Optimization of genetically encoded voltage and calcium indicators for *in vivo* imaging: GENIE Project Team updates

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Funded by Howard Hughes Medical Institute

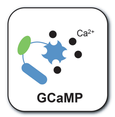
Motivation

Protein sensors are routinely used to image neuronal activity and they underlie many recent advances in cellular functional imaging. However, many sensors still have major limitations when used *in vivo*. The GENIE project engineers and optimizes fluorescent sensors for imaging of neuronal activity in the intact nervous system of experimental animals.

Summary

Chemigenetic voltage indicator improvement

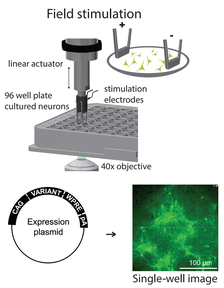
- Voltron2 showed a 65% increase in 1AP sensitivity and 3x better subthreshold membrane potential detection.
- Mutations in the voltage-sensing domain (Ace2N) were swapped between various types of voltage indicators and compared with patch-clamp.
- A new positive-going variant, Positron2, showed a 300% improvement in sensitivity.

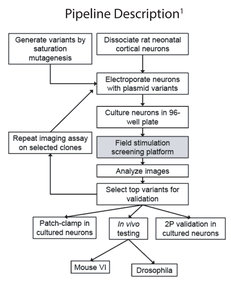


jGCaMP8s and 8m transgenic mice

- TetO-jGCaMP8s mouse has ~5X increased SNR compared to TetO-GCaMP6s. Sensitivity and fast kinetics match AAV performance.
- TIGRE jGCaMP8s/m knock-in mice stably expressed in targeted neuronal subpopulations with reduced tetTA toxicity effects.
- Mice available soon from JAX.

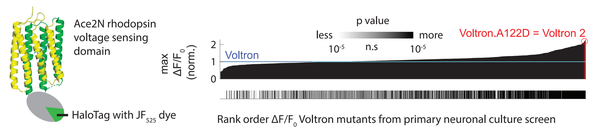
GENIE screening pipeline





Genetically encoded calcium, voltage, and neurotransmitter sensors were screened in a high-throughput neuronal culture assay at a max rate of ~2000 variants per month. The automated field-stimulation and imaging platform provided rapid testing in dissociated rat cortical/hippocampal neurons. Variants with improved sensitivity, kinetics, brightness were further validated in *in vivo* mouse and *Drosophila* assays. The suite of improved sensors (jGCaMP8s/m/f[7], Voltron2, iGABASnFR2/2n) were successfully identified using this platform.

Voltron2/Positron2

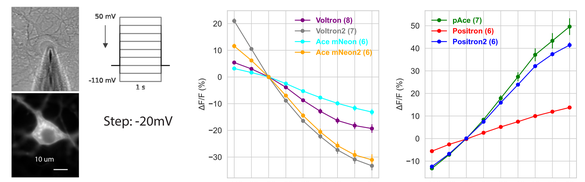


Ace2N-based voltage indicators

	Ace-mNeon		negative-going sensor[2]	
	Ace-mNeon2	N81S G229Y	negative-going sensor[6]	
	pAce	R78K S81D D92N W178F	positive-going sensor[6]	
	Voltron		negative-going sensor[3]	
	Voltron2	A122D	negative-going sensor[4]	↑ 65% sensitivity
	Positron	N81D D92N E199V	positive-going sensor[5]	
NEW	**Positron2**	R78K N81D D92N W178F	positive-going sensor	↑ 300% sensitivity

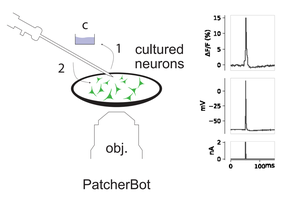
Patch-clamp head-to-head comparison

Peak fluorescence responses of negative and positive going GEVIs to voltage steps.

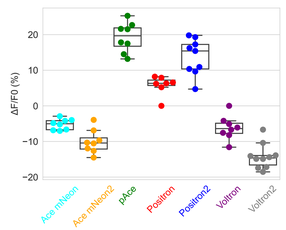


Spike detection

Current pulses (2 ms) were injected to evoke action potentials

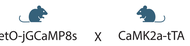


Fluorescence responses to single spikes

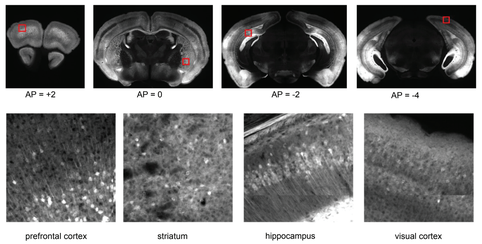


Voltron2 and Ace-mNeon2 protein sensors have been independently developed[4,6] and both have proven to be useful indicators of cellular transmembrane voltage. The Voltron and Ace-mNeon families of variants (negative and positive-going) were compared using patch-clamp and the peak fluorescence response to voltage steps was measured. Voltron2 sensitivity was 65% greater than Voltron and had 3X better subthreshold detection. Key mutations in the Ace2N domain of the sensors were shuffled and tested for novel activity. Recombining mutations from pAce and Positron led to a 300% improvement in sensitivity of a novel variant we call Positron2.

