SHBT Harvard University Speech and Hearing Bioscience and Technology

When hearing is distressing: Neural signatures of aversive sound associations

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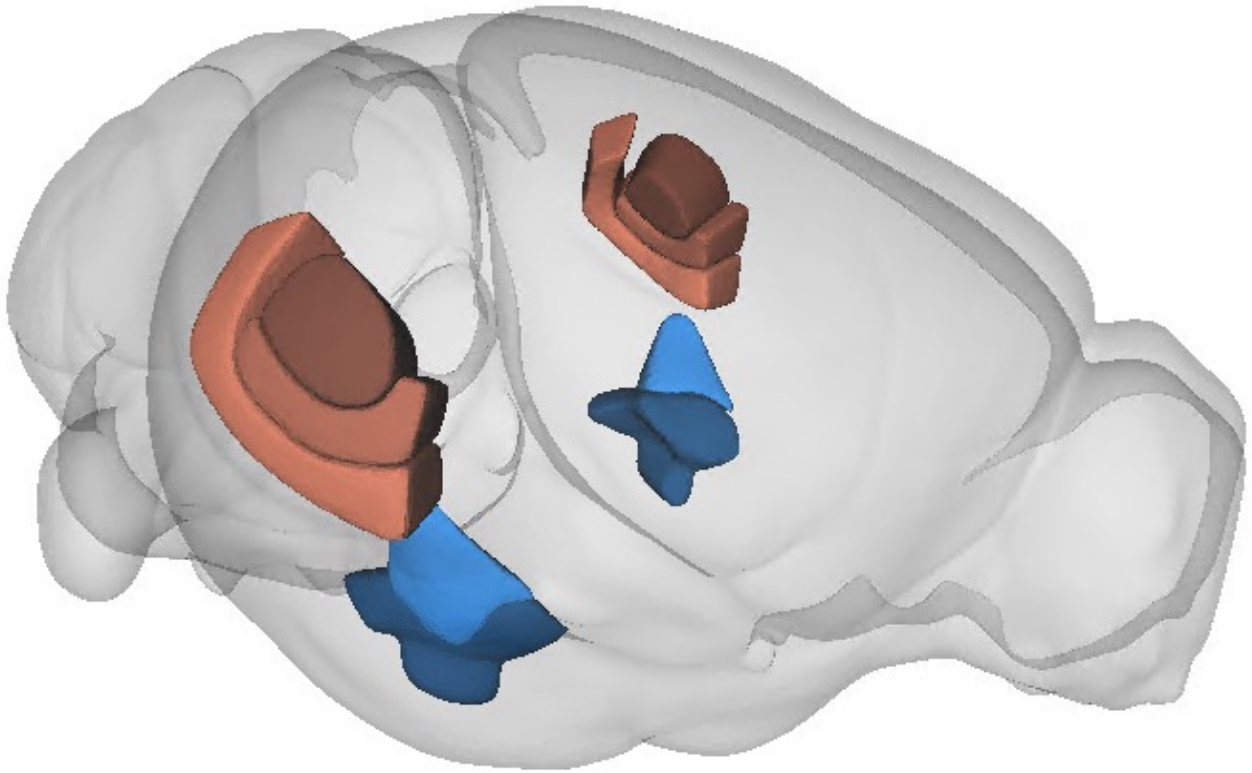
Imagine listening to the sound of waves in your favorite beach vacation

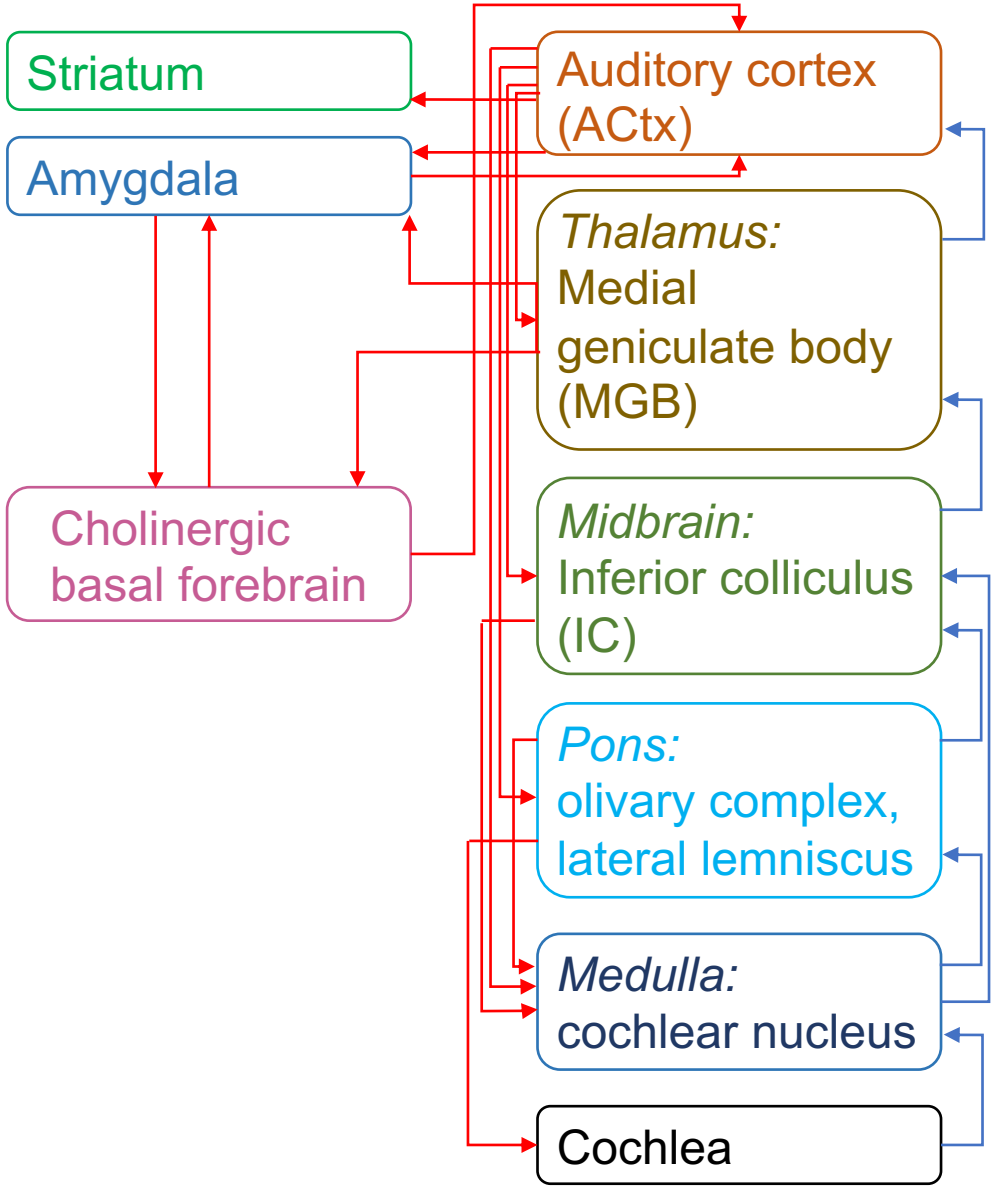
Pleasant & soothing!

Imagine listening to the same sound of waves, but when it’s a constant reminder of the consequence of a fall

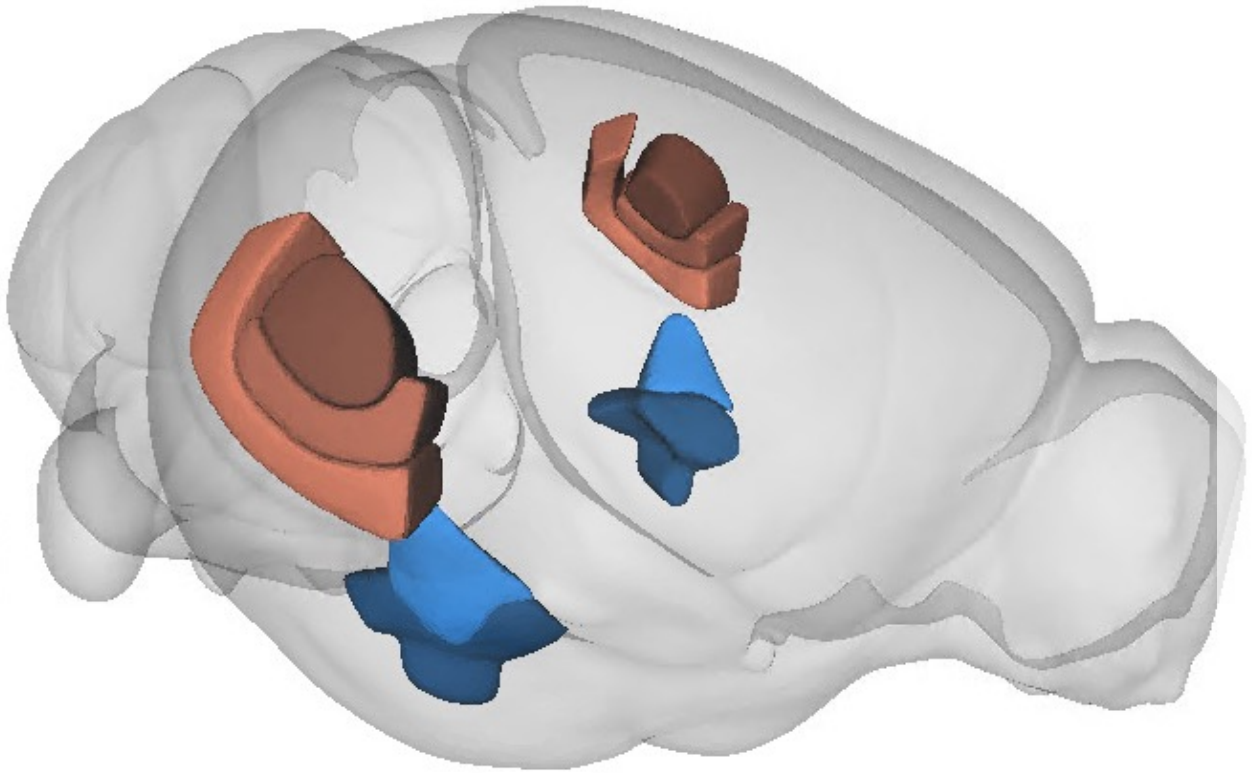
Terrifying & distressing!

