IMPACT OF PERSISTENT AND RESURGENT VOLTAGE-GATED SODIUM CURRENTS ON EXCITABILITY IN MOUSE VESTIBULAR GANGLION NEURONS

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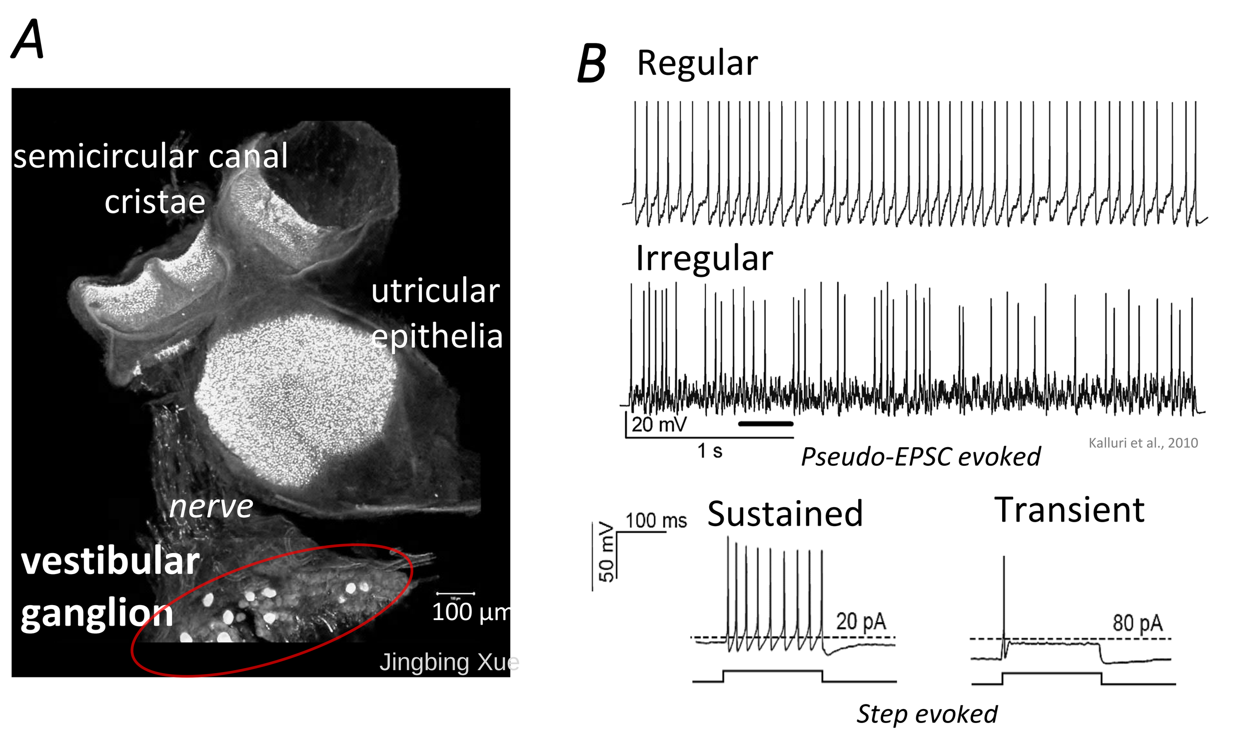
MOTIVATION: Vestibular ganglion neurons (VGN) are the cell bodies of primary vestibular afferents in the inner ear. Expression of different ion channels affects VGN **firing properties** that contribute to encoding of sensory stimuli. The impact of diverse voltage-gated sodium (**Na_V**) **currents** on firing patterns remains unknown.

APPROACH: Persistent and **resurgent Na_V currents** were recorded from isolated, cultured mouse VGN by whole-cell patch clamp. **Voltage step protocols** revealed voltage and time dependence. **Current clamp protocols** showed firing patterns. **Computational modeling** showed the impact of Na_V current components on step-evoked spiking, currents, and responses to simulated synaptic inputs.

RESULTS AND CONCLUSIONS: Persistent Na_V occurred throughout postnatal development; **resurgent Na_V currents** appeared after the first week, as firing patterns mature. Simulations suggest that both current components support sustained (regular) firing patterns by **increasing excitability**, reducing spike latency and increasing spike rate in step-evoked firing.

