Biomarkers, Immune-Mediated Disorders and Autism

By Judy Van de Water, Ph.D.

Professor, Internal Medicine
Director, NIEHS Center for Children's Environmental Health
University of California, Davis
UC Davis M.I.N.D. Institute

javandewater@ucdavis.edu

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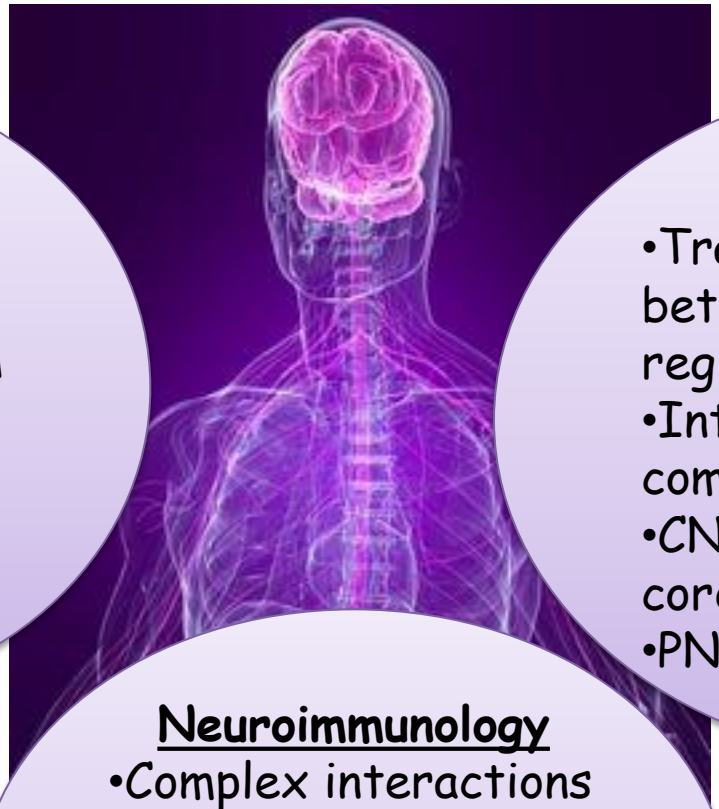
The Immune and Nervous Systems

