Pathways to New Treatments in Autism Spectrum Disorder

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- Brain and Behavior Research Foundation
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- Wiley
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- Roche Pharmaceuticals
- Novartis
- Forest
- SynapDx

Disclosures, Part 2:
VVW research group and others

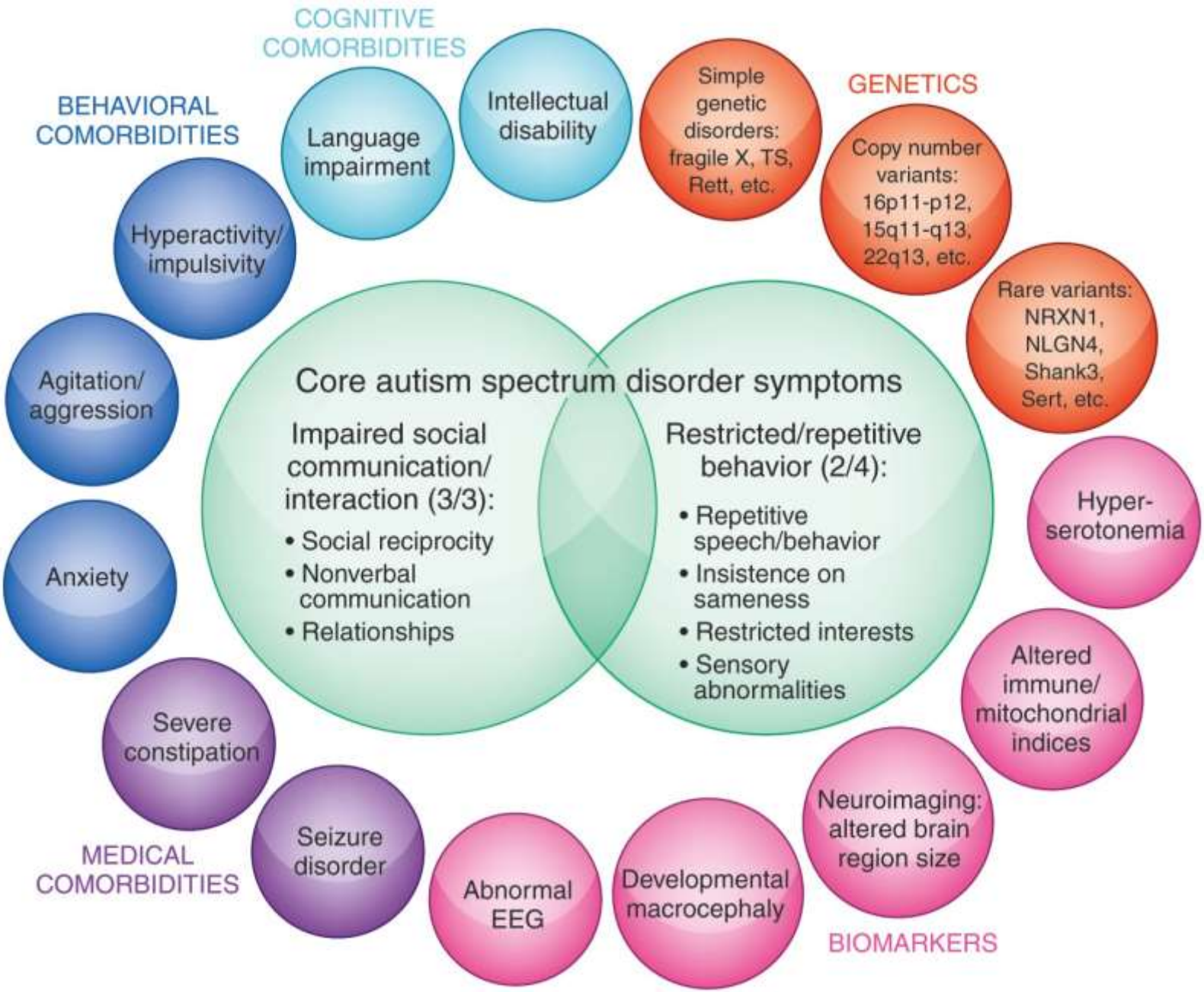
Outline

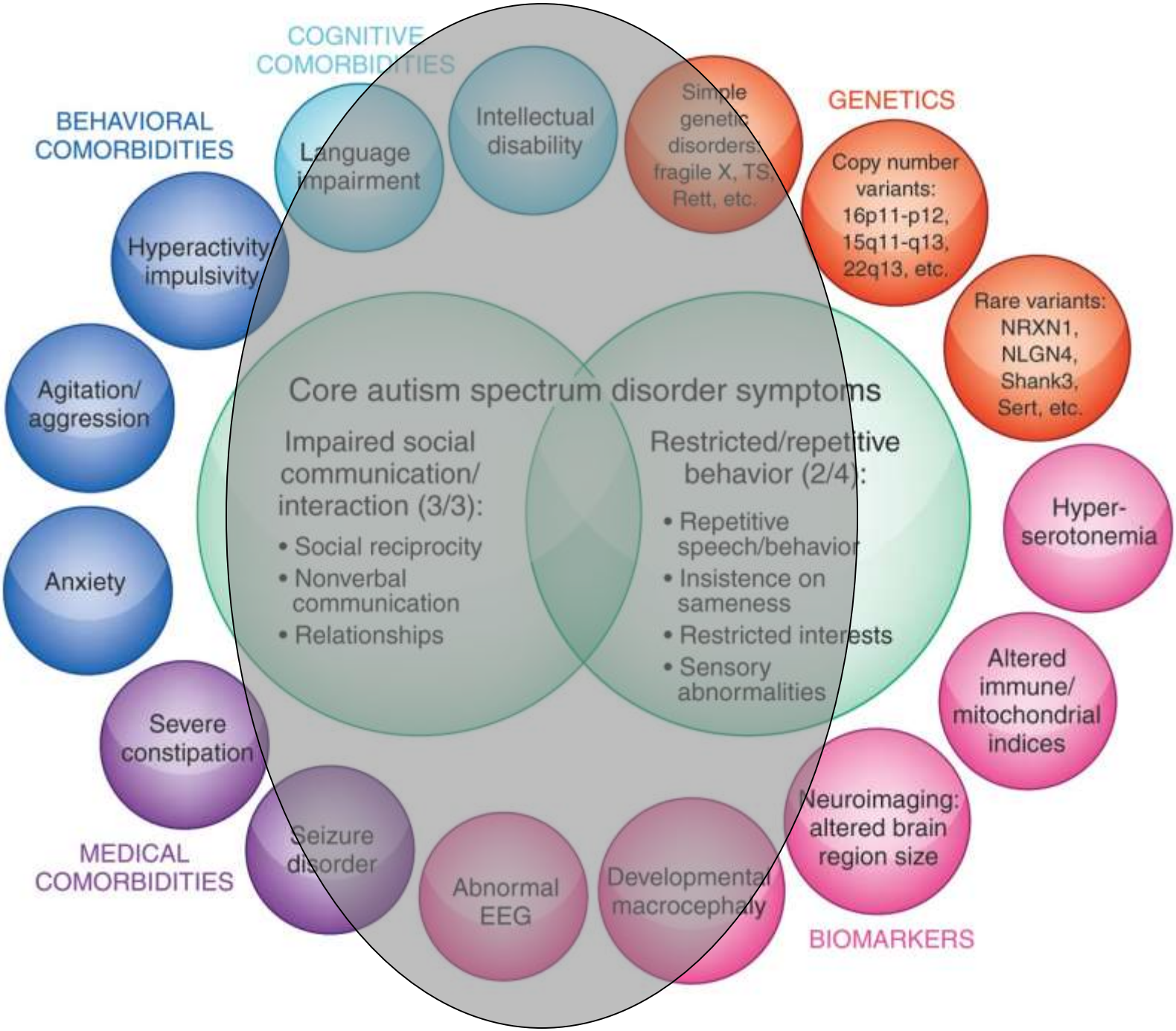
- Challenge of heterogeneity
- How have we tested treatments in ASD?
- How do we find new treatments?
  - Rare Genetic Disorders → Molecular Targets
  - Symptomatic Treatments → Circuitry Targets?
- In whom should we study new treatments?
  - Biomarker-based therapeutics?
- The ultimate goal: combined medical and behavioral treatment

What is Autism Spectrum Disorder?

"Autism is not a disease." – Isabelle Rapin

It is certainly not a single disease.


COGNITIVE COMORBIDITIES
BEHAVIORAL COMORBIDITIES
Language impairment
Intellectual disability
Simple genetic disorders: fragile X, TS, Rett, etc.
GENETICS
Copy number variants: 16p11-p12, 15q11-q13, 22q13, etc.
Rare variants: NRXN1, NLGN4, Shank3, Sert, etc.
Hyperactivity/ impulsivity
Agitation/ aggression
Core autism spectrum disorder symptoms
Impaired social communication/ interaction (3/3):
• Social reciprocity
• Nonverbal communication
• Relationships
Restricted/repetitive behavior (2/4):
• Repetitive speech/behavior
• Insistence on sameness
• Restricted interests
• Sensory abnormalities
Hyper-serotonemia
Anxiety
Altered immune/ mitochondrial indices
Severe constipation
Neuroimaging: altered brain region size
Seizure disorder
Abnormal EEG
Developmental macrocephaly
MEDICAL COMORBIDITIES
BIOMARKERS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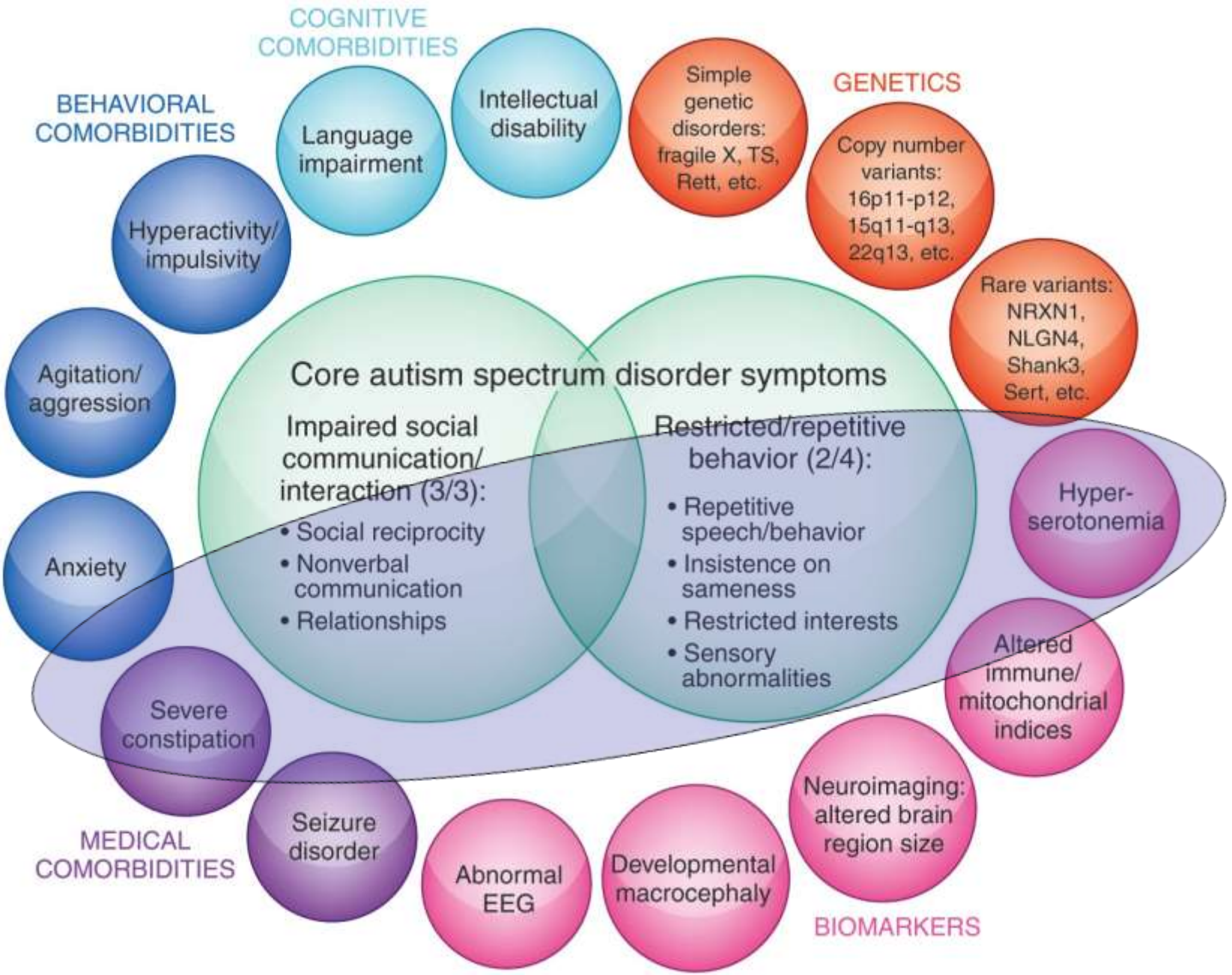
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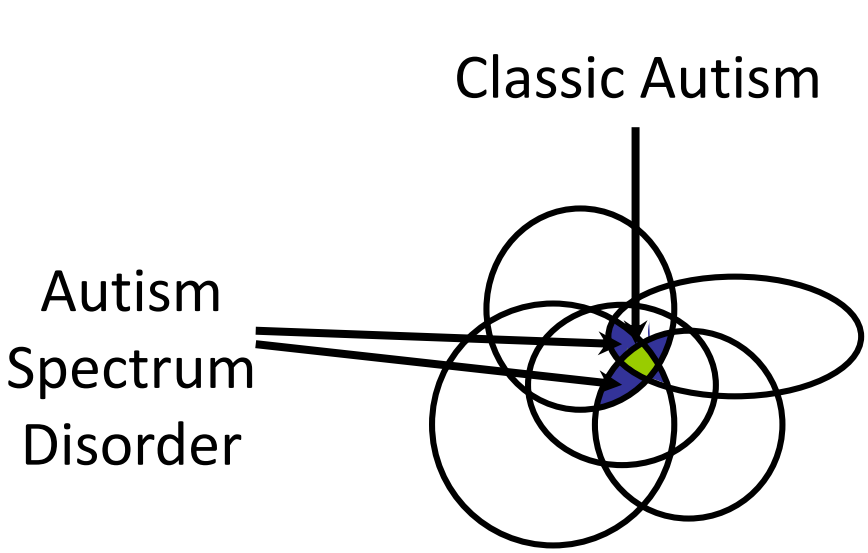
Model of Autism Risk



Rare variants/Syndromes

Fragile X (~1-2%) (*FMR1*)

Chr. 16p11 Del (~1%)

Tuberous Sclerosis (*TS1/2*)

PTEN hamartoma syndrome

Phelan-McDermid (~0.7%)

CHD8 disruption (~ 0.5%)

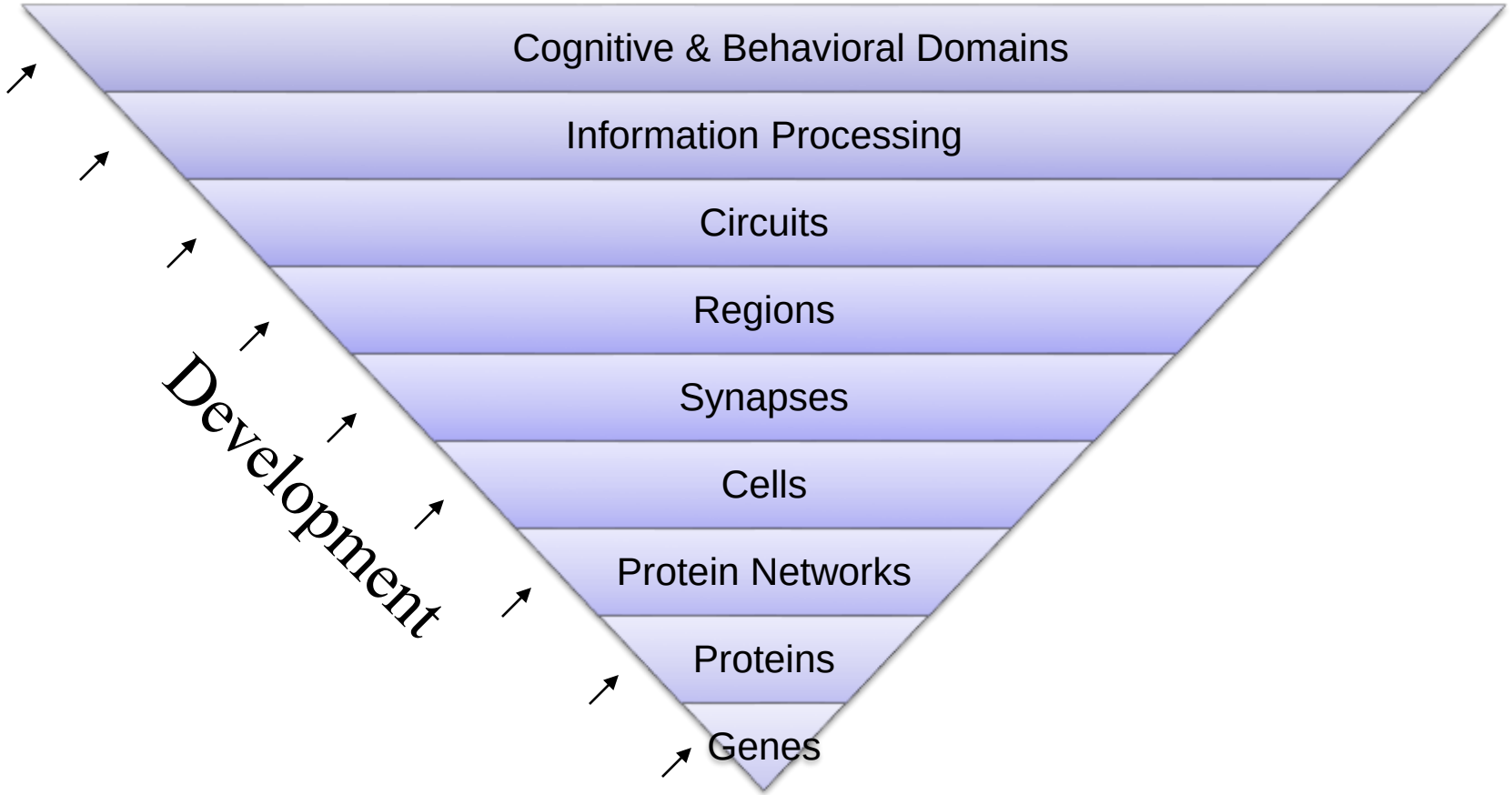
Fetal valproate exposure

Common variants

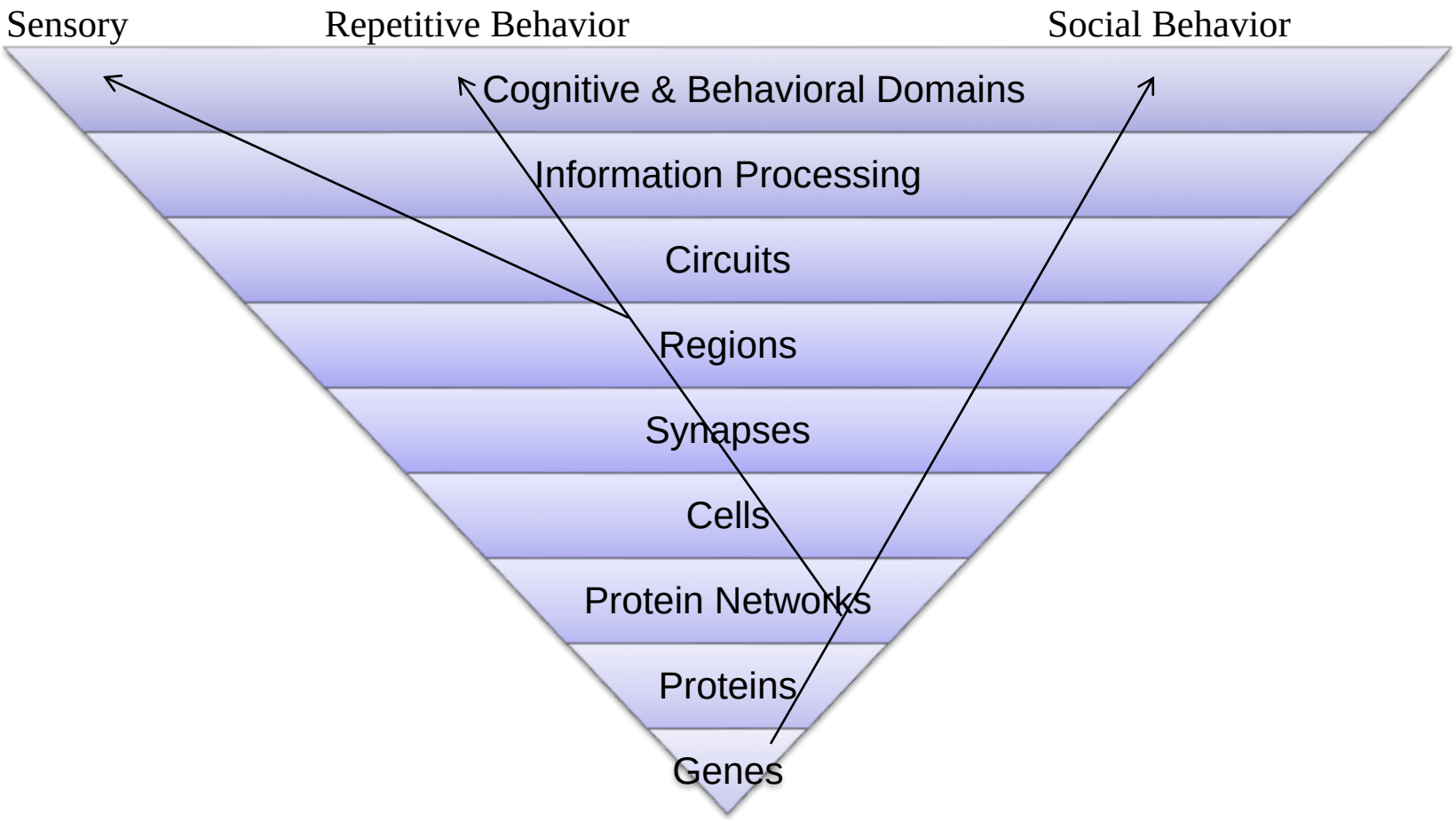
Each overlapping circle indicates a common risk factor

Slide modified from Ed Cook

How is risk realized?



