RNA expression in the primate amygdala predicts anxious temperament and its intermediate neural phenotype

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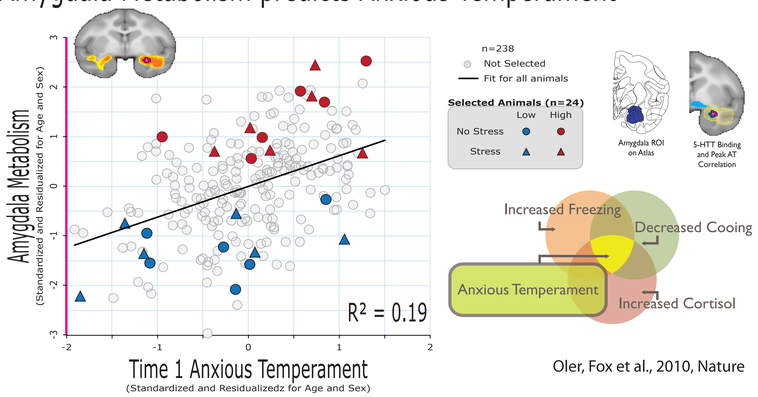
Background & Summary

We combined whole-brain FDG-PET neuroimaging with transcriptome-wide MicroArray techniques to examine the relationship between anxiety-related glucose metabolism and gene expression from the central nucleus of the amygdala (CeA) in 24 rhesus monkeys (half of whom were exposed to a mild stress). Using this unique dataset we have identified anxiety-related neural circuits (Oler, Fox et al., 2010) as well as anxiety-related gene expression. These data allows us, for the first time, to combine these two types of information in order to examine the coherence between the genetic and metabolic underpinnings of anxiety.

Amygdala Metabolism predicts Anxious Temperament

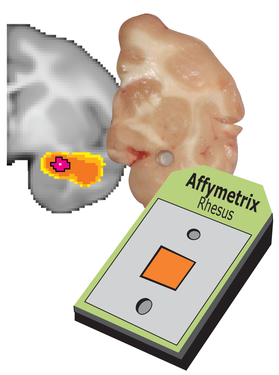


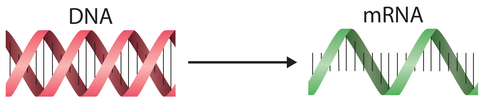
Oler, Fox et al., 2010, Nature

CeA Gene Expression Predicts Anxious Temperament

(There were no significant effects of Stress on Brain, Behavior or Genes)

$$AT = \beta_0 + \beta_1 * \mathbf{mRNA_i} + \beta_2 * StressGroup + \beta_3 * PunchSide$$





AHCTF1, ANKRD17, ARHGEF9, ASH1L, ASMTL, ASRGL1, ATP2A2, BBS5, C10orf58, C11orf41, C2orf77, C5orf53, CAMSAP1, CDC37, CHD3, CMTM4, COL4A3BP, COX16, CPT1A, CRCP, CRIP2, CYB561, DCTD, DDIT4L, DYNLRB2, EFNA3, EIF2B1, EIF4E3, EPHA4, ERC1, FAHD2A, FAM126B, FBXL5, FBXO11, FBXW11, FLRT2, FNDC3B, GADD45B, GATM, GDAP1, GIGYF2, GNB5, GOLM1, GPHN, GRINL1A, GSTT2, GTF2E2, GUSB, HAUS2, HSPA4, HTR2C, ICAM5, IFITM1, IGF2R, IPW, IRS2, ISLR2, JAK2, KCNN2, KDM2B, KIAA0564, KIAA1549, KIAA1632, LDB2, LNP1, LRRN2, LYPLAL1, MDN1, MED17, MGAT4B, MLXIP, MMRN2, MYO16, N4BP2, NAA38, NCKIPSD, NCOA1, NDUFC2, NHEDC2, NOL9, NOVA1, NPPA, NPR2, NPY1R, NTRK3, ODZ4, OSBP, PAM, PCDH17, PDCD11, PDK3, PIGG, PLA2G12A, PMM1, PNCK, POLR2E, PPID, PPM1H, PPP1R12A, PRKAG2, PTS, RAB12, RAB3GAP2, RAB3IP, RALGAPB, RBM33, RNF145, RPE, RPS6KA3, SFRS12, SIAE, SLC16A2, SLC4A10, SMARCA2, SNX12, SNX2, SPRY1, ST3GAL1, STK24, STRADA, STX3, SUMO3, SYNJ2BP, TCEAL2, TM2D2, TMEM67, TPM1, TTF1, TXNDC15, UBE2E1, UBR1, UCK1, UROD, YTHDF1, ZC3H14, ZFHX2, ZNF33B, ZNF542, ZWILCH

Animals received intravenous injections of 10 mCi [18F]-flouro-2-deoxyglucose (FDG) immediately before exposure to a human intruder presenting their profile and making no eye-contact (NEC). FDG is a glucose analog that is taken up and trapped by metabolically active cells, and is an ideal radiotracer to simultaneously study behavior and brain activity elicited by exposure to ethologically relevant situations. The time course of FDG uptake, which reflects brain activity over an approximate 30-minute period, is ideally suited to assess the sustained brain responses associated with temperament, which by definition is a disposition that is persistent and relatively context-independent.

