Tackling the Challenges of Autism Spectrum Disorders by Identifying Cellular and Molecular Targets with Research in Animal Models

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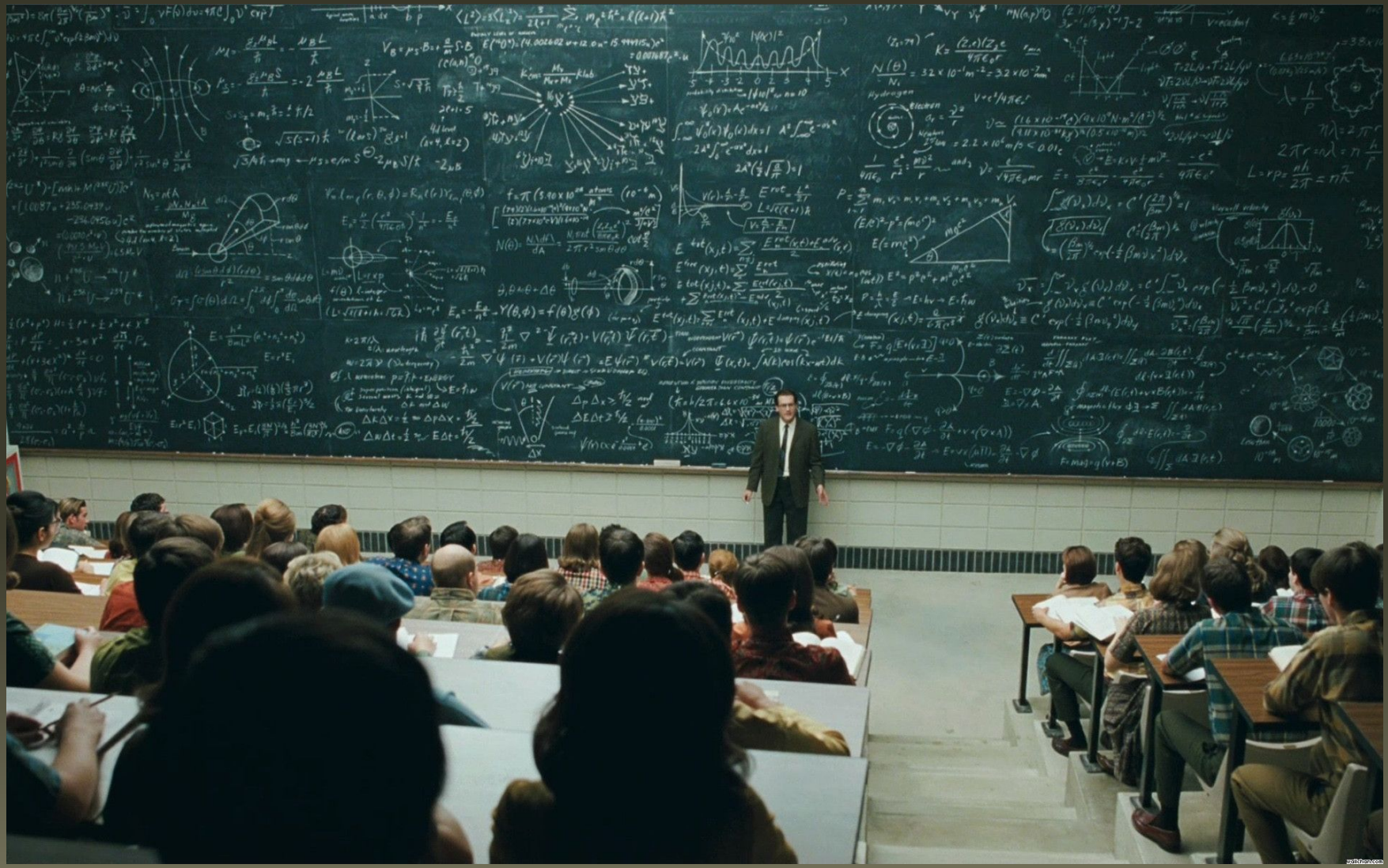
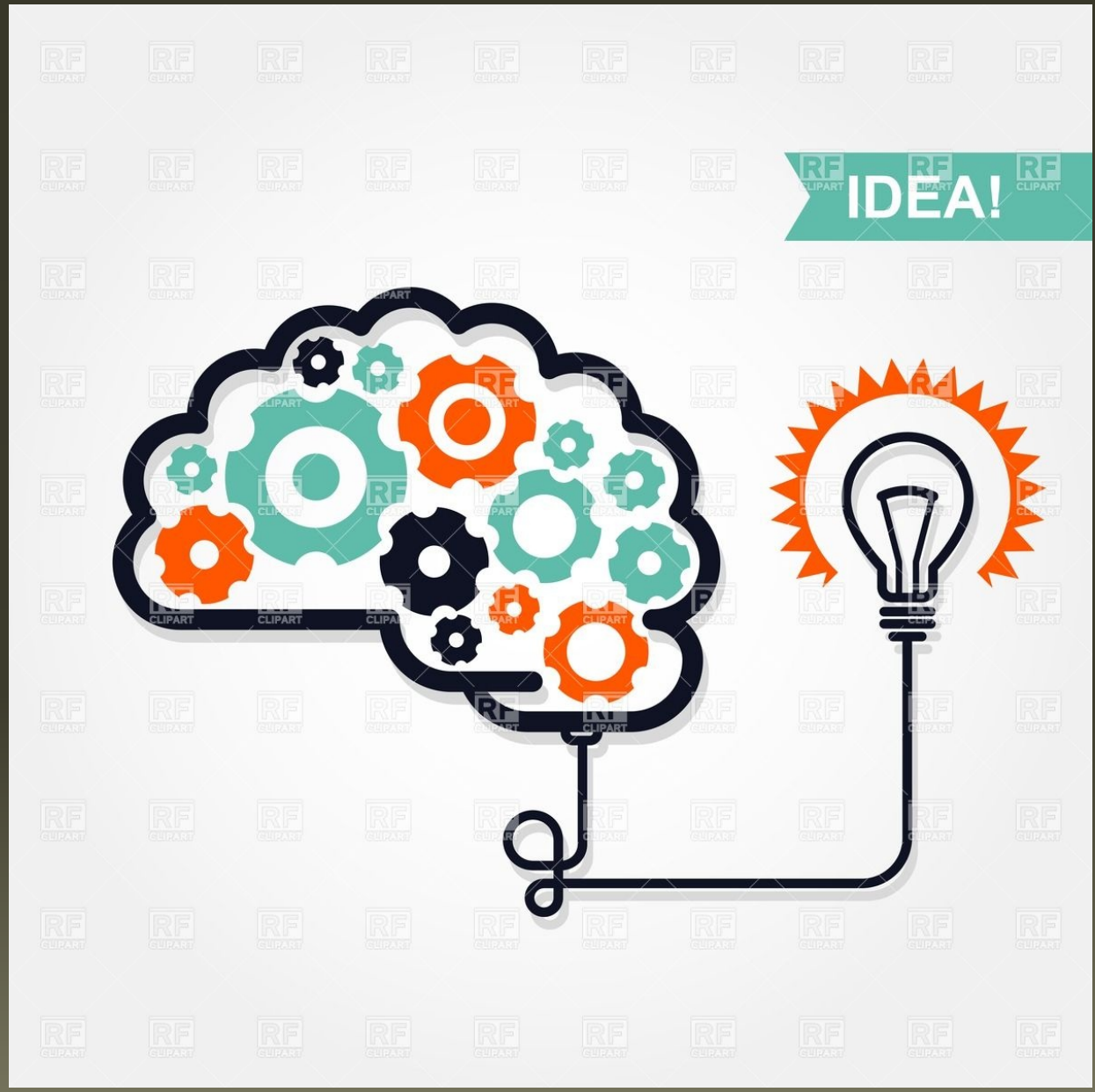
Where are we?

How do we move forward?

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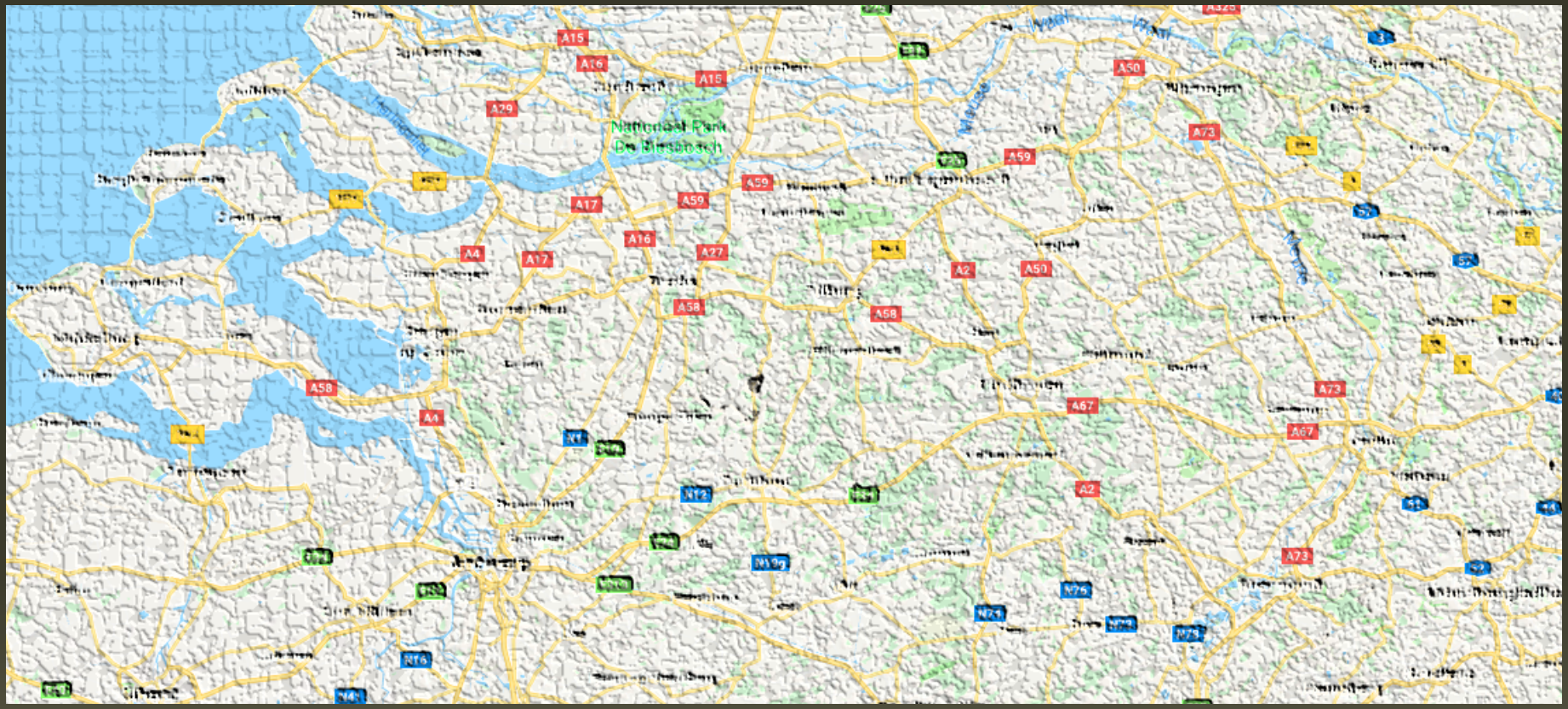
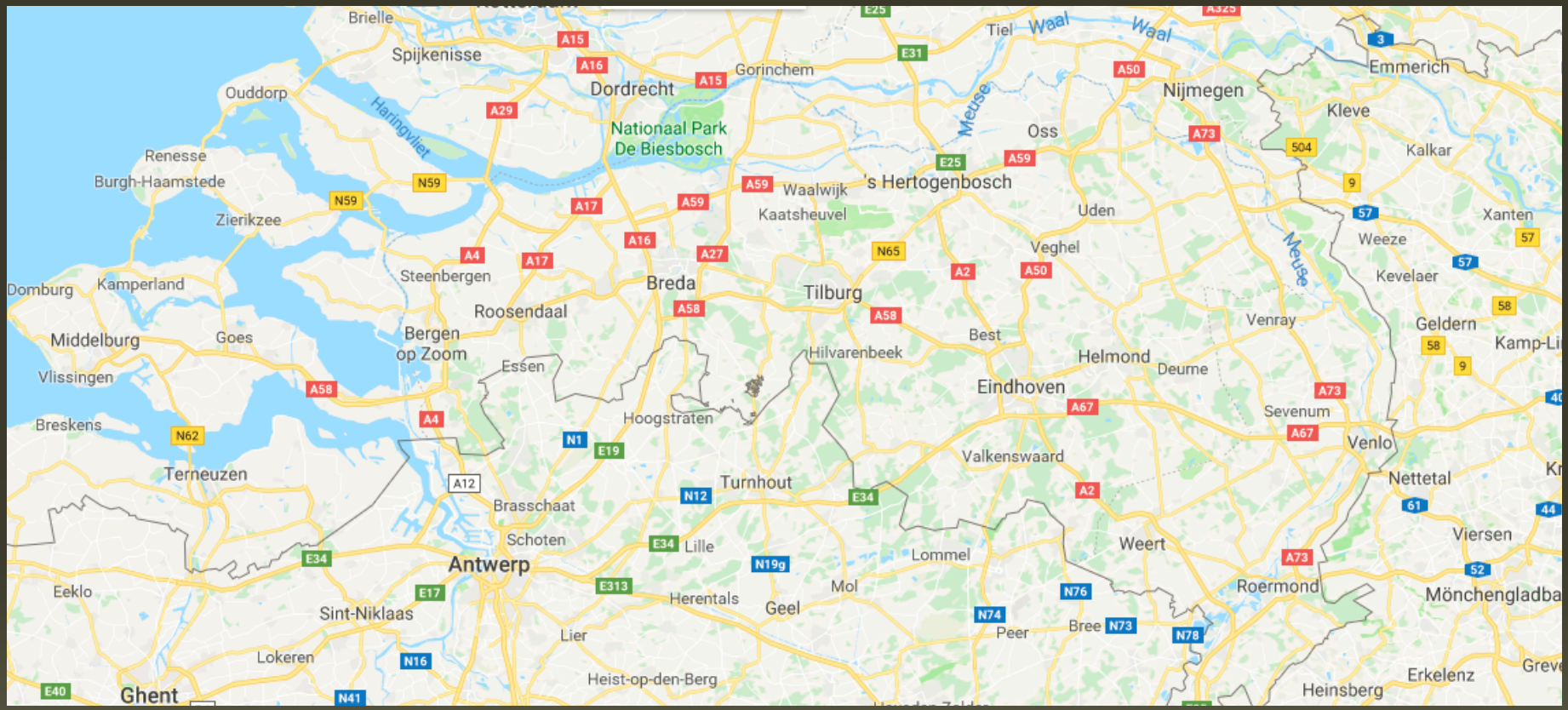


