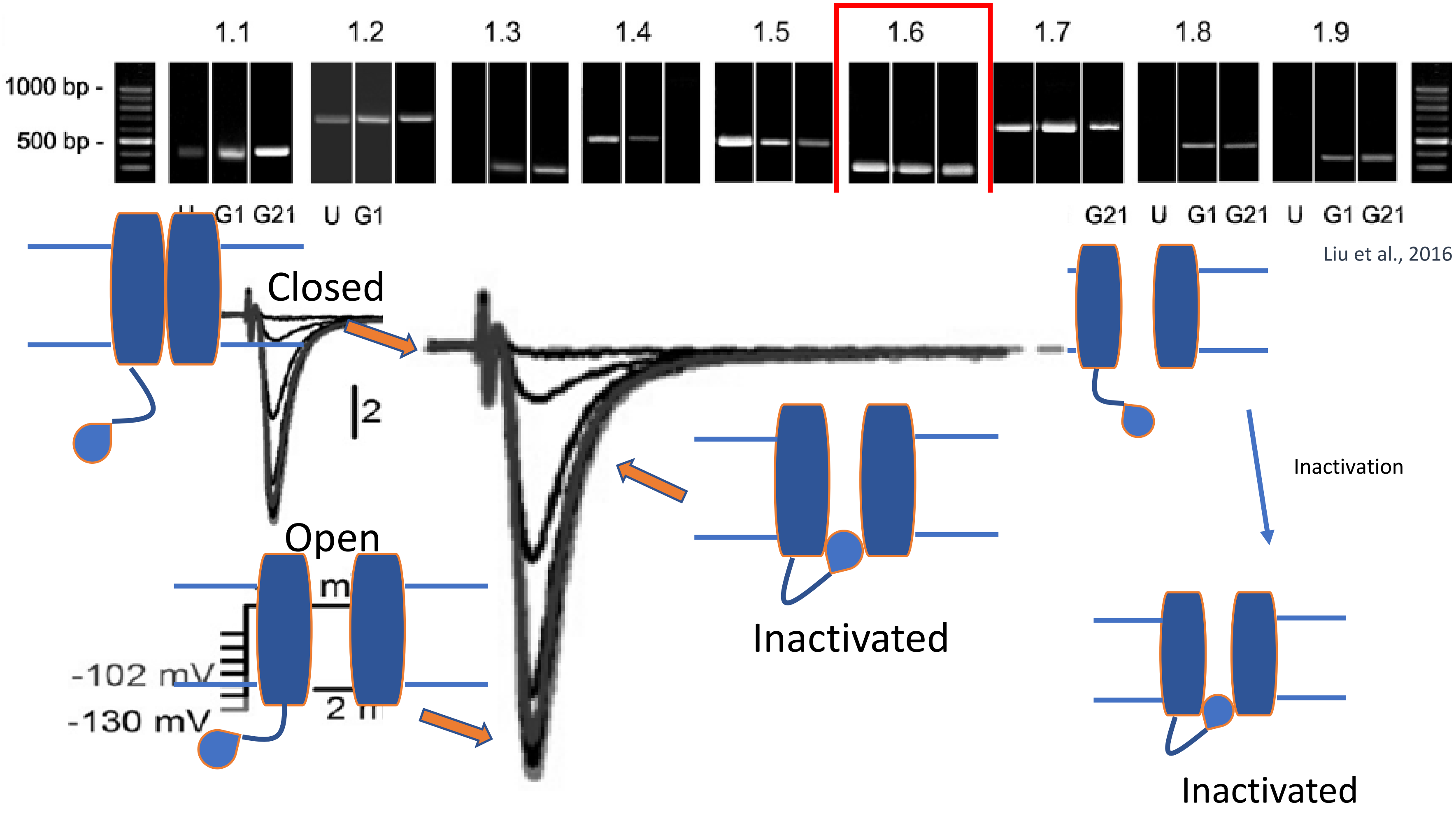


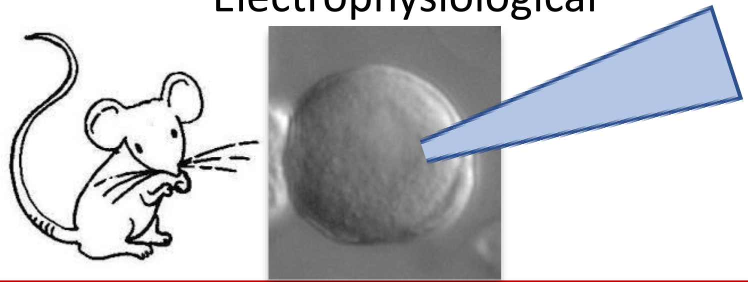
Sodium current diversity can arise from:

- Channel forming (α) subunits that carry current

- Current "modes" that reflect different channel states
  - Transient (traditional, quickly inactivating) (**Na_VT**)
  - Persistent (slowly or non-inactivating) (**Na_VP**)
  - Resurgent (blocked from inactivation) (**Na_VR**)

Today I will show results from two approaches:

Electrophysiological



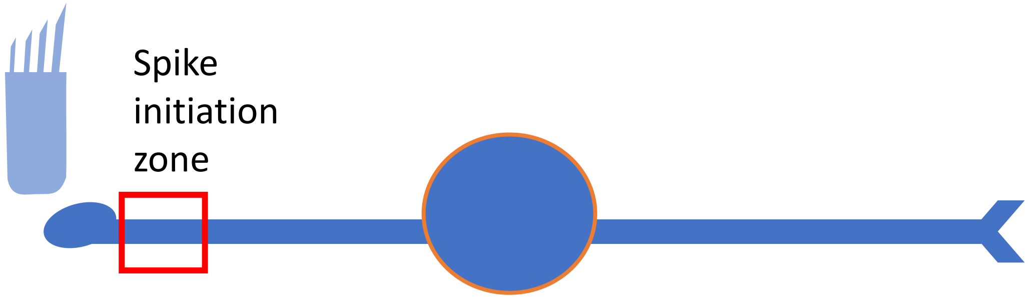
Computational

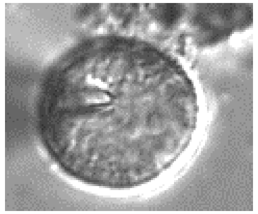
$$I_{inj} = Cm\,S\frac{dV}{dt} + I_{KL} + I_{KH} + I_{Na} + I_H + I_{leak}$$

Ephys results:

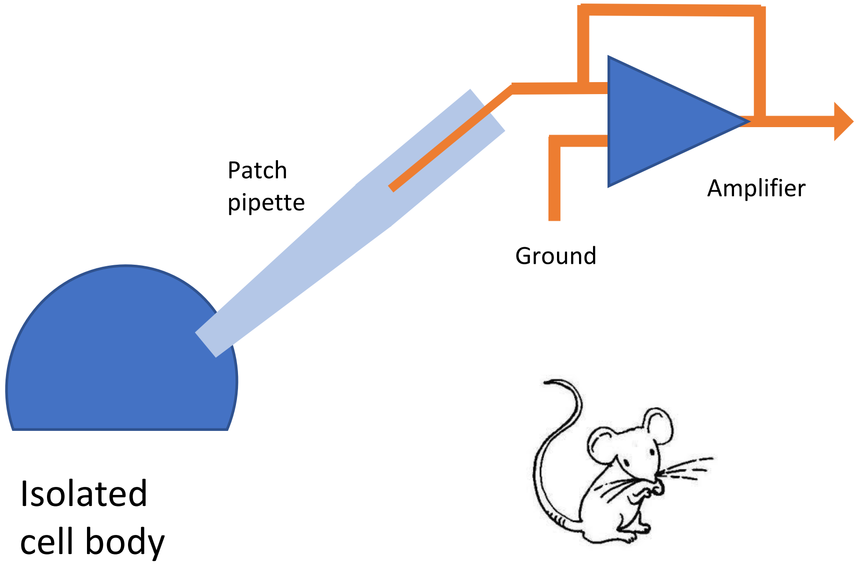
What Na current components do VGN express, and what are their influence on firing?