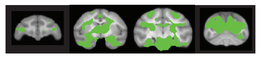
24 young Male monkeys (12 per group) were identified as expressing extreme high amygdala or low amygdala levels and sacrificed 4-5 days after the final NEC challenge. The brains were extracted and the tissue was sectioned on a block in 4.5 mm slices and then immediately frozen in a container of chilled isopentane surrounded by dry ice and stored at -70 degrees. The central nucleus region of the amygdala was collected using a 3 mm punch tool using The Rhesus Monkey Brain in Stereotaxic Coordinates (Paxinos, Huang, Petrides & Toga, 2009) and the previously identified AT-related CeA region as a guide. RNA was extracted using the RNeasy Plus Mini kit (Qiagen, Valencia, CA) from each animal and used as template for cRNA labeling using the GeneChip® 3' IVT Express kit (Affymetrix, Santa Clara, CA). The labeled cRNA from each animal was hybridized to an Affymetrix Rhesus Macaque Genome array and data were analyzed using GeneSpring GX software (Agilent Technologies, Santa Clara, CA). Some gene expression changes were confirmed using quantitative real time-PCR measuring fluorescence generated by TaqMan probes (Applied Biosystems, Foster City, CA). The same RNA used for gene chip analysis was used for qRT-PCR.

All analyses were performed using the open-source statistical package R, and the bioconductor libraries for Microarray analysis (http://www.bioconductor.org/) or Matlab and fMRIStat. We used RMA background correction, normalized across chips with a constant, ignored mismatch probes, and summarized across probes with using the median-polish technique. Resulting expression estimates for each probeset were filtered based on mean expression levels (>log2[100]). Across subject analyses were performed using a robust regression and significance was assessed using an empirical bayes method (Smyth, 2004), and corrected for multiple comparisons using FDR. Genes were annotated using publicly available annotations that were verified by BLASTing against the transcript database (http://www.unmc.edu/rhesusgenechip/). Brain data were analyzed in accordance with previously published methods.

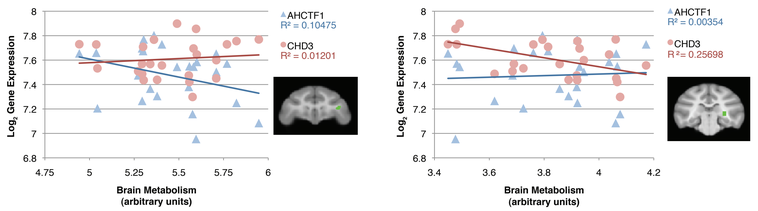
Rationale

We defined the neural circuits of AT by regressing AT on FDG-PET using a liberal significance threshold (p<.05 two-tailed uncorrected)

$$FDG_v = \beta_0 + \underline{\beta_1} * \mathbf{AT} + \beta_2 * StressGroup$$



CeA transcripts differentially relate to the neural substrates of AT.



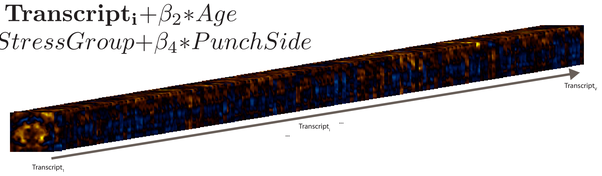
Why should CeA transcripts predict brain metabolism across the brain? This could represent different networks of genes that represent how the amygdal recieves input, projects to different regions, or shared distributed neural systems that overlap with the amygdala. We sought to determine if the relationships between gene expression and brain metabolism could help to meaningfully group genes.

Method

1) Compute a parameter-estimate to describe how each AT-related transcript predicts voxelwise brain metabolism.

$$FDG_v = \beta_0 + \underline{\beta_1} * \mathbf{Transcript_i} + \beta_2 * Age + \beta_3 * StressGroup + \beta_4 * PunchSide$$

$$\beta_{1,:} =$$



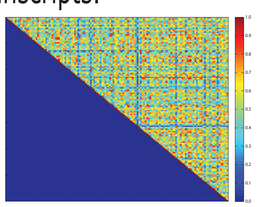
2) Compute the eta² similarity of voxelwise paramter estimates within the identified AT circuit for each pair of transcripts.

$$eta^2_{a,b} = 1 - \frac{SS_{Within}}{SS_{Between}}$$

$$= 1 - \frac{\sum_{v=1}^{V}(a_v - \frac{a_v+b_v}{2})^2 + (b_v - \frac{a_v+b_v}{2})^2}{\sum_{v=1}^{V}(a_v - \frac{\sum_{v=1}^{V} a_v+b_v}{2*V})^2 + (b_v - \frac{\sum_{v=1}^{V} a_v+b_v}{2*V})^2}$$

$$eta_{\beta_{1,i}.\beta_{1,j}} = (\quad,\quad) = S_{i,j}$$

$$\underline{S} =$$



3) Cluster transcripts based on how they predict brain metabolism.