Risk genes feed a cascade of impact



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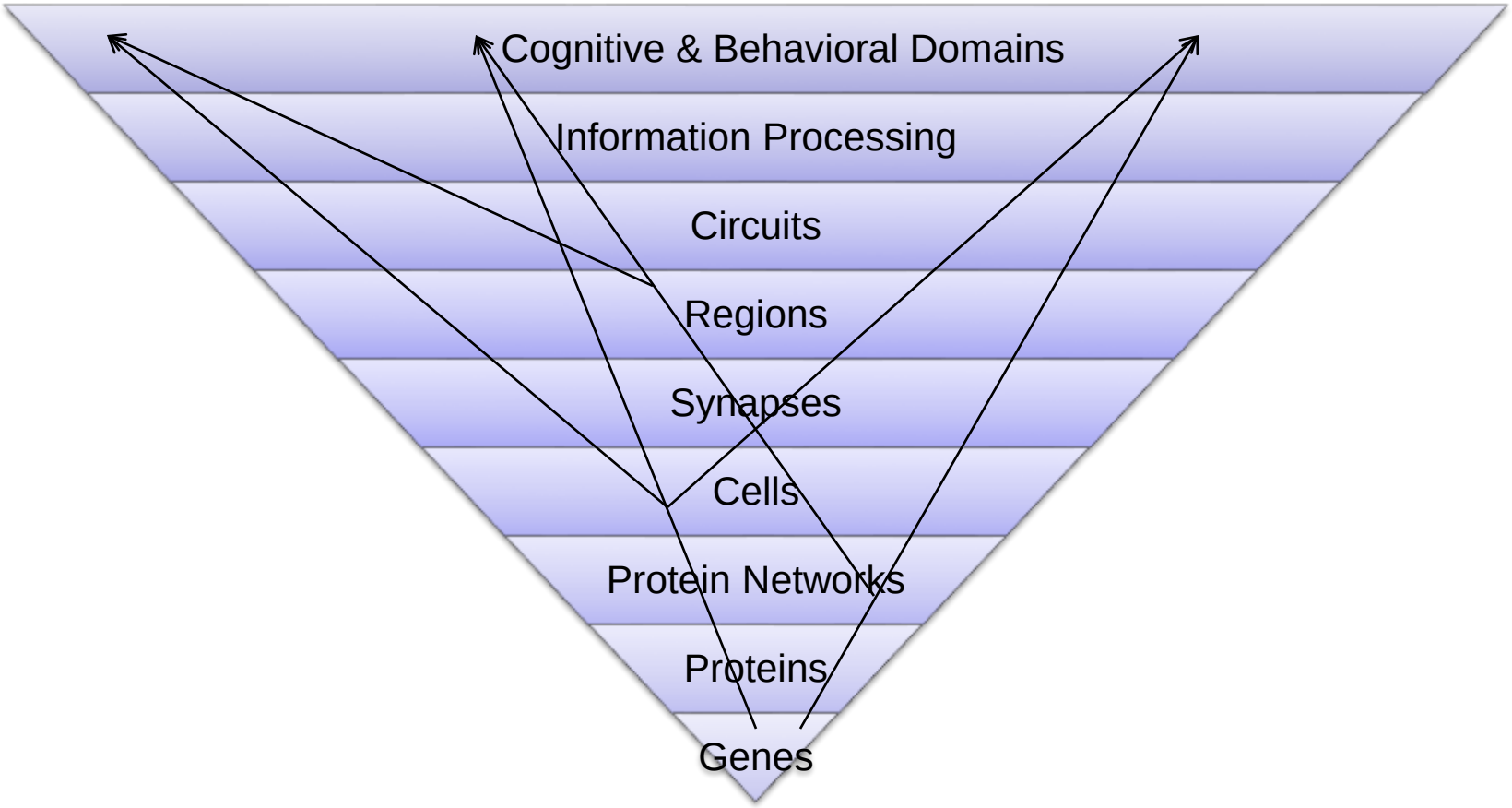

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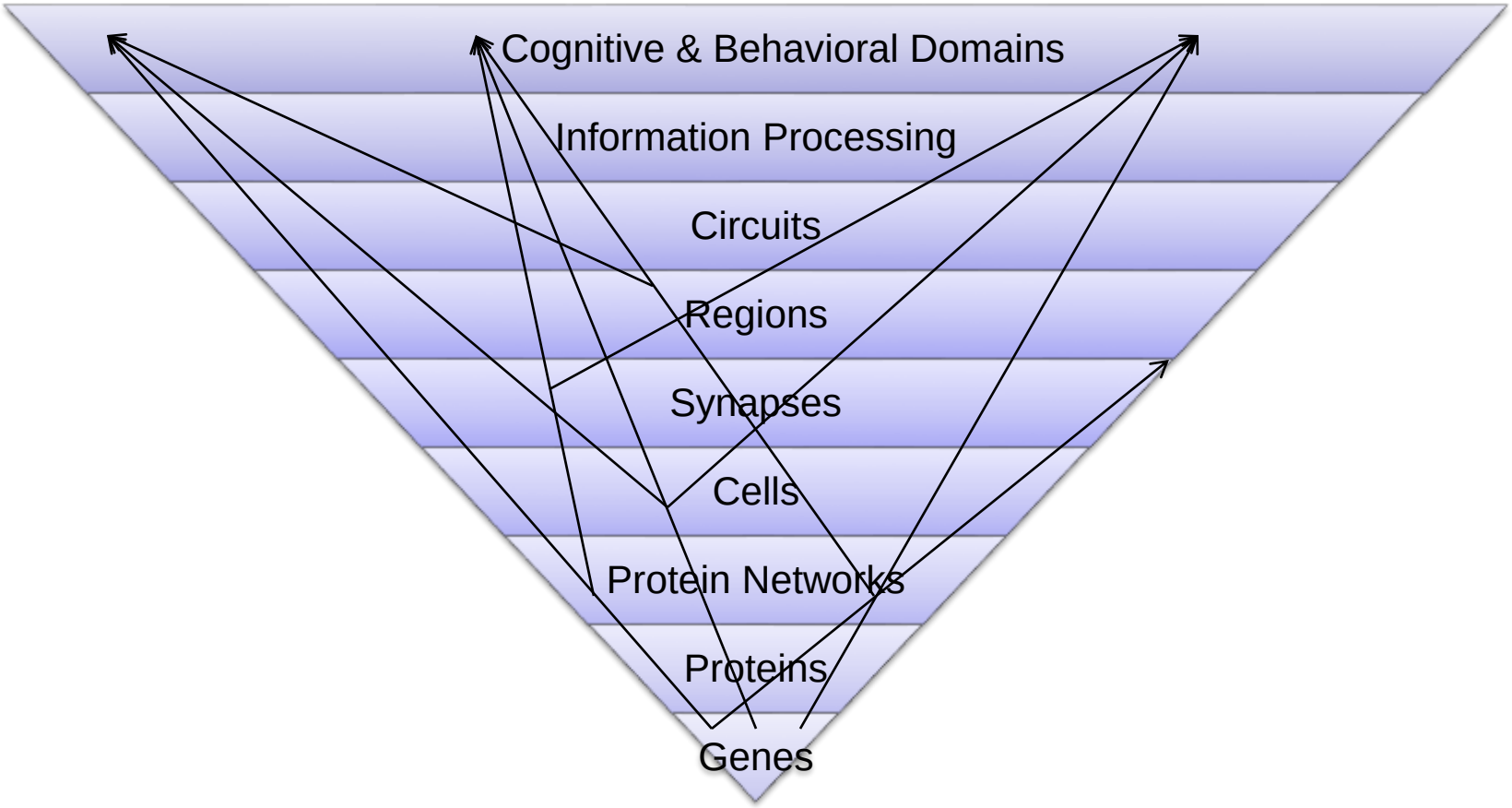
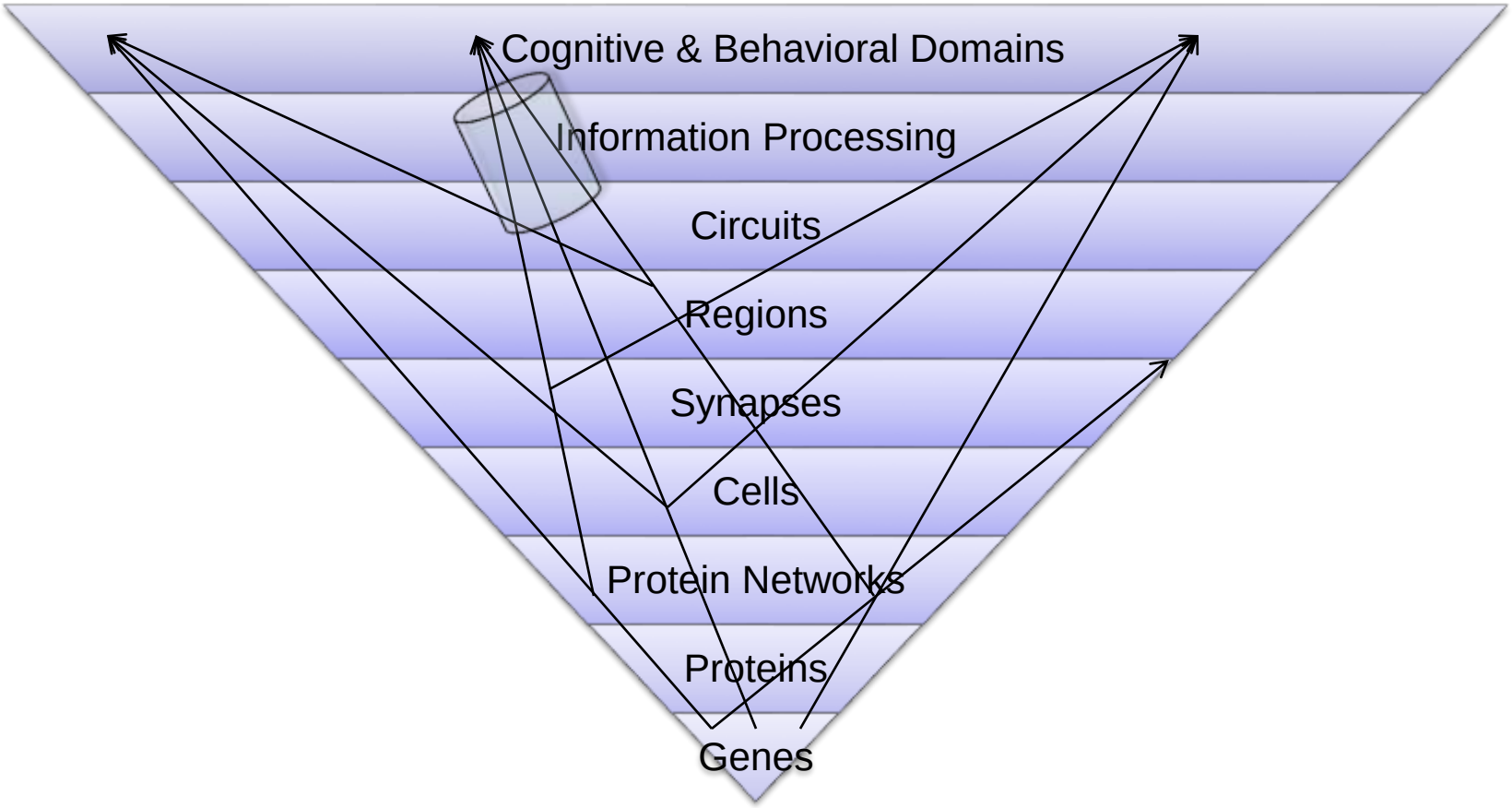
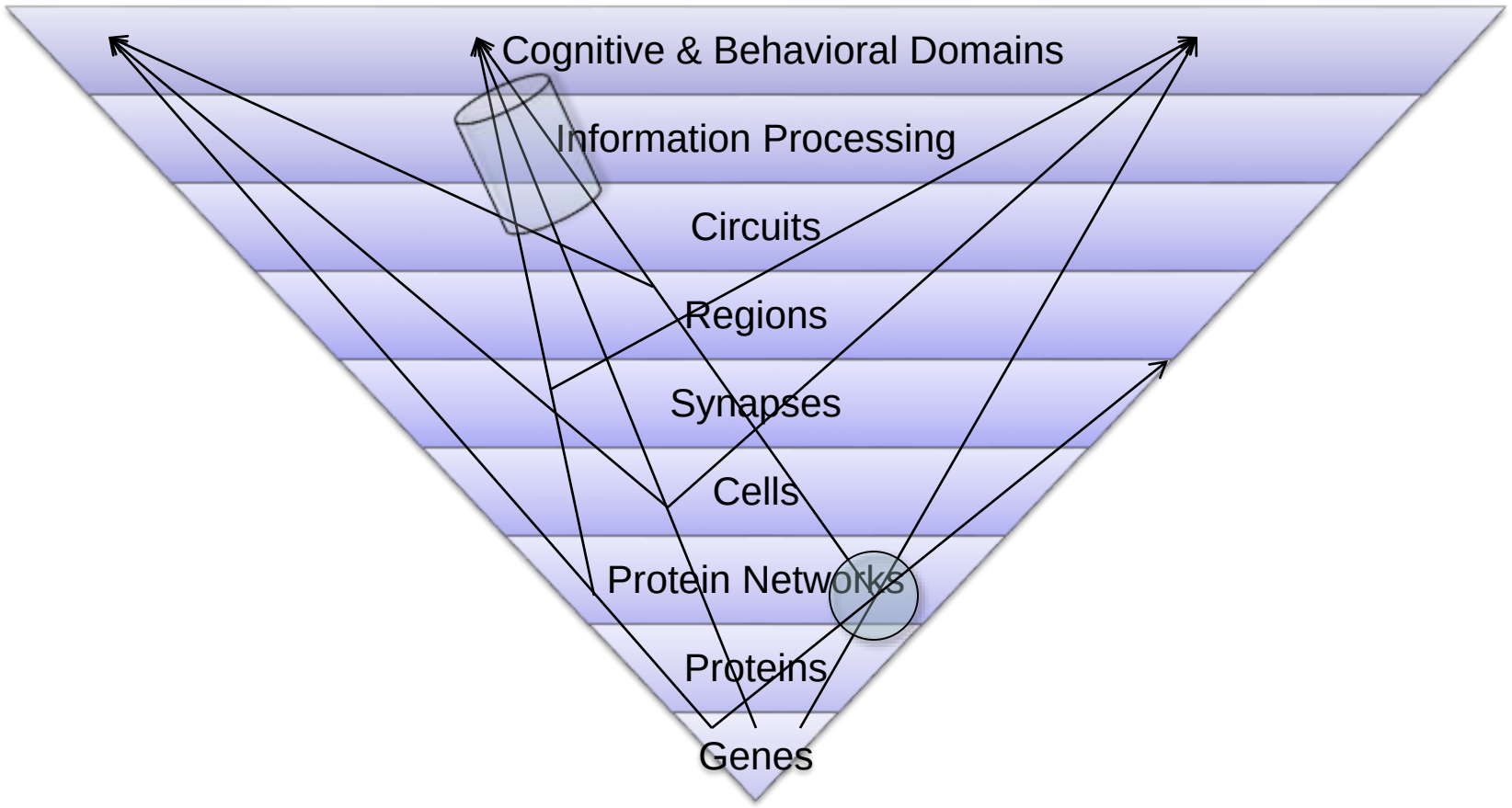