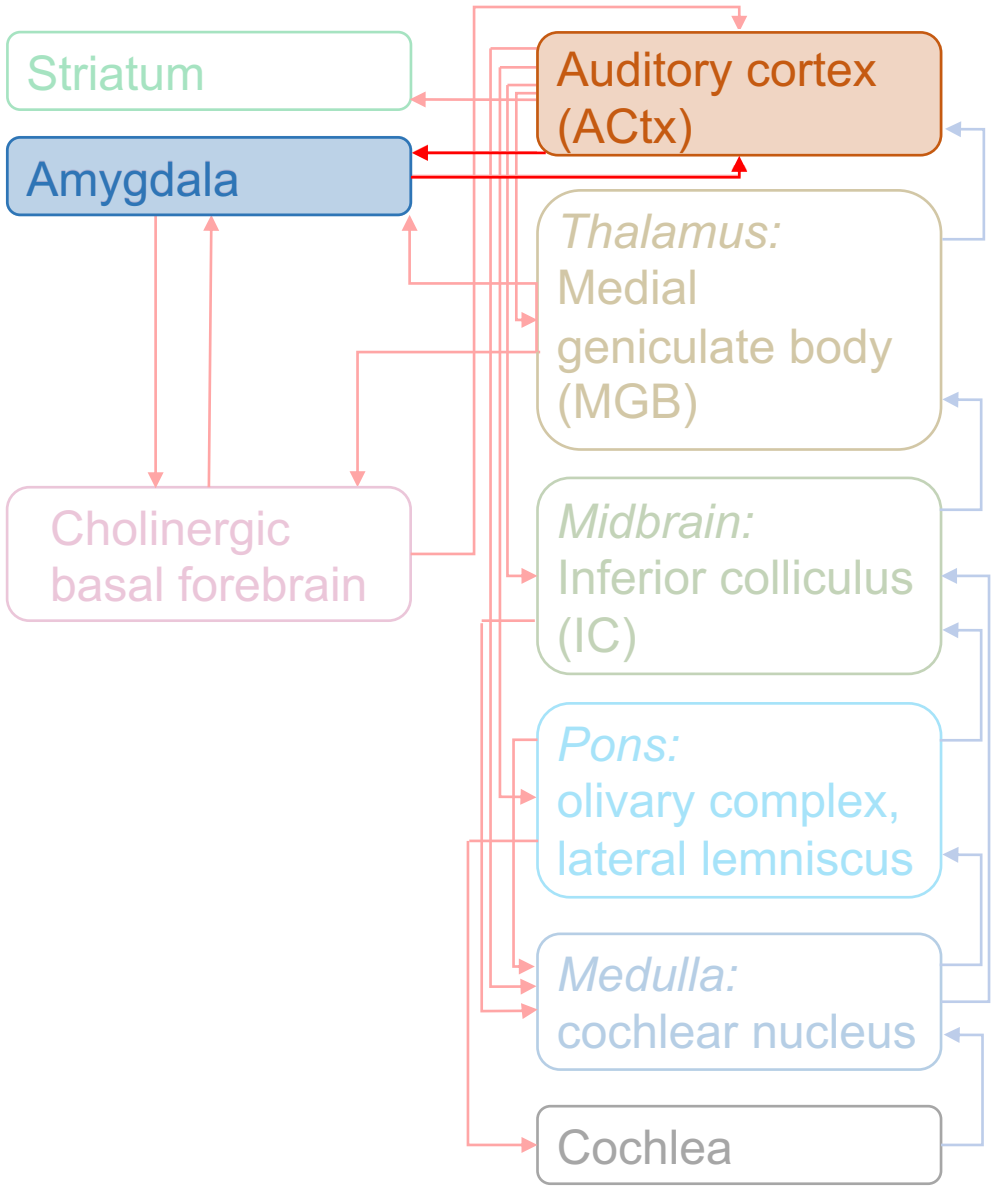
Pathways leading to affective sound processing





How do interconnected networks of ACtx and Amygdala contribute to affective sound processing?



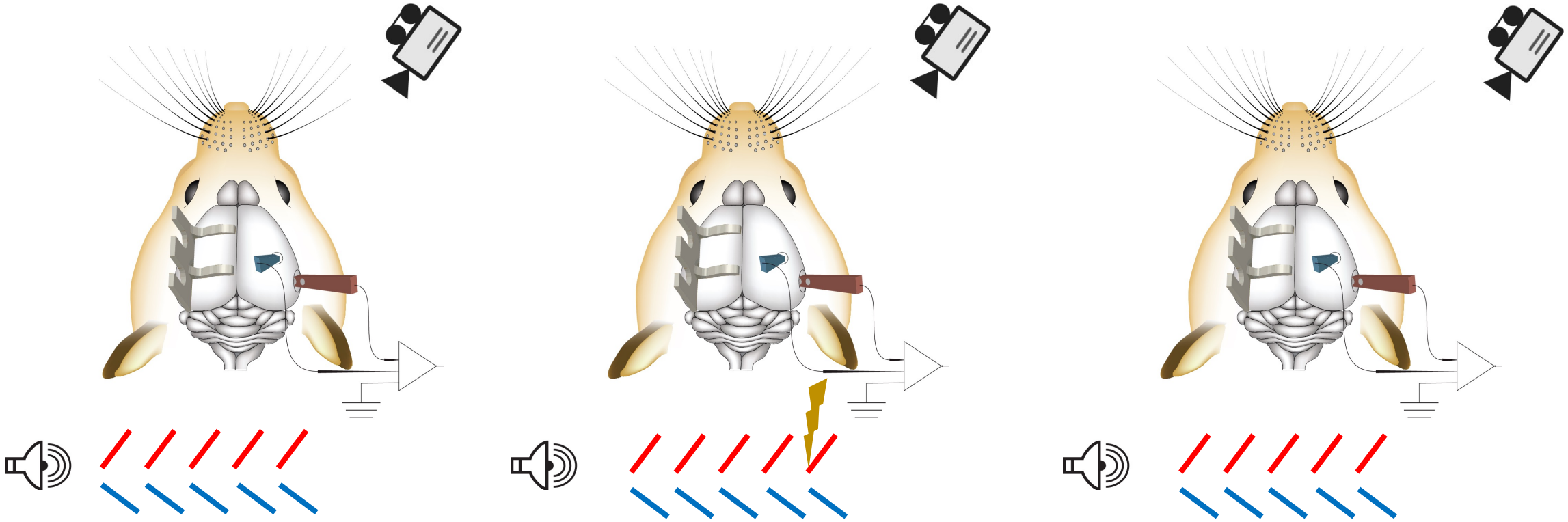


Transforming an emotionally neutral sound to a distressing sound

Day1: Habituation

Day 2: Conditioning

Day 3: Post-conditioning Recall

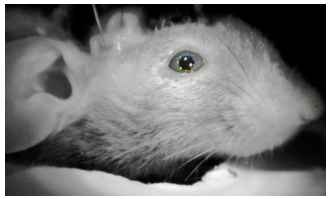


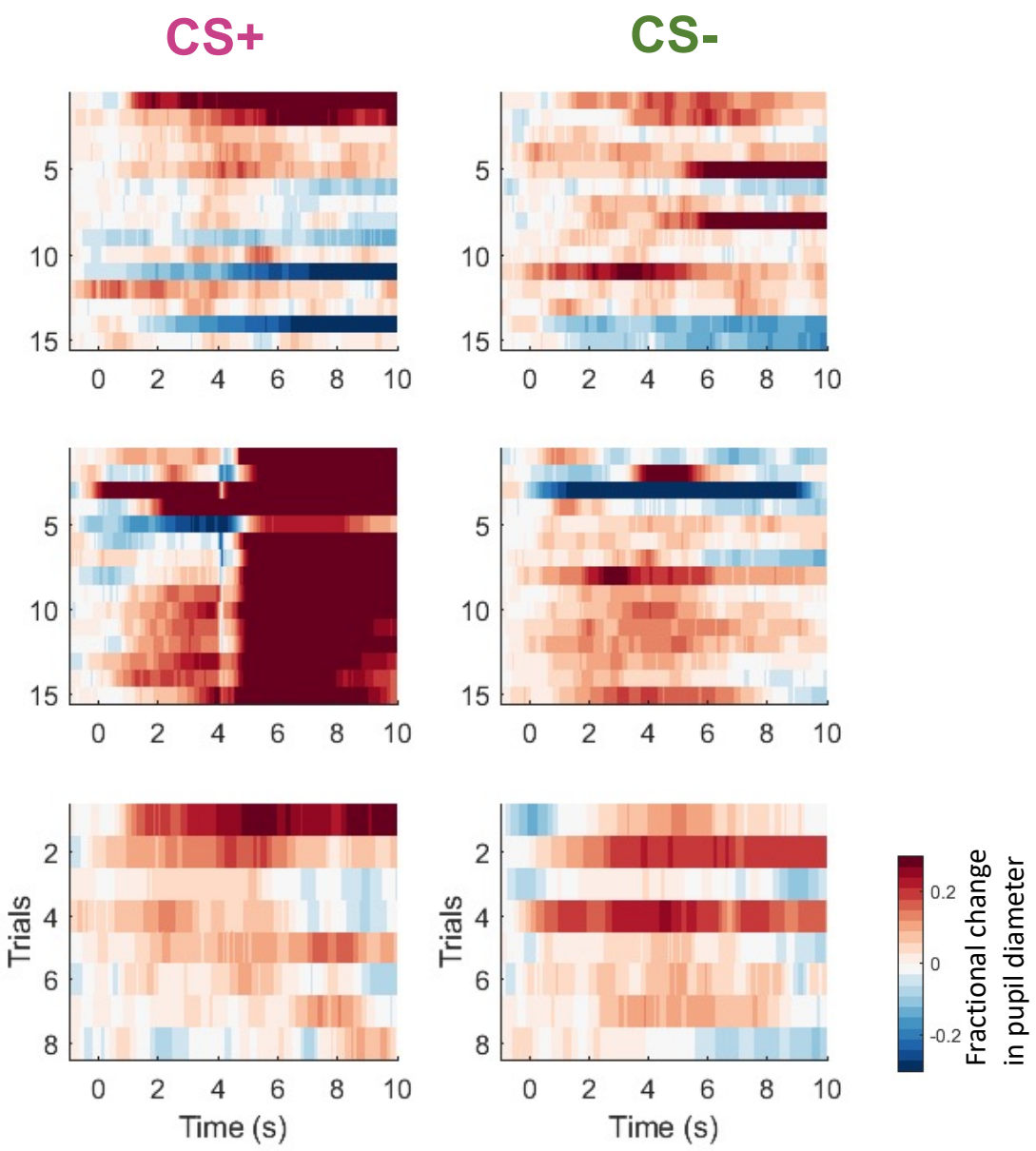
- Threat conditioning in head-fixed mice using complex sounds such as FM sweeps as conditioned stimulus (CS)
- Pupil dilation response and orofacial motions used as a threat associative learning readout
- Simultaneous recordings from higher-order ACtx (AuV/TeA) and baso-lateral amygdala (BLA) to investigate the changes in their long-range functional coupling

