Sex Differences in Addiction: Role of Hippocampal Monoamines

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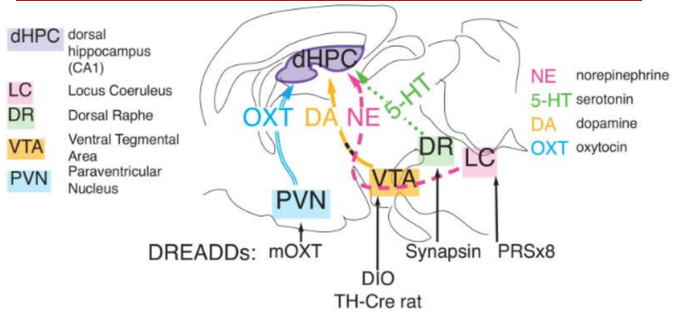
Abstract

Drug addiction is a multifaceted national crisis with an annual cost of around $740 billion dollars along with an impact on tens of thousands of lives. Current research has shown that there are sex differences in response to drug abuse, with female populations being more susceptible to drug craving and relapse, ultimately leading to dependence. Epidemiological studies also show that stress predisposes women to shorter periods of abstinence compared to men. Animal models have recapitulated these findings using rodents. However, it is unclear what molecular and neural pathways underlie resilience or susceptibility to addiction that could inform efforts to develop novel pharmacotherapies strategies. We hypothesize that norepinephrine (NE) signaling from the locus coeruleus (LC) to the dorsal hippocampus CA1 (CA1) enhances drug-seeking behaviors. Thus, inhibiting NE signaling in this circuit could offer a novel pharmacological target to prevent or reverse addiction in rodent models and in turn, humans as well. We have identified this LC to hippocampus signaling as being important for driving cocaine seeking during the first day of cocaine extinction, in a sex dependent manner and thus our interest in this pathway is two-fold: 1. We are interested in the persistence effects of manipulating LC-NE signaling to CA1 using designer receptors exclusively activated by designer drugs (DREADDs) and 2. We are in interested in ribonucleic acid (RNA) transcripts that are expressed in the hippocampus that are specific to male (resilient) or female (susceptible) rats. In order to test our hypotheses, we use a Seeking Persistence Paradigm (SPP) where male and female rats undergo 10 days of cocaine self-administration followed by extinction day 1 (ED1) testing, prior to which clozapine-n-oxide (exclusively activates DREADD receptors; CNO) is administered to either inhibit or excite signaling from LC-NE neurons to CA1. Following ED1 testing, rats are subject to 2 weeks of forced abstinence and are retested for seeking persistence (ED2), to determine the persistence effects of LC-NE to CA1 manipulations on long-term cocaine seeking persistence. A separate group of rats were tested on ED1 and tissues were fresh dissected for RNA-sequencing analysis. Our studies will provide important and novel insights into the molecular and neural mechanisms that drive addiction behaviors and could provide new pharmacological perspectives in the fight against substance abuse disorders.

Background



- **This figure (Fig. 2) shows projections to CA1 that are implicated in driving cocaine seeking persistence**
- Sagittal section of a rat brain indicating a working model of enhanced cocaine-seeking persistence
- Brain regions that project to the dHPC CA1 that are implicated in driving cocaine-seeking on ED1 prior reports using pharmacological interrogation *(summarized in table 1)*
- DREADDs indicates different promoters that can be used to drive cell-specific expression cell bodies projecting to the dHPC CA1

References

Cason, A. M., Kohtz, A.,&Aston-Jones, G. (2016). Role of Corticotropin Releasing Factor 1 Signaling in Cocaine Seeking during Early Extinction in Female and Male Rats. *PloS one*, *11*(6), e0158577. https://doi.org/10.1371/journal.pone.0158577

