Finding Patterns of Opioid Addiction in the Brain's Reward Pathway

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Background

- Opioids work by attaching to specific proteins called opioid receptors, found in the brain and spinal cord in addition to other organs of the body. The euphoric response to opioid medications is a result of an effect on brain regions involved in reward.
- Functional imaging studies have shown that activated regions of the brain during drug intoxication include the nucleus accumbens (NAc), ventral tegmental area (VTA), and frontal lobe (FL).
- The VTA is a major dopaminergic area of the brain that works closely with the nucleus accumbens, which includes important brain circuits involved in reward. The frontal lobe may be damaged during addiction, resulting in a lack of cognitive control and increased impulsivity.

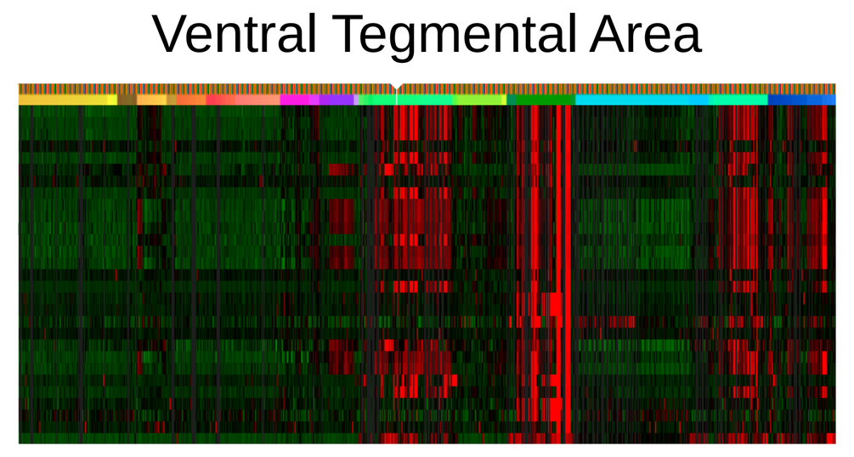
Methods

- The Allen Brain Atlas (http://www.brain-map.org) is a database used to collect gene expression data for the chosen brain regions in comparison with the gray matter of the brain using a differential search. Data for the heat maps was collected from six available donors: H0351.1009, H0351.1012, H0351.2001, H0351.2002, H0351.1015, and H0351.1016. Data for the remaining results was collected from four donors: H0351.1009, H0351.1012, H0351.2001, and H0351.2002.
- Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/) was used to compare gene lists from four chosen brain donors to identify genes that are common and different in each. A Venn diagram is created as a visual representation of the data.
- Python Anywhere (https://www.pythonanywhere.com) is a programming tool used to calculate the statistic values and variance of the fold change values of each of the four gene lists and create histograms as a visual representation.
- DAVID (https://david.ncifcrf.gov) is a bioinformatics clustering tool that subdivided the gene lists based on varying criteria related to function. Gene lists were sorted using the "official gene symbol" identifier and limited to annotations of "homo sapiens". Functional annotation tools were used to analyze the results.
- Genes of interest were entered in the STRING database (http://string-db.org) to identify potential interacting partners, pathways, and other genes relating to addiction. The database consists of networks with experimentally validated interactions.
- Genes of interest were also entered in GeneWeaver (http://www.geneweaver.org) to provide further information about the relevant genes and their functions by searching numerous experimental databases.

