

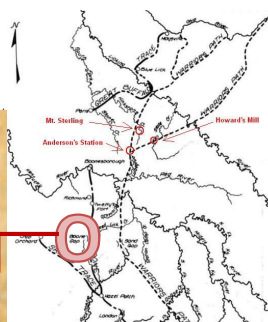
# LEARNING TO FEAR,

*and to extinguish fear.*



**Ray Luo**  
**RIKEN BSI, UCLA**

Fear is critical for survival in the real world.

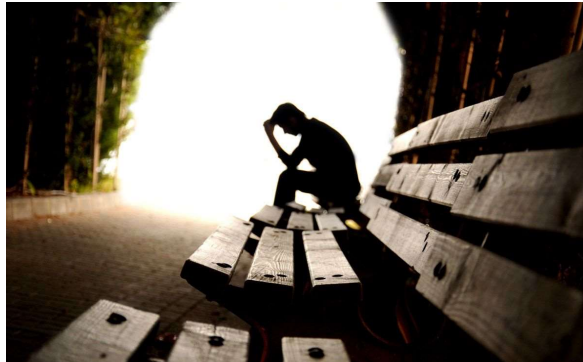


riken bsi

[rayluo.bsl.ucla.edu/projects](http://rayluo.bsl.ucla.edu/projects)

## But too much fear can destroy someone's life: Post Traumatic Stress Disorder PTSD.

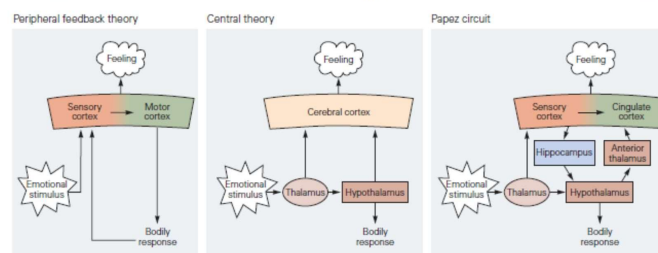
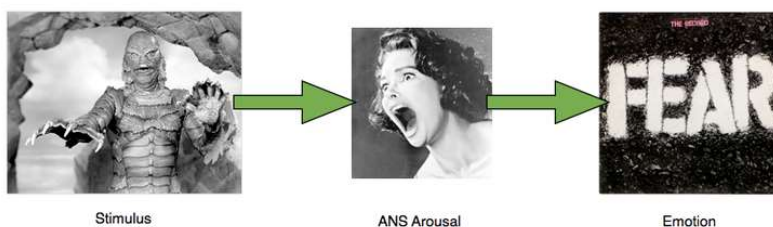
*I keep seeing  
the accident,  
over and over.  
I need help.*



riken bsi

rayluo.bol.ucla.edu/projects

## Early theories of emotion and fear implicate thalamic and cortical circuits.



LeDoux, 1996



riken bsi

rayluo.bol.ucla.edu/projects

## Klüver-Bucy syndrome: removal of temporal lobes of monkeys.

- Bizarre sexual behavior.
- Oral fixation.
- Lack fear.
- Amygdala is lesioned.

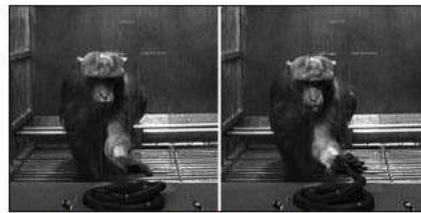


Figure 3: A monkey with Klüver-Bucy syndrome has lost his natural fear of snakes



riken bsi

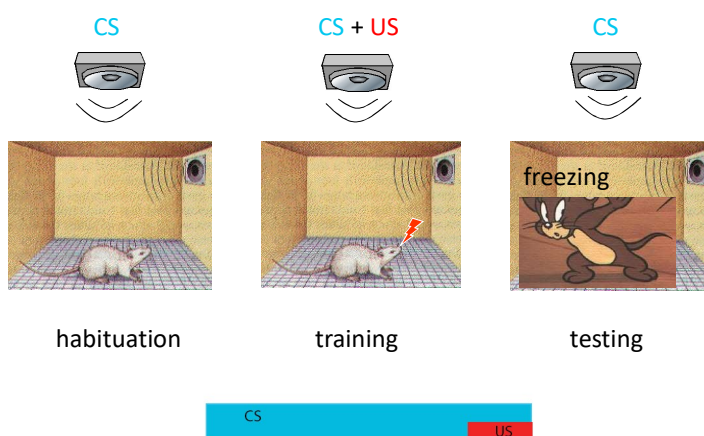
[rayluo.bol.ucla.edu/projects](http://rayluo.bol.ucla.edu/projects)

