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

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Introduction

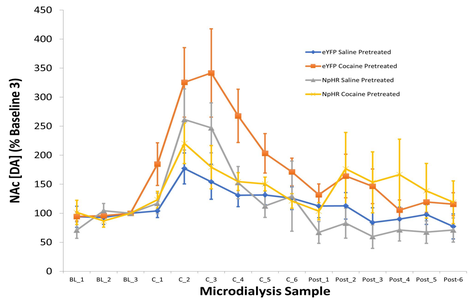
- Reward-seeking behaviors associated with human psychostimulant drug addiction are driven by dopamine (DA) neurotransmission to the ventral tegmental area (VTA). DA projections form the VTA to the nucleus accumbens (NAc) are critical for the production of psychostimulant-induced behaviors.
- Sensitization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

Mice: Male VGLuT2 IRES-Cre knockin mice (Slc17a6$^{tm2(cre)Lowl}$ /J; Jackson Laboratory, Bay Harbor,ME) were used.
Surgery: Adeno-associated viral vectors (~ 10^{12} infectious units/mL, stereotype 5, University of North Carolina vector core facility) infusions were made using a 10 µL syringe controlled by an automated infusion pump (UMP3: World Precision Instruments) attached to one of the stereotaxic manipulator arms. Mice were Bilaterally infused (200nL per hemisphere, 100nL/min) with either AAV5-EF1a-DIO-NpHR30-eYFP or AAV5-EF1a-DIO- eYFP into the LDTg. The injector needle was lowered into each LDTg (Bregma: -5.0 AP, ± 0.6 ML, -3.5 DV: Franklin & Paxinos, 2007). All mice were implanted with optic fibers during the same surgery. Bilateral 200 µm optical fibers, threaded through 1.5 mm ceramic ferrules, were angles at 10º from the midline and aimed at the dorsal order of the VTA (Bregma: -3.4 AP, ± 0.5 ML, -4.0 DV; Franklin & Paxinos, 2007). For microdialysis, mice were unilaterally implanted with a guide cannulae (CMA11; Harvard Apparatus) aimed at the nucleus accumbens core (AP +1.3, ML ±0.9, DV -3.3 from bregma and angled laterally at 10º from the vertical). All implants were secured to the skill using RelyX Luting Cement (3M) as well as stainless steel screws and dental cement.
Locomotion Testing: Mice were individually placed in an open field chamber (40 x 40 x 35cm). The position of the mouse was recorded by a camera mounted above the chamber connected to ANY-Maze video software (Stoeling Co., Wood Dale, IL). ANY-Maze software collected data on total forward locomotion, forward locomotion and time spent on the perimeter of the chamber, and forward locomotion and time spent in the center of the chamber.
Microdialysis: One day prior to the cocaine challenge injection, concentric microdialysis probes (CAMA11, 1 mm active membrane length protruding from the tip of the guide cannula; Harvard Apparatus) were lowered into the NAcc for microdialysis testing the following day. After probe insertion, mice were housed individually in 5 gal plastic buckets and kept in the same room as the locomotion testing apparatus overnight with food and water freely available. Mice were connected via a steel-spring tether to a liquid swivel and collection vial positioned outside the bucket. Probes were perfused with artificial cerebrospinal fluid at 0.3 µl/min overnight. The following morning the perfusion rate increased to 1.5 µl/min and mice remained in the plastic bucket for an additional 2 hours after which each mouse was transferred to the same open-field chamber in which it previously received locomotion testing.
Sensitization Protocol: Following pre-handling of mice, the first 3 days consisted ofhabituation Sessions before which mice received an injection of saline (10 mL/kg, i.p.). Mice were connected to Optogenetic probes, but the lasers were turned off meaning there was no inhibition of LDTg glutamate terminals in the VTA for mice that had been transfected with NpHR. Mice were then placed in the open field chamber and locomotion was recorded for 1 hour. Immediately following the habituation phase, mice either Received cocaine (15 mg/kg, i.p.) or saline injections (10 mL/kg, i.p.) for five consecutive days. During these 5 days mice were connected to optogenetic probes and lasers were turned on which inhibited LDTg Glutamate terminals in the VTA for mice that were transfected with NpHR. Again, mice were placed in an open field chamber and locomotion was tracked for 1 hour. Following this, mice had a week-long withdrawal period in which they received no treatment. Following the withdrawal period, all mice received a cocaine challenge injection (15 mg/kg, i.p.). Mice were connected to optogenetic probes, but lasers were again, turned off. The day before the challenge test mice were also connected to a microdialysis probe which collected extracellular fluid from the NAcc into vials to measure DA concentrations throughout the experiment. Mice were placed in the open field chamber for 30 minutes with no injection. Every 10 minutes vials were collected and placed on ice . After the baseline vials were collected, mice were injected with cocaine (15 mL/kg, i.p.) and vials were again collected every 10 minutes for one hour. During this time, locomotion in the open field chamber was video recorded. After this hour, vials were collected every 10 minutes for another hour. All samples were immediately frozen on dry ice and stored at -80ºC for later assessment of DA levels.
Histology: After completion of behavioral testing (9-10 weeks after AAV infusion) each mouse was transcardially perfused and coronal cryosections (40 µm) throughout the extent of the LDTg and VTA were prepared for each mouse. To verify selective VTA expression of eYFP in glutamatergic LDTg terminals of VGluT2::Cre mice, these coronal cryosections were incubated in a cocktail of primary antibodies overnight. After rinsing 3 x 10 min in PB, tissue was subsequently incubated in a cocktail of corresponding fluorescent secondary antibodies. All tissue sections were mounted on slides. All tissue sections were mounted on slides. Fluorescent images were taken with an Olympus FV1000 Confocal System (Olympus). Imaged were taken sequentially with different lasers with 10x or 100x oil immersion objectives and z-axis were collected at 2 µm. The boundaries of eYFP expression relative to the boundaries of the LDTg observed in the rostral, medial, and caudal portions of the LDTg were summarized on three LDTg sections (Bregma -4.96; -5.20; -5.40 respectively). Some sections containing the VTA and NAcc were mounted onto gelatin-coated slides, stained with cersyl violet, and coverslipped with Permount (Electron Microscopy Sciences). These sections were used to determine optic probe and canullae locations using a standard light microscopy.