Pupil dilation response as a threat association readout

Pupil tracking using DeepLabCut

Pupil dilation response provides evidence for associative learning





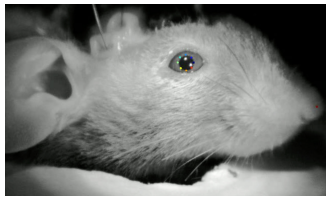
Conditioned Stimulus (CS):

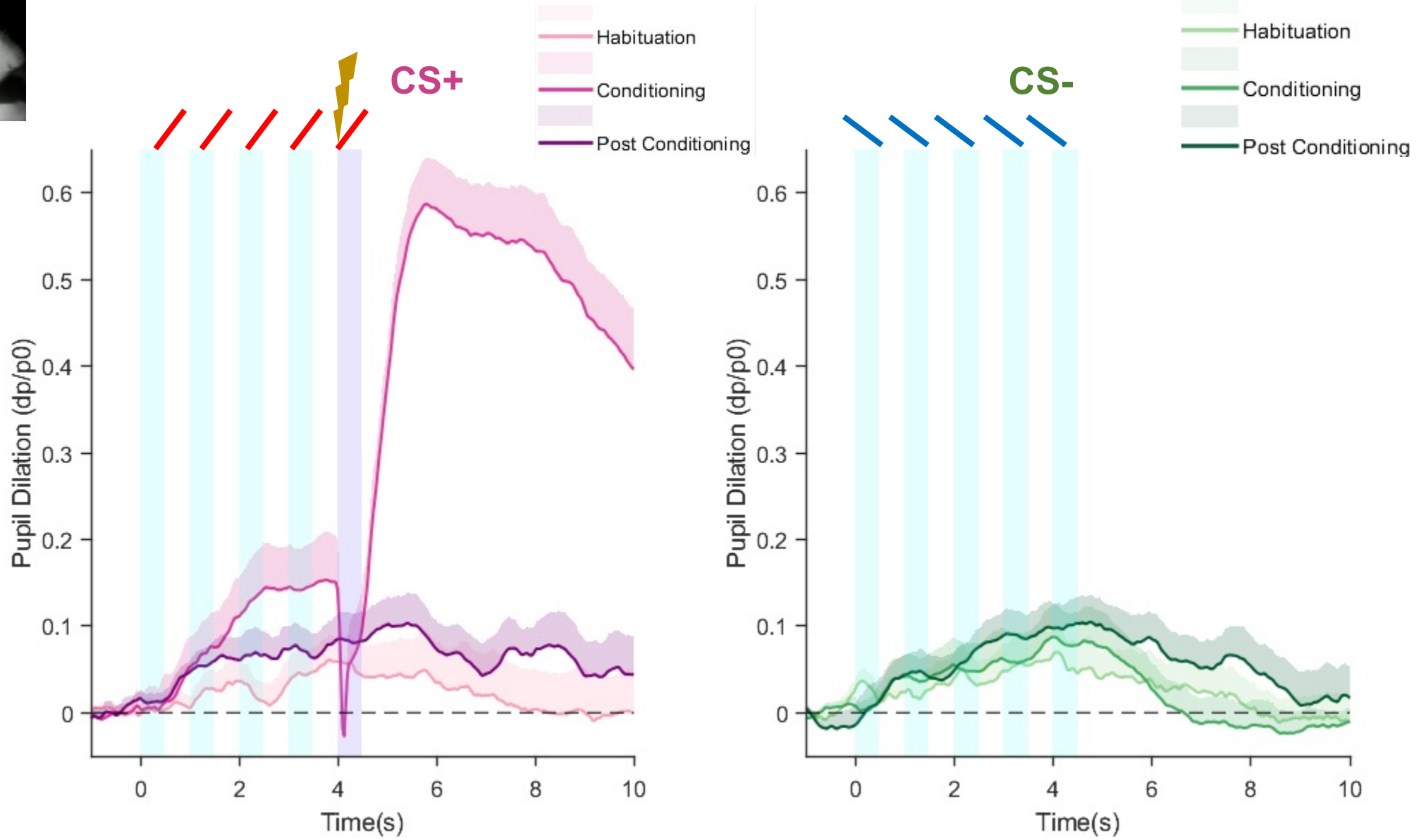
- trains of 500 ms sounds (w/ 50 ms rise and fall)
- at 1 Hz for 5 s duration (0 – 5s)
- At 70 dB SPL
- FM sweeps – log modulated up/down sweeps between 5 and 20 kHz
- 15 presentations during habituation and conditioning and 8 presentations during post-conditioning recall
- w/ ITI 20 – 180 s
- CS+/US and CS- presented in alternating fashion
- CS+ vs CS- identity counterbalanced between animals

Unconditioned Stimulus (US):

- Mildly aversive tailshock (1s, 0.4 mA AC), onset coincides with onset of last sound in the CS+ train (at 4s)

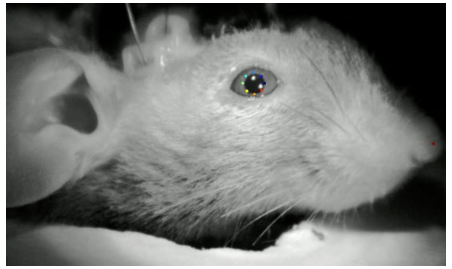
Pupil dilation response provides evidence for associative learning

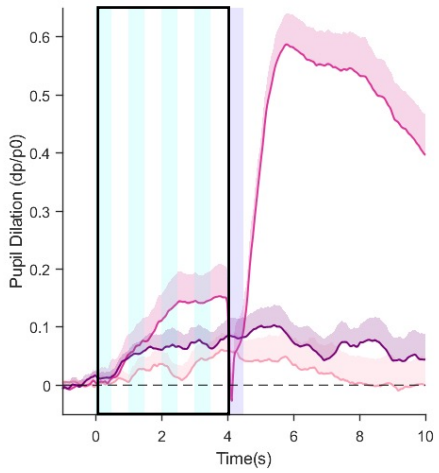


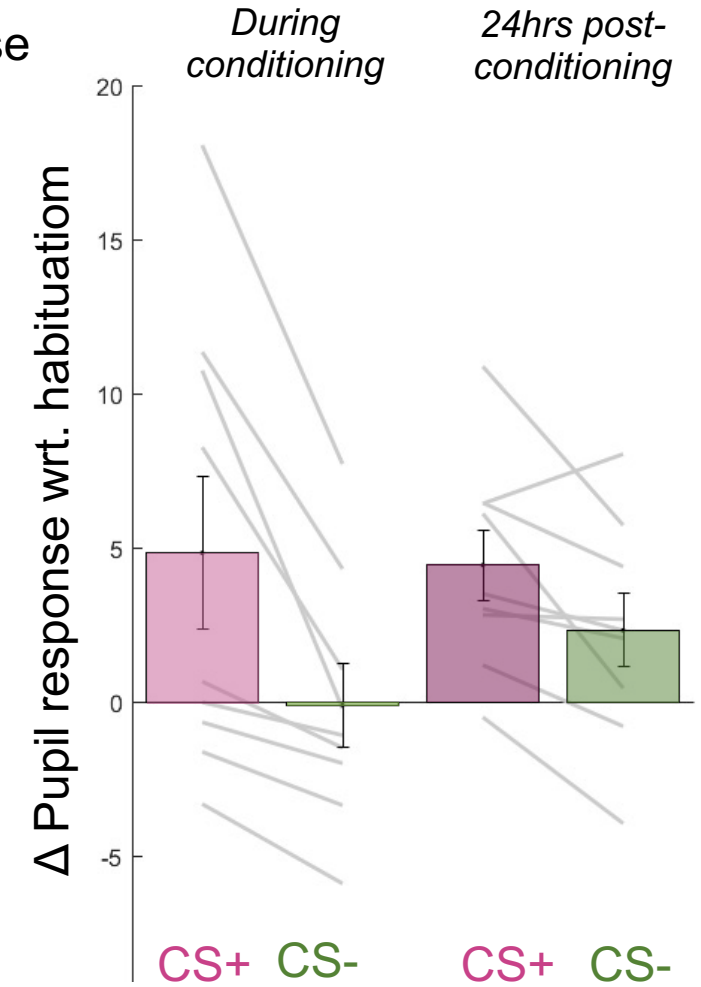


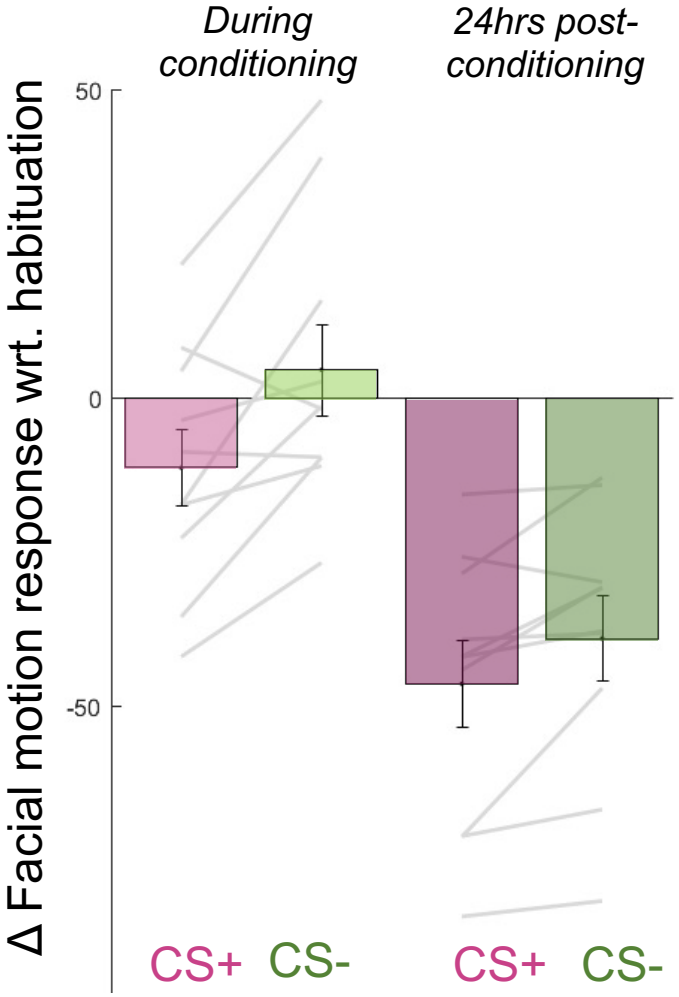
Spontaneous behaviors as a readout for threat associative learning

