Effects of cocaine on brain and behavior: An evaluation of the fragile X messenger ribonucleoprotein in dopamine D1 receptor-expressing cells of the striatum

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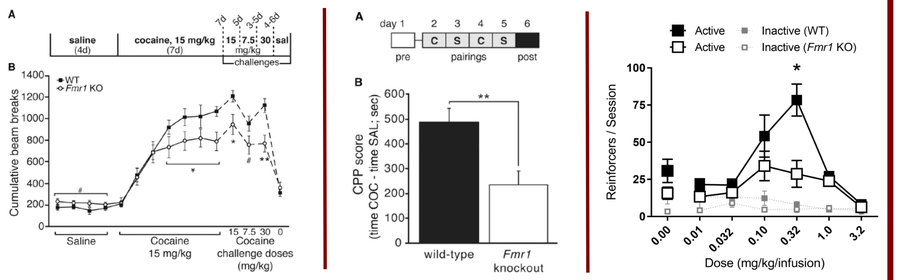
Introduction

The fragile X messenger ribonucleoprotein (FMRP; formerly fragile X mental retardation protein), an RNA-binding protein that regulates synaptic plasticity, is required for cocaine-induced synapse elimination in the striatum, a brain region critical to reward function. Moreover, loss of FMRP, either broadly or in the ventral striatum (nucleus accumbens; NAc), is capable of dampening cocaine-related behaviors. Mice lacking dopamine D1 receptors (D1Rs) do not acquire cocaine self-administration behaviors, but respond normally for a food reinforcer, and *Fmr1* KO mice, lacking FMRP, show impaired D1 receptor signaling in the striatum, suggesting that FMRP's function in these cells may specifically facilitate drug-related behavior.



Methods

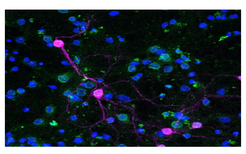
Animals: To investigate the role of FMRP's function in D1R-expressing cells of the NAc, *Fmr1* conditional knockdown (cKD) mice were generated by infusing cre-dependent *AAV2-EF1-mCherry-SICO-GFP-m-Fmr1-shRNA* into the NAc of female and male D1-BAC-Cre mice (either $Tg^{+/-}$ or $Tg^{-/-}$). Sample size was determined *a priori* using power analyses for each assay as specified in experimental methods.

Conditioned Place Preference (CPP) and Locomotor Sensitization: Mice received saline and cocaine (7.5 mg/kg, i.p.) in distinct chambers of a 3-chambered arena. On test days, mice had access to all chambers, and preference score was calculated by subtracting time spent in the saline-paired chamber from the time spent in cocaine-paired chamber. Three days following the post-test, mice were given 4 days of saline administrations followed by 7 days of cocaine administrations (15 mg/kg, i.p.). Locomotor activity was estimated using a photobeam-equipped arena (25 x 25 cm) for 30 min following injection each day. POWER ANALYSIS details: Cohen's d 0.35, α=0.05, Power 0.80; final group size=24.

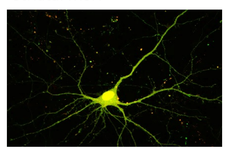
Cocaine Intravenous Self-Administration (IVSA): A separate cohort of mice received intrajugular vein catheters, and after recovery began 10 consecutive days of cocaine self administration. During 3-hr, 1X daily sessions, nose-poke behavior at the active port resulted in cue light illumination and cocaine delivery (0.1 or 1.0 mg/kg/infusion, i.v.). Acquisition criteria were met when a mouse earned $\geq$15 infusions with $\geq$70% active port preference (vs. inactive port) for 2 consecutive days with $\leq$20% variation in responding over days. POWER ANALYSIS details: (Overall: Cohen's d 0.5, α=0.05, Power 0.95; Post Hoc Analyses: Cohen's d 0.7, α=0.05, Power 0.80; final group size=35).

Primary Cortical-Striatal Co-Culture: Cultures were prepared on embryonic day 16 from D1-tdTomato WT and *Fmr1* KO mice. In some studies, whole patch-cell recordings were made in tdTom+ cells. For puncta staining studies, either D1R agonist SKF 38393 (1 µm) or vehicle (DMSO) treatment was applied for 30 min on days-in-vitro (DIV) 8, 10, and 12. On DIV16, cells were fixed in 4% paraformaldehyde / 4% sucrose.

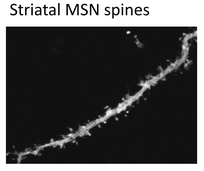
DARPP32 GluR1-NT



Striatal MSN

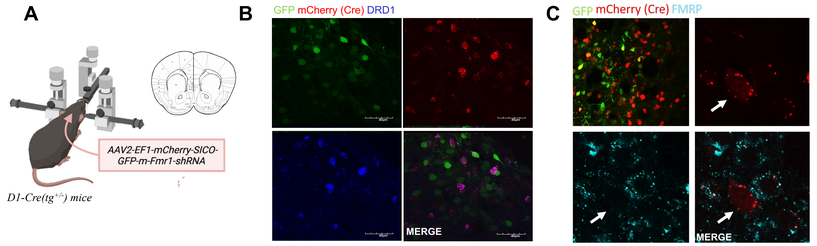


Striatal MSN spines

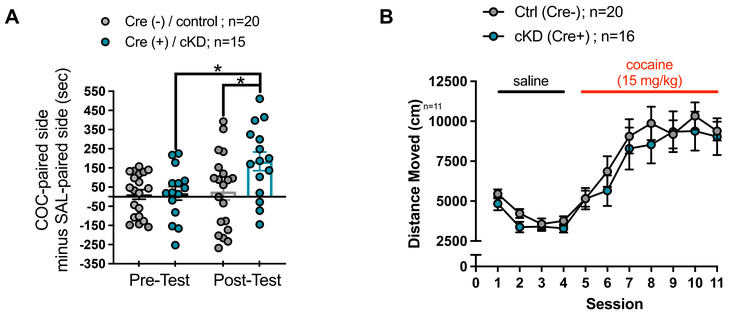


Synaptic Puncta Analysis: tdTomato-positive medium spiny neurons (MSNs) were identified, then pre- and post-synaptic markers (PSD95 and Synapsin) and individual and co-localized puncta (above a set threshold) on these cells were quantified using the SynapCountJ2 ImageJ plug-in. Thresholds were defined individually for each cell as a set number of standard deviations above the average fluorescent signal, not varying within experiment.

