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11TH ANNUAL

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Master Class for Neuroscience Professional Development

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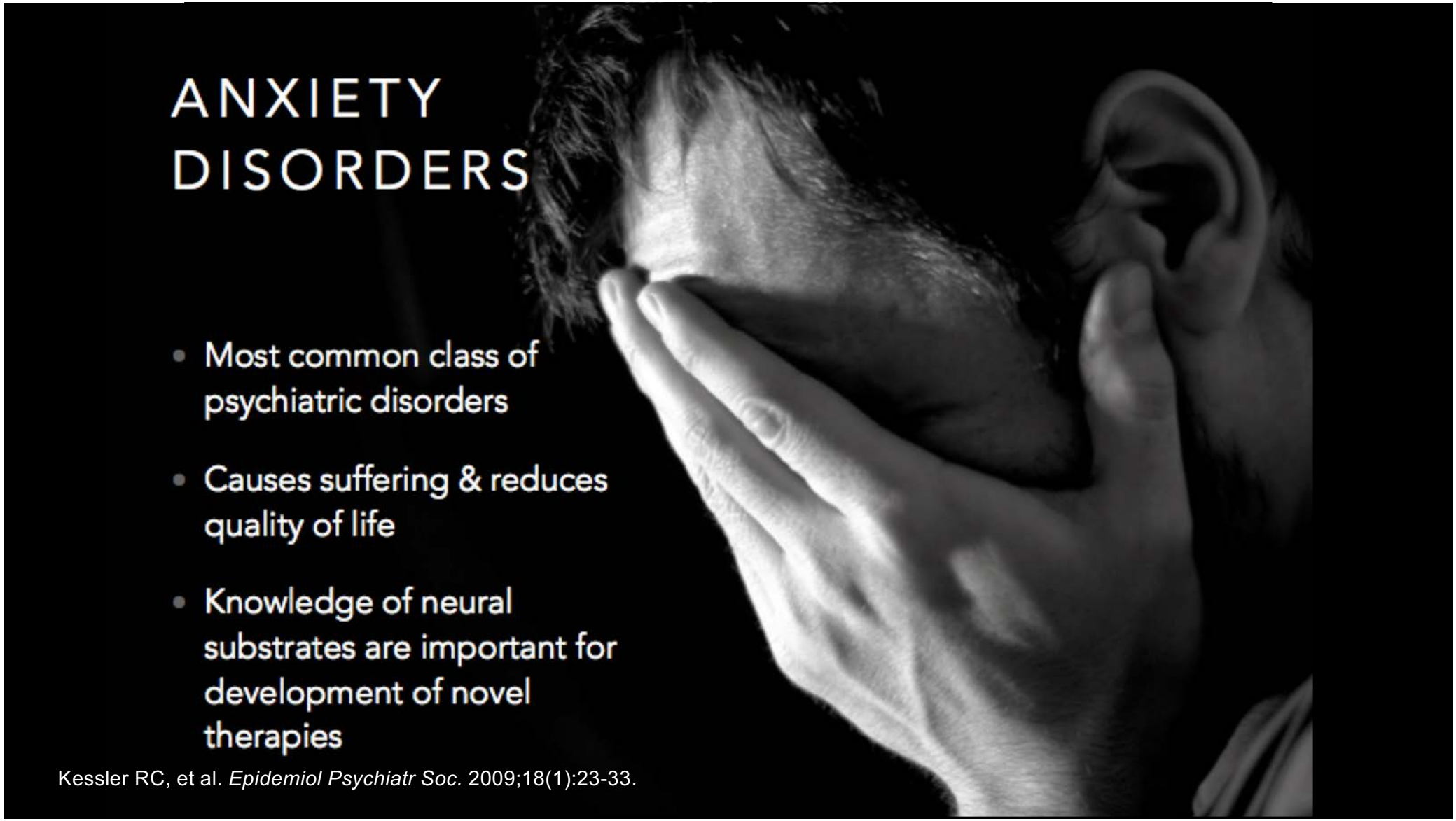
CME Outfitters

Early Life Predictors for Developing Anxiety and Depression

Ned H. Kalin, MD
Hedberg Professor and Chairman
Department of Psychiatry
University of Wisconsin School of Medicine and Public Health
Madison, WI

Learning Objective 1

Explore early risk factors for the development of anxiety and depression.



Kessler RC, et al. *Epidemiol Psychiatr Soc.* 2009;18(1):23-33.

Anxiety Disorders in Childhood

- Common, in children and adults
  - Affect up to 20% of youth
- Cause family, academic, and social dysfunction
- Prominent risk factor for psychopathology during adolescence and adulthood
- High levels of comorbidity with anxiety disorders and other disorders
- Understanding the neurobiology of anxiety early in life is needed to develop early interventions

Why Study Early-Life Risk?

Paus T, et al. *Nat Rev Neurosci*. 2008;9(12):947-957.

Heritability of Anxiety Disorders

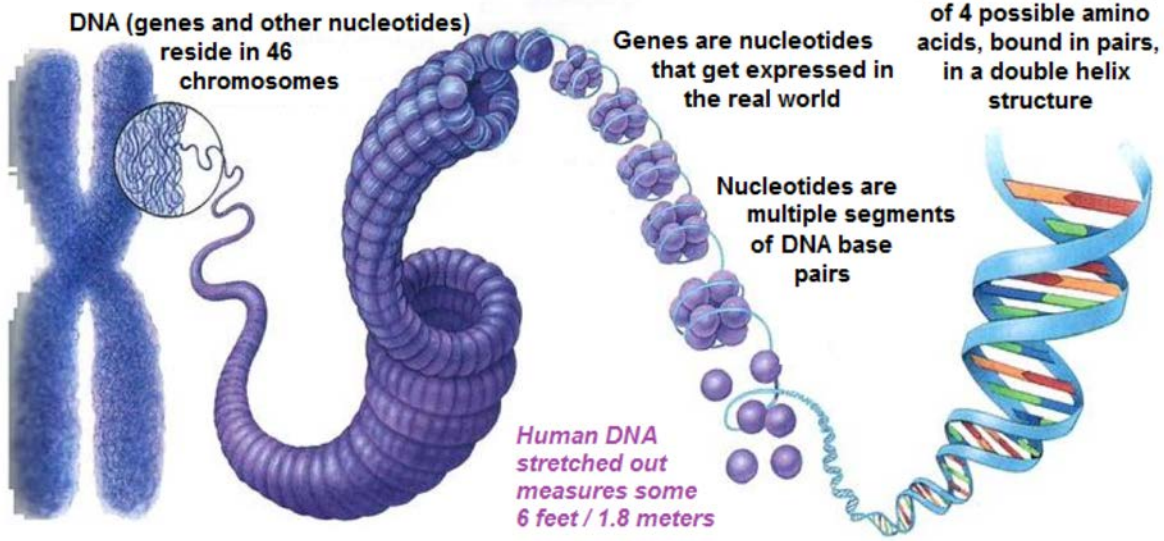
Summary and Meta-Analysis of Twin Studies for Anxiety Disorders

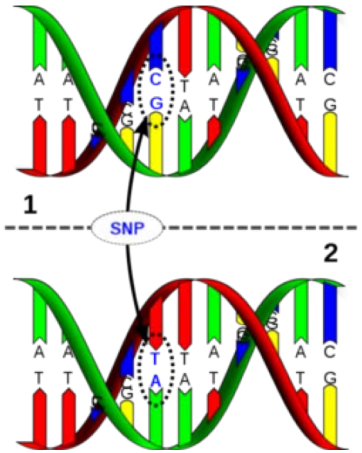
Disorder	Reference	Number of studies	N	Sex	a^2	c^2	e^2
PD	Hettema *et al.*,[9] 2001	3	9007	M, F	0.43 (0.32–0.53)	–	0.57 (0.47–0.68)
Agoraphobia	Kendler *et al.*,[10] 1992	1	2163	F	0.39	–	0.61
	Kendler *et al.*,[11] 2001	1	2396	M	0.37	–	0.63
GAD	Hettema *et al.*,[9] 2001	2	12 924	M	0.32 (0.24–0.39)	–	0.68 (0.61–0.76)
SAD	Scaini *et al.*,[12] 2014	5	20 433	M, F	0.27 (0.12–0.42)	0.04 (–0.01–0.09)	0.69 (0.59–0.79)
Animal phobia	Van Houtem *et al.*,[13] 2013	5	17 904	M, F	0.32 (0.22–0.44)	–	–
Situational phobia	Van Houtem *et al.*,[13] 2013	4	16 474	M, F	0.25 (0–33)	–	–
Blood-injury-injection phobia	Van Houtem *et al.*,[13] 2013	3	10 741	M, F	0.33 (0.28–0.63)	–	–

Confidence intervals are shown in parentheses.
Additive genetic effects (a^2) represent the genetic component of variance due to the average effects of single alleles and are known as heritability; shared environment effects (c^2) are explained by events that happen to both twins, affecting them in the same way; non-shared environment effects (e^2) are explained by events that occur to one twin but not the other, or events that affect either twin in a different way.
GAD, generalized anxiety disorder; PD, panic disorder; SAD, social anxiety disorder.

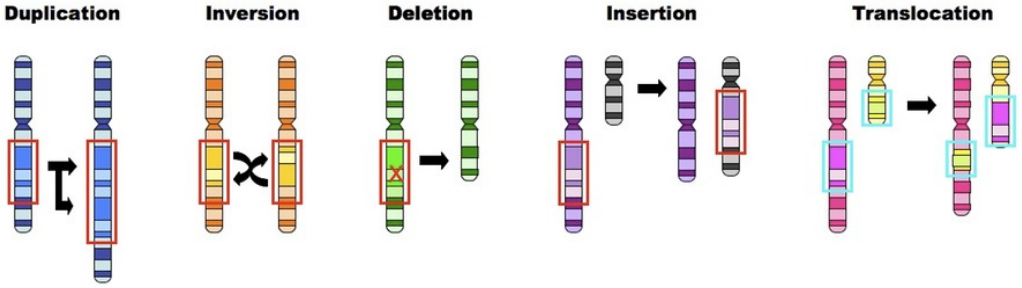
Shimada-Sugimoto M, et al. *Psychiatry Clin Neurosci.* 2015;69(7):388-401.

The Hierarchical Structure of DNA through to the Chromosome



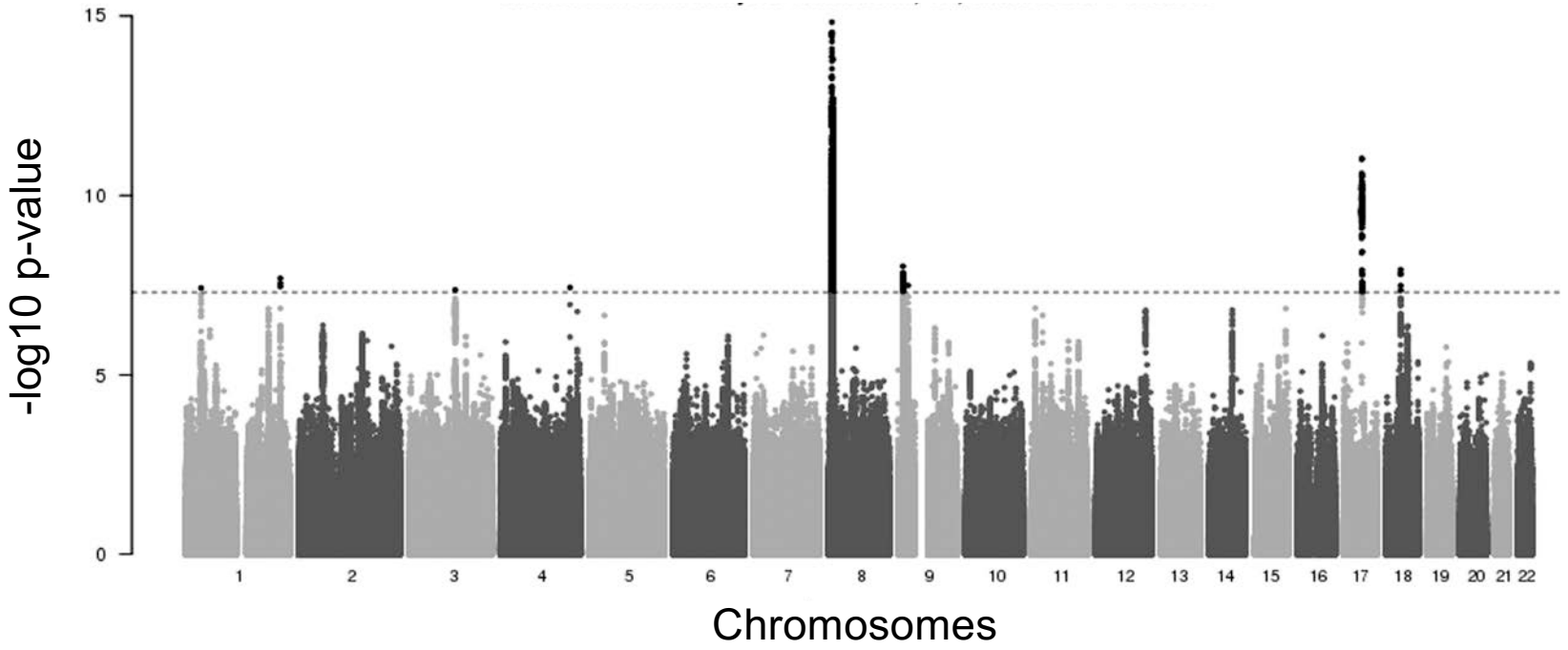


Mutations



Genome-Wide Analysis of Over 106000 Individuals Identifies 9 Neuroticism-Associated Loci

Neuroticism Meta-Analysis, SNPs n = 7,207,648



Smith DJ, et al. *Molecular Psychiatry* 2016;21:749-757.

Even Identical Twins Can Differ

Epigenetics of Stress-Related Disorders: Gene X Environment Interactions

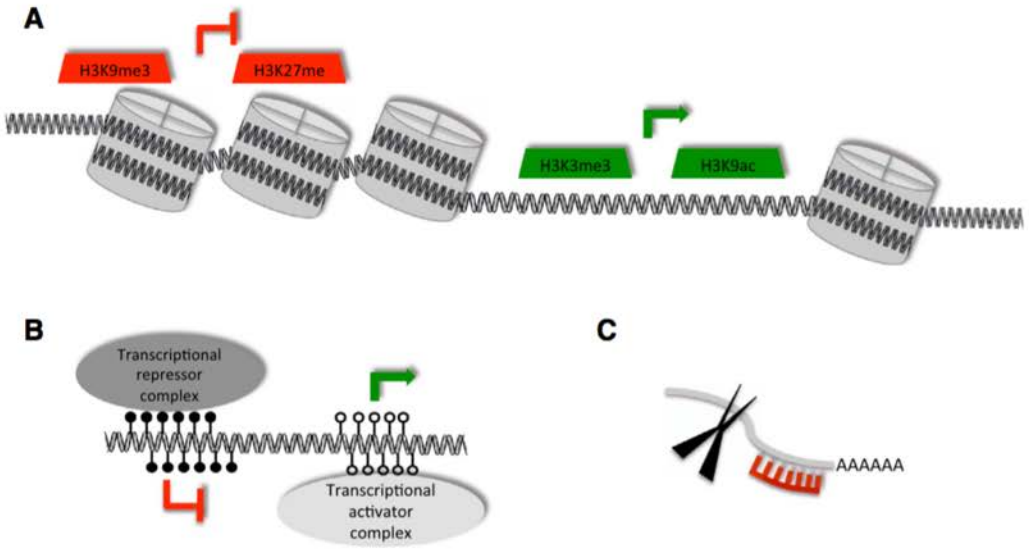


Figure 2. Schematic Representation of Main Features of Epigenetic Regulation by Post-translational Histone Modification, DNA Methylation, and Non-coding RNA

This overview explicitly reduces and simplifies the complex and multifaceted mechanisms of epigenetic regulation for clarity. More specialized reviews for a deeper description of this matter are given in the text.

(A) Histone modifications influence the condensation of the DNA around histone proteins and regulate the accessibility of functional regions to transcriptional regulators, through modification at predominantly the N-terminal tails, altering the spatial structure of the chromatin and the interaction with DNA-binding proteins. Contingent on the location and the type of modification, this can lead to a more condensed chromatin-repressing active transcription (exemplified by histone H3, lysine 27 dimethylation (H3K27me2) and histone H3, lysine 9 trimethylation (H3K9me3)) or vice versa to an open chromatin state facilitating active transcription (exemplified by histone H3, lysine 4 trimethylation (H3K4me3) and histone H3, lysine 9 acetylation (H3K9ac)).

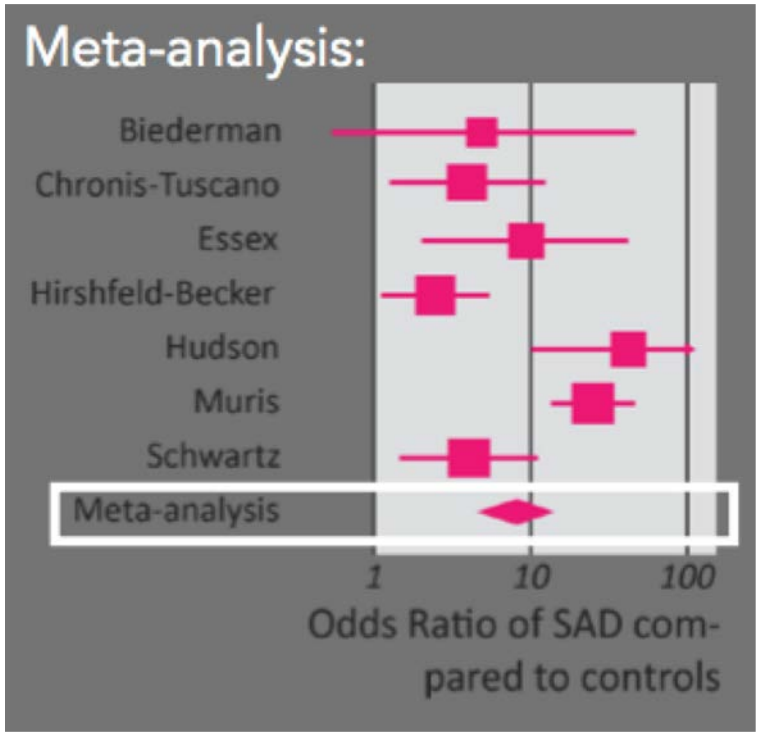
(B) DNA methylation predominantly at CG dinucleotides (CpG) can influence the spatial structure of the DNA and the binding of or repression of specific DNA-binding proteins to the DNA. The closed circles represent higher methylation at cytosine residues, and the open circles represent lower methylation. Methylation around the transcription start site in the promoter and the first exon is usually accompanied by transcriptional silencing. DNA methylation at other regulatory regions and in the gene body can also facilitate transcription. Not depicted here are other modifications such as hydroxymethylation.