Brain functions can help us understand behavior

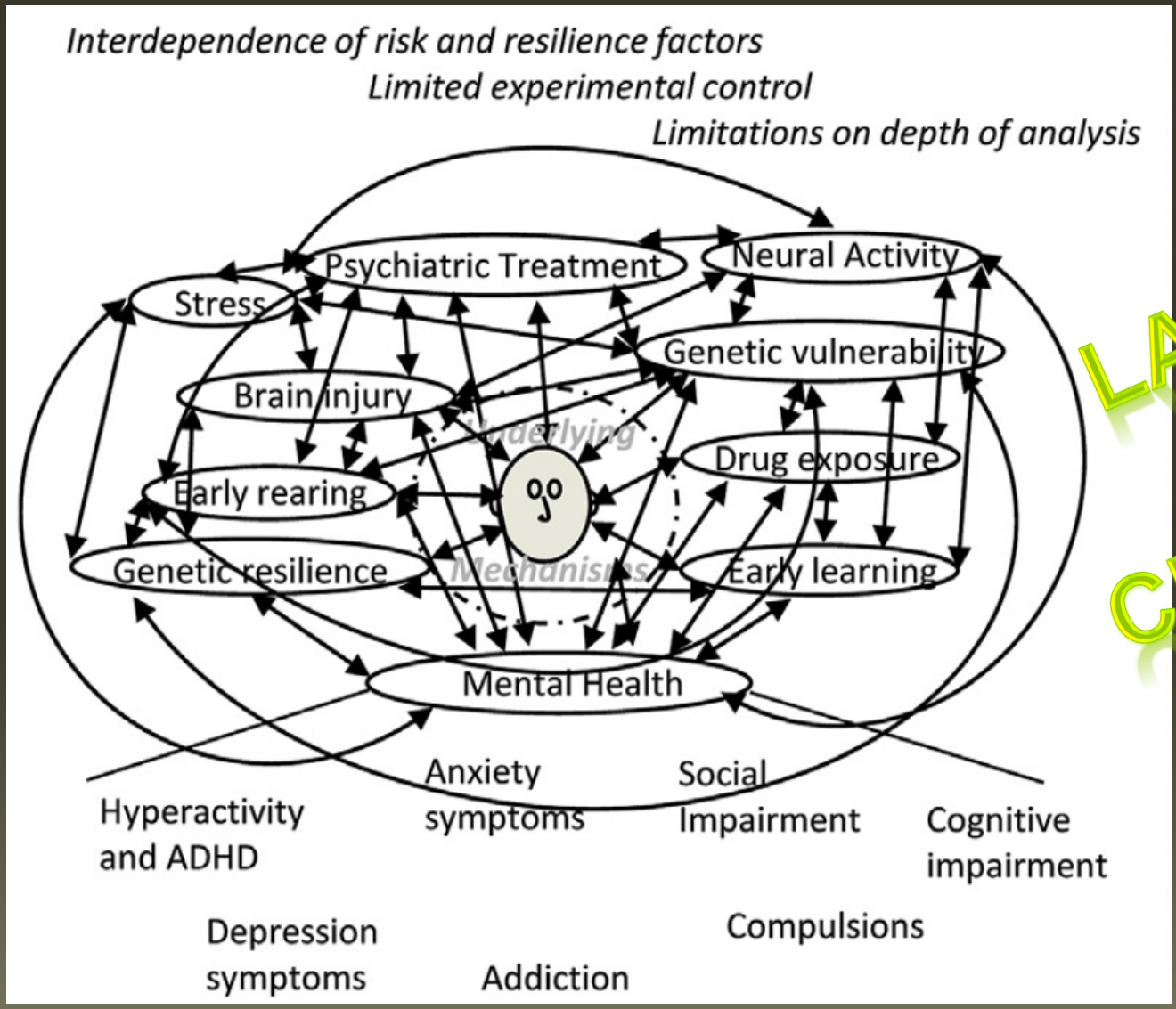
NATIONAL **FRAGILE X** FOUNDATION

FRAGILE X CLINICAL & RESEARCH CONSORTIUM



Brain functions can be understood in animal models

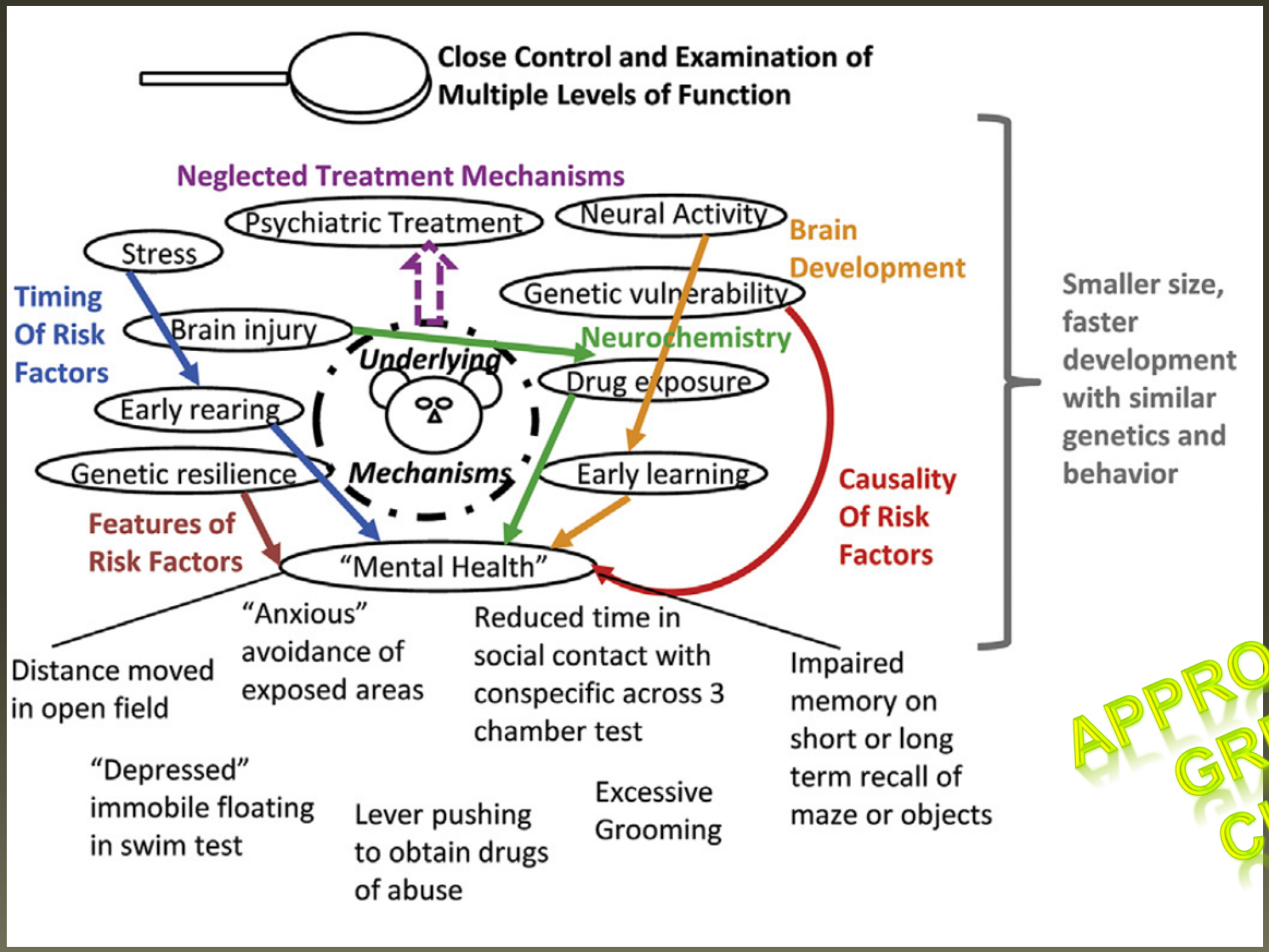
Challenges in Child Psychiatry



LACK OF CLARITY

Stevens and Vaccarino 2015 JAACAP

Animal Models for Child Psychiatry



APPROACHING GREATER CLARITY

Stevens and Vaccarino 2015 JAACAP

Autism Spectrum Disorder*

- Social deficits and repetitive behaviors/restricted interests
  - Diagnosis is based on clinical assessment– no diagnostic biomarker
  - No treatments can correct these core deficits
  - Treatments/early intervention can help improve functioning
- Higher incidence in males than females (4:1)
- Present from an early stage and causes impairment

*note: ASD can be highly integrated into a person's identity. Some individuals with ASD do not find current treatments appropriate for them

Filling in the map for Autism Spectrum Disorder

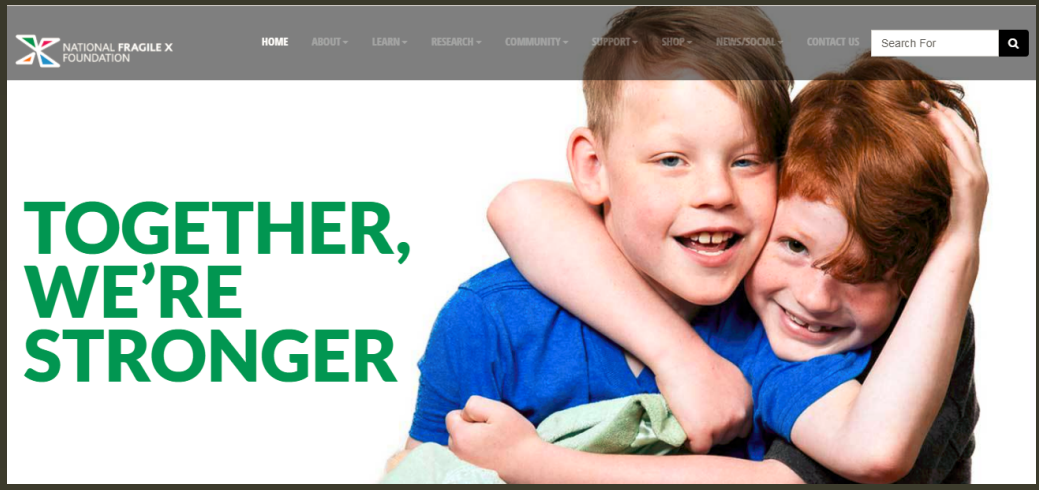
- How can we improve diagnosis and understanding?
  - i.e. when lack of diagnostic clarity limits treatment decisions
  - Better diagnostics depend on better knowledge of disorder identity and variation
  - Better understanding depends on identifying what is happening in individuals
- How can we find treatments for problems that have few?
  - i.e. autism social deficits, learning limitations
  - New, rational treatments depend on knowledge of disease mechanisms
- How can we prevent problems?
  - i.e. enhancing resilience, buffering against adversity
  - Prevention depends on knowledge of disease origins/causes and mechanisms of development

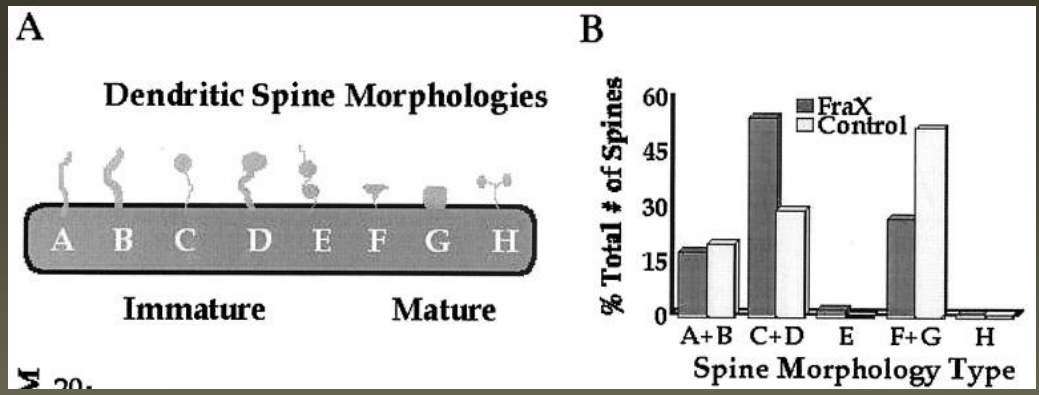
Progress made with animal models: Examples from the past 20 years

- Cell structural differences: synapses, cell populations
- Cell functional differences: transcription of genes
- Developmental events: transitions in cell signaling
- Convergence of findings with human postmortem studies
  - Helps validate causality of etiologies
  - General biological processes across species