$10^{10}$  people on Earth

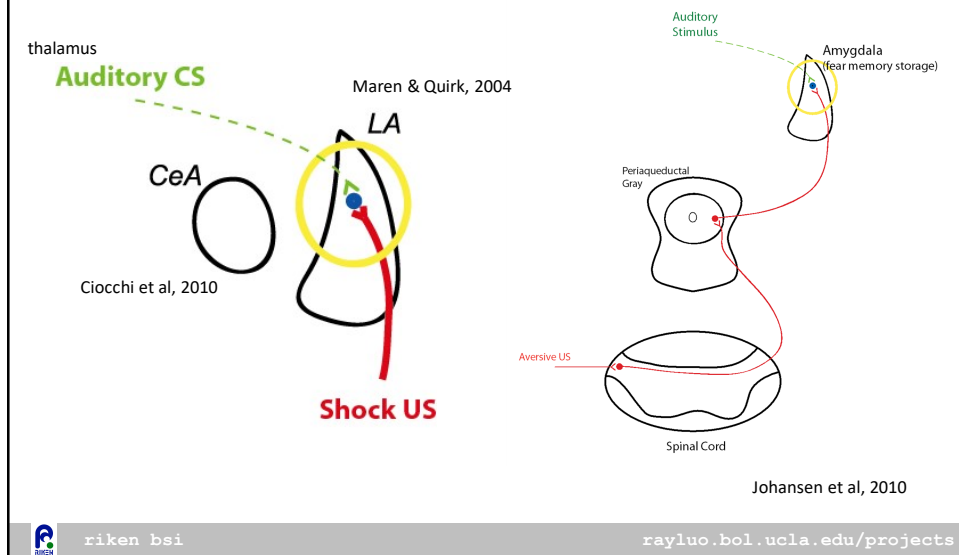




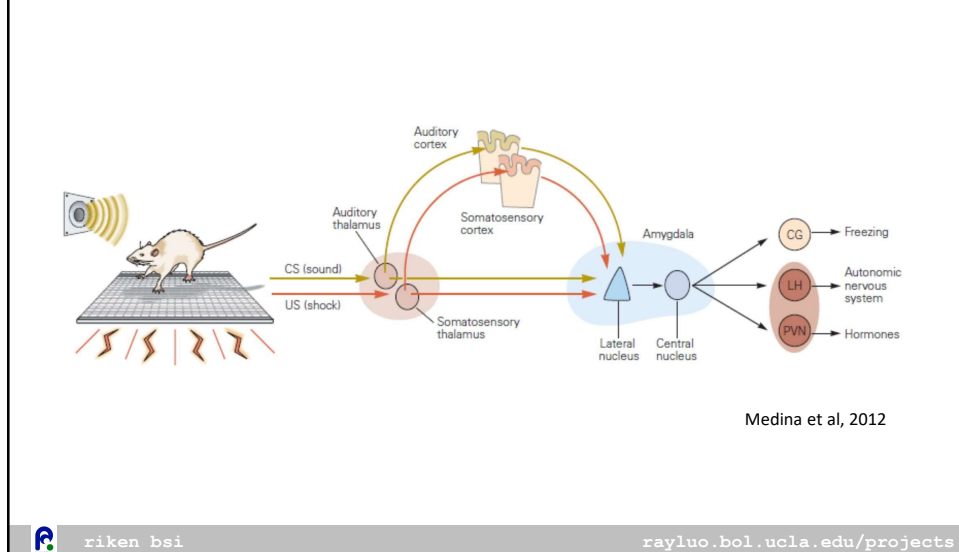
Pavlovian classical conditioning as a model for fear learning: CS tone, US shock, CR freezing.



## Microcircuitry in the amygdala mediates fear conditioning.

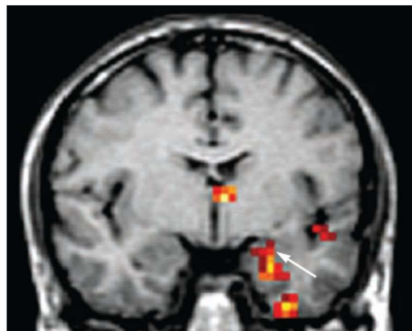


## Microcircuitry in the amygdala mediates fear conditioning.



## Human amygdala is involved in processing of fear and reward values.

- fMRI amygdala activation during CS-US pair.
- Conditioned by watching or warning of shock.
- Hippocampal lesion:
  - No explicit learning
  - But respond to CS
- Amygdala lesion:
  - No phys response
  - Recall conditioning



LaBar et al., 1998.



riken bsi

[rayluo.bol.ucla.edu/projects](http://rayluo.bol.ucla.edu/projects)

## Fear extinction is presentation of CS repeatedly without shock US.

- How do we get rid of bad memories?
- Extinction is a new form of memory.
- Extinction is context dependent.
- Renewal: return of fear in new context.
- Reinstatement: return of fear with single shock.
- Spontaneous recovery: return of fear with passage of time.

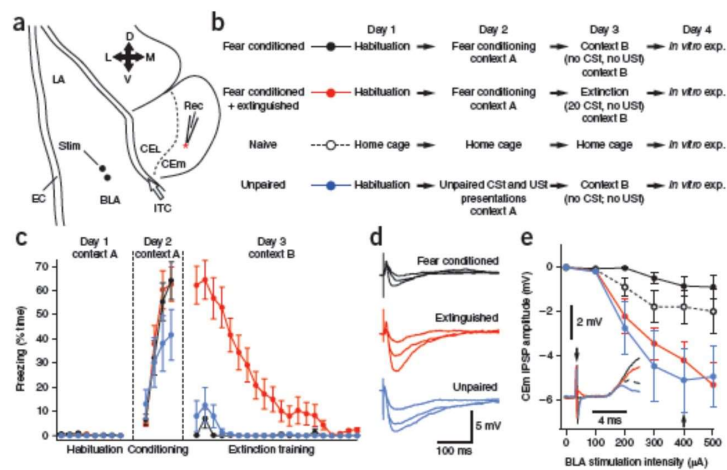


riken bsi

[rayluo.bol.ucla.edu/projects](http://rayluo.bol.ucla.edu/projects)



## Inhibition of central medial amygdala following extinction training.



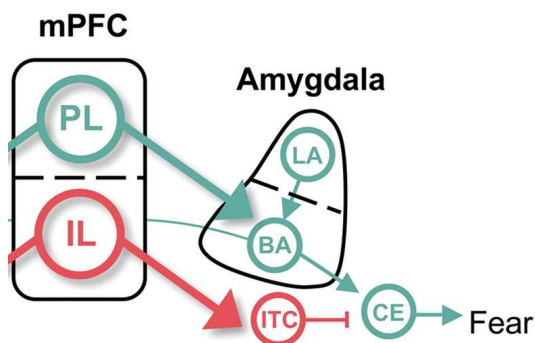
Amano et al., 2010



riken bsi

rayluo.bol.ucla.edu/projects

## Microcircuitry in the prefrontal cortex mediates fear extinction learning.



Quirk et al, 2009

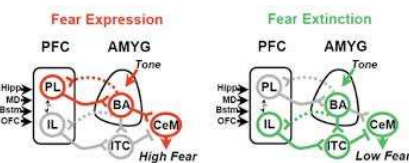
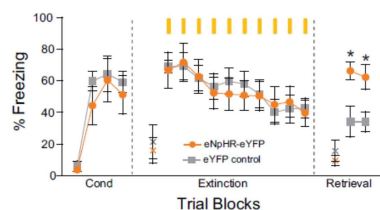


riken bsi

rayluo.bol.ucla.edu/projects

## Effects on extinction can be during acquisition or during retention.

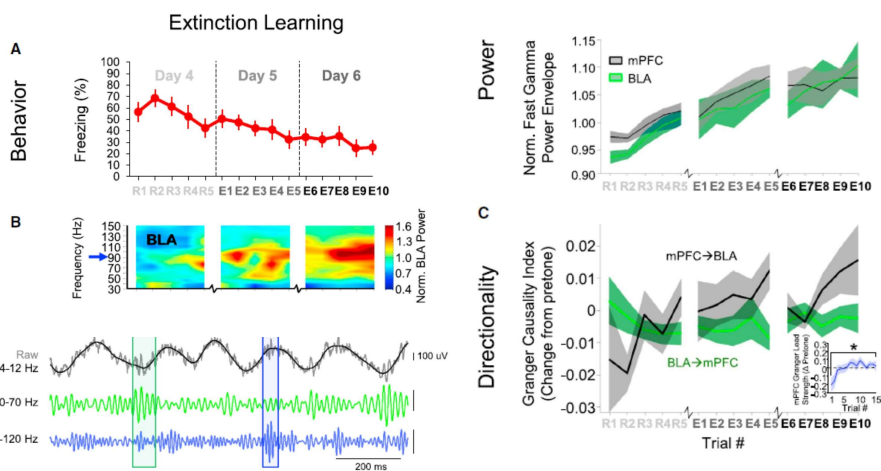
- Agonists of amygdala NMDARs facilitate extinction learning.
- BDNF activity required for long term extinction.
- Unlearning fear with immediate extinction or during reconsolidation.



