Neurobiology of Pediatric Anxiety Disorders

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Objective

- Clarify phenotype of anxiety disorders with regard to horizontal and vertical boundaries
- Brief review of some neuroanatomy
- Outline associations with various neurotransmitter, genes, and brain regions
- Examine trauma and neural development
- Brief attention to autoimmune mechanisms and PANDAS
Child Anxiety Developmental Perspective

- Infancy – Loud sudden stimuli
- Late infancy (9 months) – Strangers
- Toddlers – Separation
- Early school age – Monsters, robbers
- Mid childhood – Incompetence
- Adolescents - Humiliation
Adaptive Aspects of the Reactive Tendencies: Example in Guppies

• Inhibited guppies have fewer offspring but longer survival from predation.

• Bold guppies are preferred mates, learn to associate food with cues faster, but tend to approach predators (and have shorter lives).

Slide Courtesy of D. Hirshfeld-Becker
Neurobiology of What?

Disorder 1  Disorder 2

Disorder

Trait
Child Anxiety Disorders
General Principles

- Very high “comorbidity” with other anxiety disorders and depression
- Often remit before adulthood but adult cases of anxiety/depression often with childhood onset
- Overlap with depression. CBCL anxiety/depression subscale
Two Genetic Factors (Hettema, Arch, 2005)

- Genetic influences for Group A (GAD, Panic, Agor) and Group B (Specific phobias) with Social in between
- One common shared environmental factor of small effect
- Shared genetics with depression in a separate study
Intermediate Processes

- Increased fear potentiation and startle (Grillon et al., 2003)
- Directing attention
  - Susceptibility to emotional interference
- Attributions of other’s intentions
- Temperamental or trait anxiety
Anxiety disorder theory often involves principles of learning

- **Classical conditioning**: pairing of unconditioned and conditioned stimulus
- **Operant Conditioning**: OCD example
Brain Development

• Prefrontal regions late in its development compared to other areas
  – Grey matter density
  – Myelination to subcortical regions
Development and Anxiety

• Adolescence a period of increase onset of anxiety and depressive disorders and emotional reactivity

• Relative to period of imbalance between
  – Heightened “bottom up” subcortical limbic activity in amygdala and ventral striatum
  – Immature “top down” control from prefrontal cortex
Neurochemical Systems

- Adrenergic System
- Serotonin
- GABA and benzodiazepine receptors
- Glutamater
Adrenergic System

• Theory of increased sensitivity of beta receptors
  – Studies not supportive (Gorman et al., 1983)

• Beta blockers effective for some types of anxiety

• Some evidence for increased and decreased sensitivity of central alpha 2 receptors
OCD
Serotonergic Dysfunction

• Selectivity of serotonergic medications versus noradrenergic ones in both adults and children (DMI used as control in pediatric studies)

• Inconsistent 5-HIAA levels in CSF patients versus controls

• Induction of OCD sx by m-CPP (5-HT agonist)
GABA and Benzodiazepines

- Widely found in temporal, frontal and visual cortices

Endogenous anxiolytic that binds still sought (although anxiogenic compounds like CCK known)
Glutamatergic Dysfunction

- Animal models
- Case reports of beneficial effect of antiglutaminergic agents
- Elevated glutamine levels in CSF of OCD patients
- Association with OCD and SNPs on SLC1A1 (a glutamate transporter; Wendland et al., 2009)
Temperament Studies with Anxiety Disorders

Behavioral Inhibition
Is she just shy?

Or is it Social Anxiety Disorder?
Overlap of Temperament and Child GAD (Rettew et al., J Anx Disorder, 2006)
Behavioral Inhibition (BI)

• Defined as temperamental restraint and fear when confronted with novel people or situations
• Concept developed by psychologist Jerome Kagan at Harvard
• Thought to affect about 10-15% of 2 year olds
• Assessed through observational protocol as early as age 4 months
• “Risk factor” for later childhood and adolescent anxiety disorders
• Thought to be related to amygdala hyper-reactivity
Temperament, Anxiety and Brain Activity (Schwartz et al., Science, 2003)

Fig. 1. (A) The presentation of stimuli was divided into two phases: a familiarization phase and a test phase that consisted of alternating 24-s blocks of either novel (N) or familiar (F) faces with neutral expression. Subjects viewed a fixation cross (+) during 24-s fixation blocks. (B) Colorized group statistical map superimposed on coronal group-averaged T1 structural image in Talairach space.