Fragile X Mouse Model and Synaptic Pathology

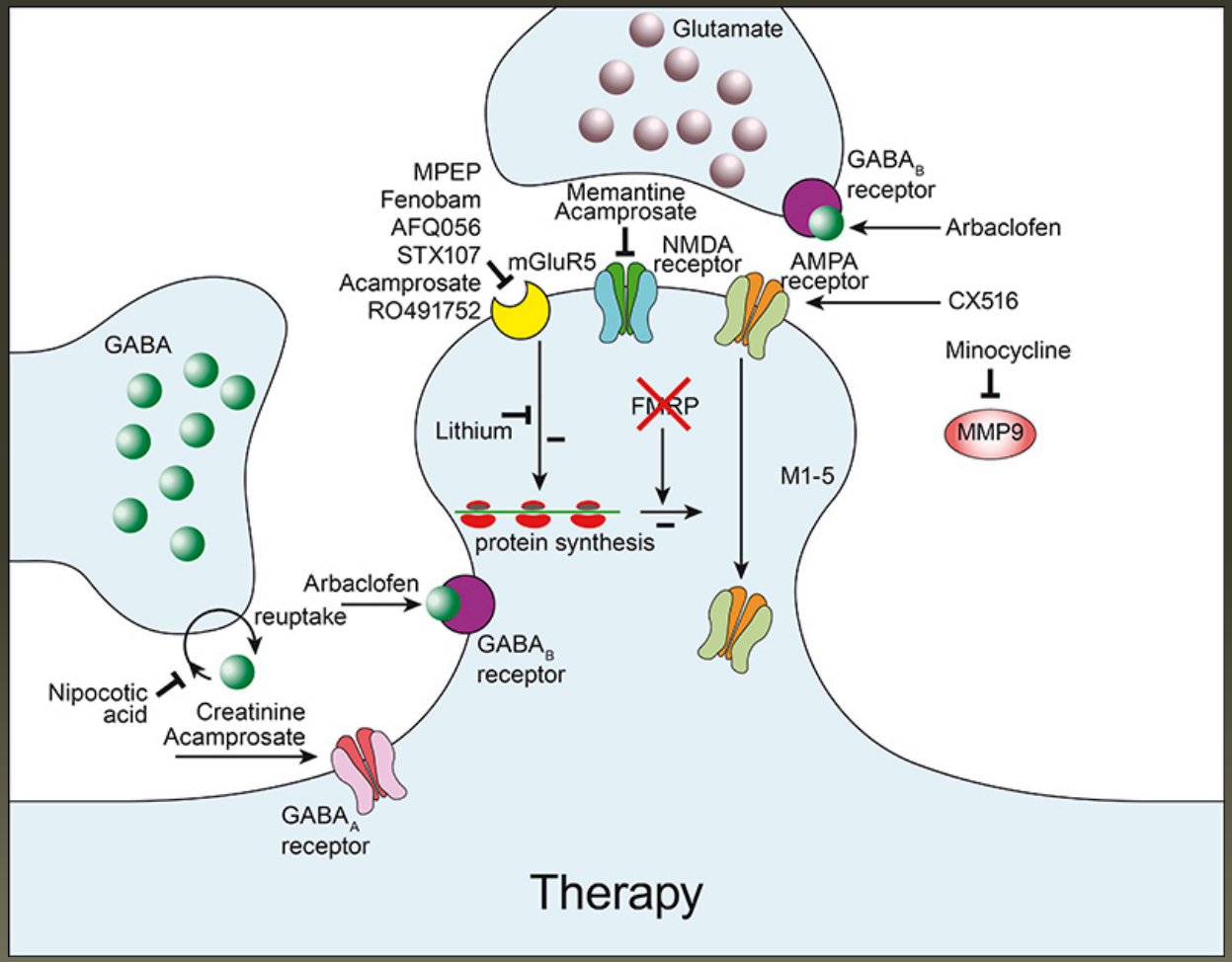
- An inherited disorder with high rates of intellectual disability and ASD
- Result from mutation of the fragile x mental retardation protein gene
- First mouse model of autism spectrum disorder
- Identified immature synapses as a major cellular deficit





Greenough et al 2000 PNAS

Fragile X Mouse Model: Synaptic function the focus of therapy

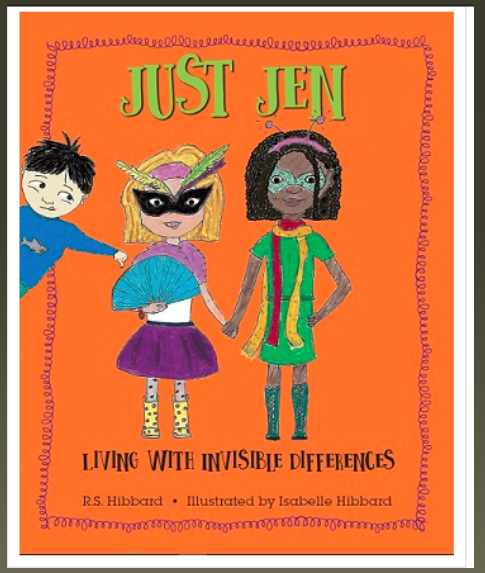


Erasmus MC Rotterdam

22q11 Deletion and Cortical Neuronal Populations

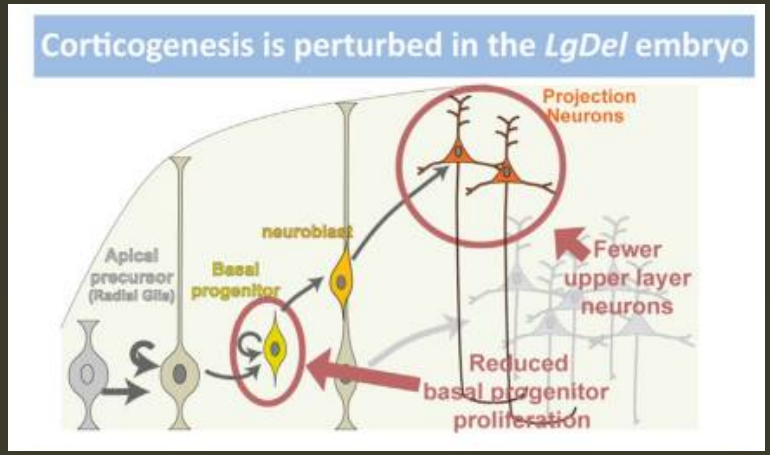
- DiGeorge or Velocardiofacial syndrome: abnormalities of brain, heart and development of other systems
- A major chromosomal abnormality associated with a high risk for ASD, schizophrenia, and/or other behavioral deficits
- Involves disruption of many genes grouped together on the human chromosome 22
- Mouse models have been created that have chromosomal disruptions, overlapping significantly with genes involved in the human genetic deficit

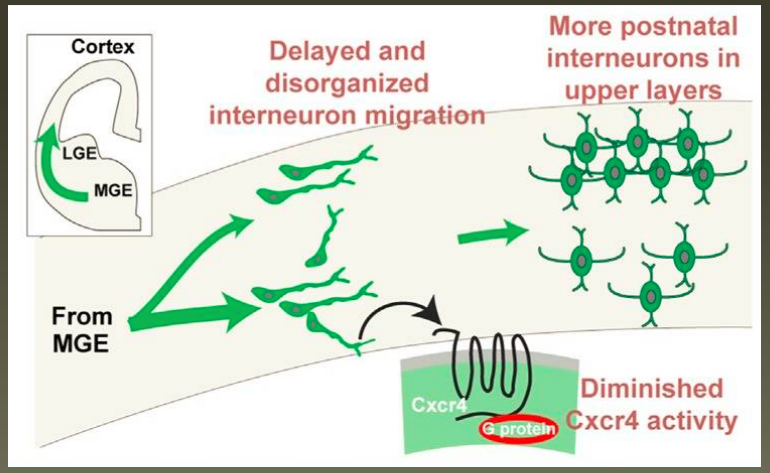
the 22q FAMILY foundation



22q11 Deletion and Cortical Neurons

- Mouse Model=LgDel (Large Deletion)
- Production of excitatory neurons is reduced, resulting in fewer neurons to communicate critical information from the neocortex to other regions
- Organization of inhibitory neurons is disrupted, resulting in cells that regulate excitatory neurons in the wrong place
- **Disrupted balance of cell populations and cortical organization**





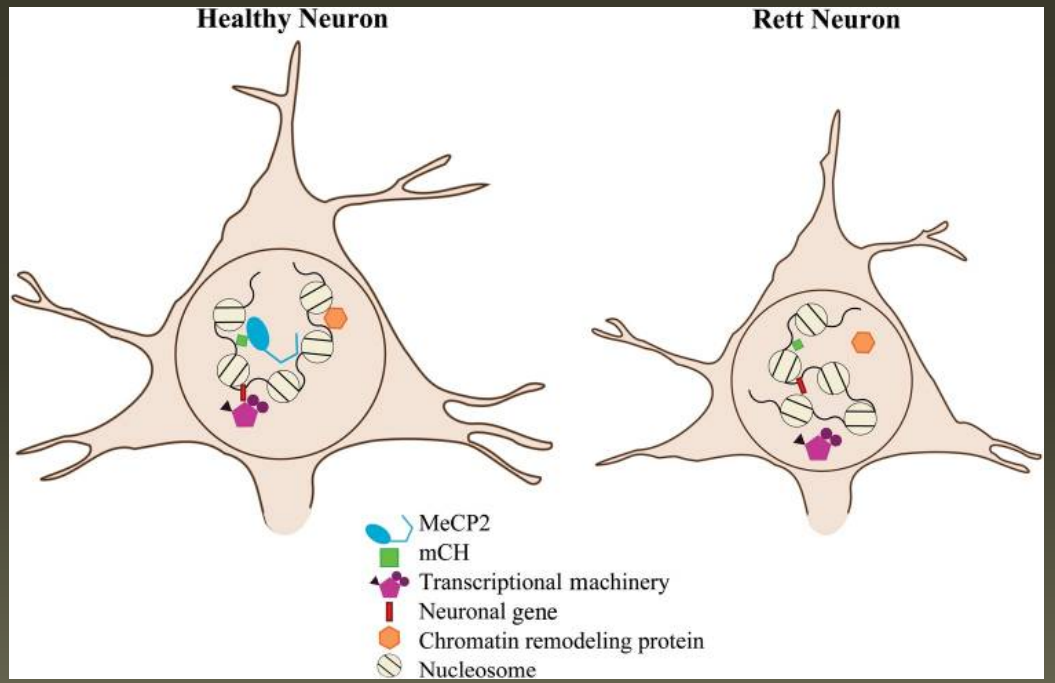
Meechan et al 2015

Rett Syndrome Risk Gene and Transcriptional Regulation

- An inherited disorder with significant motor and intellectual disability
- At times, individuals will be diagnosed with autism spectrum disorder
- Regression of skills
- More frequent in girls
- Mutation in the MeCP2 gene classically (rarely, other genes)

Rett Syndrome Risk Gene and Transcriptional Regulation

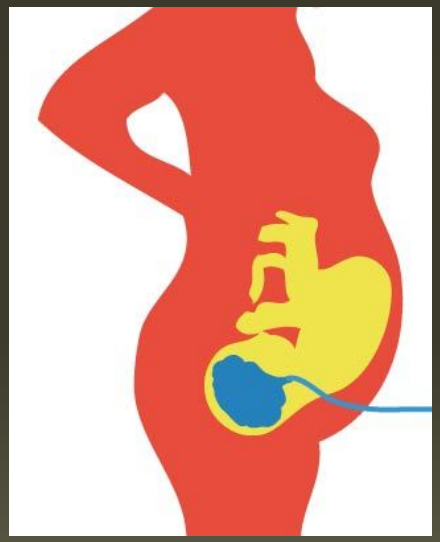
- The mutated protein does not allow for DNA to take the right form to be “read” and made into proteins (transcription), especially in brain cells
- This loss of normal protein formation contributes to multiple thing neurons and/or glia can’t do



Pohodich and Zoghbi 2015

Maternal VPA or Inflammation and Developmental Transitions

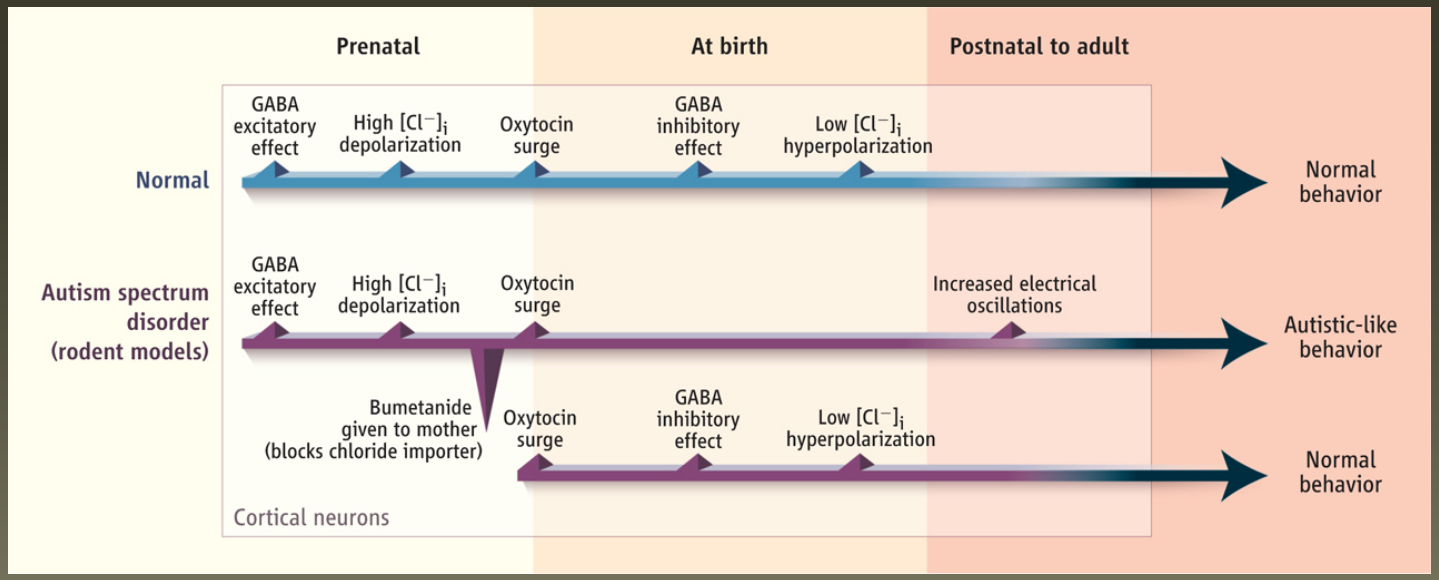
- Embryonic brain development occurs in the maternal milieu
- Maternal administration of the medication Depakote (VPA) and/or high levels of systemic inflammation during pregnancy can increase risk for autism
- Many events during this period of development are critical for later brain function



