Finding Candidate Genes for Addiction Within the Brain's Reward System

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Background

•The brain's reward system is heavily dependent on the dopaminergic pathway.

•Major brain regions involved include the Ventral Tegmental Area (VTA), Amygdala, Nucleus Accumbens, Hippocampus and Frontal Lobe.

•The VTA is responsible for dopamine production, the Amygdala and Nucleus Accumbens deal with the emotional response to a stimuli after a rise in dopamine levels, the Hippocampus stores this response as a memory and the Frontal Lobe decides what further actions to take, debating whether or not the reward is worth any possible consequences.

•Addiction becomes dangerous when dopamine signals grow weaker and the subject becomes more reliant on the source of the reward for pleasure. At the same time, the subject's ability to quit the addiction is further weakened as the Frontal Lobe becomes less active.

Methods

- The Allen Brain Atlas database (http://www.allenbrainatlas.org/) was used to obtain a large list of active genes for each of the five brain regions by contrasting them with gray matter in a differential search. This provided data for 6 different donors (H0351.2001, H0351.2002, H0351.1009, H0351.1012, H0351.1015, H0351.1016).
- Venn diagrams (http://bioinfogp.cnb.csic.es/tools/venny/) were then used to find the genes expressed in each brain region that were common to all donors, as well as to find genes only expressed in individual donors.
- These gene lists of similar and different genes were then analyzed in GOrilla (http://cbl-gorilla.cs.technion.ac.il/) and DAVID (https://david.ncifcrf.gov/).
- Genes that formed functional groups with a direct connection to the dopaminergic pathway were then filtered to form a list of genes of interest, which were then further researched with STRING (http://string-db.org/) and NCBI (http://www.ncbi.nlm.nih.gov/) to identify interacting partners and potential pathways.
- Genes with a consistently high fold-change value for all the donors were then taken for each target region, and researched via STRING and NCBI as well.
- GeneNetwork (http://www.genenetwork.org/webqtl/main.py) was used to identify covarying traits for the candidate genes.

Conclusions

•We identified genes directly related to the Dopaminergic pathway: OPRM1, DRD1, GNAL and SLC6A3

•Genes: TH and NTRK1 may have some relation to nicotine dependence but the mode of interaction is not established at this time

•The enrichment results all had somewhat weak clustering, though the P-values are still significant, ranging from 10^-3 to 10^-5

•The genes of interest with the highest fold-change values are SLC18A2, SLC6A3 and TH while the genes with the lowest fold-change values are KCSN1, SBSN and GDA

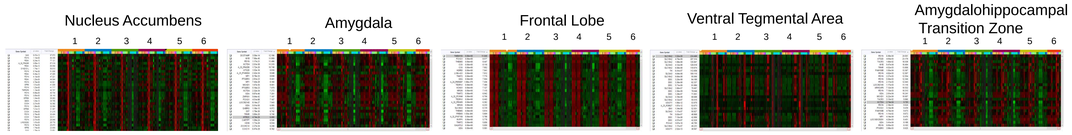
•Correlation analyses identified traits that have a greater range of expression in the dopamine receptor, DRD2 as compared to the dopamine transporter, SLC6A3

•GNAL and OPRM1 correlate with the protein expression of dopamine transporters and receptors

•Taken together, these findings support the hypothesis that dopamine signals may be inhibited thereby leading to a greater dependence on drugs, especially related to activity via OPRM1.

Results

1. Gene Expression Profiles

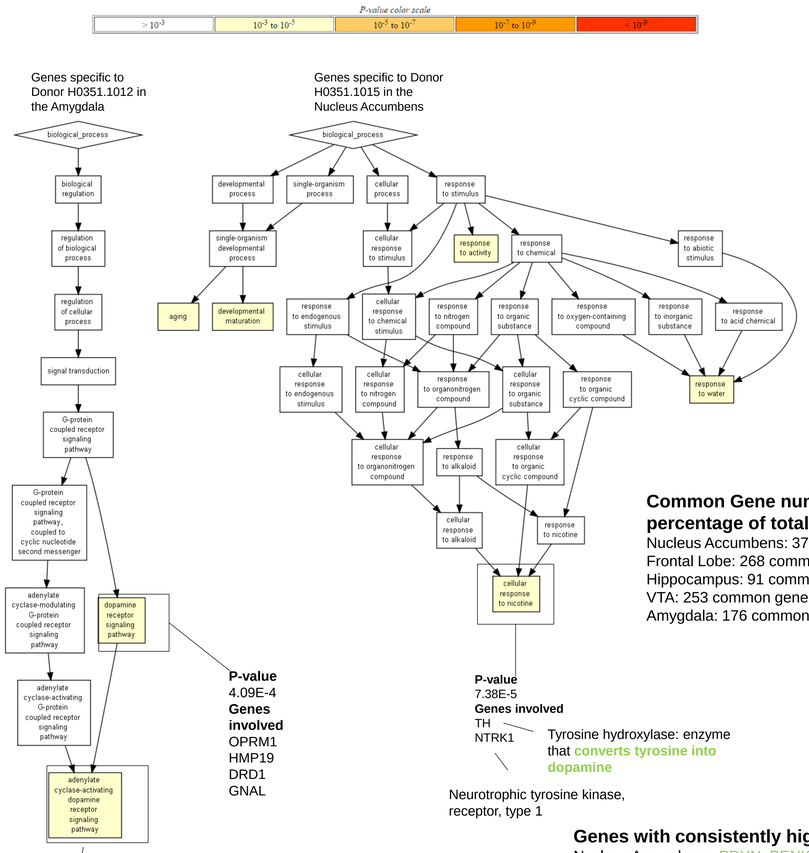


Heat maps of microarray data showing the expression profile of the top 30 genes in the Nucleus Accumbens, Frontal lobe, Amygdala, Ventral Tegmental Area and the Amygdala Hippocampal transition zone (relative to Gray Matter) for 6 donors. Each column represents a tissue sample.