Significant fMRI signal changes (arrows) are shown in the right (peak $P$ value = $2.5 \times 10^{-5}$; Talairach coordinates $x, y, z = 21, -6.5, -14$) and left ($P = 4.2 \times 10^{-4}; x, y, z = -21.5, -6.7, -18$) amygdalae (Amy) and occipito-temporal cortex (OTC). (C) Percent (%) BOLD signal change (versus fixation) in amygdala to novel versus familiar faces in adult subjects who were inhibited and uninhibited in the second year of life. One standard error of the mean is indicated.
Cortical Thickness and Anxious Temperament
Schwartz et al., Archives, 2010

- Measured reactive temperament at age 4 months
- Imaged at 18 years
- L orbitofrontal (blue) thicker in low-reactive
- R ventromedial prefrontal (red) thicker in high-reactive
Differential heritability of anxiety
Oler, Nature 2010

- Rhesus monkeys with anxious temperament
- Amygdala and hippocampus both implicated but amygdala activity less heritable
Neuroticism and Internalizing Disorders (Hettema et al., AJP, 2006)

- Genetic liability to neuroticism accounts for 1/3 to 2/3 of total genetic liability to internalizing disorders
- Disorder specific influences most prominent for unshared environment
Possible Mechanisms of Temperament/Anxiety Disorder Relations

- Spectrum
- Risk
- Scar
- Common pathway
- Combination of above
Genetics of Anxiety

Behavioral Genetics
Molecular Genetics
Epigenetics
Genetics of Anxiety (CBCL) (Lamb JAACAP 2010)
Genetic Architecture of Adolescent Neuroticism
Rettew et al., Twin Research, 2006

- Study of 3301 adolescent twins (mean age 15.5)
- Indications for different genes between girls and boys
**Table 7. Model-Fitting Results for Square Root–Transformed CBCL OCS Scores**

| Study Sample, Model† | χ² | df | P Value | AIC | Male | | | | Female | | | | | | Compared | With | Model No. | ∆χ² (Δdf) |
| USA/MOTWIN | | | | | | | | | | | | | | | | | | | | |
| ACE sex | 18.811 | 9 | .027 | 0.811 | 38 | 16 | 46 | 40 | 4 | 56 | | | | 1 | 16.349 (3)* | |
| ACE no sex | 35.66 | 12 | .000 | 11.660 | 44 | 6 | 50 | 44 | 6 | 50 | | | 1 | | |
| AE sex | 21.403 | 11 | .029 | -0.597 | 55 | 45 | 45 | 55 | | | 1 | | | 2.592 (2) | |
| NTR-7 | | | | | | | | | | | | | | | | | | | | |
| ACE sex | 17.847 | 9 | .037 | -0.153 | 57 | 0 | 43 | 55 | 0 | 45 | | | | 1 | 0.77 (3) | |
| ACE no sex | 16.617 | 12 | .098 | -5.383 | 56 | 0 | 44 | 56 | 0 | 44 | | | 1 | | |
| AE no sex | 18.617 | 13 | .135 | -7.383 | 56 | 44 | 56 | 44 | | | 2 | | 0.000 (1) | |
| NTR-10 | | | | | | | | | | | | | | | | | | | | |
| ACE sex | 12.026 | 9 | .212 | -5.974 | 57 | 5 | 38 | 51 | 2 | 47 | | | | 1 | 6.053 (3) | |
| ACE no sex | 18.079 | 12 | .113 | -5.921 | 55 | 0 | 45 | 55 | 0 | 45 | | | | | |
| AE no sex | 18.276 | 13 | .147 | -7.724 | 58 | 42 | 58 | 42 | | | 2 | | 0.197 (1) | |
| NTR-12 | | | | | | | | | | | | | | | | | | | | |
| ACE sex | 5.940 | 9 | .746 | -12.060 | 44 | 11 | 44 | 21 | 27 | 52 | | | | 1 | 4.455 (3) | |
| ACE no sex | 10.395 | 12 | .581 | 13.605 | 36 | 16 | 48 | 36 | 16 | 48 | | | | | |
| AE no sex | 17.052 | 13 | .197 | -8.948 | 54 | 46 | 54 | 46 | | | 2 | | 6.557 (1) | |

Abbreviations: See Table 4; ACE sex, model contains additive genetic (A), common environmental (C), and unique environmental (E) parameters with male and female estimates allowed to differ; ACE no sex, model contains ACE parameters but male and female estimates are constrained to be equal; AE sex, model contains AE parameters with male and female estimates allowed to differ; AE no sex, model contains AE parameters but male and female estimates are constrained to be equal; AIC, Akaike information criterion.