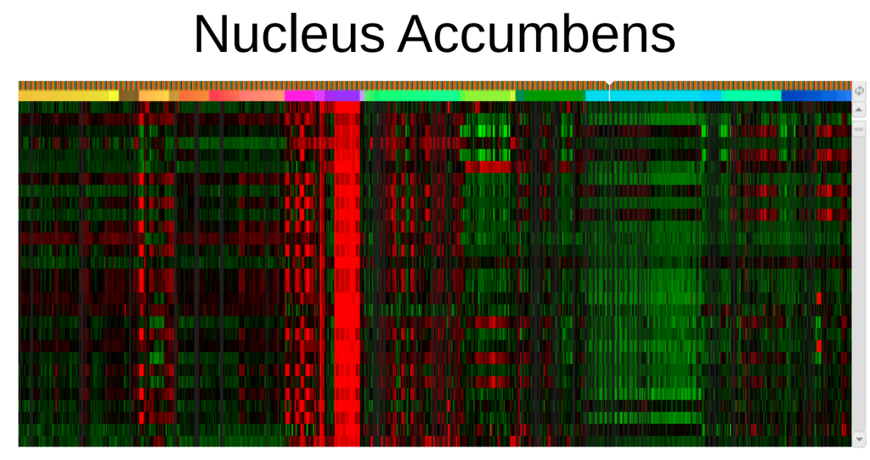
Results

1. Gene Expression Profiles

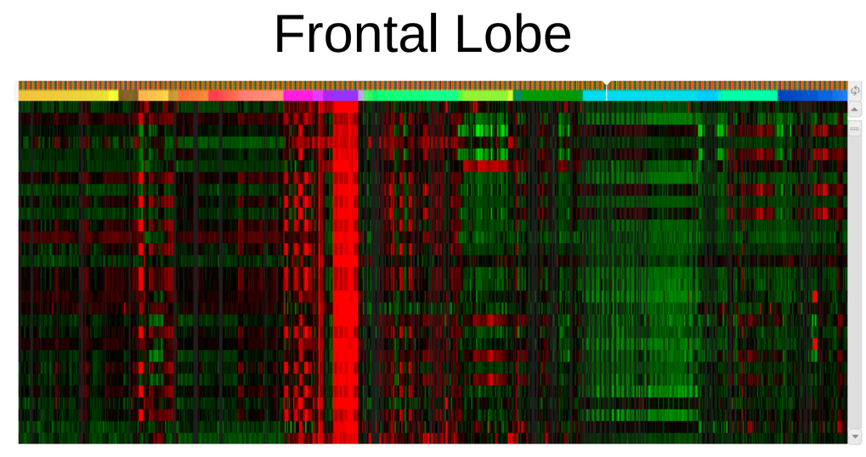
Ventral Tegmental Area



Nucleus Accumbens



Frontal Lobe

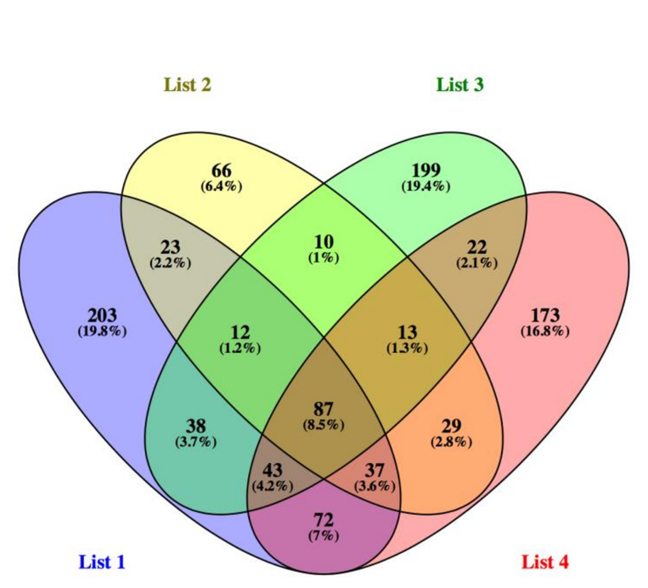


The heat maps demonstrate microarray data showing gene expression profiles of six donors (H0351.1009, H0351.1012, H0351.2001, H0351.2002, H0351.1015, H0351.1016). Each column represents a tissue sample. This data is collected from mRNA that is copied into cDNA and labeled and hybridized to an array containing all human genes. Data with a fold change of ~3 or above was used in the analysis.

Two different sample types are used for comparison: the sample under study and the control. The heat maps range in color based on the z-score over a probe. Red areas of the heat maps indicate that the expression of the sample is greater than the control (z-score of +3 and above), green areas show that the expression is less than the control (z-score of -3 and below), and black areas show that the expression is equal to the control (z-score of 0).

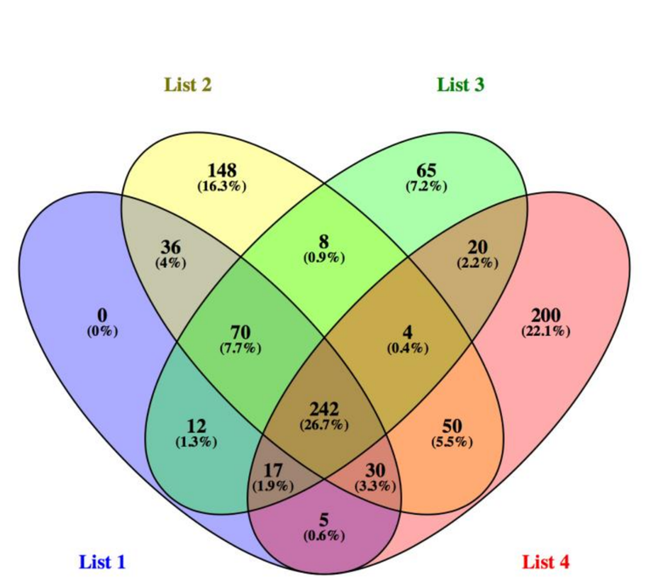
2. Gene Overlaps Among Chosen Donors in Each Region

