Mapping Cell Types in the Human Putamen from Cocaine Abusers

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Abstract

The human putamen, as part of the dorsal striatum, integrates dopaminergic inputs from the midbrain and glutamatergic inputs from the cortex. Consequently, this brain region expresses high levels of dopamine signaling components. Specifically, the dopamine transporter (DAT), as the primary target of cocaine's action, exhibits intense binding of radiolabeled cocaine in PET imaging studies. Furthermore, dopamine signaling in the putamen, as measured by dopamine D2 receptor occupancy with radiolabeled raclopride, is significantly associated with cue-evoked cravings in human subjects addicted to cocaine. This convergence of human data strongly implicates the putamen in cocaine addiction, and its association with cravings makes it an ideal region for targeting anti-addiction therapies. However, translating preclinical rodent findings to humans is challenging, since the gross anatomy of the rodent striatum differs significantly from the human striatum, as rodents lack distinction between the caudate and putamen. To address this challenge, we have mapped cell types in human postmortem putamen tissues from individuals that overdosed on cocaine and matched controls using single nuclear RNA sequencing (snRNAseq). Notably, we recognize distinct populations of dopamine D1 vs. D2 receptor-expressing neurons and large numbers of apparent oligodendrocytes. We also see scant expression of DAT, suggesting the labeling of this target in human PET studies likely results from expression on presynaptic innervating midbrain dopaminergic neurons. This is consistent with near-complete loss of DAT in the putamen of individuals with Parkinson's disease. Additional studies are necessary to identify robust changes in gene expression across cell types in cocaine abusers versus controls.

Samples & Methods

Samples

Posterior putamen from eight human brains used in this study (see table) were dissected postmortem by trained neuropathologists (D. Mash, University of Miami Brain Endowment Bank™).

Sample	Sex	Age	Race	PMI	RIN	Nicotine Use
Cocaine 1	M	45	White	18	8.3	Yes
Cocaine 2	M	41	White	14	8.2	Yes
Cocaine 3	M	49	White	15	6.6	Yes
Cocaine 4	M	34	White	24	5.6	Yes
Control 1	M	37	White	14.5	8.3	No
Control 2	M	24	White	15.5	8	Yes
Control 3	M	44	White	19	7.5	Yes
Control 4	M	34	White	11.5	6.8	Yes

Nuclei Isolation, Library Preparation and Sequencing

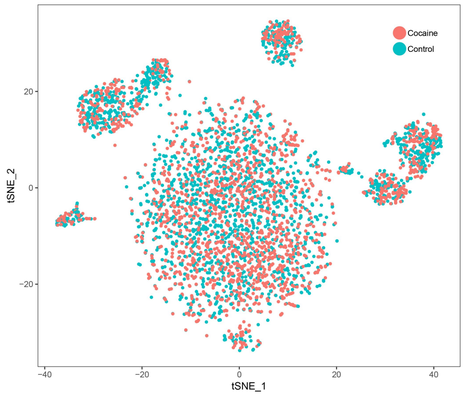
Approximately 25mg of putamen from each of four subjects per condition (cocaine overdose *vs.* controls) were combined prior to processing the tissues for nuclei isolation and sequencing. The combined samples were processed in a low detergent buffer with a Dounce homogenizer and nuclei were isolated in a sucrose buffer. Additional nuclei purification was performed via flow cytometry using a Hoechst stain, yielding >100,000 nuclei per condition. Sequencing libraries for individual nuclei were prepared by the Genomics Core within University of Iowa Institute of Human Genetics using the 10x Genomics platform. The pooled libraries were sequenced on an Illumina HiSeq 4000 instrument and de-multiplexed prior to bioinformatic analysis.

Bioinformatic Analysis

Alignments were performed against GRCh38-1.2.0 using the 10x Cell Ranger software, to generate gene-level counts. All subsequent analyses and visualizations were performed on gene-level counts using Seurat v2.3.4 in R v3.5.1.