(C) Non-coding RNAs that include, for example, miRNA can influence chromatin structure and protein binding to the DNA but also directly target transcription and translation. Depicted here is the regulation of mRNA stability through binding of miRNAs at the 3′UTR of target mRNA that can lead to a decrease in mRNA stability, a decrease in mRNA cleavage, and therefore a reduction in protein assembly.

Klengel T, Binder EB. *Neuron*. 2015;86(6):1343-1357.

Inhibited or Anxious Temperament (AT): Risk Phenotype That Precedes Anxiety and Depressive Disorders

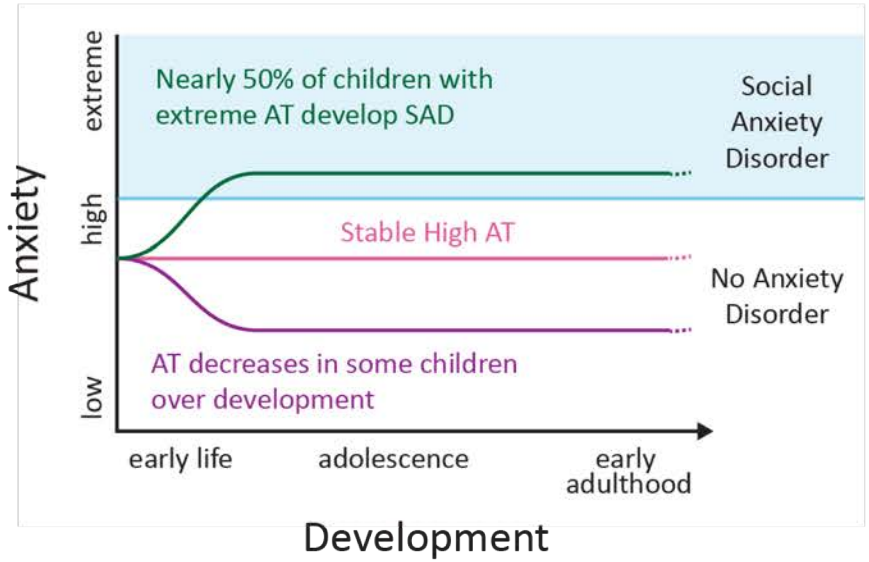
- Extreme behavioral inhibition to novel situations or strangers
- Predicts development of anxiety disorders, depression, and co-morbid drug abuse
- 3-4 fold risk to develop social anxiety disorder
- Can be identified early in life
- Inhibited monkeys and humans share behavioral and physiological features



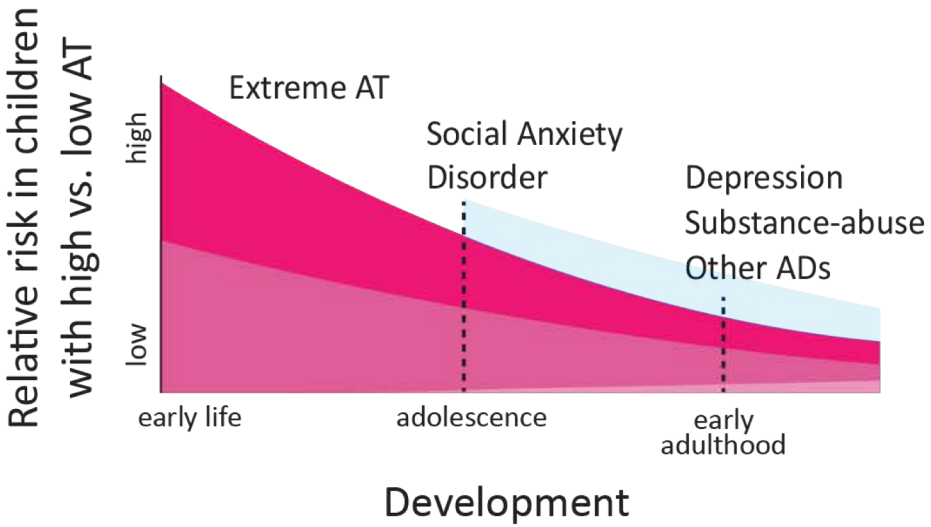
Clauss JA, Blackford JU. *J Am Acad Child Adolesc Psychiatry*. 2012;51(10):1066-1075.

Proposed Anxiety Trajectories for Children with High Anxious Temperament

a) Pathological Anxiety: Trajectories for Children with High AT

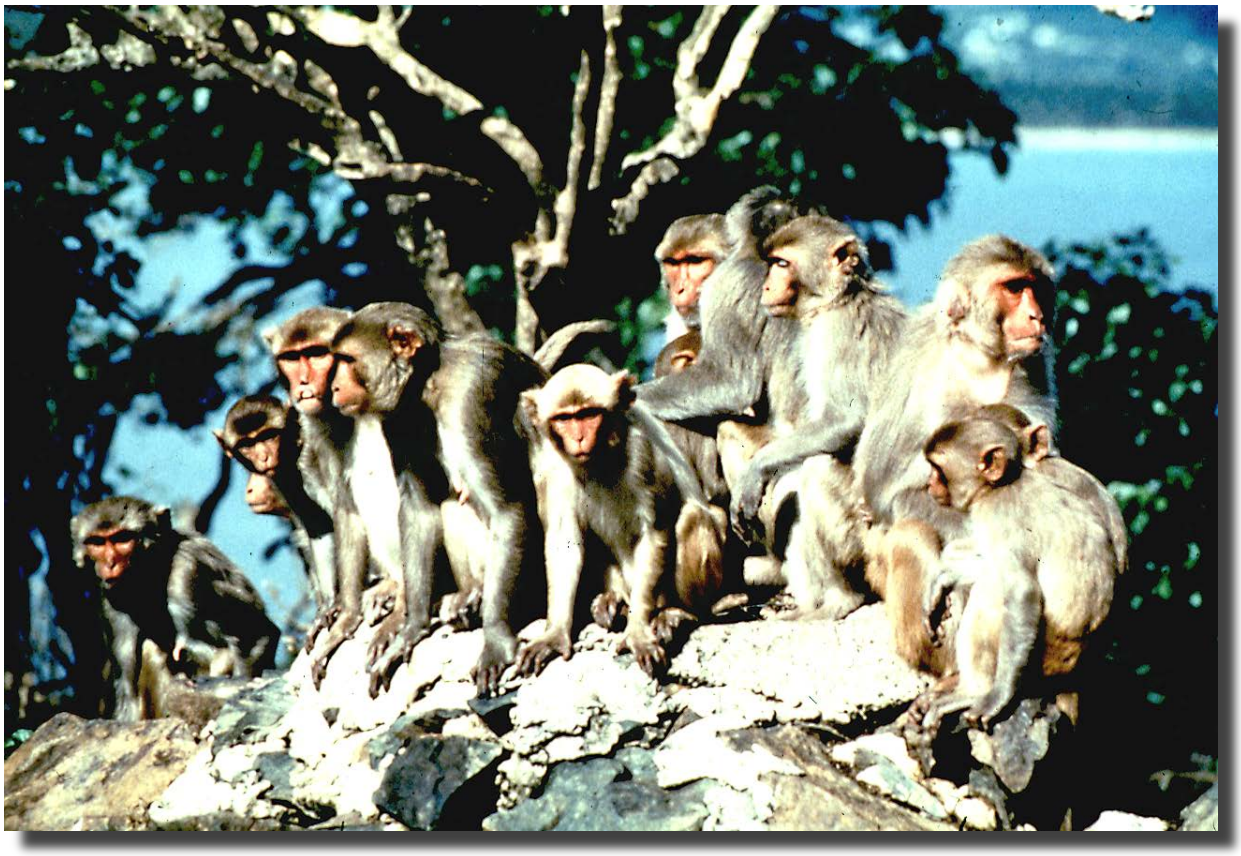


b) Extreme AT children are at risk for psychopathology across the lifespan



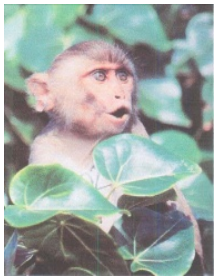
Fox AS, Kalin NH. *Am J Psychiatry*. 2014;171(11):1162-1173.

Nonhuman Primates Provide a Unique Opportunity for Studying Human Psychopathology



Anxious Temperament: A Composite Including Freezing and HPA Activity

+



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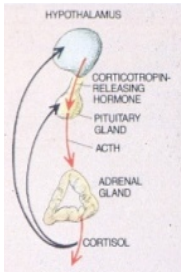
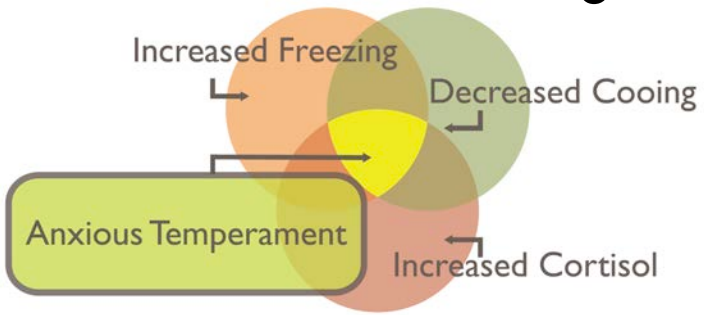
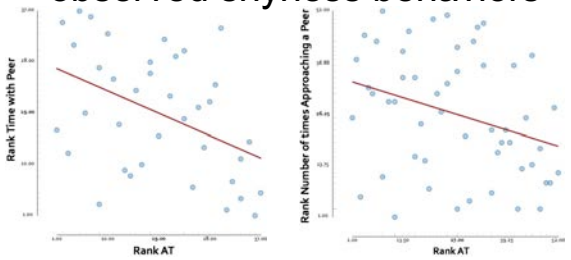


freezing ↑ cooing ↓ cortisol ↑

$$\frac{\text{freezing} \uparrow \quad \text{cooing} \downarrow \quad \text{cortisol} \uparrow}{3} = \text{AT}$$



AT predicts naturally observed shyness behaviors

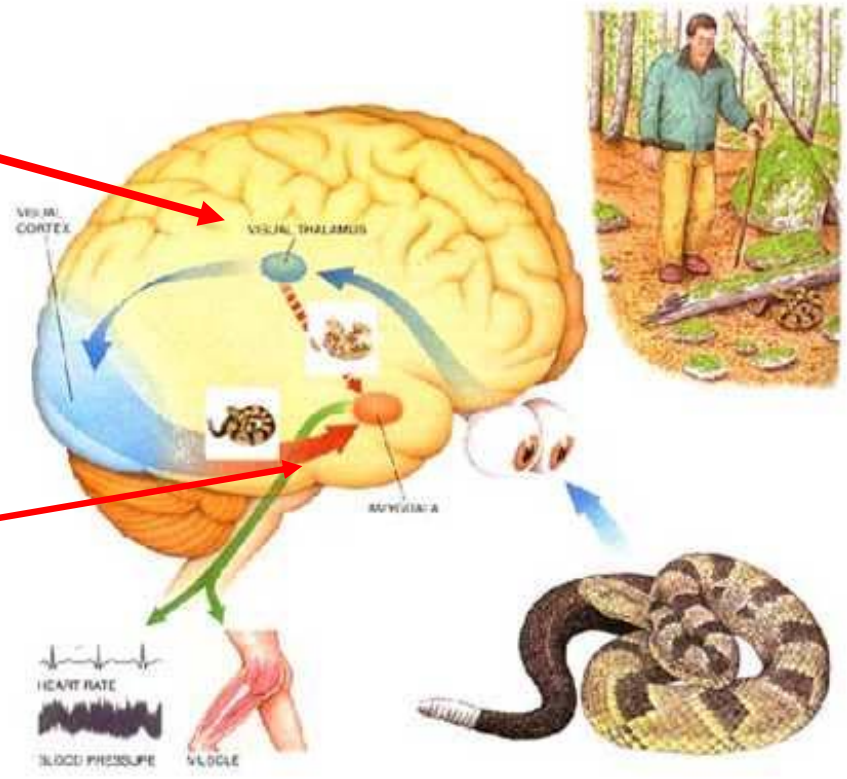


Fox AS, et al. *PLoS One*. 2008:3(7):e2570.

The Amygdala is Critical for Fear and Anxiety

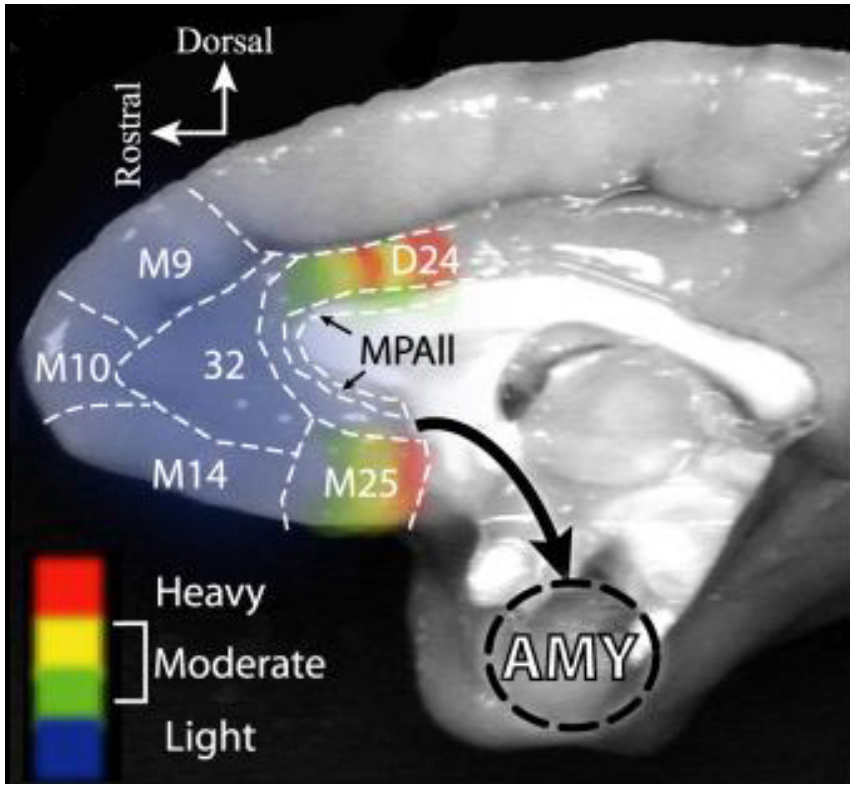
Cortical pathway
"high road"

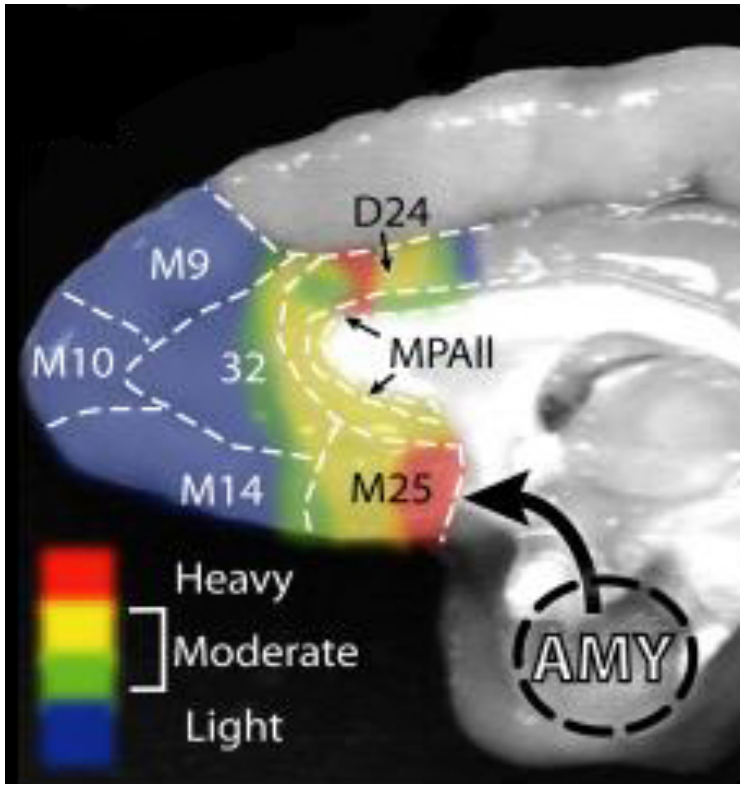
Subcortical pathway
"low road"



Ledoux AA, et al. *Front Behav Neurosci*. 2014;8:88.