Developinghumanbrain.org

Maternal VPA or Inflammation and Developmental Transitions

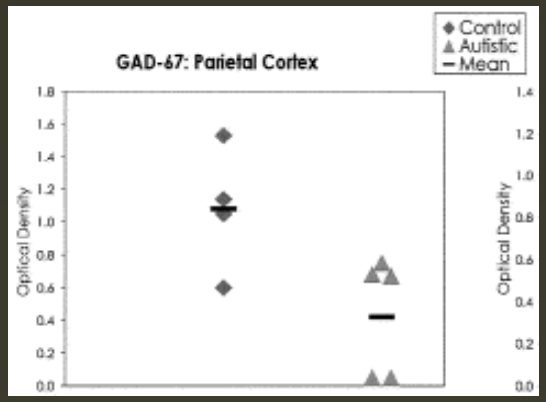
- Regulatory **inhibition** of the cortex turns on during development
- Depakote and inflammation can both disrupt that switch and lead to too much excitation in the mature brain
- Medications now in clinical trials to target the cellular components at the core of this switch: Diuretic and GABA modulator: Bumetanide (blocks the NKCC1 chloride importer)



Zimmerman and Connors 2014

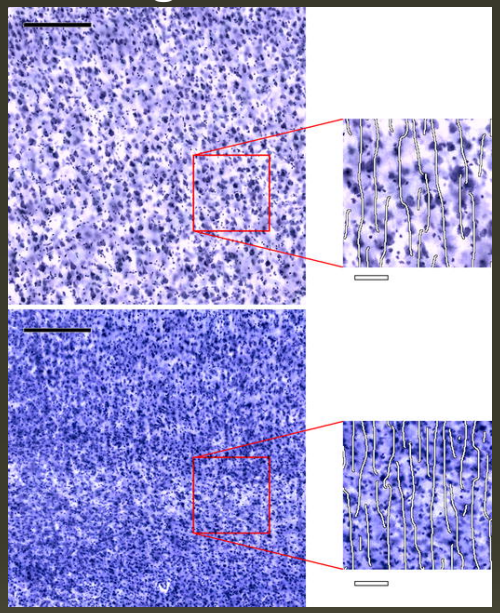
Convergence with human postmortem findings in ASD

Imbalance in Cell populations



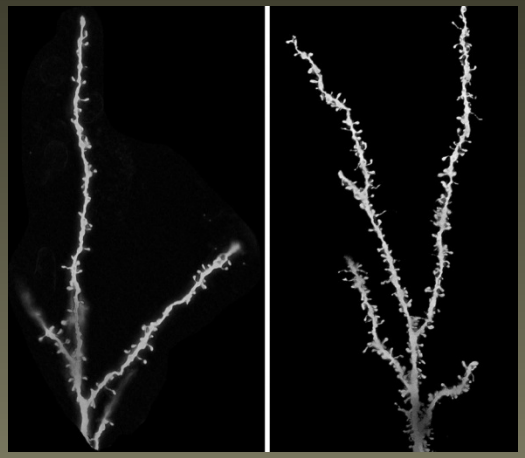
Fatemi et al 2002

Cortical Organization

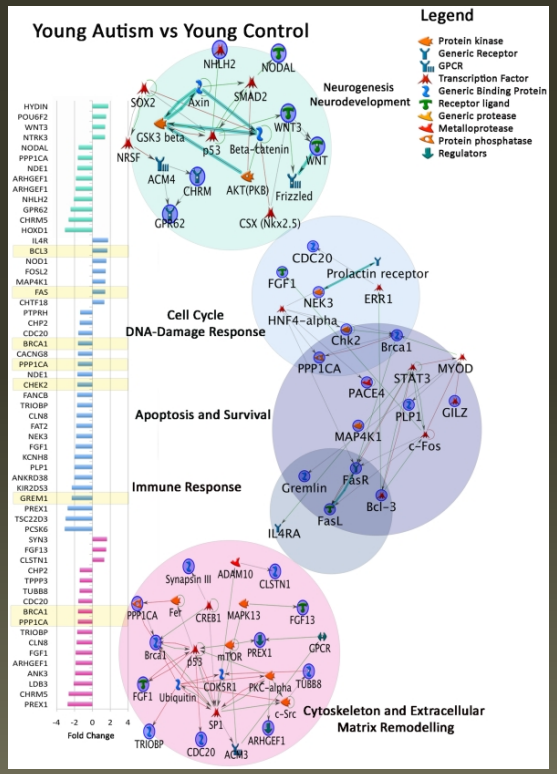


Casanova et al, 2006

Synaptic pathology



Transcriptional Changes



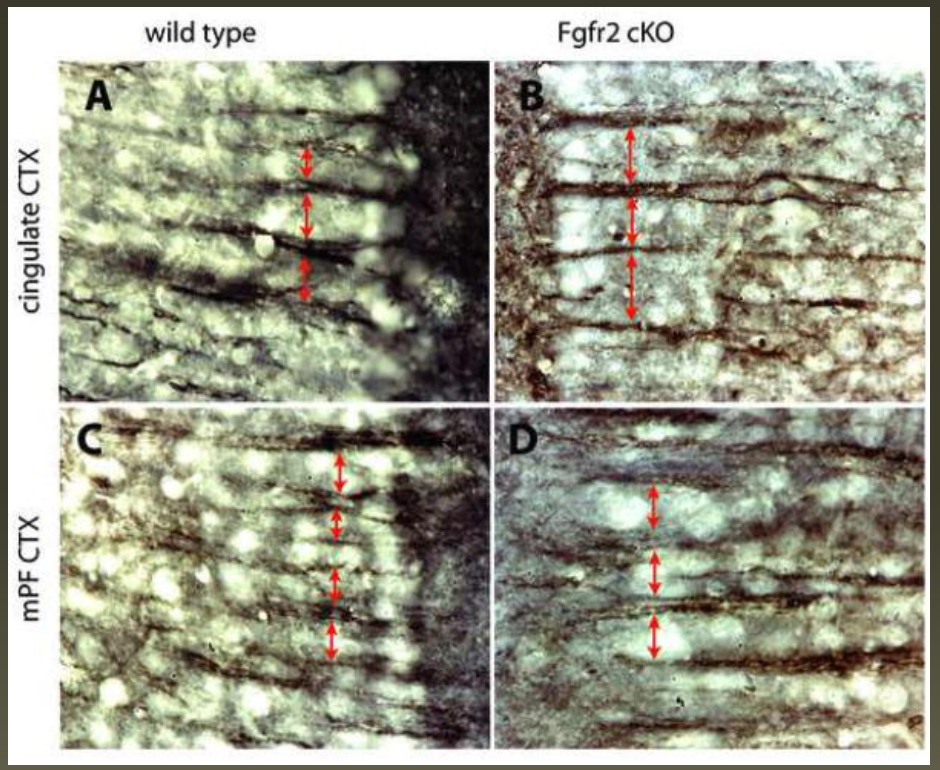
Chow et al 2012

My lab's work in animal models relevant to autism spectrum disorder

- Fibroblast growth factor: cell populations and arrangement
- Specific ASD risk gene: BCKDK and oxidative stress
- Developmental events and sex differences: prenatal stress and cellular proliferation

Fibroblast Growth Factor and Brain Formation

- Brain growth depends on multiple growth factors, including a large set of factors originally identified in fibroblasts: Fibroblast Growth Factors (FGFs)
- Common findings in autism include macrocephaly (large brain) and disrupted cortical structure
- Changing FGF signaling in mice can recapitulate both overgrowth of the brain and abnormal cortical structure



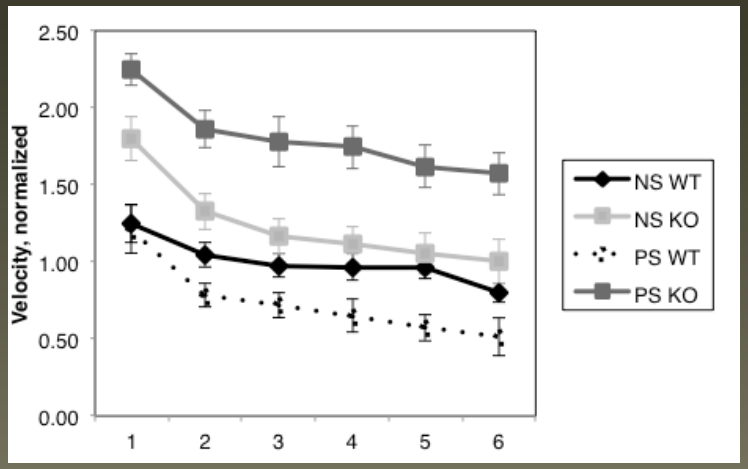
Vaccarino et al, 2009

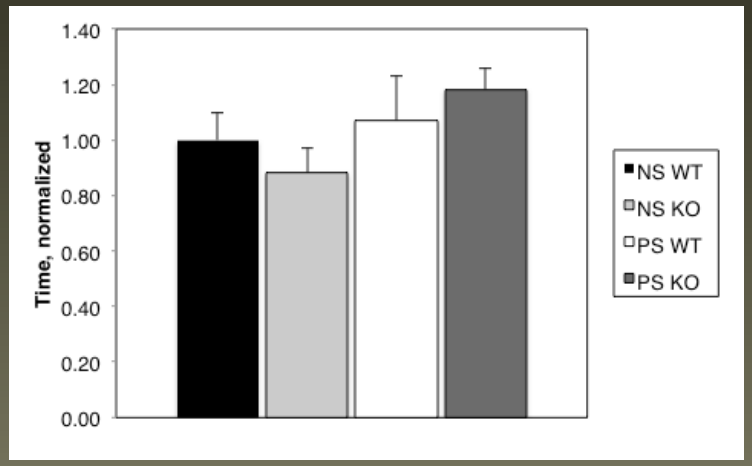
Fibroblast Growth Factor and Brain Formation

- FGF gene mutations are not implicated in autism but they affect some of the same functions as other genes linked with autism
- FGF genes also may interact with early developmental stressors to change behavior

(Prenatal stress + FGF signaling deficit= hyperactivity and impulsivity)

- These findings reveal how identifying genetic risk factors for autism may be complicated by variations in experience

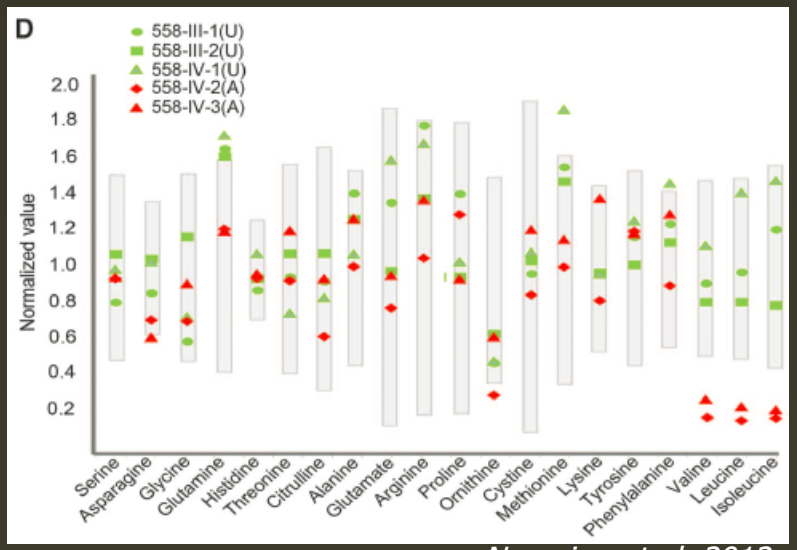




Jager and Stevens unpublished

BCKDK Risk Gene and Cellular Problems

- The BCKDK risk gene is associated with ASD and intellectual disability
- This gene mutation results in rapid depletion of branched-chain amino acids, important building blocks of proteins
- It is unclear how amino acid deficits would contribute to autism-like brain dysfunction
- The mouse model shows similar amino acid deficits but cellular problems are unclear



Novarino et al, 2012

Table 3 Concentrations of amino acids in the serum and brains of BDK$^{+/+}$ and BDK$^{-/-}$ mice

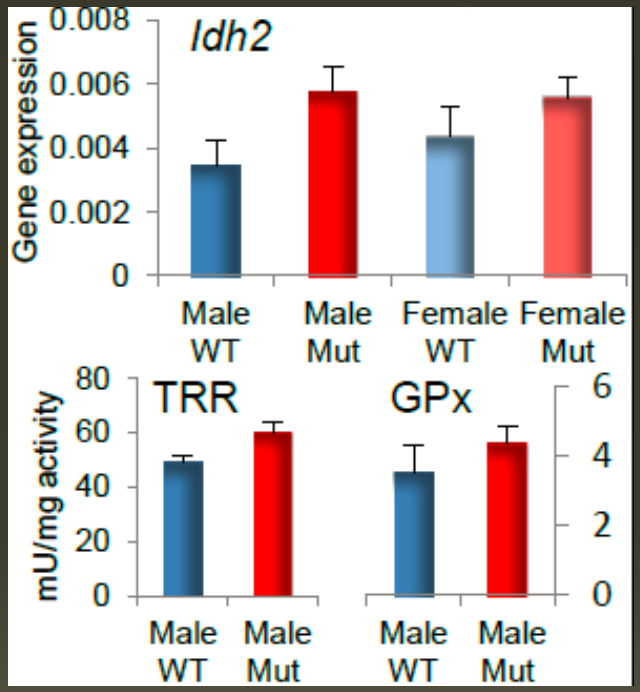
Amino acid concentrations in μmol/g of wet weight for brain and μM for serum. Values are means $\pm$ S.E.M., $n = 5$ except for isoleucine in brain ($n = 4$) (*$P < 0.0001$; **$P < 0.01$). Percentage reduction indicates how much each amino acid was reduced in BDK$^{-/-}$ mice.

