Mood and anxiety disorders

Mood disorders 10%

Anxiety disorders 15-20%

Comorbidity 30-60%

Anxiety Disorders

Generalized anxiety disorder (GAD)
Panic Disorder
Social Phobia
Specific Phobia
Post-traumatic Stress Disorder
Obsessive Compulsive Disorder
Pharmacotherapy

Mood disorders
- MAOI
- TCA
- SSRI
- Lithium
- CRF, SP antagonists

Anxiety disorders
- Benzodiazepines
- TCA
- SSRI
- CRF, SP antagonist

Anxiety and Depression

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Adaptative response</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danger</td>
<td>Fearful/anxious</td>
<td>Anxiety disorder</td>
</tr>
<tr>
<td>Loss</td>
<td>Sad/unhappy</td>
<td>Depression</td>
</tr>
</tbody>
</table>

Personality Determinants

Eysenck (1947)
Twin Studies

Genetic and Environmental Factors in Anxiety Disorders

Mouse Genetic Strategies

from Kendler et al., 1995
**Animal Models**

<table>
<thead>
<tr>
<th>Emotional stimulus</th>
<th>Human</th>
<th>Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Danger</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>• Loss</td>
<td></td>
</tr>
<tr>
<td>Emotional response</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Behavioral response</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>• Physiological response</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>• Feeling (conscious experience)</td>
<td>?</td>
</tr>
</tbody>
</table>
Validation of Animal Models

Behavioral validation
• Multiple behavioral and physiological tests
• Factor analysis (covariance analysis)

Pharmacological validation
• Response to therapeutically active drugs but not to inactive drugs
• Comparable pharmacokinetic profiles

Candidate Systems for Anxiety and Depression

<table>
<thead>
<tr>
<th>Lesion and stimulation studies</th>
<th>Pharmacological studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>GABA, Benzodiazepine</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>5-HT, 5-HT1A Agonist</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>CRF, SP, NE, Antagonist</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td></td>
</tr>
<tr>
<td>Raphe</td>
<td></td>
</tr>
</tbody>
</table>

Anatomy of the serotonergic system
5-HT1A KNOCKOUT

5-HT1A knockout mice

Anxiety-like phenotype

Effects of antidepressants on behavior and neurogenesis

Tissue-specific and developmental rescue

Conflict Tests

exploration, hunger

DANGER!

avoidance

SAFETY
Mouse Serotonin 1A Receptor (5-HT1AR)

Hippocampus
Raphe nuclei

Forebrain Rescue Strategy

Forebrain Rescue of 5-HT1AR
Does forebrain receptor reverse the phenotype of the 5-HT1A KO mice?

Forebrain rescue mice have normal anxiety-like behavior

Open Field | Elevated-Plus Maze

Novelty-Suppressed Feeding

Receptor OFF in adulthood
Shutting off receptor expression in the adult does not reverse the rescue phenotype

Open Field

Elevated-Plus Maze
Novelty-Suppressed Feeding

Receptor ON at P21

Shutting off receptor expression before P21 reverses the rescue phenotype

Open Field

Elevated-Plus Maze
Novelty-Suppressed Feeding
Summary

**Forebrain Rescue**

- Forebrain receptor sufficient to reverse knockout phenotype
- Adult receptor not required to maintain rescue
- Expression during development critical to rescue

**Evidence for developmental role of 5-HT**

- 5-HT mediates lifelong effects of altered maternal care (M. Meaney)
- 5-HT depletion or excess during postnatal period:
  - disrupted barrel and visual cortices
  - reduced hippocampal dendritic spine density (J. Haring)
  - disrupted calretinin-IR neuron morphology (J.-P. Hornung)
Increased excitability in CA1 pyramidal neurons of knockout mice

5-HT1AR function: development vs. adulthood
Mechanism of action of antidepressants