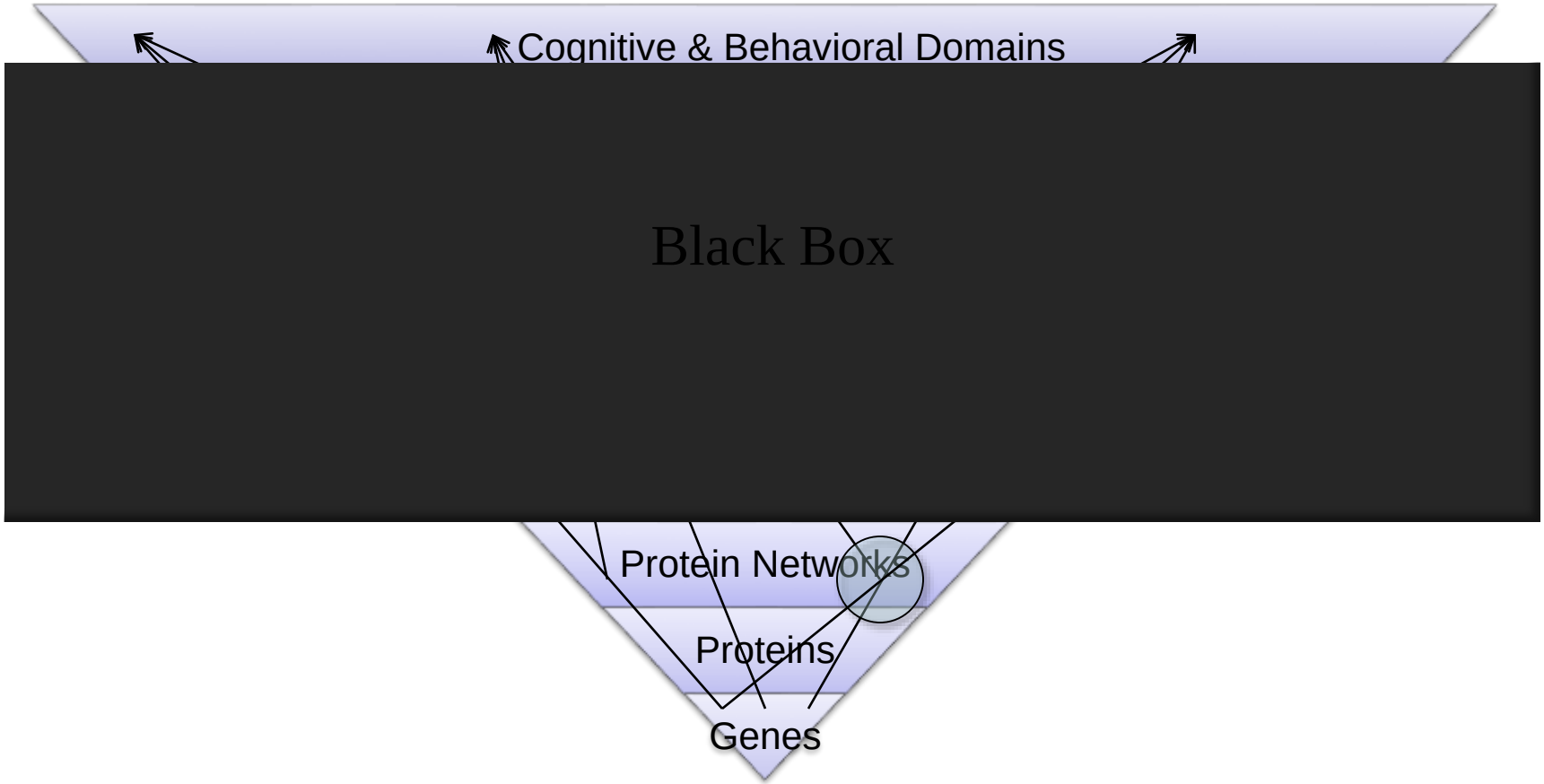
Theoretical Convergence at Behavior



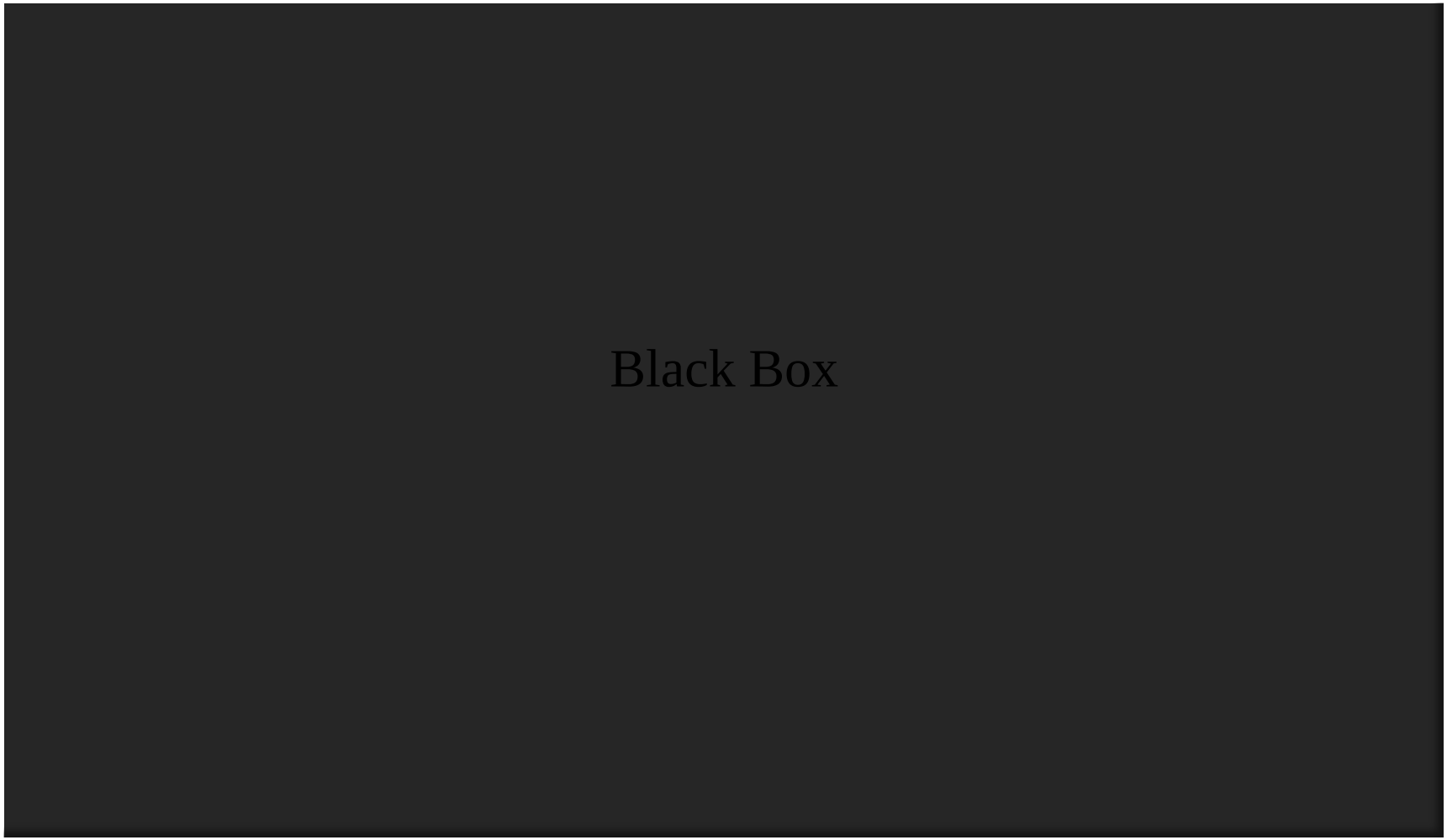
Data Convergence



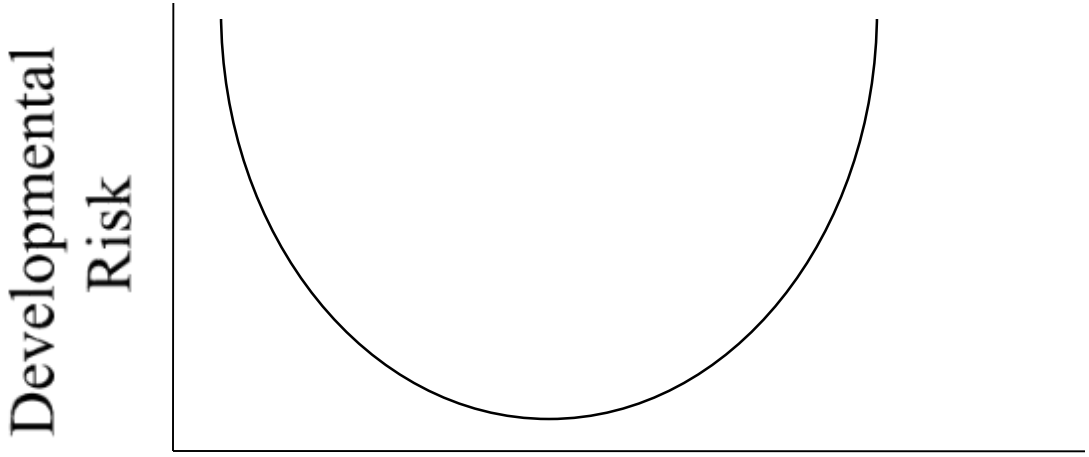
A lot remains unknown



Previous Pathway to Treatment



Risk with decreased or increased gene dosage



Developmental Disorder
Risk Gene Dosage

Examples:

MECP2 disruption / duplication

Maternal chromosome 15q duplications / deletions

Chromosome 16p duplications / deletions

…

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What treatments do we know work?

And how do we know?

Behavioral Interventions

Behavioral Interventions

- Early Intensive Behavioral / Developmental Interventions
  - Approaches based on the UCLA/Lovaas Model or Early Start Denver Model improve cognitive, language, and adaptive outcomes in certain subgroups of children.
    - Strength of evidence: Moderate
- Other behavioral interventions
  - Cognitive behavioral therapy for anxiety: Moderate
  - Social skills training: Mixed

Medications

What medication has had the most placebo-controlled trials in Autism Spectrum Disorder?

Secretin

- Strength of Evidence = high for lack of efficacy
- Lessons to be learned:
  - Hesitate to draw conclusions without randomized, controlled trials
  - Placebo effect can be powerful!
    - 22-50%

All of the evidence-based medicines treat associated synmptoms

Irritability / Agitation

- Risperidone and Aripiprazole
  - Strength of Evidence = High
  - Primary target symptoms: 'Irritability/Agitation' subscale of *Aberrant Behavior Checklist*
- **Significant side effects:**
  - Weight gain, sedation, extrapyramidal symptoms

Fung et al., *Pediatrics*, 2016

Inattention / Hyperactivity (= ADHD)

- Methylphenidate
  - 49% “much” or “very much improved” + 30% symptom reduction
- Atomoxetine
  - 48% “much” or “very much improved”
- Guanfacine
  - 50% “much” or “very much improved”

McPheeters, et al., *Pediatrics*, 2011.

Outline

- Challenge of heterogeneity
- How have we tested treatments in DD (ASD)?
- How do we find new treatments?
  - Rare Genetic Disorders → Molecular Targets = PRECISION MEDICINE
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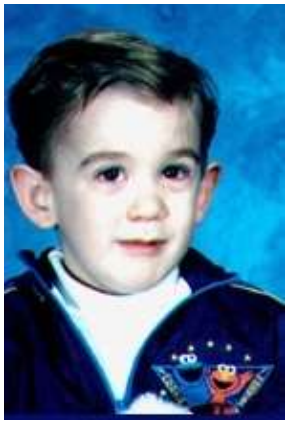
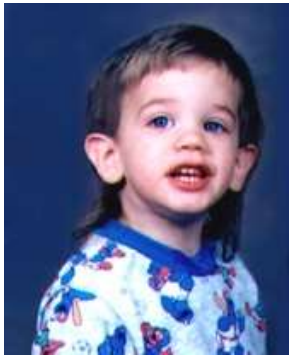
Rare ASD phenocopy syndromes

- Fragile X syndrome
- 16p11 deletion syndrome
- Maternal 15q11-q13 duplication syndrome
- Phelan-McDermid syndrome (*SHANK3* loss)
- Cowden syndrome (*PTEN* loss)
- Tuberous Sclerosis
- *CHD8* loss
- {Rett syndrome}
- …

Fragile X Syndrome (FXS)

- X-linked
- Mild to moderate intellectual disability
- Autism in ~20-30%
  - Autism Spectrum Disorder in 30-60%
  - Most patients have social difficulties
- Hyperactivity, impulsivity
- Sensory sensitivity
- Seizures
- Long face with prominent ears
- Enlarged testes (after puberty)

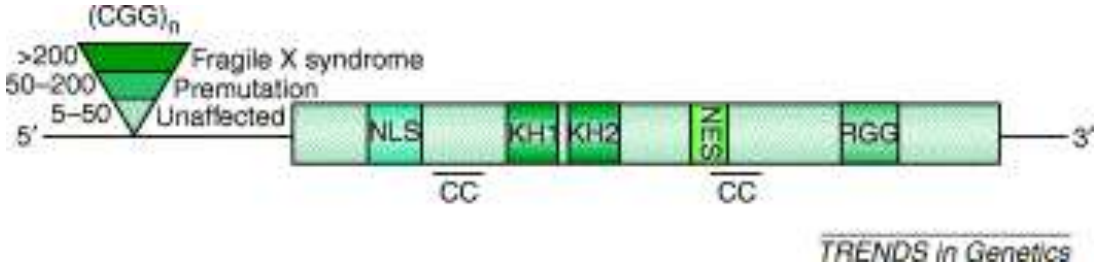




Specialchild.org

Molecular genetics: *FMR1*

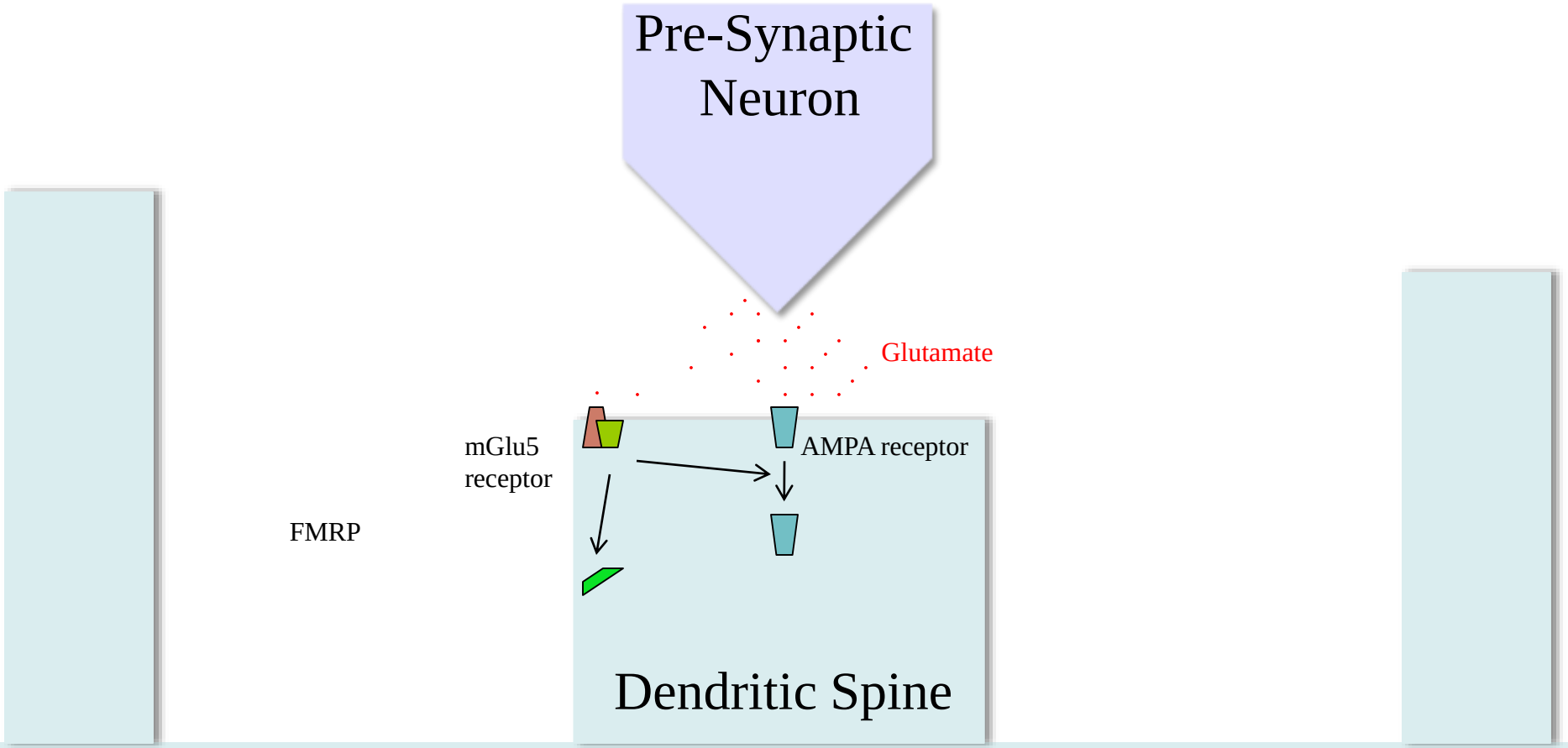
- Trinucleotide repeat (CGG) expansion



- Gene methylation → Silencing
- Encodes FMRP, an RNA chaperone

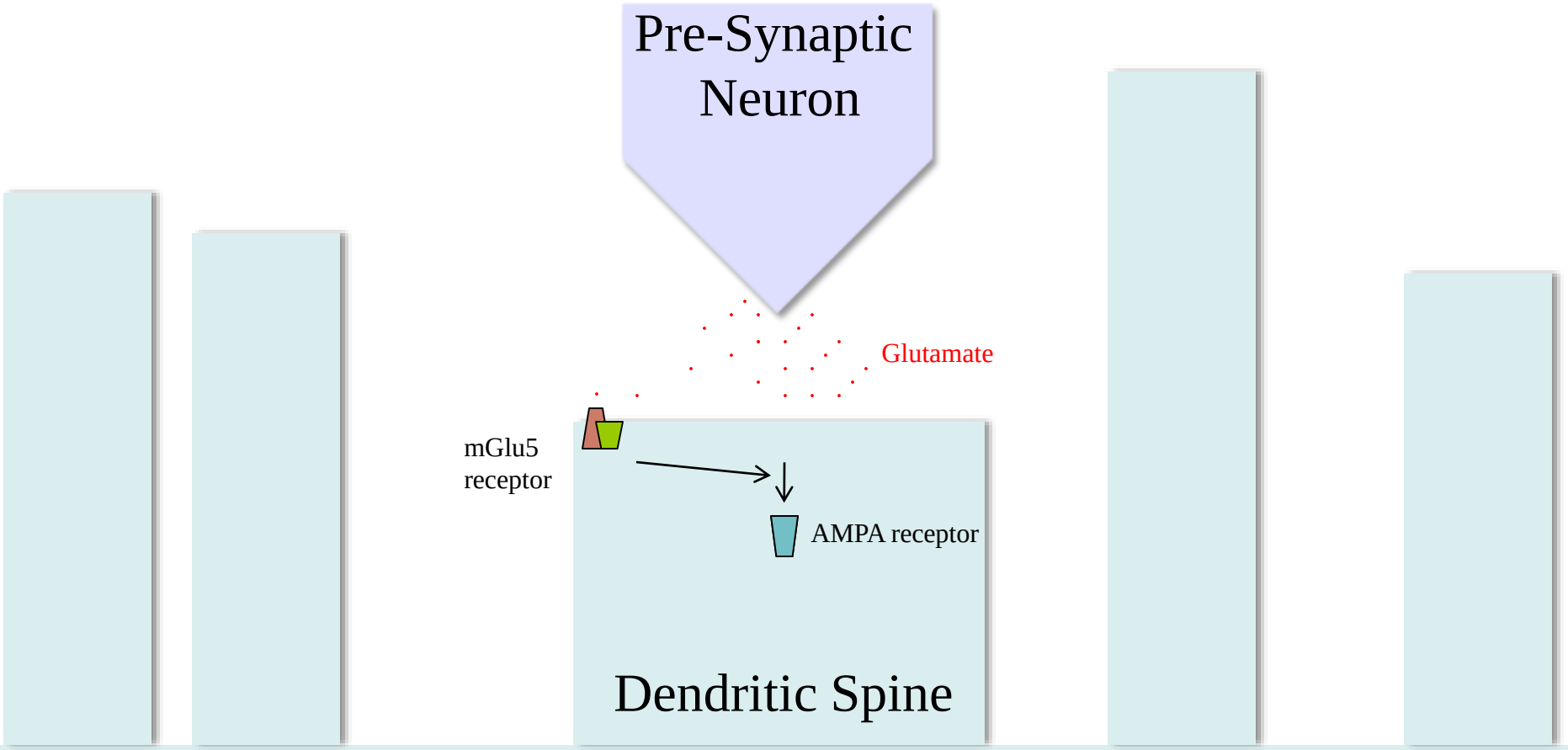
Kooy RF, *Trends Genetics*, 2003

mGlu5 receptor hypothesis



Kim Huber
Mark Bear

What if there is no FraX protein?



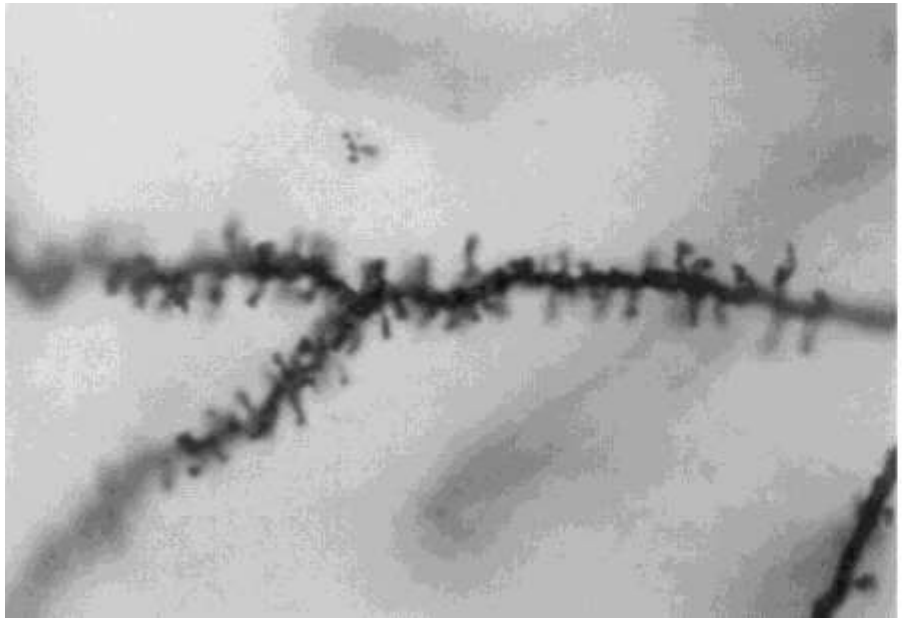
Hippocampus

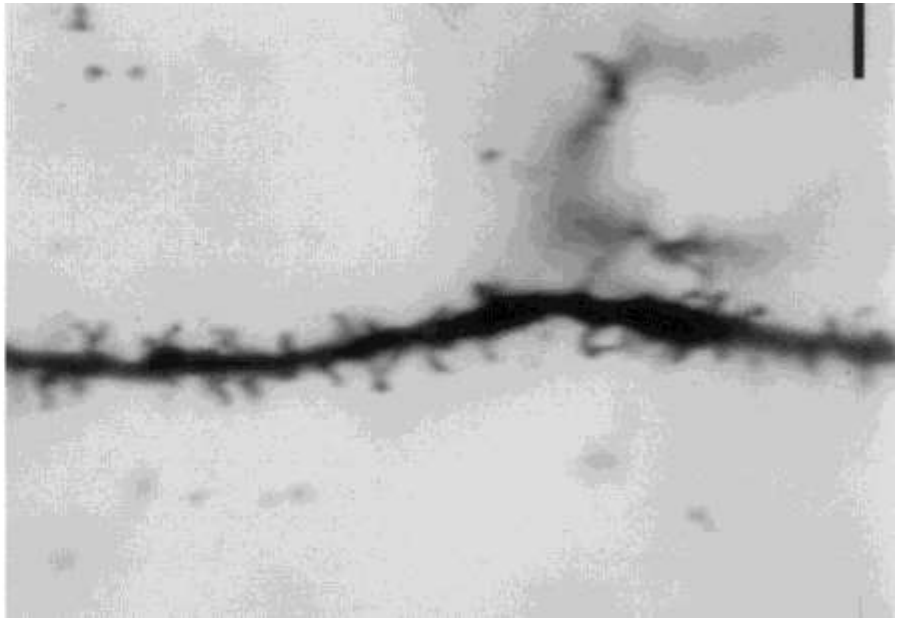
Post-Synaptic Neuron

Kim Huber
Mark Bear

How does this affect a mouse?

