Cocaine and memory: The cell type-specific role of NPAS4 in the mouse nucleus accumbens

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Background and Aims

Background: Substance use disorder (SUD) is a chronic behavioral disorder marked by drug-seeking behaviors, relapse, and negative health and social consequences. Analogous to behavioral studies in mice, when individuals receive a drug reward, an association is formed between the rewarding effects of the substance and the environment within which the drug reward was experienced. Future exposure to this environment leads to an increase in drug seeking, due to encoded contextual-associations, mediated by neuronal plasticity, within the nucleus accumbens (NAc). The majority of cells within the NAc contain dopamine D1 and D2 receptor-expressing medium spiny neurons (MSNs), which function to reinforce reward learning and drug-paired associations. However, the molecular and cellular mechanisms underlying drug-context memories in these cell types is not well understood. We previously found that cocaine conditioning activates a sparse population of cells within the NAc that express the immediate early gene transcription factor, neuronal PAS domain 4 (NPAS4). NPAS4 has been shown to control the downstream expression of various genes involved in cocaine-related memories. When NPAS4 is removed from the mouse NAc during cocaine conditioned place preference (CPP), there is a significant reduction in time spent on the cocaine-paired side of the CPP box, suggesting that the animals have trouble associating the environment with the cocaine-reward experience. As NPAS4 presence is required for drug-context associations, we hypothesized that NPAS4 acts in a cell type-specific manner within the NAc to promote cocaine-conditioned learning and memory.

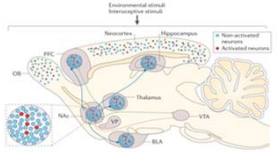
Methods and Results:

Experiment 1: Methods: To identify which cell types induce NPAS4, NPAS4 protein induction was examined in transgenic mice expressing D1- or D2-specific fluorescent-labeled cells after exposure to a drug-paired environment using IHC for NAc NPAS4.
Results: We found that ~50% of NPAS4-NAc positive cells are D1R or D2R-expressing MSNs.

Experiment 2: Methods: To determine which cell type requires NPAS4 for cocaine-reward associations, we virally reduced NPAS4 expression in D1- or D2-MSNs and conducted cocaine CPP. **Results:** While NAc D1-NPAS4 knockdown (KD) mice had normal CPP, D2-NPAS4 knockdown mice showed reduced CPP, indicating that NPAS4 expression within D2-MSNs is required for the development of cocaine CPP.

Experiment 3: Methods: To elucidate the functional role of NPAS4 knockdown in D2-MSNs, the induction of cFos, a marker of neuronal activity, was measured after cocaine CPP in the NAc of mice lacking NPAS4 within these neurons.
Results: A significant increase in FOS+ cells was observed in D2-MSNs after NPAS4 knockdown in comparison to controls. Findings indicate that D2-MSNs were activated by NPAS4 removal.

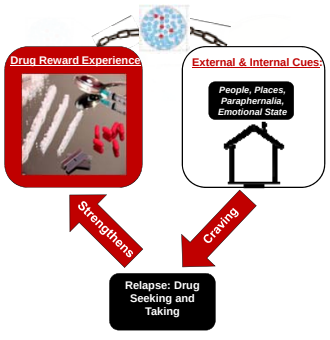
Drug-Environment Associations and Addiction Behavior



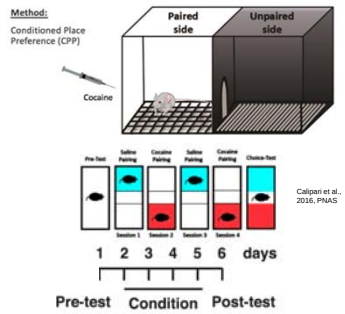
Cruz et al., 2013 Nat Rev Neuroscience

Drug Use and Epigenetics

1) Activates sparse populations of cells, which express immediate early gene transcription factors (FOS, NPAS4)
2) Activation is required for drug reward learning and memory



Behavioral Models: How do we study drug-environment associations?



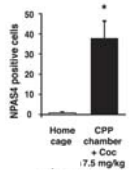
Calipari et al., 2016, PNAS

Background: NPAS4 presence required for drug-context memories

Conditioned Place Preference (CPP)

Figures A-D from: Taniguchi et al., 2017, Neuron

Neuronal PAS domain 4 (Npas4): immediate early gene transcription factor

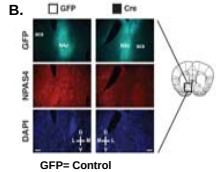


NPAS4 is a gene that was previously identified as being regulated by cues in the drug-environment.