riken bsi

rayluo.bol.ucla.edu/projects

## Fear extinction circuitry may require coordination of different sites.



Stujenske et al., 2014



riken bsi

rayluo.bol.ucla.edu/projects



## Question: How is fear learning modulated by different transmitter systems based on context?

- Noradrenergic system enhances fear memory (Soeter et al, 2011).
- Aversive events affect dopamine transmission (Badrinarayan et al, 2012).
- Serotonin depletion leads to attenuated fear response (Hindi et al, 2012).



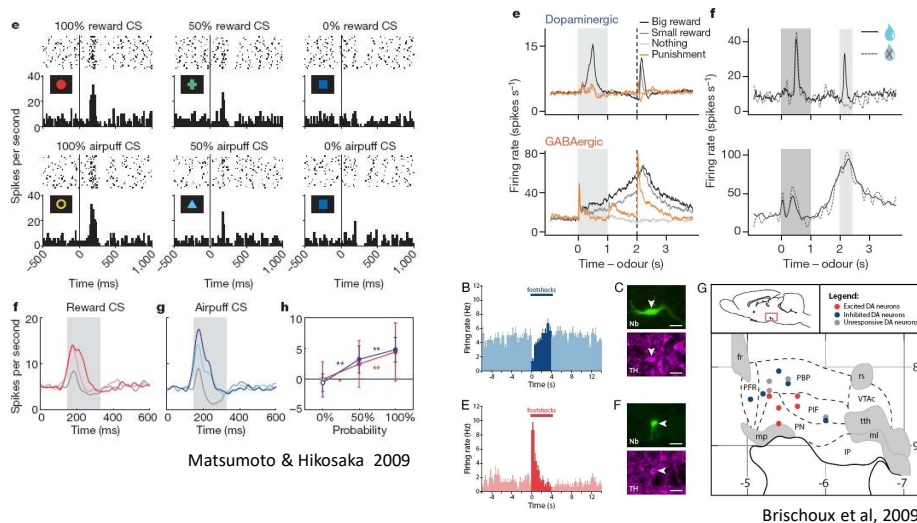
- Is dopamine system involved in fear and extinction learning?



riken bsi

rayluo.bol.ucla.edu/projects

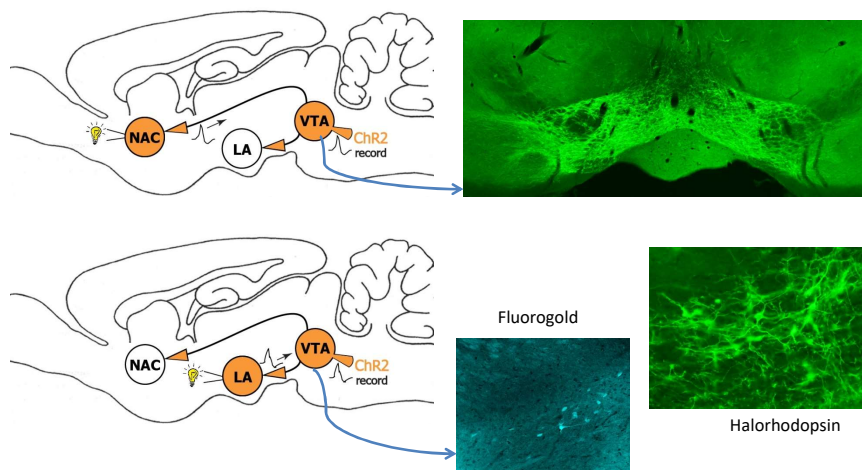
## Midbrain dopamine neurons may also respond to aversive signals.



riken bsi

rayluo.bol.ucla.edu/projects

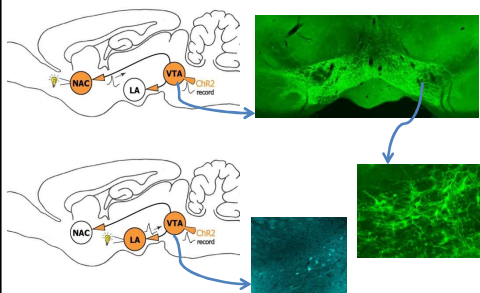
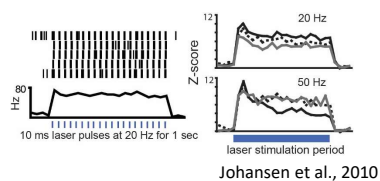
## Optogenetic identification of VTA to LA and VTA to NAc dopamine projections.



riken bsi

rayluo.bol.ucla.edu/projects

## Use optogenetics to identify dopamine cells, trace their projections, manipulate activity.



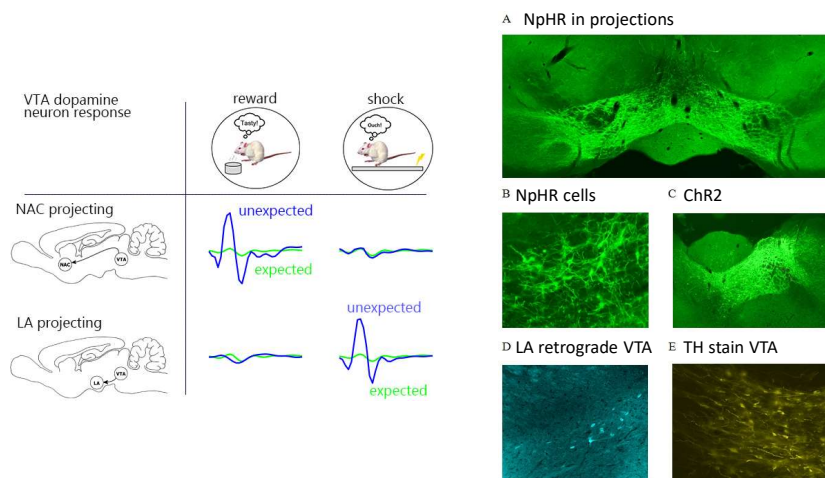
- TH-cre rats expressing a cre-dependent channelrhodopsin (ChR2) or halorhodopsin (NpHR) allows dopamine neurons – specific excitation *in vivo*.
- Virus containing ChR2 can reach terminals of transfected cells, allowing optical stimulation of their terminals at target structure to identify their projections.
- Inhibition of VTA dopamine cells during fear and reward learning using NpHR can reveal the role of dopamine neurons in appetitive and aversive learning.