Pupil dilation response

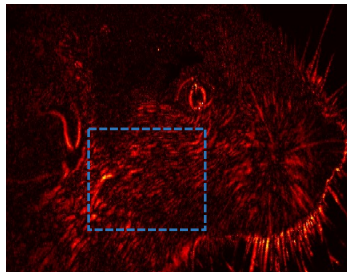


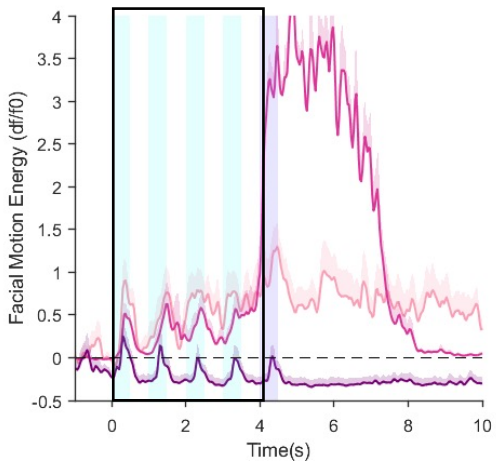






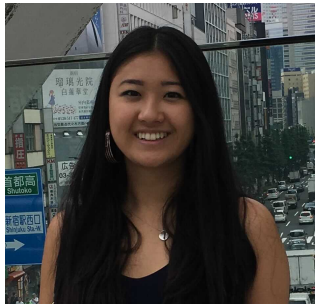
Orofacial movements



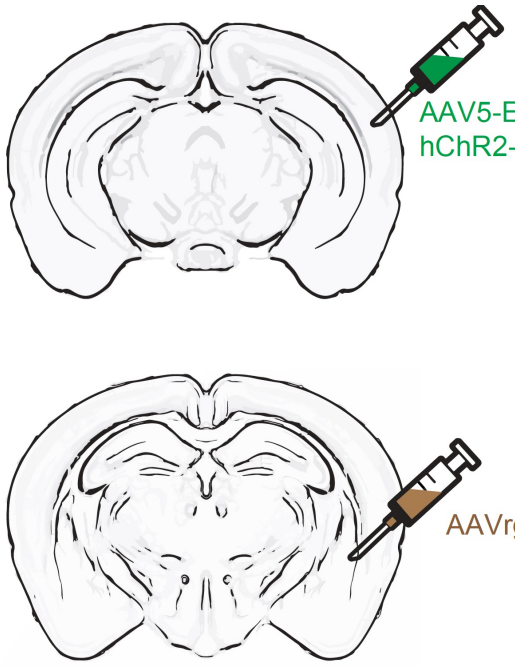


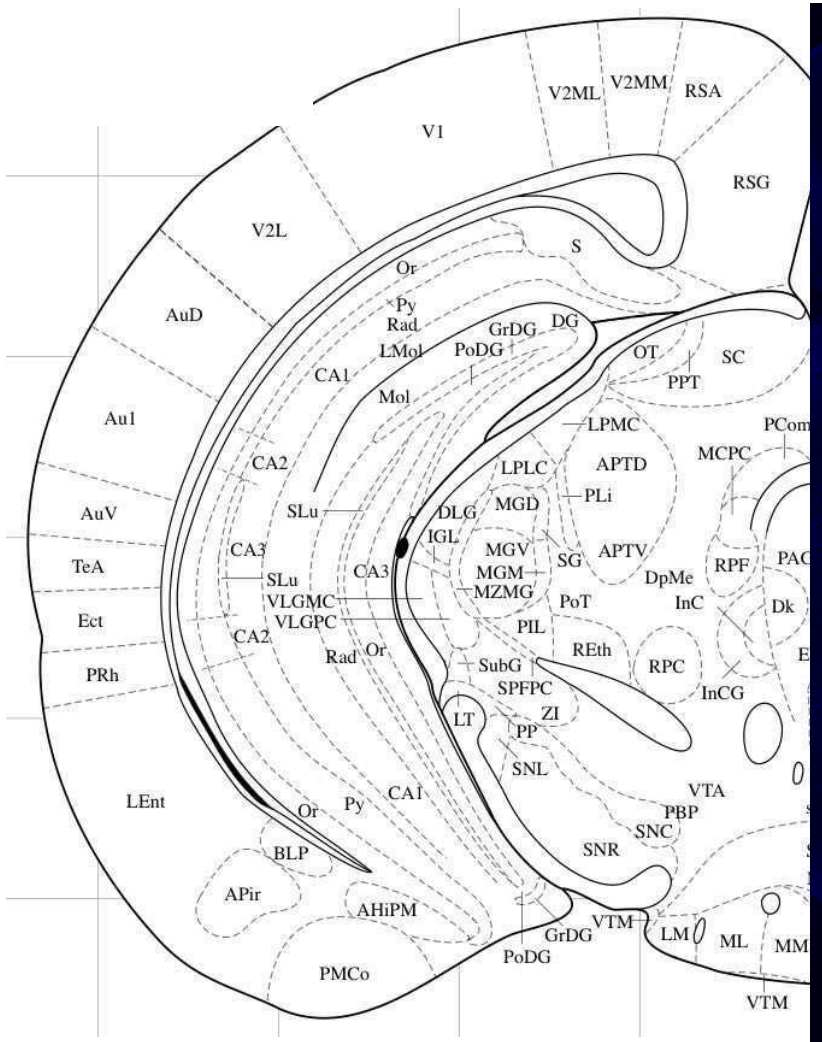
N = 10 mice

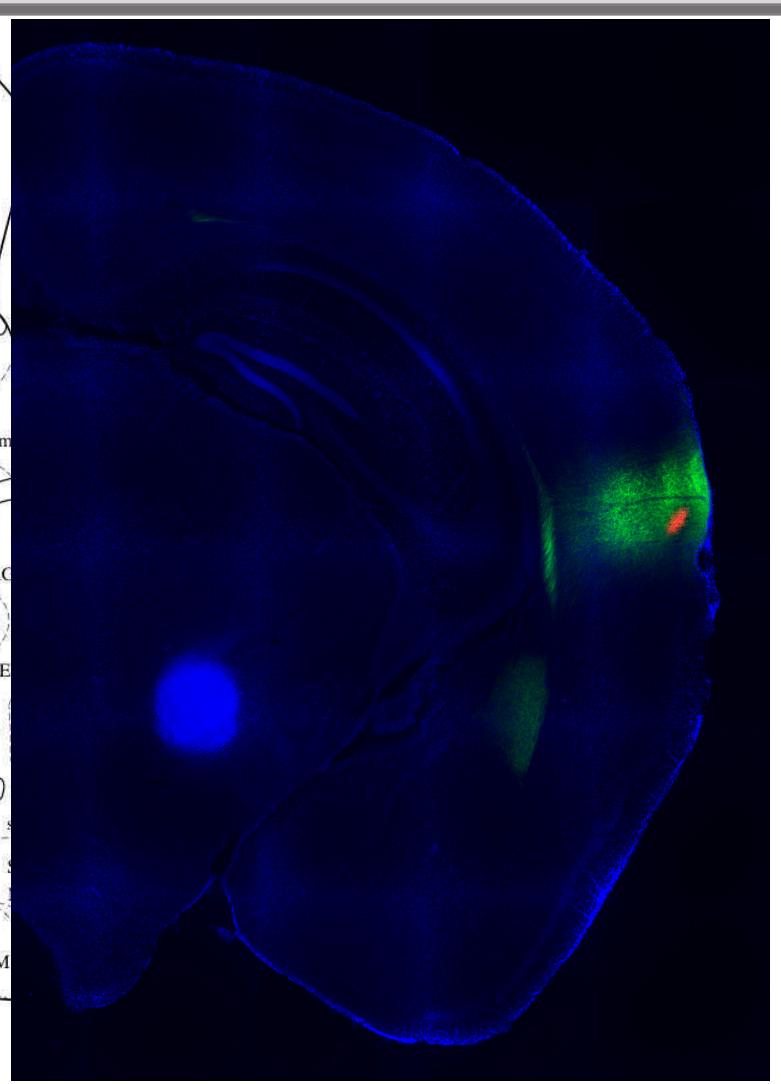
An optogenetic approach to study the higher-order ACtx – BLA circuit



Yurika Watanabe



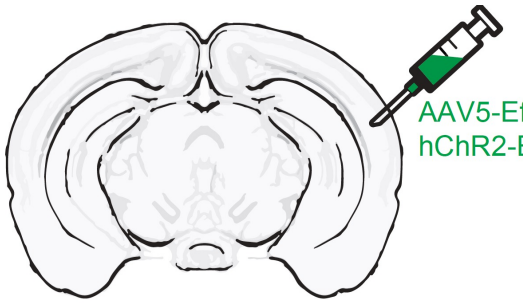


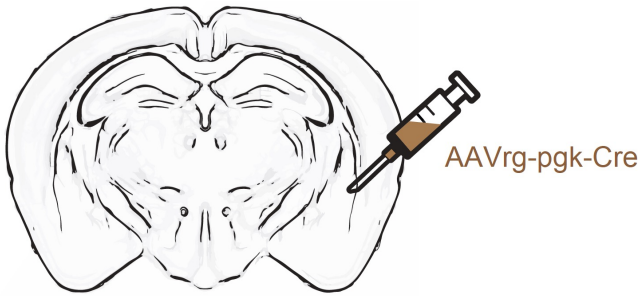


An intersectional virus strategy to selectively express channelrhodopsin in ACtx neurons that project to BLA