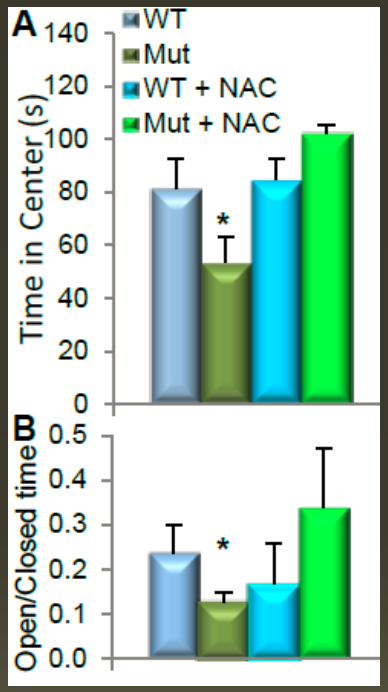
Tissue	Amino acid	Amino acid concentration BDK$^{+/+}$	Amino acid concentration BDK$^{-/-}$	Percentage reduction
Brain	Valine	173 $\pm$ 27	49 $\pm$ 14*	72
	Leucine	62 $\pm$ 6	20 $\pm$ 2*	68
	Isoleucine	30 $\pm$ 3	8 $\pm$ 1**	74
Serum	Valine	208 $\pm$ 12	105 $\pm$ 12*	49
	Leucine	149 $\pm$ 12	71 $\pm$ 9**	52
	Isoleucine	90 $\pm$ 15	28 $\pm$ 3**	69
	Threonine	138.8 $\pm$ 19.5	131.1 $\pm$ 5.8	6
	Methionine	65.5 $\pm$ 11.7	50.1 $\pm$ 5.3	24

Joshi et al, 2006

BCKDK Risk Gene and Oxidative Stress

- In the mouse model with the BCKDK mutation, markers of oxidative stress are increased
- Antioxidant dietary supplementation can rescue behavioral abnormalities of the mice with the BCKDK mutation
- The use of antioxidants may have a future role in treatment for autism





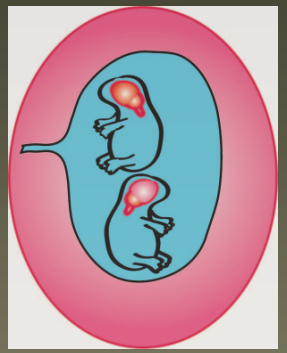
DeWitt and Stevens unpublished

Prenatal Stress, Development, and Sex Differences

- Autism and the high risk to boys arises very early in development, likely in embryonic brain
- Embryonic brain does not have large differences in hormones that later determine male and female sex characteristics
- Studying a model of embryonic brain disruption could provide clues to this higher male risk

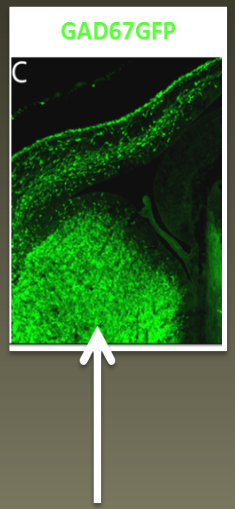
Prenatal Stress

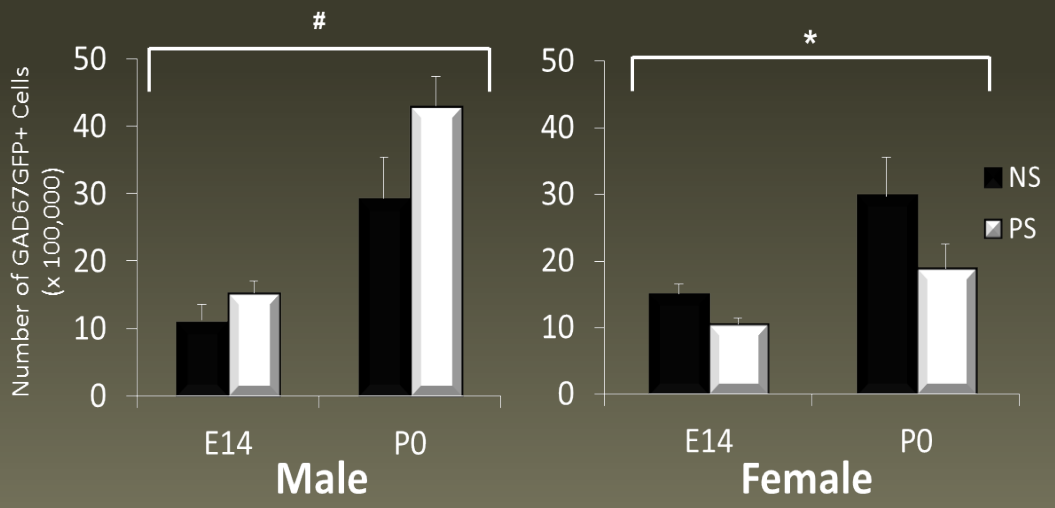
Embryonic Brain Development

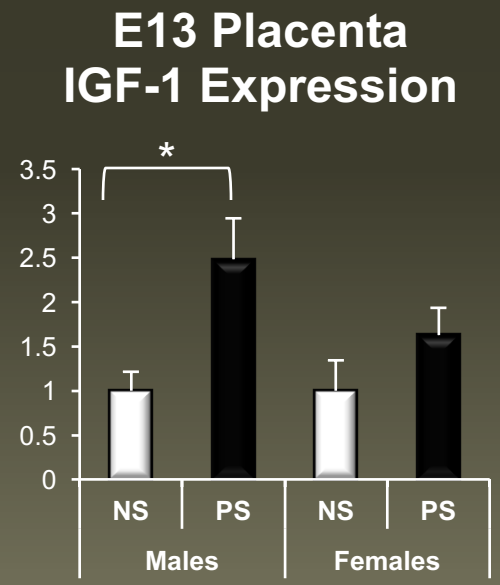


Prenatal Stress, Development, and Sex Differences

- In the prenatal stress model, we have found an overgrowth of inhibitory neurons only in males
- We have also found that a growth factor produced by the placenta, is increased only in males
- IGF-1 production may significantly influence male risk for brain abnormalities and may be a key mechanism for preventing problems







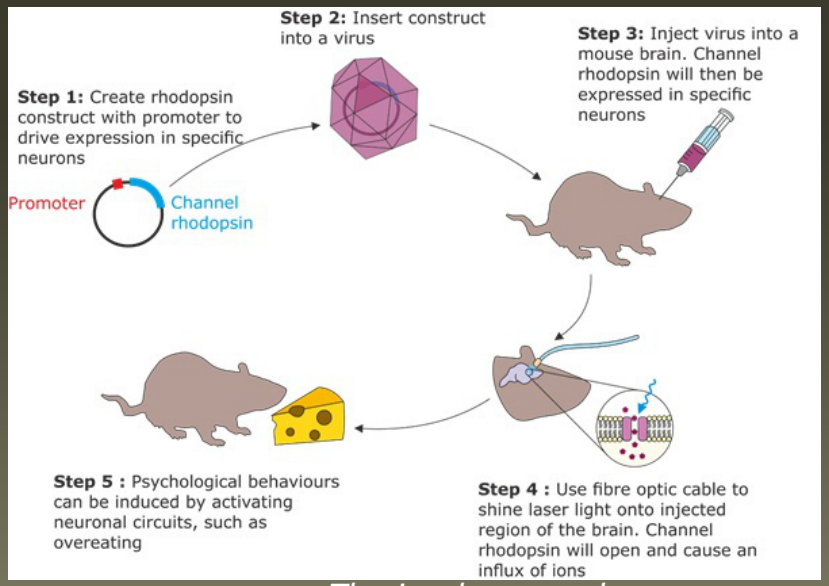
Lussier and Stevens unpublished

New Advances Coming in Animal Models

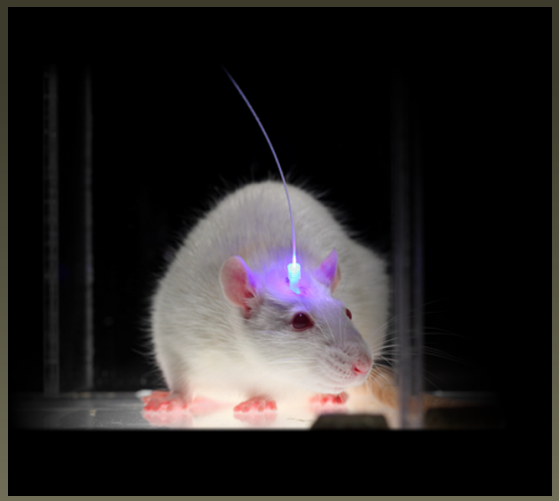
- **Circuit manipulation:** to address specific brain functions
- **High throughput screening:** medical compounds that might address autism-specific brain deficits
- **Preventive measures:** supplementation that may support health brain development in the face of autism risk

Circuit Manipulation

- Ten years ago, methods were developed to turn on specific networks within the brains of animal models
- These are ***Optogenetics***
  - "Opto": light-based stimulation
  - "Genetics": introduced through new genes in specific places in the brain
  - Enhance or reduce activity of a specific set of brain cells and change the function/behavior those cells underlie

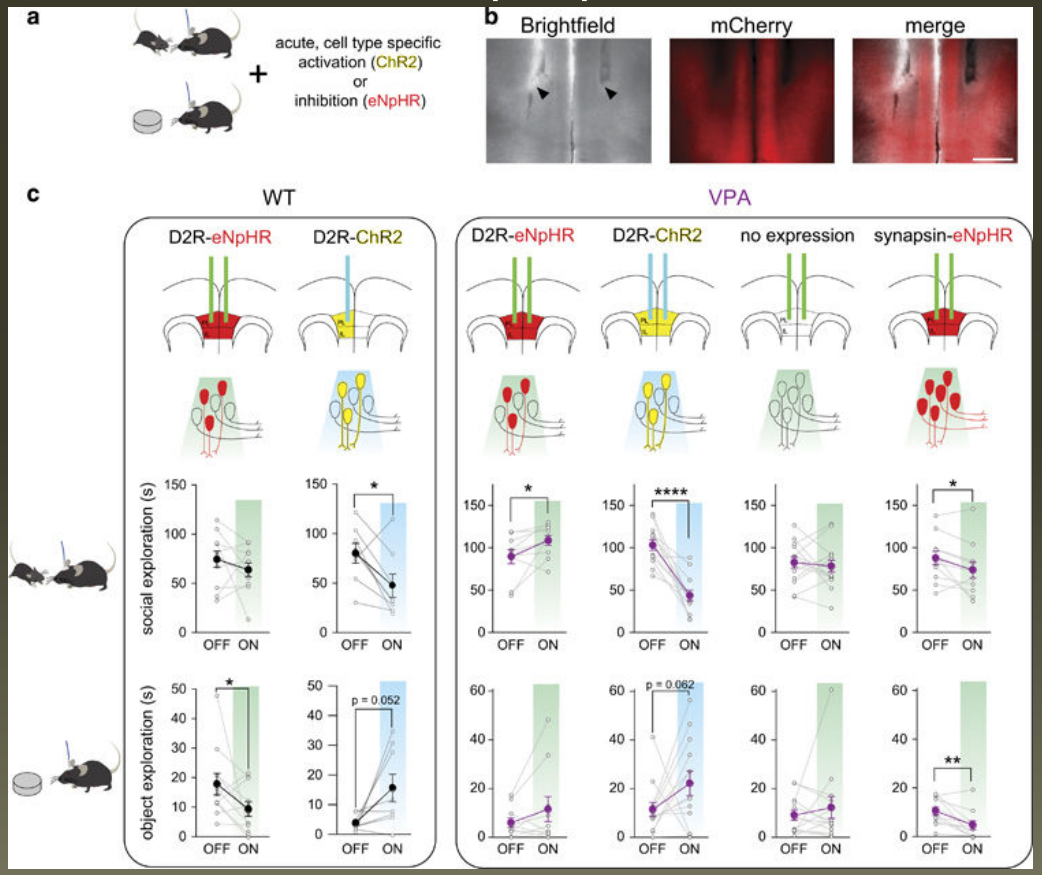


Thesignalmag.wordpress.com



Circuit Manipulation

- Across multiple autism models, activating and suppressing prefontal cortical neurons can change ***social behavior***
- These studies prepare us to perform effective neuromodulation treatments in people with ASD



Brumbach et al 2017

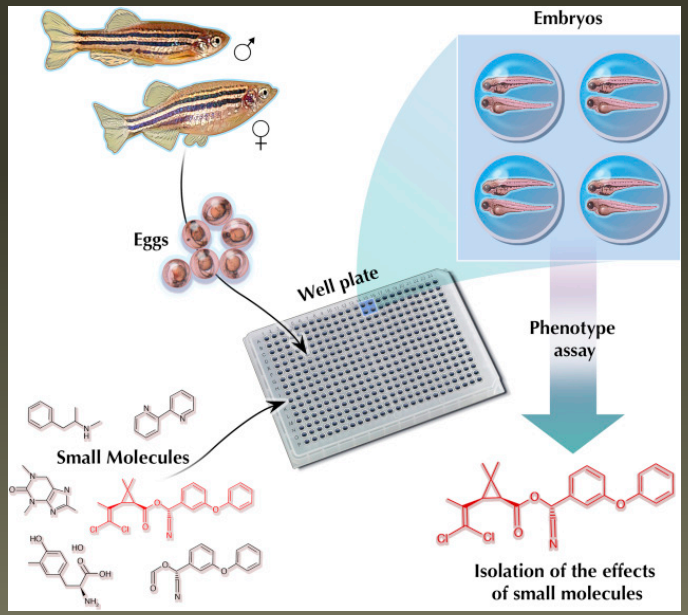
High Throughput Screening

- How can we discover if medications/compounds that already exist may be helpful for Autism?
- We need an effective way to test ***many*** compounds ***quickly*** for potential ***real benefit***