riken bsi

rayluo.bol.ucla.edu/projects

## Hypothesis: VTA to LA projections code for aversive prediction error.

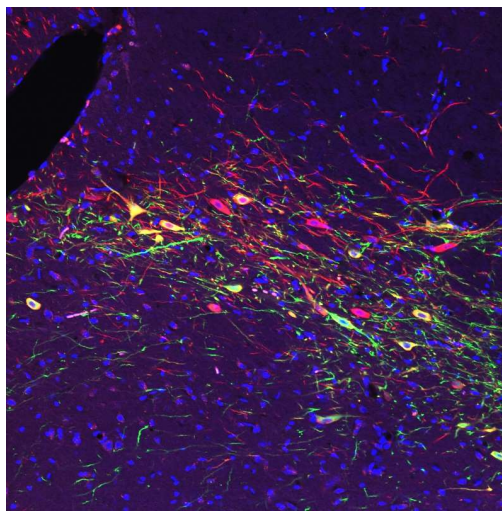
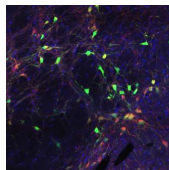


riken bsi

rayluo.bol.ucla.edu/projects

## Approach: Optogenetic manipulation of VTA dopamine cells.

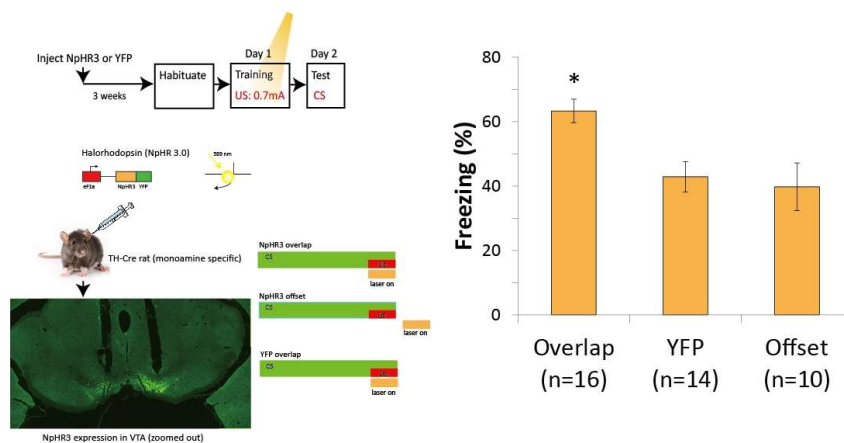
- TH (red) labeling dopaminergic cells in TH-cre animals.
- GFP (green) labeling NpHR (right) and Arch (below) infected cells.
- DAPI (blue) nucleus.



