Identifying Candidate Genes for REM Behavior Disorder

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Introduction

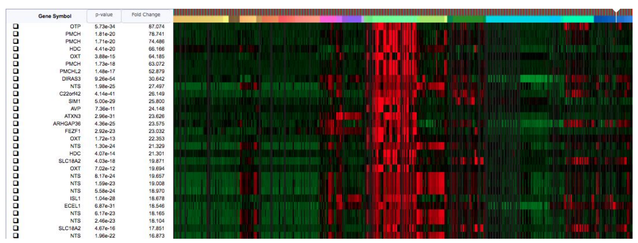
- The Hypothalamus (HTH) is a sector of the brain that is correlated with the sleep/wake cycle, maintaining balance inside the body, producing and releasing hormones, and linking the nervous and endocrine system
- The Sleep Cycle includes four stages and Rapid Eye Movement sleep (REM), where we spend about 20% of our sleep.
- Rapid Eye Movement Behavior Disorder (RBD) causes acting out, moving, and even talking out dreams during REM sleep.
- In this project, we profile gene expression data from the hypothalamus of 4 human brains, identify genes of interest, and use these to find additional candidate genes.

Methods

- The differential search option in the Allen Brain Atlas (http://www.brain-map.org) was used to track gene expression in the hypothalamus in contrast to gray matter for four different donor brains: H0351.1009, H0351.1012, H0351.1016, H0351.2002
- Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/) was used to compare tl gene lists from the four chosen brain donors to identify genes that are common and different across each donor.
- Python Anywhere (https://www.pythonanywhere.com), an online programming tool, was used to obtain statistics for the gene expression data.
- DAVID (https://david.ncifcrf.gov) was used to sort common and uncommon genes by using gene ontology classification, enrichment analysis and clustering.
- The STRING database (http://string-db.org) was used to find candidate genes relating to REM Disorder and Circadian rhythms. Networks were created based on physical interactions.
- NCBI (http://www.ncbi.nlm.nih.gov/gene) was used to obtain additional functional information for the relevant genes.

Results

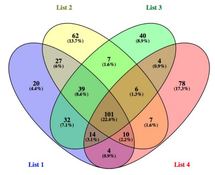
Gene Expression Profiling



Gene Symbol	p-value	Fold Change
OTP	5.73e-34	87.074
PMCH	1.81e-20	78.741
HDC	1.71e-20	74.486
OXT	4.41e-20	66.166
PMCH	3.88e-15	64.185
PMCHL2	1.73e-18	63.072
DIRAS3	1.48e-17	52.879
NTS	9.26e-54	30.642
C22orf42	1.98e-25	27.497
SIM1	4.14e-41	26.149
AVP	5.00e-29	25.800
ATXN3	7.36e-11	24.148
ARHGAP36	2.96e-31	23.626
FEZF1	4.90e-25	23.579
OXT	2.92e-23	23.032
NTS	1.72e-13	22.353
HDC	1.30e-24	21.320
SLC18A2	4.07e-14	21.301
OXT	4.03e-18	19.871
NTS	7.02e-12	19.694
NTS	8.17e-24	19.657
NTS	1.99e-23	19.008
NTS	5.58e-24	18.970
ISL1	1.04e-28	18.678
ECEL1	6.87e-31	18.646
NTS	6.17e-23	18.165
NTS	2.46e-23	18.104
SLC18A2	4.67e-16	17.851
NTS	1.96e-22	16.872