1. Background: *VGN encode head motions with different firing patterns*



Vestibular ganglion neurons innervating sensory epithelia **(A)** have **regular** or **irregular timing of action potentials (APs)**, corresponding to **(B) sustained** and **transient** firing patterns in vitro (Kalluri et al., 2010).

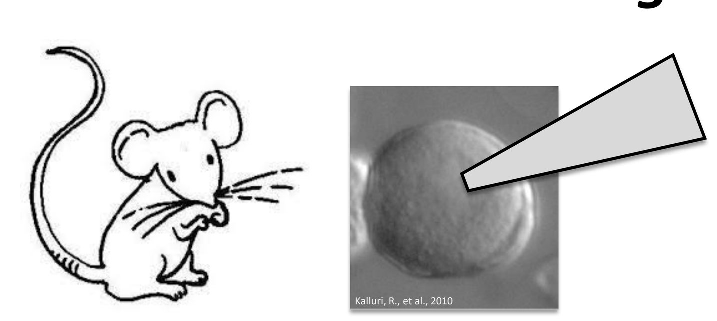
Rat VGN express multiple Na_V pore-forming (α) subunits that pass ***transient Na_V current (Na_VT)*** (Liu et al., 2016).

Na_V currents can also have **persistent** and **resurgent** forms (**Na_VP, Na_VR**) which can be significant near AP threshold, affecting neuronal excitability (Raman & Bean 1997).

Both have been described in vestibular afferent endings (Meredith and Rennie, 2020).

We are investigating whether differences in expression of ***Na_VP*** and ***Na_VR currents*** contribute to differences in regularity of firing between VGN.

2. Methods: *Whole-cell patch clamp and conductance-based VGN modeling*



Ruptured-patch whole-cell clamp from VGN cell bodies, enzymatically and mechanically dissociated and cultured overnight. *CD1 mice, P3-25*

Voltage clamp: *Steps, ramps.* External solutions: reduced Na^+, Ca^{2+} free, Cs^+ replaced K^+, +TEA. Internal: Cs^+ replaced K^+. Na_V current was isolated by subtracting data in 1 µM TTX.

