***Emerging activity dynamics and noradrenergic modulation of prelimbic cortical neuronal ensembles during heroin seeking***

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**Background**: Cue-induced drug seeking requires activation of the prelimbic prefrontal cortex (PL) that is dysregulated in substance use disorder. The heterogeneity in PL cell types has made it difficult to unveil the precise PL circuit dynamics which orchestrate drug seeking.

**Methods**: To address this, we developed a head-fixed heroin self-administration procedure to allow longitudinal tracking of PL neuronal activity during behavior. To measure the activity of PL projection neurons, we virally labeled these neurons for calcium imaging (AAVdj-CaMKIIa-GCaMP6s) and implanted a GRIN lens dorsal to PL.

**Results**: Subsequent two-photon recordings reveal both frequency and amplitude of calcium events in PL neurons are reduced following acquisition of heroin seeking, effects which persist through extinction, but then resurge during reinstatement in a manner time-locked to cue presentation. As local noradrenergic signaling rapidly increases PL activity and mediates drug-cue memory retrieval, we hypothesized that inputs from the locus coeruleus (LC) contribute to rescue of PL activity during reinstatement. We found that chemogenetic inhibition of LC→PL axon terminals blocks cue-induced reinstatement, an effect which surprisingly persisted upon a subsequent cue test. These effects were specific to drug cues, as LC→PL inhibition neither persistently suppressed stress- or drug-primed heroin-seeking reinstatement nor affected cue-induced sucrose-seeking reinstatement. Interestingly, we found suppressed cue-induced reinstatement coincides with suppressed excitatory activity in a discrete PL cell cluster which ordinarily decodes the drug-cue. Ongoing analyses aim to assess sex differences and changes in single-cell activity dynamics throughout heroin self-administration, extinction, and reinstatement.

**Conclusions**: These studies suggest LC→PL axon activation rescues excitatory activity in downstream PL projection neurons for cue-induced reinstatement of heroin seeking.

**References**: Vollmer KM*, Doncheck EM*, Grant RI, Winston KT, Romanova EV, Bowen CW, Siegler PN, Green LM, Bobadilla AC, Trujillo-Pisanty I, Kalivas PW, Otis JM. *Front Behav Neurosci* 15:744715 (2021), PMID 34776891; Grant RI, Doncheck EM, Vollmer KM, Winston KT, Romanova EV, Siegler PN, Holman H, Bowen CW, Otis JM. *eLife* 10:e65764 (2021), PMID 34184635.

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