riken bsi

rayluo.bol.ucla.edu/projects

## Optogenetic inhibition of VTA dopamine cells during shock US presentation increases fear learning.



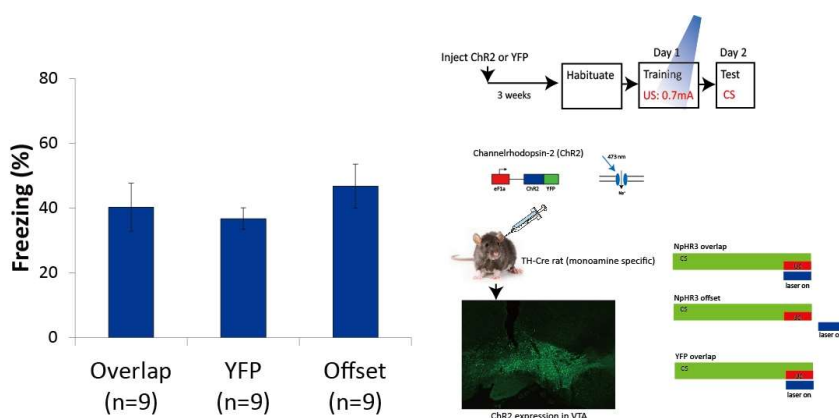
In collaboration with Luca Aquili



riken bsi

rayluo.bol.ucla.edu/projects

## Optogenetic excitation of VTA dopamine cells during shock US does not affect fear learning.



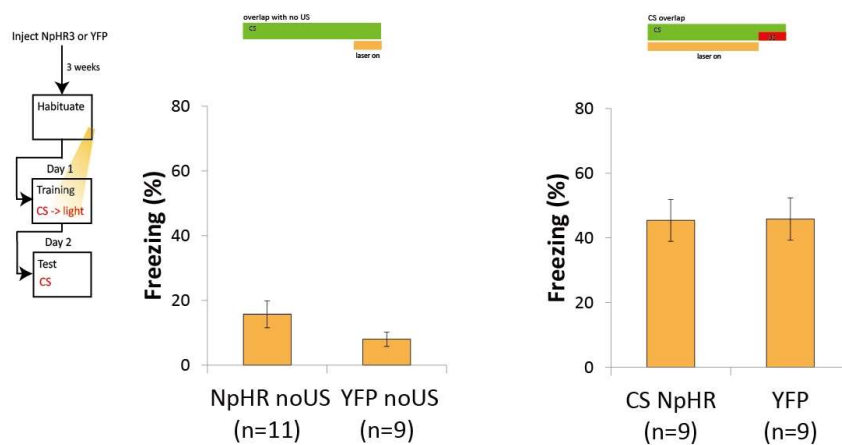
In collaboration with Luca Aquili



riken bsi

rayluo.bol.ucla.edu/projects

## Neither optogenetic inhibition during CS nor following CS alone affects fear learning.



In collaboration with Luca Aquili



riken bsi

rayluo.bol.ucla.edu/projects

## What are VTA and LA cells doing during fear learning?

learning      actual      expected

$$\Delta V \propto (\lambda - \Sigma V)$$

US

CS

Rescorla & Wagner, 1972

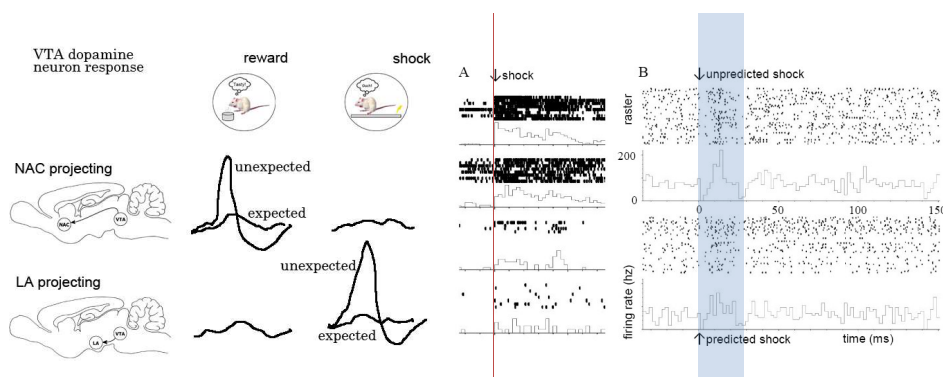
Prediction error coding can explain learning in classical conditioning.  
Model explains phenomena such as blocking and learning asymptote.



riken bsi

rayluo.bol.ucla.edu/projects

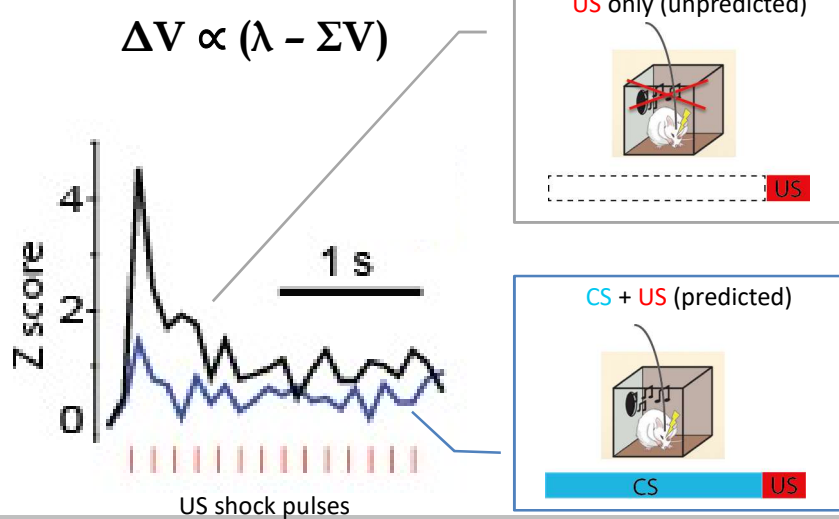
## Do VTA to LA projections code for aversive prediction error?



riken bsi

rayluo.bol.ucla.edu/projects

## Prediction error coding can explain differential responses to predicted vs. unpredicted US.



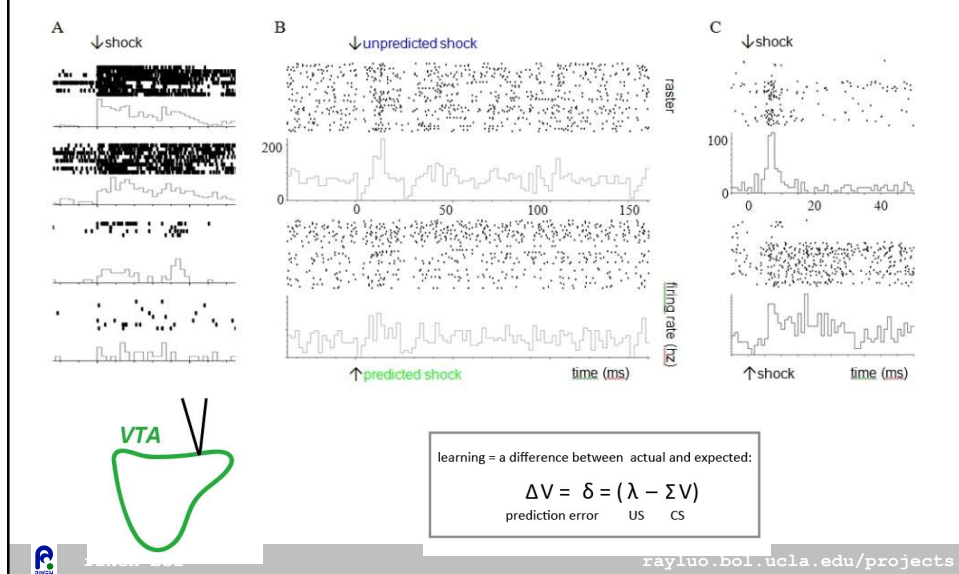
riken bsi

rayluo.bol.ucla.edu/projects

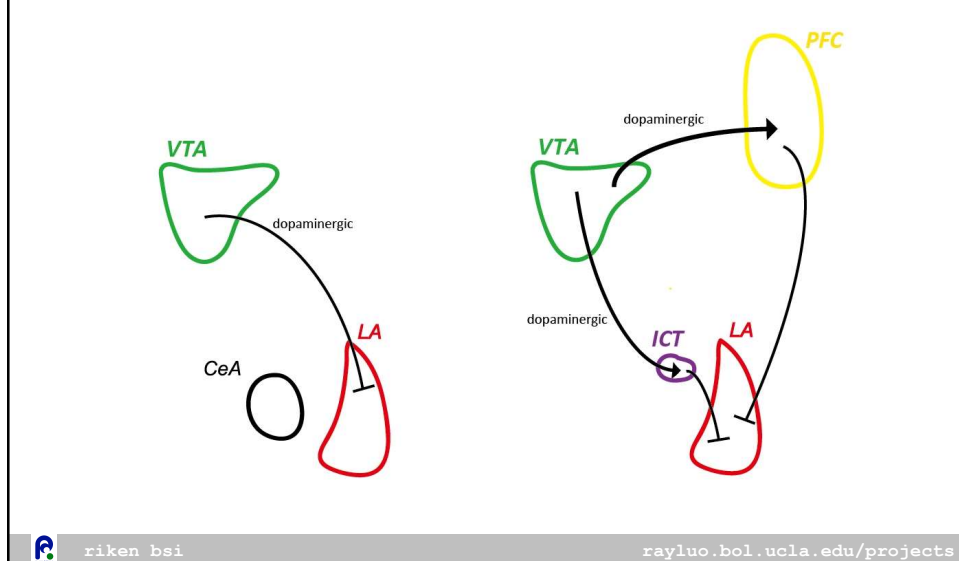
Johansen et al, 2010



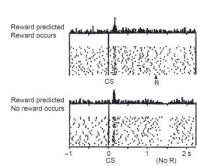
## VTA neurons respond to aversive outcomes and outcome contingencies.



