# Cholesterol Modulation of Dopamine D2 Receptor Function and Subcellular Localization

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## INTRODUCTION

- Dopamine D2 receptors (D2Rs) are Gi/o-coupled GPCRs that regulate dopamine signaling and are disrupted in Parkinson's disease, schizophrenia, and substance use disorders
- GPCR activity is highly sensitive to the lipid composition of the plasma membrane
- Cholesterol-rich membrane microdomains, known as lipid rafts, spatially organize GPCRs and their signaling partners to facilitate signal transduction
- However, the precise role of cholesterol in modulating D2R localization and function within lipid rafts remains poorly understood

## HYPOTHESIS

Cholesterol depletion impairs D2R function and membrane localization by disrupting lipid raft organization, thereby weakening D2R coupling to its downstream signaling partners

## METHOD

***In Vivo* Chronic Cholesterol Depletion:** Male Sprague-Dawley rats were implanted with osmotic minipumps delivering the cholesterol synthesis inhibitor simvastatin (5 mg/kg/day) or vehicle for 14 days

***Ex-Vivo* Fast-Scan Cyclic Voltammetry**: Dopamine release was evoked in the ventral striatum using a single electrical pulse. Presynaptic D2R function was assessed by the ability of quinpirole, a D2R agonist, to inhibit dopamine release

***In Vitro* Cholesterol Depletion:** Mouse N2A cells stably expressing HA-tagged D2Rs were treated with 3 mM methyl-β-cyclodextrin (MβCD) or vehicle for 30 min at 37°C to deplete membrane cholesterol

**cAMP Inhibition Assay:** Cells were pre-treated with quinpirole, followed by forskolin to stimulate adenylyl cyclase production. Intracellular cAMP levels were measured using a luminescence-based cAMP assay

**Sucrose Density Gradient Ultracentrifugation:** Cells were lysed in cold detergent and fractionated using discontinuous sucrose gradients. Western blotting was used to evaluate the distribution of D2Rs and their signaling partners in lipid raft (detergent-resistant) versus non-raft (soluble) membrane fractions

## CONCLUSIONS

- Cholesterol depletion via simvastatin and MβCD reduces D2R function both *in vivo* and *in vitro*
- This dysfunction is likely driven by disrupted membrane localization of D2Rs and their associated signaling partners within lipid rafts
- Agonist stimulation of D2Rs promotes Gαi2 translocation into lipid rafts, a process abolished by MβCD, indicating that cholesterol is essential for D2R-G protein coupling
- These findings suggest that modulating membrane cholesterol may represent a promising therapeutic strategy for disorders involving impaired dopamine signaling

## ACKNOWLEDGEMENTS

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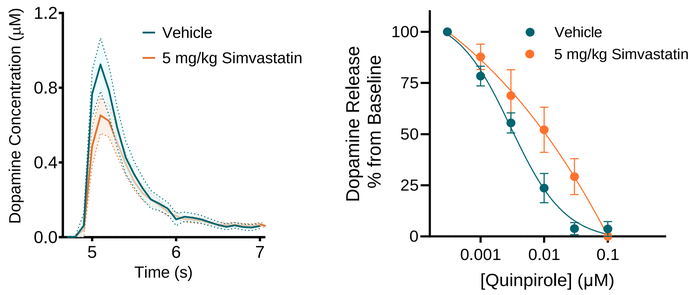
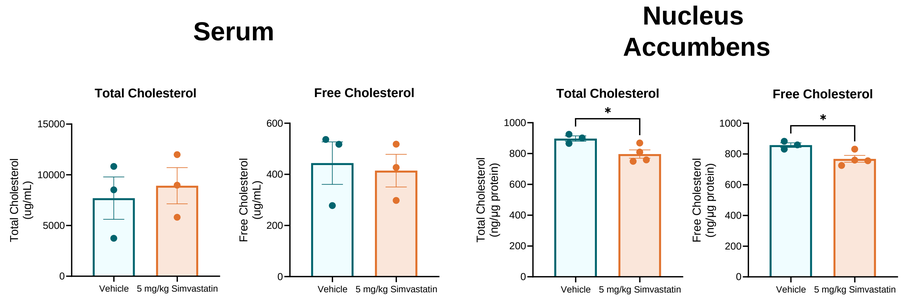
## RESULTS

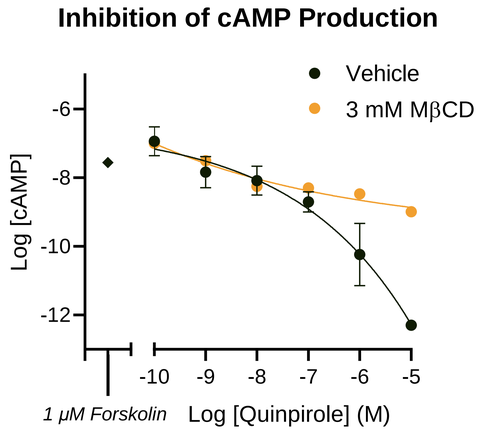
### 1. Simvastatin reduces D2R inhibition of dopamine release



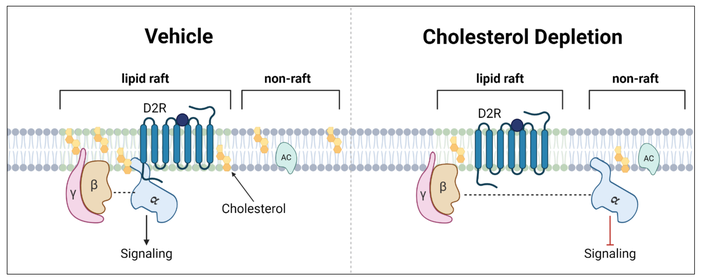

### 2. Simvastatin reduces brain, not peripheral cholesterol in rats




### 3. Membrane cholesterol depletion in mouse N2A cells impairs D2R inhibition of cAMP production




### 4. Mouse N2A cells can be fractionated into lipid raft and non-raft membrane microdomains


### 5. Acute membrane cholesterol depletion alters the membrane spatial organization of D2Rs with their associated coupling partners