*Boldface type represents the best-fitting model for that sample.
†NTR numbers indicate age (in years) of children at the time of data sampling.
‡The parameters a, c, and e are loadings of the observed phenotype on the latent factors A, C, and E and indicate the degree of relations between the latent factors and the observed phenotype. The proportion of the variance accounted for by genetic and environmental influences is calculated by squaring the parameters a, c, and e and dividing them by the total variance.

- Study of around 8500 twins age 7-12
- AE or ACE model: A=36-46; C=0-16; E=44-55
Candidate Genes in Anxiety Disorders (Stein, 2007)

- Serotonin Transporter
- B1-adrenergic receptor (introversion)
- Catechol-O-methyltransferase (COMT)
- Corticotropin releasing hormone (CRH)
- Glutamic acid decarboxylase (GAD1 and GAD2)
- Glutamate Transporter Genes (SLC1A1) in OCD
- Glutamate Receptor (GRIN2B) in OCD
- BDNF – involved in extinction learning
- HTR1AC – c/c genotype related to higher amygdala reactivity (Fakra et al, 2009)
- RGS2 – G Protein regulator – related to hippocampus functioning and reduced trait anxiety
• BDNF involved in synaptic plasticity for learning
• Extinction exercises impaired with Met carriers at Val66Met (freezing with mice, skin conductance for humans)
• Correlated with less frontomedial prefrontal cortical activity and more amygdala activity on MRI
Genotype-Environment Interactions

• Definition: Impact of a genetic effects depends upon the environment (or vice versa)

• Medical Example: Seasonal allergies
Stress, 5-HTT Gene and Depression

From Medina 2004 adapted from Caspi et al., 2003
Genotype-Environment Correlations

• Genes and environment combinations do not operate at random but rather certain environments are more likely based on particular genes
  – **Passive**: ex. Parent and child sharing “anxious” genes
  – **Evocative**: ex. Anxious child inducing more overprotective parenting
  – **Active**: ex. Anxious child preferring more solitary activities so less exposure
Hippocampus exerts inhibitory influence of HPA axis.

Amgydala excitationary influence.

Chronic stress or trauma increases amgydala branching and decreases hippocampus branching (McGowan et al., 2009).
Dysregulation of HPA Axis

- Effect mediated by levels of brain derived neurotrophic factors or BDNF (modified by antidepressants)
- This mediated by transcription factor (cAMP response element binding protein; CREB)
- Antidepressants can increase BDNF by increasing CREB (why it takes time)

from Nestler et al., 2001
Epigenetics and the HPA Axis

• Axis regulated in part by gene expression of glucocorticoid receptors in hippocampus.

• This effect mediated by epigenetic (methylation) inhibition of promoter binding sites related to neurotrophic factors.

Nestler et al., 2001; Meaney et al, 2009
Maternal Behavior and HPA Axis in Mice
Meaney et al

- Licking activates serotonin receptors that project to hippocampus
- Leads to activation of NGF1-A
- DNA demethylated and more available for transcription
- Leads to activation of promoter of glucocorticoid receptor
Neuroimaging and Anxiety
The Fear Response – LeDoux 2000

Anxiety Regions in the Brain

- Cingulated cortex
- Prefrontal cortex
- Striatum
  - 1-caudate nucleus
  - 2-putamen
  - 3-nucleus accumbens
- Orbitofrontal cortex
- Insula (cortex)
- Substantia nigra + ventral tegmental area (VTA)
- Hippocampus
- Hypothalamus
- Amygdala
- Pons
- Cerebellum
- Medulla oblongata

Database Center for Life Science (DBCLS):
http://lifesciencedb.jp/
Amygdala

- Critical region in the acquisition, storage, and expression of fear and fear memory
- 12 different regions with subunits
- Lateral, basal, accessory basal, central nuclei most involved in conditioned fear
Amygdala Neurotransmitters
Kodirov, PNAS 2006; Fakra et al., 2009

- Primarily glutamate and NMDA receptors
- Zinc related to LTP in cortical input to the amygdala by suppression of GABA inhibitory pathways
- Functioning sensitive to central serotonin
- GRP=gastrin releasing protein
5-HTTLPR Polymorphism and cingulate-amygdala interactions