Results

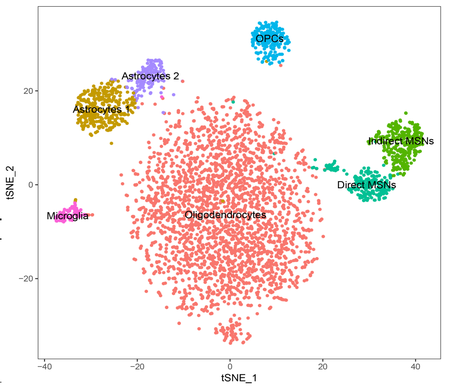
Cell Type Clusters Are Consistent Across Conditions and Reveal Cell Types Expected in the Striatum



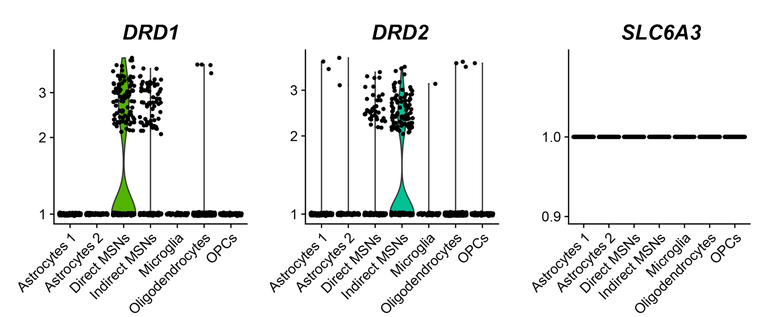
LEFT: *Nuclei isolated from the cocaine and control putamen have distinct expression patterns that correspond to specific cell types. Gene expression profiles used to distinguish cell types are not dramatically altered by cocaine, as demonstrated by the overlapping clusters between groups.*

RIGHT: *We have identified seven distinct cell-type populations in the putamen. Using canonical cell-type markers, we are able to identify major cell types expected in the striatum, including distinct populations of medium spiny neurons (MSNs). Cocaine did not dramatically alter the relative number of different cell types (see table)*

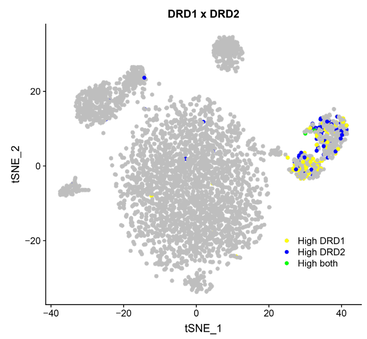
Cell Type	Cocaine (*n*)	Control (*n*)
Astrocytes 1	133	225
Astrocytes 2	49	115
Direct MSNs	113	120
Indirect MSNs	114	189
Microglia	36	60
Oligodendrocytes	1391	1504
OPCs	90	131
Total	**1926**	**2344**



DRD1 and *DRD2* Are Enriched in Different Neurons in the Putamen – DAT (*SLC6A3*) is Completely Lacking



Direct MSNs are enriched for the dopamine D1 receptor (DRD1) and the Indirect MSNs are enriched for D2 (DRD2). However, there is evidence for the expression of both dopamine receptors in both MSN subtypes. While the dopamine transporter (DAT; SLC6A3) protein is highly expressed in the putamen, the nuclei we isolated from the putamen do not originate from dopaminergic neurons and therefore lack SLC6A3 expression, consistent with the DAT observed in the putamen likely originating from innervating dopaminergic midbrain neurons.

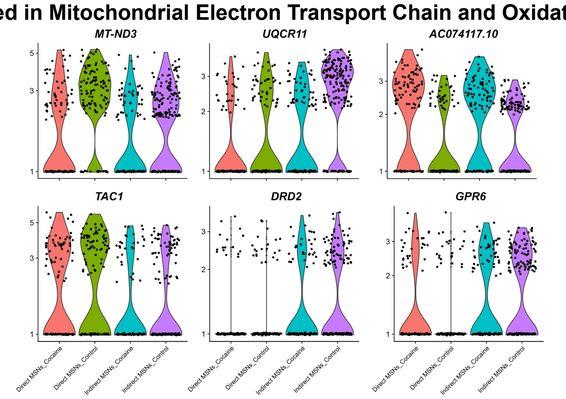


Expression of DRD1 and DRD2 occur in distinct populations of neurons. While there is evidence that Direct and Indirect MSNs express both DRD1 or DRD2, there is little overlap between the expression of these two genes.

