Analyzing Gene Expression Patterns In Associative Learning

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Introduction

- Located in the temporal lobe of the brain, the limbic system includes the Hippocampus and Amygdala which are associated with learning and memory..
- The Hippocampus is responsible for the processing and storage of short-term memory, long-term memory, and spatial navigation. Though the Amygdala is commonly known to be linked with emotions, fear, and anxiety, studies have proved its involvement in the modulation of memory consolidation.
- Purpose of this project is to analyze gene expression patterns for brain regions involved in the encoding, storage, learning, and retrieval of memories. Identify candidate gene for this behavior.

Methods

Gene expression data for the Hippocampus and Amygdala were collected from the The Allen Brain Atlas (http://www.brain-map.org) using the differential search option. Data was collected from four available donors.

Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/) was used to compare the gene lists from four chosen brain donors to identify genes that are common and different across each donor.

Statistical analysis was done in Python Anywhere (https://www.pythonanywhere.com) an online programming tool

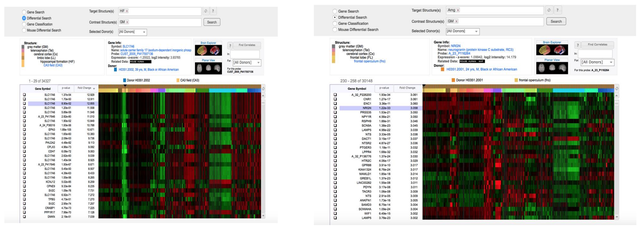
Cluster analysis and Gene Ontology classifications were obtained with DAVID (https://david.ncifcrf.gov)

The STRING database (http://string-db.org) was used to identify potential interacting partners, pathways, and other genes relating to associative learning using experimental data from gene studies.

GeneWeaver (http://www.geneweaver.org) was also used to find further information for relevant genes.

Results

Gene Expression Profiling

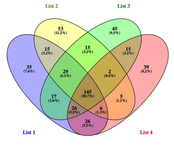


The heat maps display microarray data: fold expression of various genes in the Hippocampus and Amygdala from the profiles of four donors (H0351.2001, H0351.2002, H0351.1009, H0351.1012). Each column represents a tissue sample. This data is collected from mRNA that is copied into cDNA and labeled and hybridized to an array containing all human genes. Data with a fold change of ~3 or above was used in the analysis. 7 genes are common between the two brain regions: BCL11B, CARTPT, FOXG1, GABRQ, KCNG3, SYTL5, TRPC4.

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).

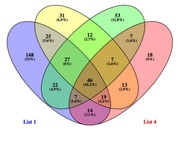
Common Genes

Hippocampus



The Venn diagrams are a visual of 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

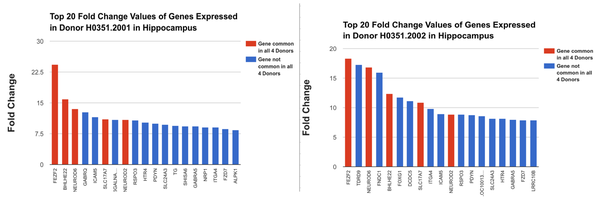
Amygdala



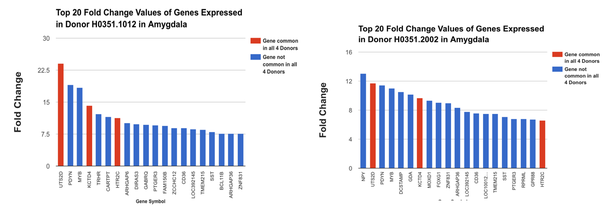
145 genes (30.7%) common in all donors

46 genes (10.2%) common in all donors

Top 20 Genes with Highest Expression Values



The graphs of the genes from the donors shown above display the 20 genes with the top fold change values in the Hippocampus. 5 out of 20 of these genes were found to be common in all four chosen donors. FEZF2 had the highest fold-change value in these two donors (H0351.2001, H0351.2002).

