Acute Membrane Cholesterol Depletion Alters Accumbal Dopamine Terminal Dynamics

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INTRODUCTION

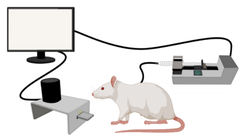
- The dopamine transporter (DAT) is the primary site of action for cocaine
- Chronic cocaine use results in the dysregulation of DAT function and, therefore, synaptic dopamine (DA) transmission, which contributes to enhanced drug-seeking and -taking behaviors
- While the mechanisms underlying cocaine-induced DAT dysfunction remain elusive, emerging evidence suggests a potential link between DAT function and brain cholesterol content
- Brain cholesterol regulates the function of membrane proteins either directly through protein-cholesterol interactions or indirectly by altering membrane fluidity, which modulates protein conformation
- Whether cocaine dysregulates DAT function by disrupting cholesterol homeostasis has not been explored

HYPOTHESIS

Cocaine self-administration significantly alters brain cholesterol homeostasis, which contributes to the altered DA transmission and DAT function observed with psychostimulant exposure

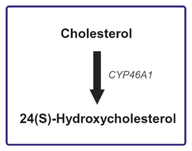
METHOD

Cocaine Self-Administration: Male Sprague-Dawley rats self-administered 40 injections of cocaine (1.5 mg/kg/infusion, 6 hrs/day) or saline (control) for five days on a fixed-ratio one (FR1) schedule

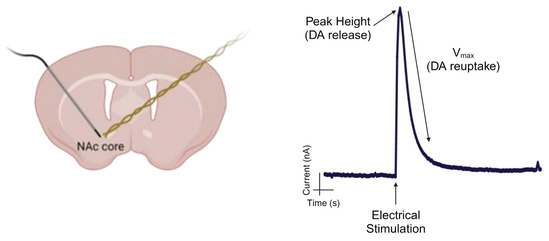


Striatal [^{3}H]DA Uptake and Cocaine Inhibition: Synaptosomes were prepared from cocaine SA and control animals. [^{3}H]DA uptake was performed to measure DAT function. To determine the potency of cocaine to inhibit DA uptake, [^{3}H]DA uptake was performed in the presence of increasing concentrations of cocaine.

Assessment of Cholesterol Metabolism: Membrane (bioactive) cholesterol content was measured using an Amplex Red cholesterol assay kit. 24(S)-hydroxycholesterol levels were determined using an ELISA kit.



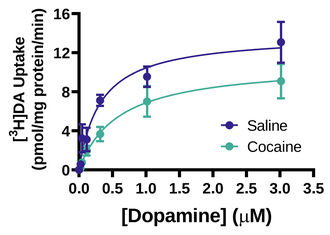
Ex-Vivo Fast-Scan Cyclic Voltammetry: Recording and stimulating electrodes were placed in the NAc core, and an electrical pulse (4ms, 750 µA) evoked DA release and reuptake. DA kinetics were determined using Michaelis-Menten modeling.

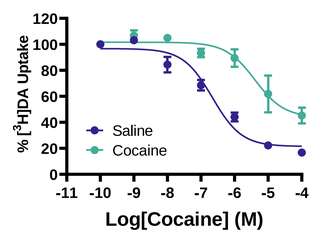


Cholesterol Depletion: Once the evoked extracellular DA response was stable, methyl-β-cyclodextrin (MβCD, 0.3, 3, or 10 mM), a membrane cholesterol chelator, was perfused over brain slices for one hour, followed by washout. Membrane cholesterol depletion was validated using an Amplex Red cholesterol assay kit.