Data Analysis: Final statistical analysis was based on n = 22 mice (n = 11 NpHR and n = 11 eYFP). NAcc DA concentration data were analyzed using a repeated measures ANOVA with time (samples collected) as the within-subjects factor and viral transfection (eYFP or NpHR) and pre-treatment condition (saline or cocaine) as the between-subjects factors. There was a separate ANOVA for baseline, cocaine injection and post injection DA content.

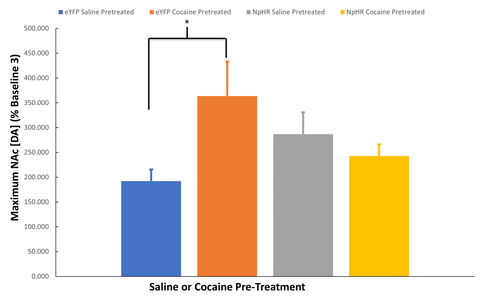
eYFP cocaine pre-treated mice showed acquisition of neurochemical sensitization compared to eYFP saline pre-treated mice while NpHR cocaine pre-treated mice did not show evidence of neurochemical sensitization compared to NpHR saline pre-treated mice



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) = 5.96, p = .04]).

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 do not show significant increases in NAc DA concentrations compared to NpHR saline pre-treated mice (interaction between sample and group [F(2.65, 23.89) = 0.91, p = .44] and group main effect [F(1, 9) = 0.20, p = .67]).

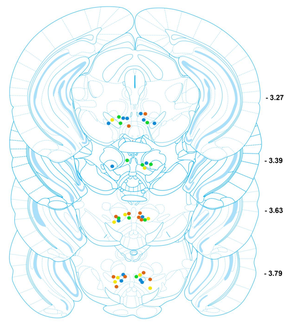
eYPF cocaine pre-treated mice showed greater DA concentrations compared to eYFP saline pre-treated mice while NpHR cocaine pre-treated mice did not show greater DA concentrations compared to NpHR saline pre-treated mice



eYFP cocaine pre-treated mice showed greater maximum DA concentrations compared to eYFP saline pre-treated mice
NAc maximum DA concentrations were significantly higher for eYFP cocaine pre-treated (M = 364, SD = 169) compared to saline pre-treated mice (M = 192, SD = 57.7), [independent samples t-test: t (10) = 2.35, p = .04]. * = p < .05

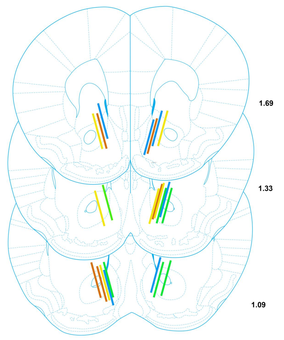
NpHR cocaine pre-treated mice did not show greater maximum DA concentrations compared to NpHR saline pre-treated mice
NAc maximum DA concentrations were not significantly higher for cocaine pre-treated mice (M = 243, SD = 57.4) compare to saline pre-treated mice (M = 287, SD = 97.7), [independent samples t-test: t (9) = 0.94 p = .34].

VTA probe placement



Bilateral VTA probe placements for optogenetics stimulation
yellow is eYFP cocaine pre-treatment, blue is eYFP saline pre-treatment, green is NpHR cocaine pre-treatment, orange is NpHR saline pre-treatment

Nac Probe Placement



Bilateral Nac probe placement for microdialysis collection
yellow is eYFP cocaine pre-treatment, blue is eYFP saline pre-treatment, green is NpHR cocaine pre-treatment, orange is NpHR saline pre-treatment

Inhibition of LDTg glutamatergic inputs to the VTA blocks neurochemical sensitization

- Halorhodopsin (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.

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 NpHR 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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