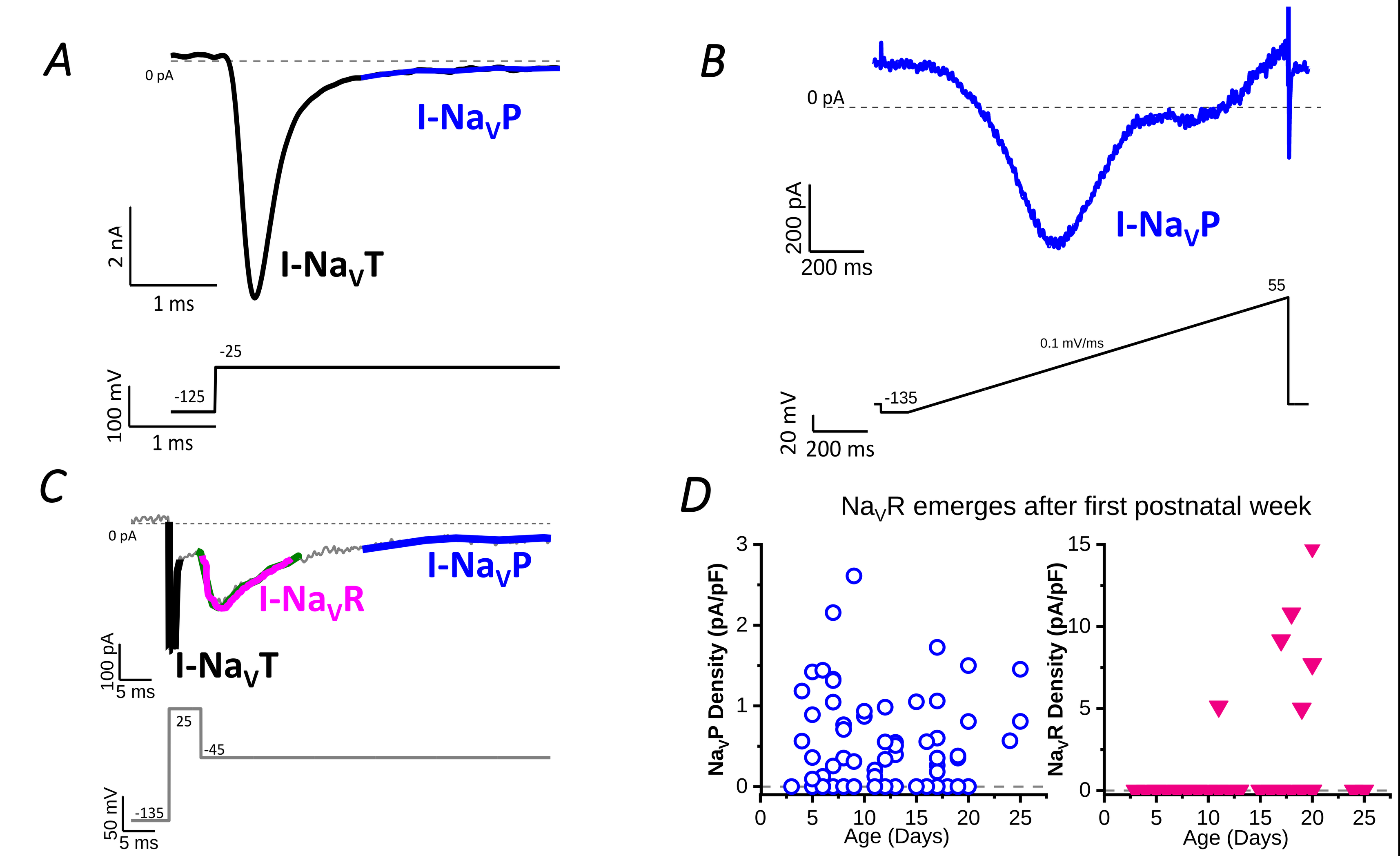
Current clamp: *Steps* to evoke spikes. Standard external solutions (high Na^+, Cl^-) internal (high K^+, Cl^-, 10 mM EGTA).

Modeling: Spike trains and currents were modeled using a Hodgkin-Huxley-based neuron model (Hight and Kalluri, 2016) altered to include Na_VR and Na_VP current components (Venugopal et al., 2018):

$$I_{inj} = Cm\,S\frac{dV}{dt} + I_{KL} + I_{KH} + I_{Na} + I_H + I_{leak}$$
$$I_{Na} = I_{NaT} + I_{NaP} + I_{NaR}$$
$$I_{NaT} = g_{NaT}(m_t{}^3 h_t)(V - E_{Na})$$
$$I_{NaP} = g_{NaP}(m_{p\infty} h_p)(V - E_{Na})$$
$$I_{NaR} = g_{NaT}((1 - b_r)^3 h_r{}^5)(V - E_{Na})$$