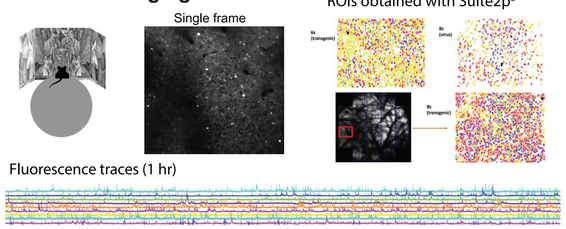
jGCaMP8 Transgenic Mice: TetO-jGCaMP8s



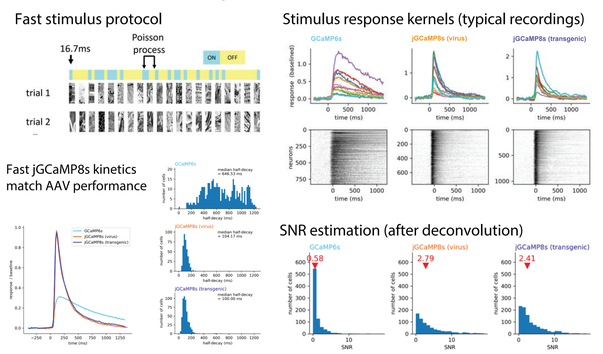
Histology



Two-Photon Imaging

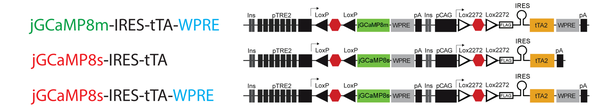


Estimation of sensor dynamics in awake mice



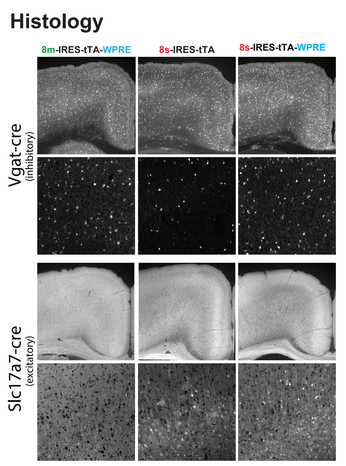
We screened 13 TetO-jGCaMP8s lines by crossing them with CaMK2a-tTA and performed histology on the double positive pups at 42 days. Three lines with good expression levels were further analyzed *in vivo*. To assess the quality of the responses, we developed a novel visual stimulation protocol based on fast visual stimulus presentation, which resulted in stimulus response kernels for each neuron. The kernels allowed us to distinguish faster and slower sensors in awake animals during normal imaging conditions, and we picked the best line to make available. The TetO-jGCaMP8s mouse had a ~5X increase in SNR compared TetO-GCaMP6s mouse[9]. The fast kinetics observed with jGCaMP8s AAV injection were maintained in the transgenic mouse. Mice will be available at JAX.

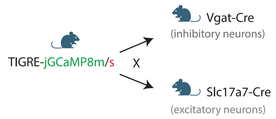
TIGRE-jGCaMP8: Knock-in mice



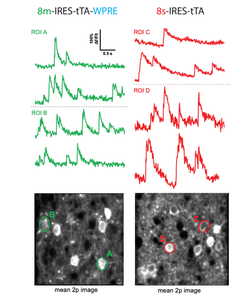
Knock-in at the tightly regulated (TIGRE) genomic locus via recombinase mediated cassette exchange

Histology





In vivo two-photon recording



100 Hz recording of spontaneous activity
250 µm depth V1; retro-orbital PHP.eB syn-Cre

We generated several knock-in strains at the TIGRE locus that co-express jGCaMP8s (or 8m) and tetTA2 in a Cre-dependent manner[10]. In these 3rd generation TIGRE lines, we inserted an internal ribosome entry site (IRES) to attenuate tetTA2 translation and thereby avoided previously reported issues associated with tetTA overexpression; offspring from pan-excitatory and pan-inhibitory crosses exhibited robust indicator expression while displaying no perinatal lethality, epileptiform activity, or signs of neurodegeneration. Both jGCaMP8s and jGCaMP8m mice displayed fluorescent dynamics with fast kinetics, as expected based on the biophysical characterization of jGCaMP8s and jGCaMP8m[7]. Mice will be available from JAX.

Reagents

- jGCaMP8 mice: JAX (*pending*)
- jGCaMP8 (plasmids/AAV): Addgene
- Voltron2/ Positron (plasmids/AAV): Addgene
- Positron2 (plasmids): Contact us above

Ongoing projects

- Chemigenetic calcium sensor
- iGluSnFR4 glutamate sensor
- Improved jGCaMP8 *Drosophila* expression

Acknowledgements

Janelia Cell Culture, Histology, Transgenic, Vivarium, and Quantitative Genomics teams specifically Deepika Walpita, Amy Hu, Monique Copeland, Ben Foster, Andy Lemire. TIGRE construct design help from Bosiljka Tasic (Allen Institute for Brain Science). PHP.eB syn-Cre virus was a gift from Tanya Daigle (Allen Institute for Brain Science)

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