Support/Conflicts

• Support for basic research by NIH RO1’s and other grants

• Support from Seaside Therapeutics and pending from Hoffmann-La Roche for clinical trials for FraX and autism

• Unapproved drugs are used under approved IRB protocols
Intellectual Disabilities

- Most common developmental disorder, 1/70 individuals, more than 1.5 million in U.S., 1.5:1, boys to girls
- Definition: Intelligence quotient (IQ) less than 70 plus 2 or more disorders of adaptive behavior with onset in childhood
- Adaptive skills: Daily living skills, communication skills or social skills
Disorders Associated with Intellectual Disability

- Behavior disorders, self-injury
- Psychiatric disorders, depression
- Motor, hearing, vision problems
- Seizures
- GI motility problems, reflux
- Overlap with autism spectrum disorders
## Levels of Intellectual Disability

<table>
<thead>
<tr>
<th>Level</th>
<th>IQ</th>
<th>%</th>
<th>Independence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>50-69</td>
<td>85</td>
<td>Most work</td>
</tr>
<tr>
<td>Moderate</td>
<td>34-49</td>
<td>10</td>
<td>With support</td>
</tr>
<tr>
<td>Severe</td>
<td>20-34</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>Profound</td>
<td>&lt;20</td>
<td>2</td>
<td>None</td>
</tr>
</tbody>
</table>
Most Common Causes of ID

Most common **genetic**: Down syndrome (Trisomy 21)

Most common **inherited**: Fragile X Syndrome

Leading global cause: Iodine deficiency causing thyroid hormone deficiency (2 billion people)

Leading causes in Baltimore City: fetal ethanol, psychosocial, lead (declining)
Autism Spectrum Disorders

- 1:110-150 children, 4:1 boys to girls
- Three related disorders: autism, pervasive developmental disorder (PDD) and Asperger syndrome
- Autism: social disability, communication disorder and repetitive, stereotyped behaviors
- As many as 70% of individuals with autism have IQ below 70
Causes of ID by IQ Level

- **Mild** 80% unidentified, psychosocial, toxins, lead, fetal alcohol, genetic?
- **Moderate** More likely genetic, e.g. Down syndrome, Fragile X
- **Severe** Genetic more likely especially
- **Profound** Associated with a syndrome
Fetal Alcohol Syndrome

Small eyes, smooth filtrum, thin upper lip
Fetal Alcohol Spectrum Disorder

- Facial anomalies, pre/post natal growth retardation, functional or structural CNS problems
- Neuronal damage/cell loss in the fetal brain
- Not inherited, but genetic predisposition
- Behavioral and learning difficulties
Ethanol Interacts with Major Neurotransmitters

Glu = Excitation

GABA = Inhibitory

NMDA = glutamate agonist
Glutamate is a flavor enhancer in food and is also the most prevalent neurotransmitter at 70% of synapses.

Glutamate is excitatory and acts at four types of receptors, the blood brain barrier keeps it from exciting the brain when consumed in restaurants.
Major Types of Receptors for Glutamate
GABA is also available as a supplement but does not cross the blood brain barrier.
Ethanol Enhances GABAergic Inhibition and Blocks NMDA Mediated Glutamate Trophic Effects
Glutamate:
Both trophic and toxic effects

High levels of ethanol can cause apoptosis and abnormal migration in the fetal brain by blocking glutamates trophic effect on neurons
Down Syndrome – Trisomy 21
Major Abnormalities in TS65Dn Mouse Model of DS

- Shorter, thinner dendritic spines
- Atrophy of cholinergic neurons in the nucleus basalis of Meynert (NBM)
- Reduced retrograde transport of nerve growth factor (NGF) into NBM neurons
- Signs of premature Alzheimer type disease in humans with DS
- Increased GABAergic inhibition

Increased number of shorter spines during normal development

Shorter, thinner spines in Down Syndrome
Improvement of Learning in Ts65 Down Syndrome Mouse Model by Inverse Agonist of GABA-A alpha 5 subtype [Braudeu et al]
Inverse Agonist

- Activation by saturating endogenous ligand
- Constitutive activity of receptor in absence of ligand

Graph:
- Full agonist
- Partial agonist
- Neutral antagonist
- Inverse agonist

Response vs. [Drug]
- Y-axis: Response
- X-axis: [Drug] (10^-10 to 10^-6)
Many Intellectual Disabilities Are Due to Impaired Synaptic Plasticity

