Introduction

Reward-seeking behaviors associated with human psychostimulant drug addiction are driven by dopamine (DA) neurotransmission to the ventral tegmental area (VTA). DA projections from the VTA to the nucleus accumbens (NAc) are critical for the production of psychostimulant-induced behaviors.

Sensitization results from repeated and repeated drug use and is characterized by persistent neuroadaptations including changes in glutamate receptor signaling at the VTA. The changes are important for turning drug use from casual to compulsive and addictive.

The laterodorsal tegmental nucleus (LDTg) provides a source of glutamate input to the VTA that excites DA signaling.

In previous studies, inhibition of LDTg glutamate inputs at the VTA using optogenetics blocks the development of locomotor sensitization.

Here we tested whether inhibition of these glutamate inputs would also block the development of neurochemical sensitization of NAc DA release which is known to accompany the development of locomotor sensitization.

Methods

Mouse: Male YAC12Sw/2J strain mice (Taconic, Germantown, NY) were used. All animal care and procedures were conducted in accordance with the approved Institutional Animal Care and Use Committee protocols.

Optogenetic Manipulation of LDTg Activity: Mice were implanted with guide cannulas (Model 006G 100; Plastics One, Roanoke, VA) 7 days prior to beginning treatment. For virus injections, mice were anesthetized with isoflurane and held in a stereotaxic frame. A stainless steel guide cannula was inserted into the LDTg (1.7 mm posterior to the bregma, 1.3 mm lateral to the midline, and 8.0 mm ventral to the skull surface). A virus containing the NpHR-mCherry or eYFP genes under control of a strong constitutive promoter was injected into the guide cannula with a 10 μl Hamilton syringe. Mice were monitored for 48 hours before being returned to their home cages. For light stimulation, mice were removed from their home cages with the guide cannula in place and held in a stereotaxic frame. Mice were placed in a 30 °C heated chamber and stimulated with a 473 nm laser beam for 30 minutes.

Locomotor Testing: Mice were habituated to the open field assay chamber for 30 min prior to testing. Locomotor activity was measured using a SmartTec apparatus (Columbus Instruments, Columbus, OH). Mice were placed individually in the open field chamber for 10 min, and total distance traveled, number of rears, and number of entries into each quadrant were recorded.

RNA-Seq Analysis: RNA was isolated from the hippocampus, striatum, and VTA of each group of mice. Total RNA was extracted using the ZR RNA prep Kit (Zymo Research, Orange, CA). cDNA was synthesized using the SuperScript III RT Kit (Invitrogen, Carlsbad, CA). Quantitative PCR was performed using the LightCycler 480 System (Roche Diagnostics, Indianapolis, IN). Gene expression was normalized to the geometric mean of three housekeeping genes (Gusb, Ldh, and Sdha).

Summary

After the cocaine challenge injection, eYFP mice that received cocaine pre-treatment showed a more significant increase in NAC DA concentration compared to eYFP saline pre-treated mice.

For NAc mice, which received inhibition to LDTg glutamate projections to the VTA, cocaine pre-treated mice did not have a significant increase in NAC DA concentrations compared to saline pre-treated mice.

These data suggest that inhibition of LDTg glutamate inputs at the VTA blocks neurochemical sensitization of NAc DA.

This suggests that LDTg glutamate inputs to the VTA are critical for the production of psychostimulant addiction.

These synapses may be an important target for interventions aimed at preventing the transition from casual to addictive drug use.

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Inhibition of laterodorsal tegmental nucleus glutamate inputs to the ventral tegmental area blocks neurochemical sensitization in mice

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Inhibition of LDTg glutamatergic inputs to the VTA blocks neurochemical sensitization

Halo rhodopsin (NpHR) is a light-sensitive chloride ion pump that hyperpolarizes the cell when exposed to green light. Yellow fluorescent protein (eYFP) is a protein marker used to identify cells in which transfection has occurred.

During optogenetic stimulation, mice that had bilateral injections of NpHR experienced inhibition of LDTg glutamate projections to the VTA, whereas mice that only had eYFP bilateral injections did not experience inhibition at these synapses.

This study found that inhibition of LDTg glutamate inputs at the VTA reduced NAc DA concentrations following the cocaine challenge injection.

eYFP cocaine pre-treated mice showed acquisition of neurochemical sensitization after cocaine administration compared to eYFP saline pre-treated mice

eYFP cocaine pre-treated mice showed marginally significant increases in NAc DA concentrations compared to eYFP saline pre-treated mice (interaction between sample and group [F(1, 75, 17.50) = 3.07, p = .08] and main effect of group [F(1, 10) = 2.56, p = .14]).

NpHR cocaine pre-treated mice did not show the acquisition of neurochemical sensitization after cocaine administration compared to NpHR saline pre-treated mice

NpHR cocaine pre-treated mice did not show significant increases in NAc DA concentrations compared to NpHR saline pre-treated mice (main effect of group [F(1, 9) = 0.26, p = .67]).

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