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Methods and Materials

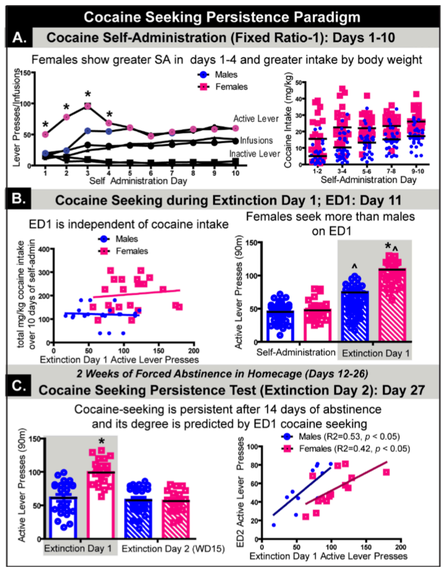


Figure 1. Cocaine Seeking-Persistence Paradigm (SPP). These data represent highly reproducible data gathered in multiple cohorts **A)** Animals are trained to lever-press for cocaine in an operant chamber containing active and inactive levers in 2-h daily sessions on a fixed ratio-1 (FR1; 0.2 mg/kg cocaine (male, ♂) 0.16 mg/kg cocaine (female, ♀) in 50 µl bolus infusions paired with a 5-s light-tone cue; 20-s time-out after each infusion) schedule until they meet criterion of 10 consecutive days of <10 infusions (SA; **n=52/group**). These cocaine doses produce equivalent cocaine cue pairings across sex by SA 5-10, per prior reports[13]. *(right)* ♀ show greater cocaine intake per body weight vs ♂. *$p<0.05$ ♀ vs ♂. **B)** Once animals reliably lever press (>10 infusions), rats are tested for initial seeking behavior (ED1, extinction day 1) as measured by active lever presses **n=(38/group)**. *(left)* ED1 lever pressing does not correlate to mg/kg cocaine intake during self-administration, which is why we control for cocaine-cue pairings and not intake with different doses for each sex[13]. *(right)* ♀ show greater cocaine-seeking behavior vs ♂ on ED1^*$p<0.05$* compared to SA (avg day 8-10). *$p<0.05$ ♀ vs ♂. All manipulations in proposed experiments (e.g. CNO) are administered prior to ED1. Rats are then returned to the homecage for 2 weeks. **D)** *(left)* Following 2 weeks of abstinence, the persistence of seeking behavior (as measured by active lever pressing) is recorded. **(n=24/group)** *(right)* Cocaine-seeking on ED1 strongly predicts cocaine-seeking persistence. **(n=10-15/sex).**