Red – Di-I coated electrode
Blue – DAPI
Green – ChR2

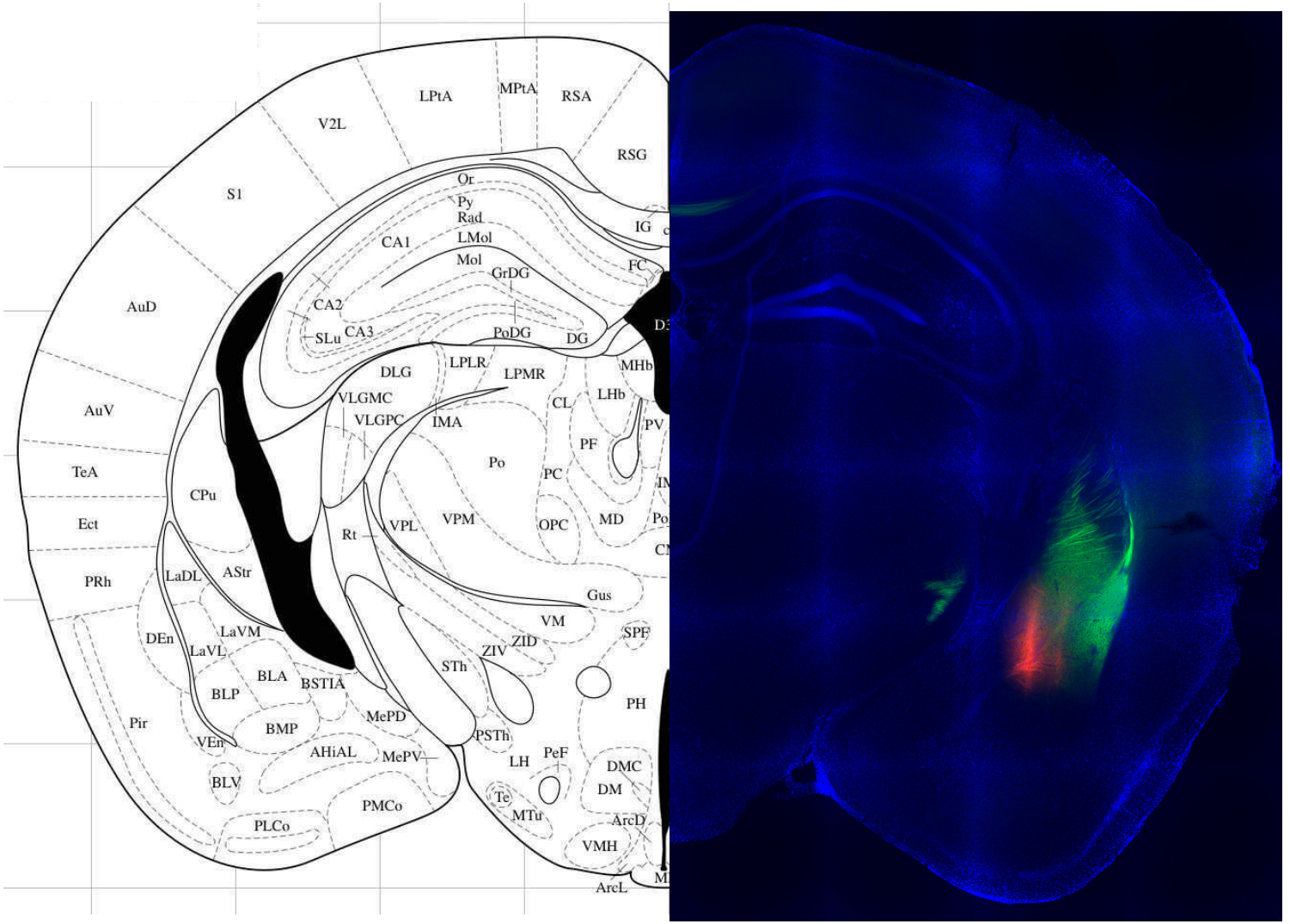
An optogenetic approach to study the higher-order ACtx – BLA circuit





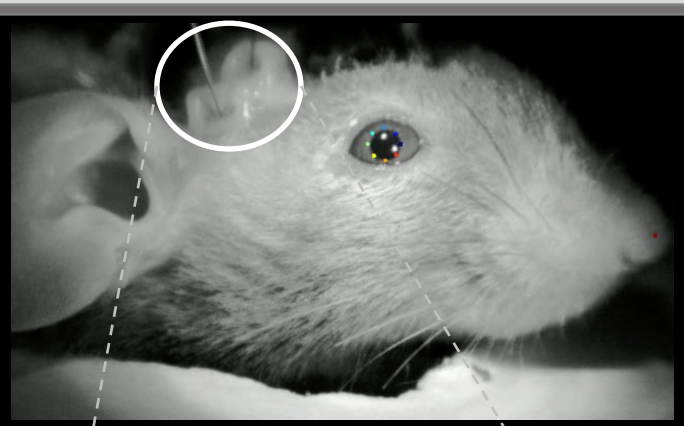
The optogenetic approach allows us to

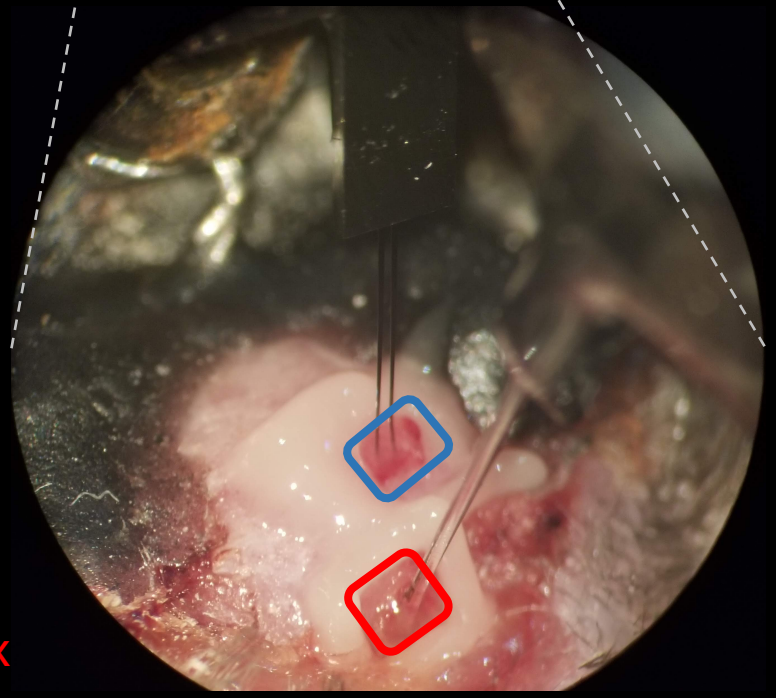
1) monitor changes in feedforward activation in BLA
2) optogenetically isolate single units in AuV/TeA that project to BLA.