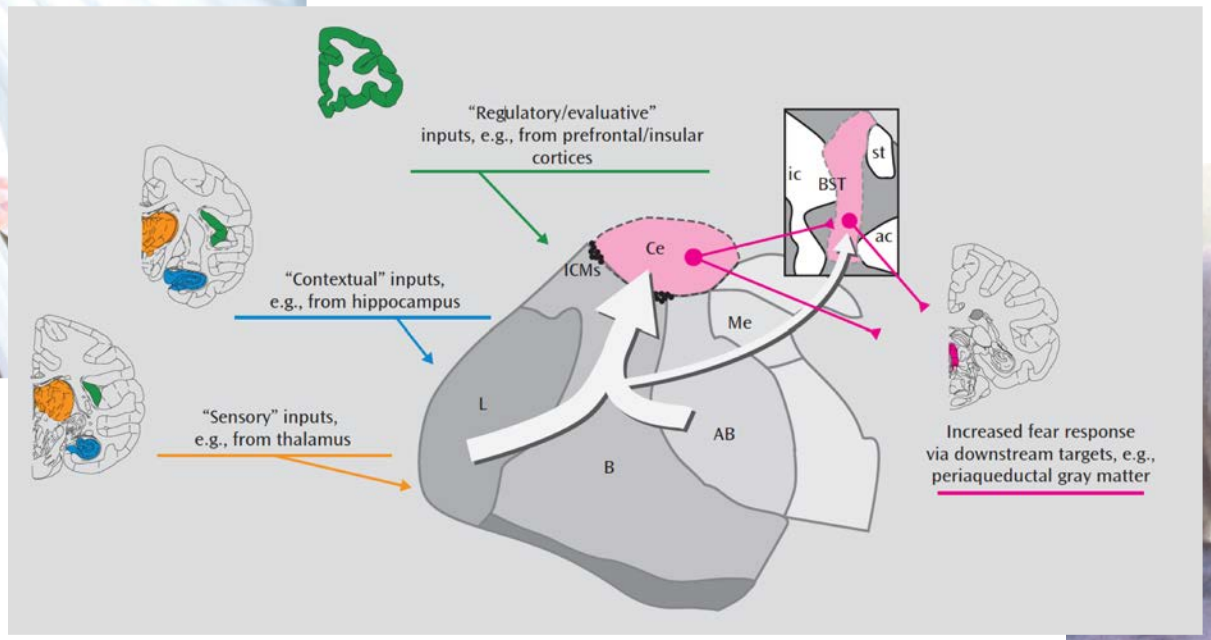
Amygdala – Frontal Connectivity: A Pathway for Emotion Regulation





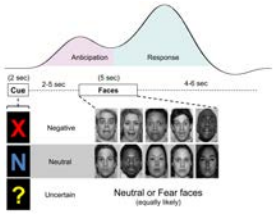
Adapted from Ghashghaei HT, et al. *Neuroimage.* 2007;34(3):905-923.

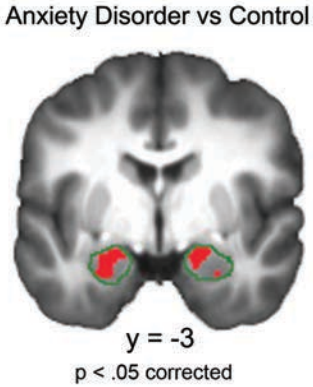
Validation of the Nonhuman Primate Model: Altered Prefrontal-Amygdala Function in Pre-adolescent Children with Anxiety Disorders and Young Rhesus Monkeys

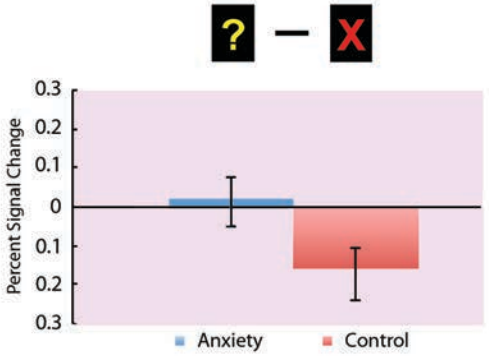


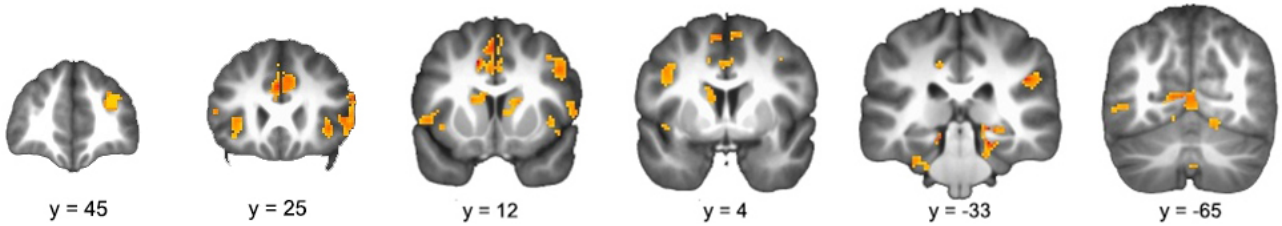
Fox AS, Kalin NH. *Am J Psychiatry.* 2014;171(11):1162-1173.

Elevated Dorsal Amygdala/Ce Activation During Uncertain Anticipation in Preadolescent Children with Anxiety Disorders







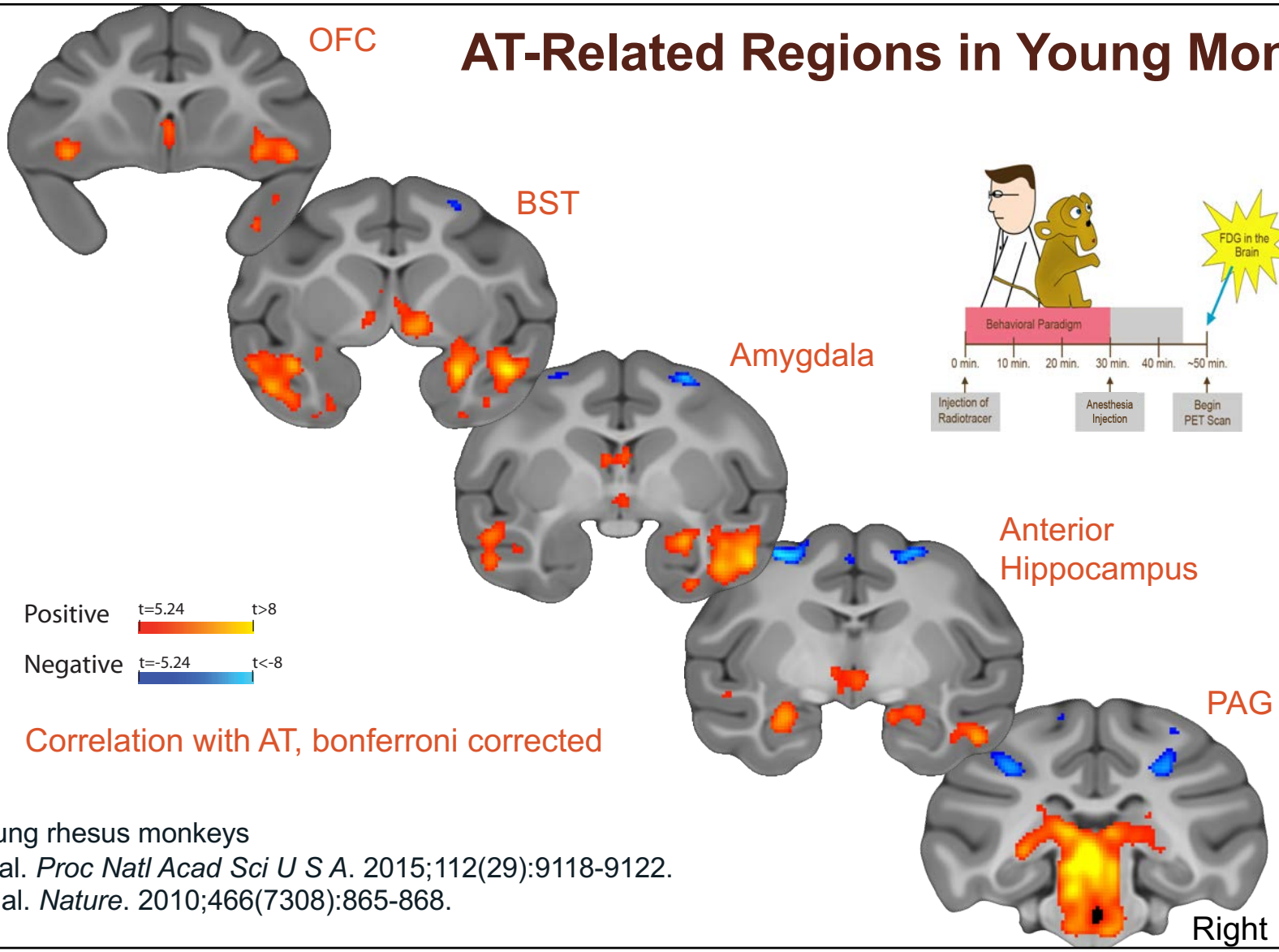


P < .005, uncorrected

n = 33 anxiety disorder, 28 control

Builds on sample from Williams LM, et al. *Neuropsychopharmacology*. 2015;40(10):2398-2408.

AT-Related Regions in Young Monkeys

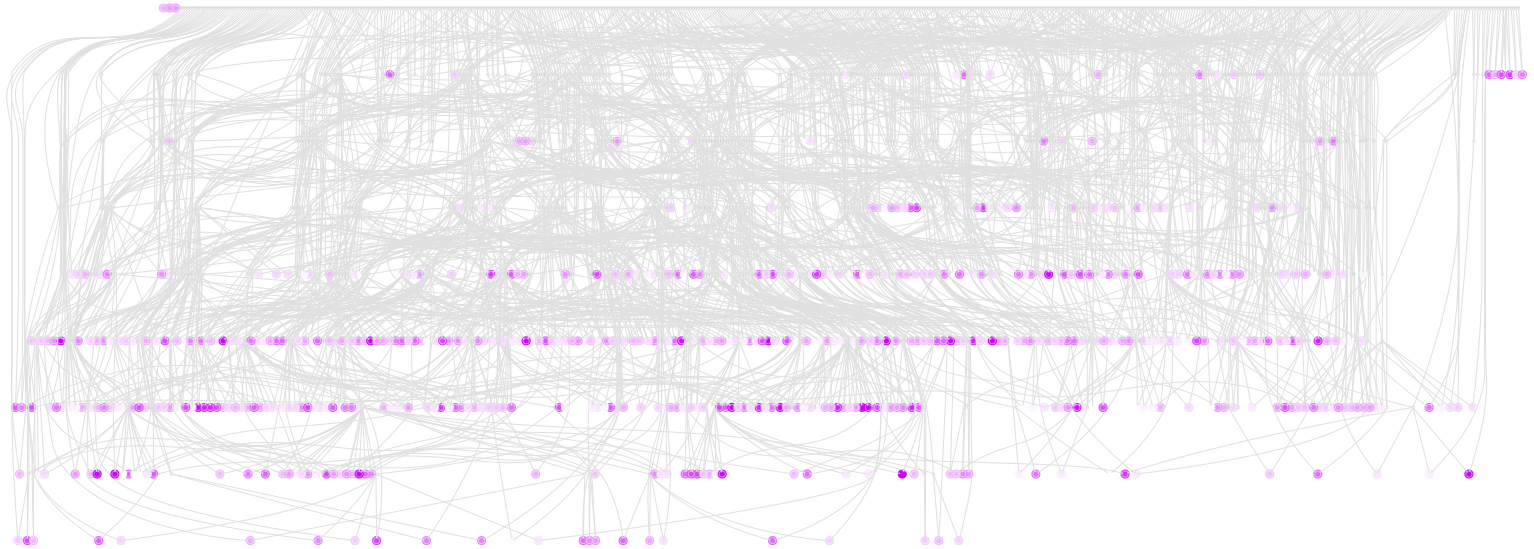


n = 592 young rhesus monkeys

Fox AS, et al. *Proc Natl Acad Sci U S A*. 2015;112(29):9118-9122.
Oler JA, et al. *Nature*. 2010;466(7308):865-868.

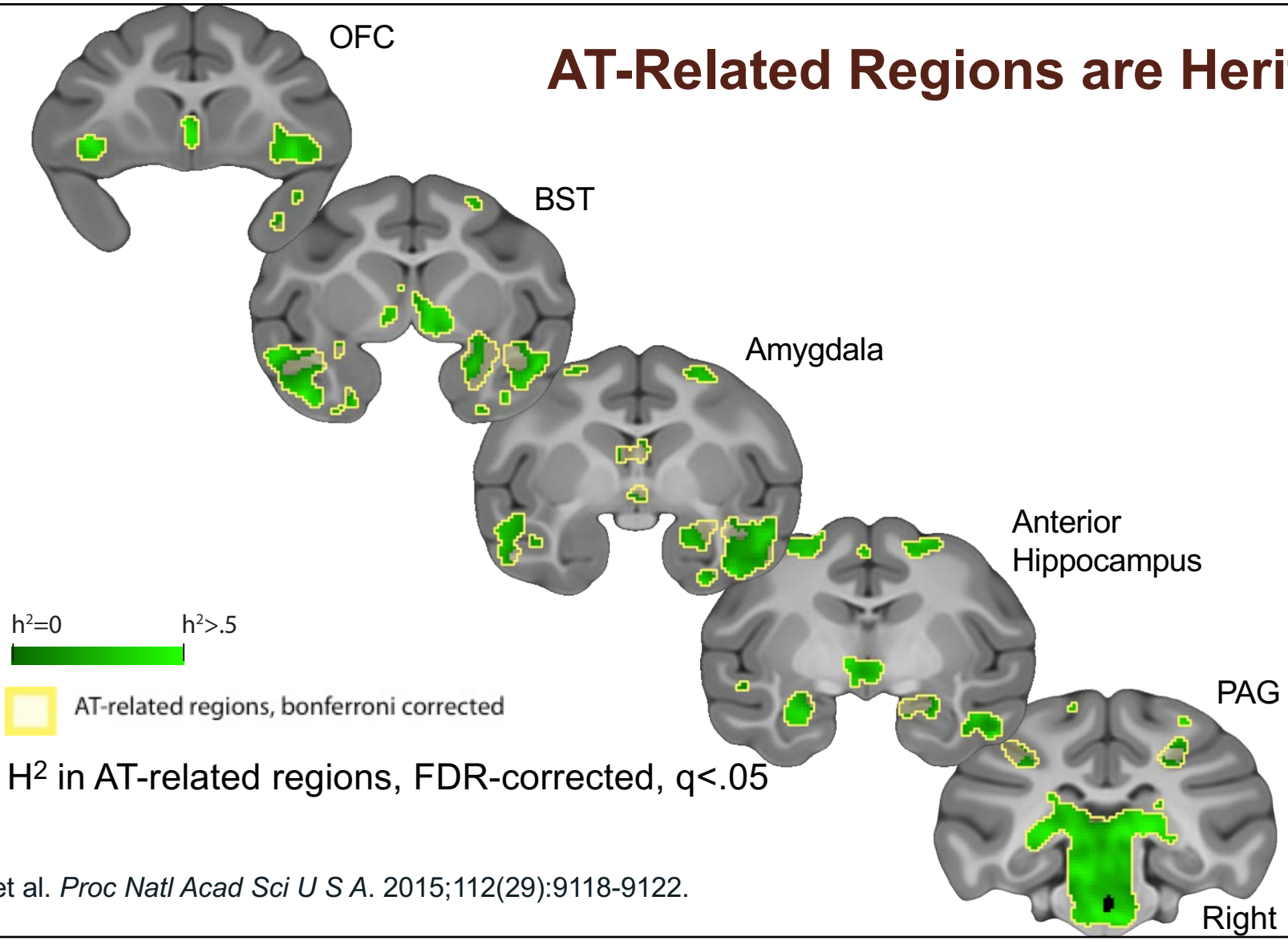
Heritability of AT

$$h^2 = .36$$



Fox AS, et al. *Proc Natl Acad Sci U S A*. 2015;112(29):9118-9122.

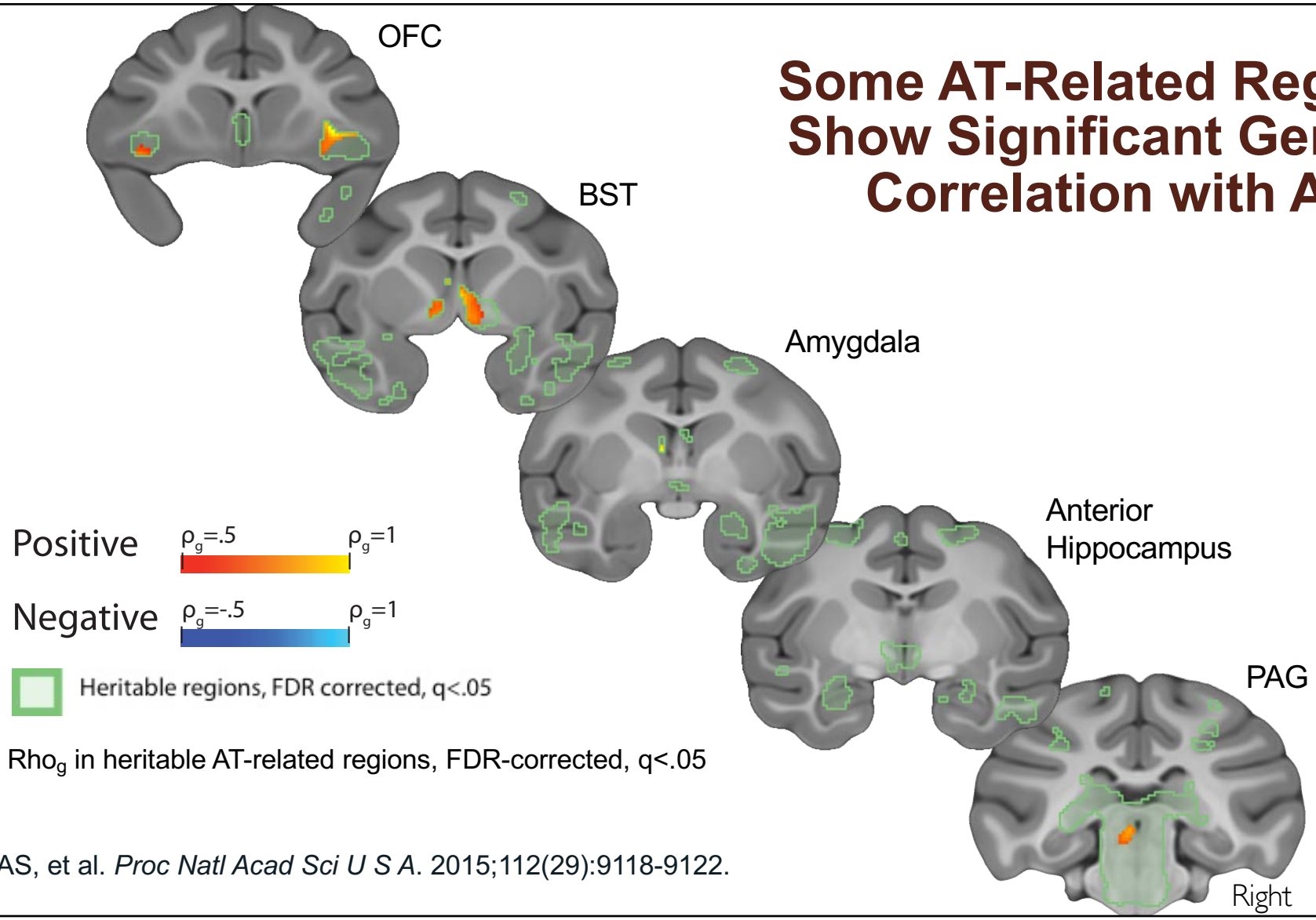
AT-Related Regions are Heritable



H^2 in AT-related regions, FDR-corrected, q<.05

Fox AS, et al. *Proc Natl Acad Sci U S A*. 2015;112(29):9118-9122.