- Change of the setpoint of monoamine transmission
- Plastic changes occurring in the limbic target areas of monoaminergic projections (Hipp., Amy., Ctx)
  - Halt hippocampal atrophy
  - Prevent stress-induced dendritic shrinkage
  - Increase hippocampal neurogenesis

Mood and Anxiety Circuits

Sensory Cortex → Transitional Cortex
Sensory Thalamus → Amygdala → dorsal Hippocampus ventral

Emotional Stimulus ⏯️ Fear Response
Hypothalamus Locus Coeruleus Central Grey
Hippocampal Atrophy In Recurrent Major Depression

Effects of stress and antidepressants on hippocampal plasticity

Adult neurogenesis: overturning the dogma

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Scientist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent</td>
<td>1965</td>
<td>Altman and Das</td>
</tr>
<tr>
<td>Song bird</td>
<td>1980</td>
<td>Nottebhom</td>
</tr>
<tr>
<td>Primate</td>
<td>1990s</td>
<td>Gould and McEwen</td>
</tr>
<tr>
<td>Human</td>
<td>1998</td>
<td>Eriksson and Gage</td>
</tr>
</tbody>
</table>
Factors that affect adult neurogenesis

<table>
<thead>
<tr>
<th></th>
<th>Increase</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>Enrichment</td>
<td>Stress</td>
</tr>
<tr>
<td>Learning</td>
<td>Task acquisition</td>
<td>--</td>
</tr>
<tr>
<td>Drugs</td>
<td>Antidepressants</td>
<td>Opiates</td>
</tr>
</tbody>
</table>

Fluoxetine (Prozac) increases neurogenesis in the hippocampus

All Antidepressants Increase Hippocampal Neurogenesis

- Tricyclics
- SSRIs
- Lithium
- ECT
- NK1 antagonist
- Vasopressin V1B antagonist
- Running, Enriched Environment
Chronic fluoxetine stimulates neurogenesis in the dentate gyrus

Survival and Differentiation of BrdU positive cells

Strategies to block hippocampal neurogenesis

**Pharmacological**: drugs that block cell division (MAM)

**Genetic**:  
1. Mutation in serotonin pathway: 5-HT1A KO mice  
2. Selective and regulatable ablation of adult DG progenitor cells

**Radiological**: selective X-irradiation of a portion of the brain containing the hippocampus
### Behavioral tests to detect antidepressant action

**Models of Acute response**

- Tail suspension test
- Forced swimming test
- Ultrasonic vocalization

**Models of Chronic response**

- Learned helplessness
- Chronic unpredictable stress
- Novelty-suppressed feeding

### Behavioral Test to Detect Chronic Antidepressant Action

**Novelty-Suppressed Feeding (NSF)**

<table>
<thead>
<tr>
<th>Latency (%)</th>
<th>5 days</th>
<th>28 days</th>
</tr>
</thead>
</table>

### Mouse Serotonin 1A Receptor (5-HT1AR)

![Hippocampus](image)
5-HT1A KO mice are insensitive to the neurogenic and behavioral effects of chronic fluoxetine

Behavioral and neurogenic effect of chronic 5-HT1A agonist

Hippocampus-specific X-ray delivery
Hippocampus-specific suppression of neurogenesis

Irradiation prevents the effects of antidepressants in the Novelty-suppressed feeding test

Irradiation prevents the effects of antidepressants in the Chronic Unpredictable Stress paradigm
X-Ray treatment does not affect CA3/CA1 physiology, stress response and cued fear conditioning.

Summary
All antidepressants stimulate hippocampal neurogenesis: marker or cause?

Stimulation of hippocampal neurogenesis is necessary for antidepressant effects.

Targeting directly the mechanisms underlying hippocampal neurogenesis may lead to novel antidepressants.