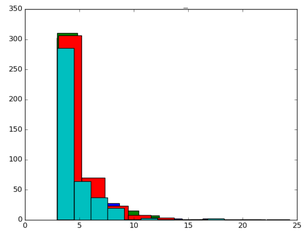


The graphs of the genes from the donors shown above display the 20 genes with the top fold change values in the Amygdala. 3 out of 20 of these genes were found to be common in all four chosen donors. There was no consistent highest fold change. UTS2D had the highest fold-change value in donor (H0351.1012).

Statistical Analysis

stats	fold_change_1	fold_change_2	fold_change_3	fold_change_4
count	414.000000	414.000000	414.000000	414.000000
mean	4.608548	4.541111	4.734249	4.476053
std	2.292733	2.077189	2.16716 7	1.914674
min	2.948000	2.969000	3.088000	2.983000
25%	3.315500	3.284750	3.406500	3.299250
50%	3.818000	3.834500	3.991000	3.777000
75%	5.006500	4.880750	5.295000	4.912250
max	22.084000	21.676000	24.331000	18.306000

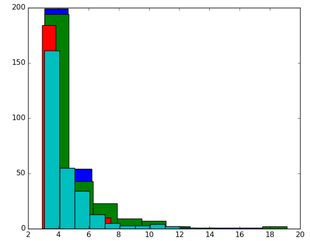
variance	
fold_change_1	5.256623
fold_change_2	4.314715
fold_change_3	4.696612
fold_change_4	3.665978



Central tendency for Hippocampus. The distribution for the highest gene expressions across all four donors are skewed to the right and clustered together near the (y-axis). For genes with a smaller fold change near the (x-axis). The mean fold change for each donor doesn't vary much, approximately ranging from 4.4 - 4.7. The standard deviation values are close to the means indicating a large spread in the data. The lowest fold change being approximately 1.9 and the highest being 2.2.

stats	fold_change_1	fold_change_2	fold_change_3	fold_change_4
count	281.000000	281.000000	281.000000	281.000000
mean	4.449281	4.760843	3.943968	4.449423
std	1.841355	2.178690	1.265508	1.666970
min	3.098000	3.101000	2.936000	3.084000
25%	3.390000	3.416000	3.200000	3.376000
50%	3.864000	4.005000	3.481000	3.838000
75%	4.870000	5.391000	4.260000	4.969000
max	18.663000	19.117000	11.997000	13.067000

variance	
fold_change_1	3.390589
fold_change_2	4.746691
fold_change_3	1.601509
fold_change_4	2.778788



Central tendency for Amygdala. The distribution for the highest gene expressions across all four donors are skewed to the right and clustered together near the (y-axis). For genes with a smaller fold change near the (x-axis). The mean fold change for each donor doesn't vary much, approximately ranging from 3.9 - 4.7. The standard deviation values are close to the means indicating a large spread in the data. The lowest being approximately 1.2 and the highest being 2.1.

Candidate Gene Analysis

Annotation Cluster 8	Enrichment Score: 2.26		Count	P_Value	Benjamin
GOTERM_BP_FAT	behavior	RT	16	2.2E-6	2.0E-4
GOTERM_BP_FAT	learning or memory	RT	8	2.0E-5	1.2E-3
GOTERM_BP_FAT	behavioral fear response	RT	3	5.5E-3	1.9E-1
GOTERM_BP_FAT	behavioral defense response	RT	3	5.5E-3	1.9E-1
GOTERM_BP_FAT	associative learning	RT	3	7.1E-3	2.3E-1
GOTERM_BP_FAT	neurological system process	RT	18	8.7E-3	2.5E-1
GOTERM_BP_FAT	fear response	RT	3	8.9E-3	2.4E-1
GOTERM_BP_FAT	learning	RT	4	1.0E-2	2.7E-1
GOTERM_BP_FAT	multicellular organismal response to stress	RT	3	3.5E-2	4.6E-1
GOTERM_BP_FAT	cognition	RT	8	5.3E-1	9.9E-1
GOTERM_BP_FAT	defense response	RT	3	9.5E-1	1.0E0

Gene Report

Help and Manual

Current Gene List: List_1
Current Background: Homo sapiens
1220 DAVID IDs
3 record(s)