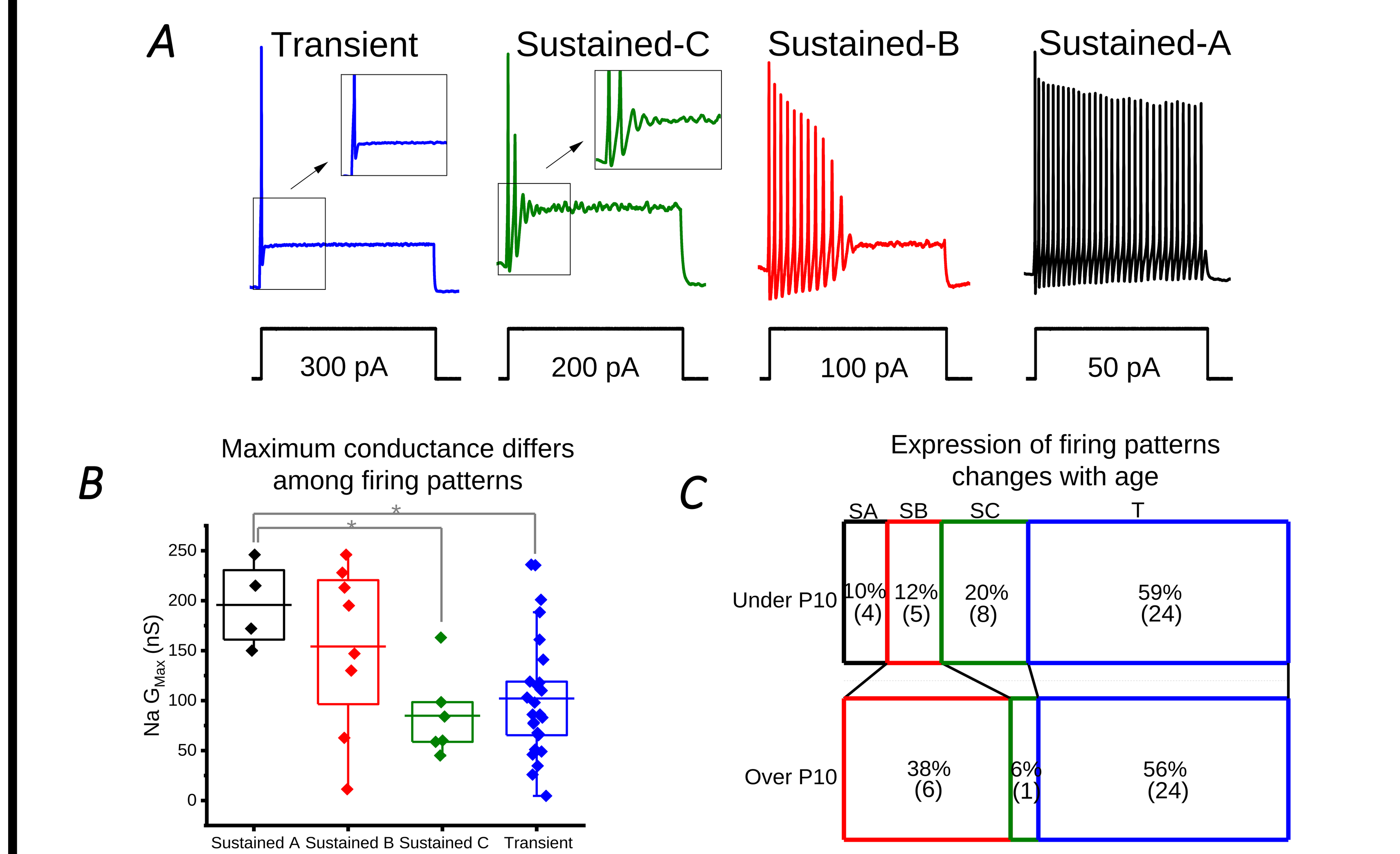
S: surface area (10 pF); **Cm**: membrane capacitance (0.9 uF/cm2); **E_{Na}**: reversal potential for sodium (60 mV); **m**: activation variable (mt: transient act, mp: persistent act); **h**: inactivation variable (ht: transient inact, hp: persistent inact, hr: resurgent inact); **b_r**: resurgent blocking variable