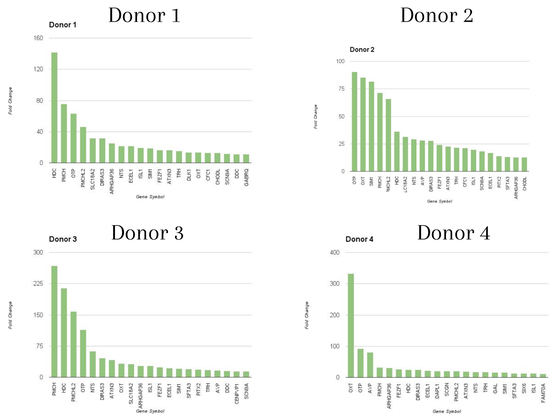
The heat map displays gene expression patterns in the hypothalamus relative to gray matter. The small varying columns at the top represent the tested four donors: H0351.1009, H0351.1012, H0351.1016, H0351.2002. The colored sections below the different donors display sub regions of the hypothalamus. Red areas of the heatmap denotes gene expression greater than control, green regions indicate under representation, and for sections that are black, expression is equal. The anterior hypothalamic, mammillary, and tuberal regions of the hypothalamus display a high concentration and conservation of highly expressed genes.

Common Genes



Among the four donors, there are 101 (22.4%) common genes found in the hypothalamus

Top 20 Genes with Highest Expression Values

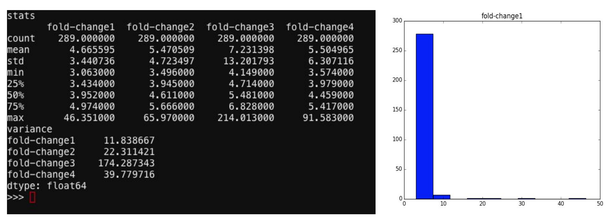


Among the 20 most highly expressed genes of the hypothalamus across four donors, 14 of these are shared between the donors (HDC, OTP, OXT, SIM1, PMCH, PMCHL2, DIRAS3, ARHGAP36, NTS, ECEL1, ISL1, FEZF1, ATXN3, TRH).

X-Axis: Gene names,

Y-Axis: Fold-change expression

Statistical Analysis



```
stats
       fold-change1  fold-change2  fold-change3  fold-change4
count    289.000000    289.000000    289.000000    289.000000
mean       4.665595      5.470509      7.231398      5.504965
std        3.440736      4.723497     13.201793      6.307116
min        3.063000      3.496000      4.149000      3.574000
25%        3.434000      3.945000      4.714000      3.979000
50%        3.952000      4.611000      5.481000      4.459000
75%        4.974000      5.666000      6.828000      5.417000
max       46.351000     65.970000    214.013000     91.583000
variance
fold-change1     11.838667
fold-change2     22.311421
fold-change3    174.287343
fold-change4     39.779716
dtype: float64
>>>
```


The means for donors 1, 2, and 4 are comparable. For donor 3, the mean is ~ 25 % greater. The standard deviation (std) for donor 3 is also high as compared to the other donors. However, the std for each of the donors is close to the mean indicating a large spread in the data. On the right of the statistics table, the histogram for donor 1 has a right skewed distribution. Few genes have high fold change values whereas most fall in the lower range. This was the case for the other donors as well.

Gene Classification

Sleep/Wake Cycle

REM Sleep Disorder

OXT	oxytocin/neurophysin I prepropeptide(OXT)	Related Genes	Homo sapiens
GOTERM_BP_DIRECT	response to amphetamine, regulation of heart rate, maternal aggressive behavior, signal transduction, positive regulation of cytosolic calcium ion concentration, heart development, female pregnancy, memory, grooming behavior, response to sucrose, positive regulation of norepinephrine secretion, response to activity, MAPK8, positive regulation of prostaglandin secretion, response to estradiol, response to retinoic acid, response to progesterone, response to prostaglandin E, social behavior, negative regulation of urine volume, positive regulation of renal sodium excretion, response to cocaine, hyperosmotic salinity response, maternal behavior, sperm ejaculation, eating behavior, drinking behavior, response to peptide hormone, response to ether, negative regulation of blood pressure, positive regulation of blood pressure, positive regulation of ossification, positive regulation of female receptivity, positive regulation of synaptic transmission, response to glucocorticoid, response to cAMP, response to electrical stimulus, regulation of sensory perception of pain, positive regulation of synapse assembly, male mating behavior, positive regulation of penile erection, positive regulation of hindgut contraction, negative regulation of gastric acid secretion, positive regulation of uterine smooth muscle contraction.		