Experimental approach





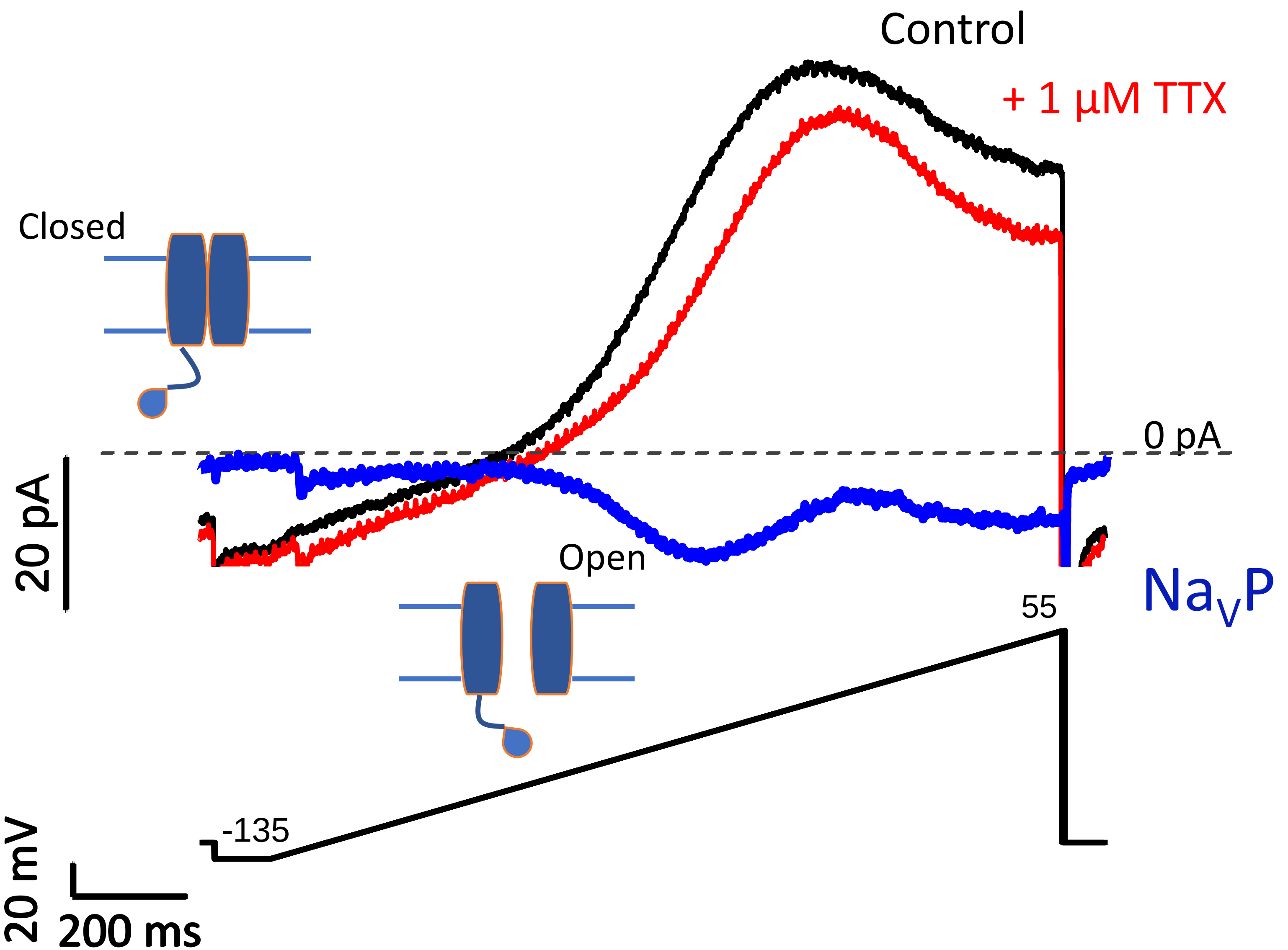
Whole-cell patch clamp electrophysiology

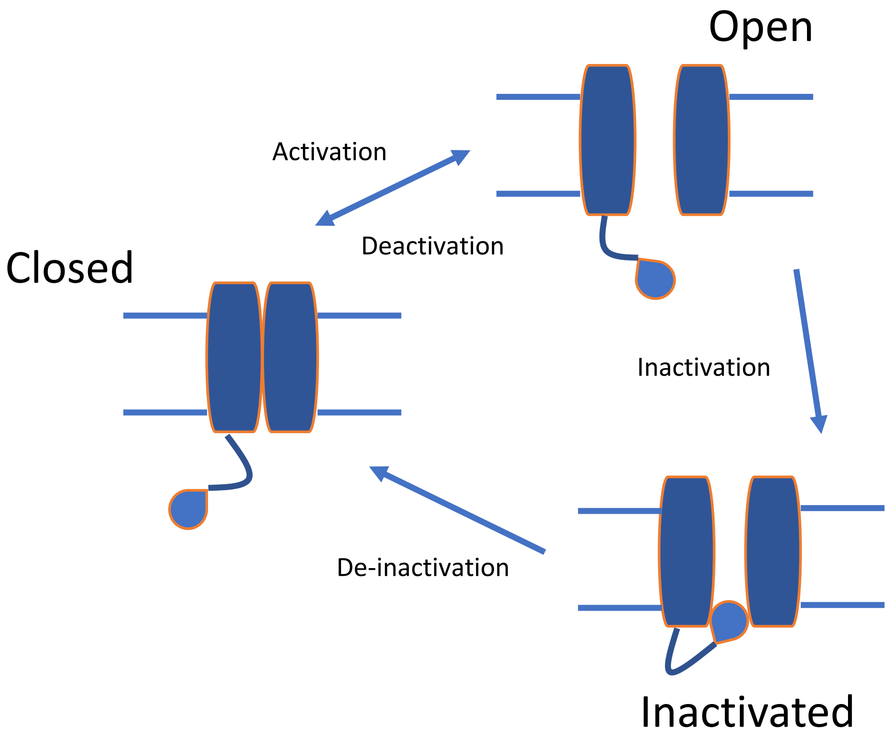


Design:

- Voltage clamp: restricted exp conditions, no K^+ or Ca^{2+}, reduced Na^+, TTX to isolate Na currents
- Current clamp: normal physiological conditions

