The Role of Prefrontal-Accumbens Circuits in the Regulation of Cognitive Flexibility and Stress-Related Pathology

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INTRODUCTION

- Prolonged social and environmental stress exposure tax the adaptive capacity (flexibility) of an individual and is widely recognized as a major determinant of risk and severity of neuropsychiatric disease.
- Impaired cognitive flexibility contributes to increased susceptibility to stress, development of substance abuse, reduced emotional control, and is one of the most widely reported cognitive symptoms across neuropsychiatric disease.
- Cognitive flexibility relies on coordinated activity of distinct populations of layer 5/6 pyramidal neurons (PN) in the prelimbic region (PrLc) and their downstream targets like the nucleus accumbens core (NAc) and medial-dorsal thalamus (MDT).
- PrLc pyramidal neurons can be primarily divided into those expressing dopamine type I (D1) or type II (D2) receptors.
- Past studies have demonstrated that deficits in cognitive flexibility produced by prolonged exposure to chronic unpredictable stress aligns with distinct (opposing) changes in the function of PrLc D1 and D2-expressing subcircuits.

Study Goal: this project tested the hypothesis that stress-induced deficits in cognitive flexibility can be restored by increasing activity in PrLc sub-circuits.

METHODS

Transgenic Animals. To selectively target D1 versus D2 pyramidal neurons, transgenic mice that express the enzyme, cre-recombinase, in D1- or D2-receptor expressing neurons were used.

Surgery and Chemogenetic Manipulation. A cre-dependent retrograde virus was infused into the NAc to promote expression of an inhibitory *DREADD* (Designer Receptor Exclusively Activated by Designer Drugs; rAAV-hsyn-d10-hm4Di-(Gi)-mcherry) or a control GFP protein within D1- or D2-expressing neurons that project to this region. A guide cannula was implanted in the PrLc to deliver the DREADD agonist, CNO (clozapine-n-oxide) to permit isolation of DREADD effects to PrLc-NAc neurons.

Attention Set Shifting Task. Cognitive flexibility will be studied using a lever-based attentional set-shifting task (ASST; Fig. 2) that resembles the clinically used Wisconsin Card Sorting Task in its sensitivity to distinct components of decision-making such as suppression of irrelevant strategies, acquisition and generation of novel strategies, and maintenance of effective strategies.

Lever Training: Following food training, animals underwent lever training until they omitted less than five total trials for two consecutive days, after which a lever preference was assessed with a bias test.

Cognitive Testing: Mice underwent a **visual cue discriminative learning test, an extra dimensional (ED) set shift test of flexibility, and finally a reversal learning test.** Mice received CNO 10-min prior to the ED shift. **Ten consecutive correct responses** determined the criterion for passing on to the next test the following day. The active lever for the set shift task was the opposite of the mouse's bias, while reversal task used the mouse's preferred lever.

RESULTS

CUS Week 1 and 3

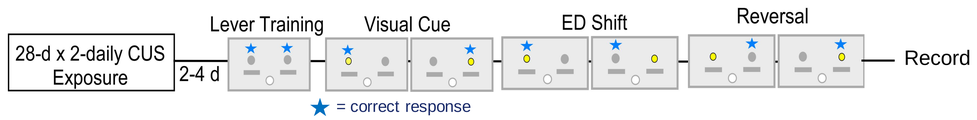
Time	1st Stressor	Duration	Time	2nd Stressor	Duration
8:00	Novel Environment	60 min	3:00	Forced Swim	10 min
10:00	Open Field	30 min	12:00	Cold Room	15 min
11:30	Restraint	30 min	12:00	Crowding on Orbital Shaker	60 min
9:00	Cage Tilt	60 min	5:00	Food Deprivation	night
8:30	Small Room Isolation	60 min	5:00	Lights on	night
8:00	Wet Bedding	8 hours	12:00	new cage/ no nest/ isolation	night
11:30	Forced Swim	10 min	12:00	Restraint	60 min

CUS Week 2 and 4

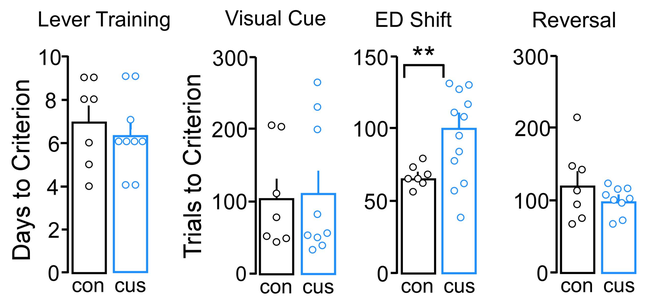
Time	1st Stressor	Duration	Time	2nd Stressor	Duration
10:00	Crowding	30 min	5:00	Cage Tilt	night
8:00	Orbital Shaker	60 min	3:00	Open Field	60 min
11:00	Small Room Isolation	60 min	5:00	New Partner	60 min
11:00	Restraint	60 min	12:00	Wet bedding in home cage	4 hours
9:30	Cage Tilt	60 min	3:30	Novel Env. (no water/bedding)	60 min
8:00	Isolation on shaker	30 min	5:00	Lights on	night
11:30	Forced Swim	10 min	12:00	Restraint	60 min

One week sample of unpredictable stressors of various durations, intensities, and in various locations (green= stress room A, red= stress room B, purple= stress room C, blue= cold room, yellow= colony). Mice received two weeks of less intense stress (top) or two or four weeks of more intense stress (bottom).