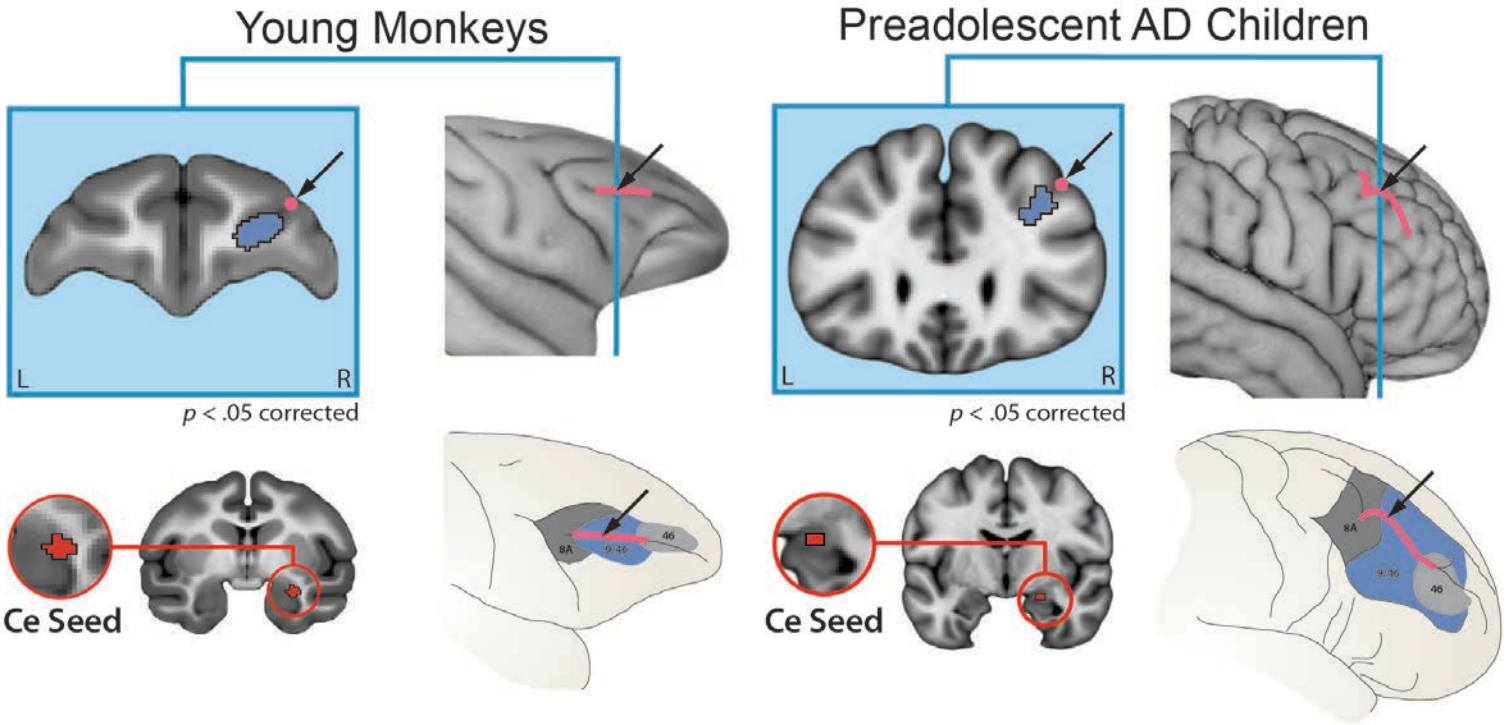
Some AT-Related Regions Show Significant Genetic Correlation with AT



Rho_g in heritable AT-related regions, FDR-corrected, q<.05

Fox AS, et al. *Proc Natl Acad Sci U S A*. 2015;112(29):9118-9122.

Evolutionarily-Conserved Decrease in dlPFC-Ce Functional Connectivity in Young Anxious Monkeys and Children



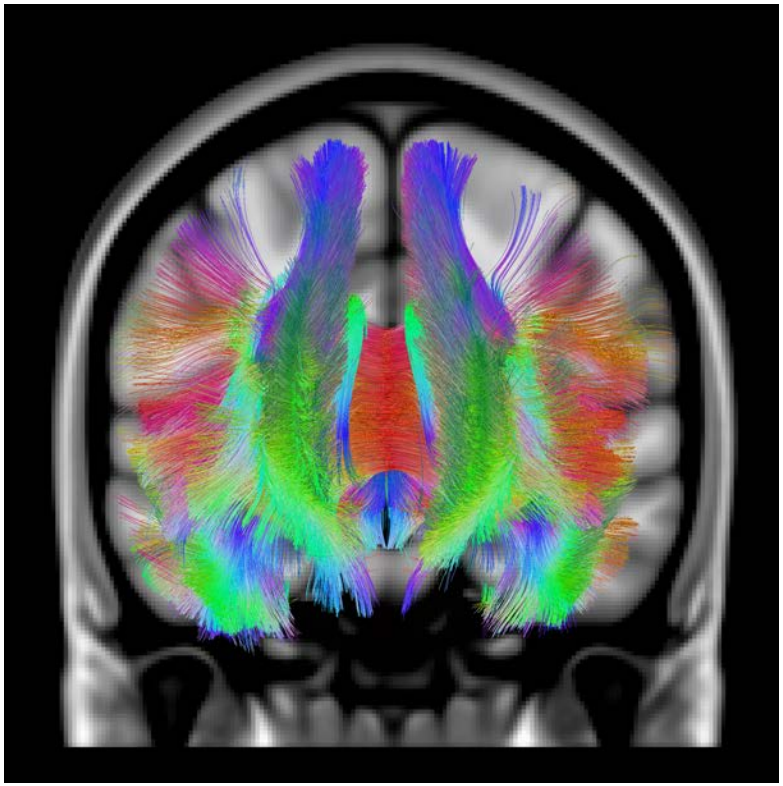
n = 89

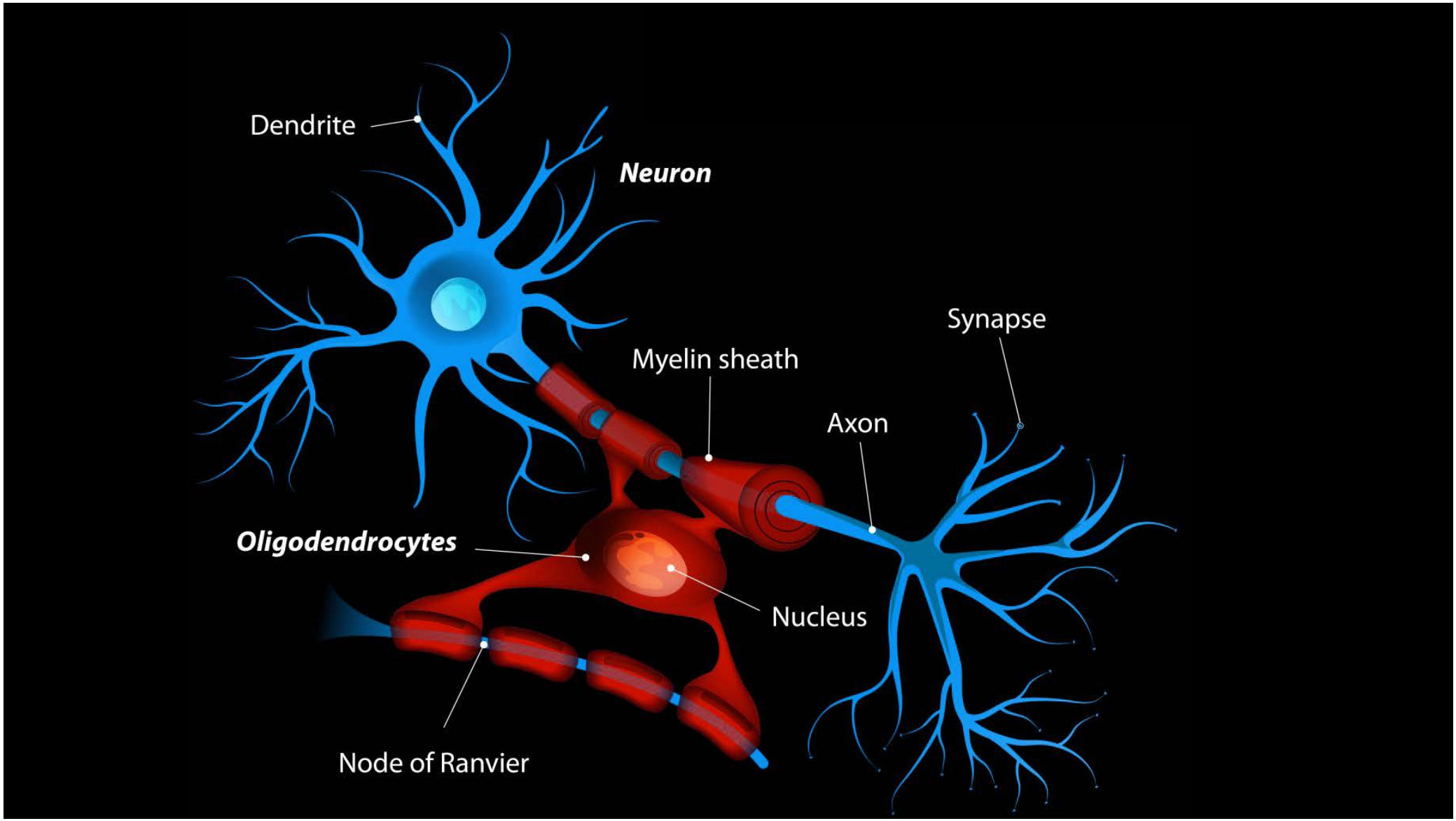
n = 14 anxiety disorder, 14 control

Birn RM, et al. *Mol Psychiatry*. 2014;19(8):853.

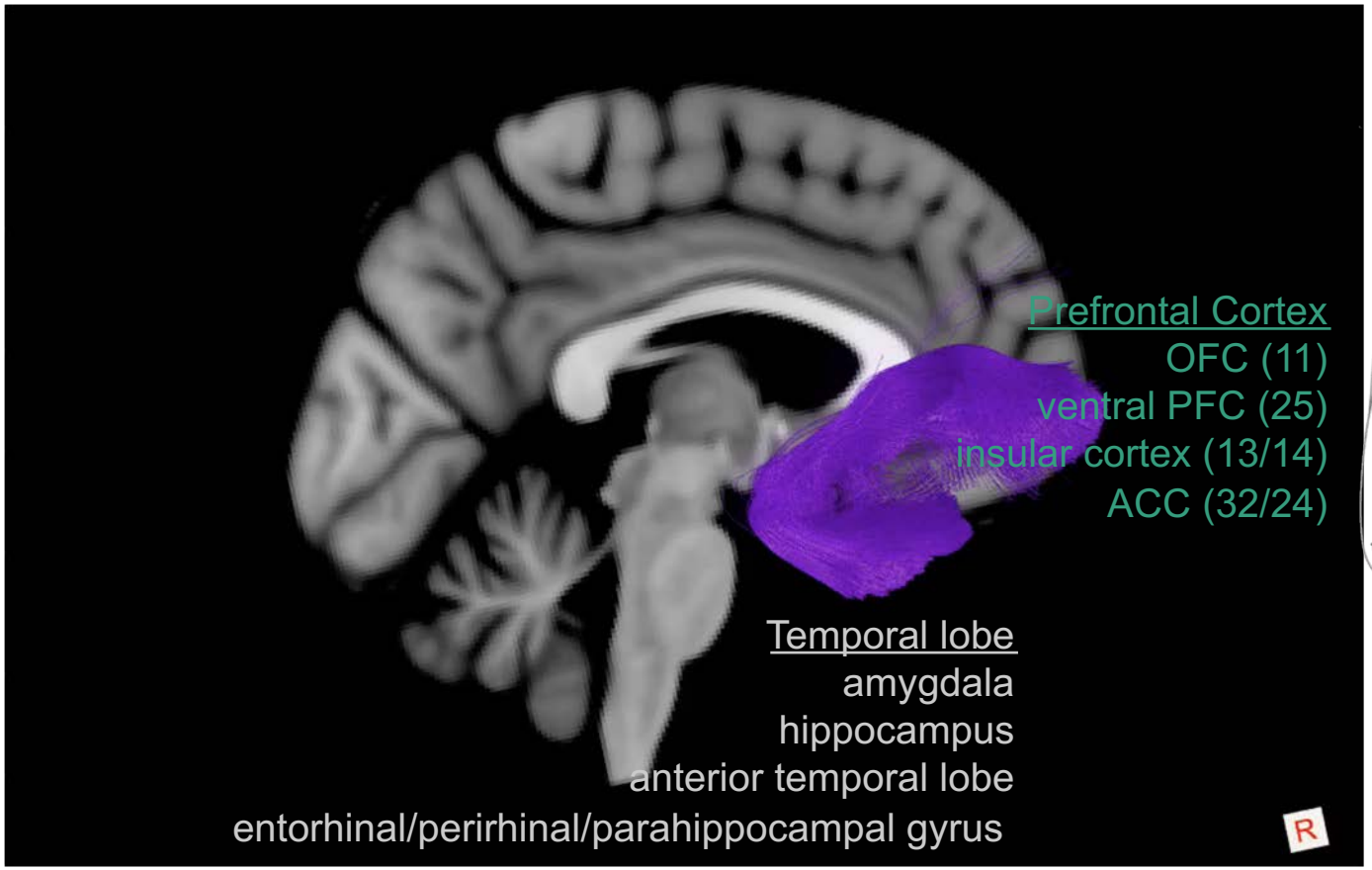
White Matter Tracts in Relation to Anxiety Disorders

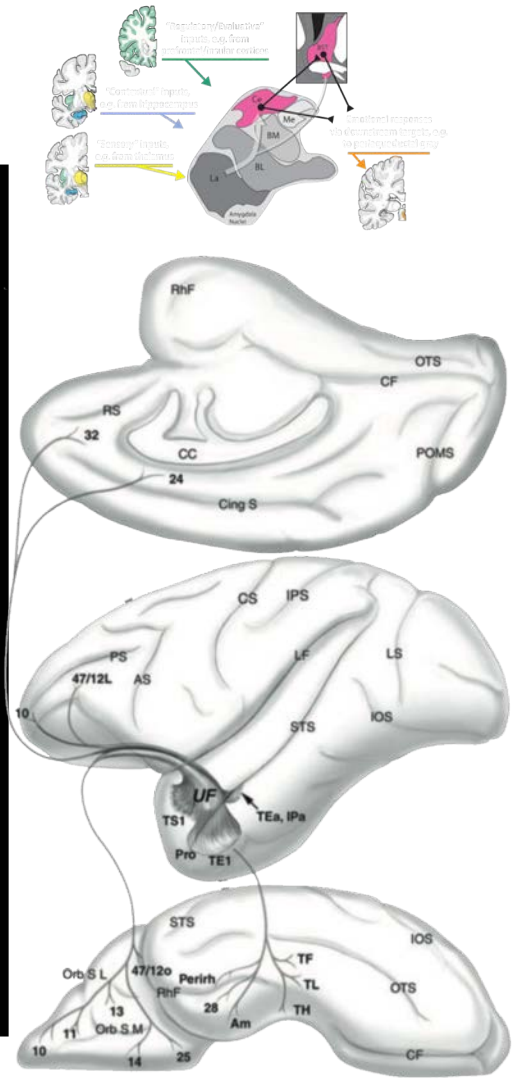
- Consists of axons and myelin
- Timing and integration of information
- 50% of the brain, yet often ignored




Dendrite
Neuron
Synapse
Myelin sheath
Axon
Oligodendrocytes
Nucleus
Node of Ranvier

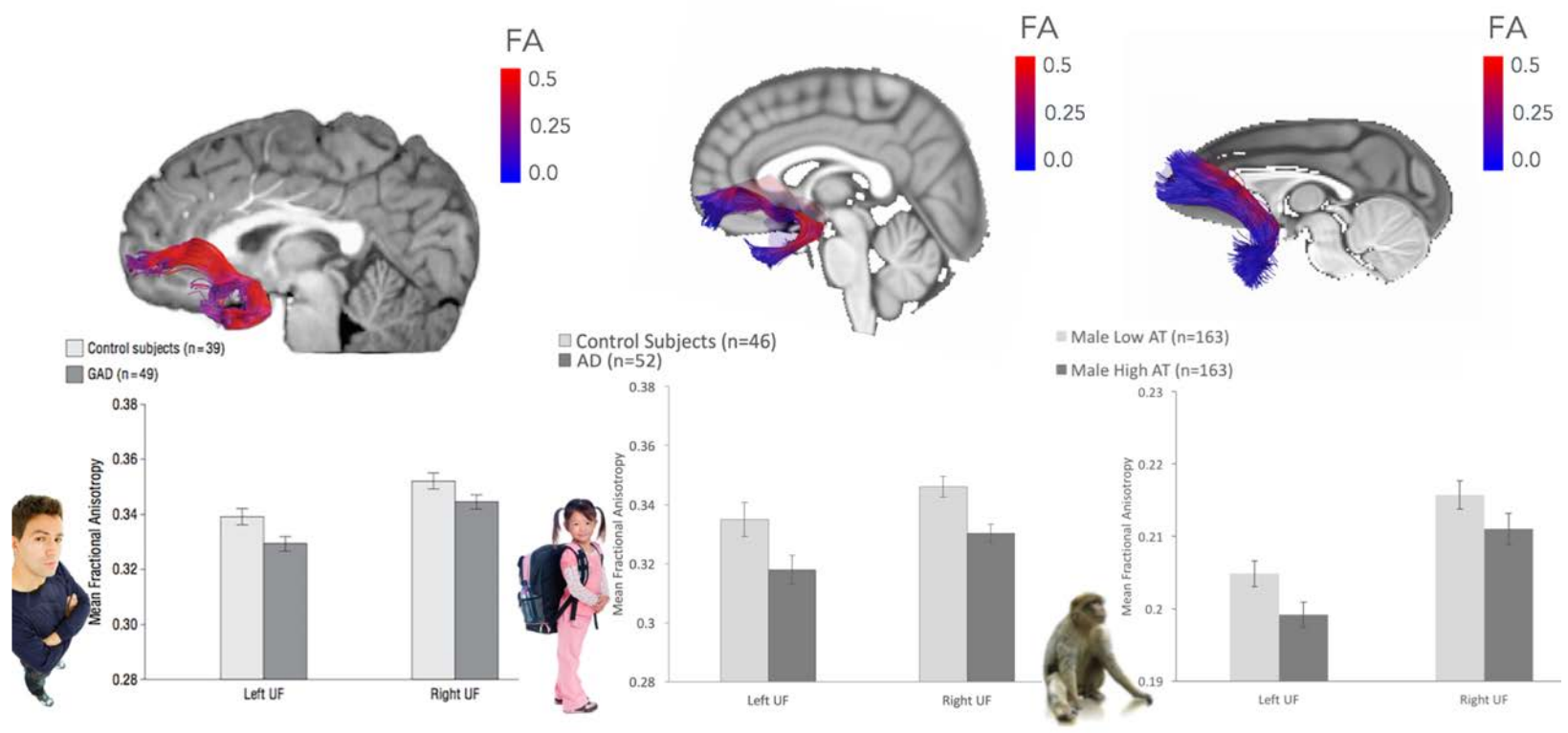
Altered Prefrontal Cortical and Amygdala Interactions Via Uncinate Fasciculus May Underlie Anxiety





Schmahmann JD, Pandya DN. *J Hist Neurosci* 2007;16(4):362-377.