Transcripts were clustered by minimizing the distance (*d*) in *S* across clusters.

$$\underline{d_{r,s}} = \sqrt{\frac{2n_r n_s}{(n_r + n_s)}} \|x_r - x_s\|_2$$

Acknolwedgements

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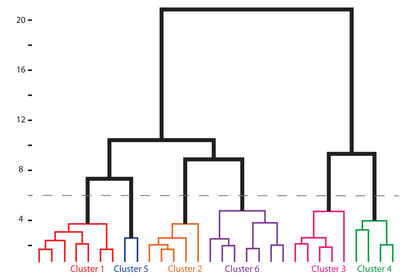
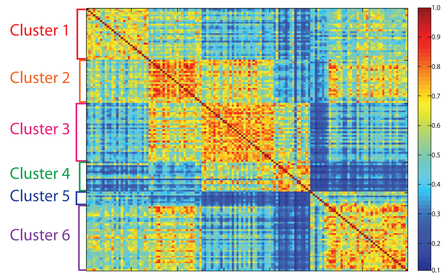
Results

Cluster Dendrogram



Similarity Matrix Sorted by Cluster



Transcript List for each Cluster

AHCTF1 ASMTL BBS5 COX16 CPT1A DDIT4L *EPHA4* FAHD2A FNDC3B GATM GUSB HTR2C ICAM5 IPW *ISLR2* KCNN2 LDB2 LNP1 NAA38 PIGG PLA2G12A POLR2E PRKAG2 RAB12 SPRY1 TMEM67 TPM1

ANKRD17 C2orf77 ERC1 FBXL5 FBXO11 GOLM1 GTF2E2 MGAT4B NOL9 ODZ4 PNCK RPE *RPS6KA3* SIAE SLC16A2 SNX12 SNX2 SUMO3 SYNJ2BP ZC3H14 ZFHX2 ZNF542 ZWILCH

ARHGEF9 ASH1L ASRGL1 C11orf41 CAMSAP1 CDC37 CHD3 CRIP2 *EIF2B1* FLRT2 GPHN *IRS2* LRRN2 MDN1 NCK-IPSD NCOA1 OSBP PCDH17 PMM1 PPID RAB3GAP2 RAB3IP RALGAPB RBM33 SFRS12 ST3GAL1 STRADA TCEAL2 TXNDC15 UCK1 YTHDF1

ATP2A2 CYB561 ***EFNA3*** ***GADD45B*** ***GIGYF2*** IGF2R KDM2B KIAA1632 MLXIP NPR2 ***NTRK3*** PDCD11 RNF145 TM2D2 ***TTF1*** UROD

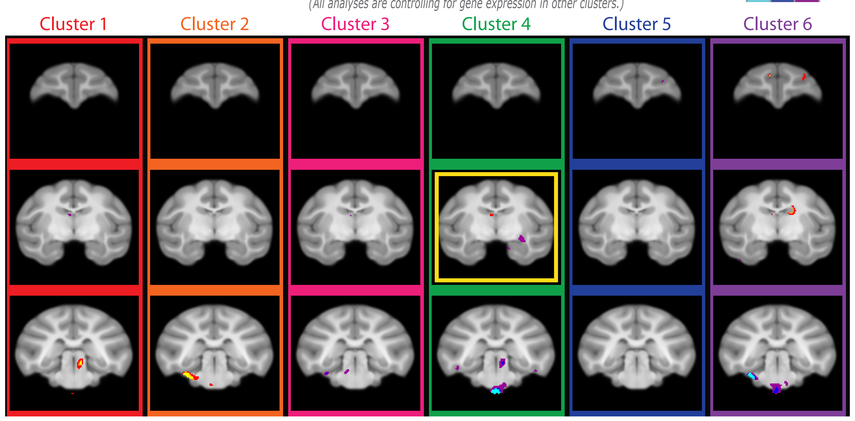
C10orf58 EIF4E3 GSTT2 IFITM1 LYPLAL1 NPPA SLC4A10

C5orf53 CMTM4 COL4A3BP CRCP DCTD DYNLRB2 FAM126B FBXW11 GDAP1 GNB5 GRINL1A HAUS2 HSPA4 *JAK2* KIAA0564 KIAA1549 MED17 MMRN2 MYO16 N4BP2 NDUFC2 NHEDC2 NOVA1 NPY1R PAM PDK3 PPM1H PPP1R12A PTS SMARCA2 *STK24* *STX3* UBE2E1 UBR1 ZNF33B

(Neurogenesis-related genes are in italic)

Voxelwise correlations between brain metabolism and average cluster transcript levels.

(All analyses are controlling for gene expression in other clusters.)



Conclusions

The atheortical approach taken here identified six clusters of CeA transcripts that differentially predict AT-related brain metabolism. The cluster that uniquely predicted amygdala metabolism was composed of 31% neurogenesis-related transcripts. These data complement our theory-based approach to understanding anxiety, where we hypothesize that a failure of neuroplastic processes underlies the development of anxiety and depression. Moreover, this method highlights the diversity of mechanisms by which gene expression in the amygdala can influence anxiety.