Closed-Loop Optogenetic System for Deep Behavioral Phenotyping

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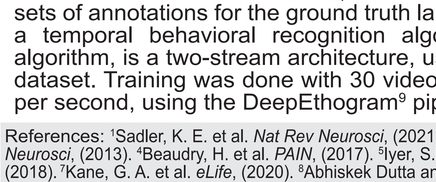
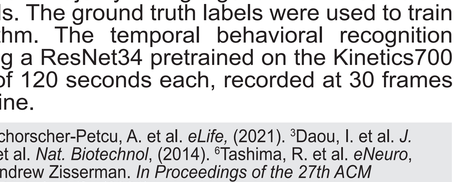
Significance

- Measures of responses to sensory stimuli in mice are generally limited to a small number of paradigms that do not capture the full rainge of responses and their features[1].
- Optogenetic methods are ideal to study specific subtypes of sensory neurons and the behavioral responses that they evoke[2-6].
- Here, we combine interests to reduce subjectivity and increase reproducibility with optogenetics to develop a new closed-loop system to monitor and record light-evoked responsess in sensory neuron subpopulations in the mouse hindpaw (CLOSER: Closed-Loop Optogenetic System for Evoked Responses). This system adds depth of information, reduces subjectivity, and increases reproducibility.
- We use CLOSER to classify behaviors and their patterns using mouse models expressing Channelrhodopsin2 (ChR2) in either heat- (*Trpv1*-lineage) or mechano- (*Cacna1h*-lineage) sensitive neurons.

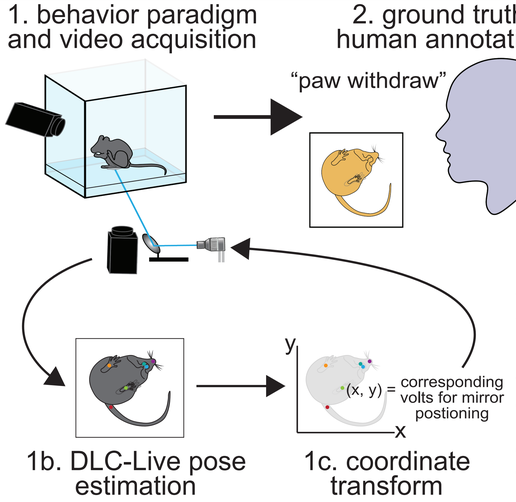
Optogenetics in Sensory Neurons



Mouse Models



Method Development



(**1b**) DeepLabCut-Live (DLC-Live)[7] continuously tracks the location of specified bodyparts (both hindpaws, both frontpaws, base of the tail, and base of the nose). (**1c**) Using a transform function, DLC-Live determined coordinates are converted into voltages to position the galvanometer mirror system for directed targeting of the laser to the right hindpaw.

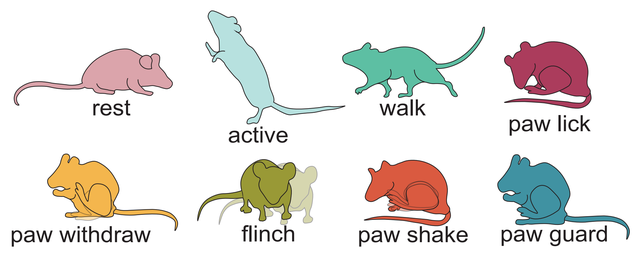
(**1**) Mice are placed in a clear box situated on an acrylic floor and allowed to move freely. Once mice are habituated, the behavior protocol is performed - the mouse is stimulated with the laser for a set number of trials with 1 min interstimulus intervals. Laser stimulation of the hindpaw only occurs if time from last stimulation, degree of movement, and paw detection confidence level, are met. All behavior experiments were video recorded for offline analysis. (**2**) Behavior annotation was performed by three trained researchers using VGG Image Annotator[8]. (**3**) The inter-annotator agreement between the three annotators was 93.8%, and we used a majority voting algorithm on these three sets of annotations for the ground truth labels. The ground truth labels were used to train a temporal behavioral recognition algorithm. The temporal behavioral recognition algorithm, is a two-stream architecture, using a ResNet34 pretrained on the Kinetics700 dataset. Training was done with 30 videos of 120 seconds each, recorded at 30 frames per second, using the DeepEthogram[9] pipeline.

References: [1]Sadler, K. E. et al. *Nat Rev Neurosci*, (2021). Schorscher-Petcu, A. et al. *eLife*, (2021). [3]Daou, I. et al. *J. Neurosci*, (2013). [4]Beaudry, H. et al. *PAIN*, (2017). [5]Iyer, S.M. et al. *Nat. Biotechnol*, (2014). [6]Tashima, R. et al. *eNeuro*, (2018). [7]Kane, G. A. et al. *eLife*, (2020). [8]Abhiskek Dutta and Andrew Zisserman. *In Proceedings of the 27th ACM International Conference on Multimedia,* (2019). [9]Bohnslav, J. P. et al. *eLife*, (2021).

Behavior Classification Results

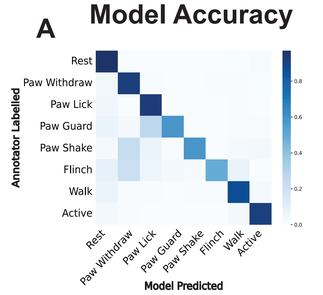
Automated behavior classification

Behavior Units of Interest

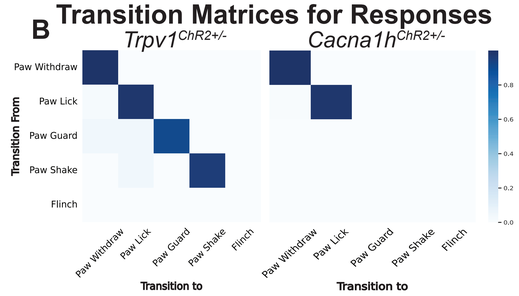


Pictograms depicting the eight behavior units of interest.