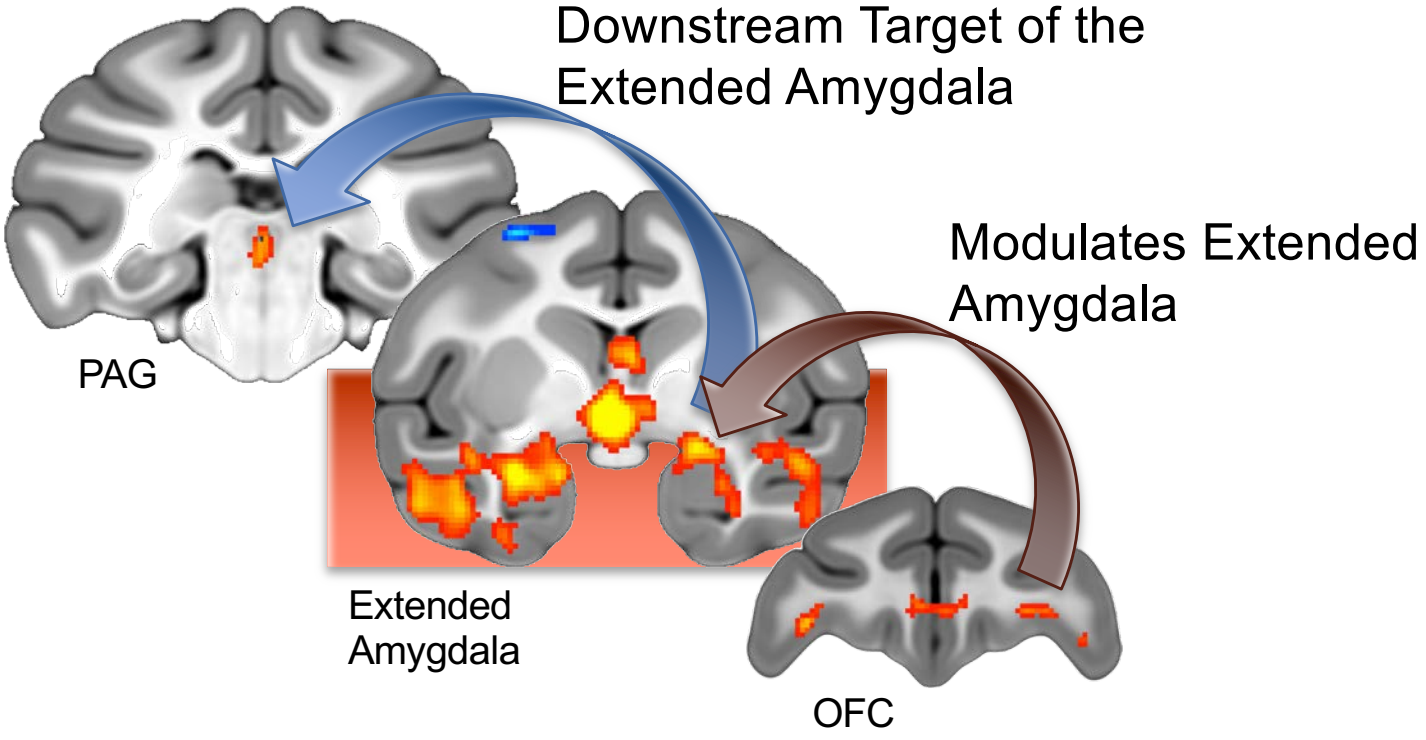
Decreased White Matter Integrity (FA) in the UF Associated with Higher Anxiety Across Age and Species



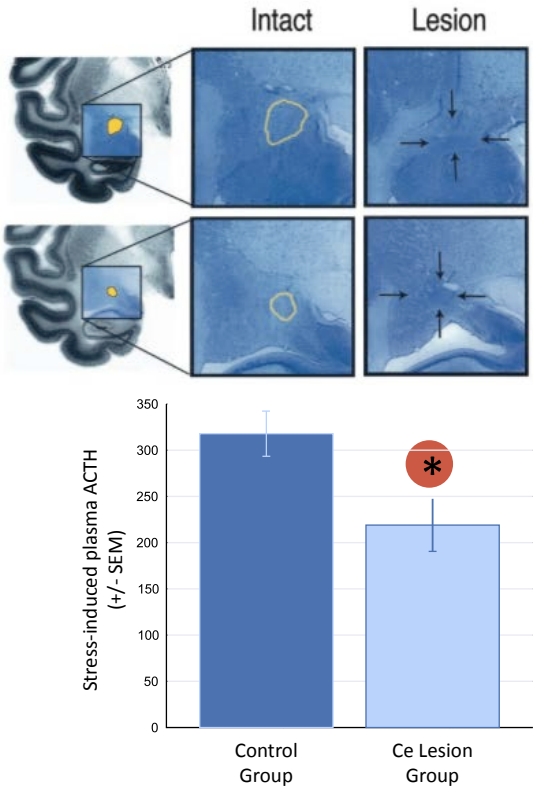
Tromp DP, et al. *Arch Gen Psychiatry*. 2012;69(9):925-934.

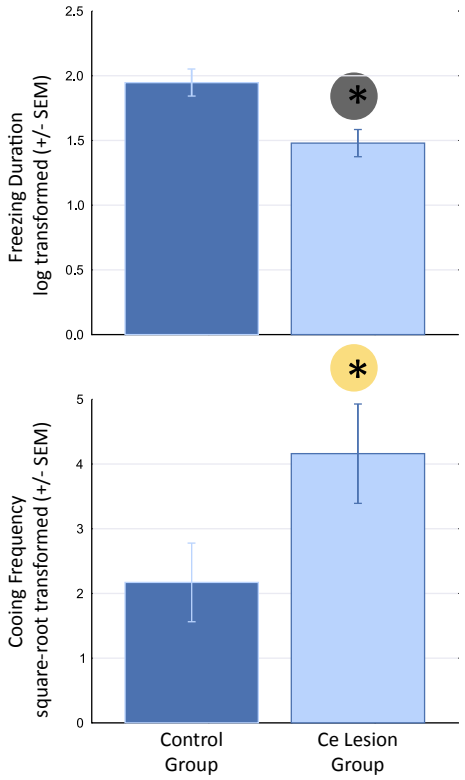
Tromp DP, et al. *Am J Psychiatry.* 2019 Jan:appiajp201818040425. doi: 10.1176/appi.ajp.2018.18040425. [Epub ahead of print]

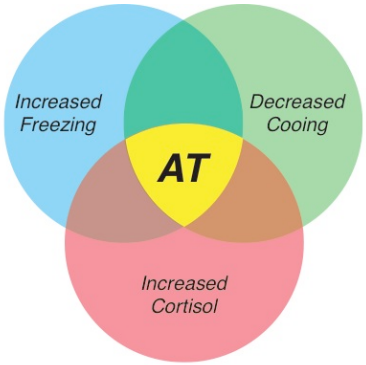
Extended Amygdala is a Core Component of AT with Downstream and Upstream Partners



Mechanistic Role of Ce: Neurotoxic Lesions Alter Components of AT





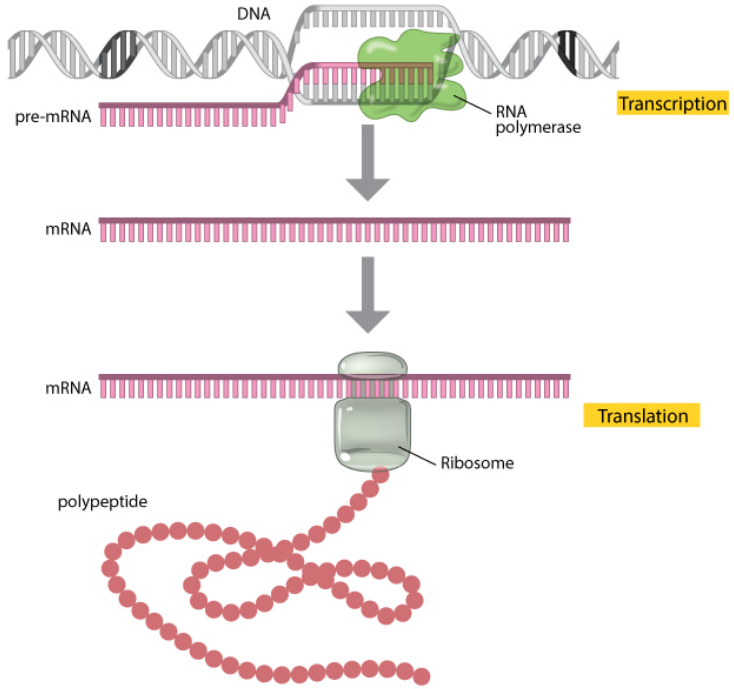


* = Control vs. Lesion p<.05

Kalin NH, et al. *J Neurosci.* 2004;24(24):5506-5515.

Performing Post-Mortem Transcriptome Studies to Identify Novel Molecular Targets

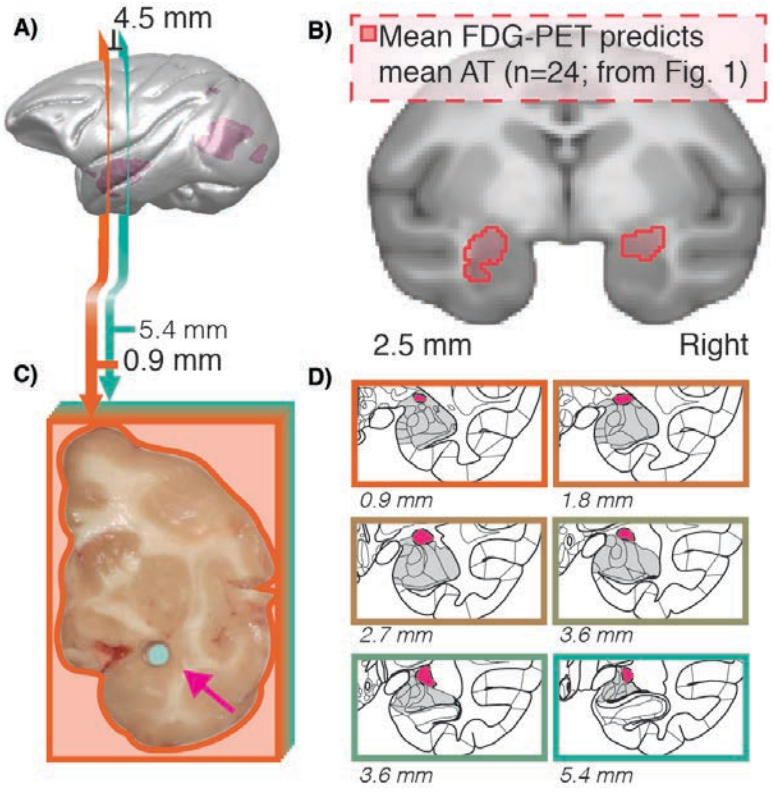
Assessing gene function measuring mRNA

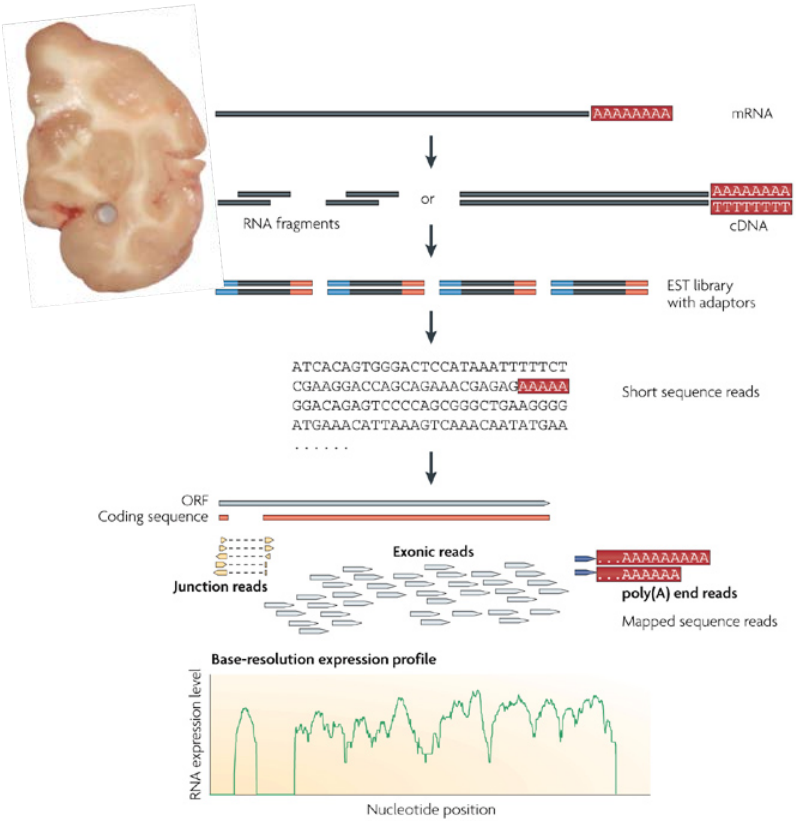


Why Study RNA?

- Reflection of the regulated step between DNA and protein
- Deficits in RNA expression and regulation is linked to numerous diseases

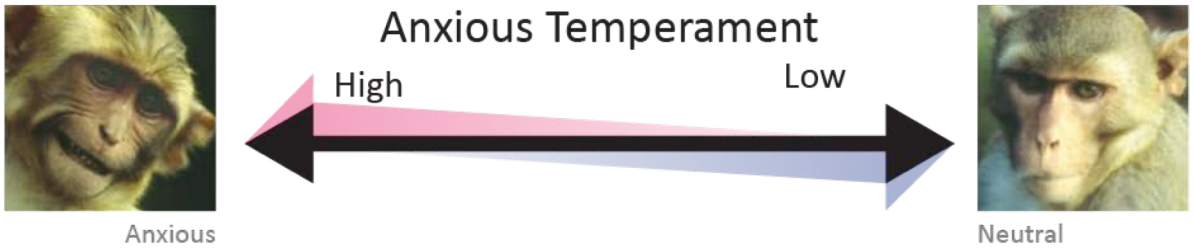
Identifying Alterations in Gene Expression Relevant to AT: At-Related Ce Gene Expression





Fox AS, et al. *Proc Natl Acad Sci U. S. A.* 2012;109(44):18108-18113.

Expression of Neuroplasticity-Related Genes in Ce is Negatively Associated with AT



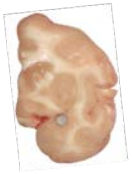
Decreased Neuroplasticity in AT-regions

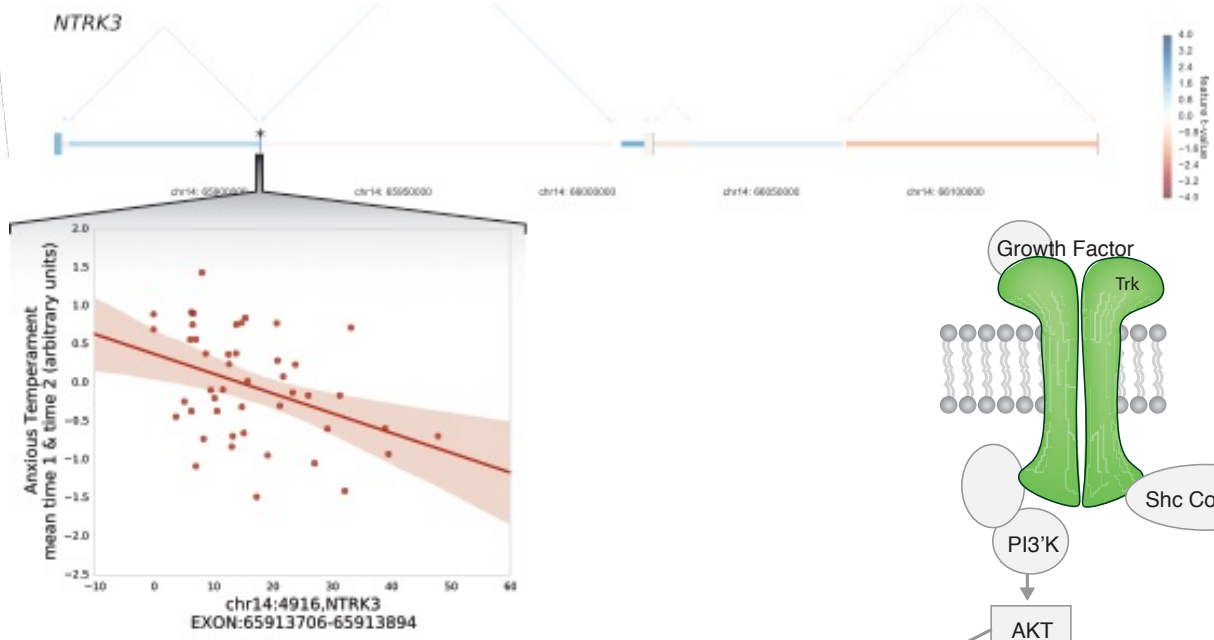
Increased Neuroplasticity in AT-regions

- 217 negatively associated with AT ($p<.05$)
  - Actin filament-based process (GO:0030029)
  - mRNA processing (GO:0006397)
  - Neurotrophin TRK receptor signaling pathway (GO:0048011)
  - Axon guidance (GO:0007411)

1. Fox AS, Souaiaia T, et al. Presented at Society of Biological Psychiatry, 70th Annual Meeting. May 14-16, 2015.; 2. Fox AS, et al. *Proc Natl Acad Sci U. S. A.* 2012;109(44):18108-18113.*; 3.* Fox AS, Kalin NH. *Am J Psychiatry.* 2014;171(11):1162-1173.

