Drugs of abuse increase SGK1 expression in the VTA

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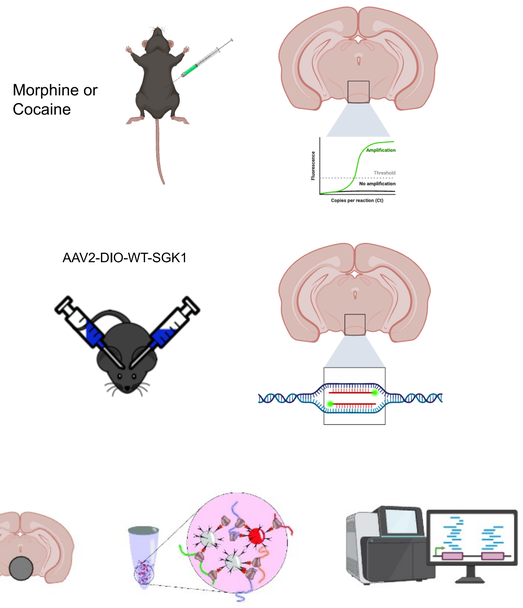
Abstract

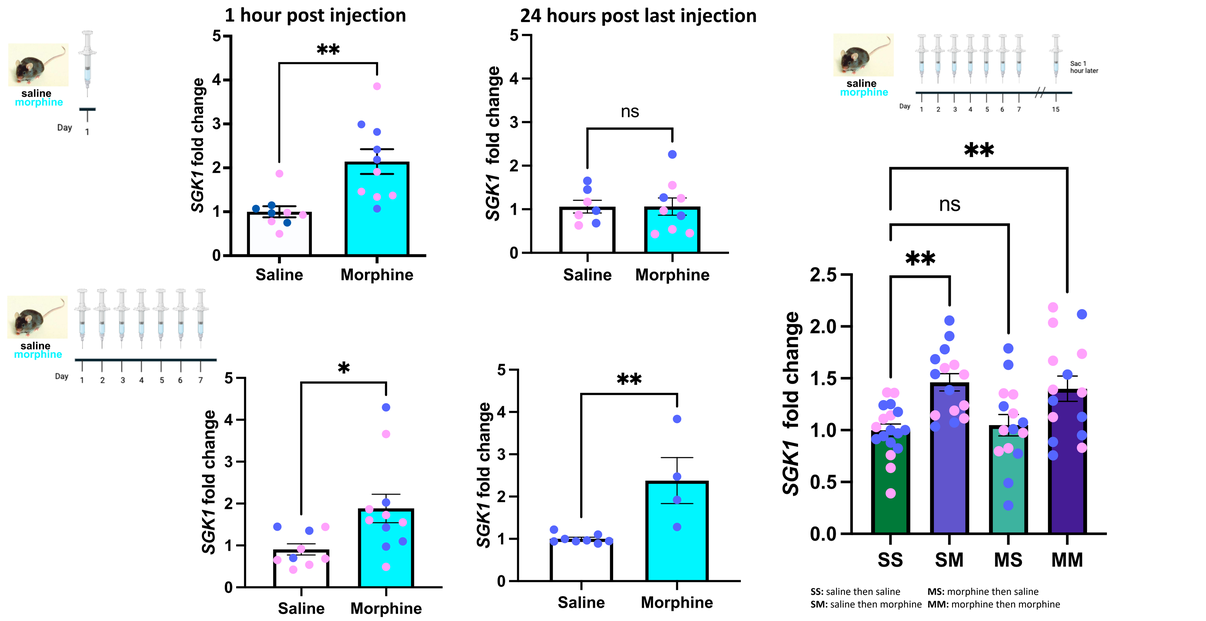
Activity within the mesolimbic dopaminergic (DA) circuitry in the brain is essential for motivated behavior and drugs of abuse can hijack this system by increasing DA signaling. Drug-induced cellular and molecular changes within the ventral tegmental area (VTA) can promote motivation to use drugs and drug-induced behavior. Our lab seeks to identify cellular and molecular changes in the VTA that promote drug responses. We found that repeated injections of morphine or cocaine increase the expression of serum– and glucocorticoid- inducible kinase 1 (SGK1) in the VTA using RNA sequencing. However, it was unclear whether similar regulation occurs with acute drug exposure and how persistent SGK1 expression changes are. To investigate this, we treated mice acutely with morphine or cocaine and isolated RNA from VTA for RT-PCR. We observed that VTA SGK1 gene expression is increased both 1 and 24 hours following both repeated and acute drug injections. We are currently exploring whether increased SGK1 expression persists during withdrawal and if it is differentially regulated by drug re-exposure. Given that the VTA is heterogenous structure containing multiple cell types besides DA neurons, we are determining which cell populations contribute to this drug-induced increase in SGK1 expression. We performed translating ribosome affinity purification studies to examine cell type-specific changes in SGK1 expression. Interestingly, we did not find an increase in SGK1 expression in either DA or GABA neuron pulldowns following morphine administration, suggesting that induction is occurring in alternative VTA cell populations or that SGK1 is not being actively translated. To assess whether SGK1 expression is occurring in specific neuronal populations or non-neuronal cells such as glia we are utilizing RNAScope analysis. Together, these studies will define how drugs of abuse alter expression of SGK1 in the VTA, a necessary first step to explore the role of this gene in drug behavior.