3. Voltage clamp: *VGN have Transient (Na_VT), Persistent (Na_VP) & Resurgent (Na_VR) currents*



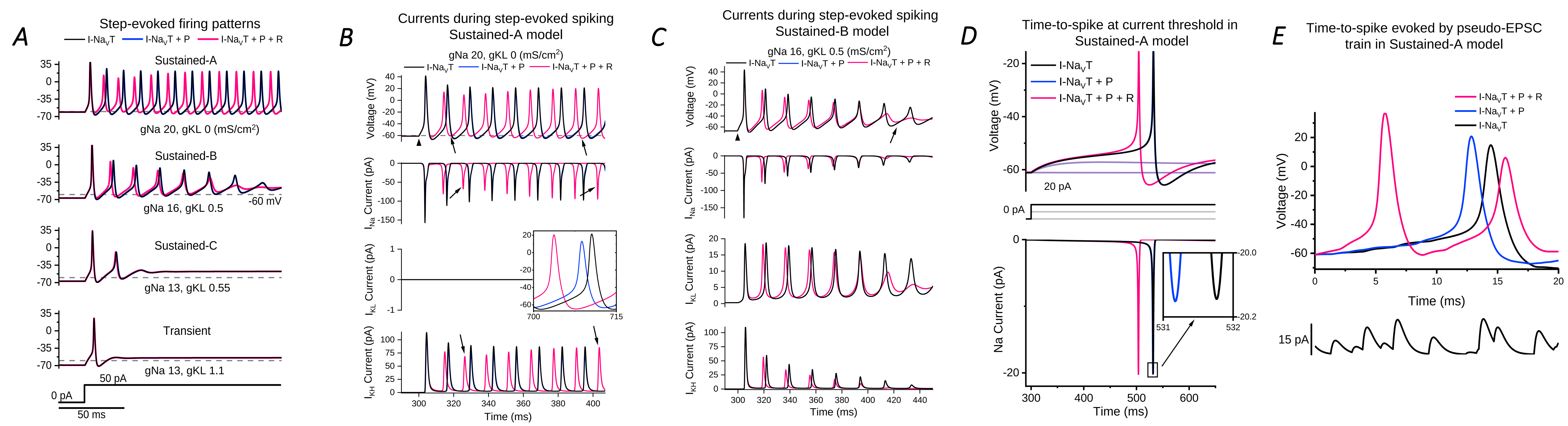
Voltage clamp: (A) All cells had I-Na_VT. Of 78 cells tested **(B)** 42 had I-Na_VP, **(C)** 6 had I-Na_VR, **(D)** where the relatively late appearance of I-Na_VR is also seen in spiral ganglion neurons (Browne et al., 2017). Not shown: Na_V1.6 blocker blocked 70% of I-Na_VT and all but residual I-Na_VP and I-Na_VR, indicating significant Na_V1.6 contribution.

4. Current clamp: *Step-evoked VGN firing patterns*



Current clamp: (A) 4 step-evoked firing patterns in VGN. **(B)** Sustained-A VGN had larger maximum Na_V conductance than Sustained C and Transient VGN (One-way ANOVA; **, p < 0.01). **(C)** Most frequent patterns in mature VGN are sustained-B and transient**.** While this reflects upregulation of I-K_{LV} (low-voltage-activated K currents; Kalluri et al., 2010), developmental changes in I-Na_V such as the addition of I-Na_VR may also play a role.

5. Modeling: *Adding Na_VP and Na_VR currents to Na_VT model VGN alters firing*



(A) Firing patterns evoked by steps are replicated by adjusting gKL (K_{LV} conductance) and gNa (see Hight and Kalluri, 2016). Adding Na_VP + Na_VR currents increases spike rate of *Sustained-A* and *-B* model VGN during positive current steps, but has no effect on firing of *Transient* or *Sustained-C* model VGN (which have greater gKL). **(B)** I-Na_VT+P+R fires faster than I-Na_VT alone , but the shorter refractory period reduces initial amplitudes of all currents (black arrows). **Inset**: I-Na_VT + P condition fires slightly faster than I-Na_VT alone. **(C)** Adding I-Na_VP + R dampens Sustained-B resonance. **(D, E)** I-Na_VT+P or I-Na_VT+P+R added to Sustained-A model **(D)** decreases spike latency to steps (top; **Inset** bottom graph) shows that I-Na_VT+P is slightly faster than control) and **(E)** increases rate for the same train of simulated EPSCs.

6. Summary

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- Na_VP and Na_VR currents have relatively negative voltage-dependence and noninactivating properties (Raman et al., 1997) that may influence firing patterns of VGN afferents. Na_VR currents were seen more frequently after the second postnatal week.
- Of the four step-evoked firing patterns in VGN, the sustained-A pattern, which may be immature, was associated with greater total Na_V conductance.
- In a model of sustained-A VGN, adding Na_VP and Na_VR currents to Na_VT current enhanced excitability: reducing the time to spike onset and increasing spike rate for step-evoked firing, and reducing the integration time for EPSCs to reach spike threshold. These effects may impact spike regularity.
- In a model of transient VGN, K_{LV} conductances reduce excitability (Kalluri et al. 2010) and adding Na_VP and Na_VR currents had no impact.

References

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