Cocaine Decreases Neuronal Expression of Genes Involved in Mitochondrial Electron Transport Chain and Oxidation

Cell Type	Gene	Fold Change	P-val (adj)
Direct MSNs	*MT-ND3*	-1.71	1.25E-06
	MT-ND2	-1.68	3.98E-06
	MT-ND1	-1.71	2.34E-05
	AC074117.10	1.82	6.80E-05
	MT-CO3	-1.68	1.32E-04
	MT-ATP6	-1.39	2.88E-03
Indirect MSNs	*UQCR11*	-1.87	7.32E-11
	NDUFB8	-1.60	4.03E-09
Complex I	*AC074117.10*	1.65	1.04E-08
Complex II	*NDUFB2*	-1.54	1.98E-08
Complex III	*SEPW1*	-1.52	3.98E-06
Complex IV	*NDUFA13*	-1.52	1.55E-05
Complex V	*COX6A1*	-1.49	3.22E-05
Oxidiation/Redox	*GPX4*	-1.43	5.86E-05
	FAM215B	1.58	9.01E-05

Comparing gene expression across Cocaine versus Control samples in each cell type reveals numerous significantly differentially expressed genes. Listed are top hits from Direct and Indirect MSNs. We also observed differentially expressed genes in Astrocytes 1, OPCs, and Oligodendrocytes cell types. The gene names are colored according to their roles in the mitochondrial electron transport chain.



Violin plots portray differences in gene expression across cell type (Direct and Indirect MSNs) and drug condition (Cocaine vs. Control). Some genes exhibit stronger cell type-specific effects (MT-ND3 & UQCR11), while others show changes in expression related to cocaine use across cell types (AC074117.10). Classic MSN markers (TAC1 & DRD2) appear unaffected by cocaine use. The potential anti-addiction drug target GPR6 appears to be bi-directionally modulated by cocaine, depending on MSN type.

Discussion

Applying flow cytometry to Hoechst-stained nuclei isolated from postmortem human brain tissues permitted efficient single nuclear RNA-seq analysis in cocaine and control putamen tissues. The majority of the nuclei we captured were from oligodendrocytes (~68%), but we were still capable of identifying distinct neuronal populations that exhibit expression profiles that are consistent with well-characterized medium spiny neurons in the striatum. Multiple cell types were affected by cocaine use, but the most evident effects were observed in neurons, particularly affecting genes related to mitochondrial function. Cocaine directly impairs mitochondrial function and this has been proposed as a mechanism for cocaine-induced cell death. Here, we lack the statistical power to detect changes in the relative number of neurons, but the gene expression changes reflect likely mitochondrial dysfunction. In searching for potential anti-addiction drug targets in the putamen, *GPR6* emerged as a target whose expression is bi-directionally modulated by cocaine in Direct *vs.* Indirect MSNs. Although not demonstrated explicitly, this likely reflects a relationship with *DRD2* signaling, as we observed significant overlap in the expression of these two genes, regardless of MSN subtype. This study provides proof-of-concept and demonstrates feasibility for studying brain tissues from drug abuse using single nuclear RNA-seq. Future studies will focus on enriching neuronal populations for study and validating mitochondrial dysfunction in these postmortem brain tissues.

Conclusions

- **We observed cell types in the human putamen that are consistent with known striatal cell types, notably Direct and Indirect MSNs**
- **We do not see expression of DAT RNA, consistent with high protein levels in the putamen likely originating from innervating dopaminergic neurons**
- **Gene expression changes in neurons associated with cocaine suggest mitochondrial dysfunction, particularly affecting genes directly involved in the electron transport chain**
- **Future studies will enrich for neuronal cells, as they appear to be most affected by cocaine use, based on the number and magnitude of uniquely differentially-expressed genes.**

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