In voltage clamp: some VGN show persistent Na_V currents

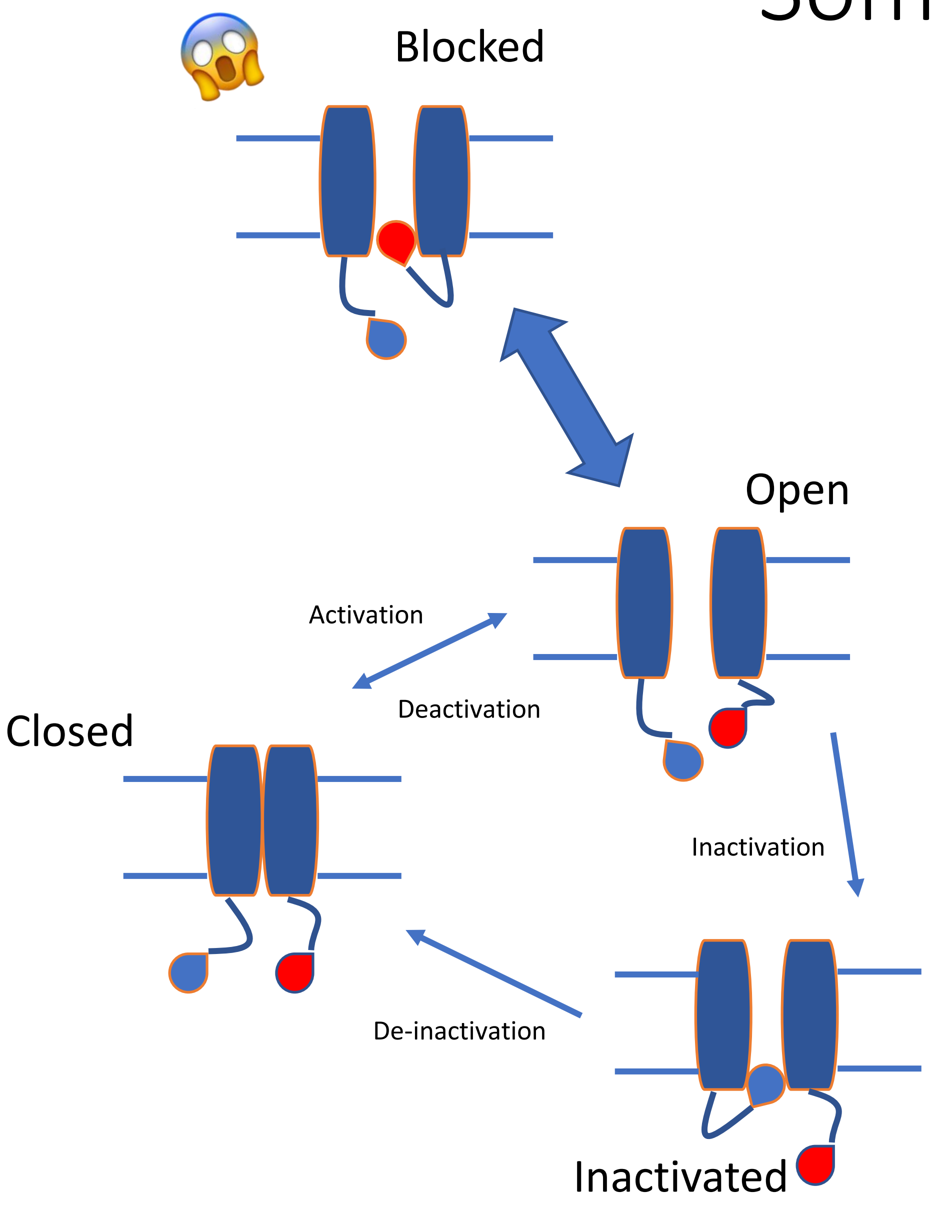


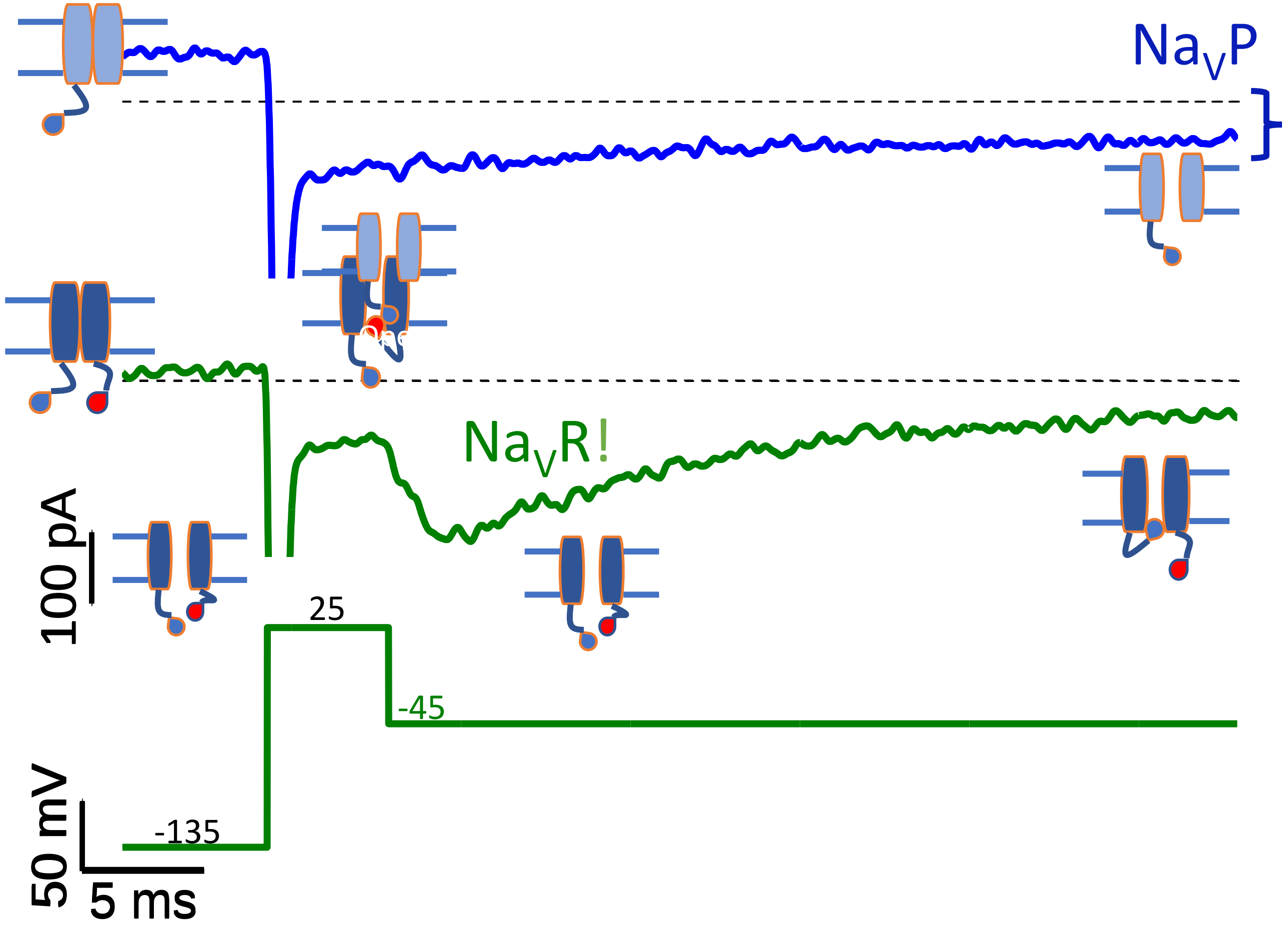


Restricted exp conditions

In 43 of 80 (54%) VGNs tested

Some VGN show resurgent Na_V currents too

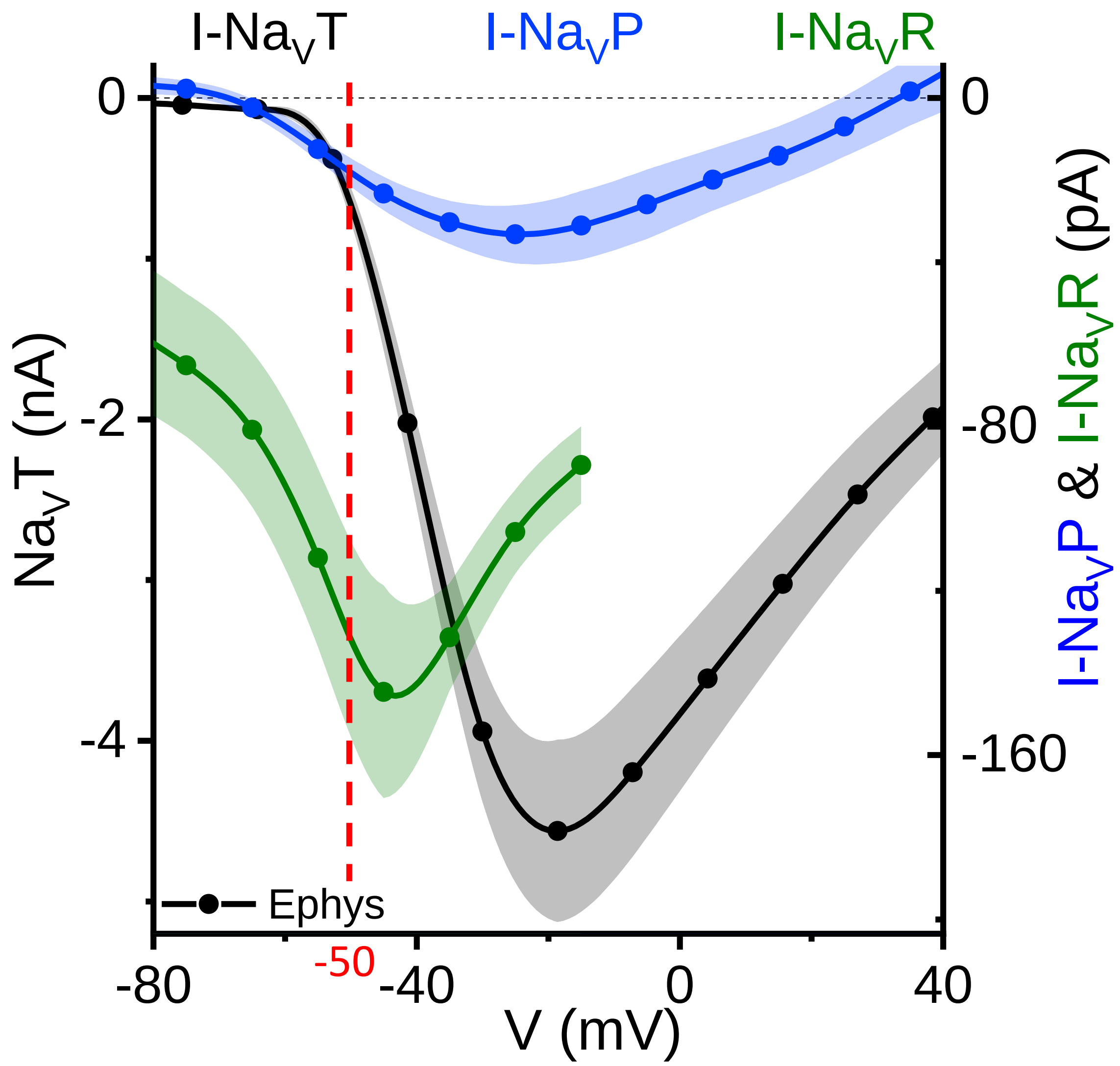




Restricted exp conditions
In 7 of 80 (8.7%) VGNs tested
5 of those 7 had Na_VP too

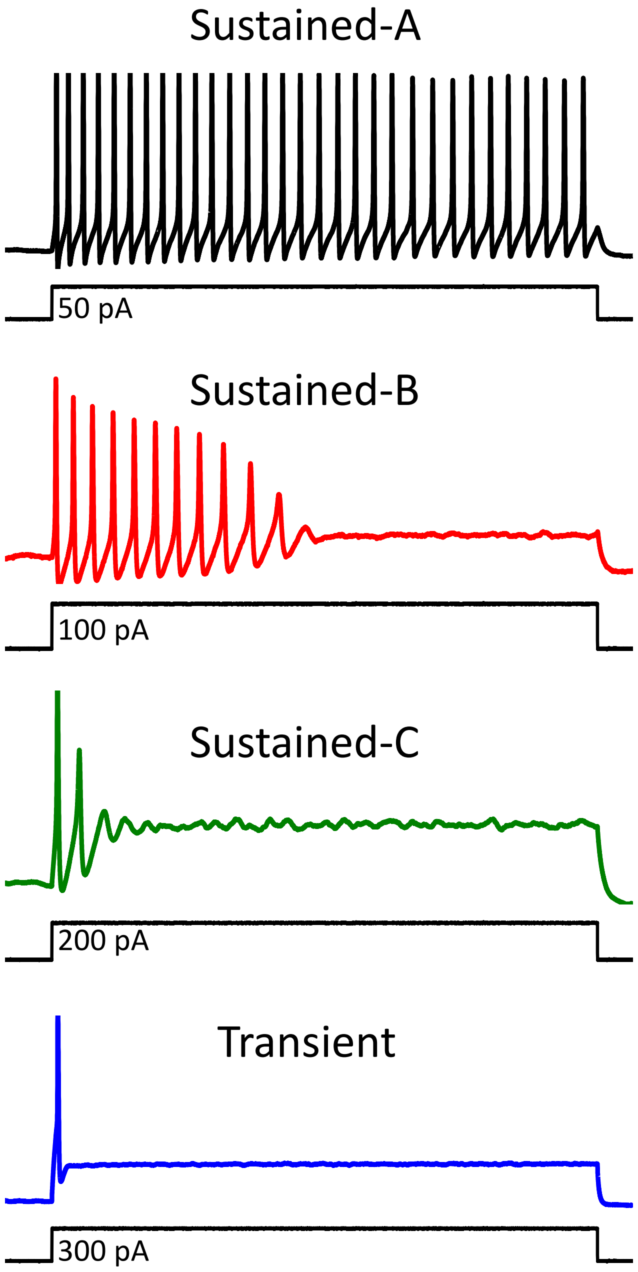
Voltage range of activation:

Subthreshold currents are significant near AP threshold, and may affect neuronal excitability.



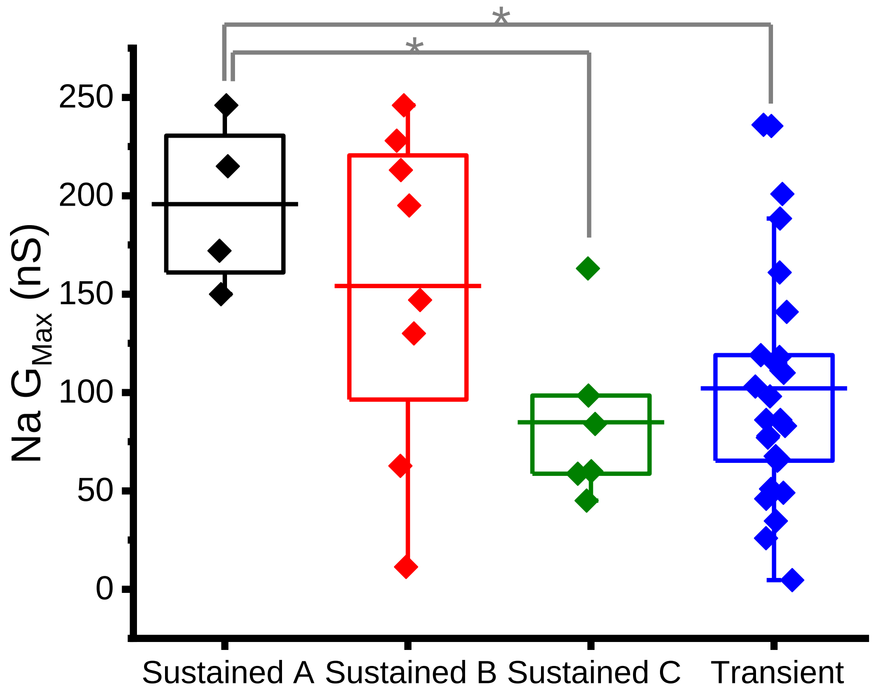
AP Threshold

In current clamp: Four firing patterns, difference in max Na conductance



Low current threshold
High excitability
Low I_{KL}
High I_{Na}

Low I_{Na}
High current threshold
Low excitability
High I_{KL}



Kalluri et al., 2010

Normal physiological conditions

Ok, cool!
But what impact do Na_VP and Na_VR currents have on firing patterns *in vitro*?