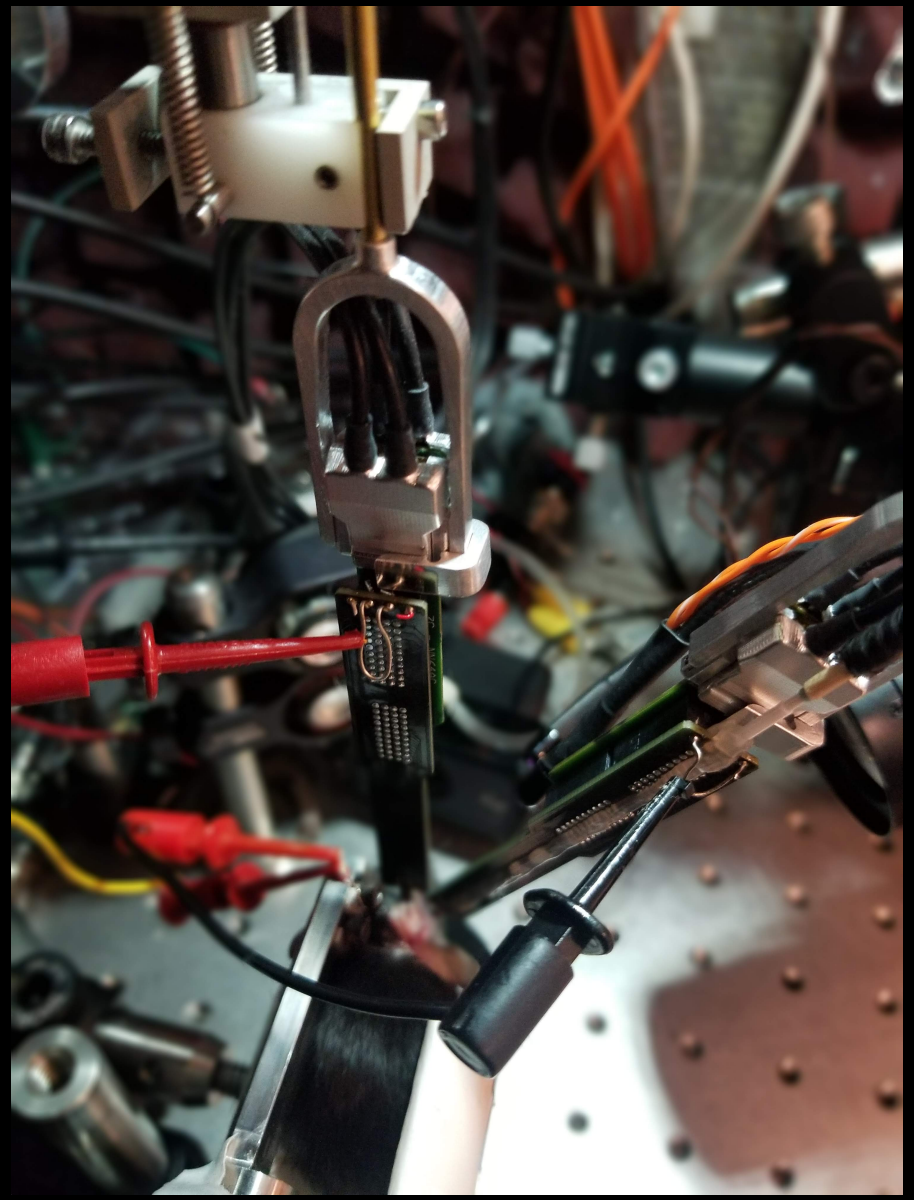


Red – Di-I coated electrode
Blue – DAPI
Green – ChR2

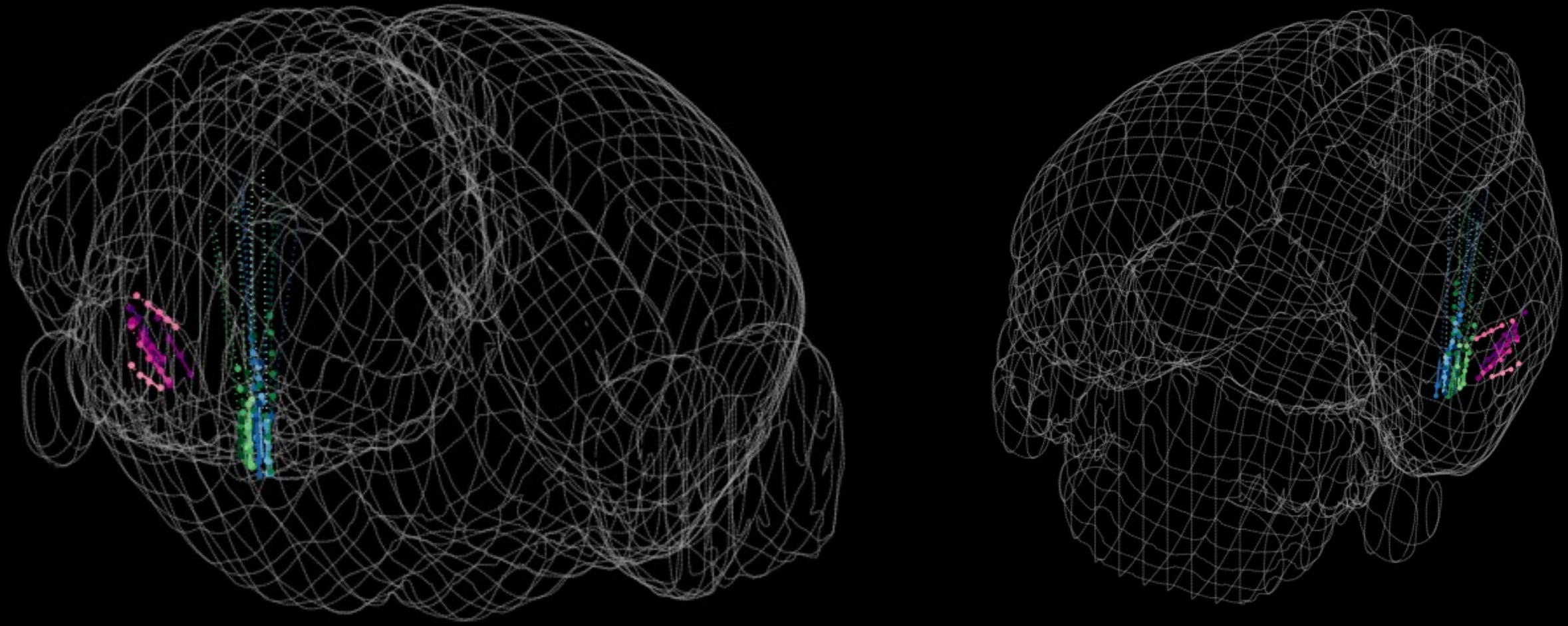
Simultaneous recordings from higher-order ACtx and BLA using an optogenetic approach







Reconstructed electrode tracks

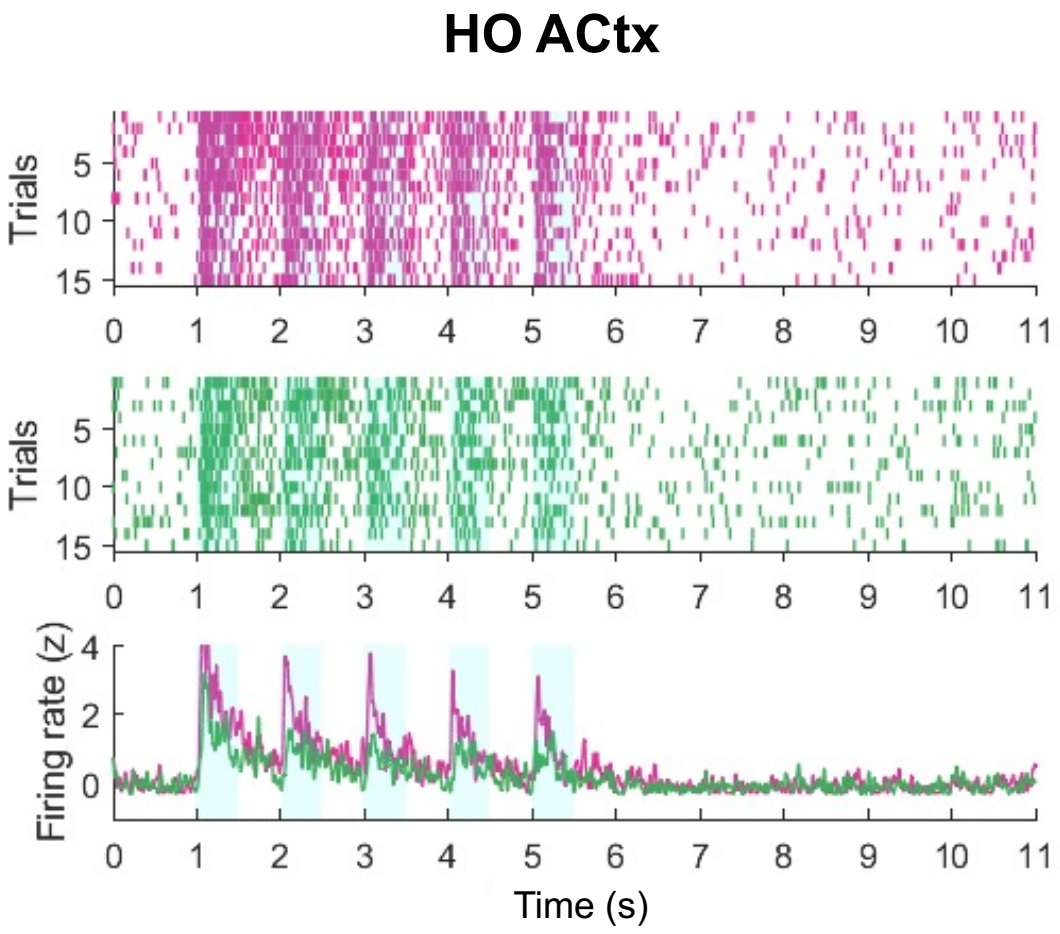


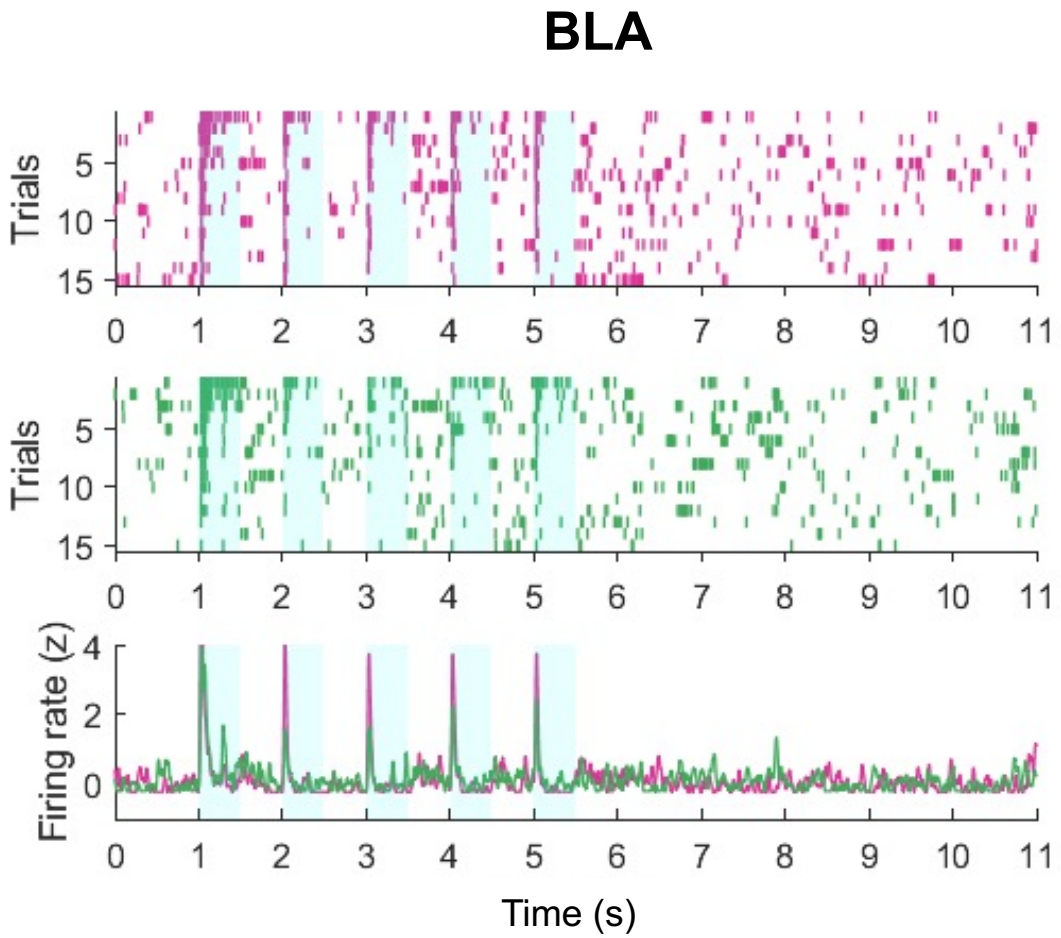
n = 21 shanks (in N = 7 animals) for which tracks have been reconstructed here using SHARP-Track

Sound-evoked responses in higher-order ACtx & BLA single units during post-conditioning recall