Ventral Tegmental Area



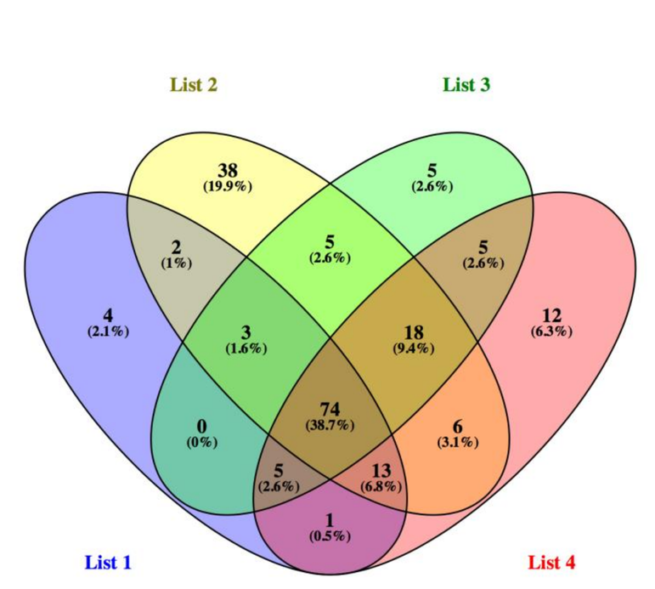
87 genes (8.5%) common in all donors

Nucleus Accumbens



242 genes (26.7%) common in all donors

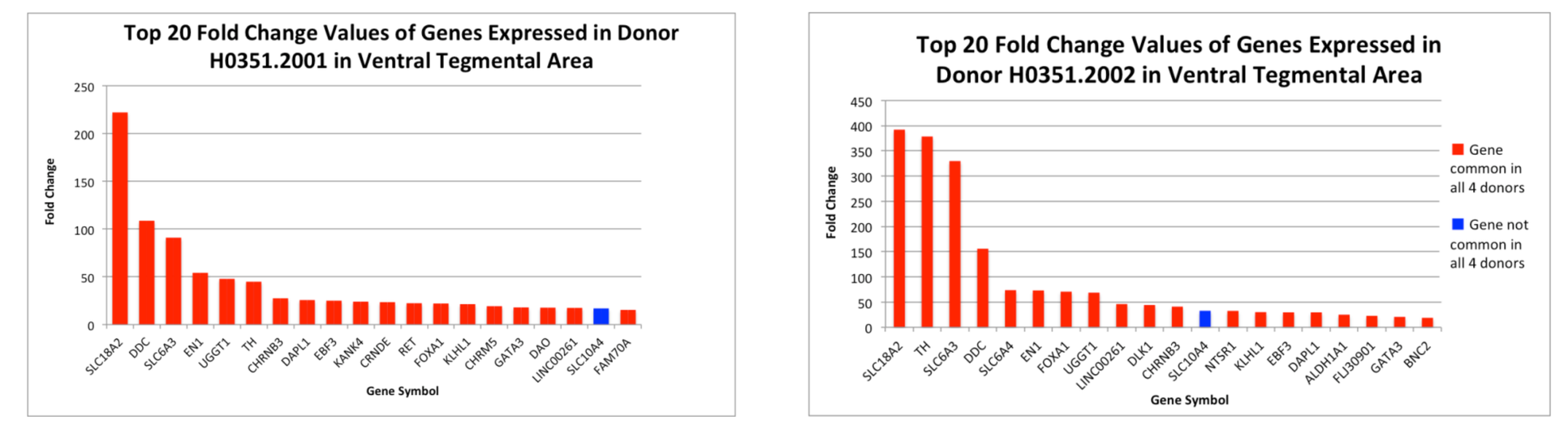
Frontal Lobe



74 genes (38.7%) common in all donors

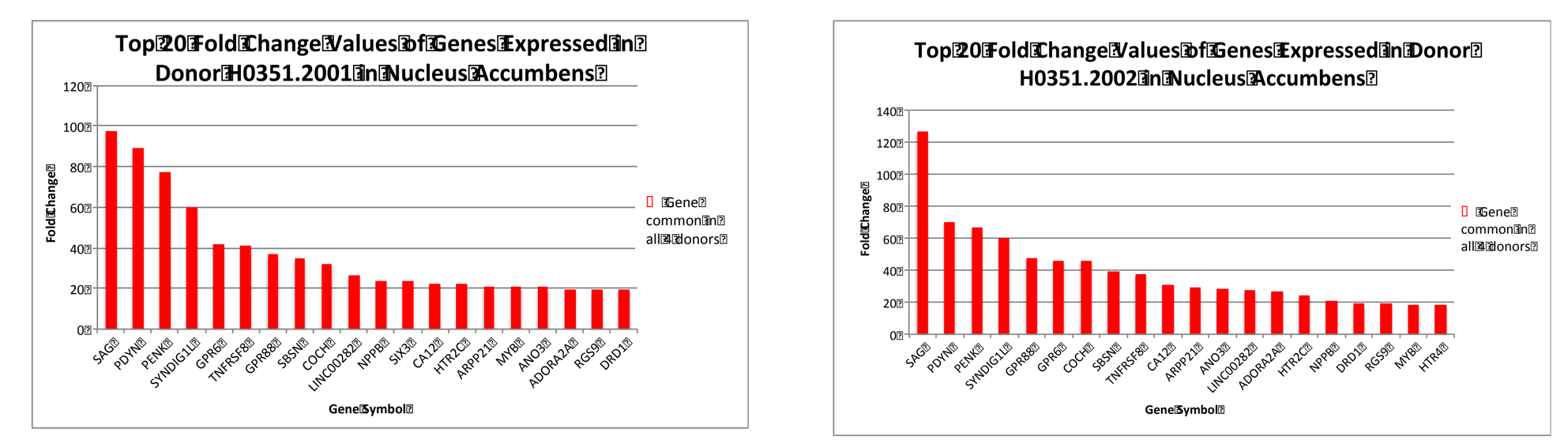
These Venn diagrams demonstrate the number and percentage of genes that overlap in each region of the brain in four chosen donors (H0351.1009; List 1, H0351.1012; List 2, H0351.2001; List 3, H0351.2002; List 4).