Concept of impaired synaptic plasticity simplifies understanding neurobiology of intellectual disabilities.
The Developing Brain is Under Construction

Like this building under construction, the immature brain has connections and structures that will be removed when it is finished.
Synaptic Connections Proliferate During The Period of Maximal Plasticity
Synaptogenesis in Human Cerebral Cortex

Correlates with Cognitive Development

Visual Cortex

Prefrontal Cortex

Auditory Cortex

P.R. HUTTENLOCHER AND A.S. DABHÖLKAR
Trajectories of Change in Cortical Thickness: Peak Cortical Thickness Delayed in Higher IQ Groups

Blue - Superior IQ  Green - High IQ  Red - Average IQ

(Superior IQ – 121-149  High IQ – 109-120  Average IQ – 83-108)

Developing Differences in Cortical Thickness Between Superior and Average Intelligence Groups


Average IQ = 83-108      Superior = 121-149
Plasticity Depends on A Balance Between Excitement and Inhibition

- Powerful excitatory circuits in the brain are required for memory, learning and reorganization
- These circuits reflect a balance of the excitatory neurotransmitter **glutamate** and the inhibitory neurotransmitter **GABA**
Architecture of Synapses is Shaped by Activity:
Dendritic spines receive only one excitatory synapse; dendritic shafts have multiple inhibitory synapses.

Excitatory synapses are more likely to be eliminated after birth than inhibitory synapses.

Glutamate and GABA Control Neuronal Excitability

**Glu** = Excitation

**GABA** = Inhibitory

**Glu** = Excitation

**GABA** = Inhibitory

Glutamate with GABA Blocked = Excitation

**NMDA** = glutamate agonist
Excitatory Synapse

Post-synaptic Glutamate Synapses

[Lau and Zukin, Nat Neurosci Rev 2007]
Glutamate Receptors Are Anchored by a Complex Protein Scaffolding

SYNGAP1 mutations cause intellectual disability and autism. This protein normally represses synaptic excitability during development. Enhanced excitability during development is associated with premature development of dendritic spines, which may stunt their growth, thereby impairing intellectual potential.
Changes in synapse activity change the shape of dendrites: Enhanced excitement causes wider, shorter dendritic spines.
Mutations in the Neurexin and Neuroligin Cell Adhesion Molecules Are Linked to Autism and Associated Neurobehavioral Disorders

Transsynaptic Signaling
by Activity-Dependent Cleavage of Neuroligin-1

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http://dx.doi.org/10.1016/j.neuron.2012.07.006

Activity-Dependent Proteolytic Cleavage of Neuroligin-1

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http://dx.doi.org/10.1016/j.neuron.2012.10.003
NMDA receptor activity at glutamate synapses cleaves neuroligin 1 via a calcium/calmodulin dependent process that is mediated by matrix metalloprotease 9. Cleavage of Neuroligin 1 destabilizes neurexin and depresses synaptic neurotransmission by reducing presynaptic release probability. Neuroligins appear to provide negative feedback to pre-synaptic nerve terminals to reduce excitatory activity to the when activity is high.
Neuroligins in excitatory synapses may fine tune activity to maintain optimal pace of synapse development.

Changes in Glutamate Receptors Support Long Term Potentiation (LTP), a Form of Synaptic Plasticity Associated with Memory Formation

[Squire and Kandel, 1999]

Rapid Stimulation Leads to Enhanced Response
LTP is Enhanced in the Immature Brain

A critical period for long-term potentiation at thalamocortical synapses

Michael C. Crair & Robert C. Malenka

Departments of Psychiatry and Physiology, Center for Neurobiology and Psychiatry, University of California, San Francisco, California 94143, USA
Activity Leads to Plasticity in Dendrites

Learning new motor skills and acquiring new sensory experiences is associated with formation of new synaptic connections in motor and sensory regions of the mouse brain.
Sequential performance of two new motor learning tasks can lead to formation of new synapses and deletion of others.