Short answer: I don't know yet.

Long answer: Recording small currents in physiological conditions is proving to be very difficult.

K currents, HCN currents, Ca^{2+} currents and a huge and fast (~20 nA) macro Na current makes it very hard to isolate a ~100 pA current, even when using TTX.

Plus we believe that Na_VP and Na_VR are being carried through the same channel (Na_V1.6) and there's no pharmacological way to isolate them from each other.

Modeling results:

Using the ephys data to develop HH model of VGN, what effects could individual sodium current components have on firing?

Model VGN shows currents shaping firing patterns

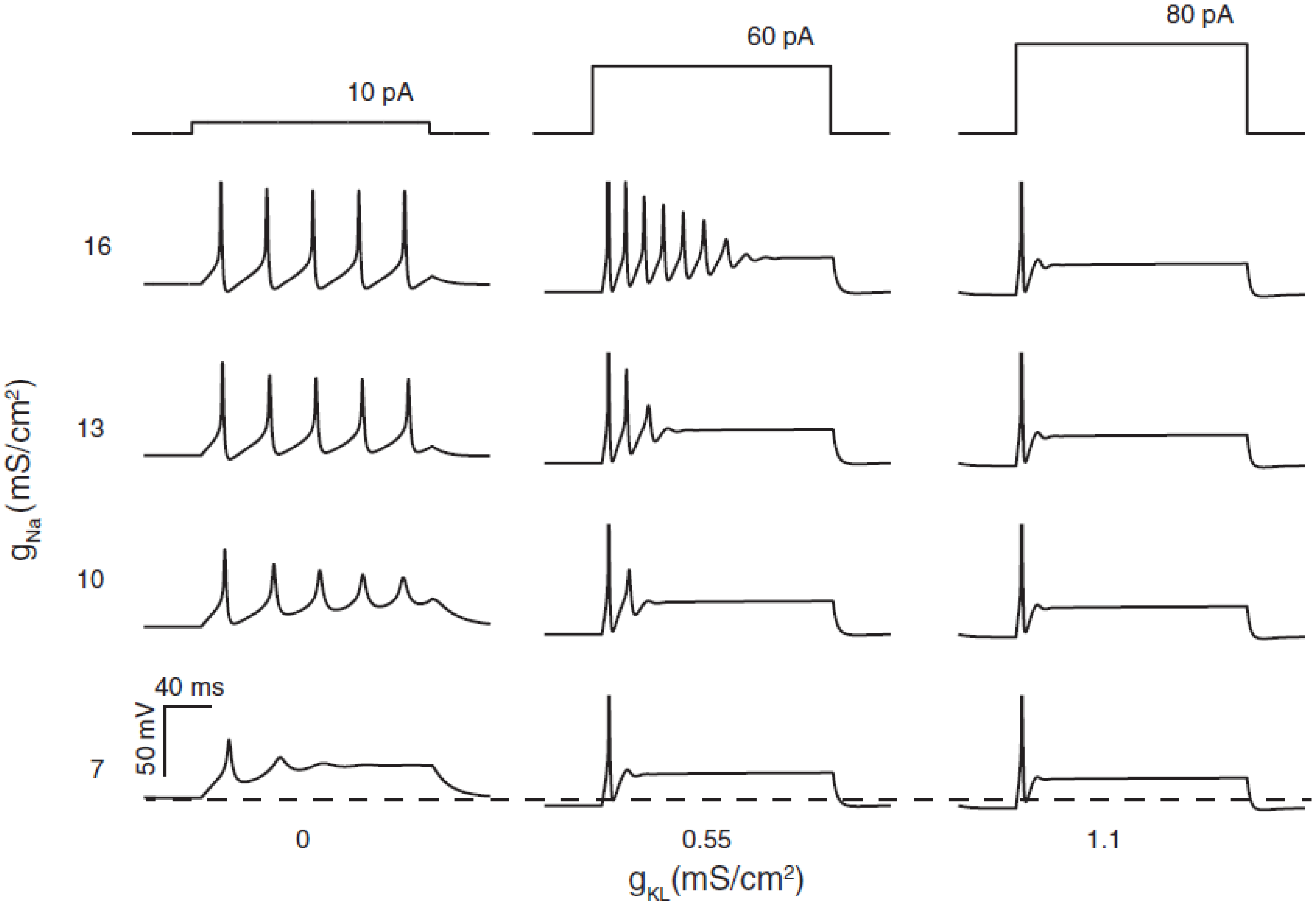
Hight & Kalluri, 2016

$$I_{inj} = Cm\, S\frac{dV}{dt} + I_{KL} + I_{KH} + I_{Na} + I_H + I_{leak}$$

Low voltage activated K conductances (Kv1 and Kv7) support spike timing irregularity

Increasing garden variety Na conductance increases spiking regularity

$$I_{Na} = \overline{g}_{Na} m^3 h S (V - E_{Na})$$



Hight AE, **Kalluri R**. A biophysical model examining the role of low-voltage-activated potassium currents in shaping the responses of vestibular ganglion neurons. *Journal of Neurophysiology* 116: 503–521, 2016.

