Anxious Temperament Related Gene Expression in the Primate Amygdala

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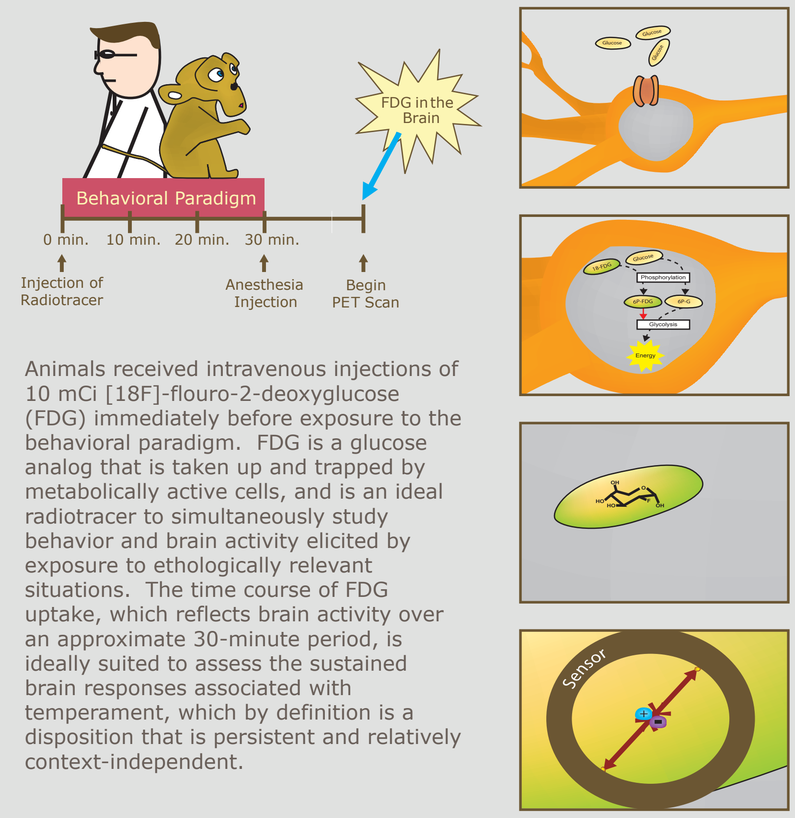
Abstract

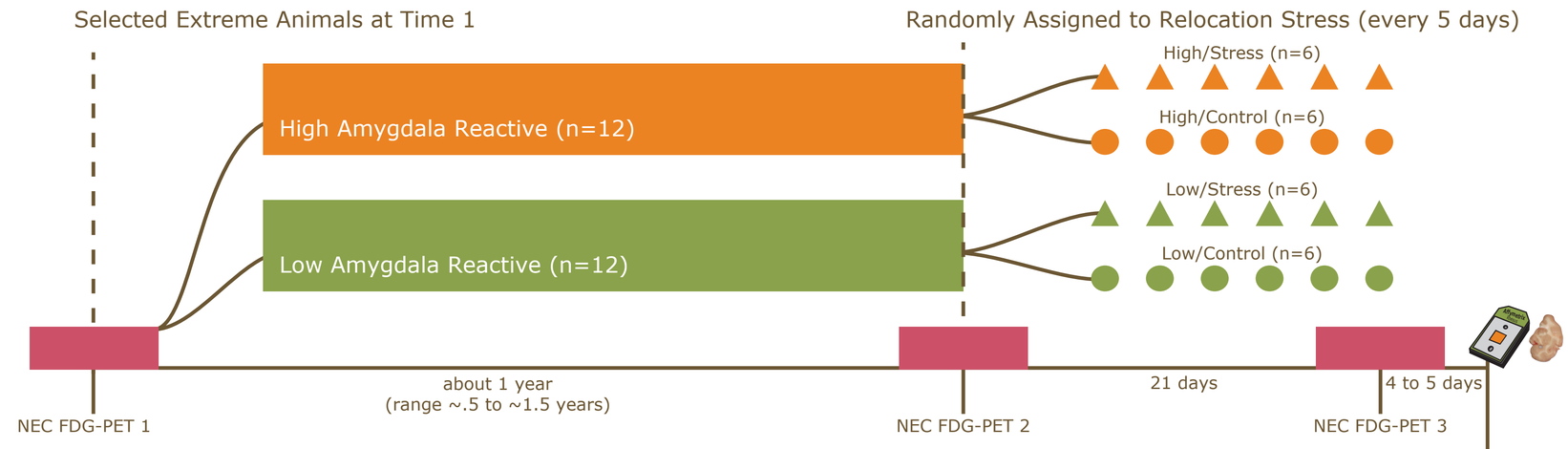
Anxious temperament (AT) is a trait-like behavioral phenotype in children that represents a risk factor for the development of later anxiety, depression and substance abuse disorders. Our group has validated a developmental rhesus monkey model of AT and, in a large (n=238) single family pedigree, demonstrated that AT is associated with increased amygdala metabolic activity measured using FDG-PET. By directly sampling tissue from the amygdala region identified to be predictive of AT in a subset of these animals (n=24), we sought to identify individual differences in mRNA levels that were predictive of individual differences in both amygdala metabolism and AT. Data from rhesus macaque microarray GeneChips (Affymetrix) revealed a number of candidate genes with expression patterns that significantly correlated with both AT and amygdala metabolism. Establishing the involvement of these candidate genes is a critical step in understanding the extent to which genetic polymorphisms and/or epigenetic modifications account for the differential expression of genes that influence amygdala metabolism and AT. This approach provides a unique opportunity to understand molecular interactions among genetic and epigenetic influences relevant to the brain circuit that underlies the childhood risk to develop stress-related psychopathology.