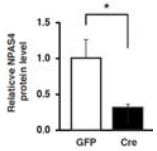
A) Figure shows a 40-fold increase in NPAS4+ cells when mice were exposed to CPP chamber for the first time relative to home cage.

IHC showed that NPAS4 was **sparsely** induced during CPP compared to total cell population of the NAc.

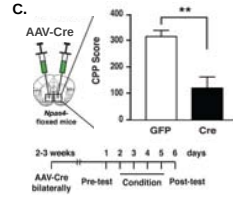
Results: NPAS4 is sparsely induced in the nucleus accumbens by cocaine conditioned place preference (CPP).

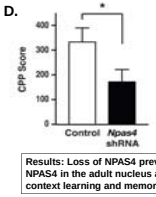


GFP= Control
CRE= NPAS4 gene knockout (KO)



B) Left: NPAS4-floxed mice were injected with Cre into NAc. Imaging highlights NPAS4 KO^{NAc}, shown by removal of NPAS4 with Cre injection, in red. Right: Graph demonstrates significant reduction in reactive NPAS4 protein level when Cre is injected compared to GFP control.





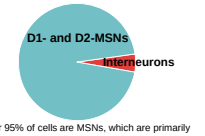
C) Deletion of NPAS4 using Cre injection into NAc of mice causes a significant reduction in preference for the cocaine-paired side during CPP on post-test day.

D) Deletion of NPAS4 when injecting NPAS4-shRNA into NAc of wild-type mice shows a similar reduction in preference for the cocaine-paired side during CPP on post-test day; indicating the efficacy of using NPAS4 shRNA to knockout NPAS4.

Results: Loss of NPAS4 prevents cocaine-context associations. NPAS4 in the adult nucleus accumbens is required for cocaine-context learning and memory.

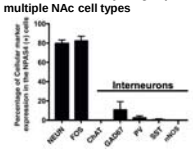
Exp 1: Cell type-specific induction of NPAS4 in transgenic mice NAc

Neuronal Distribution in the NAc:



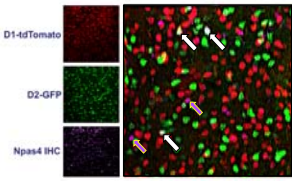
Over 95% of cells are MSNs, which are primarily made up of D1- and D2-MSN subtypes.

A. **NPAS4 is induced by drug experience in multiple NAc cell types**



A) Following cocaine and CPP chamber exposure: Plot shows percentage of NPAS4+ cells that co-express various neuronal markers in the NAc. NPAS4+ cells highly colocalize with FOS+ neurons.

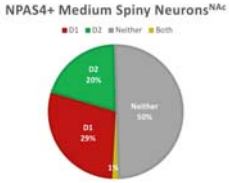
B.



B) IHC imaging of NPAS4 colocalization with NAc D1- and D2-MSNs after cocaine injection and CPP chamber exposure using D1-tdTom/D2-eGFP BAC transgenic mice, which have specific fluorescent labels to indicate designated cell types.

- In this example: Purple arrows show NPAS4+ cells that do not colocalize with D1- or D2-MSNs
- White arrows show NPAS4+ colocalization with GFP expressed in D2-MSNs

C.

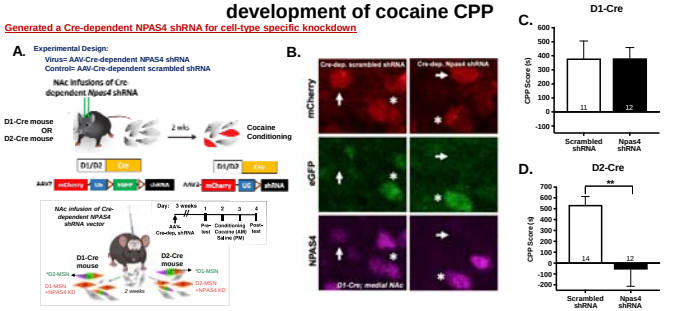


C) After cocaine injection and CPP chamber exposure, approximately 50% of NPAS4 positive cells colocalize with D1- and D2-MSNs.