Motor System Disease

Degeneration

Dysfunction
Motor System Disease

- Parkinson’s Disease
- Huntington’s Disease
- A.L.S. (Lou Gherig’s Disease)
- Primary Dystonia
- Tourette’s Syndrome
- Essential Tremor
Motor System Disease

Degeneration
- Parkinson’s Disease
- Huntington’s Disease
- A.L.S. (Lou Gherig’s Disease)

Dysfunction
- Primary Dystonia
- Tourette’s Syndrome
- Essential Tremor

Parkinson’s Disease: Clinical Characteristics

- Disease of aging:
  - Incidence: Overall 20/100,000
    Age 70 120/100,000

- Clinical Features: tremor, rigidity, slowness of movement, postural instability

- Pathology: substantia nigra pars compacta, locus coeruleus, nucleus basalis of Meynert, olfactory bulb

Control Parkinson’s Substantia Nigra

Lewy Body
Parkinson’s Disease: Environment

- 1918: influenza pandemic
- 1983: MPTP

MPTP Induced Parkinsonism

- Clinical: All cardinal manifestations of idiopathic PD.
- Pharmacology: Respond to dopaminergic drugs
- Pathology: Ventral substantia nigra, locus coeruleus, hypothalamus
- Pathogenesis: Mitochondrial dysfunction, oxidative stress
### Parkinson’s Disease: Genetics

<table>
<thead>
<tr>
<th>Locus</th>
<th>Inheritance</th>
<th>Onset</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1</td>
<td>Dominant</td>
<td>40’s</td>
<td>α-Synuclein</td>
</tr>
<tr>
<td>PARK2</td>
<td>Recessive</td>
<td>20’s</td>
<td>Parkin</td>
</tr>
<tr>
<td>PARK3</td>
<td>Dominant</td>
<td>60’s</td>
<td>??</td>
</tr>
<tr>
<td>PARK4</td>
<td>Dominant</td>
<td>30’s</td>
<td>??</td>
</tr>
<tr>
<td>PARK5</td>
<td>Dominant</td>
<td>50’s</td>
<td>Ubiquitin C-terminal hydrolase L1</td>
</tr>
<tr>
<td>PARK6</td>
<td>Recessive</td>
<td>20’s</td>
<td>??</td>
</tr>
<tr>
<td>PARK7</td>
<td>Recessive</td>
<td>20’s</td>
<td>??</td>
</tr>
</tbody>
</table>

### Parkinson’s Disease and α-Synuclein

- Mutations produce autosomal dominant PD
- Abundant in Lewy bodies
- Present in Glial Cytoplasmic Inclusions (MSA)
- Accumulates in Hallevordan-Spatz disease
α-Synuclein Biology

- Highly abundant in CNS
- Concentrated in presynaptic terminals near vesicles
- Lipid and vesicle binding
- Conformational change upon lipid binding
- Modulation of rate of recycling of the readily releasable pool

α-Synuclein Function: Vesicles
What is the nature of α-synuclein dysfunction that leads to dopamine neuron degeneration?
**α-Synuclein: Gene Targeting Strategy**

- **Wild Type Allele**
- **Targeting Vector**
- **Targeted Allele**

---

**Altered Normal Function**

- **α-Synuclein**
- **Novel Toxic Function**

- **Neurodegeneration**

---

**Lewy Body**
α-Synuclein: Knock Out Characterization

ES Cells

Northern

Mice

Western

MPTP Regimens

Acute

NECTROSIS

80 mg/kg

Sacrifice After 7 Days

Chronic

APOPTOSIS

30 mg/kg x 5 days

Sacrifice After 21 Days

α-Synuclein Null Mice: Resistance to MPTP-Induced Neurodegeneration

Nigrostriatal Nerve Terminals
α-Synuclein Null Mice: Resistance to MPTP-Induced Neurodegeneration

Nigrostriatal Cell Bodies

- Saline
- Chronic MPTP
- Acute MPTP

Common “Idiopathic” Parkinson’s Disease

Gene α-Synuclein

Environment MPTP

Diagram 1: α-Synuclein Null Mice: Resistance to MPTP-Induced Neurodegeneration

Diagram 2: Common “Idiopathic” Parkinson’s Disease

Diagram 3: Gene Environment α-Synuclein MPTP
An Inducible Model of HD

A brief introduction to HD (1):