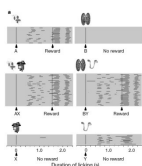
## Circuit model of fear and extinction learning: modulation by VTA dopamine neurons.



## Midbrain dopamine neurons encode rewarding as well as aversive signals.



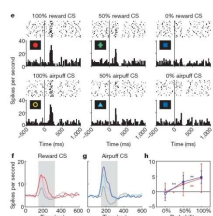
Schultz et al, 1997



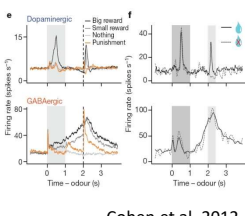
Waelti et al, 2001

Rescorla-Wagner:  $\Delta V \propto (\lambda - \Sigma V)$

Pearce-Hall:  $\Delta V \propto |\lambda - \Sigma V| \lambda$



Matsumoto &amp; Hikosaka 2009



Cohen et al, 2012

- Ventral Tegmental Area (VTA) dopamine neurons fire in response to rewards and cues that predict reward.
- Firing rates are proportional to predictability of cue for reward, and is thus high early in learning, and decreases as prediction error is decreased when well learned.
- Recent results show putative VTA dopamine neurons responsive to aversive events.
- Question 1: where do these dopamine neurons project?
- Question 2: how do these dopamine cells affect learning?



riken bsi

rayluo.bol.ucla.edu/projects

## Review Questions.

- Post Traumatic Stress Disorder (PTSD) is a failure in which of the following processes?
  - A. inability to learn fear
  - B. inability to extinguish fear
  - C. inability to generalize fear to other contexts
  - D. inability to communicate fear to other humans
- A dog is trained by pairing a neutral light cue to delivery of food until he salivates to the presentation of light by itself. Which of the following is the conditioned stimulus (CS)?
  - A. food
  - B. salivation
  - C. tone cue
  - D. light cue
- We train an animal to freeze in response to a tone, then next day we present tones only to extinguish the fear. The day after that, we put the animal in a new room and present the same tones again. What happens?
  - A. the animal stops freezing to the tone because the new room is not threatening.
  - B. the animal stops freezing initially but then remembers the fear and begins to freeze.
  - C. the animal freezes to the tone more than at the end of the extinction day previously.
  - D. the animal freezes to the tone less than at the end of the extinction day previously.



riken bsi

rayluo.bol.ucla.edu/projects

## Review Questions.

- Post Traumatic Stress Disorder (PTSD) is a failure in which of the following processes?
  - A. inability to learn fear
  - **B. inability to extinguish fear**
  - C. inability to generalize fear to other contexts
  - D. inability to communicate fear to other humans
- A dog is trained by pairing a neutral light cue to delivery of food until he salivates to the presentation of light by itself. Which of the following is the conditioned stimulus (CS)?
  - A. food
  - B. salivation
  - C. tone cue
  - **D. light cue**
- We train an animal to freeze in response to a tone, then next day we present tones only to extinguish the fear. The day after that, we put the animal in a new room and present the same tones again. What happens?
  - A. the animal stops freezing to the tone because the new room is not threatening.
  - B. the animal stops freezing initially but then remembers the fear and begins to freeze.
  - **C. the animal freezes to the tone more than at the end of the extinction day previously.**
  - D. the animal freezes to the tone less than at the end of the extinction day previously.



riken bsi

rayluo.bol.ucla.edu/projects

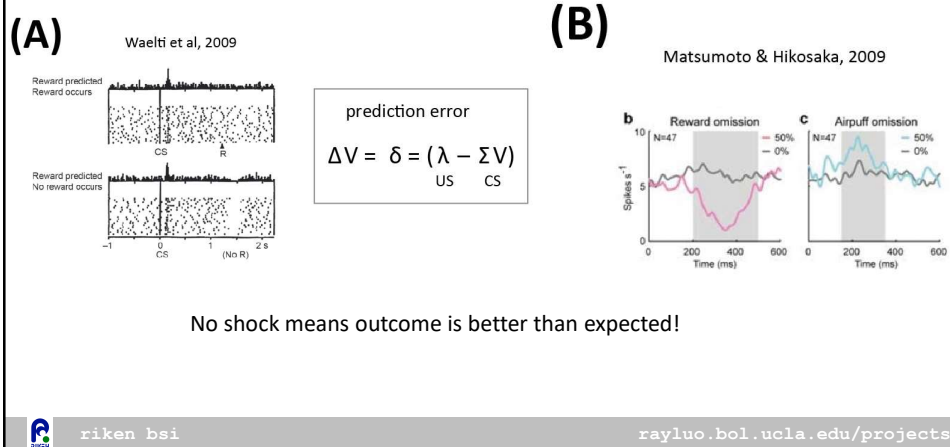
Does VTA dopamine neurons affect extinction learning?



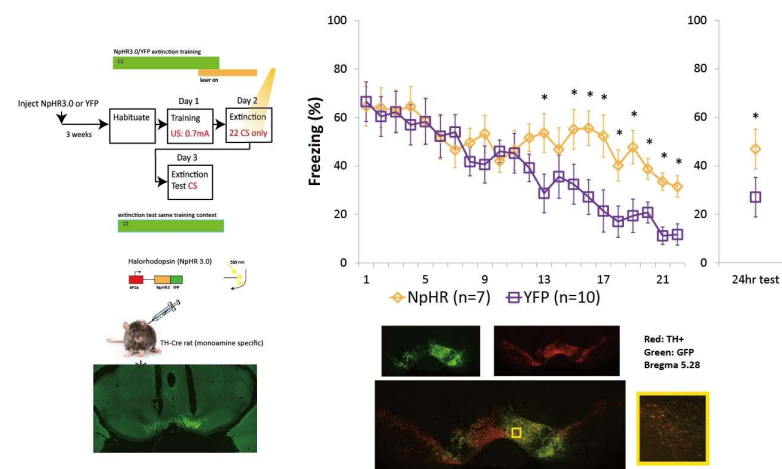
riken bsi

rayluo.bol.ucla.edu/projects

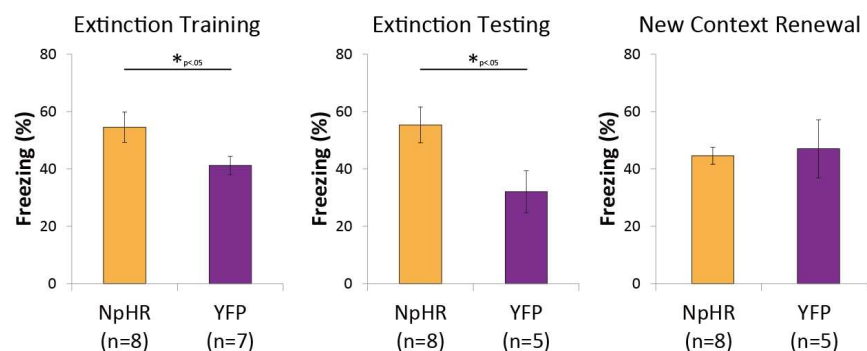
## Reward prediction error coding dopamine cells may act during shock omission period.