pmch	pro-melanin-concentrating hormone	Related Genes	Homo sapiens
GOTERM_BP_FAT	regulation of heart rate, circulatory system process, cellular ion homeostasis, cellular calcium ion homeostasis, cellular metal ion homeostasis, cell surface receptor linked signal transduction, G-protein coupled receptor protein signaling pathway, elevation of cytosolic calcium ion concentration, neuropeptide signaling pathway, cell-cell signaling, synaptic transmission, gamete generation, spermatogenesis, lactation, behavior, rhythmic behavior, circadian rhythm, feeding behavior, blood circulation, regulation of heart contraction, regulation of blood pressure, response to temperature stimulus, response to cold, response to abiotic stimulus, negative regulation of cell communication, transmission of nerve impulse, cellular homeostasis, sexual reproduction, circadian SIM1/wake cycle process, cellular cation homeostasis, cellular di-, tri-valent inorganic cation homeostasis, mammary gland development, regulation of neurological system process, negative regulation of neurological system process, regulation of synaptic transmission, dopaminergic, negative regulation of synaptic transmission, dopaminergic, multicellular organism reproduction, carbohydrate homeostasis, regulation of circadian sleep/wake cycle, REM sleep, homeostatic process, glucose homeostasis, circadian sleep/wake cycle, regulation of circadian sleep/wake cycle, regulation of circadian rhythm, positive regulation of circadian rhythm, drinking behavior, regulation of system process, regulation of circadian sleep/wake cycle, sleep, negative regulation of blood pressure, positive regulation of circadian sleep/wake cycle, sleep, positive regulation of circadian sleep/wake cycle, REM sleep, regulation of synaptic plasticity, regulation of neuronal synaptic plasticity, male gamete generation, rhythmic process, circadian behavior, positive regulation of behavior, positive regulation of response to stimulus, reproductive process in a multicellular organism, gland development, chemical homeostasis, regulation of behavior, ion homeostasis, regulation of synaptic transmission, negative regulation of synaptic transmission, neurological system process, positive regulation of multicellular organismal process, negative regulation of multicellular organismal process, cytosolic calcium ion homeostasis, regulation of transmission of nerve impulse, negative regulation of transmission of nerve impulse, metal ion homeostasis, di-, tri-valent inorganic cation homeostasis, calcium ion homeostasis, cation homeostasis, cellular chemical homeostasis,		

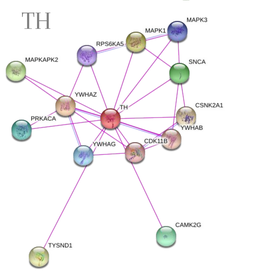
PTGER3	prostaglandin E receptor 3(PTGER3)	Related Genes	Homo sapiens
BIOCARTA	Eicosanoid Metabolism,		
GOTERM_BP_DIRECT	fever generation, transcription, DNA-templated, G-protein coupled receptor signaling pathway, adenylate cyclase-modulating G-protein coupled receptor signaling pathway, phospholipase C-activating G-protein coupled receptor signaling pathway, positive regulation of cytosolic calcium ion concentration, female pregnancy, cell death, positive regulation of gene expression, negative regulation of norepinephrine secretion, intestine smooth muscle contraction, urinary bladder smooth muscle contraction, bicarbonate transport, intracellular receptor signaling pathway, negative regulation of cAMP biosynthetic process, positive regulation of cAMP biosynthetic process, positive regulation of fever generation, response to estradiol, response to progesterone, positive regulation of urine volume, response to drug, positive regulation of vasoconstriction, positive regulation of circadian sleep/wake cycle, non-REM sleep, negative regulation of insulin secretion, maternal process involved in parturition, cellular response to lipopolysaccharide, cellular response to interleukin-1, negative regulation of platelet aggregation, negative regulation of potassium ion transmembrane transport, response to salt, negative regulation of calcium ion transmembrane transport, negative regulation of gastrin-induced gastric acid secretion, positive regulation of smooth muscle contraction involved in micturition, cellular response to forskolin, positive regulation of inhibitory G-protein coupled receptor phosphorylation, negative regulation of circadian sleep/wake cycle, wakefulness, positive regulation of myofibroblast contraction, positive regulation of colon smooth muscle contraction, positive regulation of gastric mucosal blood circulation, prostaglandin receptor internalization, positive regulation of neutrophil extravasation, negative regulation of forebrain neuron differentiation.		