Pezawas et al., 2005

- Subgenual ACC posit correlated with Amyg
- Suprigenual ACC neg correlation (feedback loop)
- Less in s individuals
- Loop explain 30% variance in Harm Avoidance scores
Intermediate Processes
Attention Orienting

- Refers to abnormal modulation of attention with regards to threat stimuli
- Assessed in the “dot probe” test which can be done in an fMRI
Attention Orienting

• When threat presented quickly, anxious adolescents showed more amygdala activation (Monk et al., Archives, 2008)

• Activation related to increased response times and subjective anxiety

• Connects subjective anxiety to specific cognitive function (attention interference with threatening stimuli) to brain activity
Attention Orienting
(JAACAP, 2008)

- In second study, used longer duration of threat stimuli (Monk et al., AJP, 2006)
- Anxious subjects had similar amygdala response but less activation in vlPFC
- Suggests modulatory role of vlPFC
Adolescence and Fear Processing (Hare et al., 2008)

- Adolescence time of heightened amygdalar activation
- Less maturity of regulatory prefrontal cortex
- More accelerator, less brake

Figure 1. Task design. Shown is the temporal layout of stimulus presentations within a scan where fear expressions were the targets and calm expressions were the nontargets. Stimuli were presented for 500 msec and followed by a variable interstimulus interval of 2000–14,500 msec.
Fear Activation and Attention
McCure et al., Archives, 2007

• Hypothesis that early developmental disruption of amygdala-PFC circuitry mediates attention biases toward threats
• Compared adolescents with GAD vs controls
• Shown happy, fearful, angry, neutral faces and asked to attend to nose width or fear
Fear Activation and Attention
McCure et al., Archives, 2007

- GAD versus control had more amygdala, vPFC, and ACC activation to fearful but not happy faces
- BUT occurred only when asked to attend to emotional state and not nose width
- Greater connectivity between amygdala and insula in patients vs controls
Panic Disorder and CO2 Sensitivity  
(Battaglia et al., Archives 2009)

- Separation Anxiety and Panic Disorder related to CO2 hypersensitivity
- Twin study showed common genetic link between SAD, adult panic, and CO2 sensitivity with minor role of parental loss
OCD Neurobiology
JAACAP Review 2008

- Cortex: Testing often shows frontal deficits
  - Recent MRI study shows higher grey density in OFC (AJP, 2008)
- Striatum: Smaller volumes in OCD
  - MRS studies shows lower higher glutaminergic activity (but lower in cortex)
- Thalamus: Larger in OCD
• Possible subgroup of pediatric OCD
• Developed from studies of Sydenham’s Chorea (70% report unwanted thoughts or rituals)
• Abrupt onset or exacerbation following A Beta-hemolytic strep infection
• Possible similar mechanism to rheumatic fever
• Also associated with mood lability, separation anxiety
• Need to show temporal relationship of symptoms to strep infection
The graphs illustrate the correlation between symptom severity and ASO titers over time for both PANDAS and NON-PANDAS conditions. The Y-BOCS scores are also shown for comparison.

For PANDAS:
- Symptom severity increases sharply from months 1 to 3, with a peak at month 2.
- ASO titers show a gradual increase starting from month 1, reaching a peak around month 3.

For NON-PANDAS:
- Symptom severity shows a more gradual increase from months 1 to 3.
- ASO titers start to rise from month 1, but do not reach as high as in PANDAS.

The graphs are courtesy of Dr. Sue Swedo.
Model of Pathogenesis for PANDAS

GABHS → Susceptible Host → Abnormal Immune Response → CNS & Clinical Manifestations

Slide courtesy of Dr. Susan Swedo
PANDAS Treatment

• Penicillin prophylaxis not shown to be generally effective (Garvey et al. 1999)

• Severe cases treated with plasmapheresis or IVIG (does not work in non PANDAS)

• Diagnosis and treatment remains debated
Clinical Implications

- Anxiety is real and in moderations is extremely adaptive
- Close inter-relations between genes and environment from birth have profound impact on brain development
- Shared neurobiology among anxiety disorders
- Close links to temperament/personality for many children
- Implications of multiple genes, brain regions, neuromodulators way beyond GABA and the amygdala that need to be further developed
- Much focus on interconnections between amygdala activation and regulation for cortical centers
The End