Computational approach

$$\boldsymbol{I_{Na} = I_{NaT} + I_{NaP} + I_{NaR}}$$

$$I_{NaT} = g_{NaT}(m_{t^3} h_t)(V - E_{Na})$$

Nonlinear voltage-dependent inactivation

Nonlinear voltage-dependent activation

$$I_{NaP} = g_{NaP}(m_{p^\infty} h_p)(V - E_{Na})$$

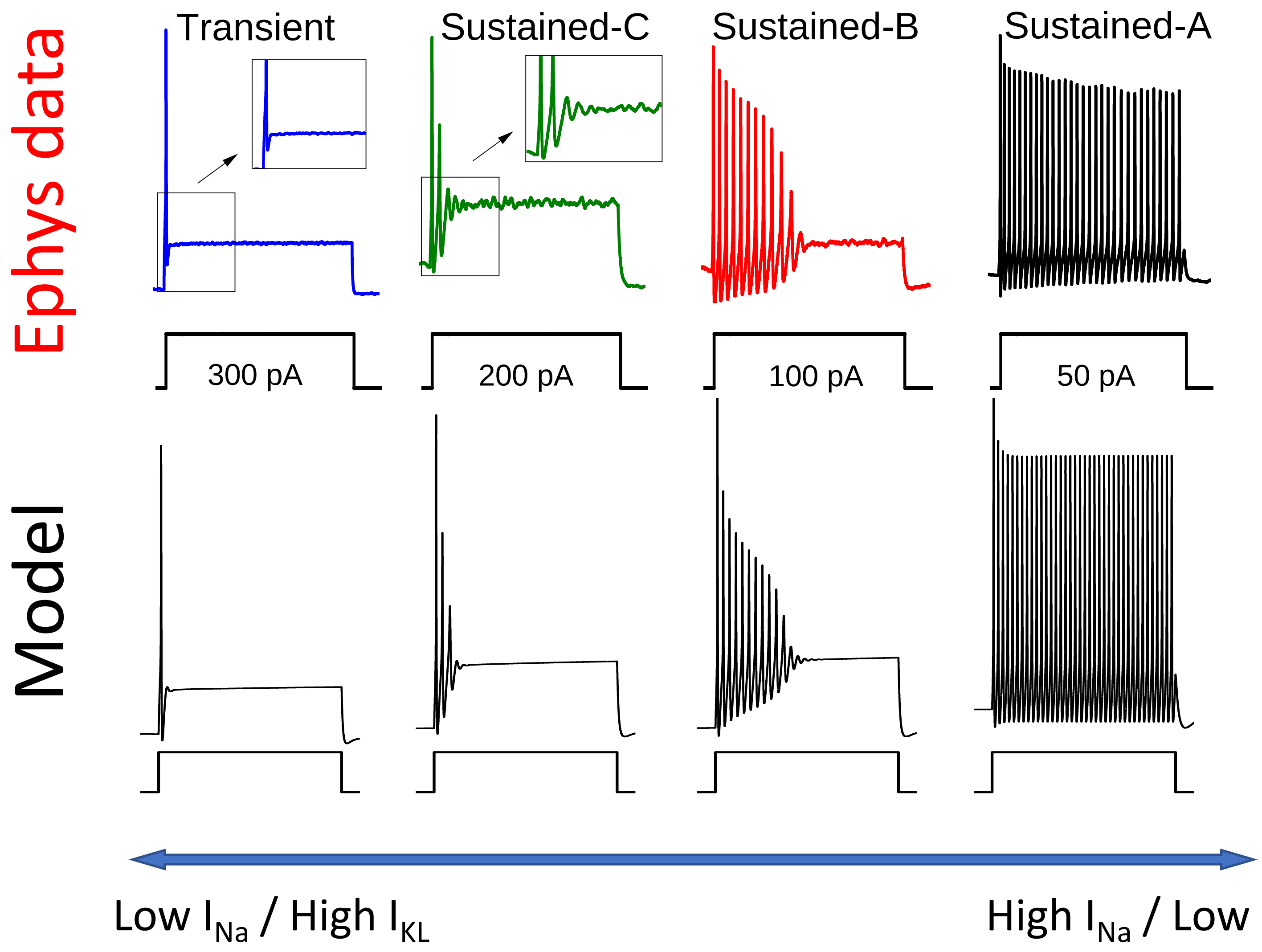
Voltage-dependent linear activation

Blocking variable

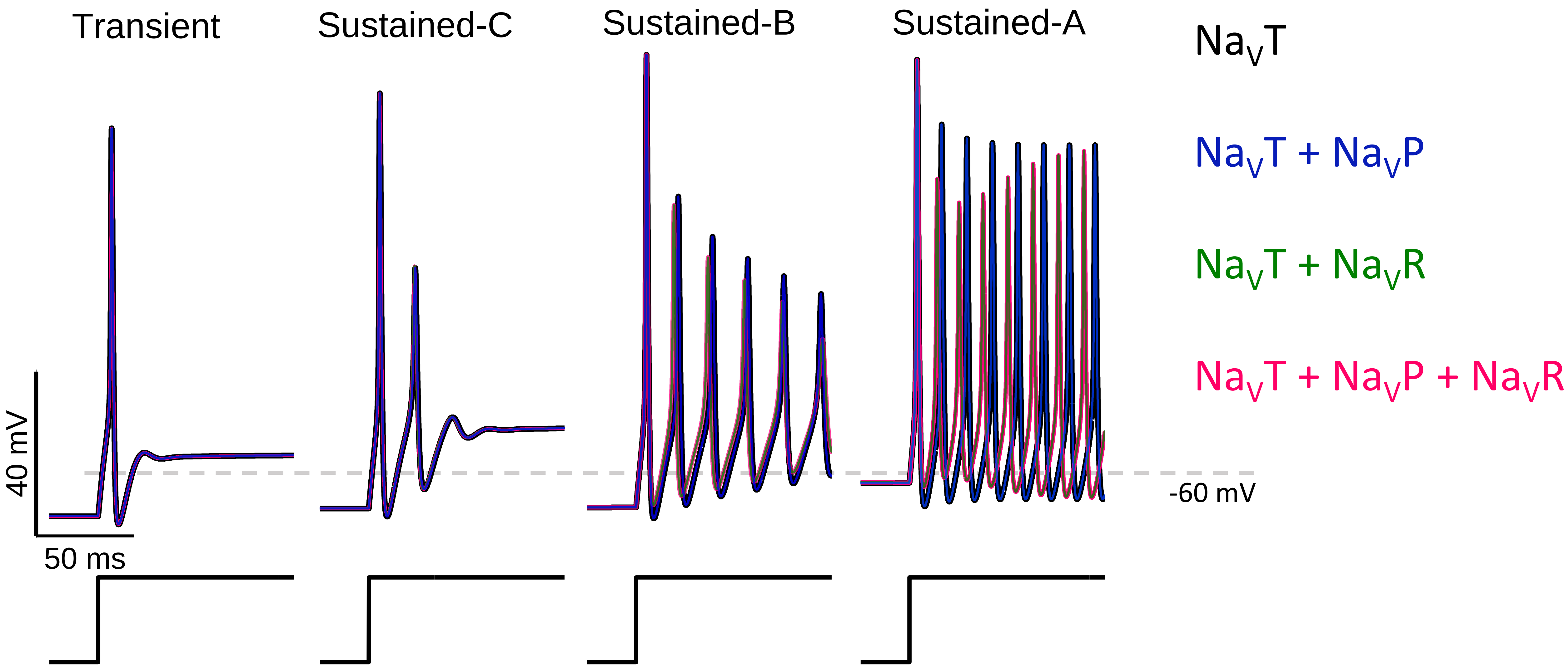
$$I_{NaR} = g_{NaT}((1 - b_r)^3 h_{r^5})(V - E_{Na})$$

Venugopal S, **Seki S**, **Terman DH**, **Pantazis A**, **Olcese R**, **Wiedau-Pazos M**, **Chandler SH**. Resurgent Na+ Current Offers Noise Modulation in Bursting Neurons. *PLOS Computational Biology* 15: e1007154, 2019.

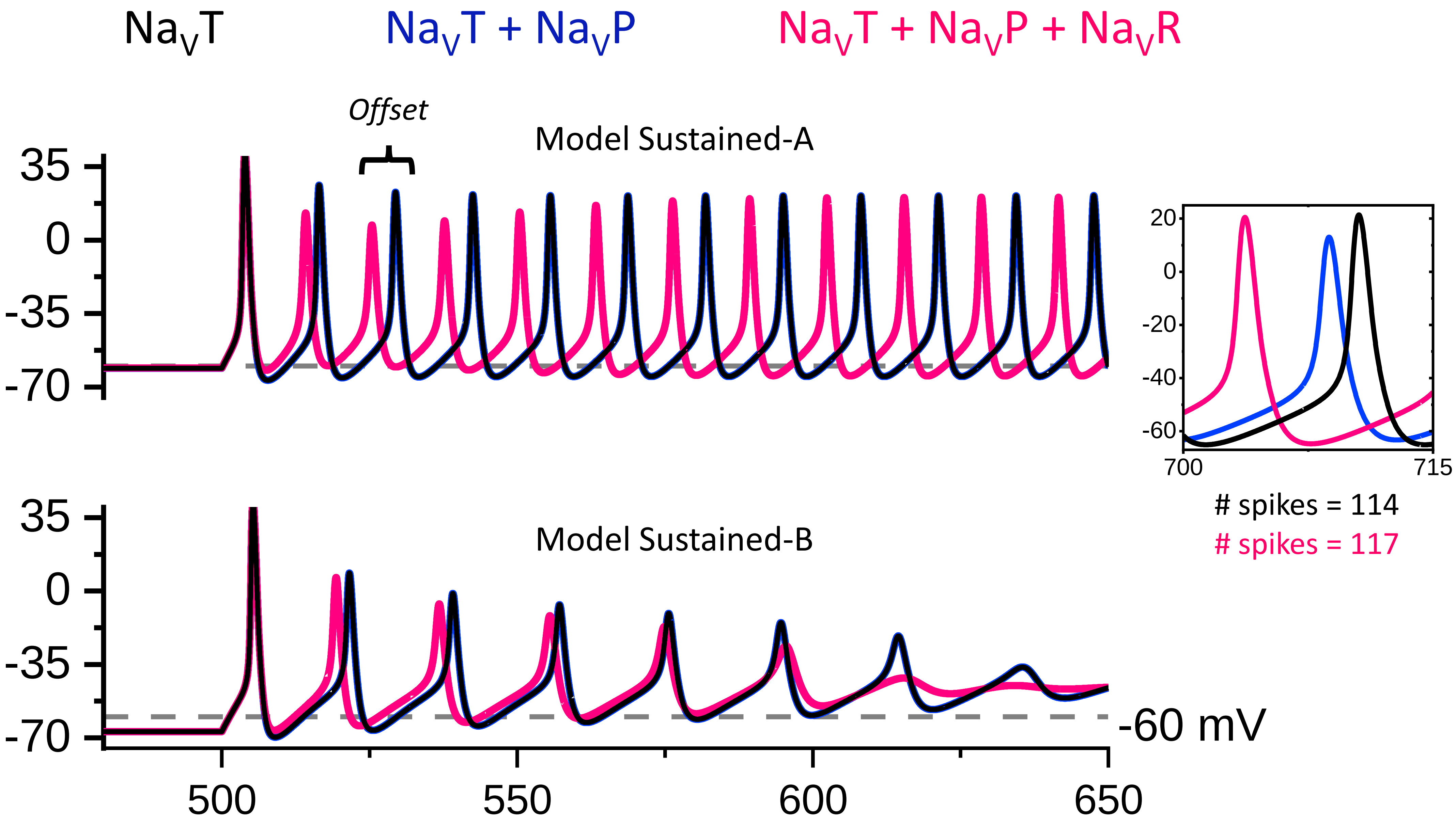
Model current clamp responses resemble those of real VGN neurons