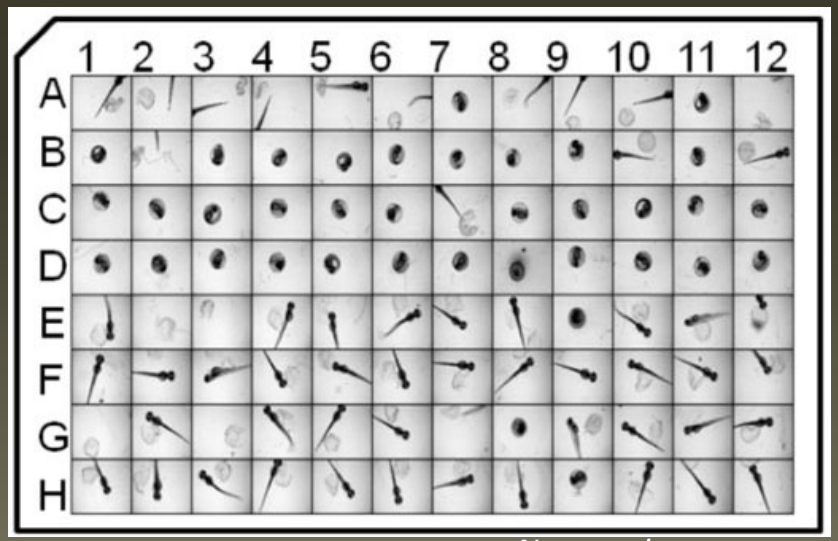
The Benefits of Fish

High Throughput Screening

- Zebrafish rapidly develop and their behavior can be easily assessed
- Zebrafish have been studied for the effects of autism risk genes on brain and behavior, identifying medications that may be “correcting”



Kithcart and MacRae 2017



Nanowerk.com

Preventive Measures

1940's-1960's Folic Acid and nucleic acid synthesis

Crider et al 2011

1980's Dietary Folate Supplements and Neural Tube Defects

Late 1980's and 1990's Folic Acid RCTs
↓ NTDs by 70-100%

CDC
Centers for Disease Control and Prevention

Early 1990's: CDC recommends Supplementation for women

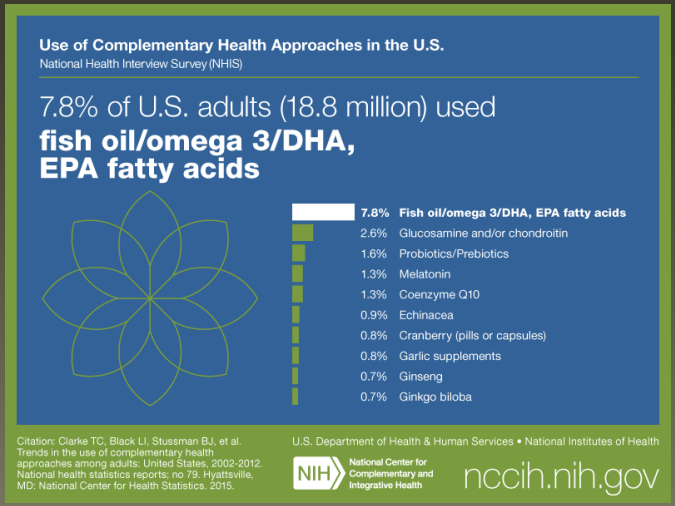
Mandatory Fortification by 2000

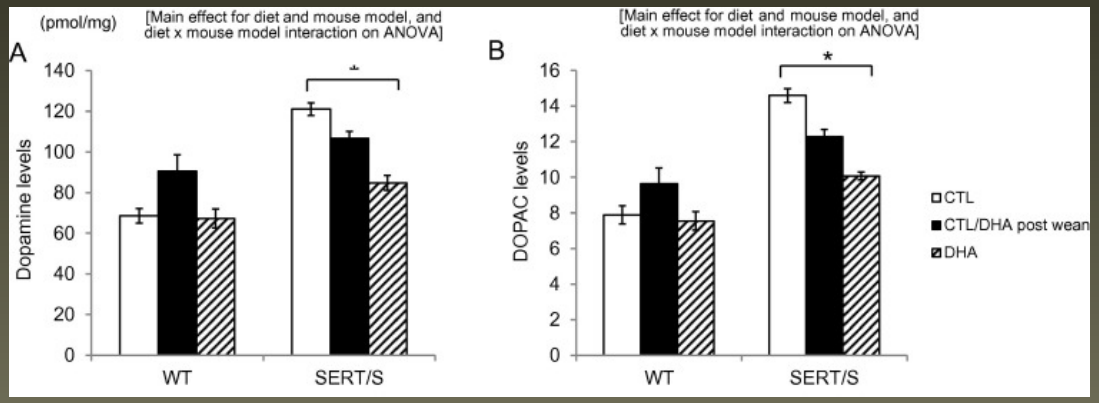
Brumbach et al 2017

Preventive Measures

- Animal models of autism have been used to assess preventive measures
  - DHA Fatty Acids given during prenatal and early postnatal brain development reverse some behavioral and neurochemical abnormalities in mouse models

- This approach may identify other essential elements that may support healthy brain development generally





Matsui et al 2018

Navigating the map for Autism Spectrum Disorder

- Improving diagnosis and understanding
- Finding treatments for problems that have few
- Preventing problems

AUTISM SOCIETY

Improving the Lives of All Affected by Autism

Iowa

Acknowledgments

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- *Nat'l Center for Advancing Translational Science (Yale Center for Clinical Investigation)*
- *APIRE/Wyeth Pharmaceuticals*
- *NARSAD YI Award: Dr. Mortimer D. Sackler Developmental Psychobiology Research Program*

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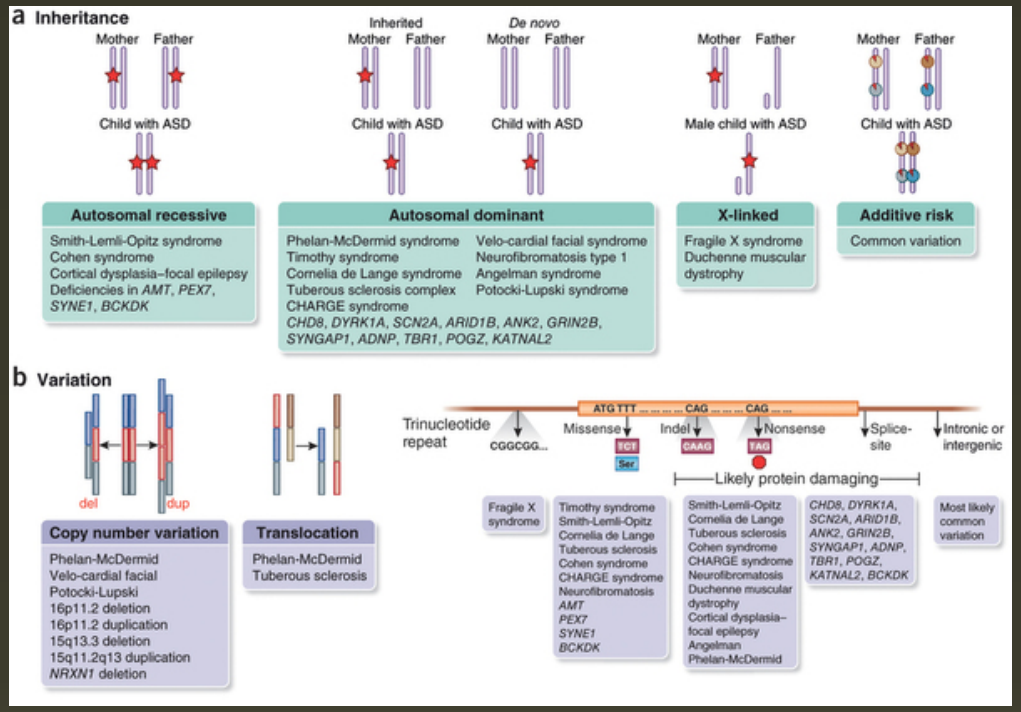
ASD Etiology

Genetics:

- 90% heritability
- More than 20 known single gene causes (with variable penetrance). Single gene disorders such as
  - Fragile X syndrome (boys)
  - Rett syndrome (girls)
  - 22q11 syndrome (velocardiofacial)
  - tuberous sclerosis
  - Mutations in pten, CTNAP2, NRG1
- GWAS and linkage studies have found genes that increase risk (e.g. serotonin transporter)
- Higher rate of de novo and inherited copy number variations (CNVs)



De la Torre-Ubieta L et al Nature Medicine

Environment: very likely plays a role by interaction with risk genes

- Perinatal complications and prenatal exposures (valproic acid, maternal immune activation)
- severe early neglect
- other unknown etiologies

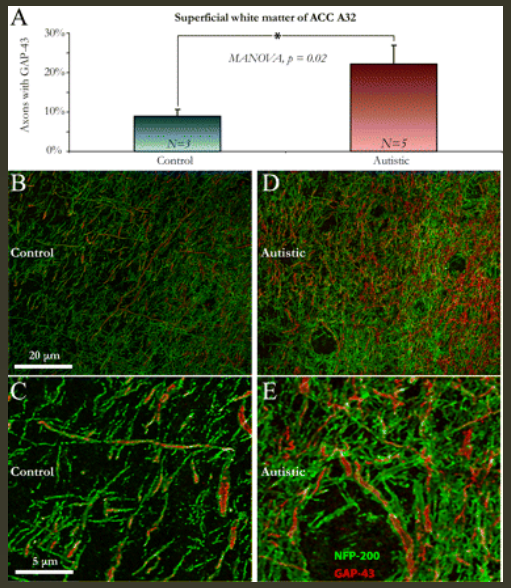
Table 1. The known and putative ASD-related genes and environmental factors contributing to the ASD

Genes related with ASD	Location	Gene name
ADNP [124]	20q13.13	Activity-dependent neuroprotector homeobox
ANK2 [125]	4q25-q26	Ankyrin 2, neuronal
ARID1B [126]	6q25.3	AT rich interactive domain 1B (SWI1-like)
ASH1L [127]	1q22	Ash1 (absent, small, or homeotic)-like (Drosophila)
ASXL3 [128]	18q11	Additional sex combs like 3 (Drosophila)
CHD8 [129]	14q11.2	Chromodomain helicase DNA binding protein 8
DYRK1A [130]	21q22.13	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A
GRIN2B [131]	12p13.1	Glutamate receptor, inotropic, N-methyl D-aspartate 2B
POGZ [132]	1q21.1	Pogo transposable element with ZNF domain
PTEN [133]	10q23	Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)
SCN2A [134]	2q24.3	Sodium channel, voltage-gated, type II, alpha subunit
SETD5 [135]	3p25.3	SET domain containing 5
SHANK3 [136]	22q13.3	SH3 and multiple ankyrin repeat domains 3
SUV420H1 [137]	11q13.2	Suppressor of variegation 4-20 homolog 1 (Drosophila)
SYNGAP1 [138]	6p21.3	Synaptic Ras GTPase activating protein 1
TBR1 [139]	2q24.2	T-box, brain 1
Environmental factors		**Risk factors**
Prenatal viral infection [140-143]		Influenza, rubella, and cytomegalovirus, etc.
Zinc deficiency [144, 145]		
Abnormal melatonin synthesis [146, 147]		
Maternal diabetes [148]		
Prenatal and perinatal stress [149]		Stress hormones, psychological stress, etc.
Toxins [150, 151]		Valproic acid, thlidomide, organophosphate, etc.
Advenced parental age [152]		

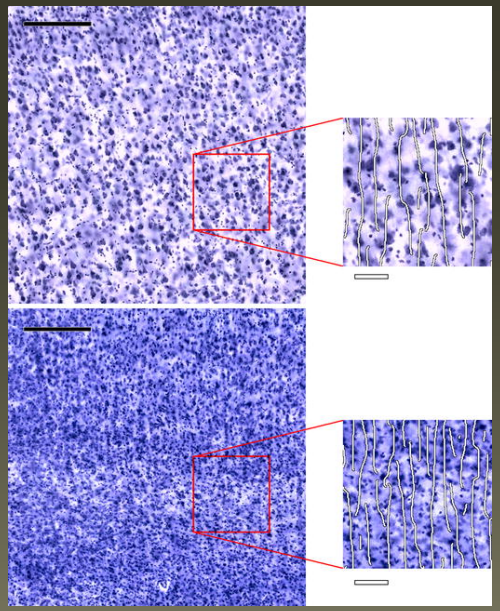
Park et al 2016

ASD Pathophysiology

- Multiple theories of brain process dysfunction
  - specific social cognition deficits and anxieties
  - learning deficits- not able to process/integrate right level of details
  - sensory processing deficits
- Neurobiological
  - Hyperserotonemia is common
  - Macrocephaly more common (but may be macrosomia)
  - Cortical connectivity- more local, less distant
  - Cortical minicolumn structure
  - Many other postmortem studies with abnormalities in microglia, astrocytes, GABAergic cells, serotonergic projections
- Theorized cellular pathology
  - synaptic production/pruning
  - neural cell fate/growth may be disrupted
  - possible excitation/inhibition imbalance
  - Possible role for immune/inflammatory processes in the brain