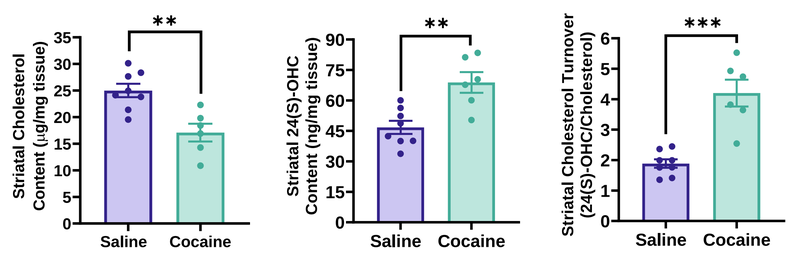
RESULTS

1. Cocaine self-administration decreases [^{3}H]DA uptake and cocaine inhibition of [^{3}H]DA uptake

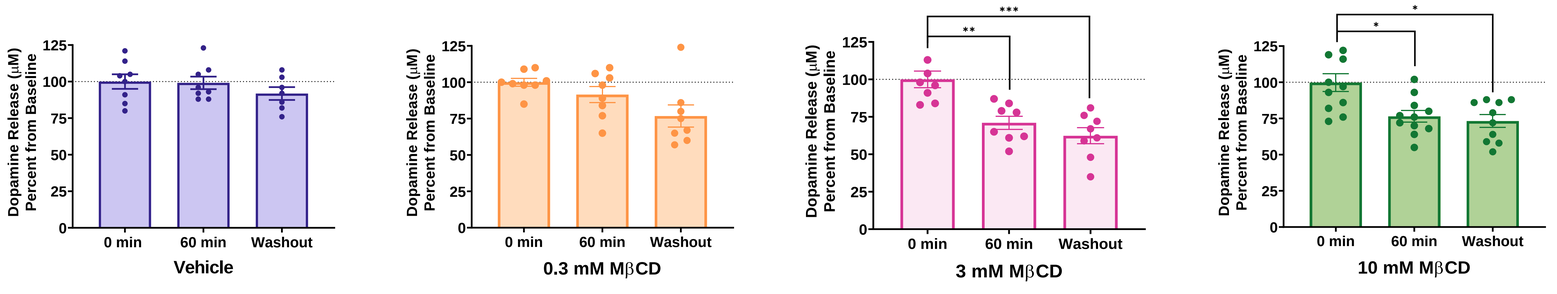




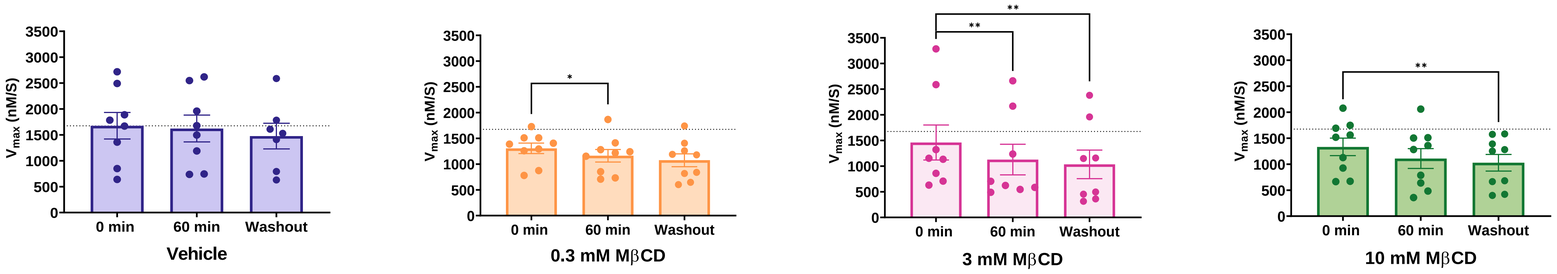
2. Cocaine self-administration decreases striatal cholesterol content via accelerated turnover



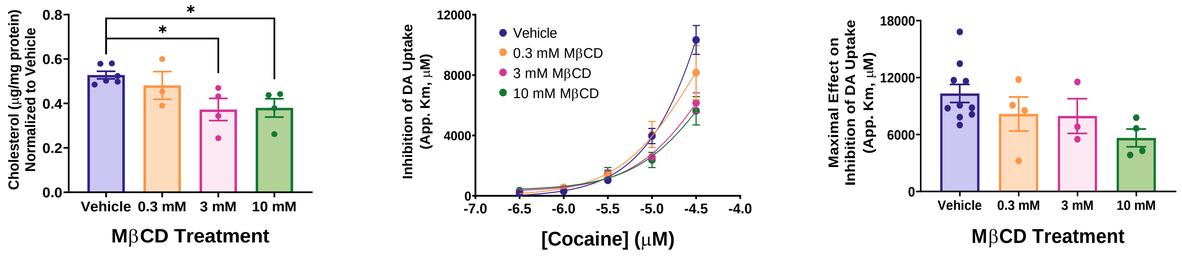
3. Membrane cholesterol depletion irreversibly decreases evoked DA release



4. Membrane cholesterol depletion irreversibly decreases the maximal velocity of DAT



5. Membrane cholesterol depletion irreversibly decreases striatal cholesterol content and cocaine inhibition of DA uptake



CONCLUSIONS

- Membrane cholesterol depletion directly reduces DAT function and alters DA terminal dynamics
- Therefore, dysregulated DAT function in cocaine SA animals may be partly mediated by the disruption of brain cholesterol metabolism, highlighting this interaction as a potential therapeutic avenue for further investigation
- Further, this study underscores the need for tools to monitor brain cholesterol metabolism in individuals with psychostimulant use disorder

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