- Altered dendritic spine density and shape
- Hyperactivity (mild)
- Social deficits (subtle and inconsistent)
- Impaired learning (subtle and inconsistent)
- Inducible seizures

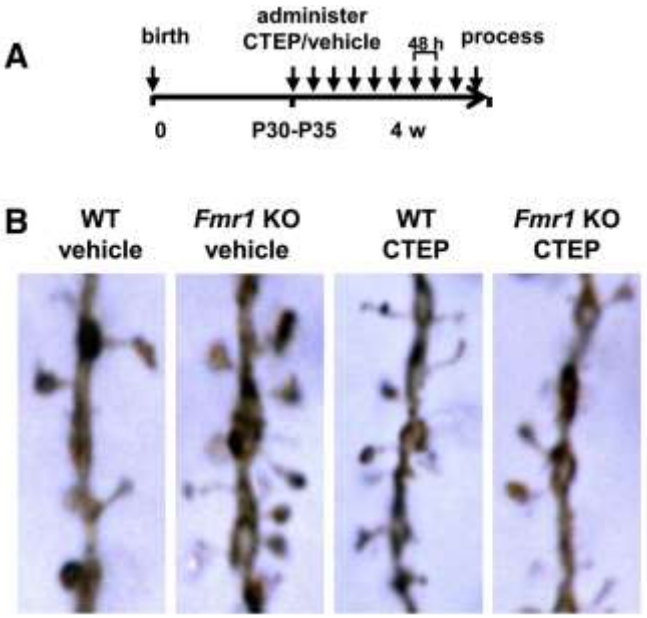




Comery TA, *PNAS*, 1997

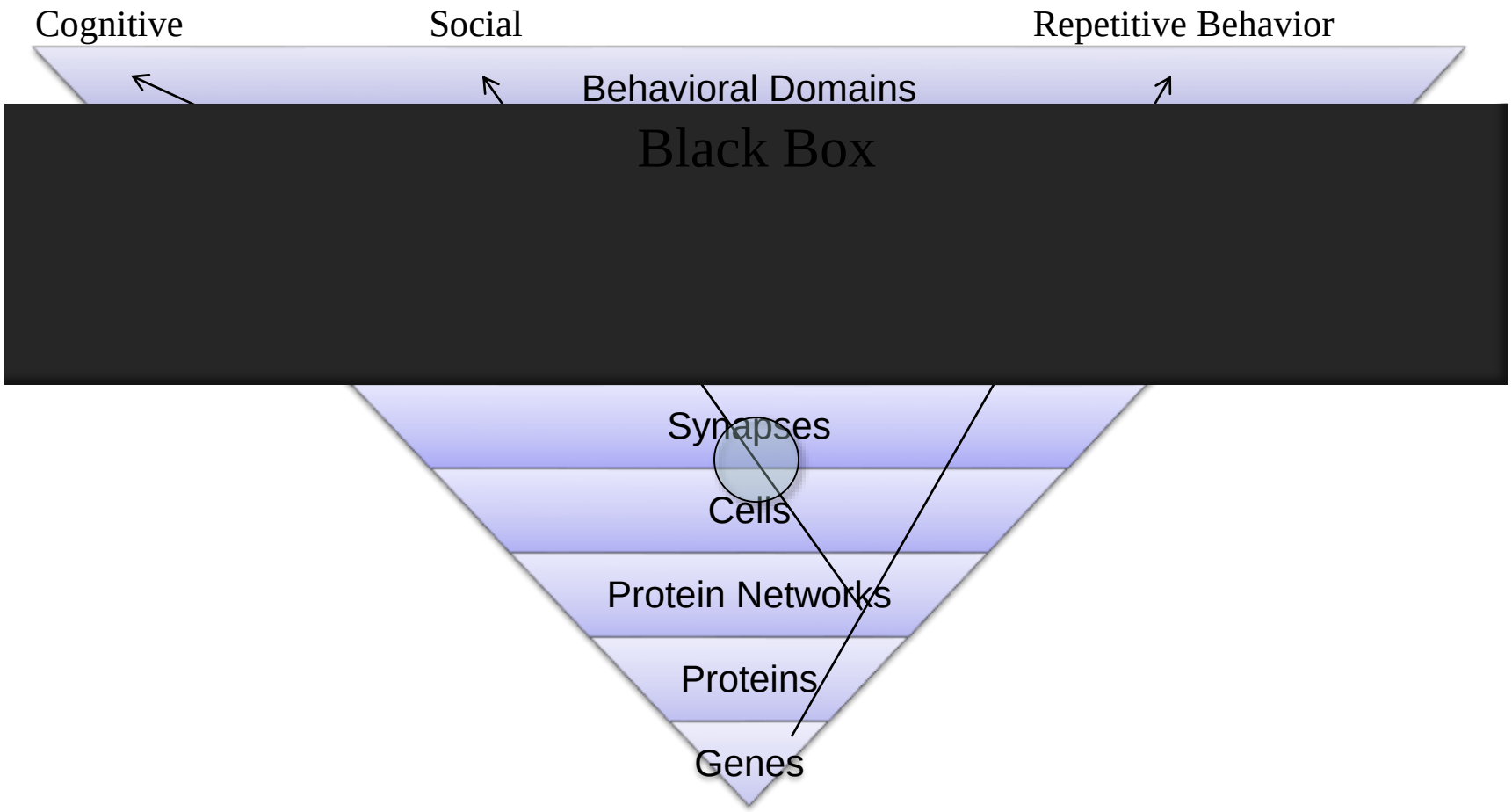
Pharmacological Rescue

- Novel mGluR5 negative allosteric modulator = CTEP
  - Roche compound
- Rescues brain and behavior
  - Decreased dendritic spines
  - Improved hyperactivity
  - Improved learning (subtle)
  - Improved auditory sensitivity
  - Decreased seizures
  - No obvious negative effects on health

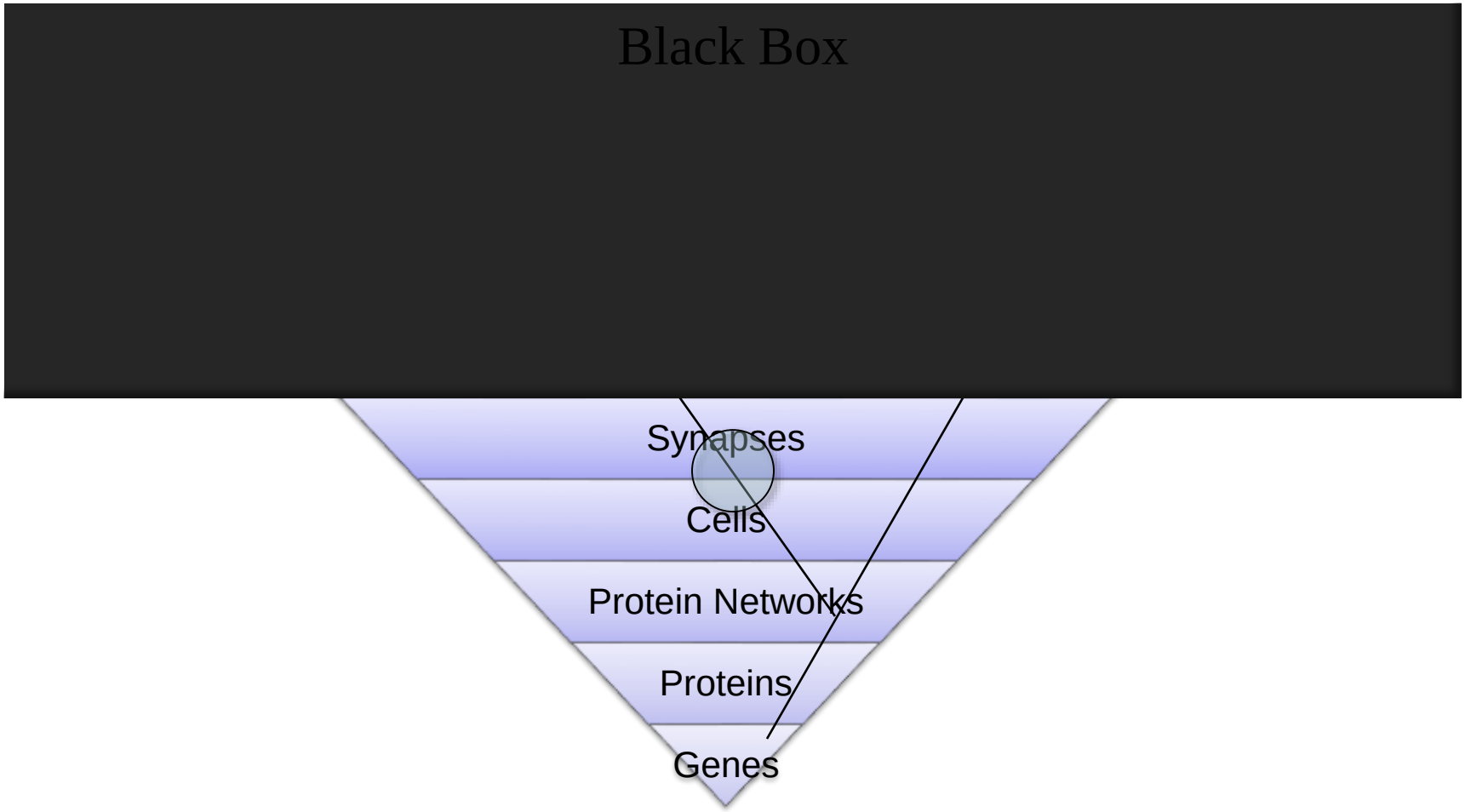


Michalon et al., *Neuron*, 2012

Cascade


Cognitive
Social
Repetitive Behavior
Behavioral Domains
Black Box
Synapses
Cells
Protein Networks
Proteins
Genes

Cascade



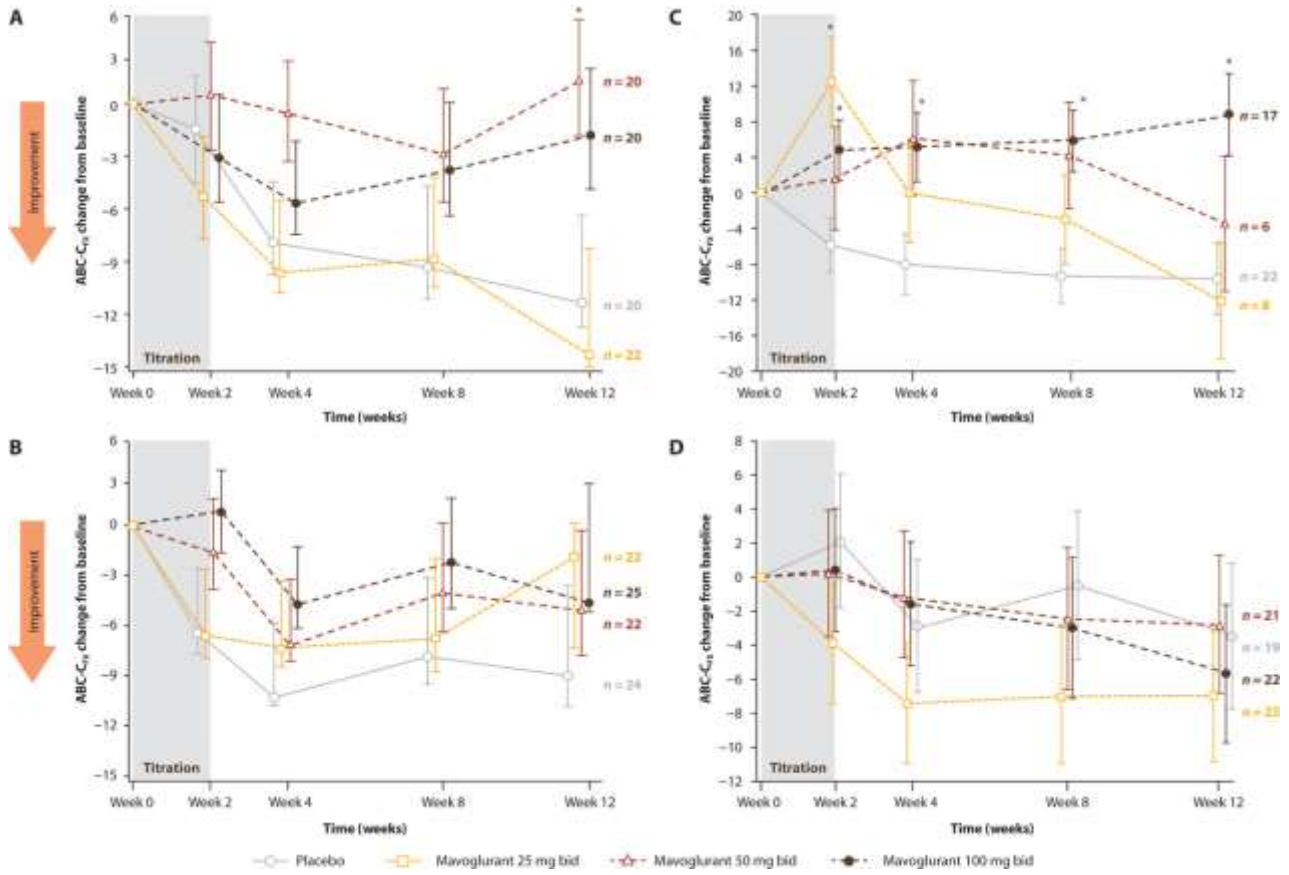
What about humans?

Large Scale Trials in Adults and Adolescents…

Complete Methylation | Partial Methylation

Adults

Adolescents

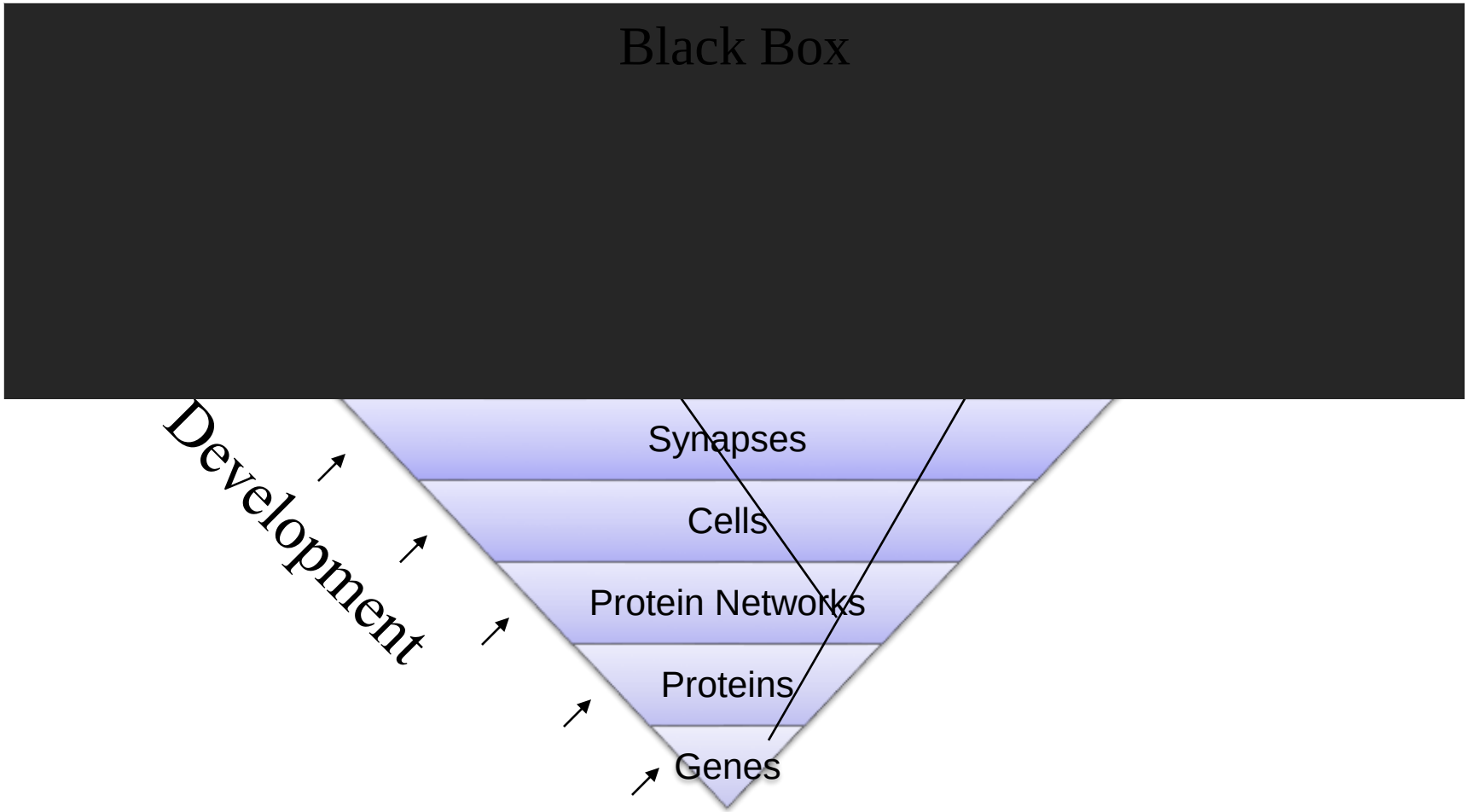


Berry-Kravis et al., *Sci Transl Med*, 2016

Why did these trials fail?

- Novartis and Roche FXS programs closed
  - Placebo effect?
  - Wrong target?
  - Wrong Drugs?
  - Wrong Doses?
  - Wrong Ages?
  - Wrong Outcome Measures?
  - {Wrong Species?}
- Child study now funded
  - NINDS NeuroNext: Berry-Kravis, Abedutto et al.

Risk genes feed a cascade of impact



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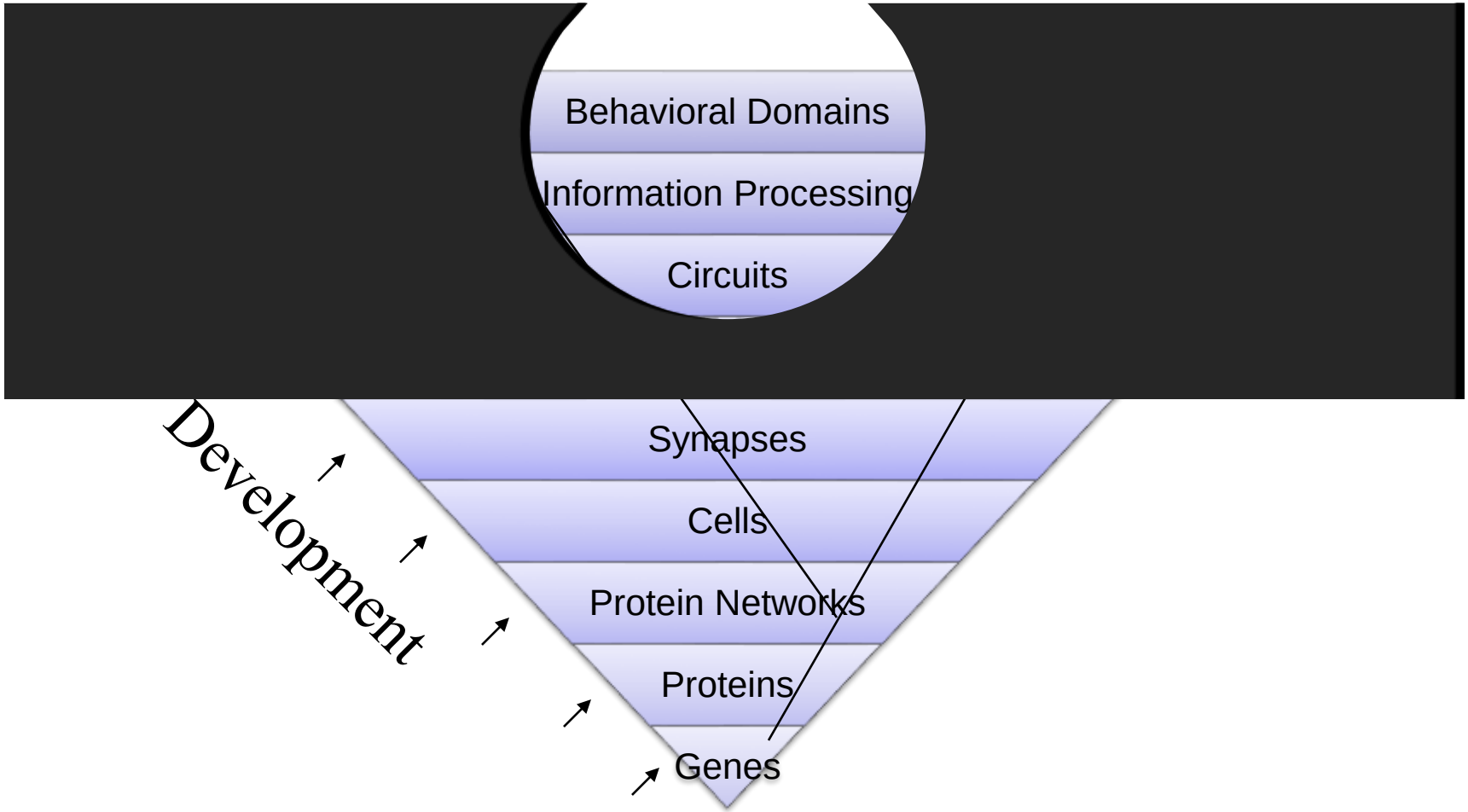

Promise of Precision Medicine

- Potential for profound benefit in a given syndrome
- Opportunity to learn how to study successful treatments of neurodevelopmental disorders
- Possibility that treatments extend to subset of non-syndromal children with ASD

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Precision medicine sounds great but…

Where have we had success in ASD treatment so far?