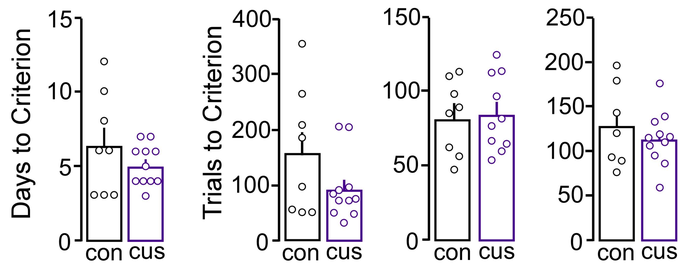
Attentional Set-Shifting Experimental Time line



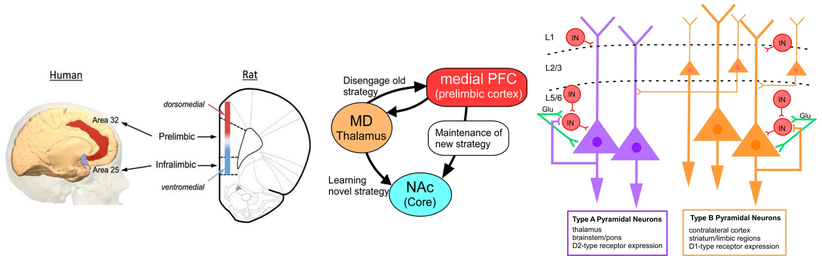
CUS impairs cognitive flexibility in male mice



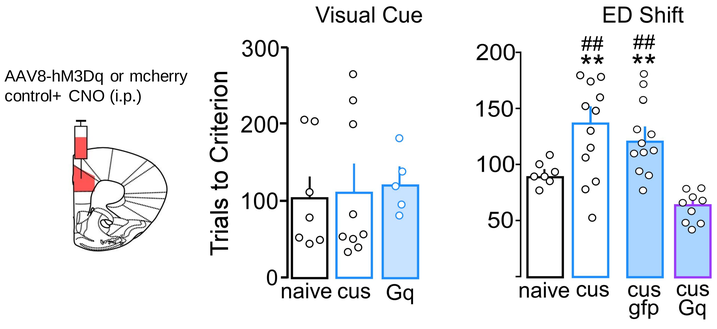
CUS does not impair cognitive flexibility in female mice



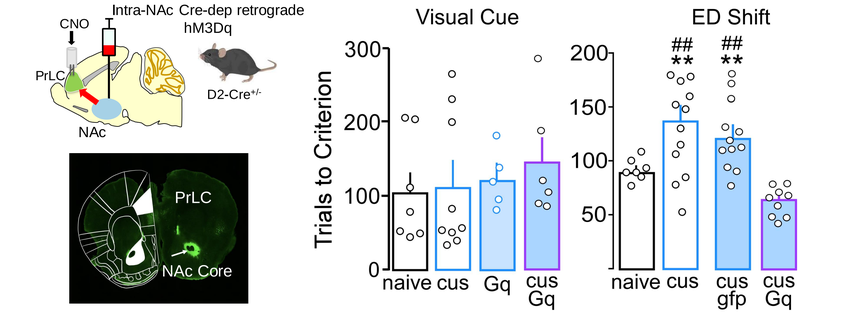
Prefrontal cortex regulates cognition through complex sub-circuits



Activation of the prelimbic cortex restores CUS-induced deficits in flexibility in males



Activation of the NAc projecting D2R-expressing pyramidal neurons restores CUS-induced deficits in flexibility



SUMMARY/CONCLUSIONS

- Current data show that our regimen of CUS significantly impairs cognitive flexibility in males but not females. This lack of effect highlights potential resilience in females to stress-related deficits in cognition that is observed in women.
- Deficits in cognitive flexibility were rescued by increasing neural activity in prelimbic cortical pyramidal neurons regardless of their downstream target, indicating that deficits in flexibility are due in part to reduced neural activity in this region.
- Selective activation of prelimbic-accumbens pyramidal neurons expressing the dopamine type II receptor was sufficient to restore CUS-induced deficits in flexibility, highlighting a role for this subcircuit in flexible decision-making under naïve and pathological states.

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