3. Top 20 Genes with Highest Fold Change Values



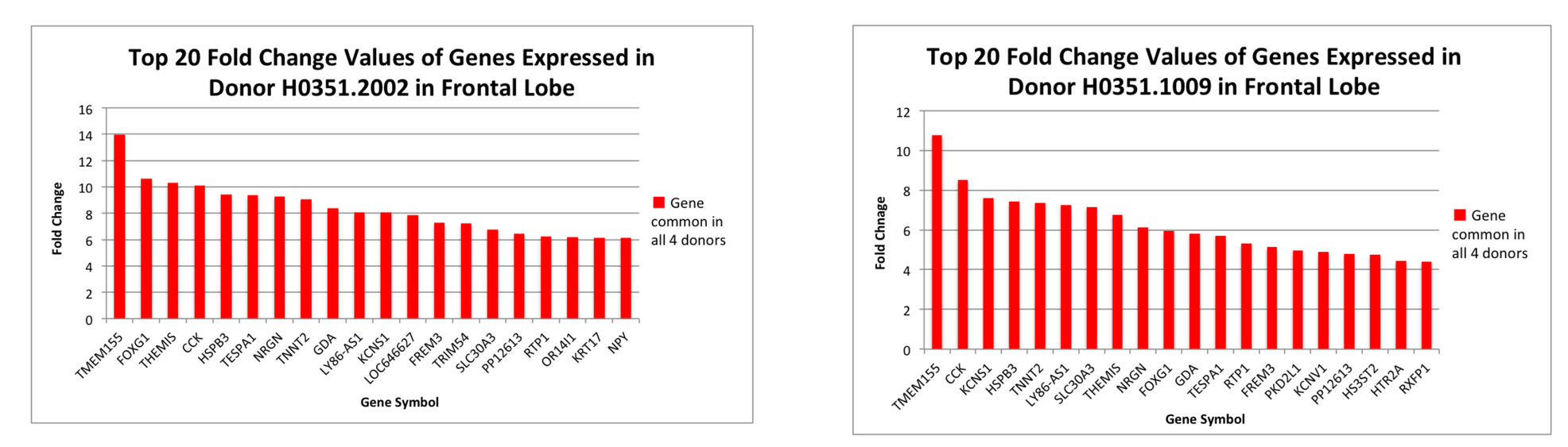
The graphs of the genes from the donors shown above display the 20 genes with the top fold change values in the VTA. 19 out of 20 of these genes were found to be common in all four chosen donors.

SLC18A2, DDC, SLC6A3, EN1, and TH were all genes that consistently had the highest fold changes and were found in all four of the chosen donors.



In the nucleus accumbens, 20 out of 20 of the genes with the top fold change values in all four of the donors (including H0351.1009 and H0351.1012) were common in all donors, two of which are shown above.

SAG, PENK, PDYN, and SYNGIG1L consistently had the highest fold change values and were common in all four donors.



In the two donors of the frontal lobe shown above, 20 out of 20 of the genes with the top fold change values were common in all of the donors.

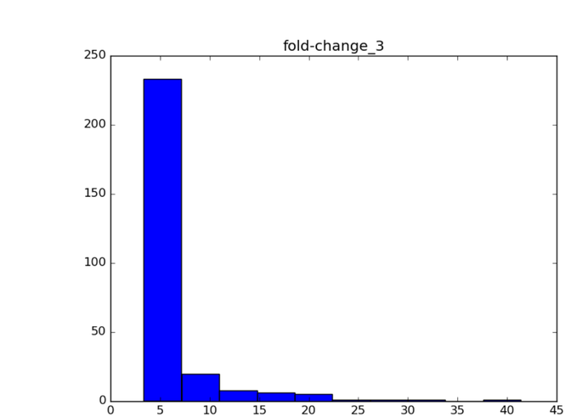
TMEM15S, FOXG1, CCK, and LY86-AS1 were all genes that consistently had the highest fold change values and were common in all four donors.

4. Statistics and Variance of Genes

Ventral Tegmental Area

stats	fold-change_1	fold-change_2	fold-change_3	fold-change_4
count	276.000000	276.000000	276.000000	276.000000
mean	7.380185	9.970536	5.865236	8.955087
std	10.023990	32.448725	4.580648	23.333101
min	3.633000	2.993000	3.363000	3.574000
25%	4.095500	3.298250	3.689250	3.896500
50%	4.790500	3.860000	4.288500	4.461500
75%	6.284750	5.596250	5.820000	5.927250
max	108.695000	378.537000	41.387000	275.509000

variance	
fold-change_1	100.478580
fold-change_2	1052.919780
fold-change_3	20.982337
fold-change_4	544.433621



Histogram of Donor H0351.1009

Fold-change_1 represents Donor H0351.2001, Fold-change_2 represents Donor H0351.2002, Fold-change_3 represents Donor H0351.1009, Fold-change_4 represents Donor H0351.1012

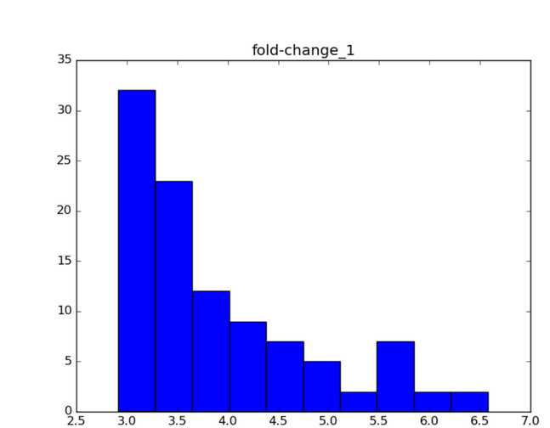
In the VTA, the frequency of genes (y-axis) is heavily skewed for genes with a smaller fold change (x-axis). The mean fold change for each donor varies slightly, ranging from about 5.9-10. The standard deviation, however, varies drastically among donors, the lowest fold change being about 4.5 and the highest being 32.4.

In the NAc, the distribution of fold changes was similarly represented. The mean fold change was approximately the same in each donor, each about 7. The standard deviation in each donor was also approximately the same, each about 8.