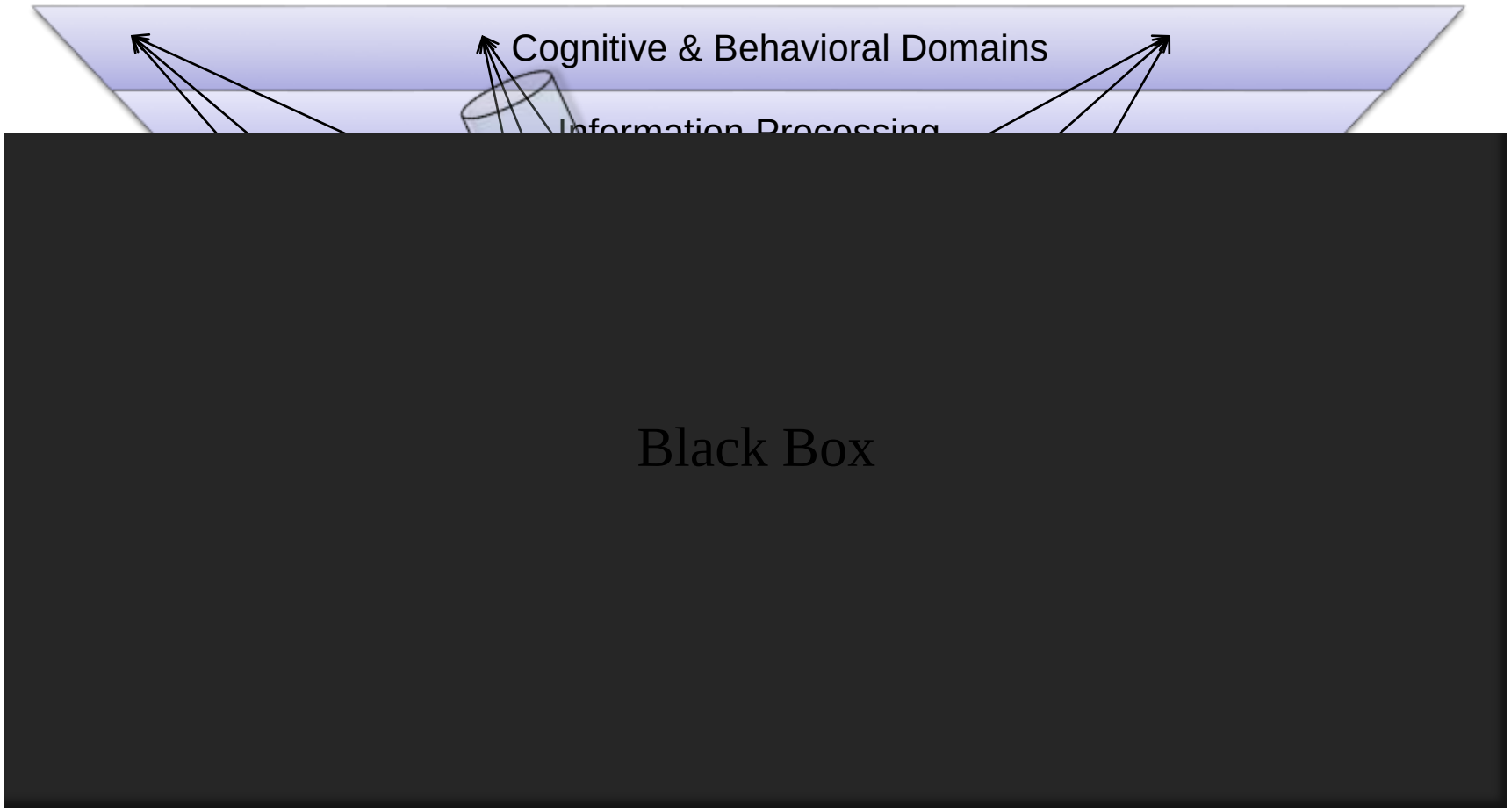
Examples of potential symptomatic treatments

- Constipation
- Epilepsy
- Language
- Cognition
- Irritability / agitation
- Anxiety
- Attention Deficit Hyperactivity Disorder
- Tics / stereotypies
- Sociability

Assertion

- Treatments that benefit the majority of children with ASD are likely to be focused on symptoms, not pathophysiology
  - Target a universal system or circuit that has a conserved function across species
    - Research Domain Criteria approach (RDoC)
    - You can find these via risk genes!
- These treatments are unlikely to be “cures” or even be “disease modifying”

Cascade?

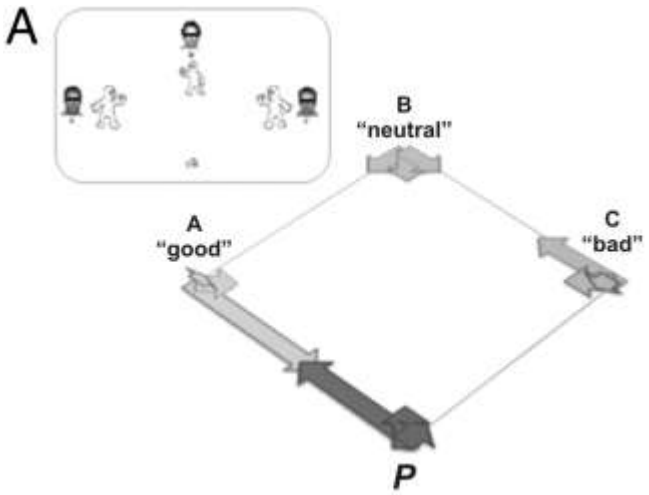


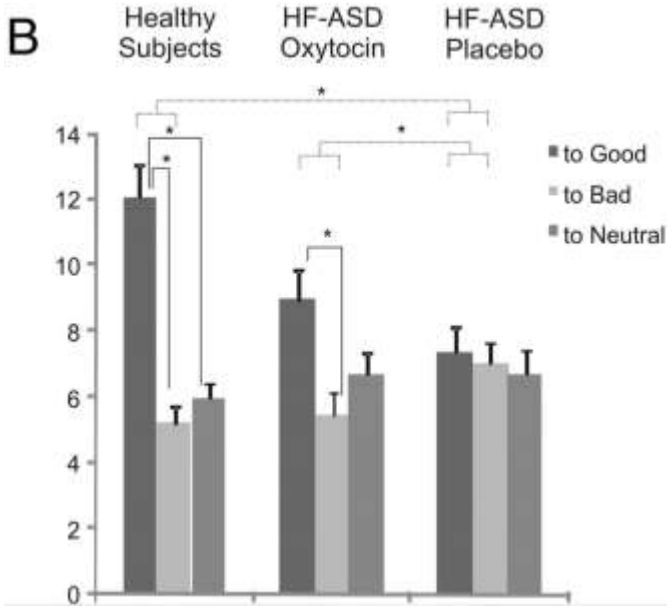
Oxytocin

- Hypothalamic neuropeptide hormone
  - Best known for role in parturition and lactation
  - Also maternal behavior, pair bonding, trust
  - Vasopressin = sister (brother) hormone
- Autism Spectrum Disorder (Yamasue talk)
  - ? Inconsistent plasma oxytocin findings
  - Mixed genetic data pointing to oxytocin receptor
    - Allelic association
    - Rare deletion
    - Increased methylation

Can oxytocin impact social behavior in humans?

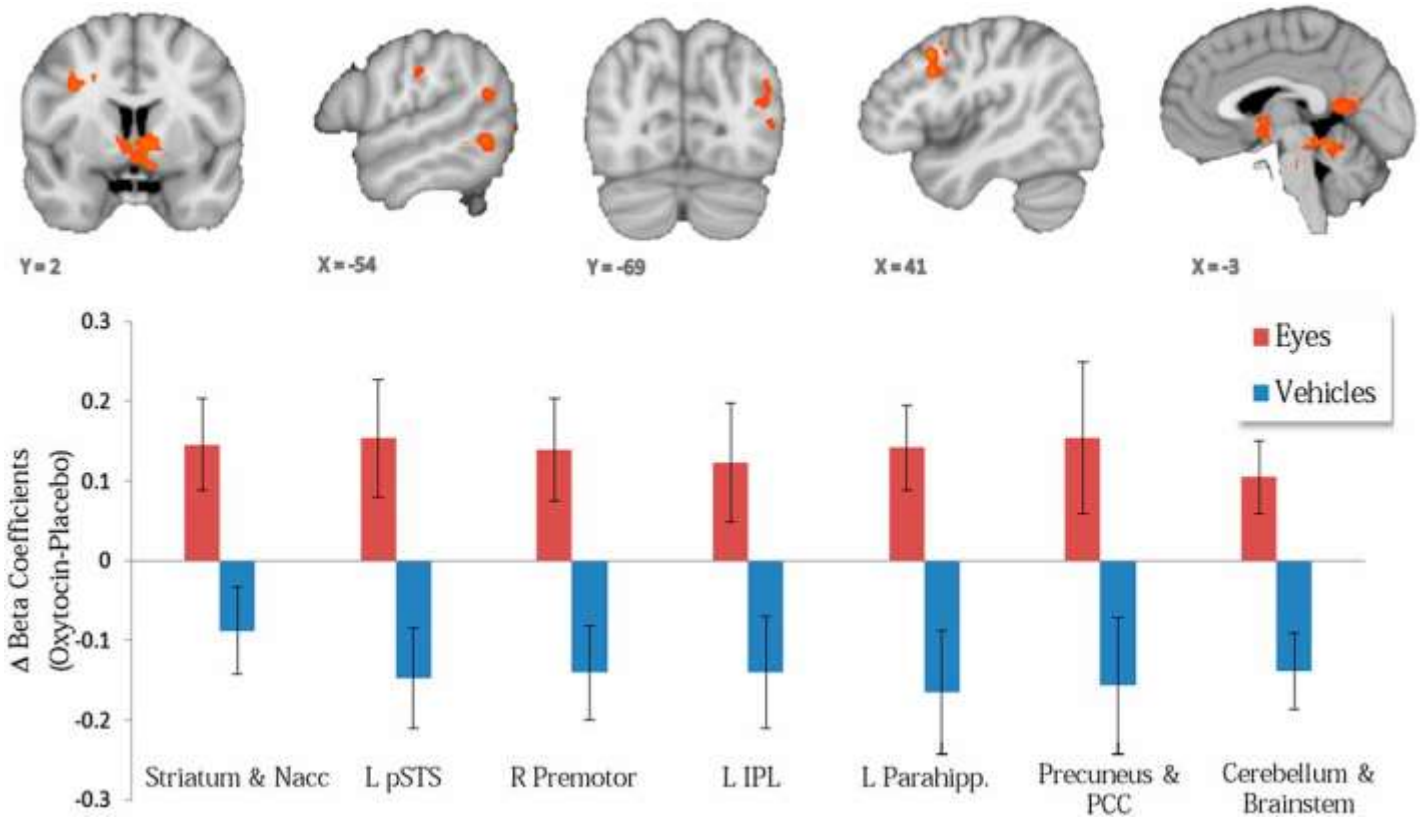
- Intranasal delivery





Andari et al., *PNAS*, 2010

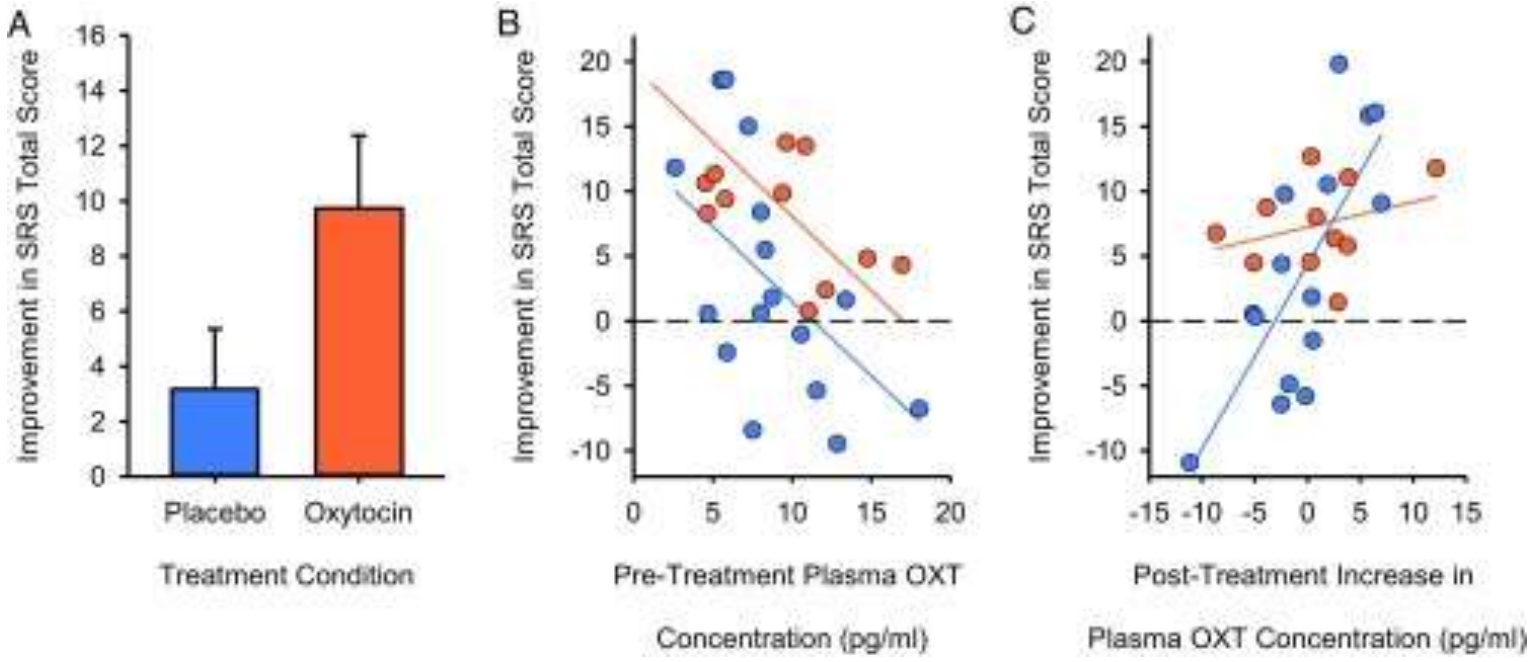
What is happening in the brain?



Gordon et al., *PNAS*, 2013

Pilot trials of longer-term treatment…

- 35 participants, 4-week randomized treatment



Parker et al., *PNAS*, 2017

What's next for oxytocin?

- Real world testing
  - Acute vs. chronic administration?
  - Pair with social skills intervention?
  - What outcome measures?
  - Who responds?
    - Biomarker analyses
- Pending = 290 participants with ASD over 6 months, completed March 2018
    - Duke, Columbia, Vanderbilt, UW, MGH, Mount Sinai

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What if a treatment benefits more than 1%, but less than the majority of children with ASD?

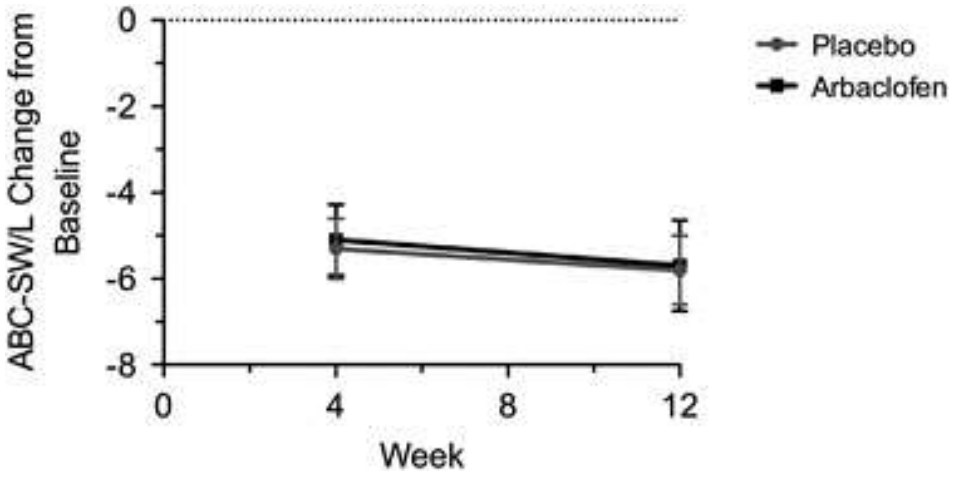
Arbaclofen parallel-group randomized, controlled trial, n = 150

		Intent to Treat population		
		Change from baseline (LSMean ±SEM)		
		STX209	Placebo	P-value
ABC-Social Withdrawal	(lower score better)	-5.3 ±0.87	-6.1 ±0.83	0.477
CGI-Improvement		3.1 ±0.12	3.3 ±0.12	0.305
CGI-Severity		-0.7 ±0.10	-0.3 ±0.10	0.009
ABC-Irritability		-3.6 ±0.91	-3.3 ±0.86	0.805
Sensory Profile	(higher score better)	10.9 ±2.12	7.5 ±2.01	0.250
VABS-Communication		2.6 ±0.91	1.4 ±0.88	0.365
VABS-Socialization		4.4 ±1.19	2.0 ±1.15	0.152

Disclosure: Seaside Therapeutics

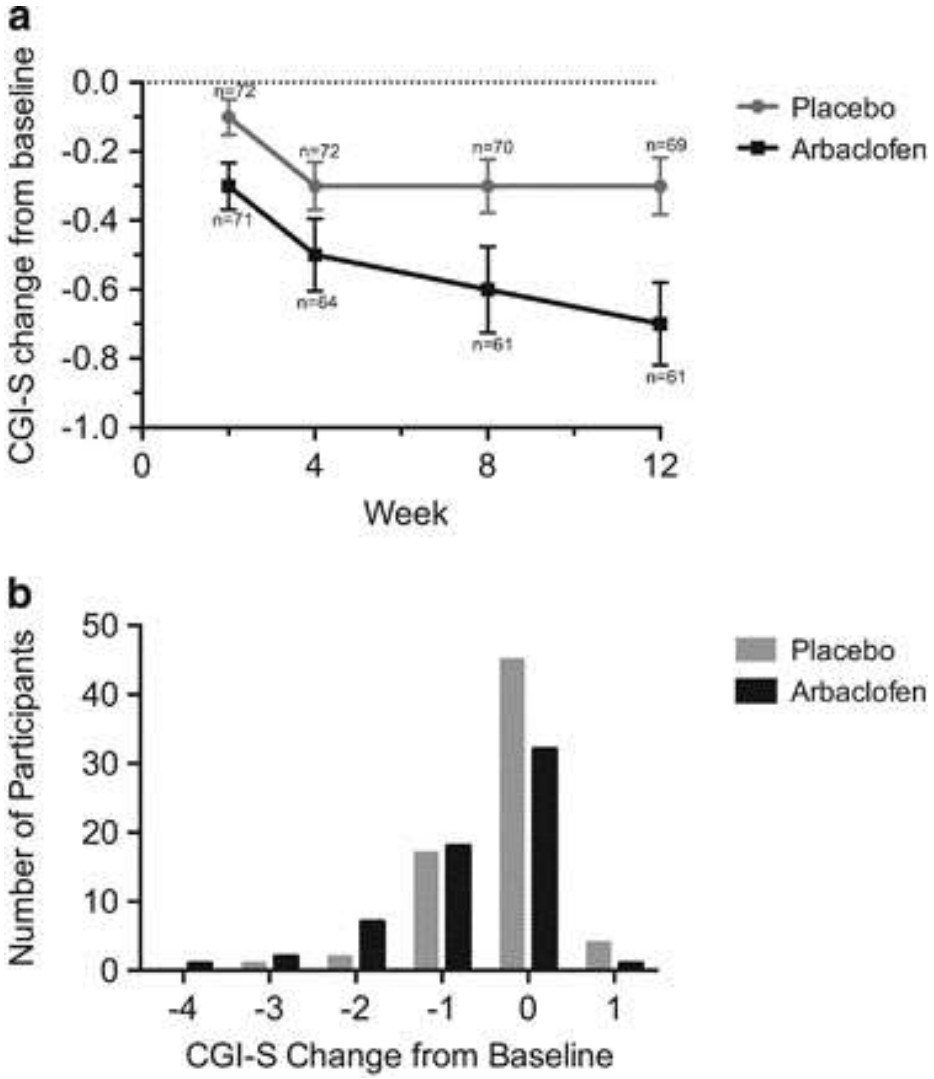
Veenstra-VW et al., 2017

Social Withdrawal / Lethargy



Veenstra-VW et al., 2017

Clinical Global Impression - Severity



Veenstra-VW et al., 2017

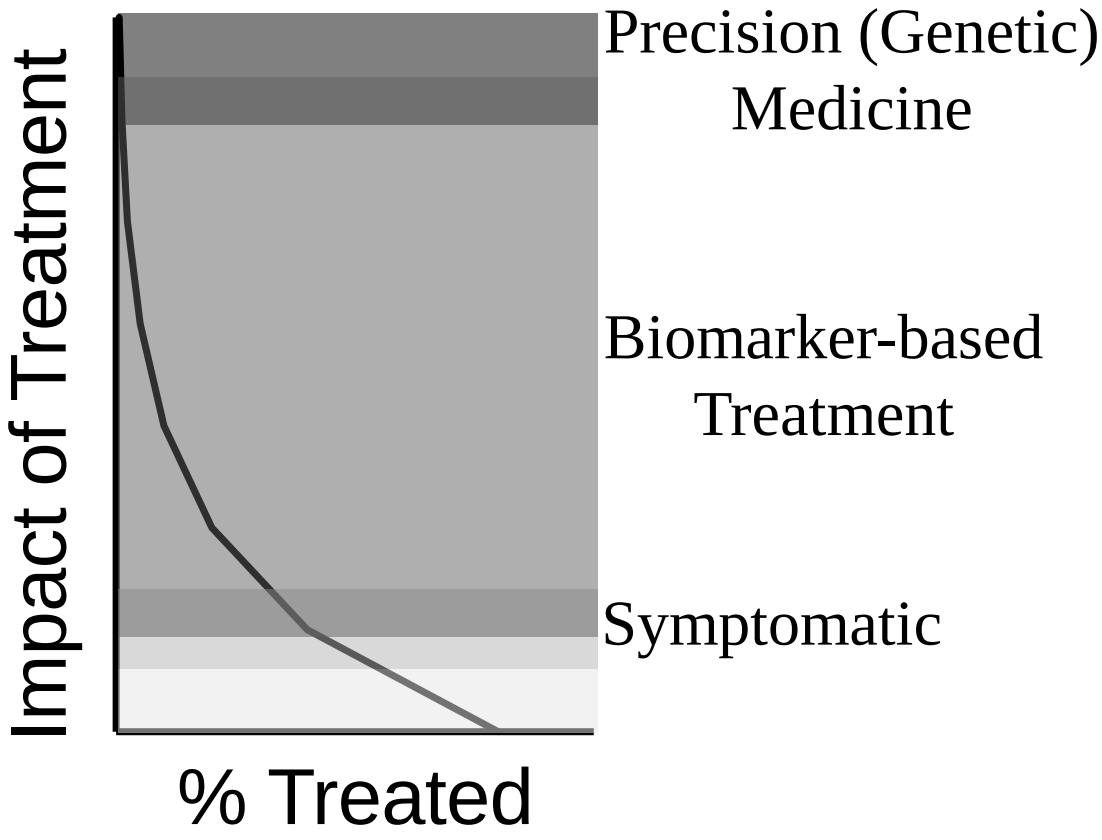
What can we conclude?