[18F]-flouro-2-deoxyglucose (FDG) NEC

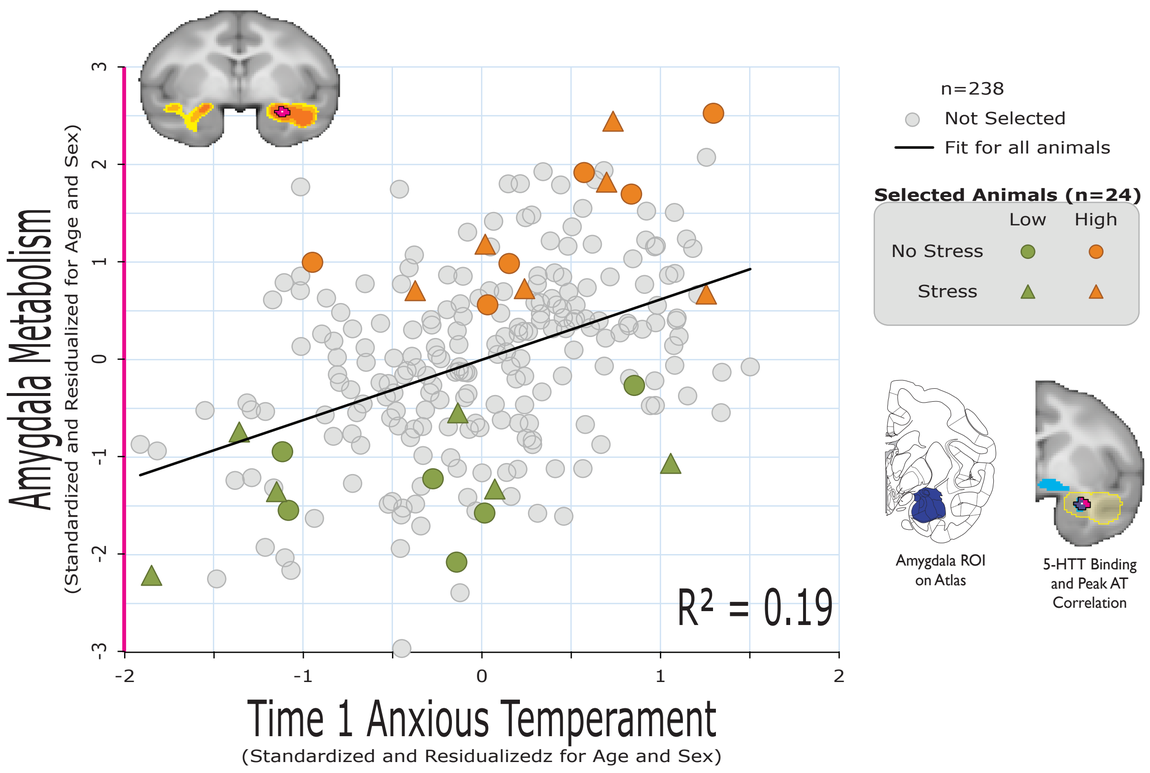


Animals received intravenous injections of 10 mCi [18F]-flouro-2-deoxyglucose (FDG) immediately before exposure to the behavioral paradigm. FDG is a glucose analog that is taken up and trapped by metabolically active cells, and is an ideal radiotracer to simultaneously study behavior and brain activity 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.