Ce NTRK3 Gene Expression Predicts AT in 46 Young Non-Human Primates



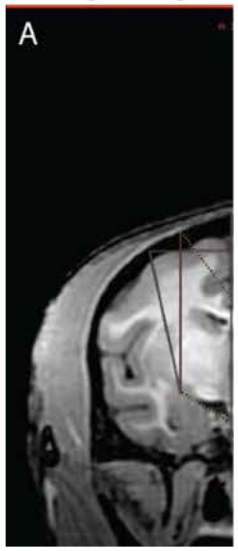


Fox AS, Souaiaia T, et al. Presented at Society of Biological Psychiatry, 70th Annual Meeting. May 14-16, 2015.

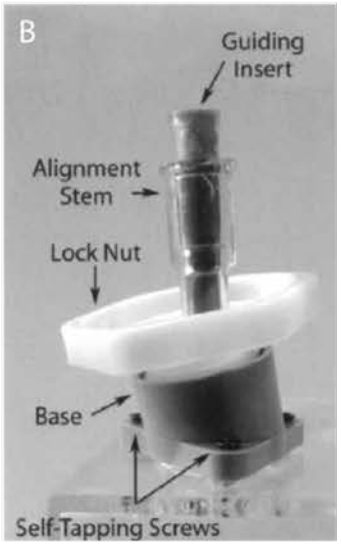
Combining gene manipulation studies with behavioral and human imaging measures as a "proof of concept" for testing new drug targets in primates.

Real-Time MRI for Site Specific Delivery of Viral Vector (AAV2)

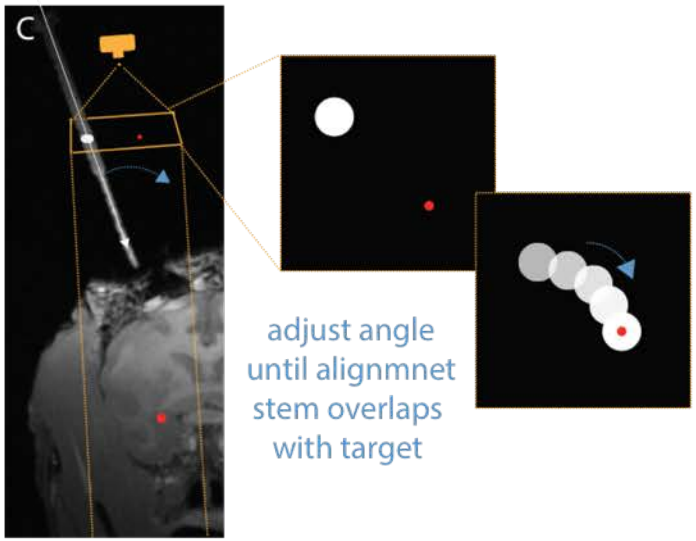
presurgical targeting



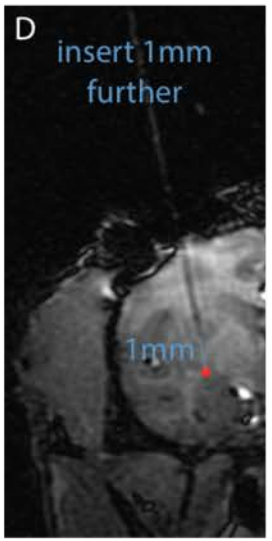
Navigus port placement



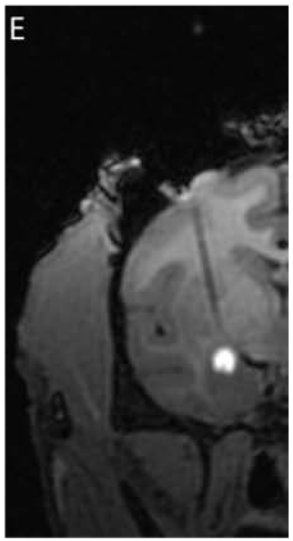
alignment guide & intraoperative trajectory planning



depth assessment

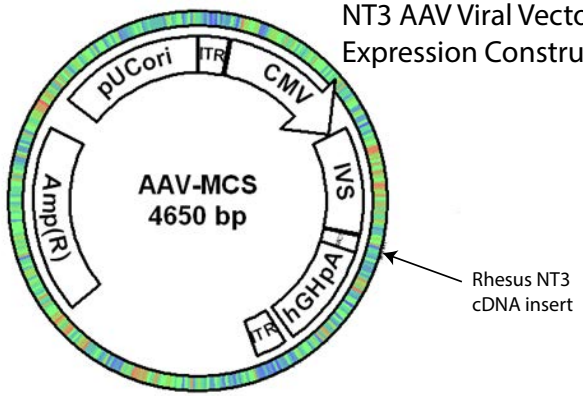


infusion monitoring

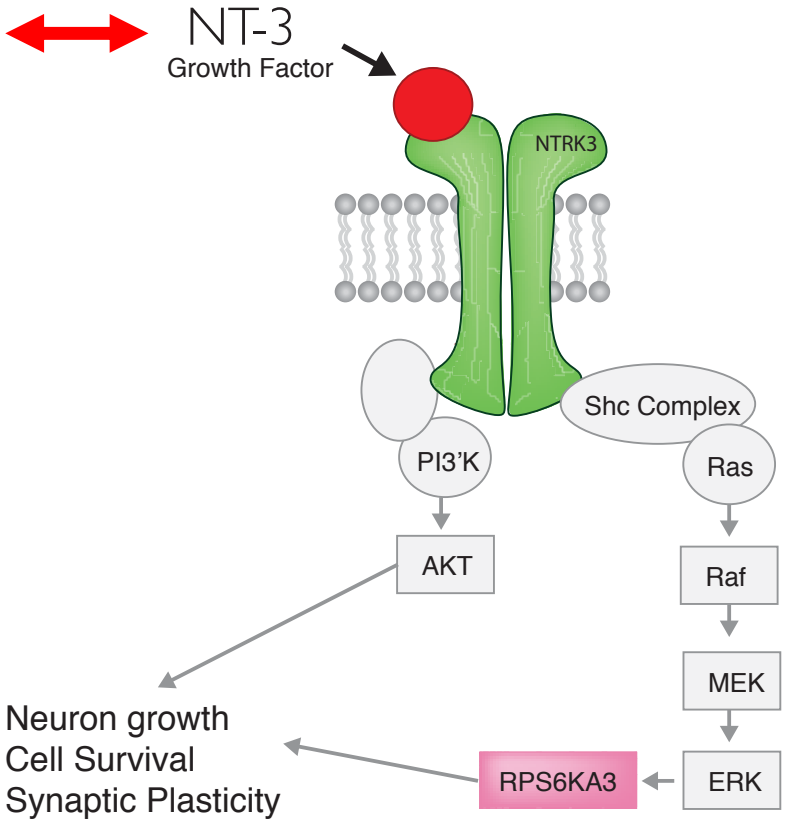


Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.

Testing the Neuroplasticity Hypothesis: AAV5 Virus to Overexpress NT-3

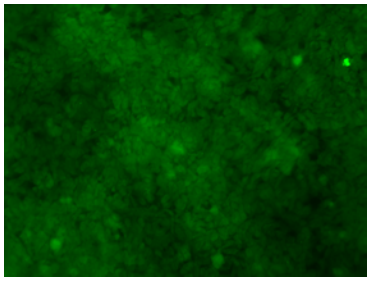


NT3 AAV Viral Vector
Expression Construct

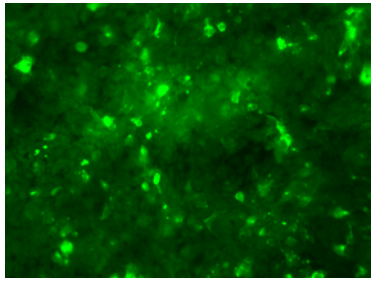


Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.

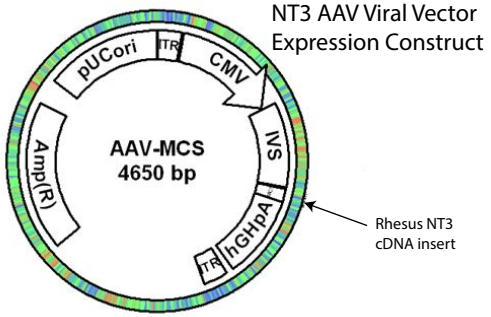
NT-3 Overexpression



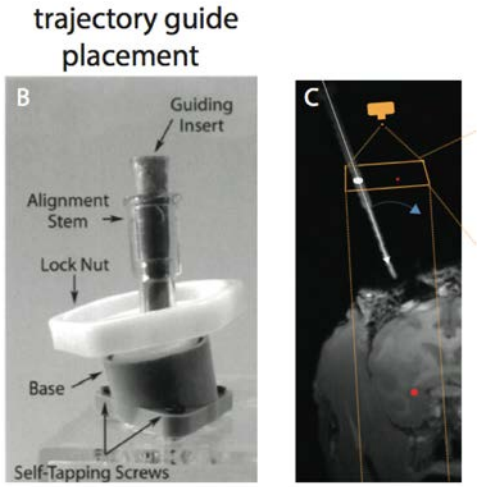
Control

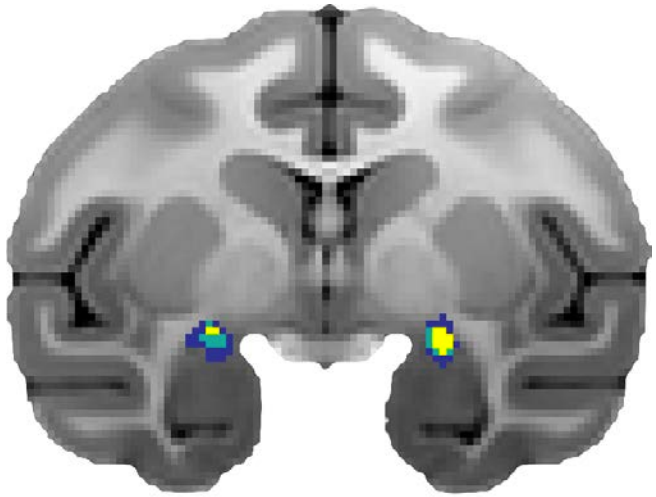


AAV5-NT3



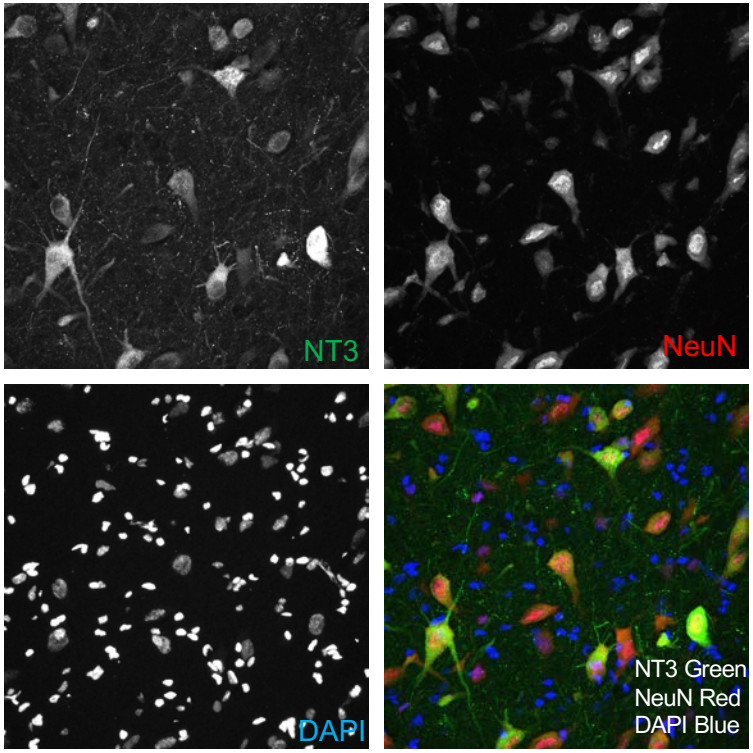
Infusion Overlap (n = 4/5)





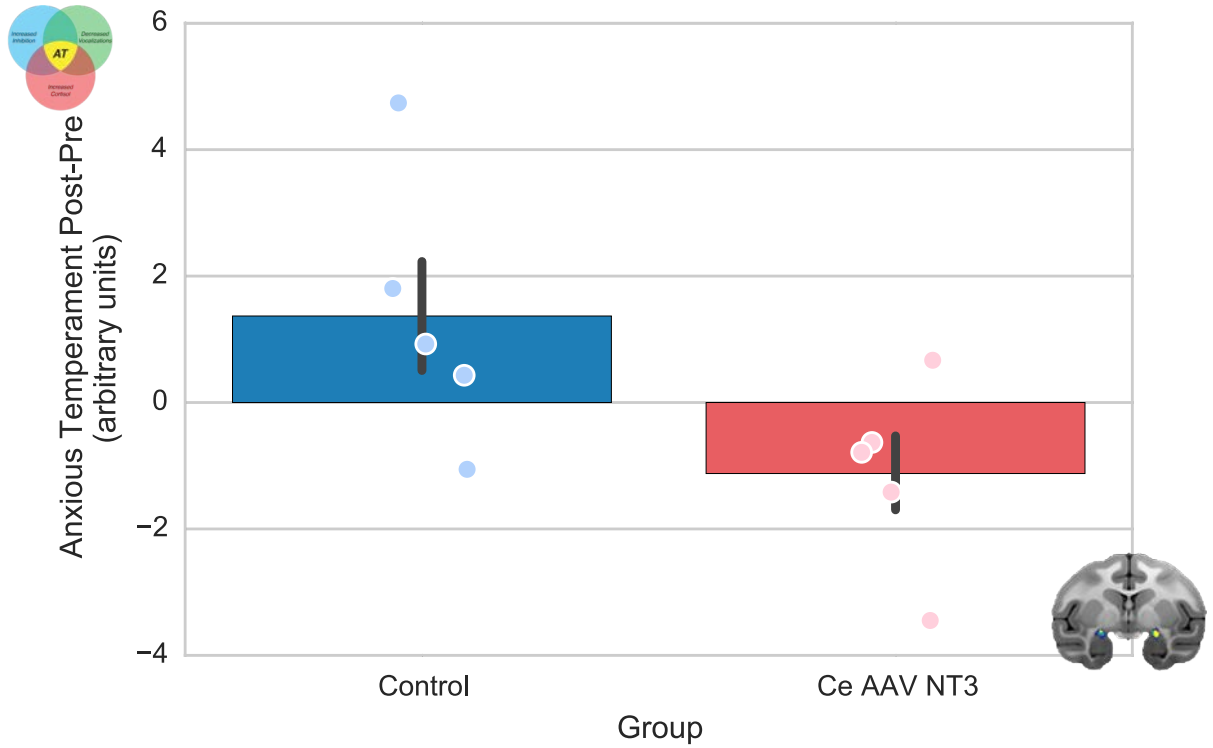
Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.

Overexpression of NT3 in Neurons of the Dorsal Amygdala



Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.

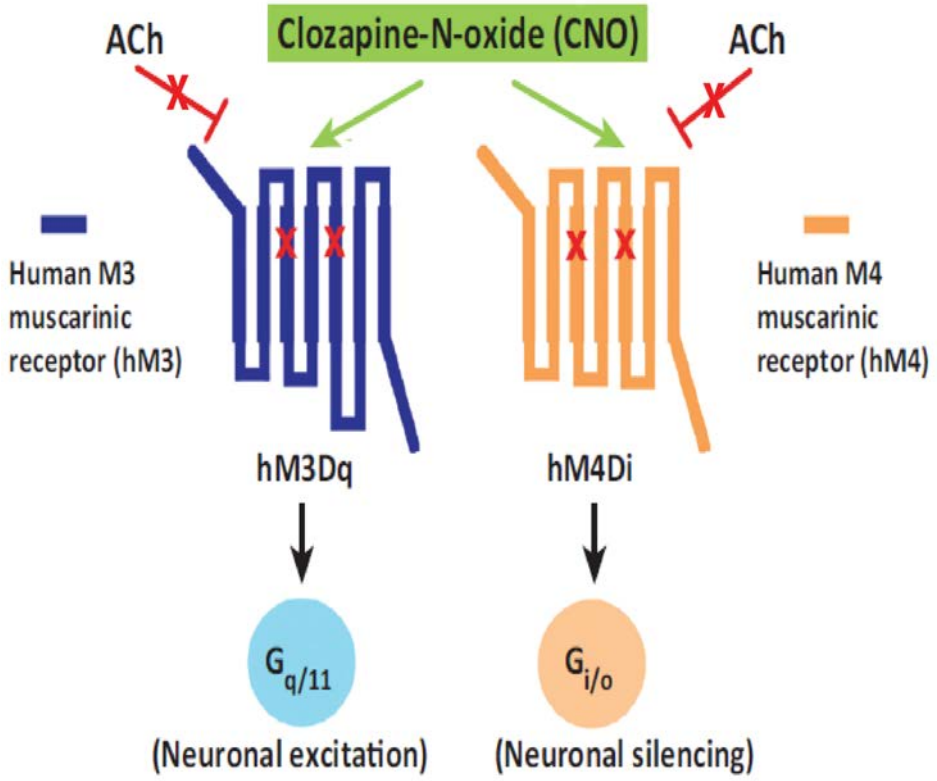
Ce AVV5 NT3 Decreases AT



AT is significant p<.05 one-tailed significant, t=-2.515, p=0.066; t=-3.013, p=0.039, two-tailed paired t-test

Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.