- Not much → needs replication
- Probably not a *general* treatment for autism
- We need a way to identify who responds
  - Clinical profile: higher IQ, better language
  - Biomarker (MEG/EEG, gene expression, cell signaling) studies pending

What would an ideal biomarker be?

- Quantifiable
- Reliable
- Replicable
- Heritable?
- Bimodal?
- Changes with treatment?
- Connected to pathophysiology?

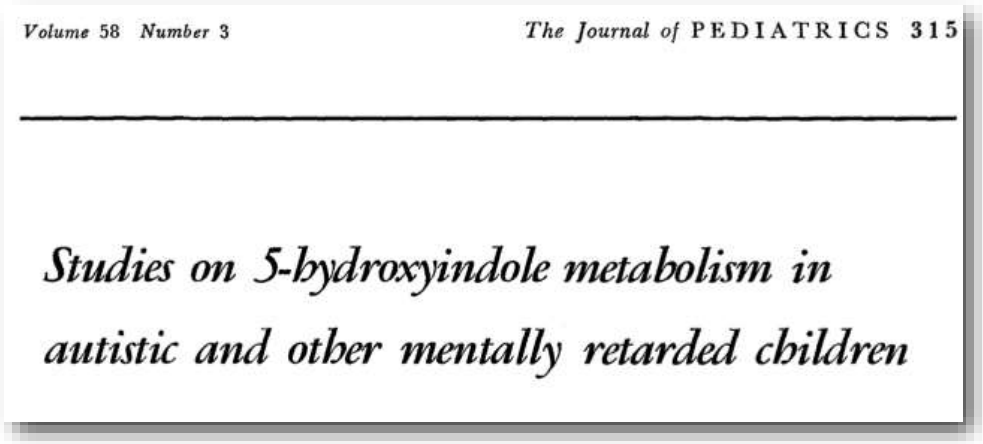
Where would biomarkers fit?



Potential Biomarkers

- Genetic
  - Disruption of genes bound by FMRP (Huber, 2012)
- Neuroimaging
  - Auditory evoked response on MEG, EEG (Roberts, various)
- Somatic
  - Generalized overgrowth in ~15% of ASD (Chawarska, 2011)
- Immune
  - Elevated IL-1β, IL-6, IL-8, IFN-γ (Masi, 2015)
- Maternal
  - Maternal antibodies in ~20% of ASD (Braunschweig, 2013)

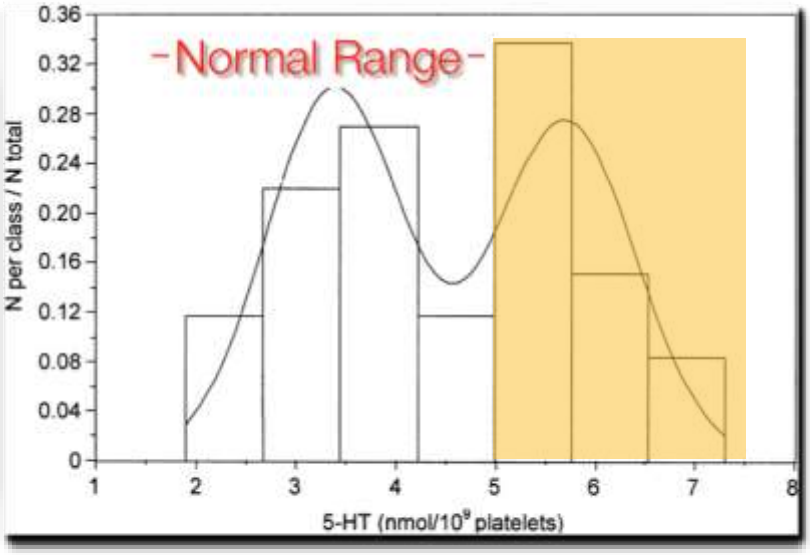
Hyperserotonemia in Autism: Half a Century of Mystery



Volume 58 Number 3 The Journal of PEDIATRICS 315

Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children

SCHAIN AND FREEDMAN, 1961

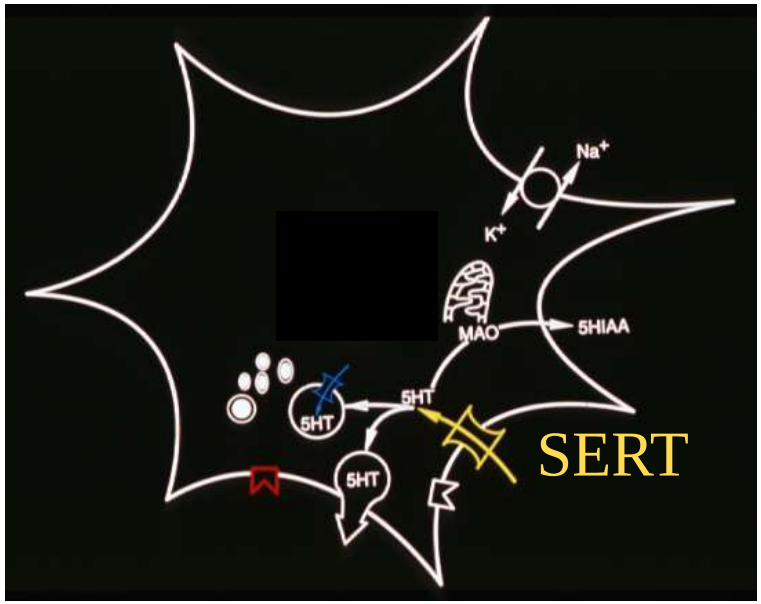


Mulder et al, 2004

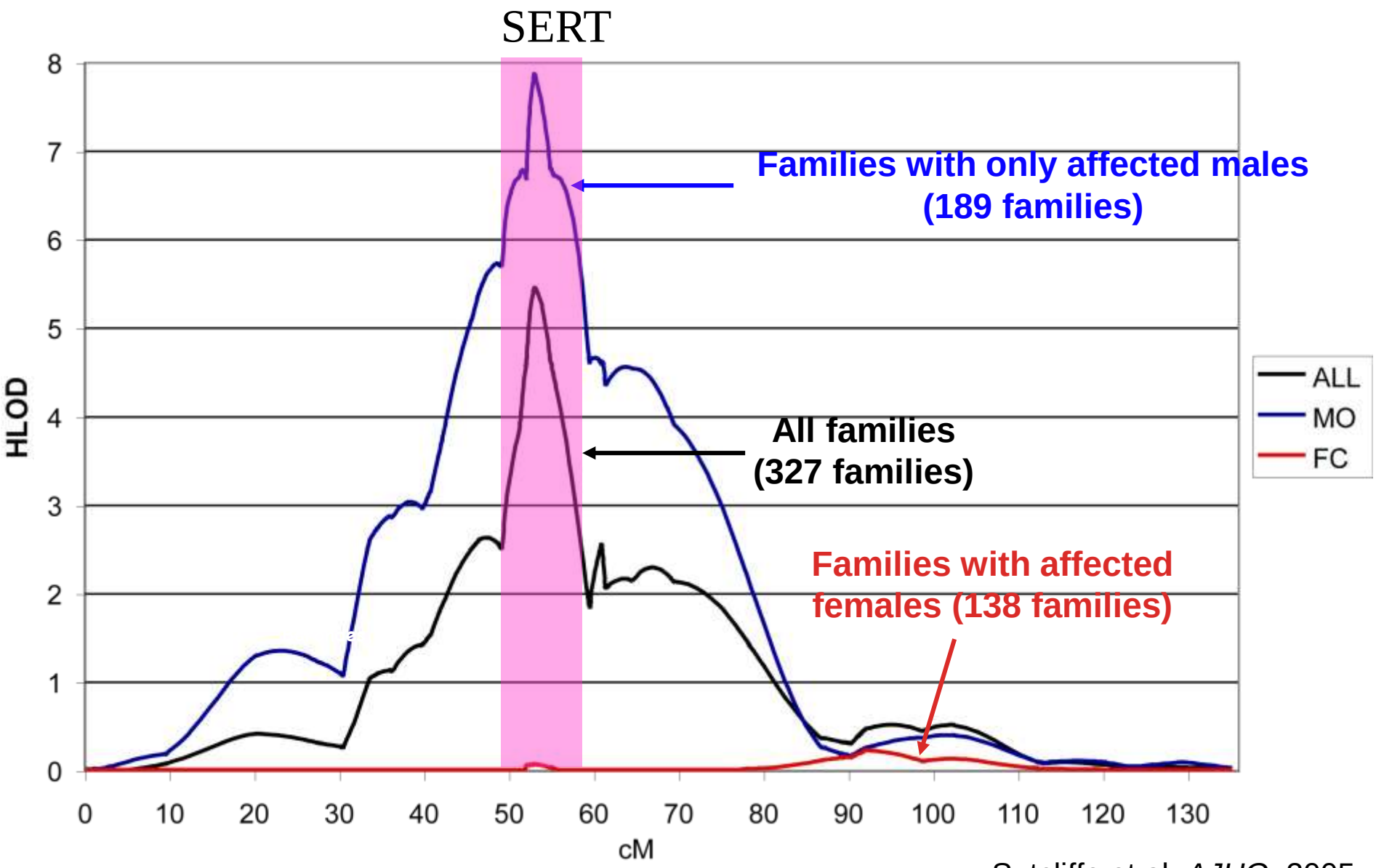
- HYPERSEROTONEMIA IN ~25-30% OF CHILDREN WITH AUTISM

Hyperserotonemia in Autism

- >99% of blood 5-HT is in the platelet
  - Taken up by serotonin transporter (SERT)
- Whole blood 5-HT is highly heritable
  - Broad heritability ~ 0.99



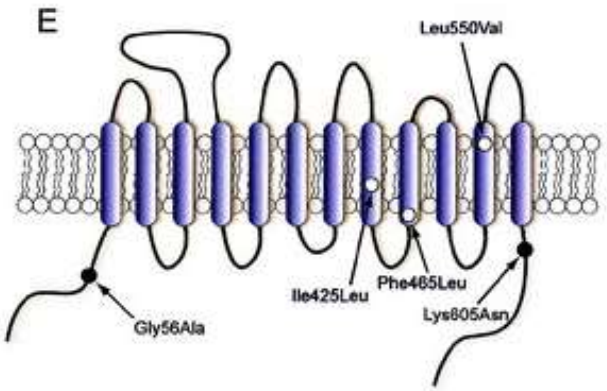
Genetic Linkage of Autism on Chromosome 17q



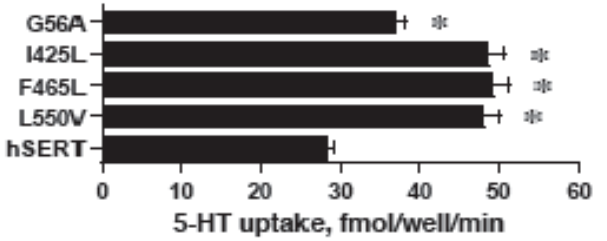
Sutcliffe et al, *AJHG*, 2005

Multiple Rare SERT Variants Identified in Autism Probands

- Rare amino acid variants
  - Conserved amino acids

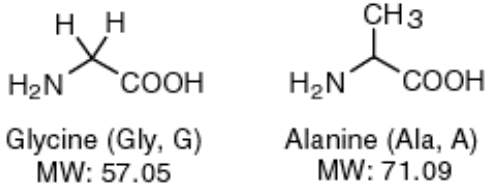


- Variants increased 5-HT uptake in lymphoblastoid cell lines and transiently transfected HeLa cells
  - Ala56 ↑ ~30%



Prasad et al, 2009

SERT Gly56Ala

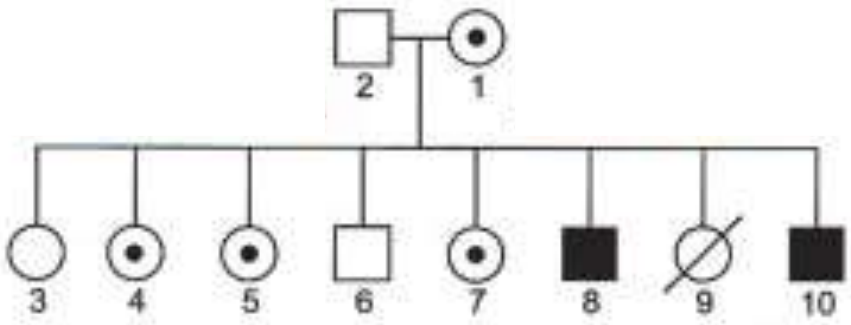


- 3:1 transmission rate to affected males
  - Rigid-compulsive symptoms (P = 0.0085)
  - Sensory aversion (P = 0.0005)

Human SERT	G	–	–	–	–	–	–	A	G	D	D
Mouse SERT	S	–	–	–	–	–	–	A	G	D	E
Rat SERT	S	–	–	–	–	–	–	A	G	D	E
Cow SERT	G	–	–	–	–	–	–	A	G	D	D
Chicken SERT	G	P	C	S	G	M	G	E	A	E	D
Fly SERT	V	T	–	–	–	–	–	D	–	–	P
Human DAT	S	S	T	–	–	–	–	L	T	N	P
Mouse DAT	N	S	T	–	–	–	–	L	I	N	P
Rat DAT	N	S	T	–	–	–	–	L	I	N	P
Cow DAT											
Zebrafish DAT	S	S	S	–	–	–	–	L	R	N	P
Human NET	L	A	–	–	–	–	–	–	–	–	–
Mouse NET	L	A	–	–	–	–	–	–	–	–	–
Rat NET	–	–	–	–	–	–	–	–	–	–	–
Cow NET	L	A	–	–	–	–	–	–	–	–	–
Chicken NET	S	S	N	–	–	–	–	L	L	P	A

Caveats

- SERT does not have *de novo* variants in ASD
- SERT variants found in some unaffected family members



- Amino acid variants add to complexity of multiple functional SERT variants
  - Enable studies in mice

Construction of mSERT Gly56Ala Knock-In Mouse

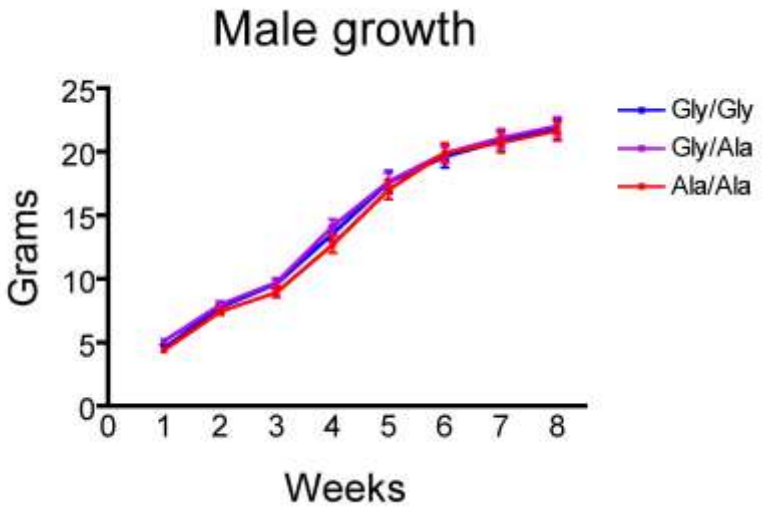
loxP

129S6/SvEvTac

X

129S4/Jae Protamine-Cre Transgenic Mouse

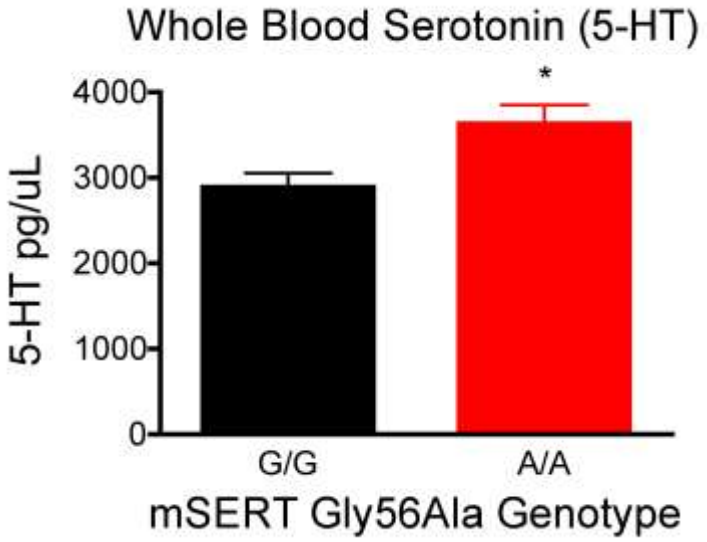
⇓



Normal growth.
No health problems.