Hypothesis and Methods

When/where is SGK1 expression increased following drug exposure?

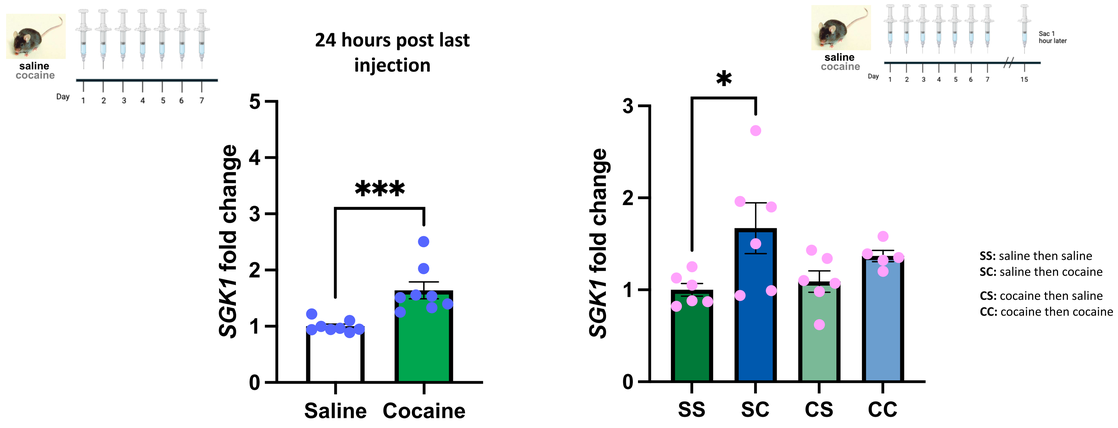


When does morphine treatment increase SGK1 expression in the VTA?



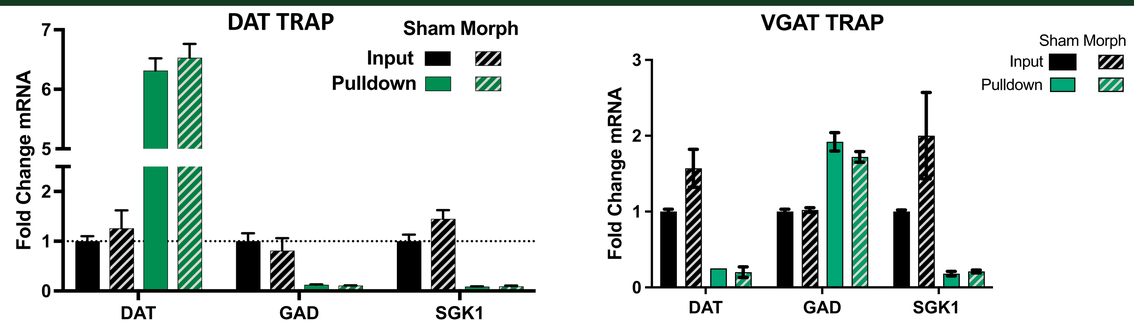
SGK1 expression in mouse VTA is increased following acute and chronic morphine exposure (15mg/kg, 20mg/kg). Only chronic administration is sufficient to increase SGK1 expression 24 hours post last injection. However, this does not persist after a period of forced abstinence. Data are represented as mean ± sem (n= 7-20 mice per group, 2-way ANOVA, *p<0.05).