Frontal Lobe

stats	fold-change_1	fold-change_2	fold-change_3	fold-change_4
count	101.000000	101.000000	101.000000	101.000000
mean	3.872386	5.113317	3.909030	4.405614
std	0.918703	1.670388	1.192413	1.226790
min	2.918000	3.568000	2.963000	3.242000
25%	3.178000	3.934000	3.142000	3.593000
50%	3.561000	4.521000	3.462000	3.986000
75%	4.263000	5.400000	4.016000	4.656000
max	6.576000	10.639000	8.500000	9.230000

variance	
fold-change_1	0.844015
fold-change_2	2.790197
fold-change_3	1.421849
fold-change_4	1.505014



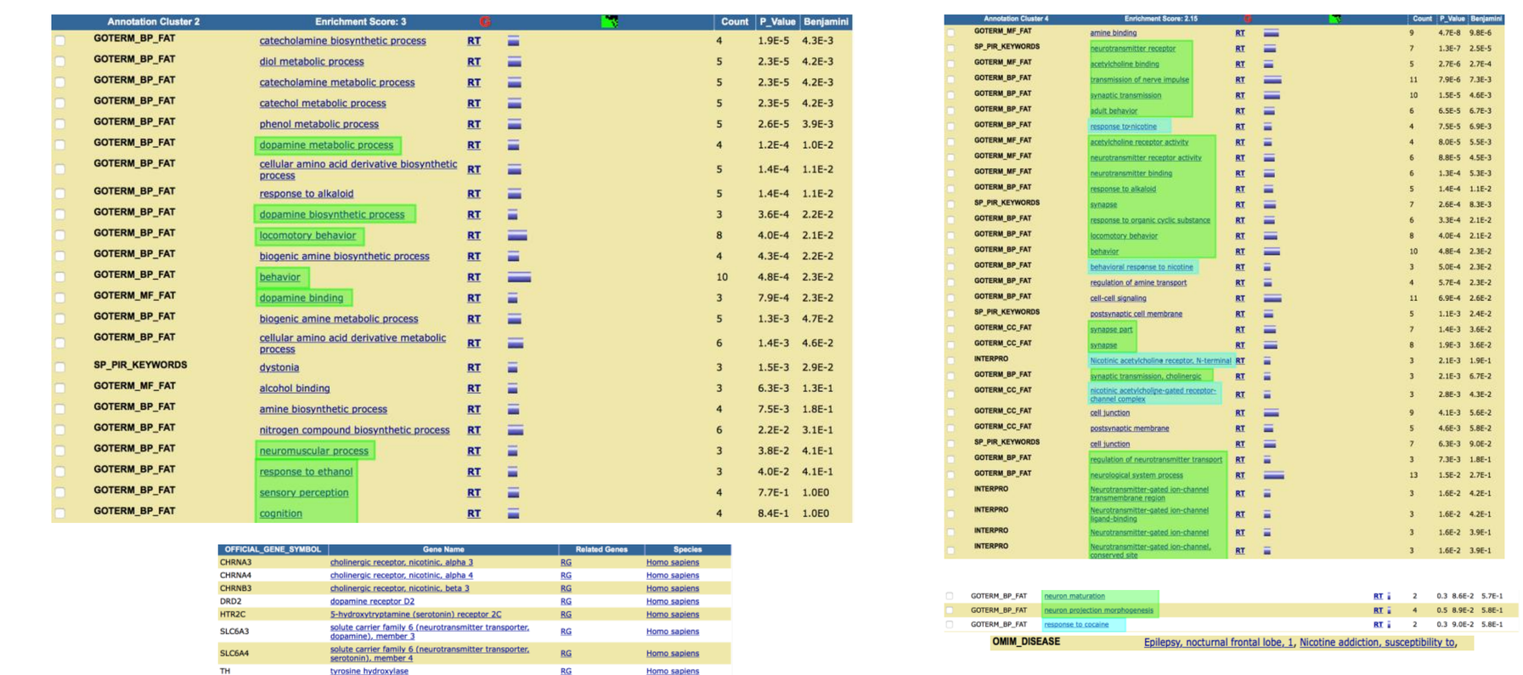
Histogram of Donor H0351.2001

The fold-change columns represent the same donors as above.

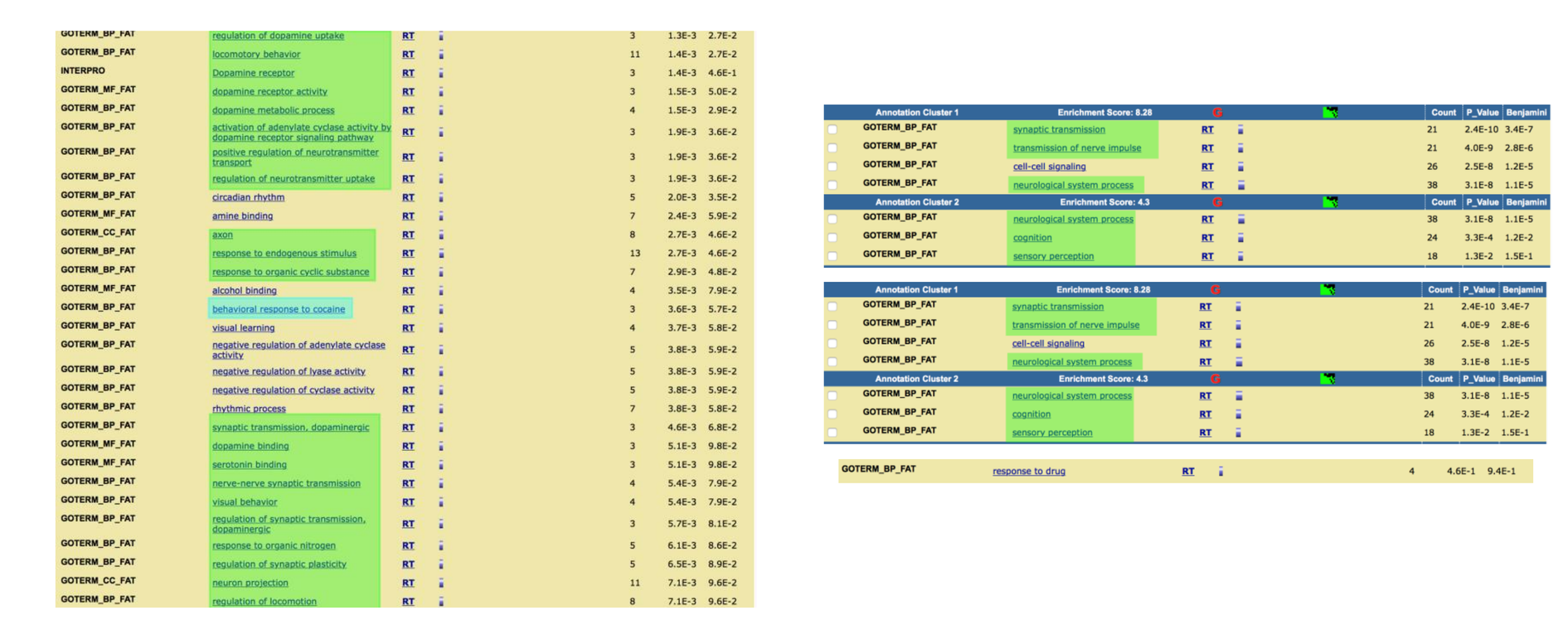
In the frontal lobe, the frequency of genes is less skewed. However, overall, the fold change values are smaller than those in the VTA and NAc. The maximum fold change value in the frontal lobe was 10.6.