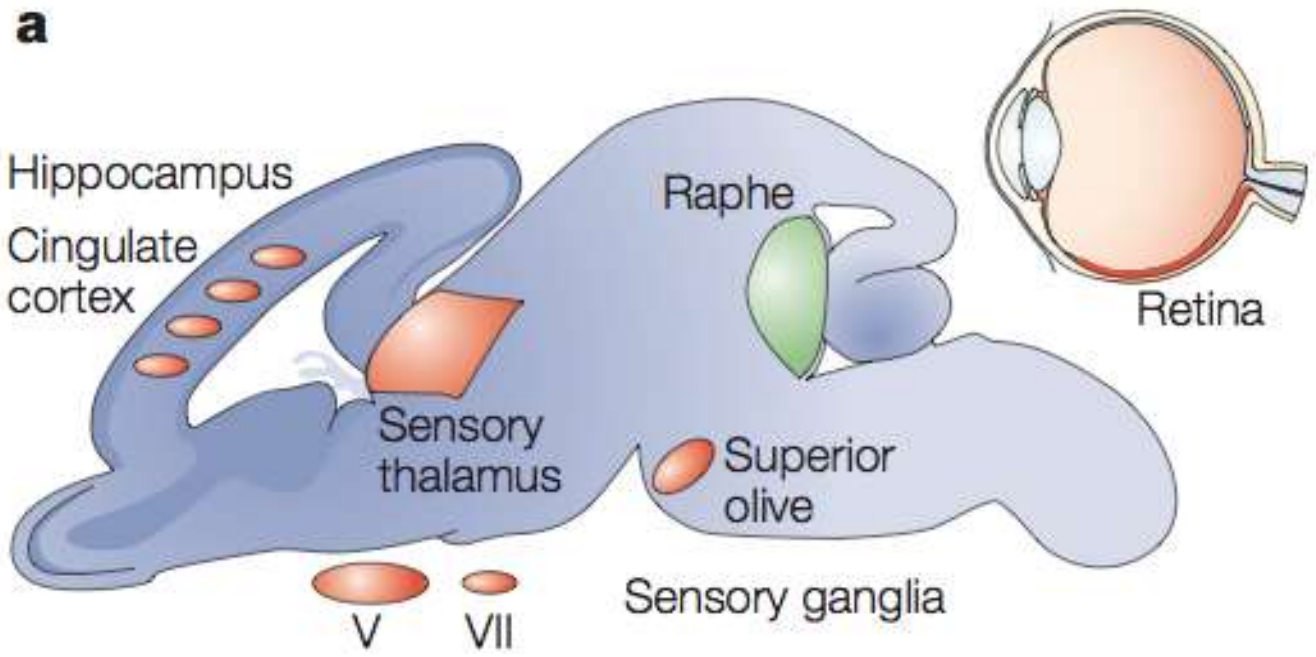
Veenstra-VW et al., 2009

Hyperserotonemia



$P < 0.05$

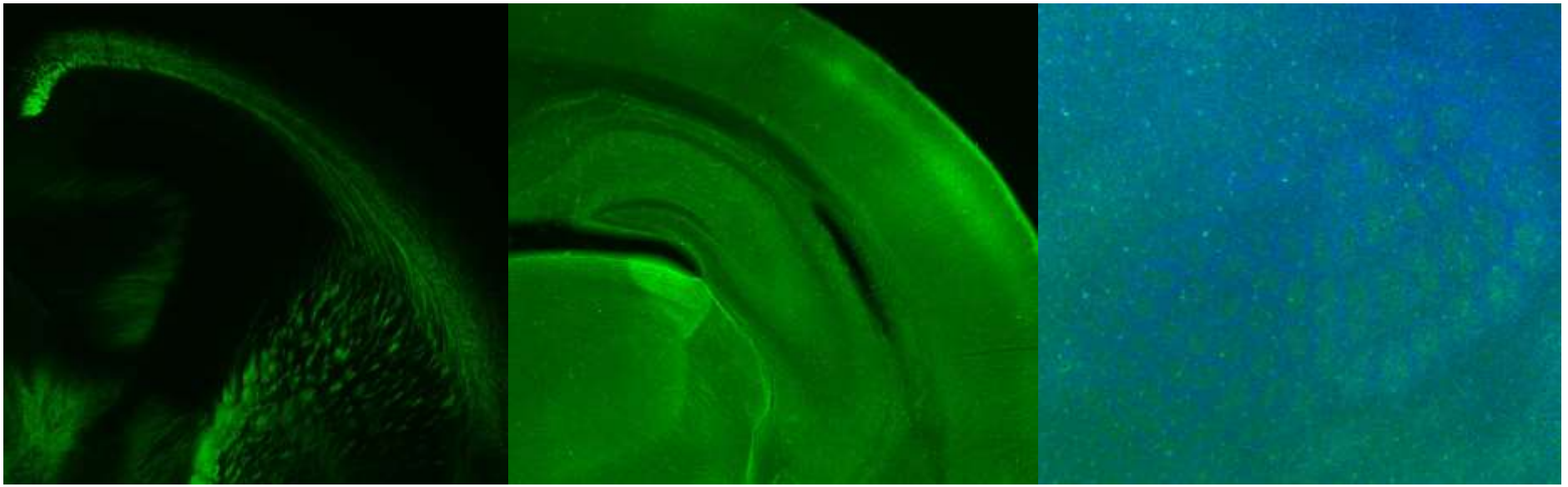
SERT transiently expressed in sensory regions during development



Gaspar et al., 2003

Hypothesis: Altered 5-HT signaling during development alters the formation of sensory-related brain structures, leading to sensory aversion.

Important Time Points in Somatosensory Cortex Development



E15: Transient TCA SERT expression begins

P2: TCAs form rows in S1

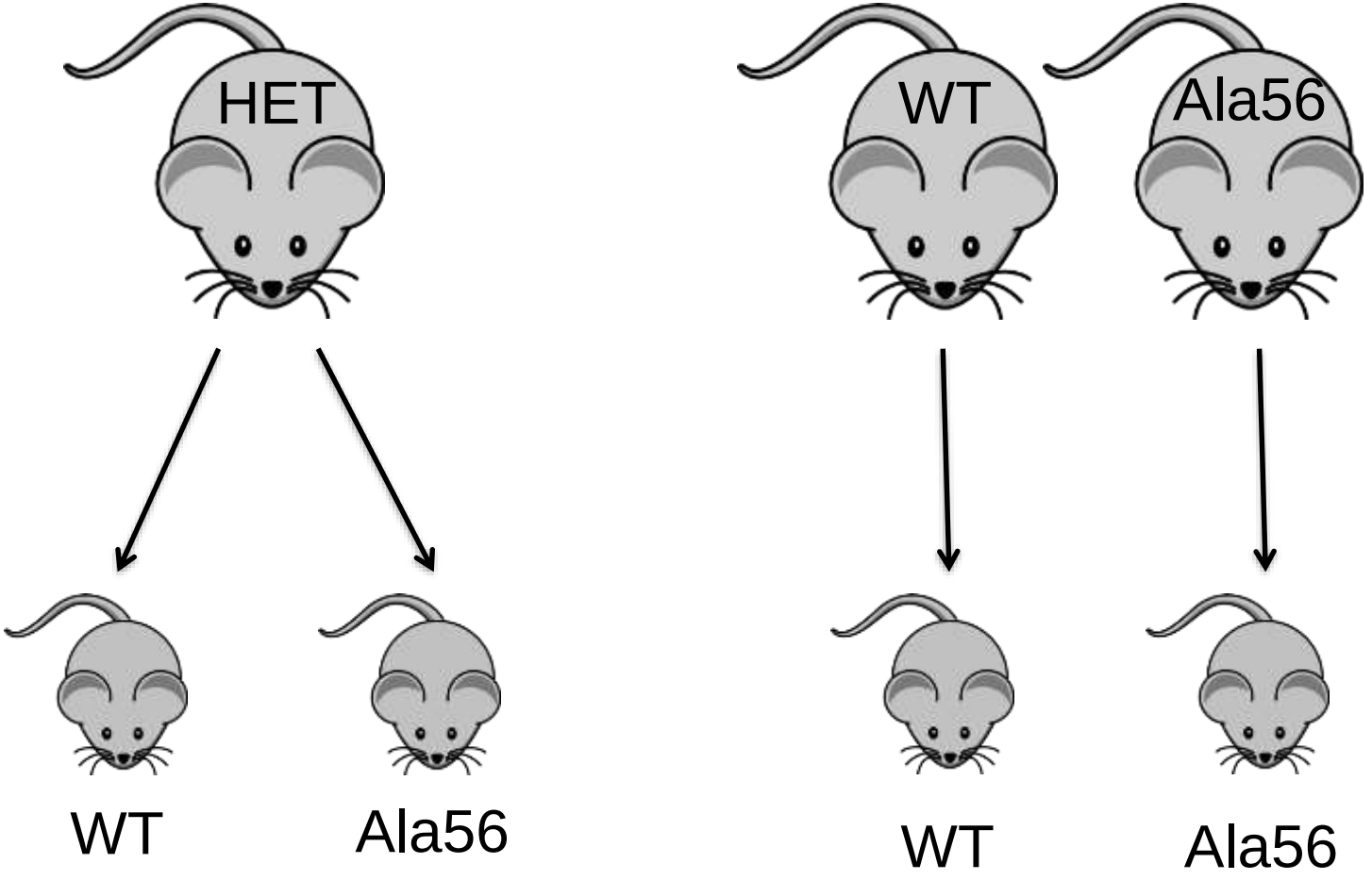
P5-7: Layer IV neurons form barrel septa

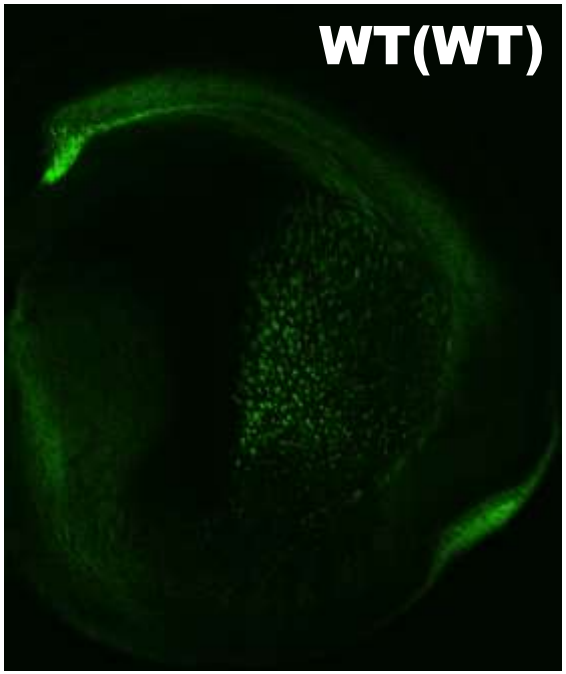
E18: TCAs reach cortical plate

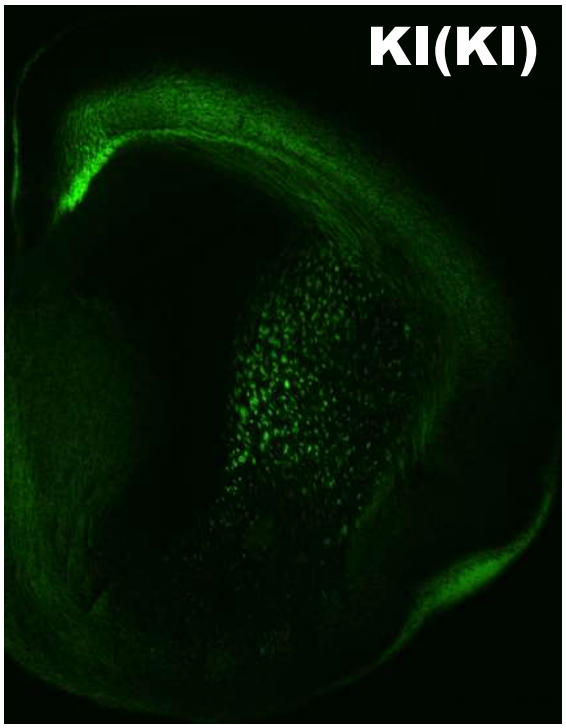
P3-4: TCAs segregate into barrels

Chris Muller

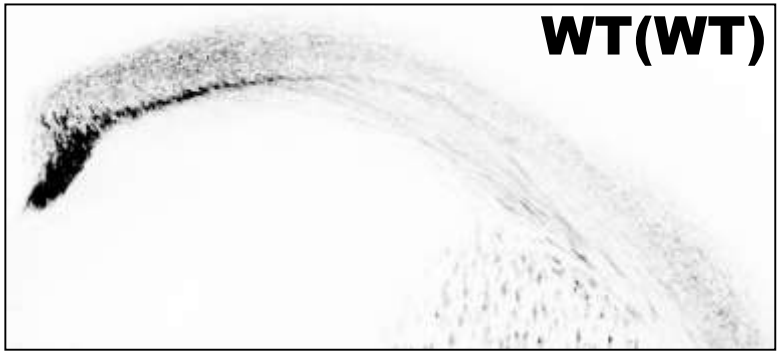
Breeding Schemes Used to Generate Mice for thalamocortical projection experiments

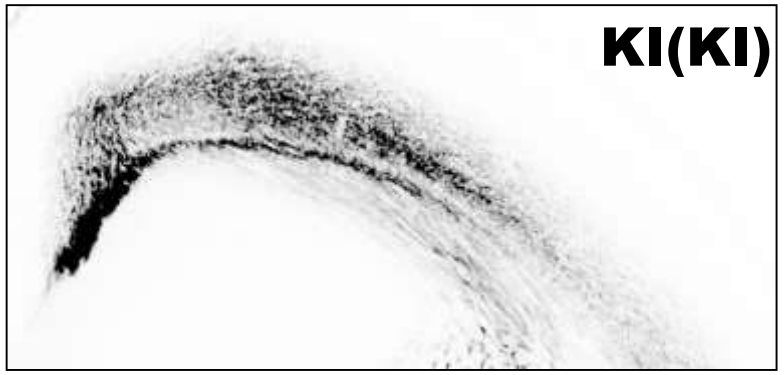




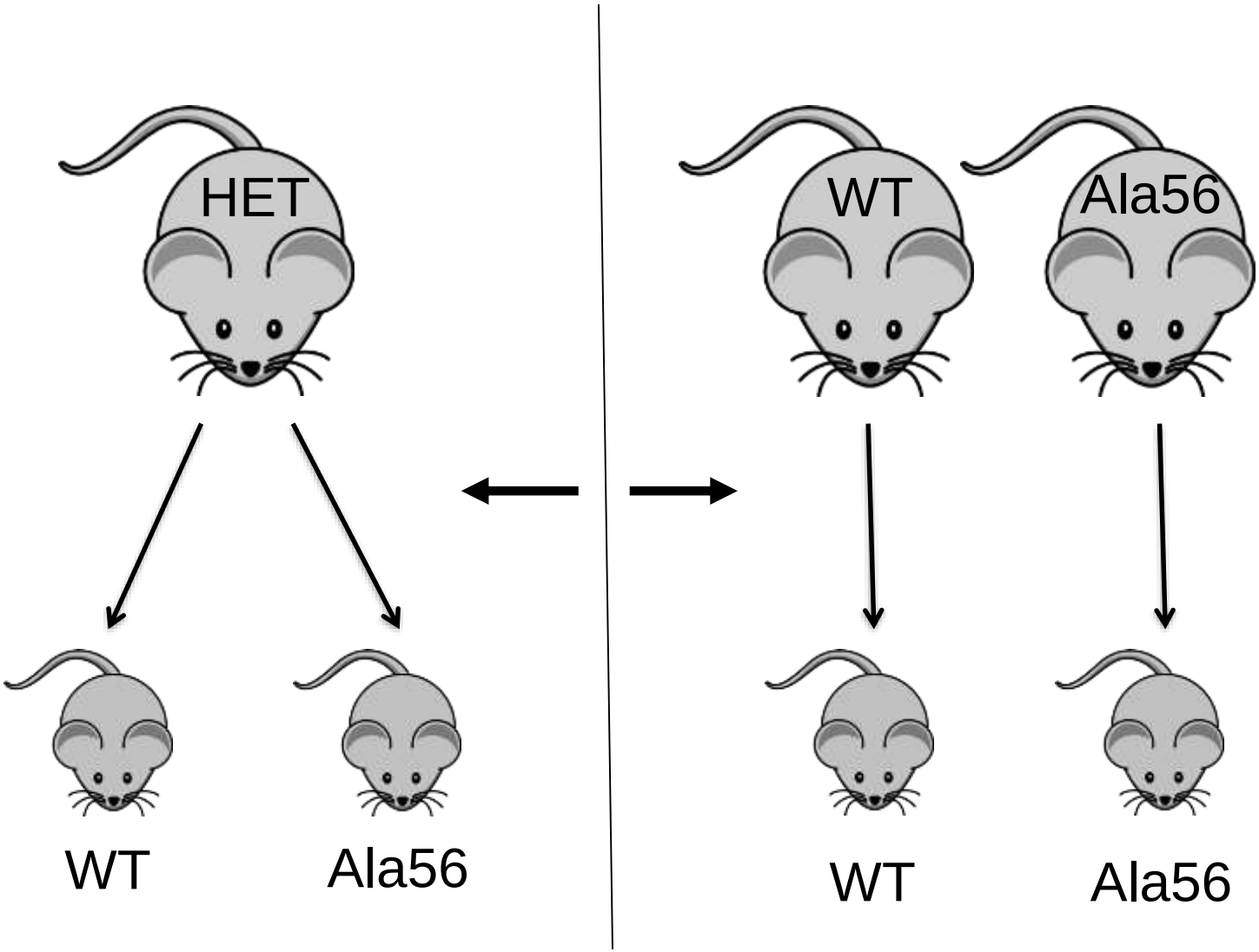


Embryonic Day 18.5

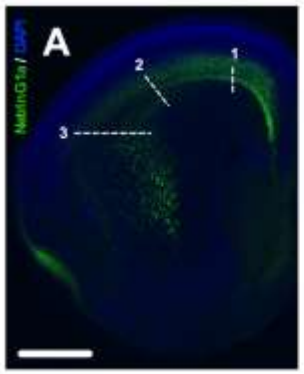




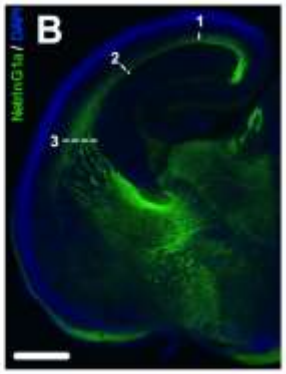
Breeding Schemes Separated



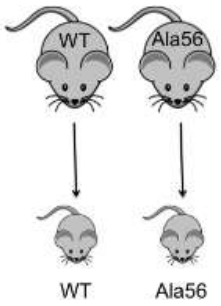
Maternal Genotype Effects

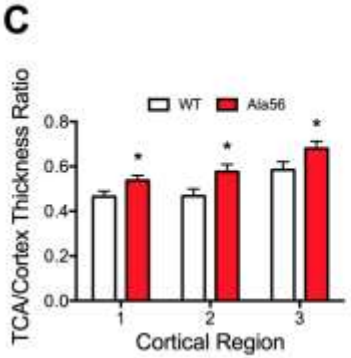


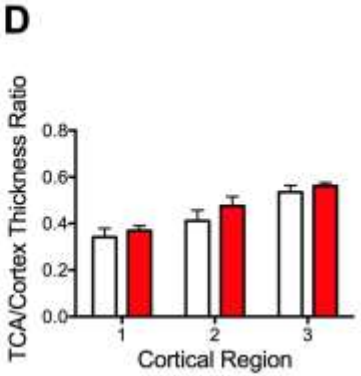
Rostral

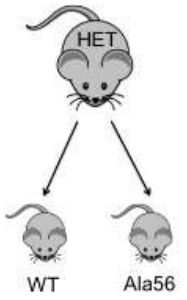


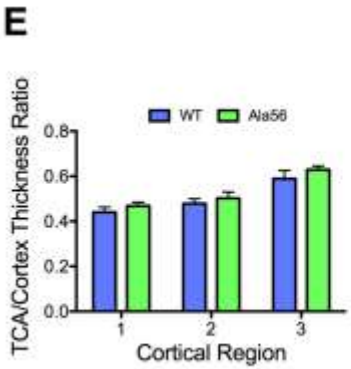
Caudal

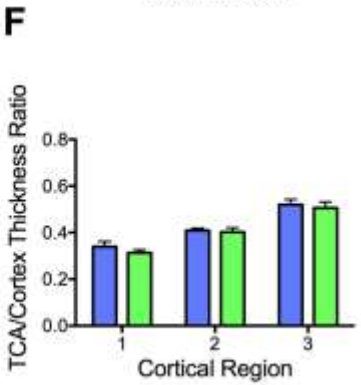






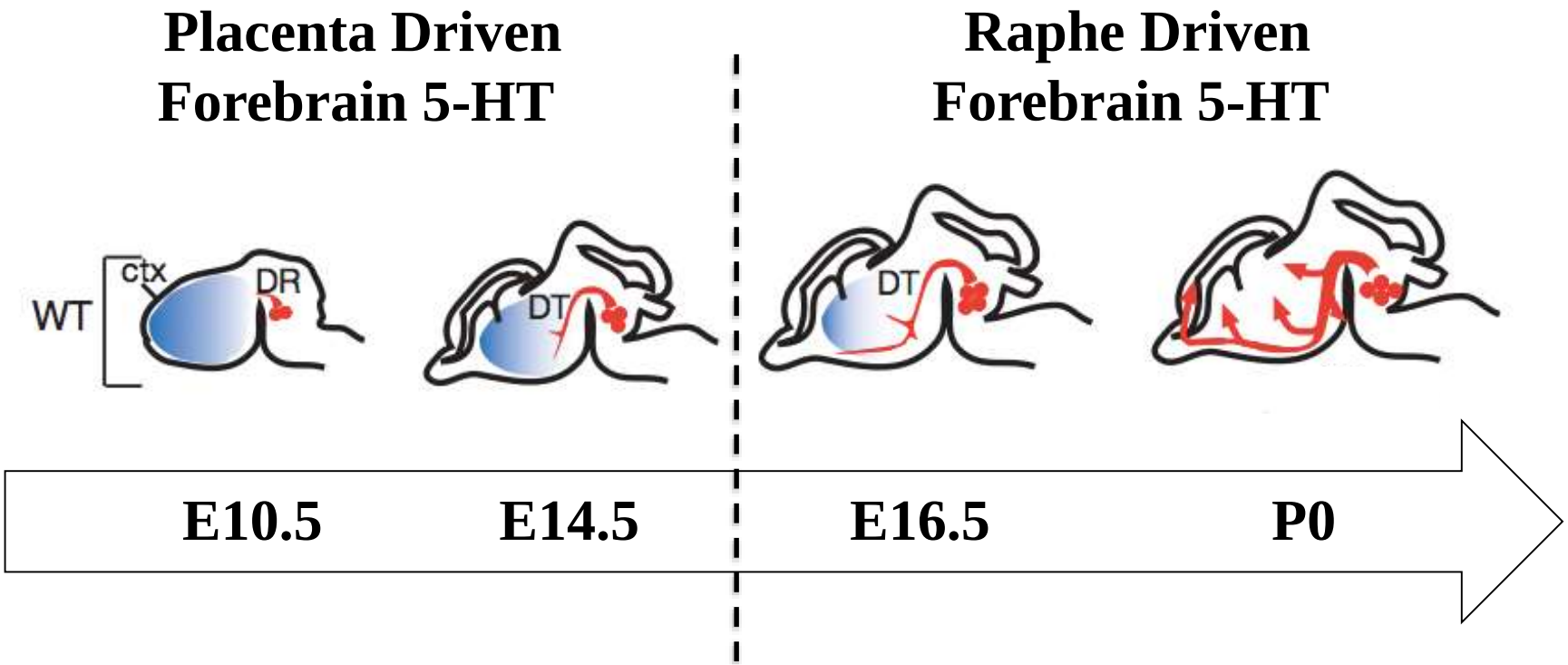






Muller et al., 2017

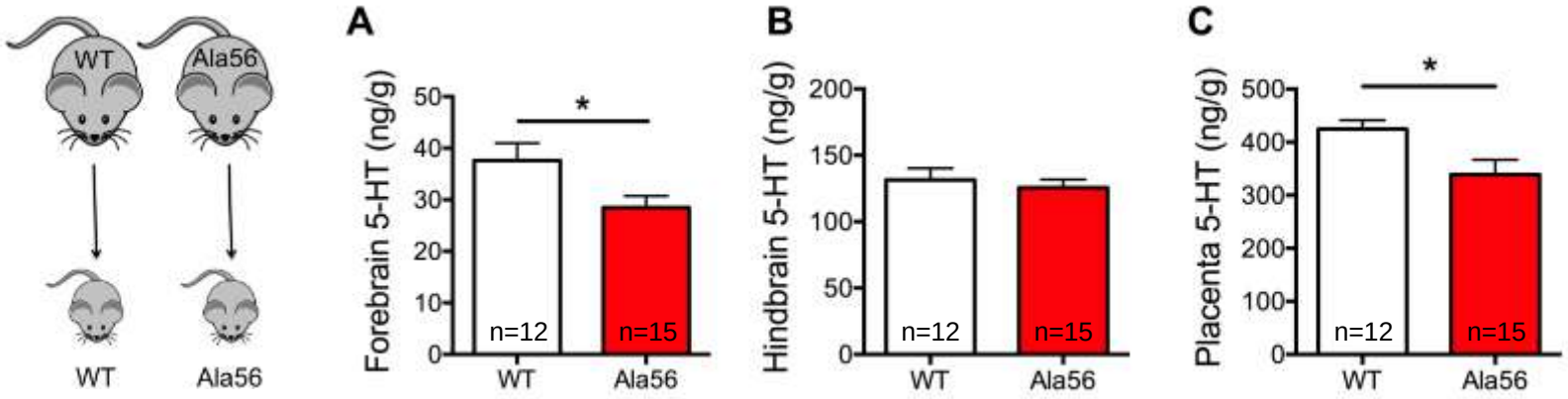
Timeline of Serotonergic Development in the Fetal Forebrain