CS+

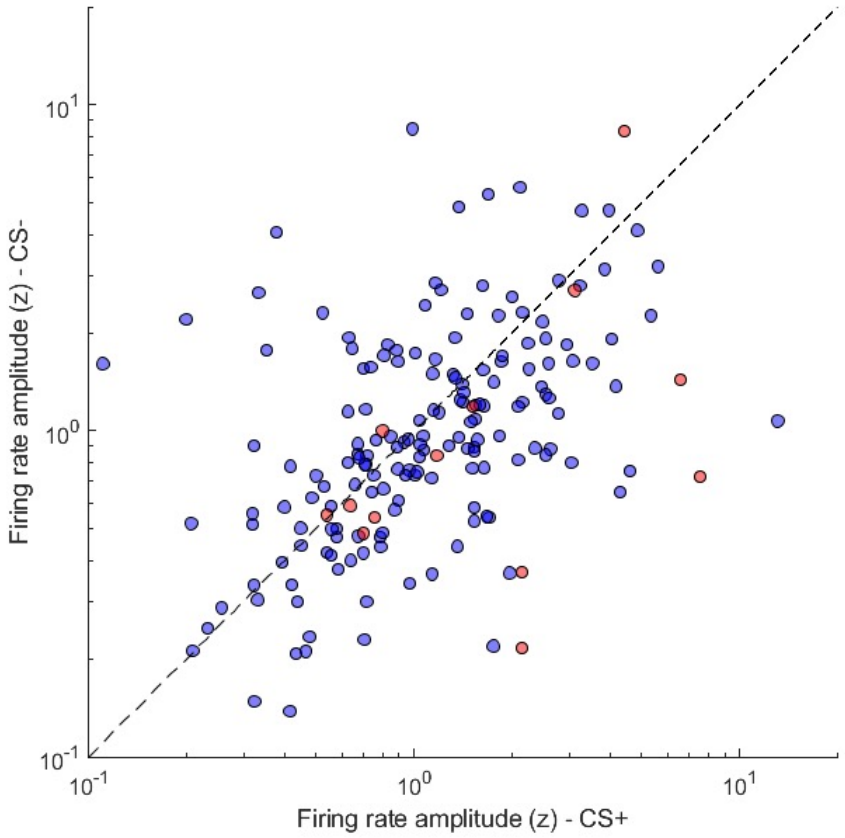
CS-





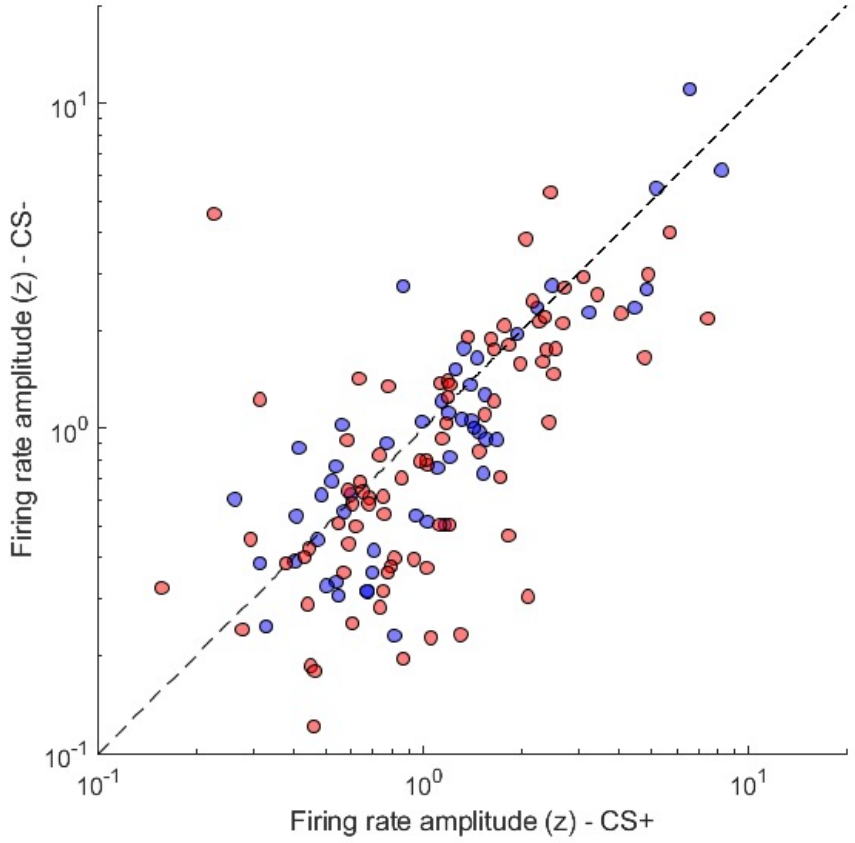
Enhanced sound-evoked responses to CS+ in higher-order ACtx & BLA single units during post-conditioning recall

HO ACtx



n = 180 total sound responsive cells (N =7 mice)
m = 40 c-amy cells (jitter-threshold = 0.5 ms)

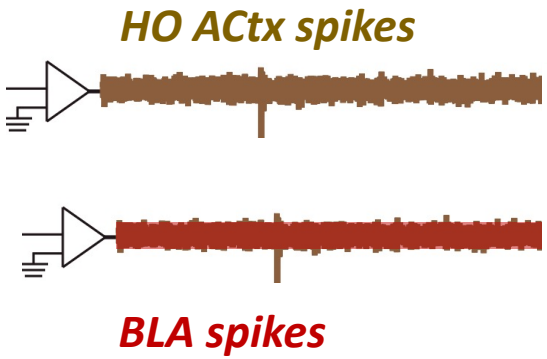
BLA



n = 135 total sound responsive cells (N =7 mice)
m = 82 laser excited (82/135)

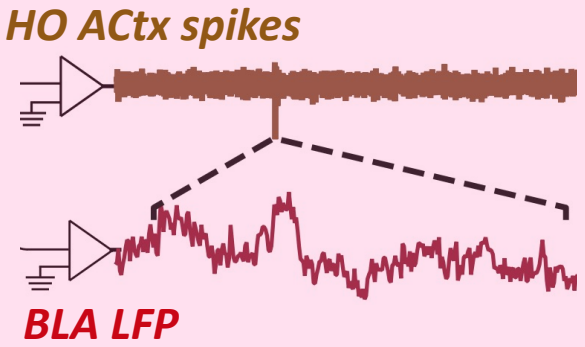
Investigating the transient changes in HO ACtx – BLA functional connectivity

Cell – cell connectivity



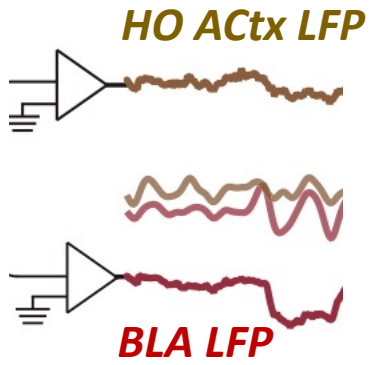
- Can be used to infer functional connectivity based on lag at which there is a significant spike cross-correlation
- But low probability of finding two cells with significant interactions

Spike-triggered LFPs



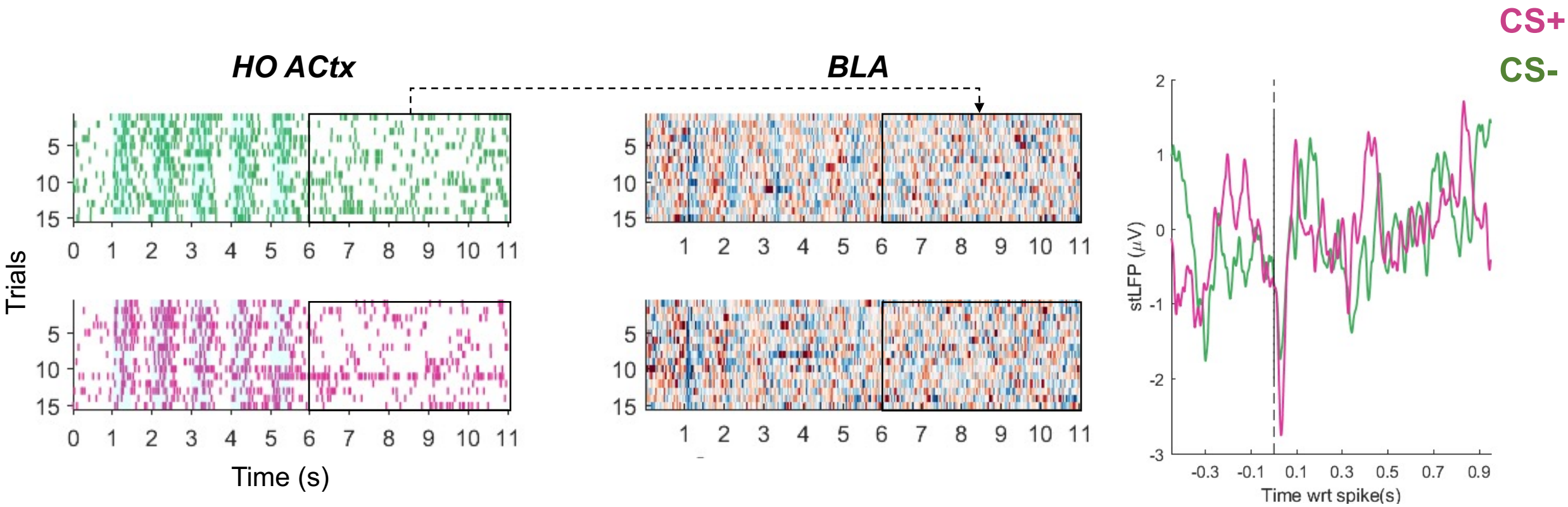
- Can be used to assess the strength of synaptic activations at one site (reflected in the LFP at that site) caused by the spiking output at the other site
- In general, it is more sensitive than other cell-to-cell connectivity methods for detecting weak connections because it detects subthreshold responses, and it relies on the postsynaptic response of multiple cells (hundreds of cells) and not just one

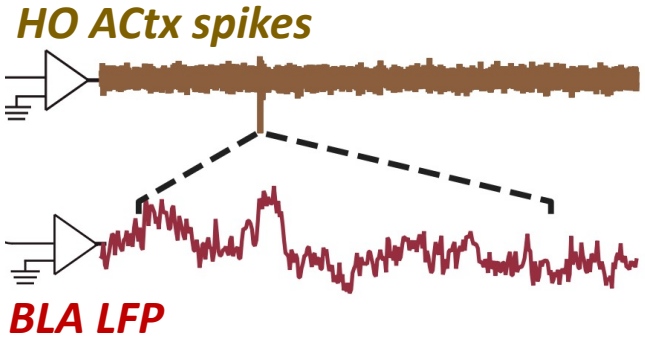
LFP-LFP coherence



- LFPs recorded simultaneously from two regions display synchrony if their peaks and valleys align (phase coherence) or if their amplitudes correlate (power correlation)
- But will lose temporal resolution and harder to compute these measures if the time window of interest is short

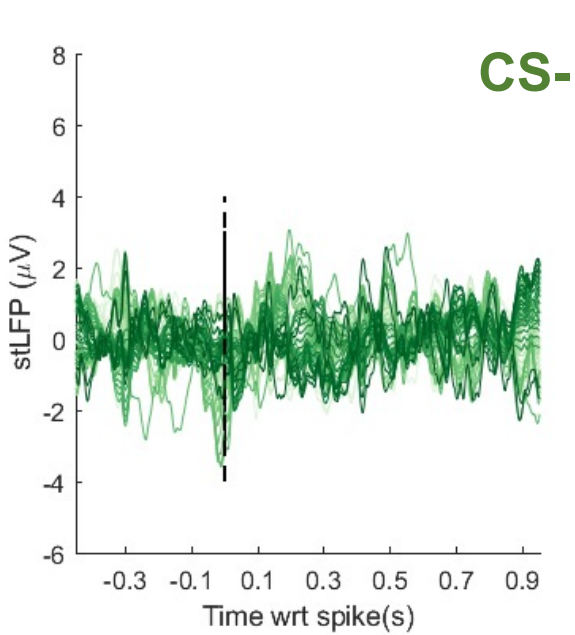
Resting state functional coupling using cross-regional spike-triggered LFP (stLFP)

