Effects of Amygdalar CaMKII Activity on Extinction and Reconsolidation of a Cocaine-Associated Memory

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INTRODUCTION

- Memories of environmental cues associated with drug use contribute to relapse to drug-taking, which is prevalent in addiction.[1,2]
- The amygdala is involved in the formation of memories associated with drug use.[3]
- Manipulating protein kinase activity within the amygdala regulates both the reconsolidation and extinction of drug-induced memories and may serve as a potential treatment for addictive disorders.[4,5]
- Proteomic approaches may be beneficial for identifying proteins that are differentially expressed following extinction and reconsolidation of a drug-cue associated memory[6]
- Calcium/calmodulin-dependent protein kinase II (CaMKII), has been shown to modulate synaptic activity, contributing to memory storage, and may be involved in the formation of drug-associated memories.[7,8]
- The involvement of CaMKII in drug-cue memory formation in the basolateral amygdala (BLA) was investigated via phospho-proteomic analysis and a drug reinstatement paradigm

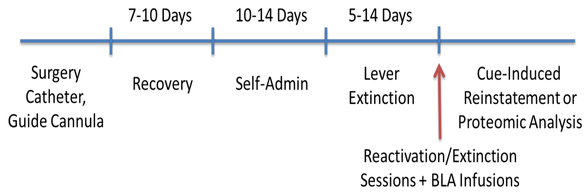
METHODS

Subjects: Male Sprague-Dawley rats (275-325 g) were maintained on a regular light/dark cycle, and given *ad libitum* access to water. Rats were maintained at 90% of their free-feeding weight throughout behavioral experiments.

Surgery: All rats were implanted with a chronic indwelling catheter into the right jugular vein for cocaine self-administration. In experiment 2, rats also received bilateral guide cannulae targeting the BLA .

Behavioral Procedures: Rats were trained to self-administer cocaine (1 mg/kg) accompanied by a 10 s light+tone cue (CS) on an FR1 schedule of reinforcement on an active lever. Rats underwent 10-14 daily 1 h sessions until reaching acquisition of self-administration (≥8 infusions for each of the last 3 consecutive SA sessions) . Responding was then extinguished during 5-14 daily 1 h sessions, in which both levers were available but had no programmed consequences. After meeting extinction criteria (<25 active lever presses over 2 consecutive days) rats underwent either cue reactivation (3 CS presentations) or one of two extinction durations (60 or 120 CS presentations). In experiment 1, rats were euthanized by focused microwave irradiation 15 min after memory session. Brains were dissected and the amygdala was lysed, subjected to tryptic digestion and enriched for phosphopeptides. Samples were analyzed using unbiased, label-free quantitation using a nano-UPLC system coupled to an orbitrap mass spectrometer. A subset of the phosphopeptides were further analyzed for quantitative differences using targeted, multiple reaction monitoring (MRM) mass spectrometry. In experiment 2, immediately following the reactivation/extinction sessions, rats underwent a bilateral infusion of the CaMKII inhibitor KN62 (340 or 680 ng/side) or its vehicle into the BLA. 24 hours after infusions, rats underwent a 1 h cue-induced reinstatement session. Responses on the active lever resulted in presentation of CS but no cocaine infusion. The placement of cannula in the BLA was confirmed using standard histological techniques.

Statistical Analysis: For proteomic data, the normalized average intensity across transitions for each phosphopeptide was computed for each sample and these values were averaged within groups. Group averages were compared statistically using an SRMstats restricted analysis and p-values corrected for multiple comparisons and p<0.05 considered significant. Behavioral data was analyzed using repeated measures 2-way ANOVA and p<0.05 considered significant.