Adding Na_VP + Na_VR hastens spiking in model sustained-A and –B VGN

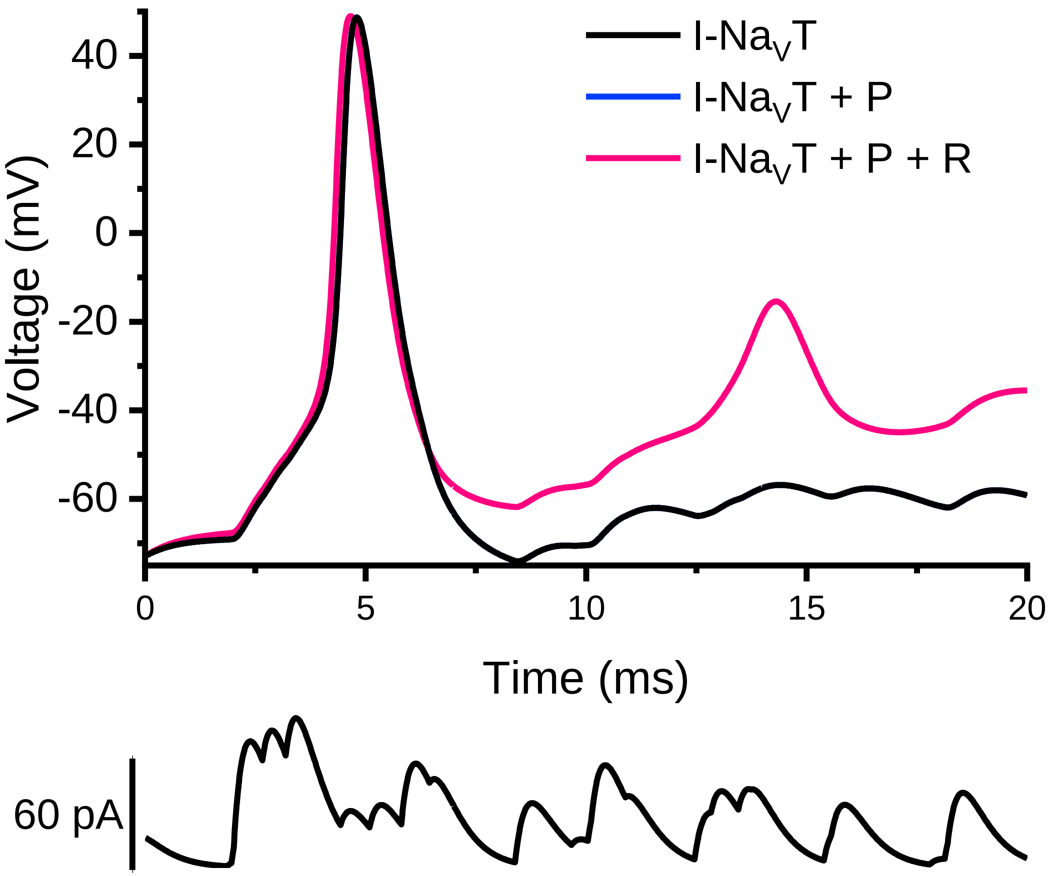


Adding Na_VP + Na_VR hastens spiking in sustained-A and –B VGN

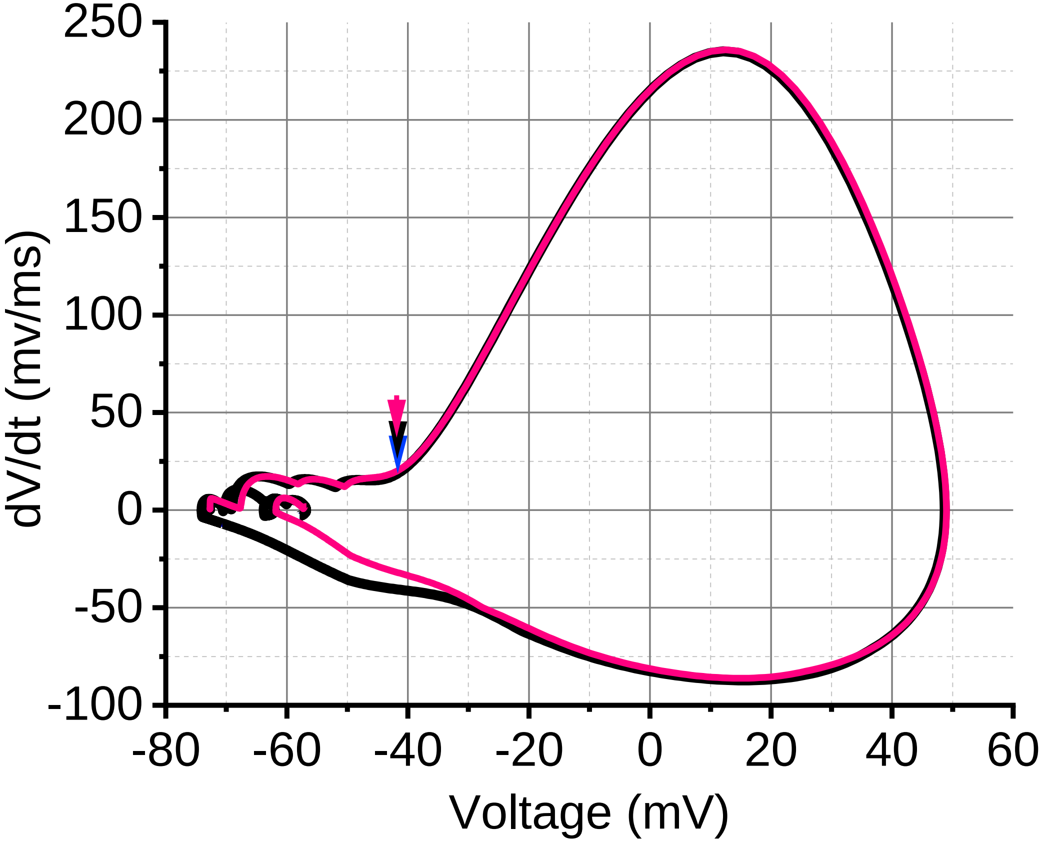


Simulating EPSC-evoked firing:
Na_VP + Na_VR increases excitability but does not alter AP waveform in model Transient VGN