The mean fold change is relatively similar in all donors, ranging from 3.9-5.1. The standard deviation is also nearly the same, ranging from 0.9–1.7.

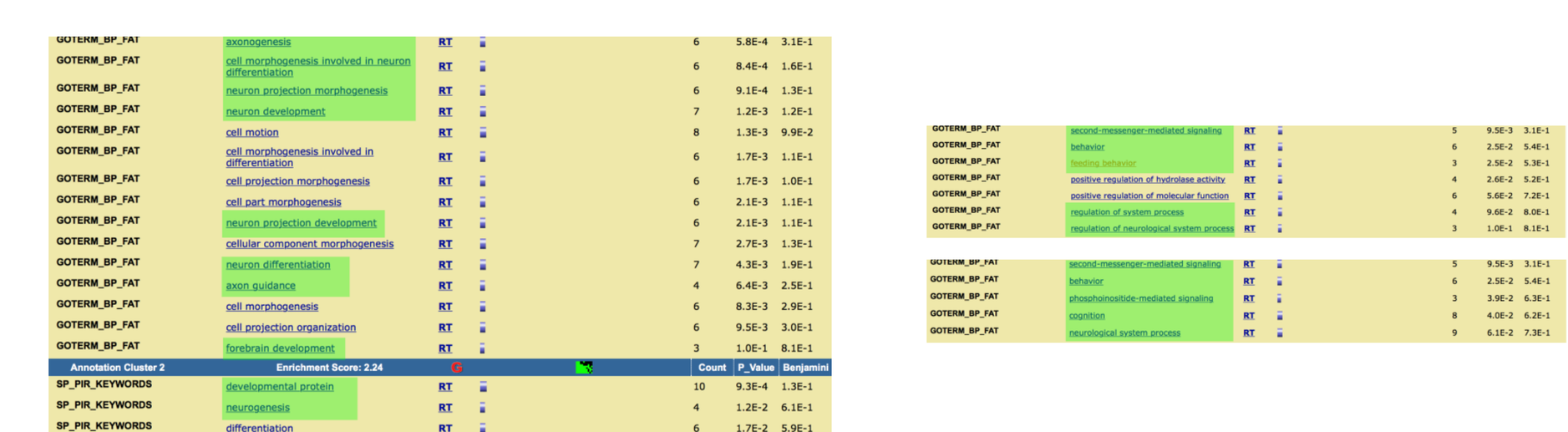
5. Genes of Interest



Results from the Ventral Tegmental Area



Results from the Nucleus Accumbens



Results from the Frontal Lobe

An analysis of the genes common of all four chosen donors revealed a large number of relevant genes of interest. Terms highlighted in green represent general genes of interest, while terms highlighted in blue represent addiction-related genes of interest.

Many of the genes of interest included dopamine-related processes and binding, behavioral responses, neurological system processes, neurotransmitters, and responses to drugs (nicotine, cocaine, alcohol).

Relevant genes generating addiction-related responses in all three regions of the brain included CHAT, CHRNA3, CHRNA4, DRD1, DRD2, HTR2C, SLC6A3, OPRD1, OPRM1, and PPP1R1B.

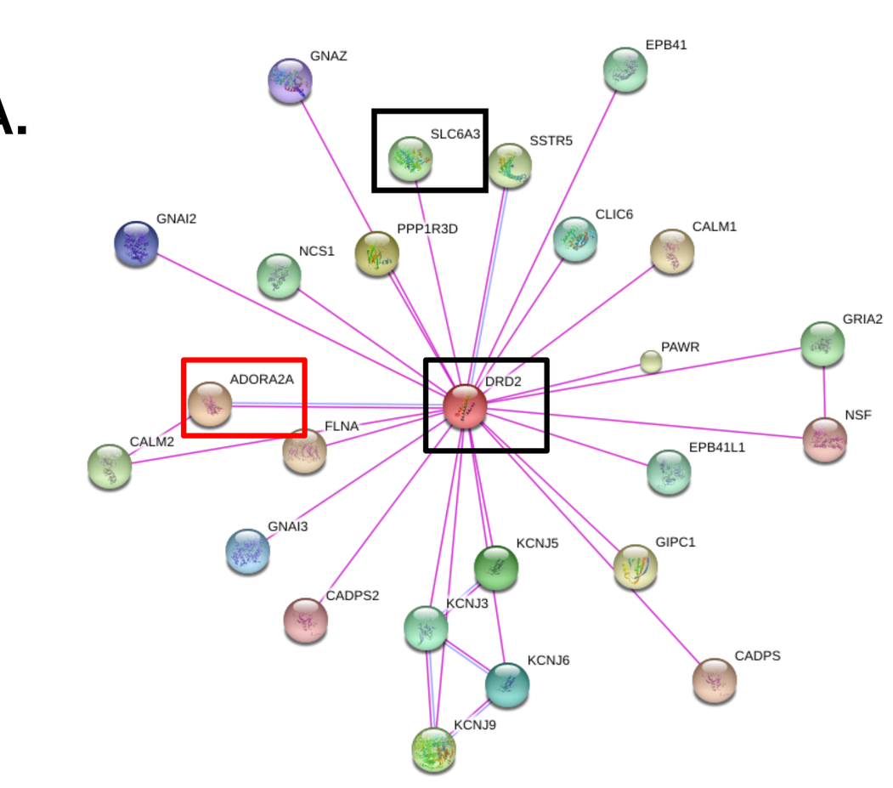
6. Protein Interaction Networks and Results