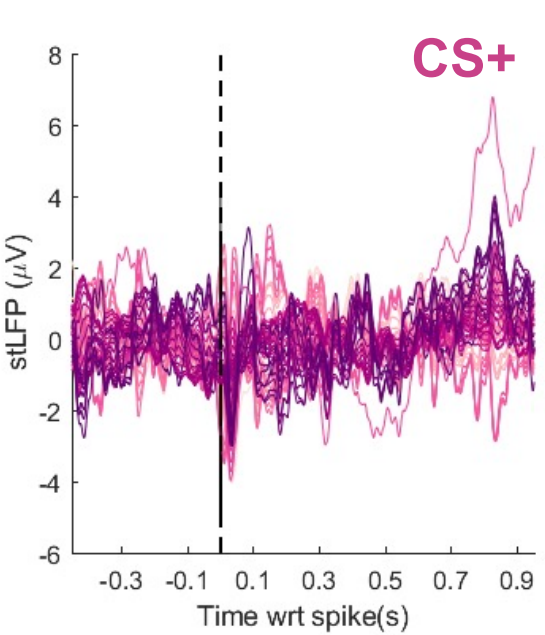


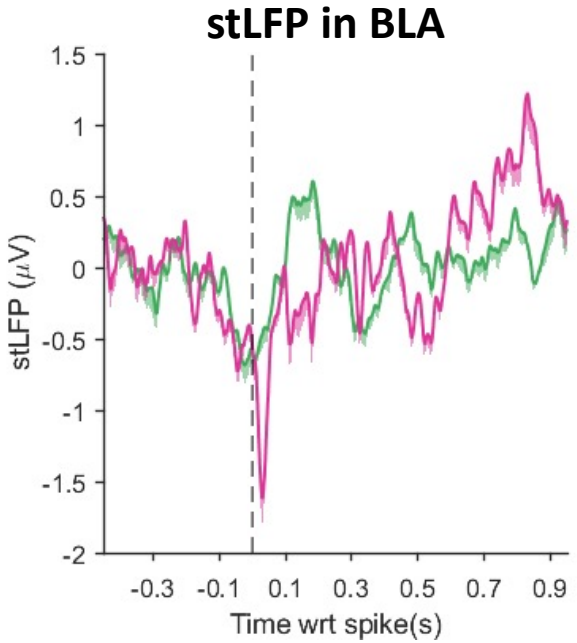


- The resting state stLFP (6 – 11 s) is computed to evaluate the strength and directionality of functional coupling between HO ACtx and BLA, while getting rid of the dominant contribution of common inputs to both regions which might occur during the sound evoked period (1 – 5 s)
- For each spike from a unit in HO ACtx in a 5 s time window right after the sound offset (6 – 11 s), the LFP in BLA is aligned wrt. the spike and the stLFPs are averaged across all the spikes in the window

Transiently enhanced functional coupling from higher-order ACtx -> BLA on the post-conditioning day following presentation of the sound paired with aversive reinforcement

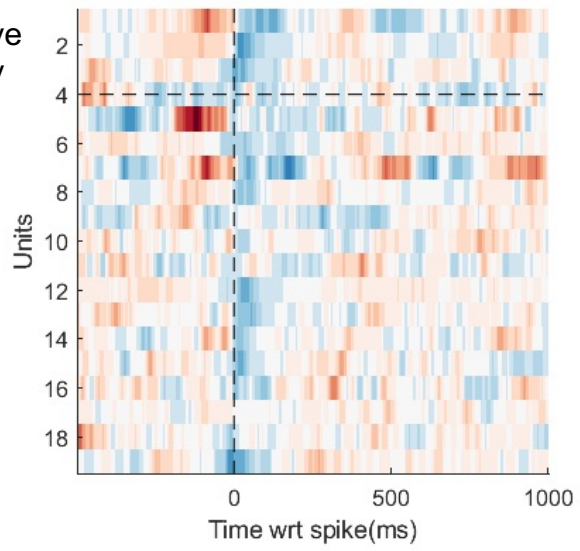


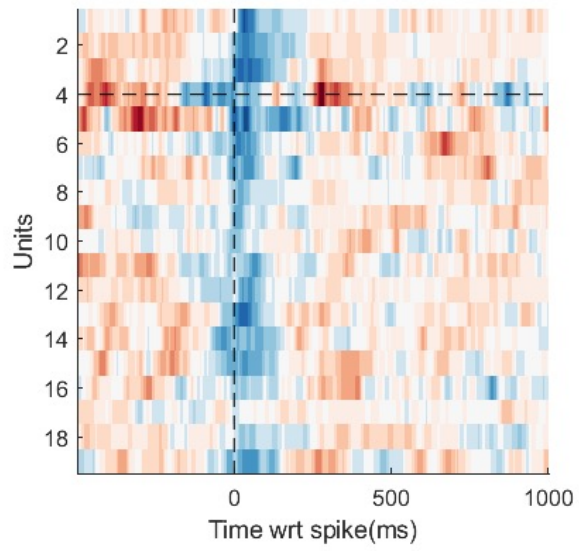


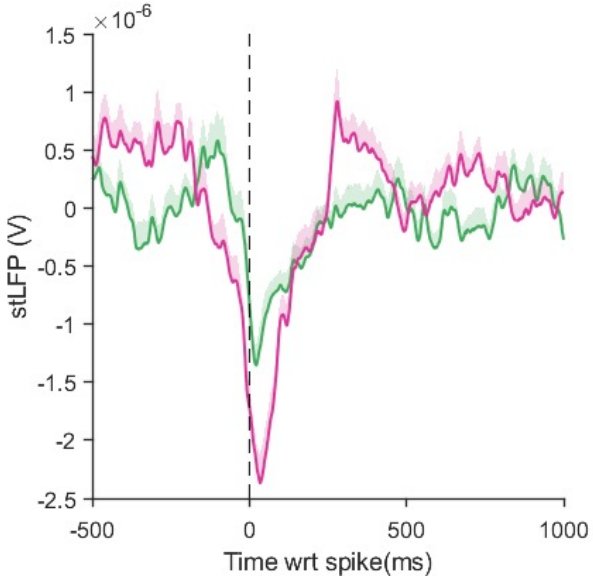


Each trace on the left and center plot correspond to one channel in BLA, and the plot on the right shows the average stLFP (mean across all channels in BLA) for an example unit in HO ACtx

Putative C-Amy

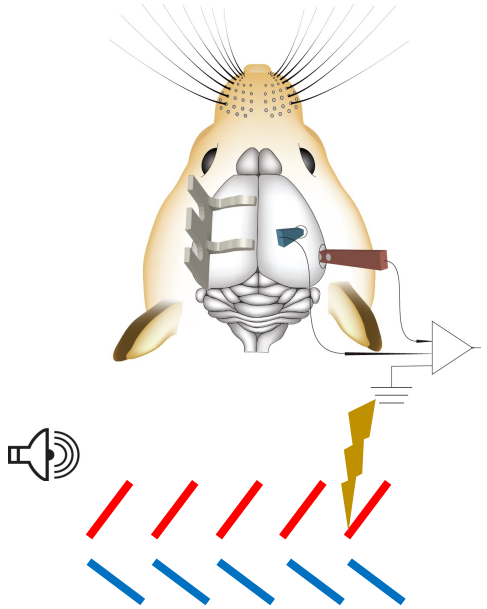


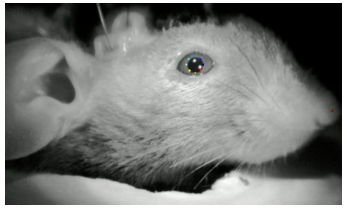


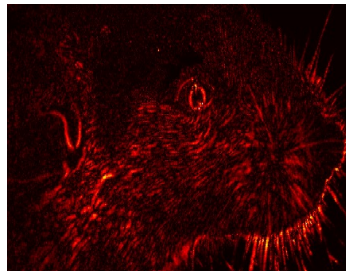


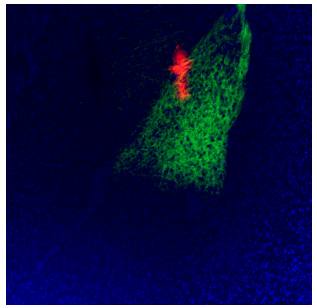
stLFP across cortical units suggests enhanced functional coupling from HO ACtx -> BLA on the post-conditioning day following presentation of the sound paired with aversive reinforcement.

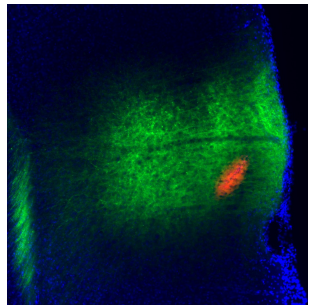
Summary: Distributed network interactions between higher-order ACtx and BLA contribute to affective sound processing

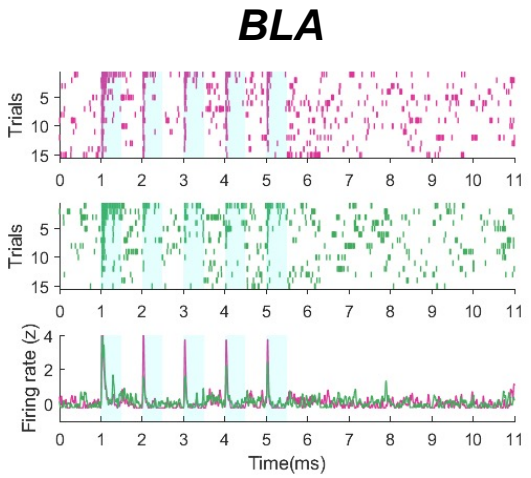


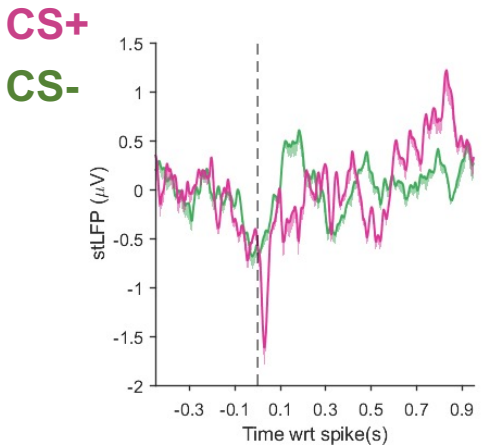


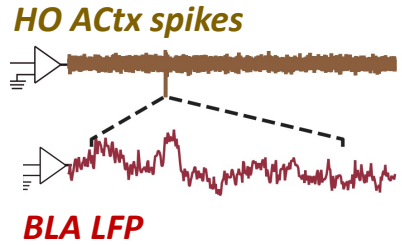










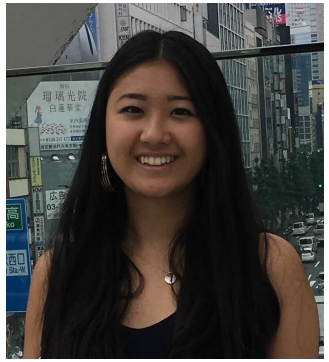


- A neutral sound is transformed to a distressing sound after learned associations
- Spontaneous behavioral measures obtained using orofacial videography provide evidence for the threat associative learning

Simultaneous recordings from HO ACtx and BLA reveal that

- these distributed networks of neurons show an enhanced response to the sound paired with the aversive reinforcement, and
- their functional coupling contributes to the retrieval of the aversive sound memories

Acknowledgements



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Thank you all for coming to my poster!
Any feedback or questions?