- George Huntington, 1872
  - Age of onset: 40 to 50 yrs
  - Progressive
    - Motor: chorea, dystonia
    - Psychiatric: depression, anxiety, suicide
    - Cognitive changes: declarative memory, dementia
  - Autosomal dominant inheritance

Striatal specific atrophy of HD

![Brain Image]
A brief introduction to HD (2):

- The HD gene, IT15 (HDCRG, 1993)
  - Promoter: housekeeping gene?
  - Conserved to Fugu puffer fish
  - Gene KO studies
    - Nasir et al., Zeitlin et al., Duyao et al.
- Gene product, huntingtin (htt)
  - Large protein in complex
  - Function: unknown
  - Ubiquitous expression
  - Cleavage by calpain and caspase-3

A brief introduction to HD (3):

- Triplet repeat expansion (C-A-G)
  - Unaffected, n=4 to 35; Pathogenic, n > 37
  - Triplet repeat disorders (17)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Inheritance Pattern</th>
<th>Repeat Normal Length</th>
<th>Expanded Length</th>
<th>Affected Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragile X</td>
<td>FMR1</td>
<td>X-linked dominant</td>
<td>CGG 6-52</td>
<td>230-1000</td>
<td>Brain enlargement, connective tissue</td>
</tr>
<tr>
<td>Fragile XE</td>
<td>FMR2</td>
<td>X-linked (?), dominant</td>
<td>GCC 7-35</td>
<td>230-750</td>
<td>Brain enlargement, connective tissue</td>
</tr>
<tr>
<td>Myotonic Dystrophy</td>
<td>DMPK</td>
<td>Dominant CTG 5-37</td>
<td>50-3000</td>
<td>Wide range of organ systems.</td>
<td></td>
</tr>
<tr>
<td>Friedrich's Ataxia</td>
<td>Frataxin</td>
<td>Recessive GAA 7-22</td>
<td>200-900</td>
<td>DRG (sensory neurons), spinocerebellar tract</td>
<td></td>
</tr>
<tr>
<td>SBMA</td>
<td>Androgen receptor</td>
<td>X-linked recessive CAG 9-36</td>
<td>38-62</td>
<td>Spinal chord-motoneurons</td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>Huntingtin</td>
<td>Dominant CAG 6-34</td>
<td>36-121</td>
<td>Striatum and cortex</td>
<td></td>
</tr>
<tr>
<td>SCA1</td>
<td>Ataxin-1</td>
<td>Dominant CAG 6-44</td>
<td>39-82</td>
<td>Cerebellar purkinje cells, fronto-temporal lobes</td>
<td></td>
</tr>
<tr>
<td>SCA2</td>
<td>Ataxin-2</td>
<td>Dominant CAG 15-31</td>
<td>36-63</td>
<td>Cerebellar dentate neurons, brain stem</td>
<td></td>
</tr>
<tr>
<td>SCA3</td>
<td>Ataxin-3</td>
<td>Dominant CAG 12-41</td>
<td>62-84</td>
<td>Cerebellar dentate cells, striatum, brain stem</td>
<td></td>
</tr>
<tr>
<td>SCA6</td>
<td>CACNA1a</td>
<td>Dominant CAG 4-18</td>
<td>21-33</td>
<td>Cerebellar purkinje cells, inferior olive, dentate</td>
<td></td>
</tr>
<tr>
<td>SCA7</td>
<td>Ataxin-7</td>
<td>Dominant CAG 4-35</td>
<td>37-306</td>
<td>Macula, cerebellum, visual cortex</td>
<td></td>
</tr>
<tr>
<td>SCA12</td>
<td>PP2A-B?</td>
<td>Dominant CAG 7-28</td>
<td>65-78</td>
<td>Cortex and cerebellum</td>
<td></td>
</tr>
<tr>
<td>DRPLA</td>
<td>Atrophin-1</td>
<td>Dominant CAG 6-36</td>
<td>49-84</td>
<td>Cortex, striatum, cerebellum</td>
<td></td>
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<tr>
<td>SCA17</td>
<td>TBP</td>
<td>Dominant CAG 29-42</td>
<td>47-55</td>
<td>Cerebellum, striatum, cortex</td>
<td></td>
</tr>
<tr>
<td>SCA8</td>
<td>Unknown</td>
<td>Dominant CTG 16-91</td>
<td>107-127</td>
<td>Cortex and cerebellum</td>
<td></td>
</tr>
<tr>
<td>SCA17</td>
<td>TBP</td>
<td>Dominant CAG 29-42</td>
<td>47-55</td>
<td>Cerebellum, striatum, cortex</td>
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<tr>
<td>SCA8</td>
<td>Unknown</td>
<td>Dominant CTG 16-91</td>
<td>107-127</td>
<td>Cortex and cerebellum</td>
<td></td>
</tr>
</tbody>
</table>
CAG repeat length influences age of onset