GHRH	growth hormone releasing hormone(GHRH)	Related Genes	Homo sapiens
BIOCARTA	Ghrelin,		
GOTERM_BP_DIRECT	adenylate cyclase-activating G-protein coupled receptor signaling pathway, cell-cell signaling, positive regulation of cell proliferation, cAMP-mediated signaling, adenohypophysis development, growth hormone secretion, positive regulation of cAMP biosynthetic process, response to food, positive regulation of multicellular organism growth, positive regulation of insulin-like growth factor receptor signaling pathway, positive regulation of circadian sleep/wake cycle, REM sleep, positive regulation of growth hormone secretion.		

Gene ontology classification filtered the common gene list and found genes of interest relating to sleep, circadian rhythm, and specifically REM sleep. These genes were used in a subsequent network analysis to identify additional candidates (PTGER3, GHRH, OXT, TH, PMCH) that may be involved in the sleep pathways.

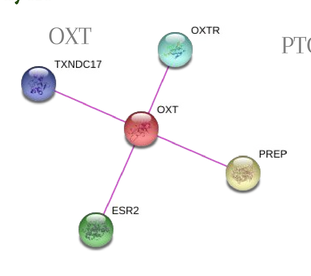
Network Analysis

Sleep/Wake Cycle

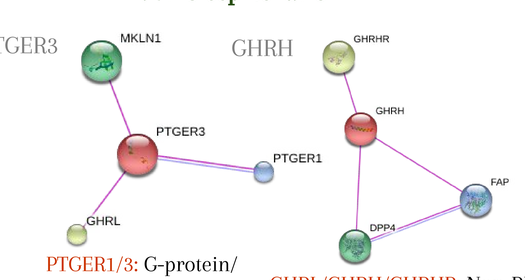


TH: sleep/wake cycle, learning & memory, locomotory, feeding, mating/social behavior,
SNCA: Alzheimer's, Parkinson's,
RPS6KA5: Circadian entrainment,
MAPK1/3: Alzheimer's, Prion disease

REM Sleep Behavior



OXT/OXTR: Calcium/hormonal signaling,
ESR2: Estrogen signaling
PREP: Renin–angiotensin system (hormone regulation),
TXND17: Cytokine/inflammation regulation



PTGER1/3: G-protein/ calcium signaling pathway
MKLNI: Cell adhesion

GHRL/GHRH/GHRHR: Non-REM sleep, regulation of circadian sleep/wake cycle
DPP4: Behavioral fear response, psychomotor, locomotory exploration
FAP: Cell adhesion

Conclusions

- Network analysis based on physical interactions for the genes associated with REM sleep behavior and Circadian rhythm have themes in signaling and hormonal regulation.
- The TH network is the largest and has a high degree of interconnectivity compared to the others.
- These genes (MAPKAP2, YWHAZ, PRKACA, YWHAG, YWHAZ, TH, CDK11B, YWHAG) mainly link to cell cycle and signaling. Several are also associated with neurological disorders such as Prion disease, Conduct disorder/ADHD and Segawa syndrome, a genetic disorder with Parkinson-like symptoms.
- Of the candidate genes, one of the more interesting for further study is DPP4, which occurs in the REM sleep behavior network.
- DPP4 is linked to psychomotor and locomotory exploration. During a normal sleep cycle, motor responses shutdown to prevent physical movement, supporting a role for DPP4 in this disorder which includes movement and speech during REM sleep.