Investigating the Role of the Basal Forebrain in Susceptibility to Addiction

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Abstract

Drug addiction places a heavy burden on a whole spectrum of people ranging from adolescents to the elderly. Though we know that both environmental and genetic factors contribute to drug addiction, we do not have a cure, owing in part to an incomplete understanding of how drug use causes persistent changes in the brain that lead to addiction. Progress has been hampered by a lack of unbiased approaches to enable discovery of neuronal circuits and molecular pathways that play a causative role in the development and maintenance of addiction. **Our project aims to identify circuits that contribute to addictive phenotypes *before* the development of addiction in order to identify or treat at-risk populations.** Using a novel genetic strategy that enables permanent tagging of neurons activated by an experience (TRAP2), we've identified a number of unexpected brain sites whose activity is highly correlated with that of regions known to play a role in addiction, such as the ventral tegmental area. Interestingly, the **basal forebrain and hippocampus** were significantly over-represented in this population, suggesting that activity throughout these regions may play a key role in the early stages of addiction. We investigated the contribution that these ensembles may play in in a variety of addictive behaviors using chemogenetic inhibition.

Background

- Addiction is characterized by drug seeking and use that is compulsive, or difficult to control, *despite harmful consequences*[1]
- Methamphetamine (MA) abuse remains a critical public health problem with no FDA approved pharacotherapies[2]
- In the U.S. alone, 5.4% of people have reported using MA in their lifetime, and roughly 10% of those were diagnosed with a methamphetamine use disorder[3]
- GWAS studies have tried to find SNPs in genes associated with MA addiction[4]

Our lab takes an alternative approach by using an unbiased whole-brain screen approach to identify the neuronal ensembles that contribute to addictive phenotypes before the development of addictive-like behaviors.

Methods & Results

Experimental procedure

TRAP initial drug dose → Run behavioral experiments → Identify active neurons

Targeted Recombination in Active Populations (TRAP2)

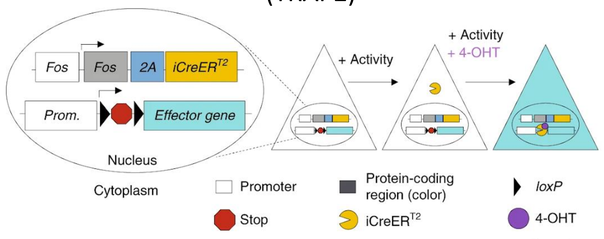


Figure 1

TRAP2[5] permanently labels neurons in response to an experience (in this case, the first drug exposure) through co-administration of drug and 4-hydroxytamoxifen.

Whole Brain Activity Visualization

Figure 2

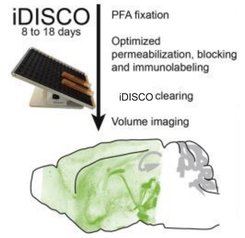


Figure 3

iDISCO[6] is a method for both immunolabeling and 3D imaging of whole brain samples.

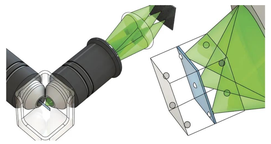
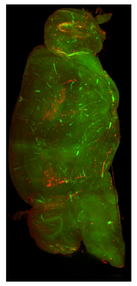
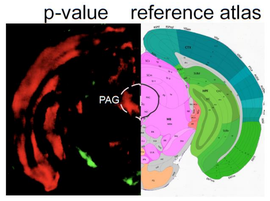


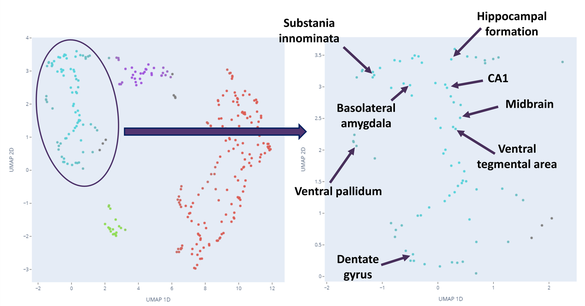
Figure 4



Lightsheet microscopy uses a light-sheet, rather than point scanning, to image with cellular resolution in intact tissue. We use a software called ClearMap[7] to quantify labelled TRAP neurons in >300 anatomically defined brain regions by aligning the images to the Allen Brain Atlas.

p-value reference atlas



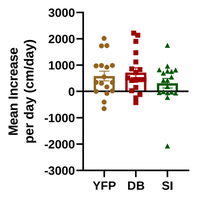


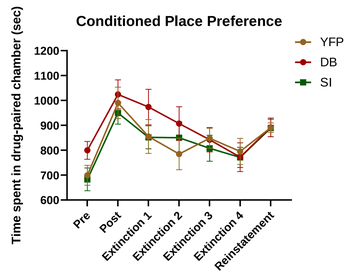
UMAP[8] plot of brain regions from mice TRAPed after a single exposure to methamphetamine with all brain regions (left) and zoomed into circled cluster (right).

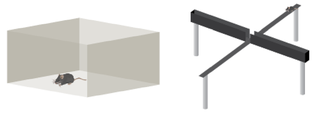
Behavioral tests with basal forebrain inhibition

Figure 5

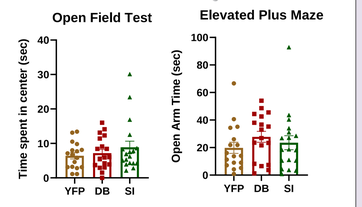
Animals in every group developed a place preference and locomotor sensitization.

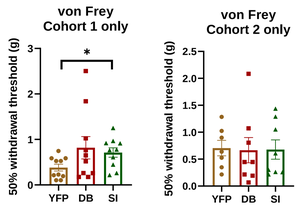






No differences were found between groups for anxiety-related measures.





SI animals in cohort 1 (left) showed a difference in meth-induced hyperalgesia compared to YFP animals. The YFP animals of cohort 2 (right) did not show withdrawal hypersensitivity, so effects of the inhibition were inconclusive.

Summary

- Using an unbiased whole brain screen approach of neuronal activity allows us to reveal clusters of brain regions with correlated activity following a single exposure to methamphetamine
- Using the TRAP2 method after an initial dose of drug showed key brain regions historically studied in addictive-like behaviors in these clusters
- The basal forebrain and hippocampus were highly over-represented in these same clusters suggesting these regions as promising targets in regard to development of addictive-like behavior
- Inhibition of cholinergic cells in the basal forebrain had no behavioral consequences for the development, extinguishing, or reinstatement of CPP, nor any effects on locomotor sensitization or withdrawal-induced anxiety
- We did observe that inhibition of these cells prevented development of meth-induced hyperalgesia
- This suggests that basal forebrain cholinergic cells may be integrated into networks associated with methamphetamine addiction, particularly for the development of withdrawal-induced hyperalgesia

Future Directions

- We are currently replicating and analyzing withdrawal-induced pain behaviors such as the hot plate, thermal probe test, and acetone evaporation test to further investigate our findings related to hyperalgesia
- We also plan to track the neuronal dynamics of these ensembles using in vivo imaging methods such as fiber photometry[9] and miniscope imaging[10].

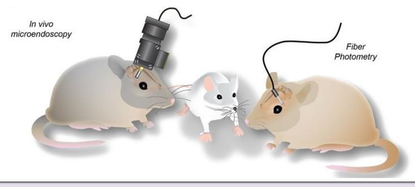


Figure 6

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Figures:
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