Results: ~50% of NPAS4-NAc positive cells are D1R- or D2R-expressing MSNs.

Exp 2: NPAS4 expression in NAc D2-MSNs is required for the development of cocaine CPP

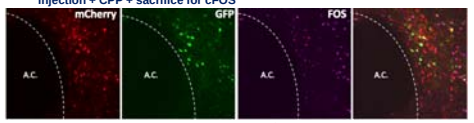
Generated a Cre-dependent NPAS4 shRNA for cell-type specific knockdown



A) Top figure shows the viral construct used to remove NPAS4 from the NAc, which works by expressing mCherry (red), a U6 promotor, and eGFP (green). eGFP physically separates the U6 promotor from the shRNA sequence. After infusion, when virus enters a cell that expresses Cre recombinase, which will occur in the D2 cells of a D2-Cre mouse for example, Cre removes GFP, the cells are only red, and the shRNA is expressed (either scrambled or NPAS4 KD). **B)** IHC imaging showing the workable utility of the Cre-dependent shRNA vector within a D1-Cre mouse. Left column arrows: D1-cells with Cre-dep. scrambled shRNA infusion have undergone recombination, have had eGFP removed, and still express NPAS4. Right column arrows: D1-cells with Cre-dep. NPAS4 shRNA infusion have undergone recombination, have had eGFP cassette removed, and do not express NPAS4. Asterisks in this image mark cells that are not D1, such as D2 cells on both the left and right columns, to show that they do not undergo Cre-mediated recombination because they do not have Cre, and therefore, express mCherry (red), eGFP (green), and NPAS4 (purple). **C)** Cell type-specific knockdown of NPAS4 in D1-Cre mice NAc has no effect on CPP. **D)** Knockdown of NPAS4 in D2-MSNs of D2-Cre mice NAc causes a significant reduction in preference for the cocaine-paired chamber during cocaine CPP.

Exp 3: FOS expression indicates NPAS4 KD influences NAc D2-MSNs cell activation to decrease cocaine reward learning

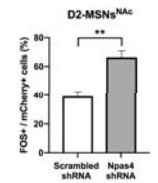
A. **Cre-dependent shRNA in the NAc of D2-Cre mice with cocaine injection + CPP + sacrifice for cFOS**



Given 7.5 mg/kg cocaine + CPP conditioning → Sac for cFOS → Mark FOS+ cells → Mark FOS+/mCherry+ cells → no GFP indicates D2-MSN

A) Imaging of Cre-dep shRNA in NAc of D2-Cre mice sacrificed an hour after CPP chamber to examine neuronal activity marker FOS, which is induced by cocaine injection + CPP chamber exposure. Looked for cells expressing FOS, then looked for FOS+ cells also expressing mCherry, which indicates that cells took up the virus, then looked for cells that lost eGFP (No GFP= Cre-positive cells, indicating cells are D2-MSNs in the D2-Cre mice).

B.



B) There is a significant increase in FOS+ cells co-occurring in D2-MSNs after NPAS4 KD in the NAc of D2-Cre mice using NPAS4 shRNA, indicating that D2-MSNs are activated by NPAS4 removal.

Conclusions

- NPAS4 is sparsely induced in the NAc during cocaine CPP
- Total knockout of NPAS4 in the NAc prevents cocaine-context associations (reduces cocaine CPP)
- NPAS4 is induced by a drug experience in multiple NAc cell types, however ~50% of NPAS4 induction occurs in D1- and D2-MSNs
- NPAS4 knockdown in NAc D2-MSNs decreased cocaine reward learning during CPP
- NPAS4 knockdown in NAc D2-MSNs increased FOS+ cell colocalization in these neurons, indicating that NPAS4 knockdown in NAc D2-MSNs increases D2-MSN activity

Future Directions:

- Examine how NPAS4 influences cell function of D1-MSNs using FOS+ cell expression
- Record additional electrophysiology data after NPAS4 KD in D2-MSNs to confirm NPAS4-dependent changes in synaptic transmission within the NAc
- Analyze the cell type-specific roles of NPAS4 in D1- and D2-MSNs for deficits in operant learning and cue-induced reinstatement during cocaine self-administration in rats

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