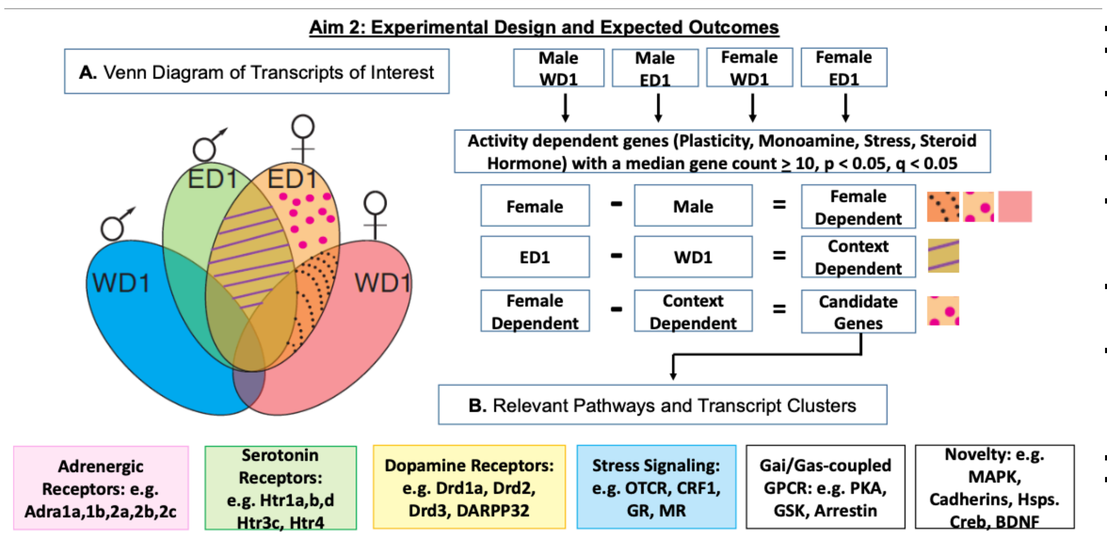
SPP Testing

- LC-NE input to CA1 on cocaine seeking-persistence in male and females
- The role of the LC-NE signal appears to be more strongly involved in females based on prior data (Table 1)
- Male (n=11/condition) and female (n=11/condition) rats received a micro infusion of an AAV containing a PRSx8 promoter driven HM4Di or HM3Dq DREADD bilaterally
- A similar virus that expresses only the mCherry tag will be used in control animals
- We have shown that our PRSx8 promoter-driven AAV DREADD is specific to LC-NE neurons and that we can induce activation of LC-NE neurons by applying CNO. Rats remained in their homecages for 6 weeks following DREADD infusion
- Rats were then intracranially implanted with cannula targeted to CA1
- Rats were assessed for the effects of inhibiting or activating LC(NE)->CA1 input on cocaine CPP
- Rats were returned to their homecages for 2 weeks, and then were reassessed for CPP as in prior reports to determine persistent effects as secondary violation
- The preliminary results indicated that inhibiting LC(NE)-CA1 decreases cocaine SPP in females (Table 1) and that excitation of the LC(NE)-CA1 induces cocaine-seeking behavior in male rats that resembles the behavior of female rats
- We hypothesize that there will be a leftward shift in the relationship between LC and CA1 firing with DREADD intervention in females, and that Gq stimulation by DREADDs will produce a rightward shift in the relationship between LC and CA1 firing in males
- The analysis of RNA transcripts will represent potential therapeutic targets, in particular in females, to reduce cocaine-seeking behavior

	Cocaine Seeking Persistence	Males		Females	
	Extinction Day 1 Manipulation	**Extinction Day 1 Injection**	**Extinction Day 2 (WD15)**	**Extinction Day 1 - Injection**	**Extinction Day 2 (WD15)**
Systemic	Oxytocin, 0.3 or 1.0 mg/kg IP	↓	No Effect	↓	↑
	CRF-1 antagonist, 10 or 20 mg/kg IP	↓		↓↓	
	S-Propranolol 10 mg/kg IP	↓		↓	↓↓ Persistent
Intra-CA1	5HT1A+1B Antagonists	↓	↓ Persistent	↓	↓ Persistent
	β-Adrenergic Antagonists	No Effect	No Effect	↓↓	↓↓ Persistent
	(Dorsal Raphe Synapsin hM4Di) + CA1 CNO	↓	↓ Persistent	↓	↓ Persistent
	(Locus Coeruleus PRSx8 hM4Di) + CA1 CNO	No Effect	No Effect	↓↓	↓↓ Persistent
	(Locus Coeruleus PRSx8 hM3Dq) + CA1 CNO	↑	↑	↓	↓ Persistent

Table 1: Long-term or transient effects of different pharmacological and pharmacogenetic interventions on ED1. We have tested several different pharmacological manipulations both systemically administered and administered directly to the dorsal hippocampus on ED1. Effects to reduce or increase cocaine seeking behavior compared to saline administered controls are reported by arrows indicating the direction of effects. Only significant effects are reported p <0.05, n=6-12/group. Blank cells indicate untested effects.

Results and Future Direction



- **Fig 3 (Aim 2) shows the expected outcomes and analysis of:**
- **A. Prediction of transcripts differentially expressed in conditions of interest (e.g. sex/ED1)**
- *Sex differences in the transcriptome:* In human post-mortem brain tissue, ~448 genes show sex-biased expression. In adult mice whole brain, ~600 genes show sex-biased expression
- As such, we predict a similar number of transcripts (~400) to display sex-biased expression independent of experimental condition in the rat
- *Cocaine-Seeking dependent differences in the transcriptome*: Many signaling pathways have been implicated in the development of addiction, primarily studied in the ventral tegmental area and nucleus accumbens. This proposal will extend these findings to CA1
- *Sex-biased signaling*: CRF1 signaling in males is biased towards **β**-arrestin-(Rho, Akt, Src) and CRF1 endosomal internalization, whereas in females, CRF1 receptor activation is associated with Gş-PKA-CREB induction
- This sex difference has been identified as a contributing factor in driving enhanced tonic LC activity in response to stress in females. We have previously shown that systematic administration of CRF1 antagonists reduce ED1 cocaine-seeking more effectively in female rats than in males (Table 1). Thus, we expect that transcripts downstream CRF1Rs will be upregulated in SPP
- **B. Cluster analysis of associated transcripts**
- *Monoamine receptor transcription*: Prior reports indicate that exposure to cocaine induces transcription of adrenergic, serotonergic, and dopaminergic receptors in nucleus accumbens. We predict that at least some of these receptors may have increased transcription following ED1
- *Monoamine Signaling*: Our preliminary data indicate that receptor signaling in CA1 can be attenuated to reduce cocaine SPP, therefore we predict that transcripts downstream G-protein coupled receptors will reflect activity dependent induction following ED1
- *Stress signaling, novelty, memory, and addiction phenotypes*: Our model uniquely examines plasticity related changes during initial abstinence that contribute to cocaine-seeking persistence. Strongly implicated genes based on prior reports would be downstream of the mammalian target of rapamycin complex 1 (mTORC1), cAMP-responsive element binding (CREB), including CRF, brain-derived neurotrophic factor (BNDF), and c/EBP

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