Network found between DRD2 and SLC6A3

DRD2: dopamine receptor D2; activity is mediated by G proteins which inhibit adenylyl cyclase
-gene common in all donors in both the VTA and NAc

SLC6A3: solute carrier family 6 (neurotransmitter transporter, dopamine), member 3; terminates action of dopamine

A.



ADORA2A:
-gene found to have a response to amphetamine and to be involved in the dopaminergic pathway

adenosine A2a receptor; activity of this receptor is mediated by G proteins which activate adenylyl cyclase

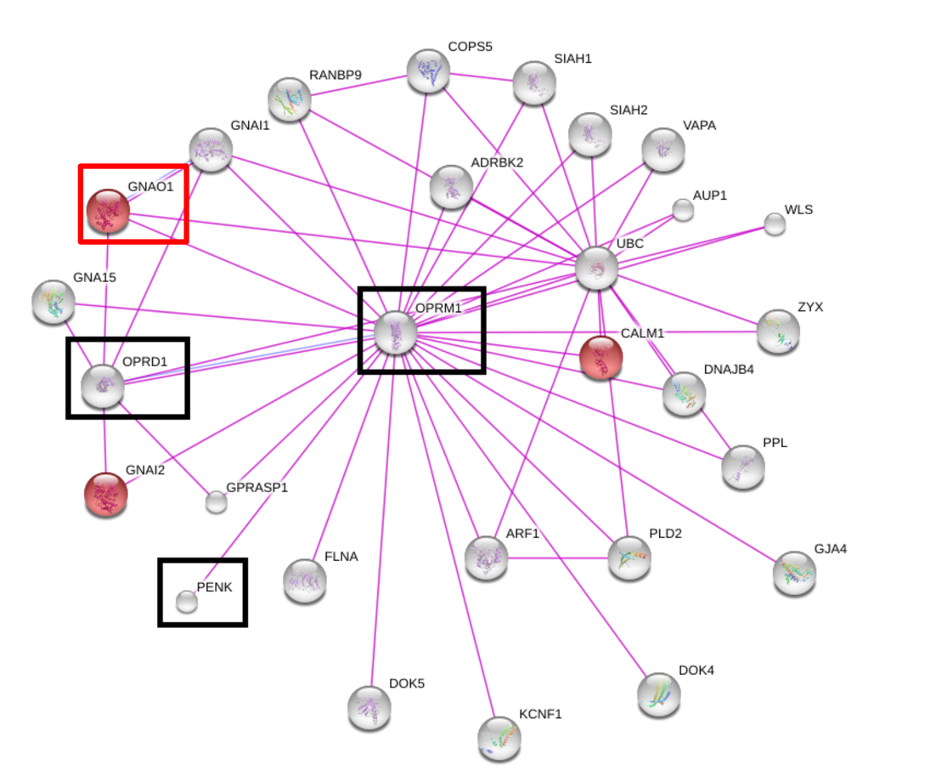
Network found between OPRM1, OPRD1, and PENK

OPRM1: opioid receptor, mu 1

OPRD1: opioid receptor, delta 1; inhibits neurotransmitter release by reducing calcium ion currents and increasing potassium ion conductance

PENK: proenkephalin; Met- and Leu-enkephalins compete with and mimic the effects of opiate drugs; play a role in pain perception and responses to stress

B.