When does cocaine treatment increase SGK1 expression in the VTA?



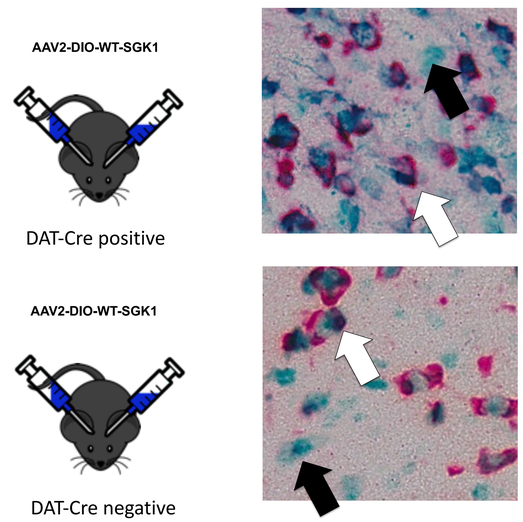
SGK1 expression in mouse VTA is increased following chronic cocaine exposure (20mg/kg). However, this does not persist through a forced abstinence period. Data are represented as mean ± sem (n= 5-10 mice per group, 2-way ANOVA, *p<0.05). Abstinence study was females only.

Is SGK1 expression increased in VTA dopamine or GABA neurons?

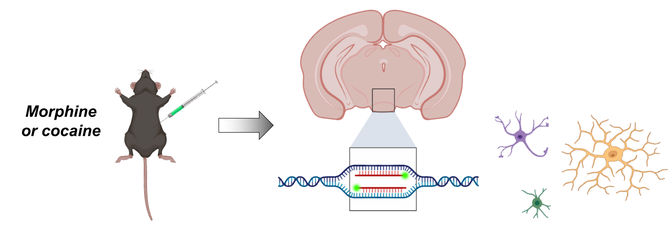


SGK1 expression in mouse VTA is increased following morphine pelleting. However, morphine pelleting does not increase SGK1 expression in dopamine or GABA neurons. Data are represented as mean ± sem (n=2-3 mice per group, 2-way ANOVA)

Validate SGK1 RNAScope probe using an AAV2-DIO-WT-SGK1 in DAT-Cre mice



Can we determine in which cells SGK1 expression is increased following drug?



DAT-Cre +/- or +/+ mice underwent stereotaxic surgeries to overexpress WT-SGK1 in VTA dopamine neurons. RNAScope duplex for SGK1 and DAT. White arrows indicate SGK1 signal in DAT-positive cells and black arrows indicate SGK1 signal in DAT-negative cells. Images taken in brightfield.

Summary/Future Experiments

- Cocaine and morphine increase SGK1 expression in whole VTA following acute or chronic treatment
- SGK1 expression is not increased in dopamine or GABA neurons following morphine pelleting
- Only chronic treatment of morphine, not acute, is sufficient to increase SGK1 expression 24 hours following last injection
- SGK1 expression does not persist during forced abstinence following chronic administration of drug
- Determine if expression of other genes are correlated with an increase expression of VTA SGK1 to better understand the role of increased SGK1 expression
- Determine in which cell types SGK1 expression is increased following drug exposure

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