Dendritic spine shape changes with potentiation or depression.
Long term potentiation and depression shape dendritic spines
Cognitive Disorders Due to Impaired Signaling and Plasticity

Glutamate Receptors

Post-Synaptic

Membrane

NF-1: Neurofibromin: Ras/GTPase activating protein (GAP)

Tuberous Sclerosis: Hamartin/Tuberin: GAP proteins for Ras-like Rheb

Fragile X: FMRP, RNA binding protein, mGluR receptors

Rubinstein-Taybi: CREB binding protein

Coffin-Lowry: Rsk-2, CREB

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Pb++ - Glutamate, PKC

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Pb++ - Glutamate, PKC
Rett Syndrome
Rett Syndrome

Signs of neuronal dysfunction: acquired microcephaly, stereotypic movements, seizures, intellectual disability, abnormal breathing, autonomic dysfunction, dystonia, and ataxia.

Neuropathology: Reduced gray matter, neurons packed too close together (Armstrong et al).
Brain Growth Decelerates In Rett Syndrome During The Period of Maximal Synapse Proliferation
Rett Syndrome Is Due to Mutations in MeCP2, a Transcriptional Factor

MeCP2 silences transcription and is important for neuronal differentiation

Action of MeCP2 on Gene Expression is Activity Dependent

(Martinowich, et al, Science 302:890-893,
Glutamate Receptors Higher in Cortex of Young Girls with RTT

NMDA Receptors Higher in 2 Year Old with Rett Syndrome v. Control

NMDA Receptors Lower in 10 Year Old with Rett Syndrome v. Control

Density of Synapses is reduced in Rett.

Ratio of NMDA receptors/synapses is higher in Rett.
Other Disorders of Glutamate in Rett Syndrome

- Increased glutamate levels in postmortem brain tissue from girls with RTT [Wenk et al, 1997]
- Increased glutamate/glycine in brain on MRS in patients with RTT [Pan et al, 1999]
- Increased glutamate transporters in brain tissue on microarray [Colantuoni, 2001]
- Increased levels of glutamate in CSF [Riikonen, 2003]
MR Spectroscopy Shows Elevated Glutamate/Glycine Peak in Younger Girls with RTT

(Naidu et al 2008, Kirby Imaging Center)
Activity-dependent Refinement of Synaptic Connections

Synchronous firing of 2 axons on the right leads to enhanced glutamate receptor activation, post-synaptic depolarization, and trophic “feedback.”

Mecp2 deficient neurons are more vulnerable to excitotoxic death and fail to form stable synapses; one common factor in these pathologies could be altered levels of growth factors, e.g. BDNF.

The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression, Chang et al, Neuron 49:341-348, 2006.
Olfactory Biopsies Demonstrate a Defect in Neuronal Development in Rett’s Syndrome

Gabriele V. Ronnet, MD, PhD,1,2 Donald Leopold, MD,3 Xiaohe Cai, MD,1 Kristen C. Hoffbuhr, PhD,4 Linda Moses, BA,4 Eric P. Hoffman, PhD,4 and SakkuBai Naidu, MD2,5

Mecp2 Deficient Mice Have Immature Post-Synaptic Densities and Fewer Dendritic Spines

[Fukuda et al 2005]
MeCP2 Defect Prevents Postnatal Burst in Synaptogenesis, Causing Microcephaly
Rett Syndrome Impairs Synaptic Plasticity

- Synapses
- Neuronal Circuits
- Gene Transcription
- Signal Cascades

Diagram showing connections between Rett Syndrome, synaptic plasticity, synapses, neuronal circuits, signal cascades, and gene transcription.
Clinical Trials for RTT Aimed at Synaptic Targets at KKI

- **Dextromethorphan**: Competitive antagonist at NMDA Channel
- **Donepezil**: Acetylcholinesterase inhibitor, compensates for deficit in cholinergic nucleus basalis in RTT; promotes development of axons and dendrites as well as activity-dependent plasticity in neuronal networks

Dr. Sakku Naidu FDA-NIH R01 Grant and IRB approved clinical trial of dextromethorphan in RTT
Neurofibromatosis Type 1: Mild/Moderate Learning Disorders
Neurofibromatosis and Tuberous Sclerosis

Mutation in GAP enhances Ras and GABAergic Inhibition in NF-1 Mouse

NF-1: Ras/GTPase-activating protein (GAP)

TS-2: GTPase-activating protein

mRNA, Synaptic Protein

New Therapies Under Study for NF-1

**Lovastatin** - used to reduce cholesterol, also inhibits P21Ras/mitogen activated protein kinase and improves cognition in mice with NF-1

**Rapamycin** – In trial for plexiform neuromas

**Humanized antibody against VegF** - In trial for tumors
Fragile X Syndrome

10% of inherited ID

• 1/1500 males, 1/2500 females

• Mild to moderate ID

• ADHD and social avoidance

• Long narrow face, prominent jaw, large ears and testicles

• Reduced expression of fragile X MR protein (FMRP) due to expanded CGG repeats
Fragile X Mice

Dendritic spines of pyramidal neurons from FMR1 knockout mice are longer than those in wild-type mice, and thin and tortuous.