Schematic of experimental timeline

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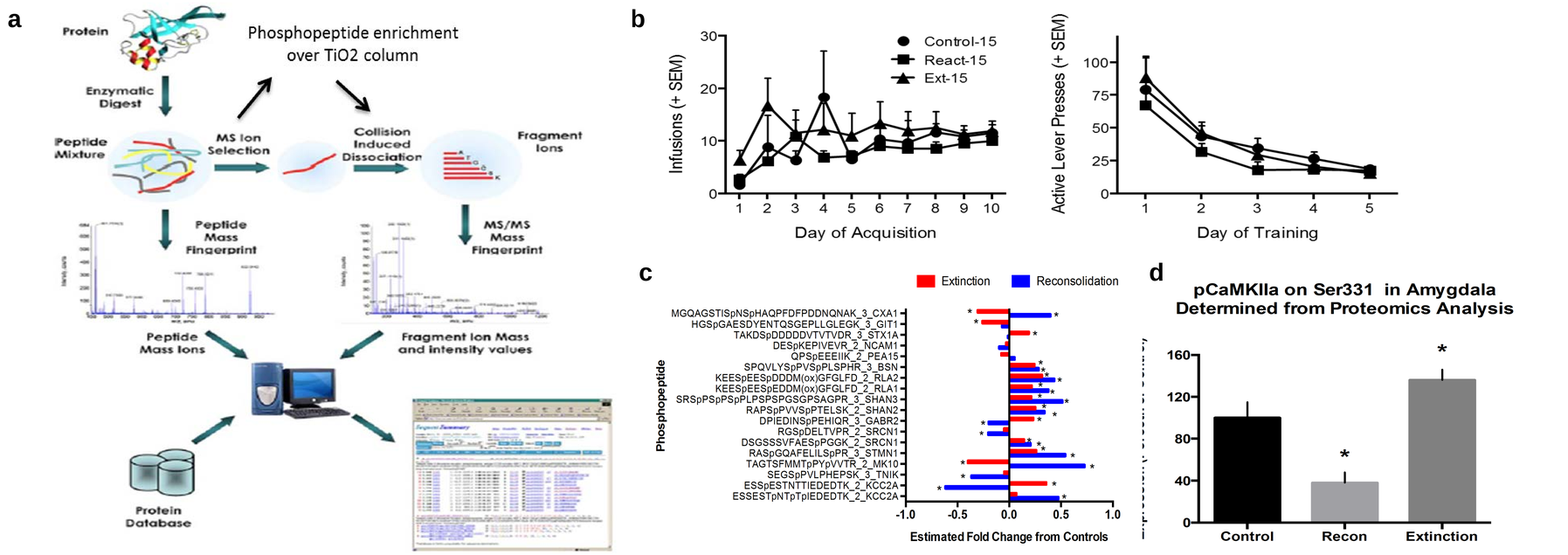
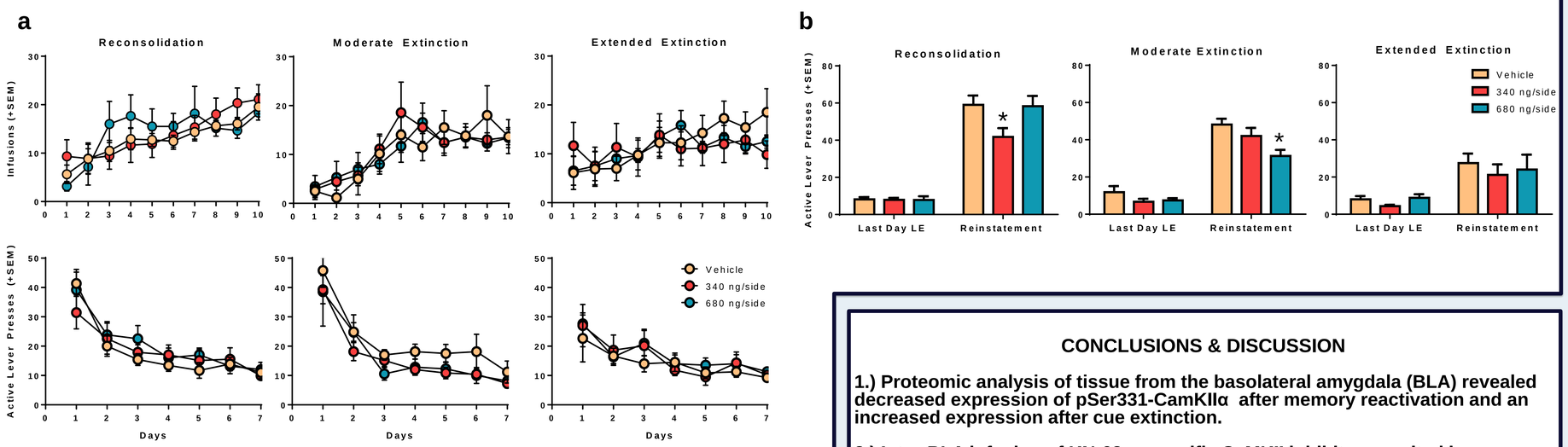


Experiment 1: Proteomic Analysis of BLA Following Drug-Memory Manipulations. 15 min after memory test sessions, brains were dissected and BLA tissue was subjected to proteomic analysis. **(a)** Schematic of proteomic analysis.[6] **(b)** Number of infusions earned during acquisition and number of active lever presses during lever extinction. Repeated measures ANOVA revealed no significant differences across treatment group. **(c)** Fold change of various phosphorylated proteins following reconsolidation or extinction relative to controls. **(d)** CaMKIIα phosphorylation at Ser331 was significantly upregulated following extinction and significantly downregulated in response to memory reconsolidation (n = 8 per group, p <.001).



Experiment 2: Effects of CaMKII Inhibition on Cocaine-Seeking Behavior. Rats trained to self-administer cocaine had the drug-associated memory reactivated to induce reconsolidation or underwent one of two extinction durations. Immediately following these sessions, rats were infused with KN-62 (340 ng/side or 680 ng/side) or its vehicle in the BLA. 24 hours later, rats were subjected to a cue-induced reinstatement session. **(a)** Number of infusions earned during acquisition and number of active lever presses during lever extinction. Repeated measures ANOVA revealed no significant differences across treatment group. **(b)** Levels of cue-induced reinstatement were significantly lower in rats that received a low dose of KN-62 immediately following drug-cue memory reactivation sessions compared to vehicle controls. The effect of moderate levels of drug-cue memory extinction were significantly enhanced by a high dose of KN-62, whereas extended extinction alone was sufficient to reduce drug-seeking independent of CaMKII inhibition (n = 6-11 per group, * p<.05).

CONCLUSIONS & DISCUSSION

1.) Proteomic analysis of tissue from the basolateral amygdala (BLA) revealed decreased expression of pSer331-CamKIIα after memory reactivation and an increased expression after cue extinction.

2.) Intra-BLA infusion of KN-62, a specific CaMKII inhibitor, resulted in decreased responding on a cue-induced reinstatement test both in rats that had undergone cue reactivation and cue extinction.

3.) Inhibition of CaMKII may interfere with the reconsolidation of drug-cue associated memory, thus impacting its stability.

4.) Blocking activity of CaMKII may facilitate extinction of drug seeking behavior.

5.) Inhibitors of CaMKII might be useful adjuncts to extinction training in the treatment of addiction.

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