Reversibly Manipulating Neural Circuits in Primates: Proof of Concept for Humans

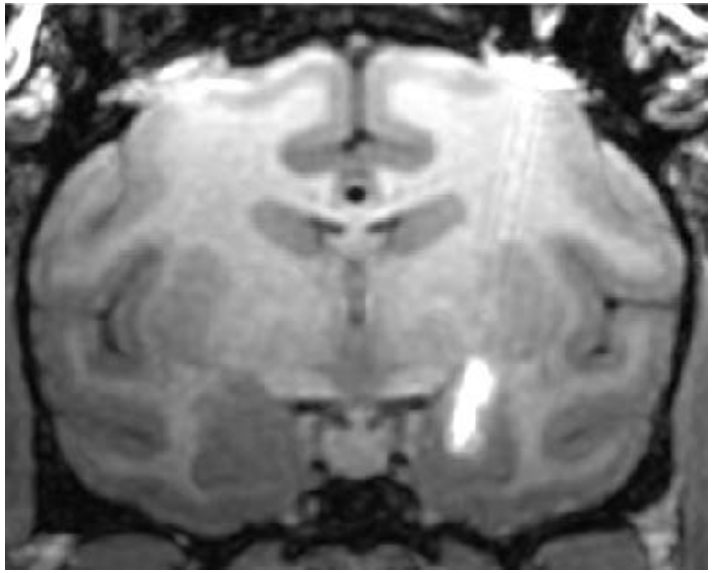


In Vivo PET Assessment of DREADDs: Clozapine Binding

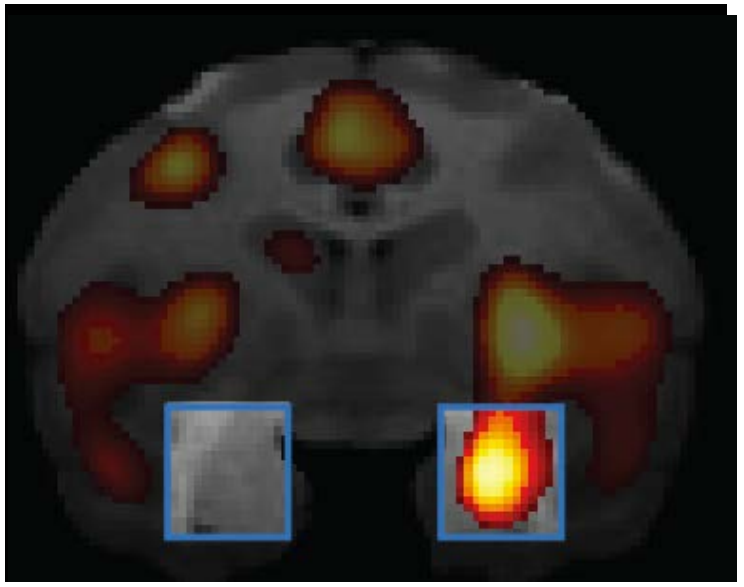
Right hemisphere injections: Amygdala (24 µL) and Putamen (24 µL)

AAV2/5-hSyn-hM4Di – 6.95 x 10^{13} gc/ml

iMRI

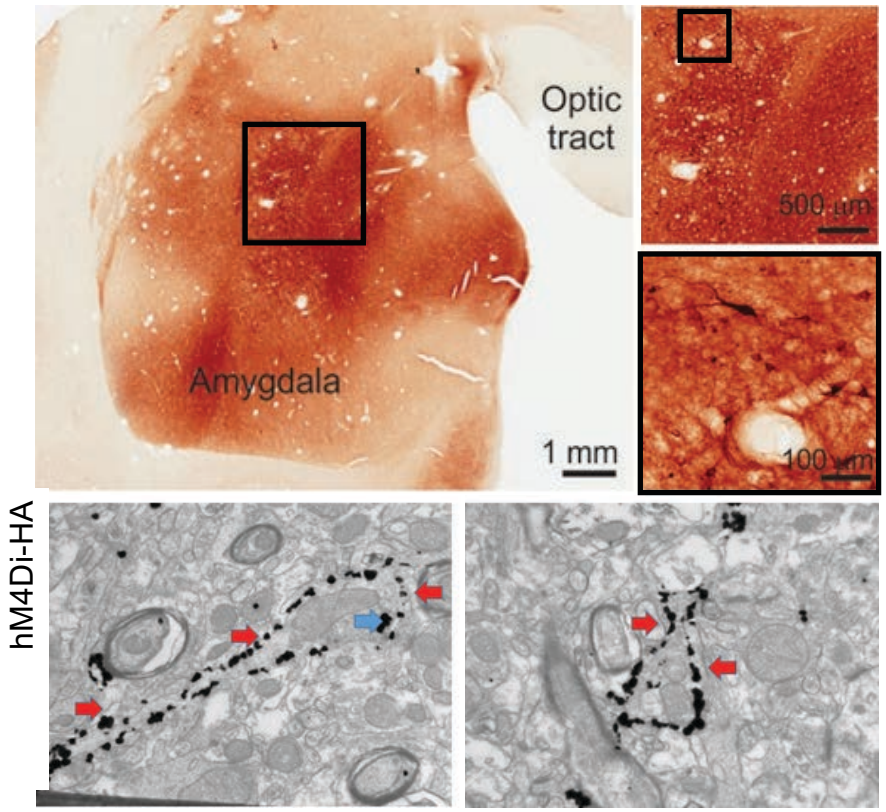


[^{11}C]clozapine PET



Subject 43

DREADDs Expression in the Primate Amygdala



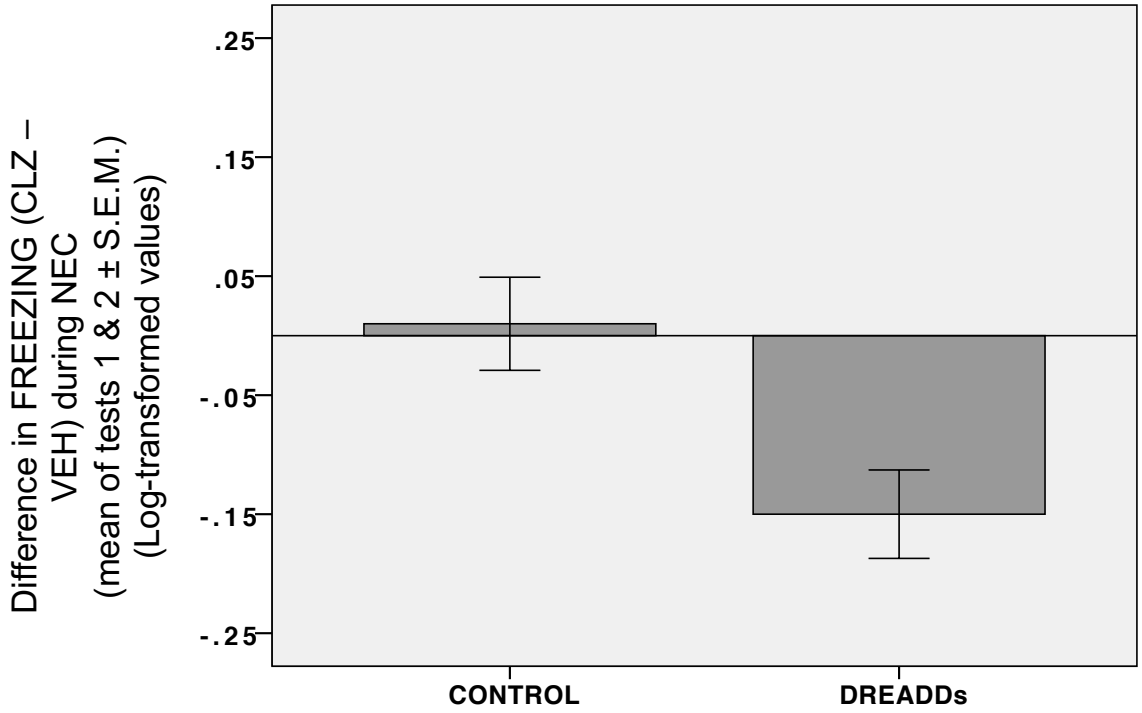
AAV2/5-hSyn-hM4Di
– 6.95 x 10^{13} gc/ml

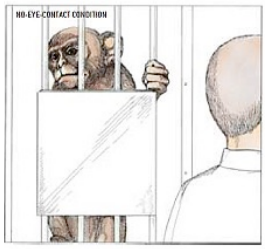
EM localization of DREADDs expression

- plasma membrane
- intracellular space

In collaboration with Adriana Galvan

Effect of hM4Di DREADDs activation on Freezing during NEC condition





group T-test on difference score

$t_8 = 2.966$, ***p* = 0.018***

N = 10, 5 Control and 5 Experimental

Call to Action

- It is important to take into account complex interactions between genetic and early life environment
- Identify early risk associated with sub-syndromal trait-like anxiety
- Early interventions have the potential to be preventative
- New treatments should be based on scientific knowledge focused on molecular alterations in specific neural circuits

DEPARTMENT OF Psychiatry
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

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RESEARCH INSTITUTE
UNIVERSITY OF WISCONSIN


Lisa Williams


Jonathan Oler


Pat Roseboom


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Richie Davidson


Rasmus Birn


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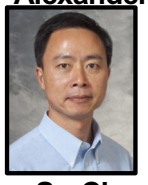
Su-Chun Zhang


Do Tromp


Rothem


Margaux Kenwood


Josh Cruz


Greg Rogers


Marina Emborg


Wally Block


Sascha Mueller


Rachel Puralewski


Nakul Aggarwal


Miles Olsen


Marilyn Essex


Brad Christian


Alex Converse

Wisconsin National Primate Research Center
UNIVERSITY OF WISCONSIN–MADISON

Harlow Center for Biological Psychology

WAISMAN CENTER

UCDAVIS
UNIVERSITY OF CALIFORNIA


Drew Fox


Alex Shackman

BCM
Baylor College of Medicine

UNIVERSITY of ROCHESTER MEDICAL CENTER


Jeff Rogers


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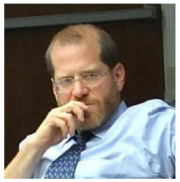
SUNY DOWNSTATE Medical Center


Jim Knowles


Tade Souaiaia

NIH National Institute of Mental Health

VANDERBILT UNIVERSITY


Danny Pine


Jenny Blackford

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We thank the staff at the Wisconsin National Primate Center, the Harlow Center for Biological Psychology, the HealthEmotions Research Institute, the UW Department of Psychiatry, Waisman Laboratory for Brain Imaging and Behavior. This work has been supported by National Institutes of Health grants MH046729, MH081884, MH084051, MH018931, Wisconsin National Primate Research Center, P51OD011106 / P51RR000167, and the Neuroscience Training Program.

Questions & Answers

Don’t forget to fill out your evaluations to collect your credit.

A Review and Meta-Analysis of the Genetic Epidemiology of Anxiety Disorders

John M. Hettema, M.D., Ph.D.

Michael C. Neale, Ph.D.

Kenneth S. Kendler, M.D.

Objective: The authors conducted meta-analyses of data from family and twin studies of panic disorder, generalized anxiety disorder, phobias, and obsessive-compulsive disorder (OCD) to explore the roles of genetic and environmental factors in their etiology.

Method: MEDLINE searches were performed to identify potential primary studies of these disorders. Data from studies that met inclusion criteria were incorporated into meta-analyses that estimated summary statistics of aggregate familial risk and heritability for each disorder.

Results: For family studies, odds ratios predicting association of illness in first-degree relatives with affection status of the proband (disorder present or absent) were homogeneous across studies for all disorders. The calculated summary odds ratios ranged from 4 to 6, depending on the disorder. Only for panic disorder and generalized anxiety disorder could the authors identify more than one large-scale twin study for meta-analysis. These yielded heritabilities of 0.43 for panic disorder and 0.32 for generalized anxiety disorder. For panic disorder, the remaining variance in liability could be attributed primarily to nonshared environment. For generalized anxiety disorder, this was true for men, but for women, a potentially significant role for common familial environment was also seen.

Conclusions: Panic disorder, generalized anxiety disorder, phobias, and OCD all have significant familial aggregation. For panic disorder, generalized anxiety disorder, and probably phobias, genes largely explain this familial aggregation; the role of family environment in generalized anxiety disorder is uncertain. The role of nonshared environmental experience is significant, underscoring the importance of identifying putative environmental risk factors that predispose individuals to anxiety.

Hettema JM, et al. *Am J Psychiatry*. 2001;158:1568-1578.

Childhood Normative Fears and Development

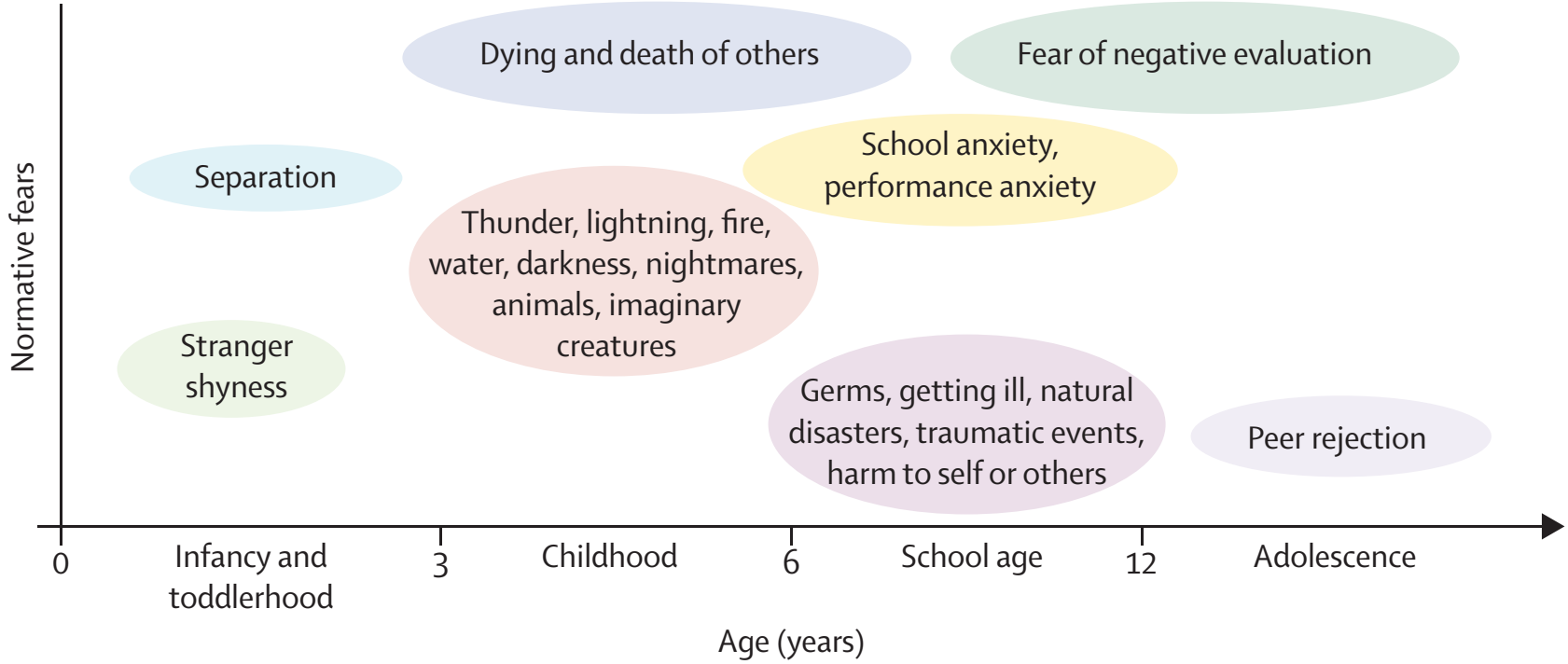
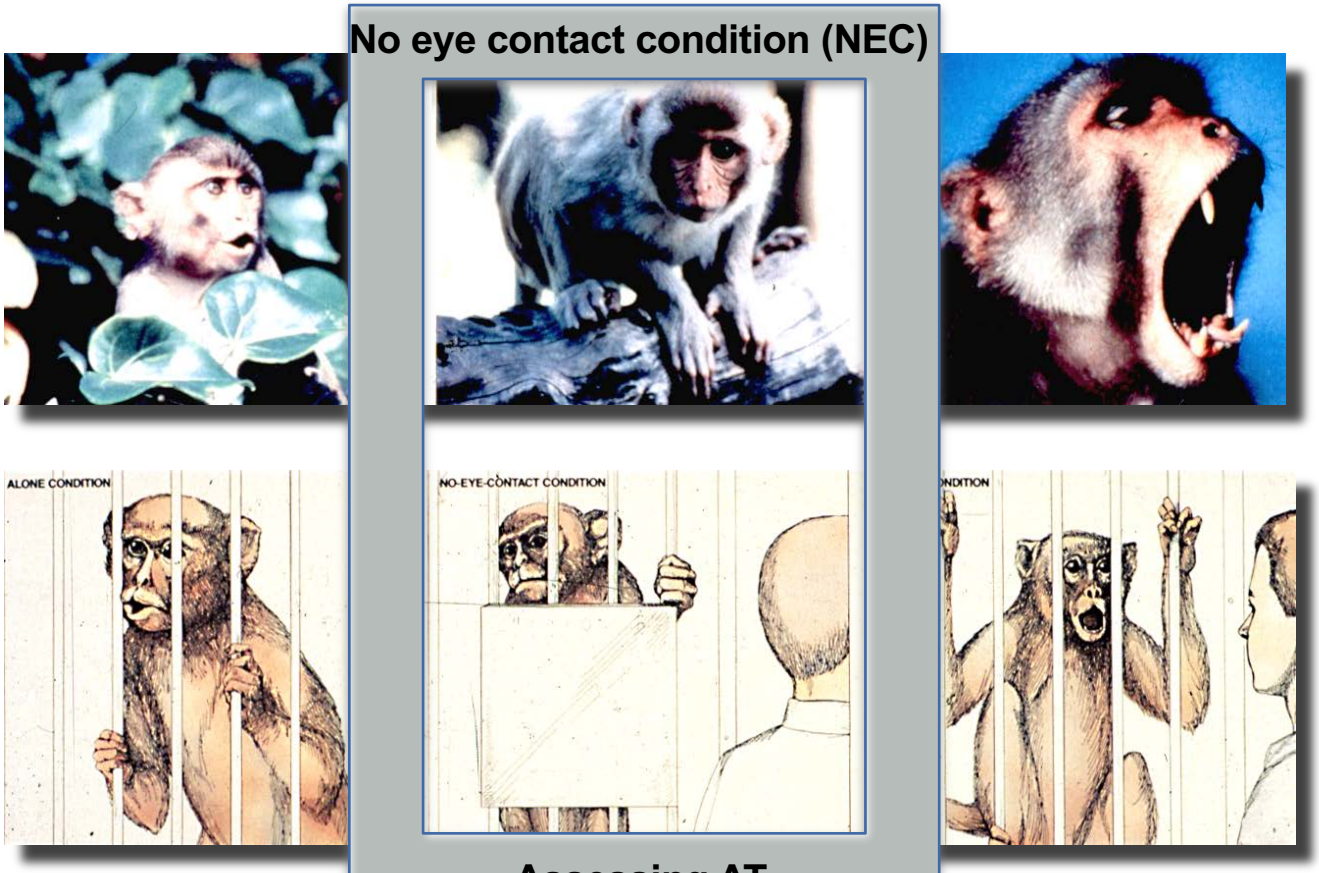