[Comery, et al, PNAS 1997;94:5401.]
Box 2 | Correlates of synaptic strength

Enhanced LTD in FraX

- Spine head
- Synaptic vesicles
- PSD
- AMPAR
- NMDAR

Spine brightness (integrated fluorescence) vs. Spine volume
PSD size, AMPAR content vs. Spine volume
Number of docked synaptic vesicles vs. PSD size
Major Types of Receptors for Glutamate
FMRP Inhibits Long Term Depression: In FraX Mouse, Reduced AMPA Activity Increases LTD

Potential Therapies for Fragile X Syndrome

• **Ampakine Compound CX516** - Positive modulator of AMPA type glutamate receptors.

• **mGluR5 receptor antagonists** - 2-methyl-6-(phenylethynyl) pyridine reverses neuronal changes in mouse and fruit fly models and some human trials.

• Rbaclofen reduces presynaptic release of glutamate via activating GABA-B receptors
Investigational Therapies for Fragile X Syndrome

- Fragile X Syndrome (FXS) and Autism Spectrum Disorders
  - FXS is universally associated with autistic spectrum symptoms, and is the most common genetic cause of Autism
  - mGluR theory of FXS (Bear et al., 2004)

Rbaclofen inhibits presynaptic glutamate release

mGluR5 inhibitor reduces post-synaptic glutamate signaling

Seaside Therapeutics
GABA_B Receptors inhibited by Rbaclofen
KKI Clinical Trials Center

- IRB Approved Trials of Arbaclofen for Fragile X and Autism
- Starting up trial of mGluR antagonist
Tuberous Sclerosis

- Ash-leaf spots, other skin lesions
- Tubers

Mental Retardation
Seizures
Infantile Spasms
Retinal Lesions
Heart, Kidney tumors
Tuberous Sclerosis

TSC1-Hamartin
TSC2-Tuberin mutations lead to up-regulation of Ras homologue GTPase: Rheb

(Goh and Weiss, Annals of Neurology, 60:506, 2006)
Rapamycin Prevents Epilepsy in a Mouse Model of Tuberous Sclerosis Complex

Ling-Hui Zeng, MD, PhD, Lin Xu, PhD, David H. Gutmann, MD, PhD, and Michael Wong, MD, PhD

Annals of Neurology 2008;63:444-453

- Early treatment with rapamycin prevented development of epilepsy and premature death in Tsc1GFAP KO mouse
- Late treatment suppressed seizures and prolonged survival
FMR1 and TSC2 mutations normalize LTD in a genetic cross experiment.

Phosphatase and Tensin Homolog PTEN Mutations

Figure 1  Frontal and profile views of subject two with a PTEN mutation (D252G) at 3.5 years of age showing macrocephaly. (Photograph reproduced with permission.)
Growth factors, hormones

- PI3K
- Akt
- PTEN
- AMPK
- TSC2
- TSC1
- mTOR
- S6K
- S6
- 4E-BP1
- eIF4E

Protein synthesis
Phosphatase and Tensin Homologue (PTEN) Conditional Knockout Mouse

PTEN Mutations increase length and number of dendritic spines

C. Normalized fEPSP slope

WT

KO

Control

PTEN cKO

B.

D.
Cognitive Disorders Due to Impaired Signaling and Plasticity

**NF-1:** Neurofibromin: Ras/GTPase activating protein (GAP)

**Tuberous Sclerosis:** Hamartin/Tuberin: GAP proteins for Ras-like Rheb

**Fragile X:** FMRP, RNA binding protein, mGluR receptors

Cretinism: Silenced transcription
Neurobiology of Intellectual Disabilities

Synapses

Signal Cascades

Neuronal Circuits

Gene Transcription

Intellectual Disabilities Due to Impaired Synaptic Plasticity
Neuroscience Laboratory Team

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