## Optogenetic inhibition of VTA dopamine cells during period of expected US presentation.



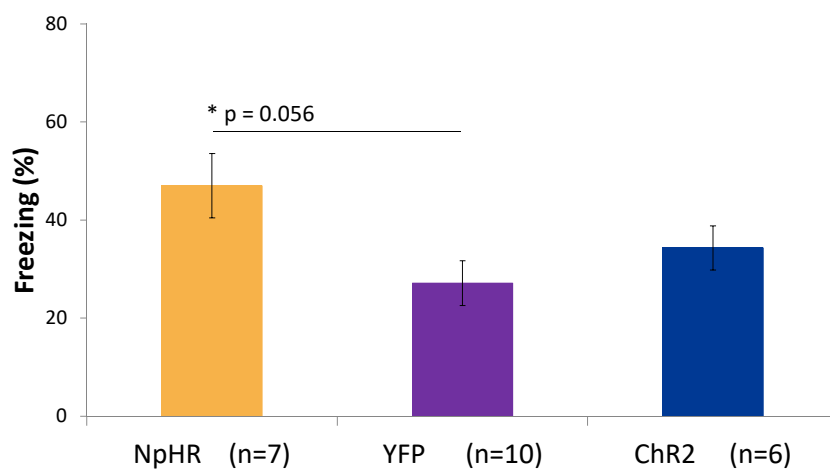
Extinction learning is reduced, but renewal of fear in a new context is unaffected.



riken bsi

rayluo.bol.ucla.edu/projects

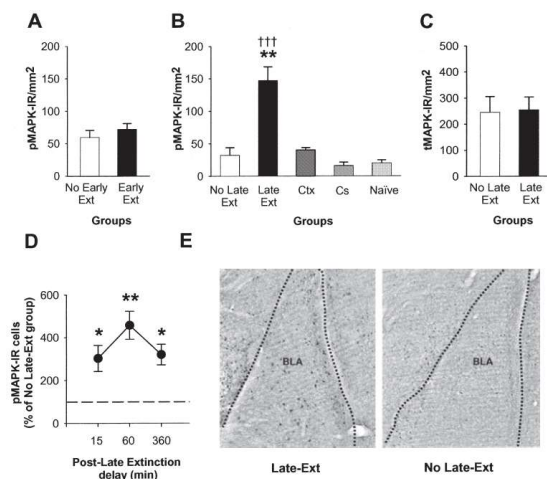
Optogenetic activation of VTA dopamine cells during expected US does not affect extinction.



riken bsi

rayluo.bol.ucla.edu/projects

## Extinction of fear is associated and requires BLA MAPK phosphorylation.



Herry et al, 2006

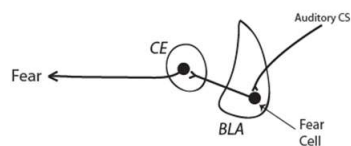


riken bsi

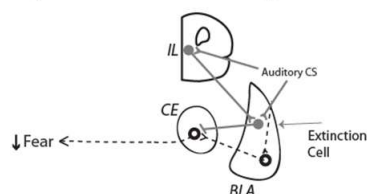
rayluo.bol.ucla.edu/projects

## Optogenetic inhibition of VTA dopamine neurons during omission period of fear extinction.

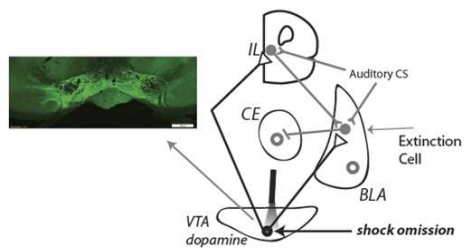
### A) Fear circuit



### B) Extinction learning circuit



### Optogenetic approach

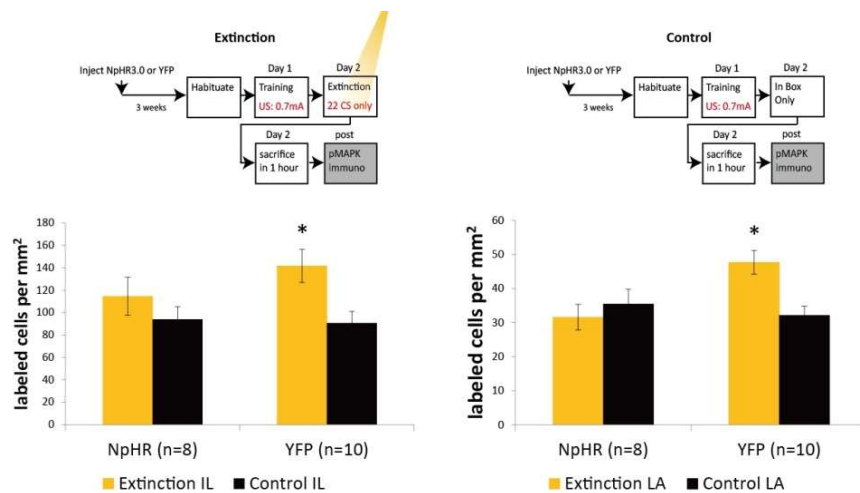


riken bsi

rayluo.bol.ucla.edu/projects



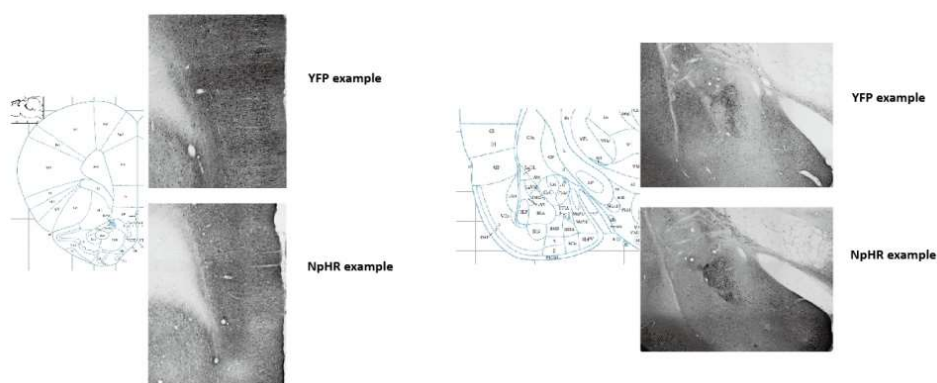
## Inhibition of VTA dopamine during omissions in extinction reduces MAPK phosphorylation in BLA & IL.