Effect of CAG expansion on htt

• CAG expansion is translated
  – CAG encodes for glutamine (Q)

• No loss of function
  – Patients homozygote for HD mutation

• Dominant gain of function

Mouse models of HD:

• Bates’ R6 transgenic (R6/2)
  – Mangiarini et al. (1995) Cell

• cDNA transgenic

• N171 transgenic

• YAC transgenic

• (CAG)n knock-in
  – Shelbourne et al. (1999) Hum Mol Genet 8, 763-74
  – Lin et al. (2001) Hum Mol Genet 10, 137-44
Intracellular aggregates

- Davies et al., 1997
  - Intranuclear aggregates
  - "Dark bodies" (Roizin, 1977)
- DiFiglia et al., 1997
  - Intranuclear aggregates
  - Cytoplasmic aggregates
- Scherzinger et al., 1997
  - Ribbon-like morphology

Question:

- Is continuous expression of the transgene required for the HD-like phenotype?
- Is the HD-like phenotype reversible?

Inducible mouse model
A model with a switch
Tet-regulatable system

Expression pattern of polyQ-htt

Intranuclear & cytoplasmic aggregates
Neuropathology in HD94 mice

HD mice demonstrate a progressive clasping phenotype

Summary

- Expression of exon1 CAG94 leads to an HD-like phenotype
Protocol

- 2 mg dox/ml 5% sucrose
- Duration: 16 weeks
  - Halt progression
  - Amelioration
Behavioral Reversal in HD94 mice
Summary

- Expression of exon1 CAG94 leads to an HD-like phenotype
- Abolishing gene expression for 16 weeks leads to a reversal of aggregate formation and motor phenotype

Primary striatal cultures from HD94 mice

- Kinetics of aggregate formation and reversion
- Possible mechanisms for aggregate reversion

Aggregate formation in vitro
Aggregate Formation in vitro

A

Inclusion-positive neurons (%)

β-Tub HD94-htt C C C HD94
DIV1 DIV2 DIV3

B

Inclusion-positive neurons (%)

0 2 4 6 8 10 12 14 16

Aggregate Formation and Reversion in vitro

Without Tet
Tet from DIV3
Tet from DIV4

Inclusion-positive neurons (%)

0 2 4 6 8 10 12 14 16

Lactacystin inhibits aggregate reversion

Tet Lact
DIV7 DIV9 DIV9

*
Summary (2)

- **In vitro**
  - “Soluble” exon1CAG94 clears within 2 to 3 days of shutting down gene expression.
  - Aggregates clear within 5 days of shutting down gene expression (3d + 2d).
  - Aggregate clearance is inhibited in the presence of lactacystin (proteasome).

Tissue specificity of the pathology

Tissue-specific expression of mutant htt