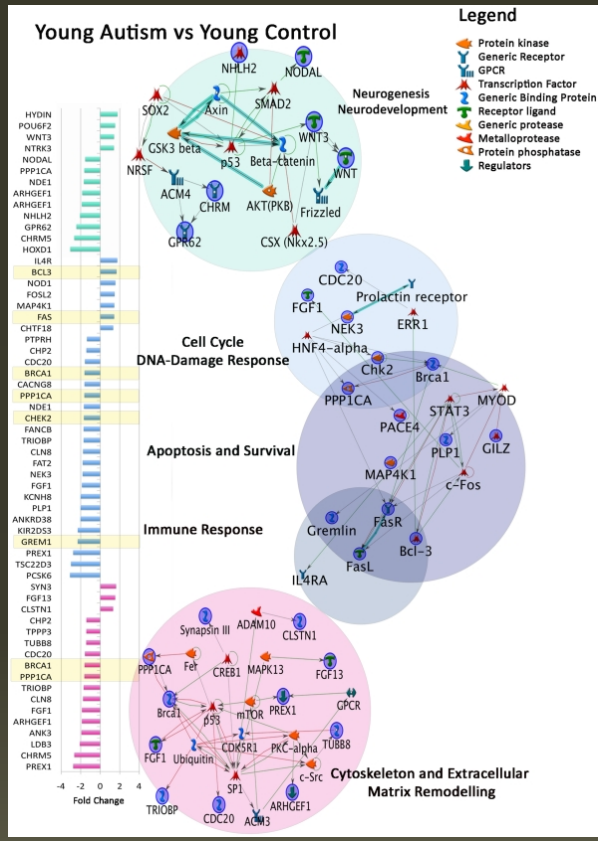
Zikopoulos and Barbas 2010



Casanova et al, 2006

Changes in brain gene expression

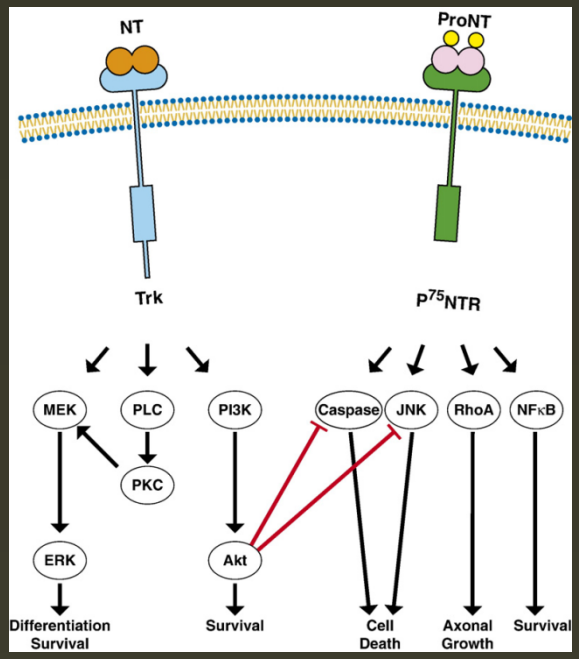
- In young people with autism, genes related to early development are more altered, and, in older people, expression of genes related to signaling and repair are more altered in prefrontal cortex (Chow et al 2012)
- Some alteration in genes that regulate other genes:
  - Transcription factors (TFs) that control whether other genes are transcribed are themselves changed:
    - Some TFs that respond to sex-hormones are altered in prefrontal cortex (Sarachan and Hu 2013). May inform sex differences.
    - TF1 and its downstream GABAergic target genes are altered in cingulate cortex (Thanseem et al 2011; 2012)
    - The TF, Lmx1b, and presynaptic proteins for serotonin neuron development are altered in anterior cingulate cortex (Nakamura et al 2011)
  - Levels of regulatory RNAs in prefrontal cortex and cerebellum (Ziats and Rennert 2013) as well as abnormally high and low RNA editing in cerebellum (Eran et al 2012)



Chow et al 2012

Changes in brain gene expression

- Some alteration in:
  - Growth factor gene profiles in fusiform gyrus and cerebellum (Sajdel-Sulkowska et al 2011; Garcia et al 2012)
  - Expression of enzymes for anti-oxidant production (Muratore et al 2013)
  - Mitochondrial proteins (Tang et al 2013, Anitha et al 2012)
  - Extracellular matrix protein in adult neurogenic zone (Pearson et al 2013)
  - Receptors for axon guidance in cortex (Suda et al 2011)
- Negative findings in:
  - No altered glutamate metabolism in cingulate cortex (Shimmura et al 2013)
  - No leukemia related viruses (Lintas et al 2011)

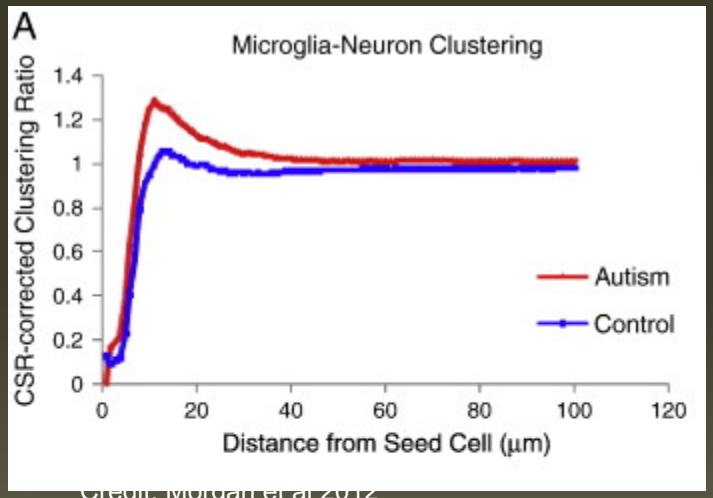


Credit: Buckley et al 2007

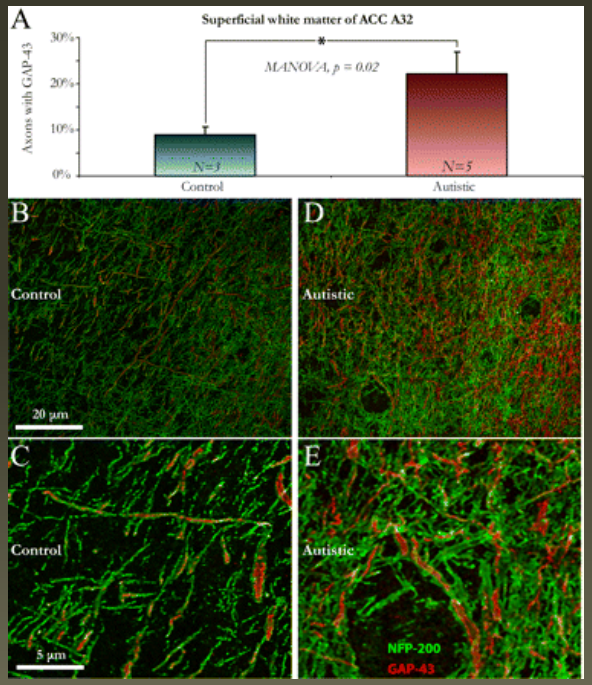
There are inconsistencies in the findings listed above– this may emphasize how varied the causes of autism are

Changes in cell structure of the brain

- Microglial cells cluster more around neurons in prefrontal cortex of people with autism (Morgan et al 2012)
- Higher ratio of Von Economo neurons to pyramidal neurons (Santos et al 2011)
- Decreased long-distance pathways and excessive connections between neighboring anterior cortical areas (Zikopoulos and Barbas 2010)



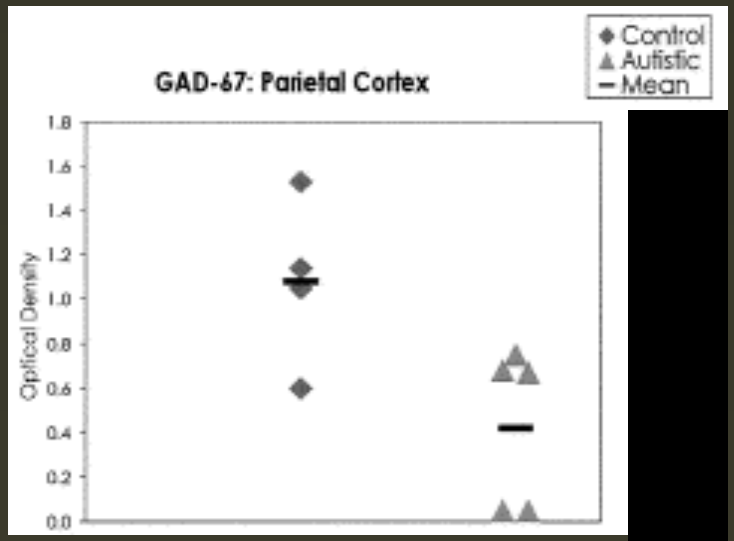
Credit: Morgan et al 2012



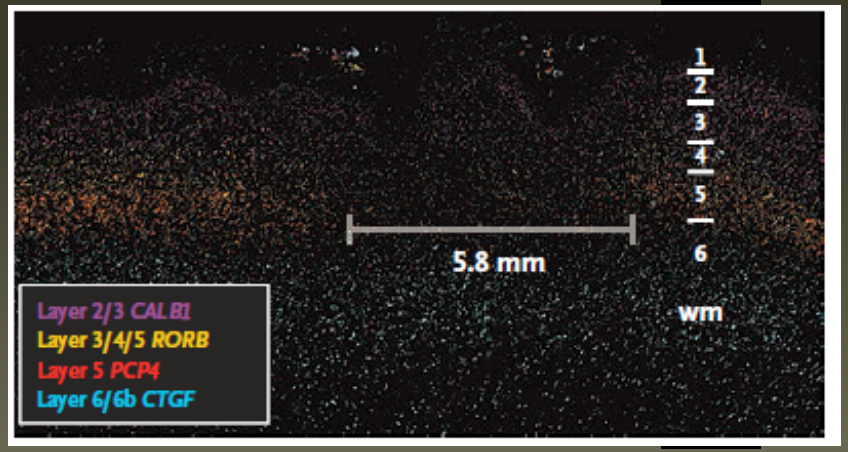
Credit: Zikopoulos and Barbas 2010

Changes in brain cell types and structure

- Inhibitory neurons and their expression of GAD65/67 reduced (Fatemi et al 2002; Lawrence et al 2010; Yip et al 2009) as well as expression of receptors for GABA that on other cells (Blatt and Fatemi 2011)
- Glial fibrillary acidic protein increased in cerebellar vermis (Fatemi et al 2011)
- Increased serotonin projections into forebrain (Azmitia et al 2011)
- Microglia reduced in multiple cortical regions (Tetreault et al 2012)
- Gene expression is disrupted in patches of cortex in postmortem tissue of children with autism (Stoner et al 2014)



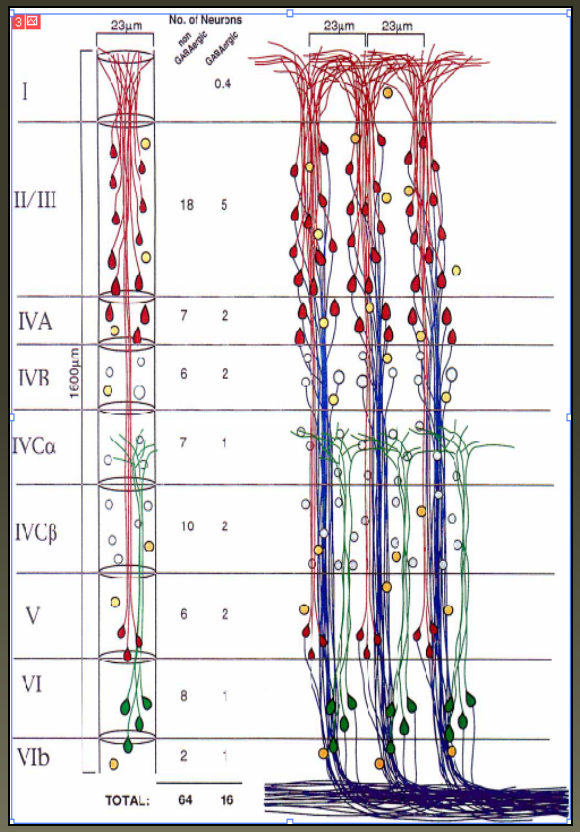
Credit: Fatemi et al 2002



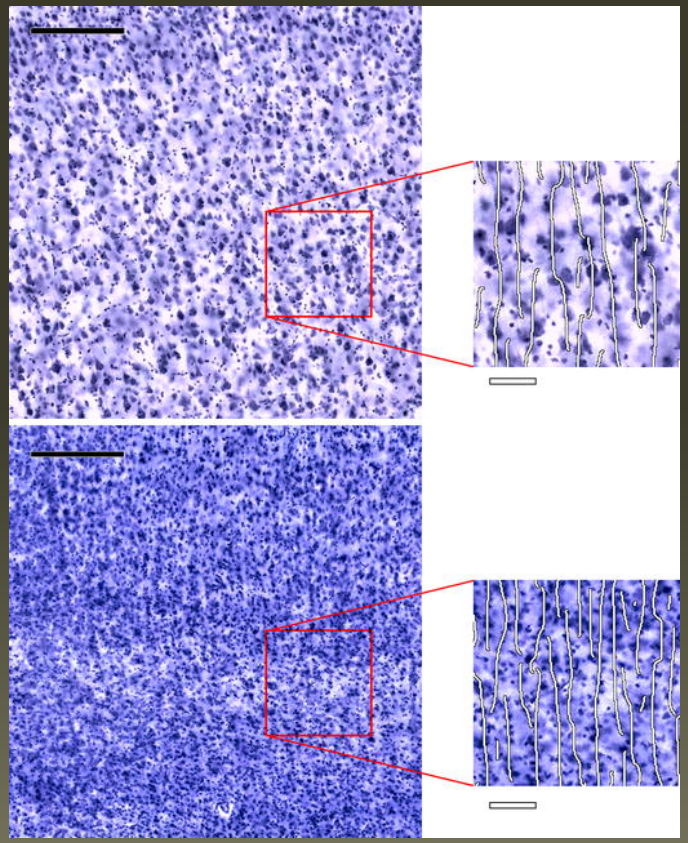
Stoner et al 2014

Cortex and Neuropsychiatric Disorders

- Neuropathology of Autism:
  - Cortical minicolumns formed around excitatory neurons have been shown to be more densely packed in autism