A Model Accuracy



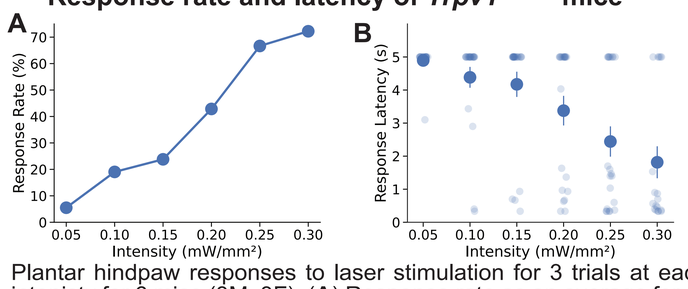
B Transition Matrices for Responses



(**A**) Class-balanced accuracy of the behavior classification model. (**B**) Transition matrices for response behaviors following laser onset for both mouse models.

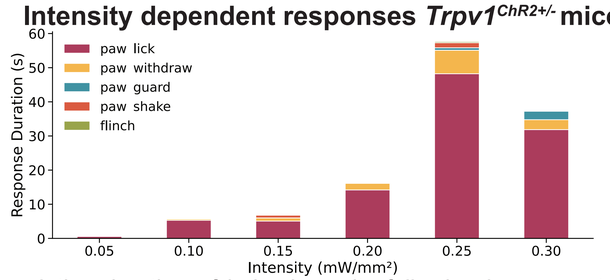
Selective activation of noxious, heat-sensitive neurons or low-threshold mechanoreceptors in mouse hindpaw skin

Response rate and latency of *Trpv1*$^{ChR2+/-}$ mice



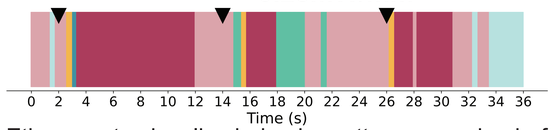
Plantar hindpaw responses to laser stimulation for 3 trials at each intenisty for 6 mice (3M, 3F). (**A**) Response rate as an average for all trials across all mice. (**B**) Latency to respond to laser stimulation at each intensity with a 5 sec cutoff time; individual trial response (dot), average data (diamond) and SEM (vertical line).

Intensity dependent responses *Trpv1*$^{ChR2+/-}$ mice



Cumulative duration of behavior units following laser onset for all mice. Each color block represents time spent in that behavior unit.

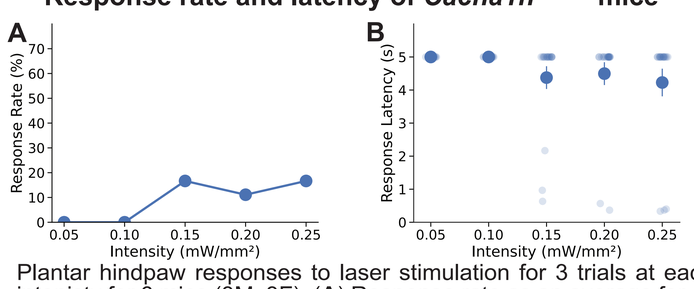
Ethogram for a *Trpv1*$^{ChR2+/-}$ mouse stimulated at 0.25mW/mm²



watch the video!

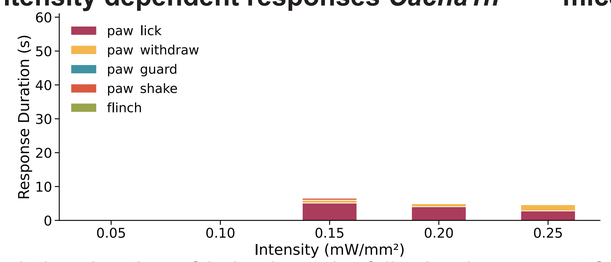
Ethogram to visualize behavior patterns occuring before (2 sec) and after (10 sec) laser stimulation of the hindpaw. Black arrows indicate laser onset. Color blocks represent identified behaviors units.

Response rate and latency of *Cacna1h*$^{ChR2+/-}$ mice



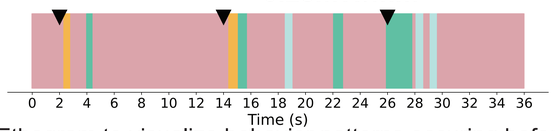
Plantar hindpaw responses to laser stimulation for 3 trials at each intenisty for 6 mice (3M, 3F). (**A**) Response rate as an average for all trials across all mice. (**B**) Latency to respond to laser stimulation at each intensity with a 5 sec cutoff time; individual trial response (dot), average data (diamond) and SEM (vertical line).

Intensity dependent responses *Cacna1h*$^{ChR2+/-}$ mice



Cumulative duration of behavior units following laser onset for all mice. Each color block represents time spent in that behavior unit.

Ethogram for a *Cacna1h*$^{ChR2+/-}$ mouse stimulated at 0.25mW/mm²



watch the video!

Ethogram to visualize behavior patterns occuring before (2 sec) and after (10 sec) laser stimulation of the hindpaw. Black arrows indicate laser onset. Color blocks represent identified behaviors units.

Resources

- The manuscript detailing the development of CLOSER is in preparation and will be available on BioRxiv by the end of the year
- All data and code used for this project will also be availble on Github through the Lipscombe Lab repository
- Code developed by David Sheinberg for video acquisition will be available through his Github repository

This work was funded by grants NS055251 (DL), T32MH020068 (RM).