riken bsi

rayluo.bol.ucla.edu/projects

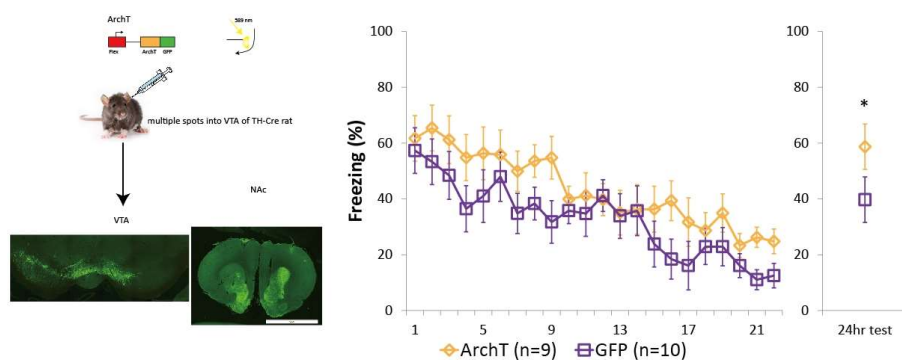
## Inhibition of VTA dopamine during omissions in extinction reduces MAPK phosphorylation in BLA & IL.



riken bsi

rayluo.bol.ucla.edu/projects

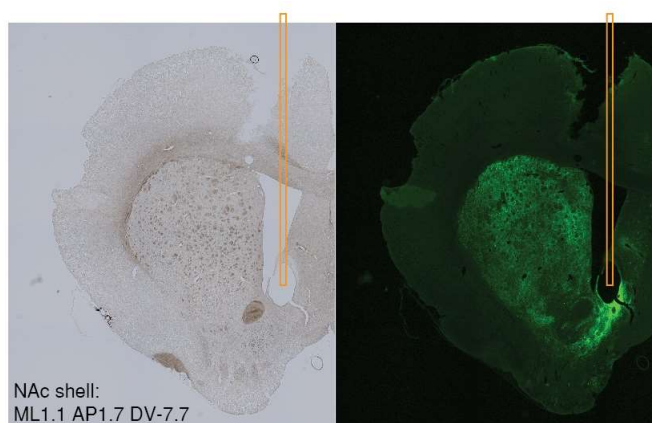
## Inhibition of dopamine terminals in nucleus accumbens (NAc) during omitted shock period abolishes extinction learning.



riken bsi

rayluo.bol.ucla.edu/projects

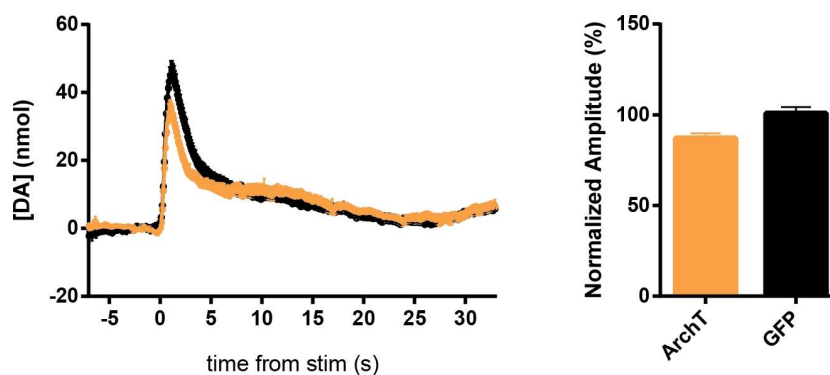
## Recording dopamine release in nucleus accumbens (NAc) with fiber optic manipulation of terminals.



riken bsi

rayluo.bol.ucla.edu/projects

## Light inhibition of dopamine terminals in NAc reduces evoked amplitude.



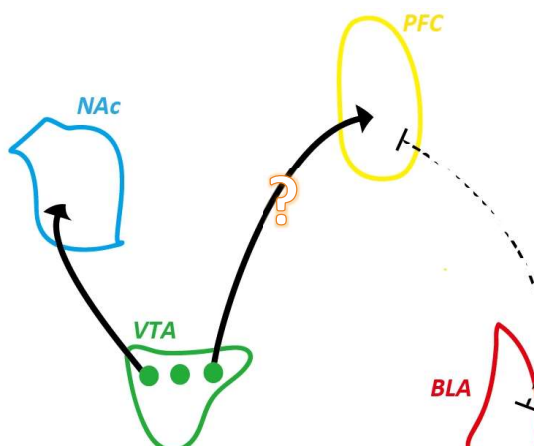
Upshot: light manipulation really reduces dopamine release during omission of shock.



riken bsi

rayluo.bol.ucla.edu/projects

## Extinction is modulated by differential dopamine projections to different areas.



Implication: PTSD treatment must be target to specific cell populations.



riken bsi

rayluo.bol.ucla.edu/projects



## RIKEN Brain Science Institute Josh Johansen laboratory (Neural Circuitry of Memory).



Ray Luo	Mami Kimura	Mai Iwasaki	Lindsay Preston	Akira Uematsu	Takaaki Ozawa	<b>Joshua Johansen</b>	Edgar Ycu	Baozhen Tan	Jenny Koivumaa	Jake Ormond Touqueer Ahmed
------------	----------------	----------------	--------------------	------------------	------------------	----------------------------	--------------	----------------	-------------------	-------------------------------------

**Not shown:** Ashwani Kumar, Hiroki Hamanaka, Yanqiu Tao, Anna Krejcirikova, Lifeng Yeh



riken bsi

[rayluo.bol.ucla.edu/projects](http://rayluo.bol.ucla.edu/projects)

## Questions?



riken bsi

[rayluo.bol.ucla.edu/projects](http://rayluo.bol.ucla.edu/projects)