Conditional knockdown of FMRP in D1R-expressing cells of the NAc

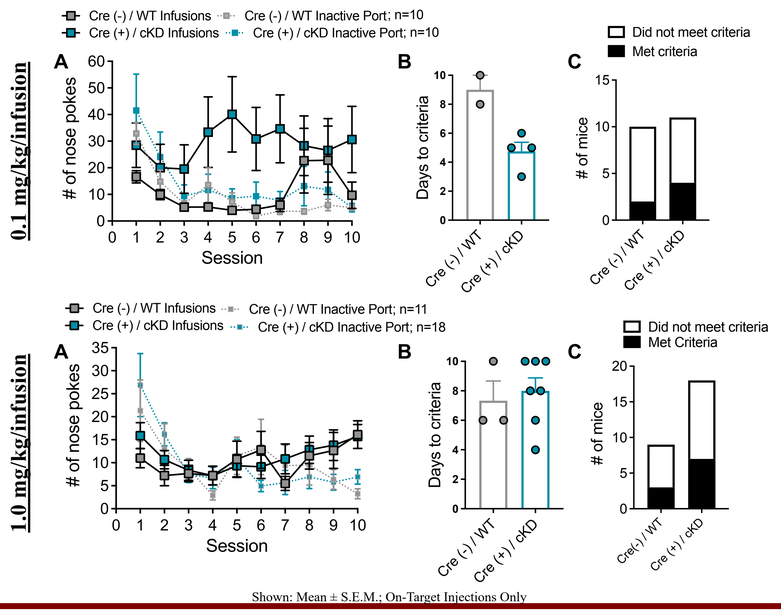


Preliminary Data: Loss of FMRP in D1R-expressing cells of the NAc increases CPP, but has no effect on locomotor sensitization



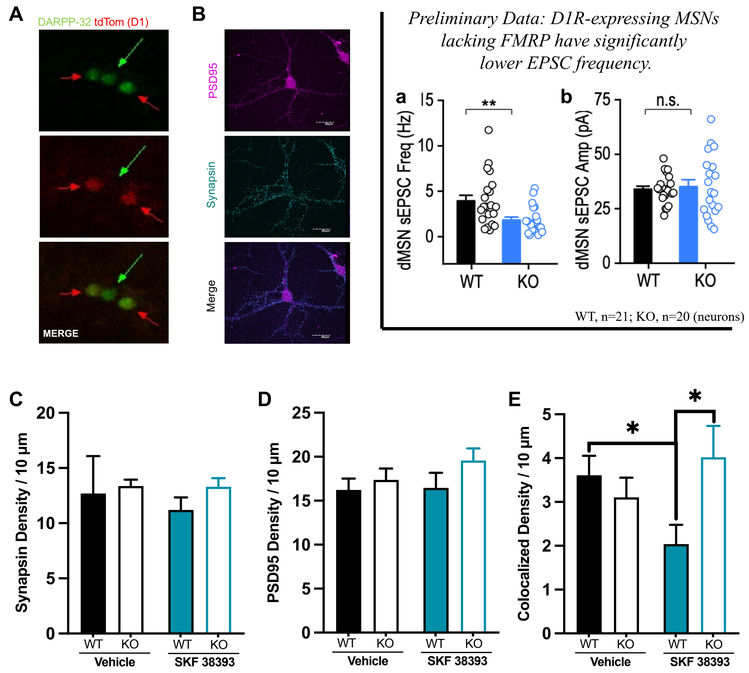
Shown: Mean ± S.E.M.; On-Target Injections Only

Preliminary Data: Loss of FMRP in D1R-expressing cells of the NAc facilitates acquisition of low dose cocaine self-administration



Shown: Mean ± S.E.M.; On-Target Injections Only

Preliminary Data: FMRP is required for synapse elimination in D1R-expressing cells after repeated D1R agonist exposure *in vitro*



Conclusions

- Global loss of FMRP impairs adjustments in cocaine self-administration that preserve reinforcement level, resulting in decreased self-administration under increased cost conditions.
- Loss of FMRP in D1R-expressing cells of the NAc increases cocaine CPP and propensity to self-administer a low dose, suggesting that it functions in this cell type to suppress drug-taking, or alternatively, its absence in D1R, but not D2R, -expressing cells alters the normal balance of these pathways in NAc during early drug exposure.
- Repeated exposure to a D1R agonist reduces colocalized puncta density in WT, but not *Fmr1* KO, striatal D1R-expressing cells *in vitro*, suggesting that D1 signaling drives synapse elimination on D1R-expressing cells and requires FMRP.

References

Huebschman, J. L., Corona, K.S., Guo, Y., Smith, L. N. (2020). The fragile X mental retardation protein regulates striatal medium spiny neuron synapse density and dendritic spine morphology. *Frontiers in Molecular Neuroscience*, 13, 1-11. doi: 10.3389/fnmol.2020.00161.

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