Heat maps are colored to indicate the z-score over a probe ranging from green (z-score of -3 and below) through black (0) to red (z-score of +3 and above). Genes with an expression level threshold of ≥ 2-fold were considered in the analysis.

The distribution pattern for all of the gene expression data were heavily right-hand skewed because a relatively small number of genes were highly expressed whereas, the majority exhibited low expression (~ 2- fold).

2. Enrichment analyses



P-value
7.2E-4
Genes involved
OPRM1
DRD1
GNAL

Guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type

Opioid receptor, mu 1: The MOR also has an important role in **dependence to other drugs of abuse, such as nicotine, cocaine, and alcohol via its modulation of the dopamine system**
-Related to drug dependence

Dopamine receptor, d1: D1 receptors regulate neuronal growth and development, **mediate some behavioral responses**, and modulate dopamine receptor D2-mediated events
-Related to response and connected to DRD2

P-value
4.09E-4
Genes involved
OPRM1
HMP19
DRD1
GNAL

P-value
7.38E-5
Genes involved
TH
NTRK1

Tyrosine hydroxylase: enzyme that **converts tyrosine into dopamine**

Neurotrophic tyrosine kinase, receptor, type 1

Common Gene number and percentage of total
Nucleus Accumbens: 378 common genes, 30%
Frontal Lobe: 268 common genes, 50%
Hippocampus: 91 common genes, 6%
VTA: 253 common genes, 16%
Amygdala: 176 common genes,16%

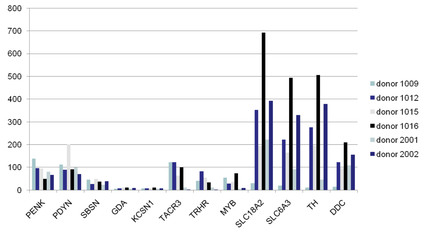
Genes with consistently higher expression levels
Nucleus Accumbens: PDYN, PENK, SBSN
Frontal Lobe: GDA, KCSN1
Hippocampus: PDYN, TACR3, TRHR, MYB,
Ventral Tegmental Area: SLC18A2, SLC6A3, TH, DDC

-PDYN and PENK are both related to opioid reception and Oprm1

-SLC6A3 is a dopamine transporter where variations can result in increased dependence on alcohol, cocaine and nicotine.

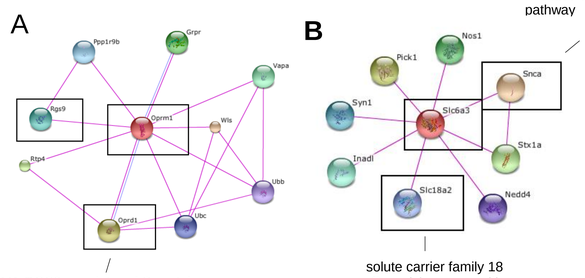
-DDC is related to the production of dopamine

3. Gene Candidates



Fold change values (y-axis) for candidate genes (x- axis) that are expressed in all six donors.

4. Candidate genes and interactions

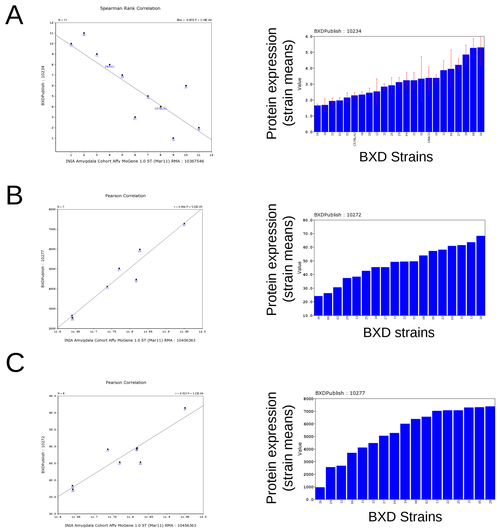


OPRM1-RGS9: regulator of G-protein signaling 9; **Inhibits signal transduction** by increasing the GTPase activity of G protein alpha subunits thereby driving them into their inactive GDP-bound form.
-Related to signal transduction in nervous system

SLC6A3-SNCA: synuclein, alpha; May be involved in the **regulation of dopamine release and transport**
-Related to dopaminergic pathway

solute carrier family 18
-One of the genes with a consistently high fold change value

5. Covarying Traits in GeneNetwork



A. Oprm1 covaries inversely (rho = -0.855, P=3.18e-04) with the protein expression of the dopamine transporter DAT and SLC6A3 (left). Bar graph represents the BXD strain means (+/- SEM). GeneNetwork Trait ID: 10234
B & C. GNAL covaries (r = 0.966, P = 5.03e-5 and 0.923, P = 3.23e-04) with two studies related to the expression of SLC6A3 (B) and DRD2 (C). Bar graphs represent the BXD strain means. GeneNetwork trait IDs 10272 and 10277