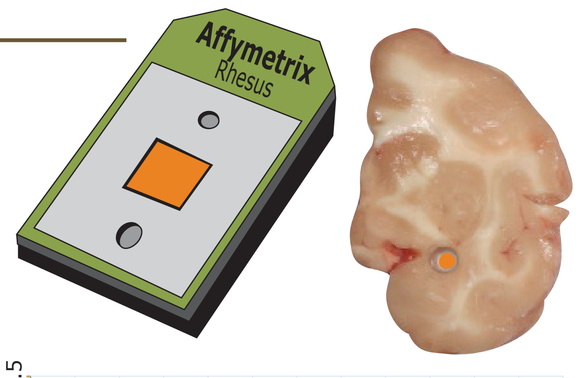
Study Design



Time 1: Correlation between AT and Amygdala Metabolism

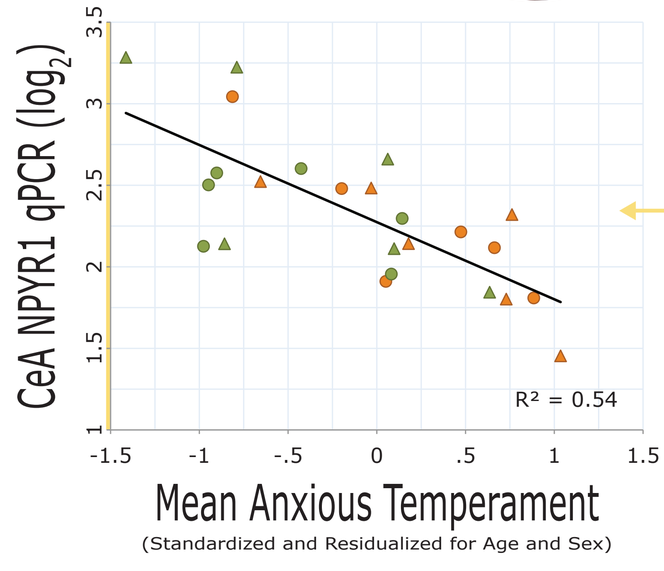


Time 3: MicroArray Experiment



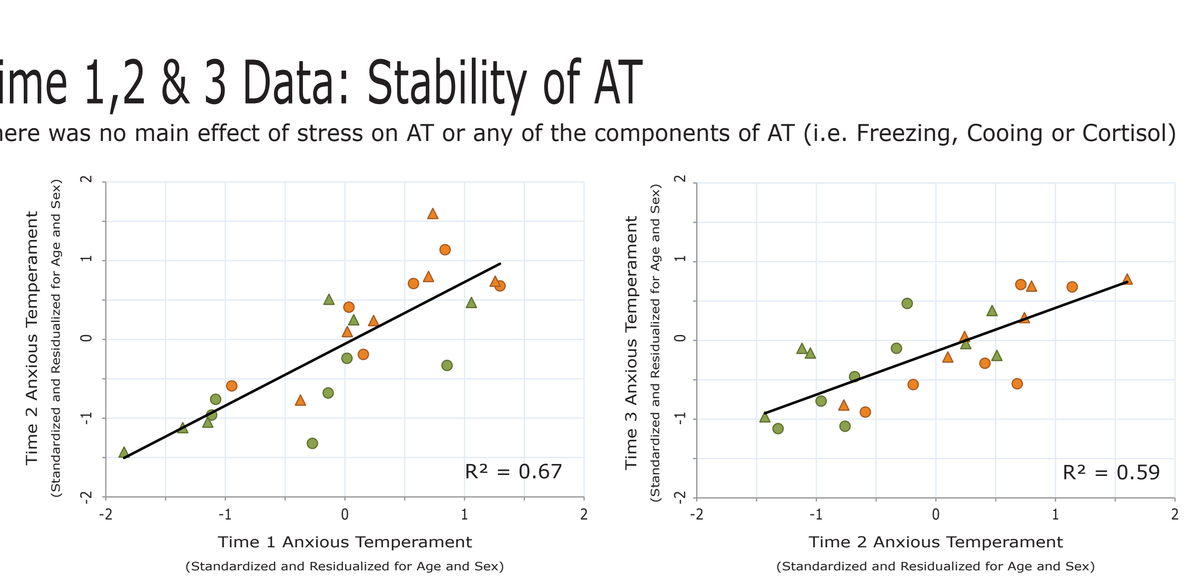
All analyses were performed using the open-source statistical package R, and the bioconductor libraries for Microarray analysis (http://www.bioconductor.org/). We used RMA background correction, normalized across chips with a constant, ignored mismatch probes, and summarized across probes with using 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/).