Modified from
Bonnin et al. 2011

Decreased Forebrain and Placenta 5-HT at E14.5

Maternal Genotype Effects



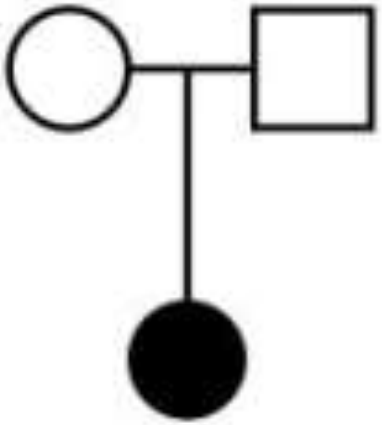
Muller et al., 2017

Translation to Human?

- How to approach?
  - Genetics
    - SERT Ala56 is rare
    - Other SERT variants are common but complex
  - Biomarker
    - Hypothesis: Maternal whole blood 5-HT levels will be associated with proband phenotype.
    - No access to mid-pregnancy blood 5-HT in longitudinal sample
    - No access to maternal blood 5-HT in a general population sample

Translation to Human?

UIC ACE:
105 Families

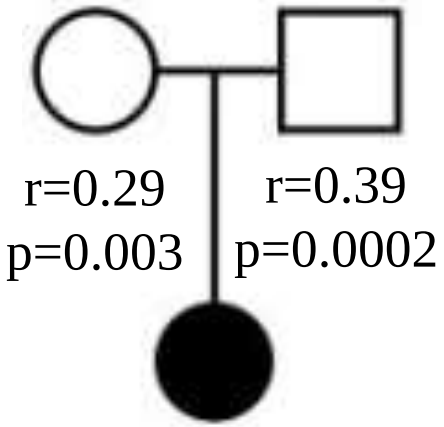


ASD
Diagnosis

Ed Cook, Steve Guter

Recall, blood 5-HT levels are heritable

UIC ACE:
105 Families

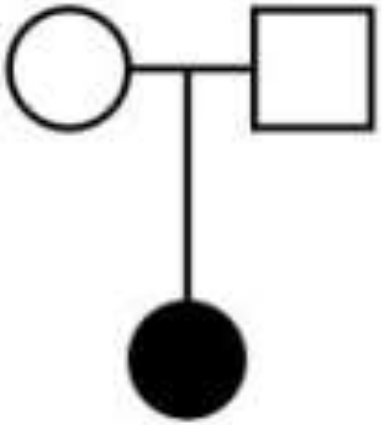


ASD
Diagnosis

1. Must correct for any significant proband effects
2. Paternal levels may control for genetic background and relatedness

Pilot Findings

UIC ACE:
105 Families



ADOS Social (-)		Ø
ADOS Repetitive (-)		Ø
PPVT (+)		Ø
EOWPVT (+)		Ø
VABS Total (+)		Ø
VABS Comm (+)		Ø
VABS Living Skills (+)	ASD Phenotype Ø	Ø

<u>Observational or Anchored Measures</u>:

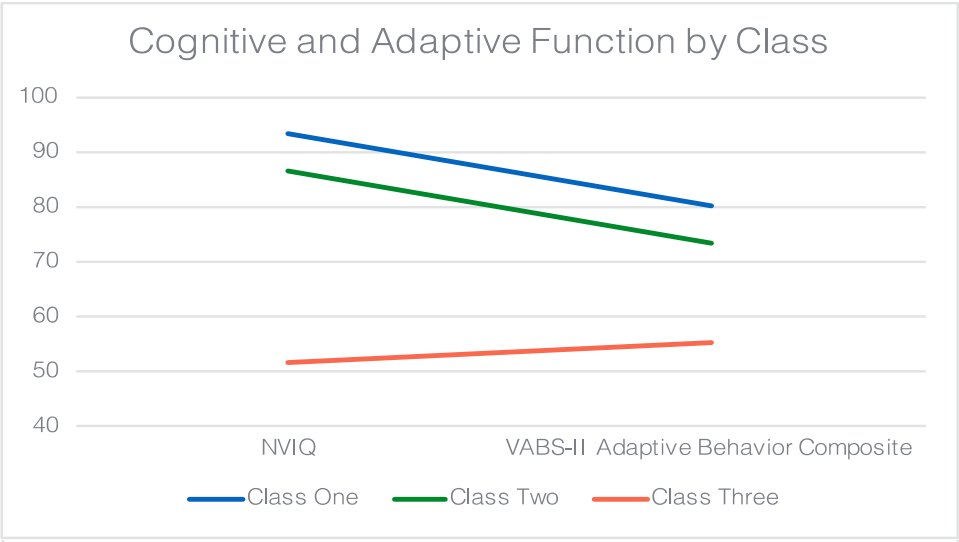
Autism Diagnostic Observation Schedule (ADOS)

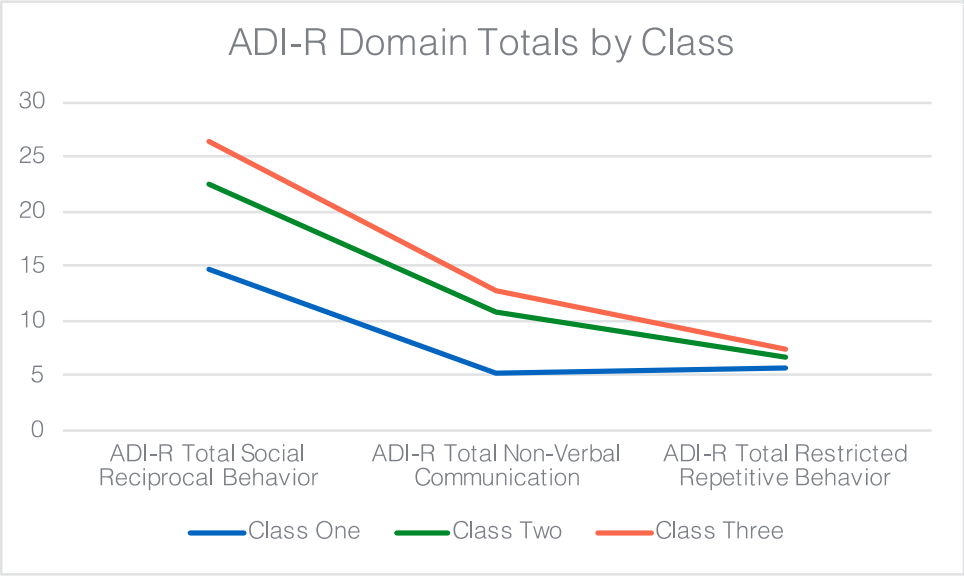
Peabody Picture Vocab (PPVT) / Expressive 1-Word Vocab (EOWPVT)

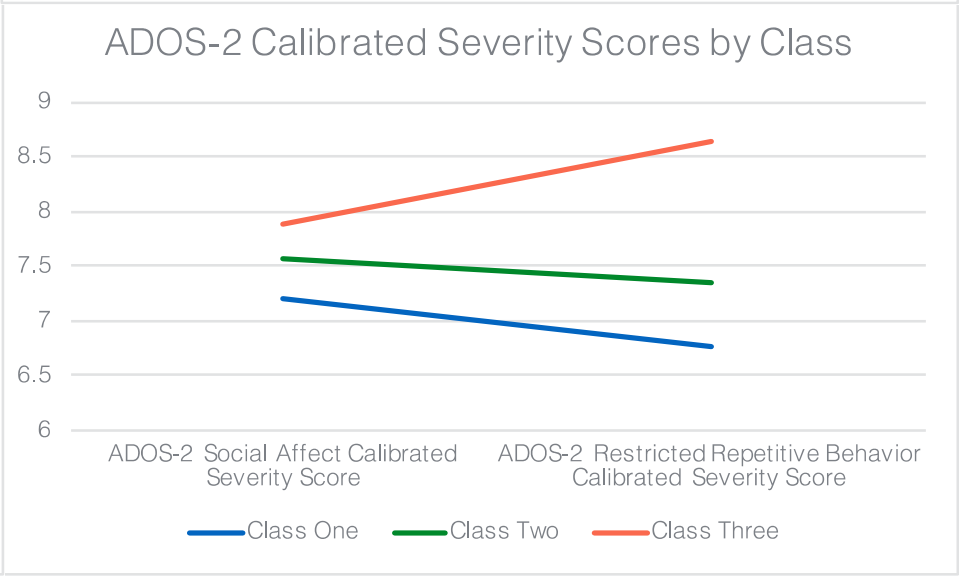
Vineland Adaptive Behavior Scales (VABS)

Latent Class Analysis

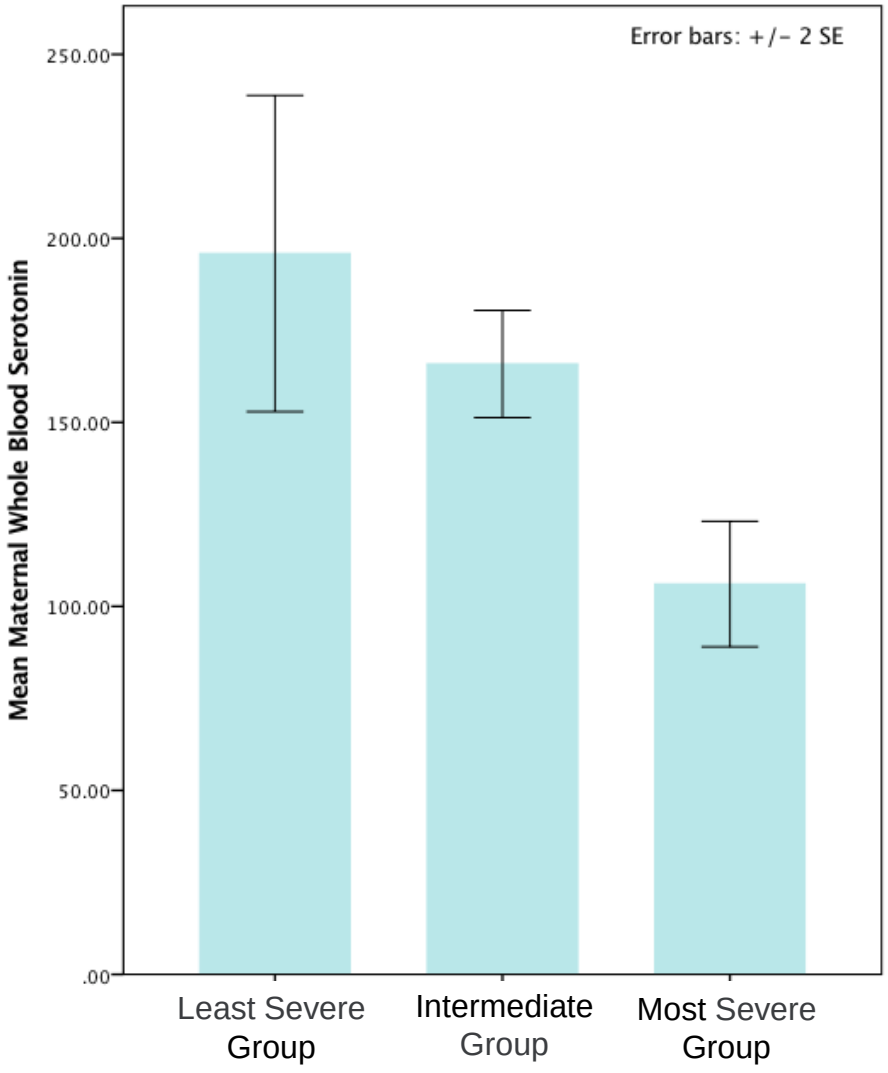
Class One	Mildest ASD Severity Average IQ Average Adaptive Skills
Class Two	Moderate Severity ASD Low Average IQ Borderline Adaptive Skills
Close Three	Highest Severity ASD Intellectual Disability Lowest Adaptive Scores







Montgomery et al., 2017



	N	Mean Maternal 5-HT	Std. Err
Least Severe Group	19	195.86	21.48
Intermediate Group	74	165.87	7.29
Most Severe Group	13	106.10	8.53

Mean Maternal 5-HT was significantly different across the classes of severity classes, Welch's $F(2, 32.457) = 16.948$, $p < .001$.

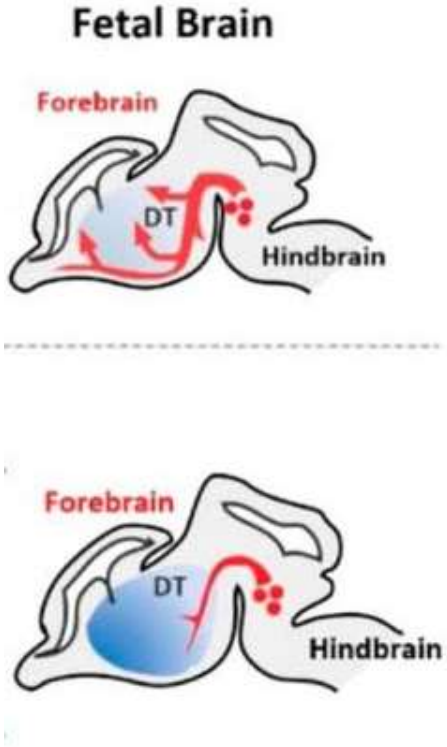
Lots left to do…

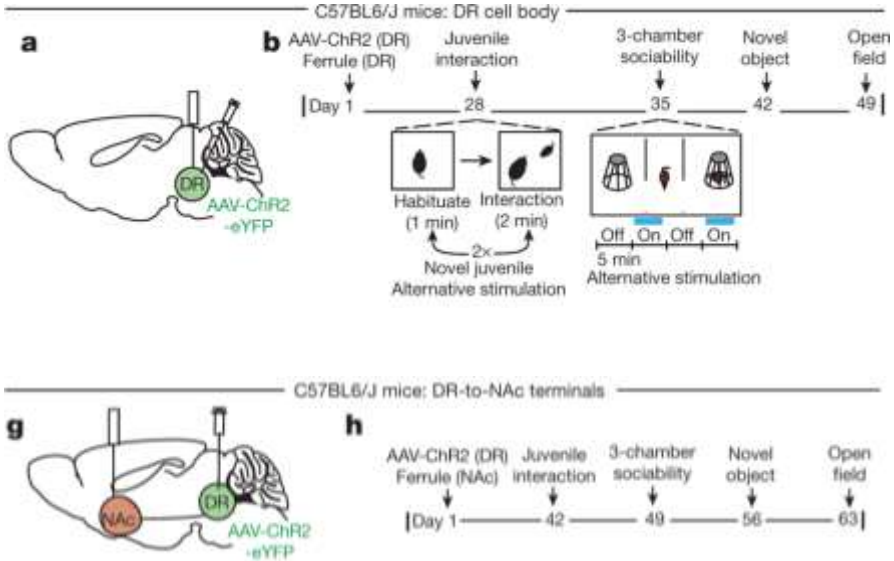
- Mice
  - Placental (and pregnancy) mechanism
  - Maternal gene x embryo gene interaction
  - Consequences on brain and behavior
- Humans
  - Replication
  - Non-ASD population
    - Prospective
    - Retrospective

Mechanisms?

Exogenous 5-HT regulates developing 5-HT system

5-HT projections to N Acc modulate sociability





Goeden et al., *J Neurosci*, 2016

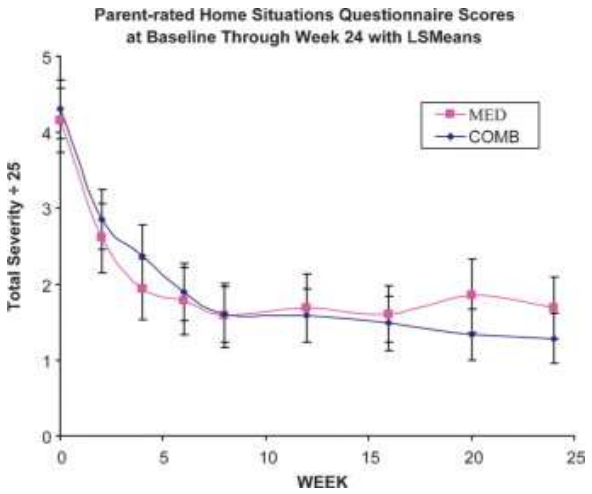
Walsh et al., *Nature*, 2018

How to intervene?
When to intervene?
In whom to intervene?

Outline

- Challenge of heterogeneity
- How have we tested treatments in ASD?
  - Known potholes
- How do we find new treatments?
  - Rare Genetic Disorders → Molecular Targets
  - Symptomatic Treatments → Circuitry Targets?
- In whom should we study new treatments?
  - Biomarker-based therapeutics?
- The ultimate goal: combined medical and behavioral treatment

Additive Effects of Behavioral Therapy and Risperidone?



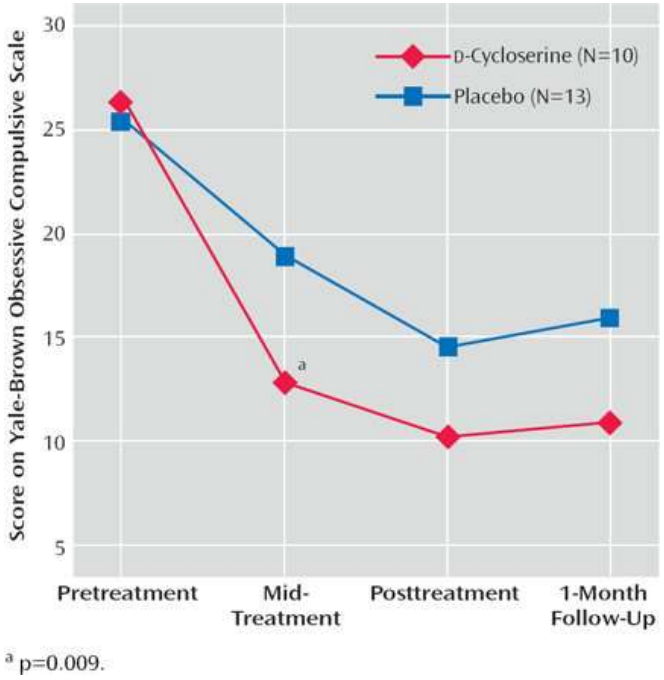
P = 0.006

Is this clinically significant?

Aman et al, *JAACAP*, 2009

Synergistic Effects of D-cycloserine and Extinction

- D-CS facilitates behavioral therapy
  - Taken 1 hour before therapy session
  - Data in OCD, PTSD, Social Phobia, Specific Phobia



Wilhelm et al., *Am J Psychiatry*, 2008

Overview

- DSM-defined Autism Spectrum Disorder is not a disease
- Current treatments not based on neurobiology
- “Placebo” effects are common & complex
- Promise of precision medicine
- Circuit-based treatments → larger population?
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- The Sackler Foundation

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