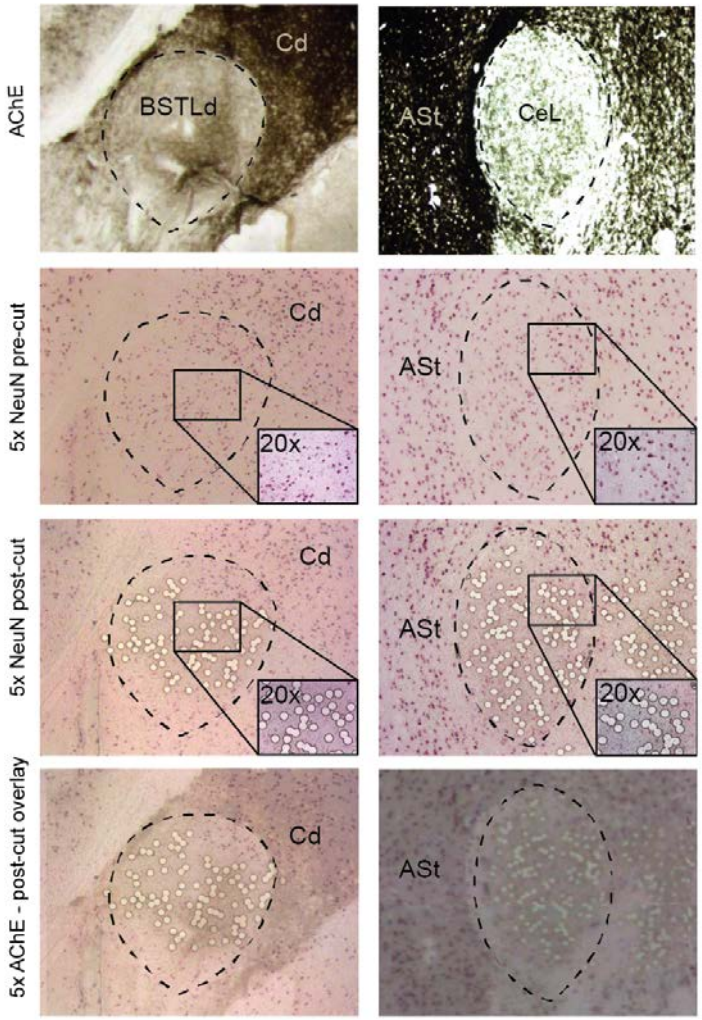
Figure adapted from Beesdo-Baum K, Knappe S. *Child Adolesc Psychiatri Clin N Am.* 2012;21:457-478.
Craske MG, Stein MB. *Lancet*. 2016;388(10063):3048-3059.

Human Intruder Paradigm: A Method to Assess Adaptive Defensive Responses as Well as Anxious Temperament



Kalin NH. *Sci Am*. 1993;268:94-101.

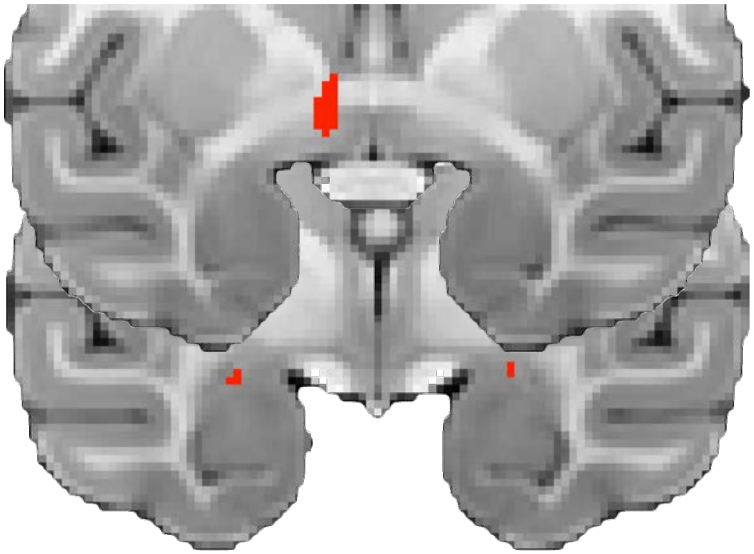
Capturing Ce and BST Neurons with LCM for RNAseq



Neurons from 47 animals from CeL have already been collected.

Mean = 650 neurons
std = 66

Ce AVV5 NT3 FDG-PET Change is Associated with Freezing



Ce / BST Metabolism

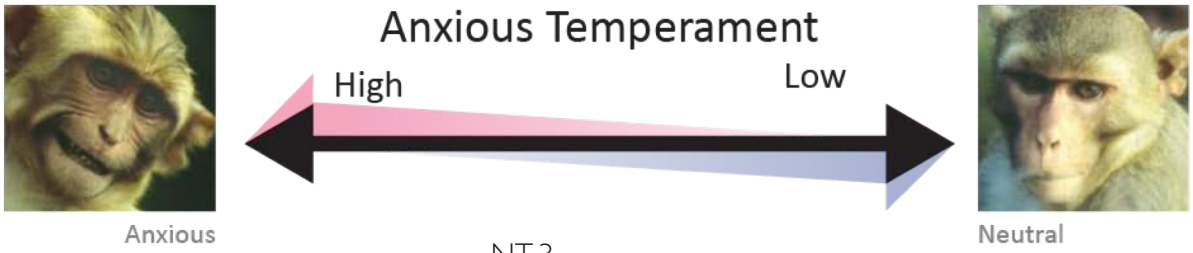
and correlated

Anxious Temperament

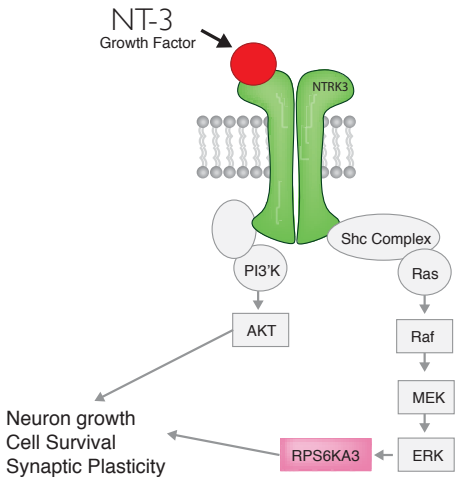
Group by time interaction (pos): p<.05, two-tailed AND negatively correlated with pre-post change in AT, p<.05, two-tailed

Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.

Support for Our Extended Amygdala Neuroplasticity Hypothesis in Mediating AT



Decreased Neuroplasticity in AT-regions

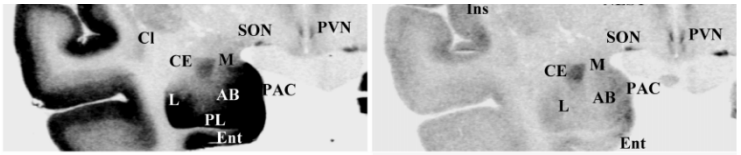


Increased Neuroplasticity in AT-regions

Fox AS, et al. *Proc Natl Acad Sci U. S. A.* 2012;109(44):18108-18113.
Fox AS, Kalin NH. *Am J Psychiatry.* 2014;171(11):1162-1173.

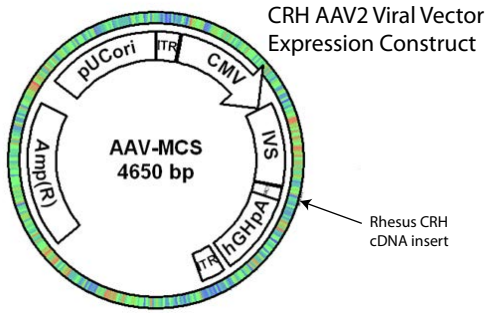
Testing Mediators Of AT: Corticotrophin Releasing Hormone (CRH) In Ce

CRH Receptors are expressed in Ce

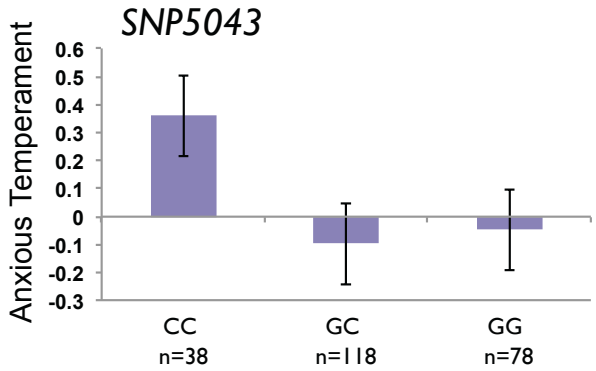


CRHR1 CRHR2

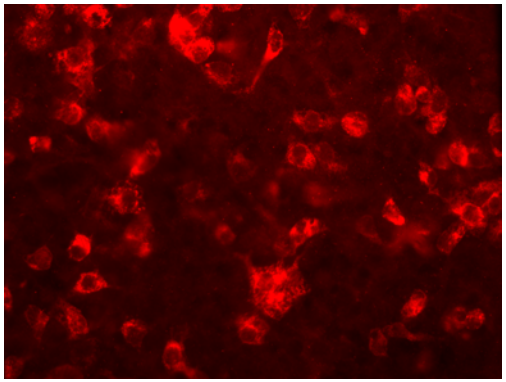
Sanchez et al., 1999



CRHR1 SNPs are associated with AT

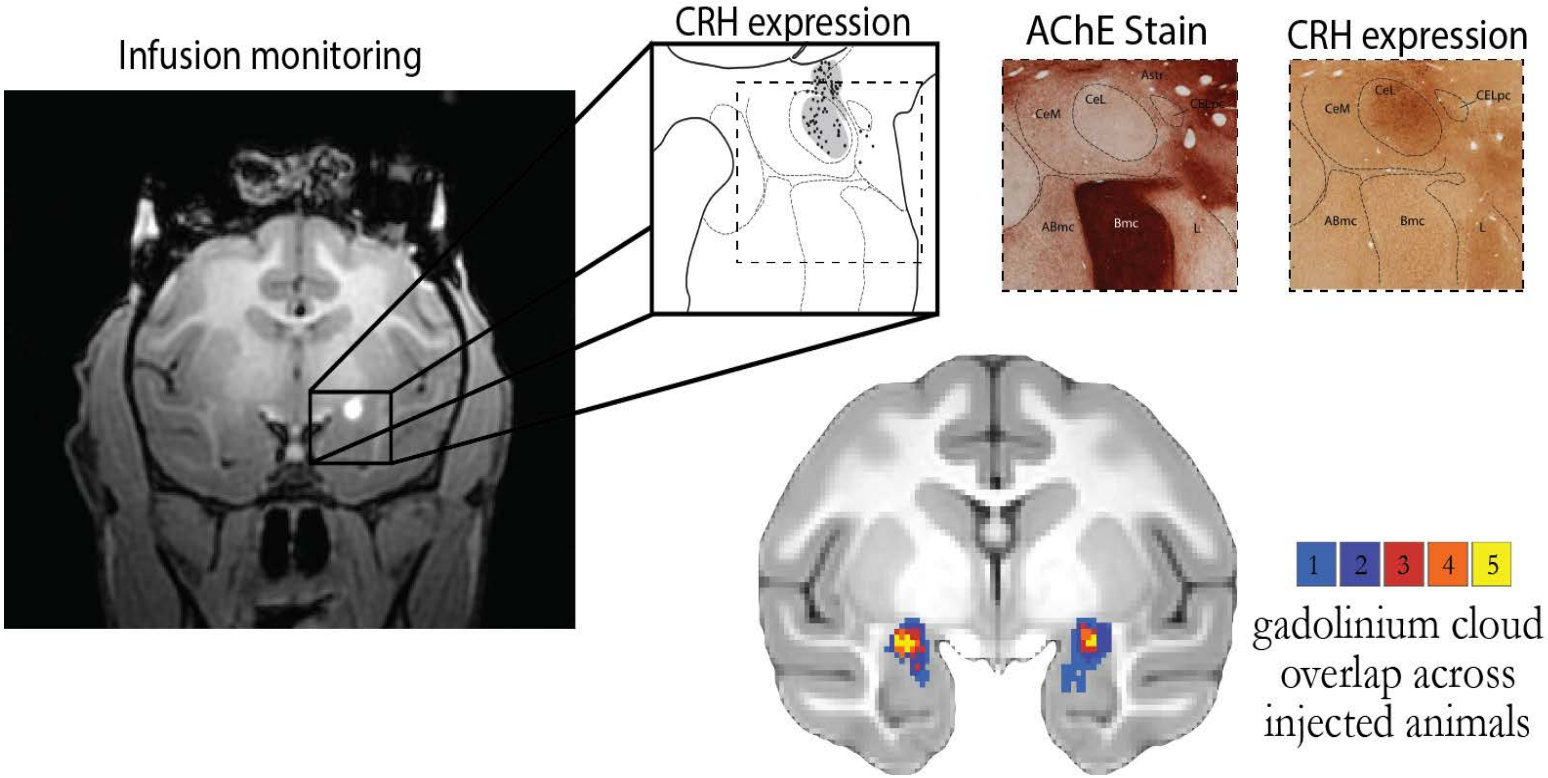


CRH expression in HEK293 Cells infected with CRH AAV2 construct



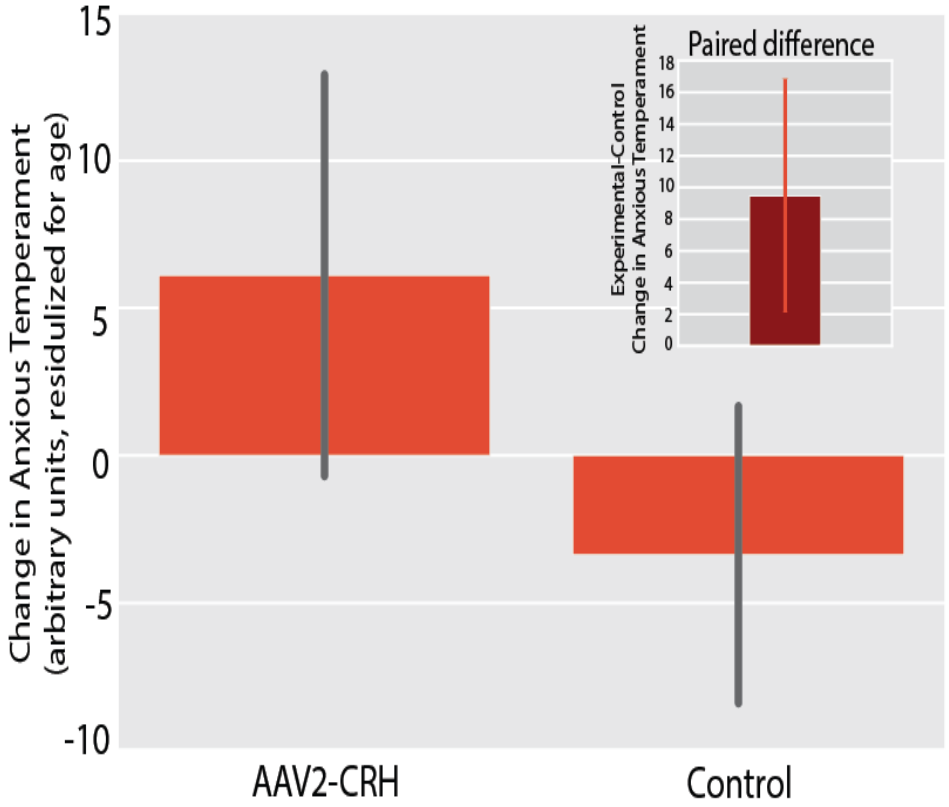
Rogers J, et al. *Mol Psychiatry*. 2013;18(6):700-707.

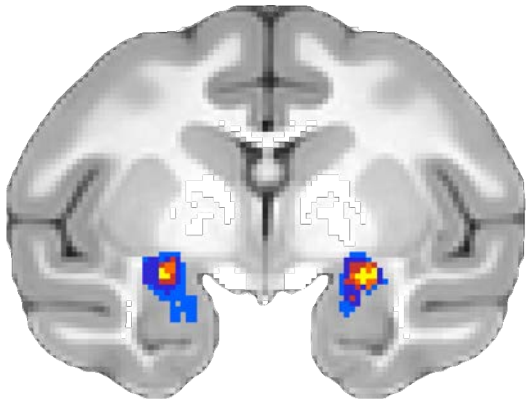
Overexpression of CRH in the Ce



Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.

Ce CRH Overexpression Increases AT





N = 5, per group

p <. 05, one-tailed

Kalin NH, et al. *Biol Psychiatry*. 2016;80(5):345-355.