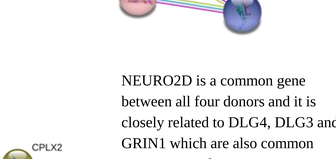
Download File

OFFICIAL_GENE_SYMBOL	GENE NAME	Related Genes	Species
GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	RG	Homo sapiens
GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate 2B	RG	Homo sapiens
NEUROD2	neurogenic differentiation 2	RG	Homo sapiens

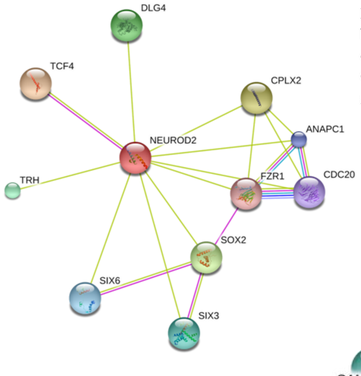
Gene ontology (GO) was used to classify the common genes from the venny graph. The (GO) term biological process (BP) were exclusively used to seekout genes of interest and genes that were relevant to the hippocampus. The gene report for associative learning provided three genes: GABRA5, GRIN2B, and NEURO2D.

Protein Interaction Network

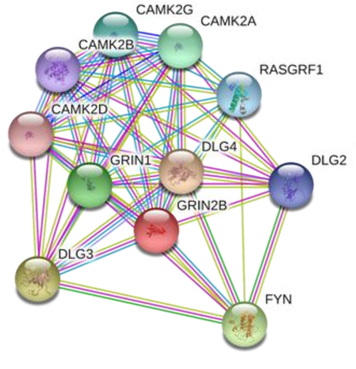
GABRA5 is a common gene between all four donors and it is closely related to GPHN, GABRA4, and GABARAP which are also common genes among donors.



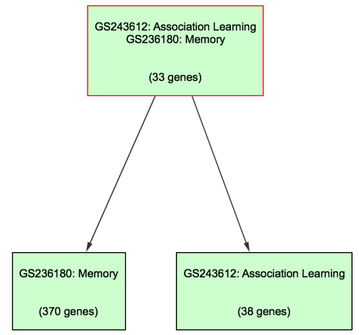
NEURO2D is a common gene between all four donors and it is closely related to DLG4, DLG3 and GRIN1 which are also common genes among donors.

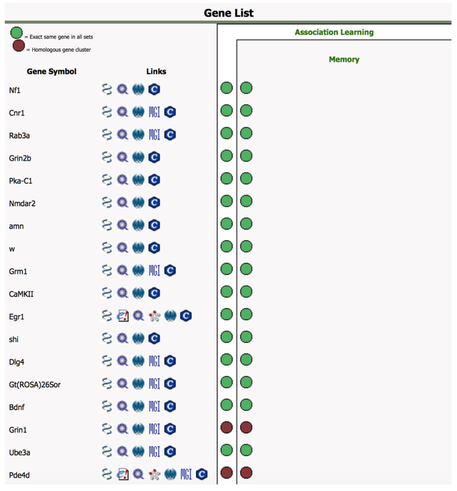


GRIN2B is a common gene between all four donors and it is closely related to DLG4, DLG3 and GRIN1. GRIN2B is a protein coding gene and it helps makes up NMDA receptor channels.



Geneset Analysis





There were 20 common genes expressed between two gene sets connected with associative learning.

Conclusions

- The Gene expression patterns on the heatmaps for the Hippocampus are highly similar across all four donors whereas, for the Amygdala, it is more variable
- The three common genes between four donors that were involved in the associative learning are NEUROD2, GRIN2B, and GABRA5. NEUROD2 is a transcription regulator for neuron differentiation. GRIN2B is a NMDA receptor subtype of glutamate-gated ion channels. GABRA5 is a subunit of a GABA receptor which are the major inhibitory receptors in the mammalian brain.
- Amygdala results: There was no relationship found at this time for gene expressed in the Amygdala and learning/memory, although there are genes associated with a broad range of neural processes that were found using an integrative genomics database.

Key References

http://www.ncbi.nlm.nih.gov/pubmed/8532847