Background

- Opioid 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, ventral tegmental area (VTA), and frontal lobe (FL).
- The VTA is 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


Results

1. Gene Expression Profiles

   - The heat maps demonstrate microarray data showing gene expression profiles of six donors (H0351.1009, H0351.1012, H0351.2001, H0351.2002, H0351.1015, and 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

   - 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, and H0351.2002: List 4). Of these proteins, GNAO1 and OPRM1 are genes activated only in morphine addiction in DAVID (GNAO1, GNAI1, ADORA2A, CALM1, CALM2, GNAI3, GNAQ, GNAQ, GNA11, GNA13, SSTR6, EPBH1, EPB41L1, FLNA, GNAQ). These processes include dopamine signaling, opioid response, and neuronal signaling. Four of these proteins (GNAQ, GNA2, GNA13, and GNAZ: KEGG Pathway) are also associated with long-term depression.

3. Top 20 Genes with Highest Fold Change Values

   - The graphs of the genes from the donors shown 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.

4. Statistics and Variance of Genes

   - 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 H0351.1015) were common in all donors, all of which are shown above. SAG, PENK, and SYNGV1 consistently had the highest fold change values and were common in all four donors.

5. Gene of Interest

   - An analysis of the genes common of all four chosen donors revealed a large group of highly related 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, CRHR1, CRHR2, DR1, DR2, HTTR2, SLC6A3, OPN1, OPND1, and PPI7.B8.

6. Protein Interaction Networks and Results

   - Network found between DRD2 and SLC6A3

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

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

   - Network found between OPN1, OPND1, and PENK

   - OPN1: opioid receptor, mu 1

   - OPND1: 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 opioids drugs; play a role in pain perception and responses to stress

   - TH and PNDV1 can be seen in this visual representation of a cocaine addiction KEGG pathway.

   - The DR2 network consists of 23 proteins, 30 edges, and has few interconnections. The DR2 network has been implicated in neuro-related processes - GPCR1, NSF, ADORA2A, CALM1, CALM2, GNAI3, GNAQ, GNAQ, GNA11, GNA13, SSTR6, EPBH1, EPB41L1, FLNA, GNAQ. These processes include dopamine signaling, opioid response, and neuronal signaling. Four of these proteins (GNAQ, GNA2, GNA13, and GNAZ: KEGG Pathway) are also associated with long-term depression.

   - The OPN1 network consists of 28 proteins, 53 edges, and has moderate interconnectivity. Ten of these proteins function in similar neuronal processes as the DRD2 network - GNA1, GNA2, OPN1, PENK, PHC1, S1H1, S1H2, CALM1, and UBC.

   - The two networks have 3 proteins in common - CALM1, FLNA, and GNA2.

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

   - Out of the 36 genes found in the cocaine and amphetamine addiction KEGG pathway genes, DHC, TH, OPN1, and SLC6A3 were genes that were consistently found to have the highest fold-change values in all four donors in the VTA and NAc.

   - In the VTA, the frequency of genes (y-axis) is heaviest 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, about 7. The standard deviation in each donor was also approximately the same, each about 8.

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 gene 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 SLC6A3, GNAQ, GNAQ, 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 DRD1, OPN1, PENK, and GNA1.
- 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 DRD1, OPN1, PENK, and GNA1.
- The DRD2 and OPN1 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.
- GNA1 and OPN1 are genes activated only in morphine addiction and therefore may be specific to opioid addiction.

- Finding Patterns of Opioid Addiction in the Brain’s Reward Pathway
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