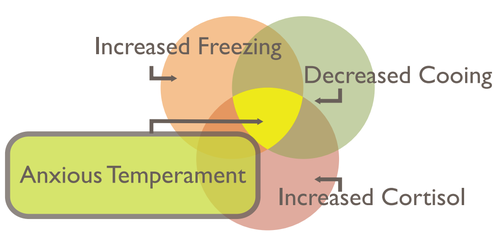
We examined genes in the "Neuroactive ligand-receptor interaction" pathway in the KEGG database (ko04080). Results demonstrated a number of significant correlations corrected for multiple comparisons within this pathway for correlations with both AT and Amygala FDG.



Time 1,2 & 3 Data: Stability of AT

There was no main effect of stress on AT or any of the components of AT (i.e. Freezing, Cooing or Cortisol)





"Neuroactive ligand-receptor interaction" (KO04080)

There are no significant effects of stress on gene expression within this pathway.

KO04080: Significant correlations with mean AT

Human Gene ID	Gene Symbol	Gene Description	logFC	AveExpr	t	P.Value	corrected P-Value
3358	HTR2C	5-hydroxytryptamine (serotonin) receptor 2C	-0.36	10.52	-5.96	5.19E-06	0.00022
4886	NPY1R	neuropeptide Y receptor Y1	-0.34	7.49	-4.40	2.26E-04	0.00649
4889	NPY5R	neuropeptide Y receptor Y5	-0.22	8.15	-3.94	6.87E-04	0.01477

KO04080: Significant correlations with mean amygdala metabolism

Human Gene ID	Gene Symbol	Gene Description	logFC	AveExpr	t	P.Value	corrected P-Value
5746	PTH2R	parathyroid hormone 2 receptor	0.19	8.07	4.26	0.00030985	0.02665
2917	GRM7	glutamate receptor, metabotropic 7	-0.13	9.42	-3.73	0.00113339	0.04115
9568	GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	-0.24	11.36	-3.61	0.00151119	0.04115
2558	GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	-0.20	10.22	-3.50	0.00199034	0.04115
2742	GLRA2	glycine receptor, alpha 2	-0.29	7.25	-3.42	0.0023924	0.04115

MicroArray Methods:

24 young Male monkeys (12 per group) were identified as expressing extreme high AT or low AT 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) 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). 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 annotated transcripts

Significant correlations with both mean AT and mean amygdala metabolism

Human Gene ID	Gene Symbol	Gene Description	logFC	Mean AT				Mean Amygdala			
				AveExpr	t	P.Value	corrected P-Value	logFC	t	P.Value	corrected P-Value
51422	PRKAG2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit	-0.22	8.26	-7.37	2.2E-07	0.00144	-0.15	-4.19	0.00037	0.04318
8660	IRS2	insulin receptor substrate 2	-0.15	10.96	-6.32	2.3E-06	0.00407	-0.10	-4.62	0.00013	0.02719
4616	GADD45B	growth arrest and DNA-damage-inducible, beta	-0.19	8.53	-5.50	1.6E-05	0.00813	-0.14	-5.76	8.1E-06	0.01047
23195	MDN1	MDN1, midasin homolog (yeast)	-0.17	6.67	-5.30	2.5E-05	0.01042	-0.11	-4.13	0.00042	0.04513
25758	C11orf41	chromosome 11 open reading frame 41	-0.13	7.83	-4.93	6.2E-05	0.01584	-0.12	-4.26	0.00031	0.04157
4616	GADD45B	growth arrest and DNA-damage-inducible, beta	-0.19	7.49	-4.65	0.00012	0.02217	-0.14	-5.76	8.1E-06	0.01047
51422	PRKAG2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit	-0.24	8.08	-4.49	0.00018	0.02935	-0.15	-4.19	0.00037	0.04318
317649	EIF4E3	eukaryotic translation initiation factor 4E family member 3	-0.17	6.94	-4.20	0.00037	0.04133	-0.10	-4.20	0.00036	0.04293
4878	NPPA	natriuretic peptide precursor A	-0.32	7.11	-4.14	0.00042	0.04432	-0.31	-4.38	0.00023	0.03924
83877	TM2D2	TM2 domain containing 2	-0.11	9.58	-4.13	0.00044	0.04488	-0.11	-5.41	1.9E-05	0.01353
9079	LDB2	LIM domain binding 2	-0.29	9.68	-4.09	0.00048	0.0455	-0.18	-4.36	0.00024	0.03924

Acknolwedgements

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