Model Transient



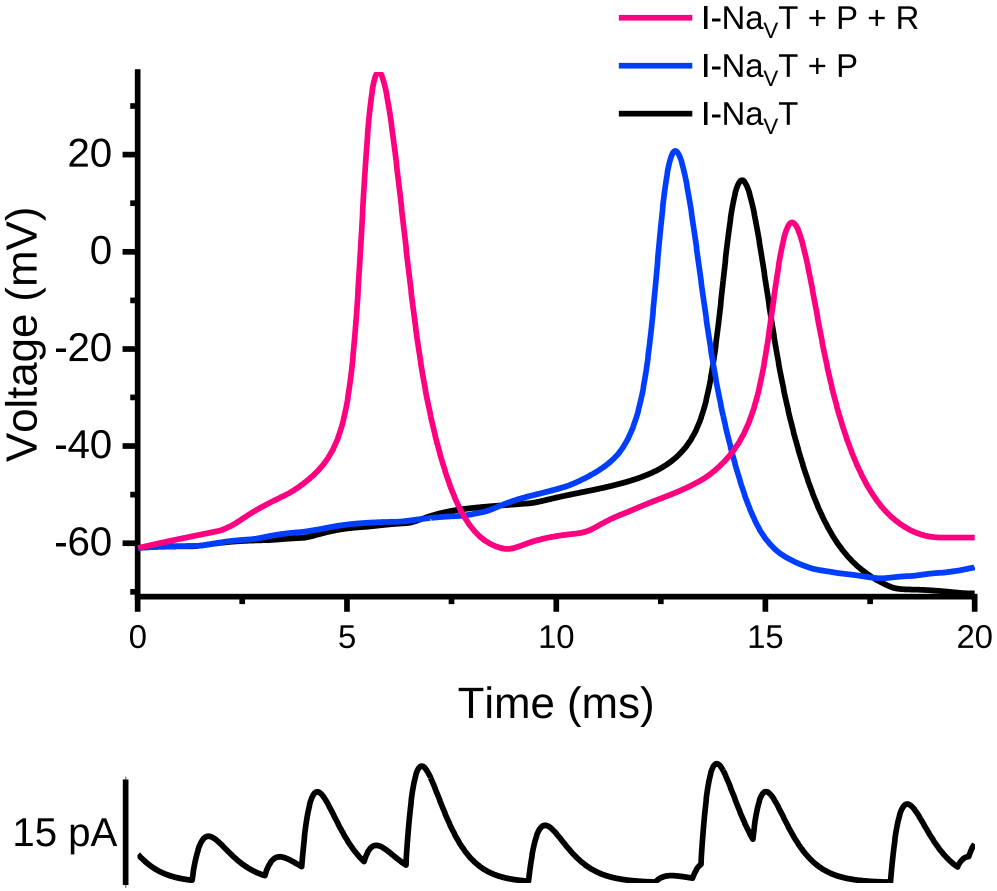
Adding Na_VP + Na_VR induced rebound spike



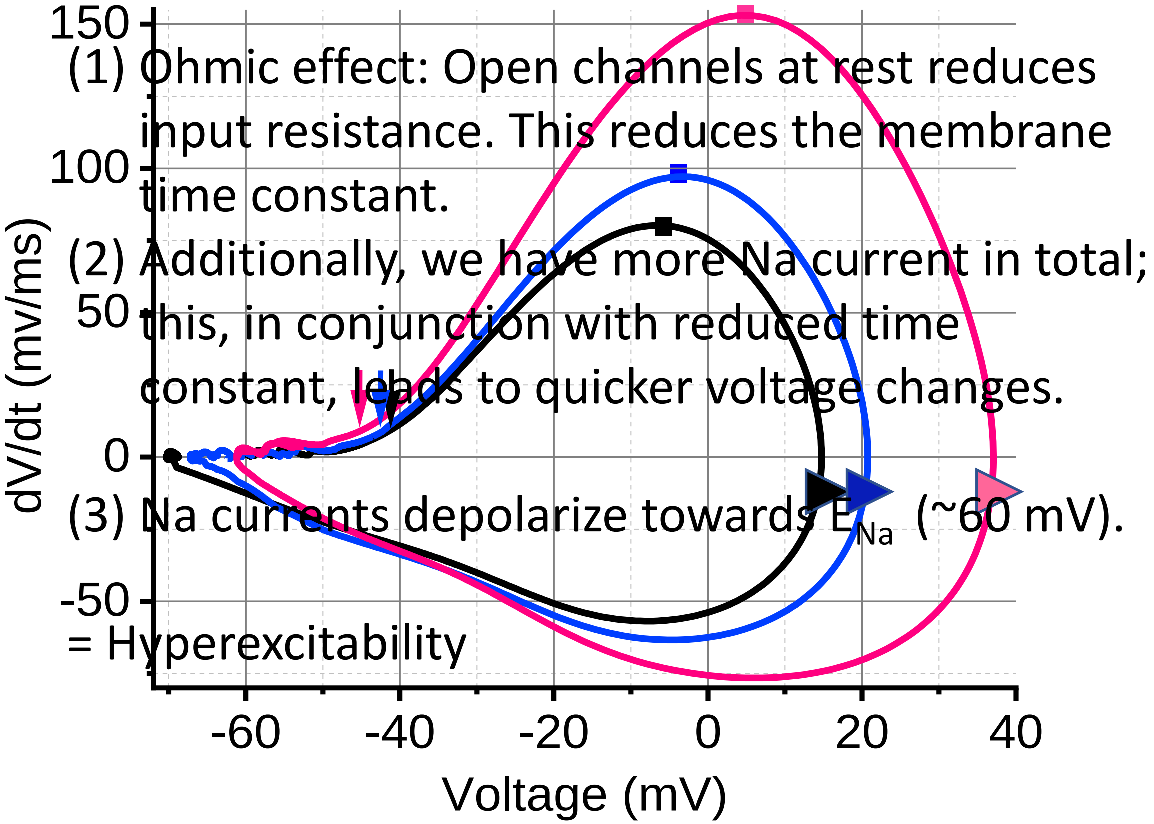
But had little influence on AP waveform

Na_VP and Na_VP + Na_VR increases excitability and alters AP waveform in model Sustained-A VGN

Model Sustained-A



Adding Na_VP and Na_VP + Na_VR reduced time-to-spike



(1) Ohmic effect: Open channels at rest reduces input resistance. This reduces the membrane time constant.

(2) Additionally, we have more Na current in total; this, in conjunction with reduced time constant, leads to quicker voltage changes.

(3) Na currents depolarize towards E_{Na} (~60 mV).

= Hyperexcitability

And influenced action potential height (triangles), rate of AP depolarization (squares), and voltage threshold for spiking (arrows)

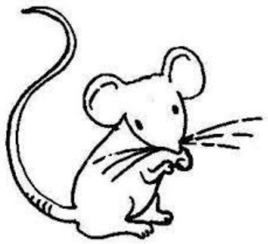
In summary:

- $Na_V P$ are present in approximately half of VGN tested and $Na_V R$ is far less frequent (>10%).
- We are unable to directly test whether this influences firing pattern, indirectly spike time regularity.
- Using a model, we predict what impact $Na_V P$ and/or $Na_V R$ could have on different firing patterns.
- $Na_V P$ and $Na_V R$ seem to have greatest impact on model Sustained-A VGN (lowest I_{KL}).
- $Na_V P$ and $Na_V R$ <u>increases excitability</u> by decreasing refractory period, reducing time-to-spike, increasing rate of depolarization and spike height.

Next:

Testing the possible ramifications this effect may have on sensory encoding.

Questions?



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hhmi Howard Hughes Medical Institute

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