GNAO1:
-gene found to have a response to morphine and drug

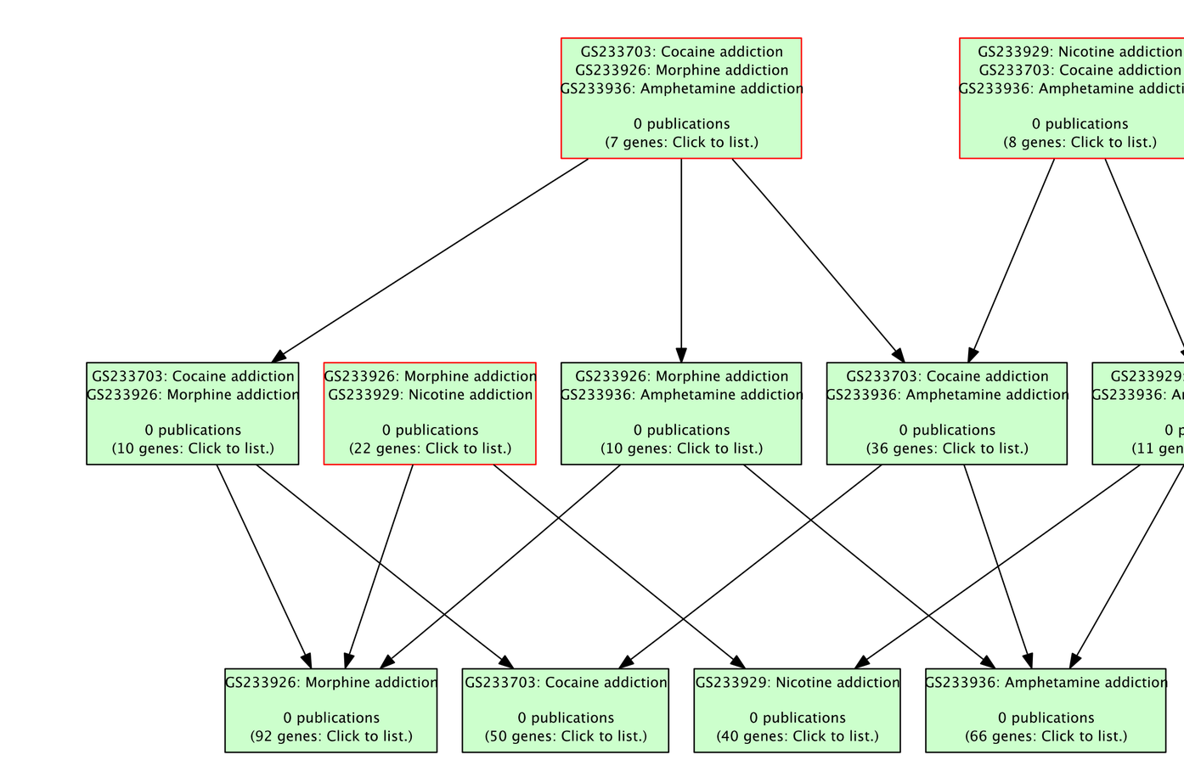
guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O; G proteins are involved as modulators or transducers in various transmembrane signaling systems

A. The DRD2 network consists of 25 proteins, 30 edges, and has low interconnectivity. Fifteen of these have been implicated in neuro-related processes - GIPC1, NSF, ADORA2A, CALM1, CALM2A, NCS1, GRIA2, GNA12, GNA13, SLC6A3, SSTR5, EPB41, EPB41L1, FLNA, GNAZ. These processes include dopamine signaling, opioid response, and neuronal signaling. Four of these proteins (GRIA2, GNA12, GNA13 and GNAZ: KEGG Pathway) are also associatied with long-term depression.

B. The OPRM1 network consists of 28 proteins, 53 edges, and has moderate interconnectivity. Ten of these proteins function in similar neuronal processes as the DRD2 network - GNAO1, GNAI1, GNAI2, OPRM1, PENK, UBC, SIAH1, SIAH2, CALM1, and UBC.

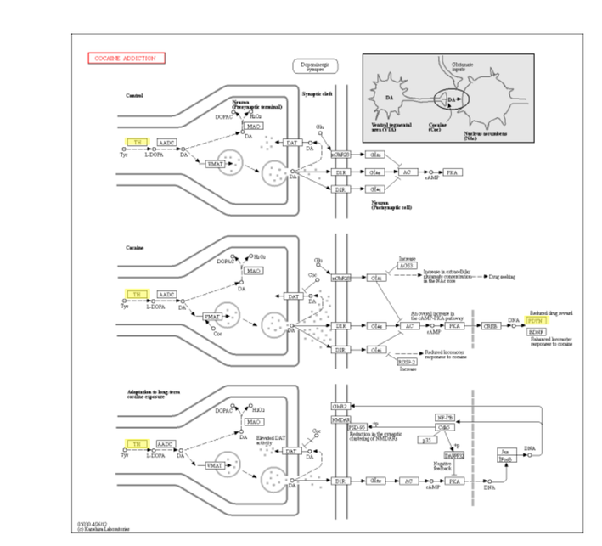
The two networks have 3 proteins in common - CALM1, FLNA, and GNAI2.

7. KEGG Pathway Genes



Out of the 7 genes that were found in GeneWeaver genesets and KEGG pathways of cocaine, morphine, and amphetamine addiction, **DRD1** was a gene of interest. No genes of interest were found in the genesets of nicotine, cocaine, and amphetamine addiction.

Out of the 36 genes found in the cocaine and amphetamine addiction KEGG pathway genesets, **DDC, TH, PDYN, AND SLC18A2** were genes that were consistently found to have the highest fold-change values in all four donors in the VTA and NAc.



TH and **PDYN** can be seen in this visual representation of a cocaine addiction KEGG pathway.

Conclusion

- An informatics approach was used for profiling gene expression patterns to identify other genes that may be involved in opioid addiction.
- In the VTA, NAc, and frontal lobe, the genes with the highest fold change values tended to be common in all four chosen donors.
- The genes of interest with the highest fold change values include SLC18A2, DDC, SLC6A3, TH, PENK, and PDYN.
- There is a high conservation of gene expression patterns pertaining to addiction in all three brain regions relevant to the reward pathway, especially in the VTA and NAc.
- Gene interaction networks were found between DRD2, SLC6A3, and ADORA2A, as well as between OPRM1, OPRD1, PENK, and GNAO1.
- The DRD2 and OPRM1 networks both have genes that overlap in KEGG pathways for long-term depression, indicating possible comorbidity between substance abuse and depression. Both networks also indicate a high level of G-protein signaling.
- DRD1 is a dopamine receptor that is activated in morphine, cocaine, and amphetamine addiction.
- GNAO1 and OPRM1 are genes activated only in morphine addiction and therefore may be specific to opioid addiction.