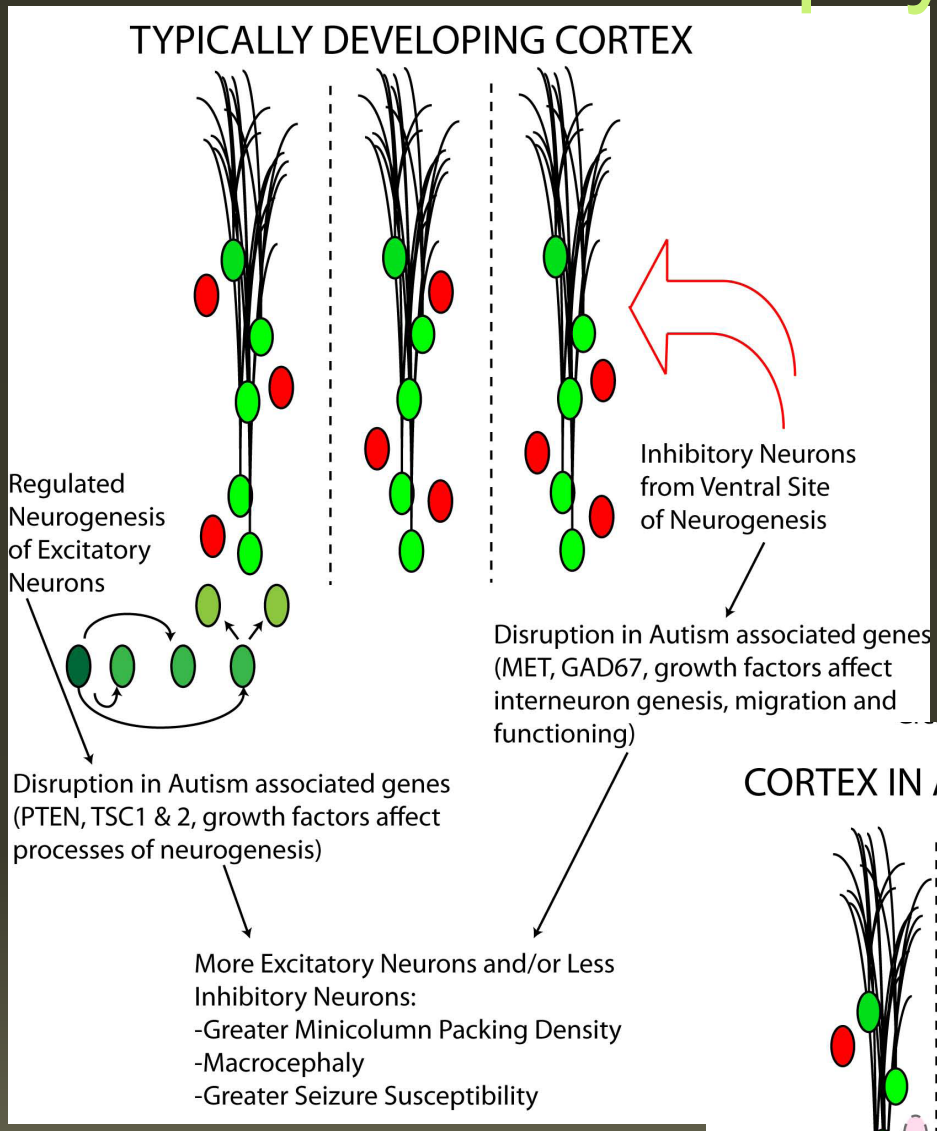


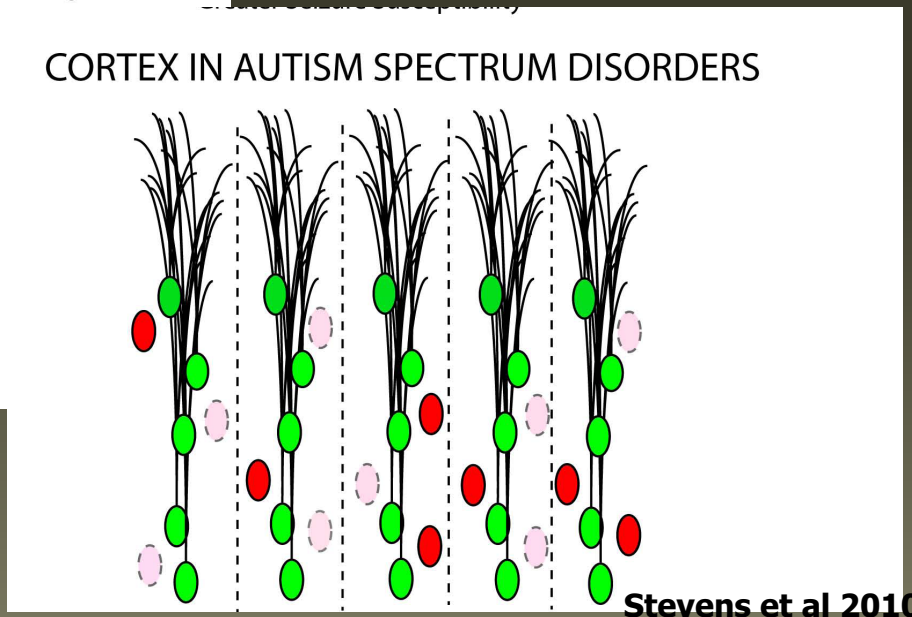
Peters and Yilmaz, 1993



Casanova et al, 2006

Cortex and Neuropsychiatric Disorders





Stevens et al 2010

Blood measures

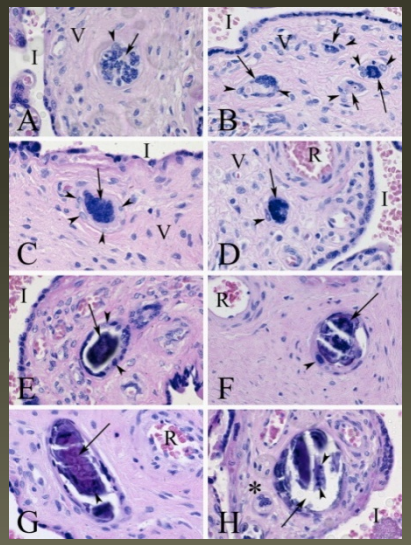
- Most consistent finding= Serotonin abnormalities
  - Blood platelets have high serotonin and alterations in proteins that interact with serotonin (transporters and receptors)
  - These alterations are mirrored in the brain of people with autism:
    - Changes in serotonin break-down products in cerebrospinal fluid
    - Changes in serotonin transporters and receptors that can be studied through external brain imaging (like PET scans)

- In some subsets of people, there is dysfunction in immune cells
  - Altered immune cell number, altered cytokines and chemokines produced and received by immune cells and altered responses to immunological challenges
  - In a select group of patients, there may be problems with mitochondria in blood cells: the “powerplant” of the cell

Other tissues

- Gastrointestinal tract
  - Studies show some people with autism have abnormal GI epithelial function (enzymes) typically in people with GI comorbidities
- Placenta
  - Some results suggest that the placenta from people with autism may have more pockets of abnormal cell structure
- Theses are isolated studies that require further investigation and replication to appreciate their implications for brain dysfunction

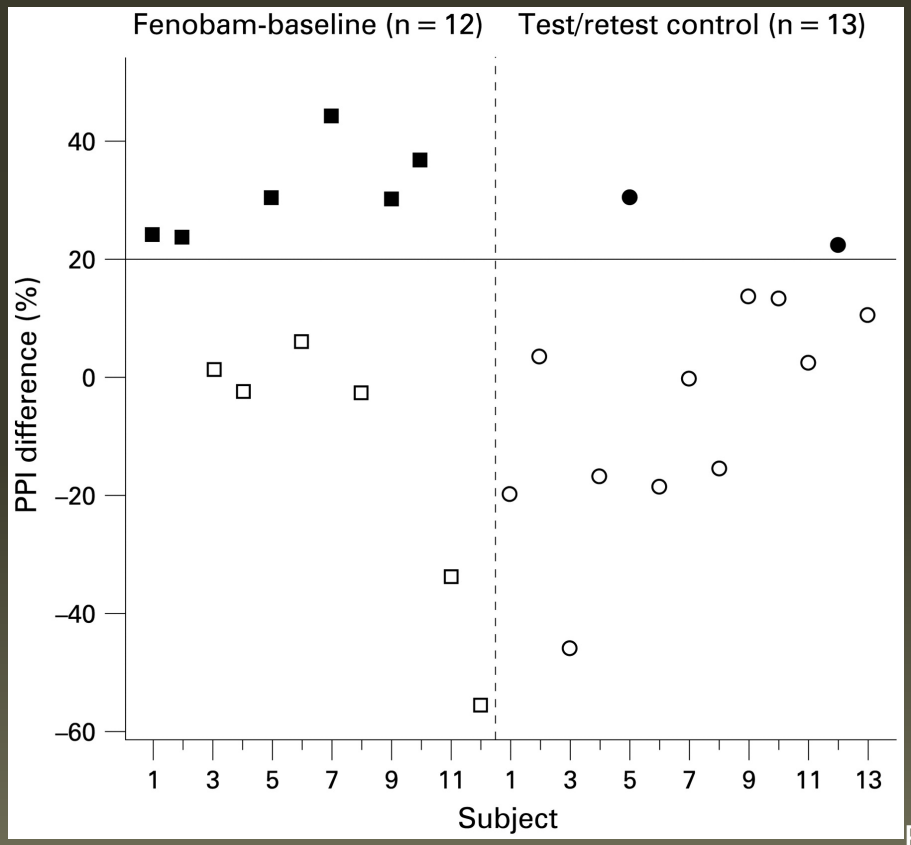
Credit: IHSSadvocates



Credit: Walker et al 2013

Future directions

- Therapies based on neurobiological findings:
  - Glutamate antagonist in Fragile X: Fenobam

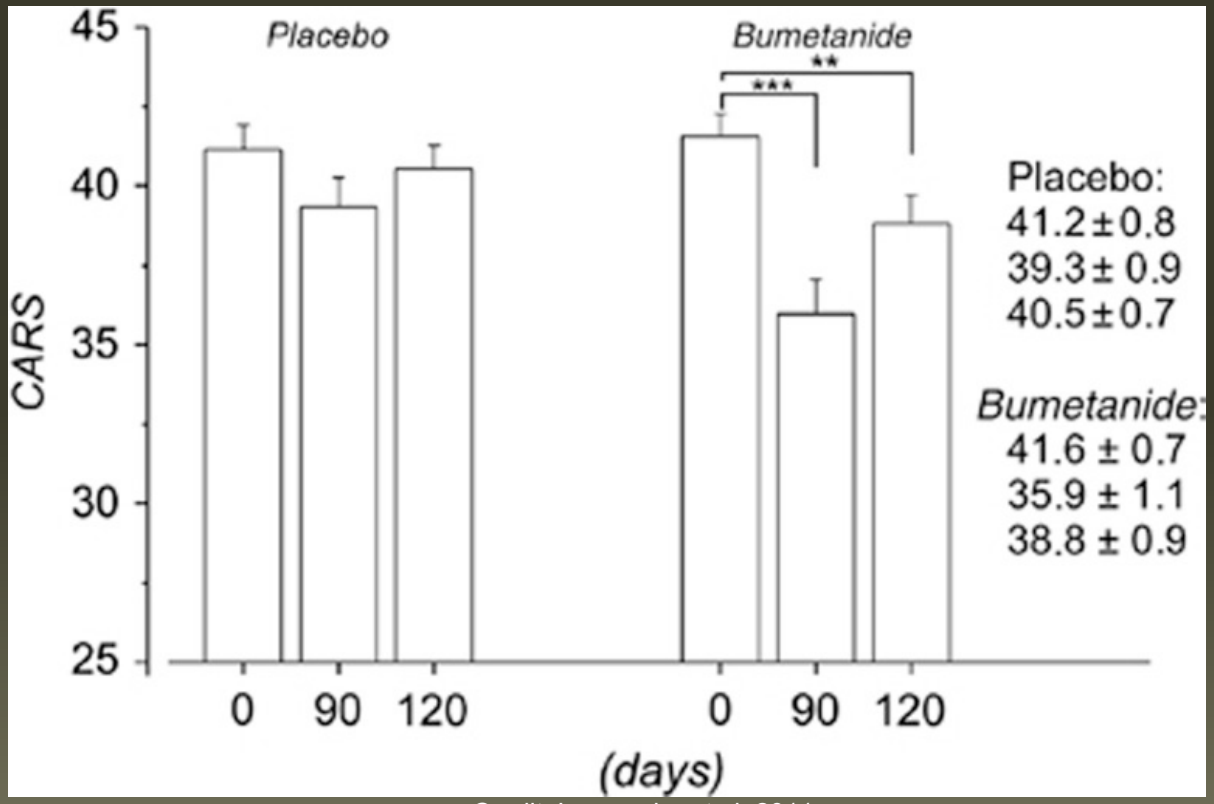


"In nine of the 12 cases, calmed behaviour was observed with improvement in eye contact, ability to interact, anxiety and/or motor overactivity"

Berry-Kravis, E et al. 2009

Future directions

- Therapies based on neurobiological findings:
  - Diuretic and GABA modulator for children: Bumetanide (blocks the NKCC1 chloride importer)



Credit: Lemonnier et al. 2011

"Bumetanide reduced the severity of the symptoms, the parents used literally the same words to stress that the children are more 'present' with enhanced communication with their environment"