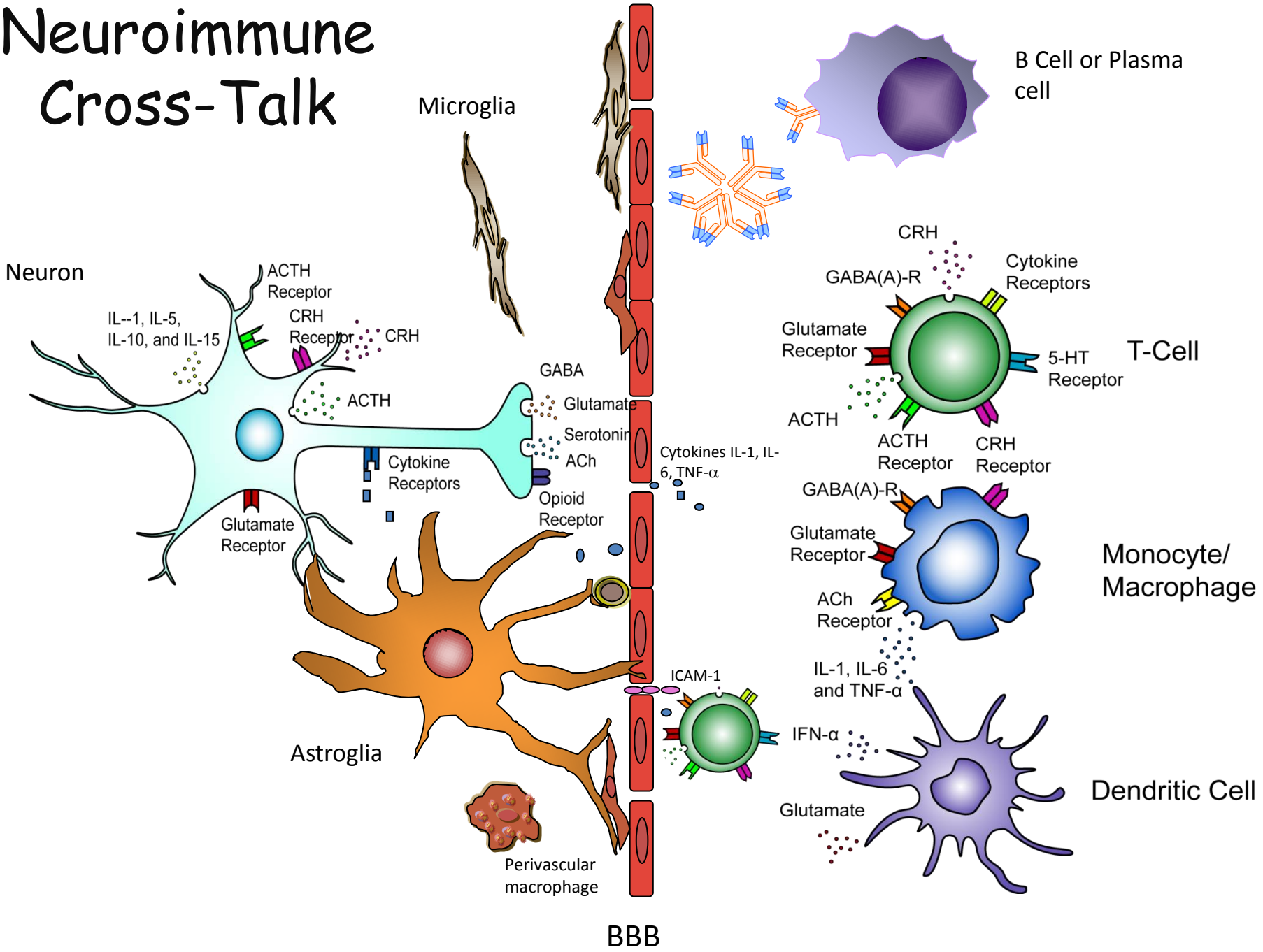


Immune System
- Body's natural defense mechanism
- Detection of wide variety of foreign agents

Nervous System
- Transmit signals between different regions of the body
- Interactions between complex neural pathways
- CNS: brain and spinal cord
- PNS: sensory neurons

Neuroimmunology
- Complex interactions between the two systems during homeostasis, response to injuries, and development.

Neuroimmune Cross-Talk

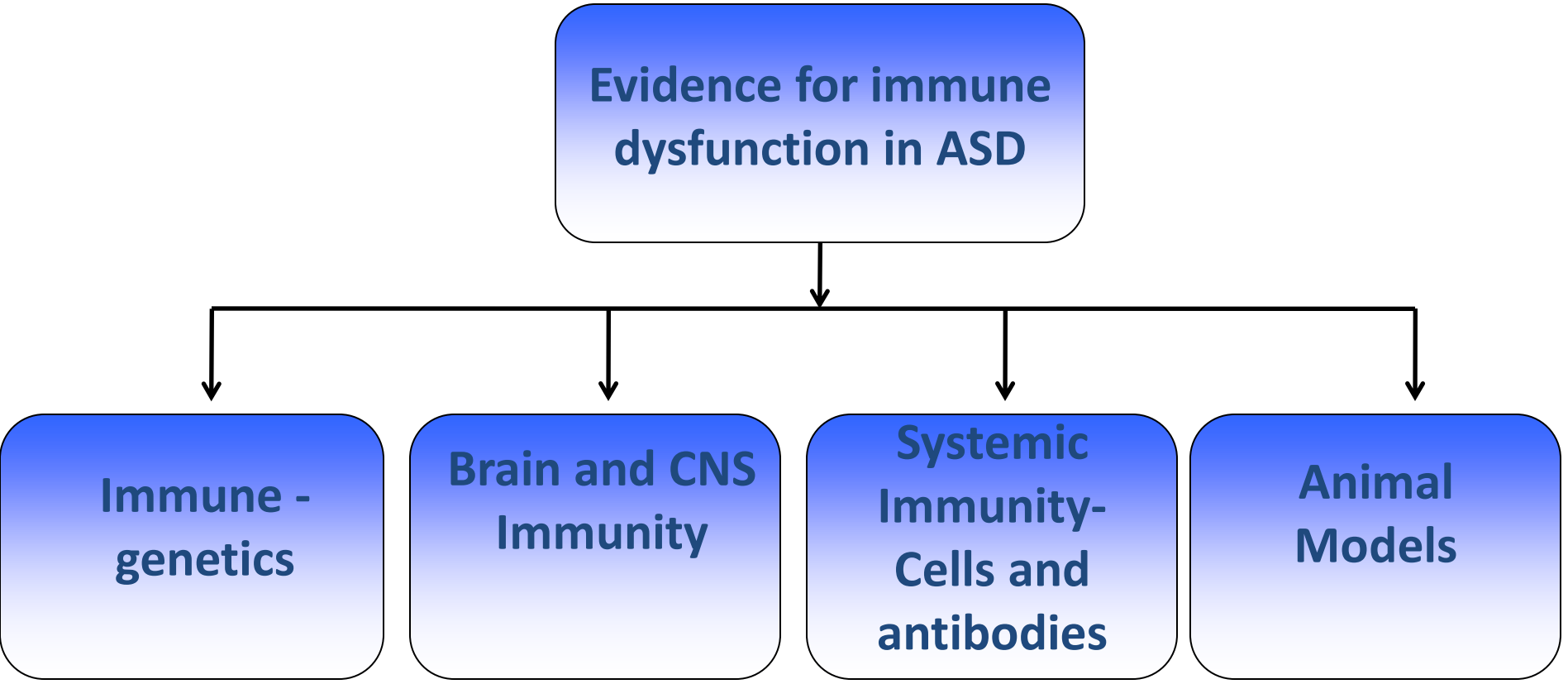


Autism and the Immune Response

What we know now

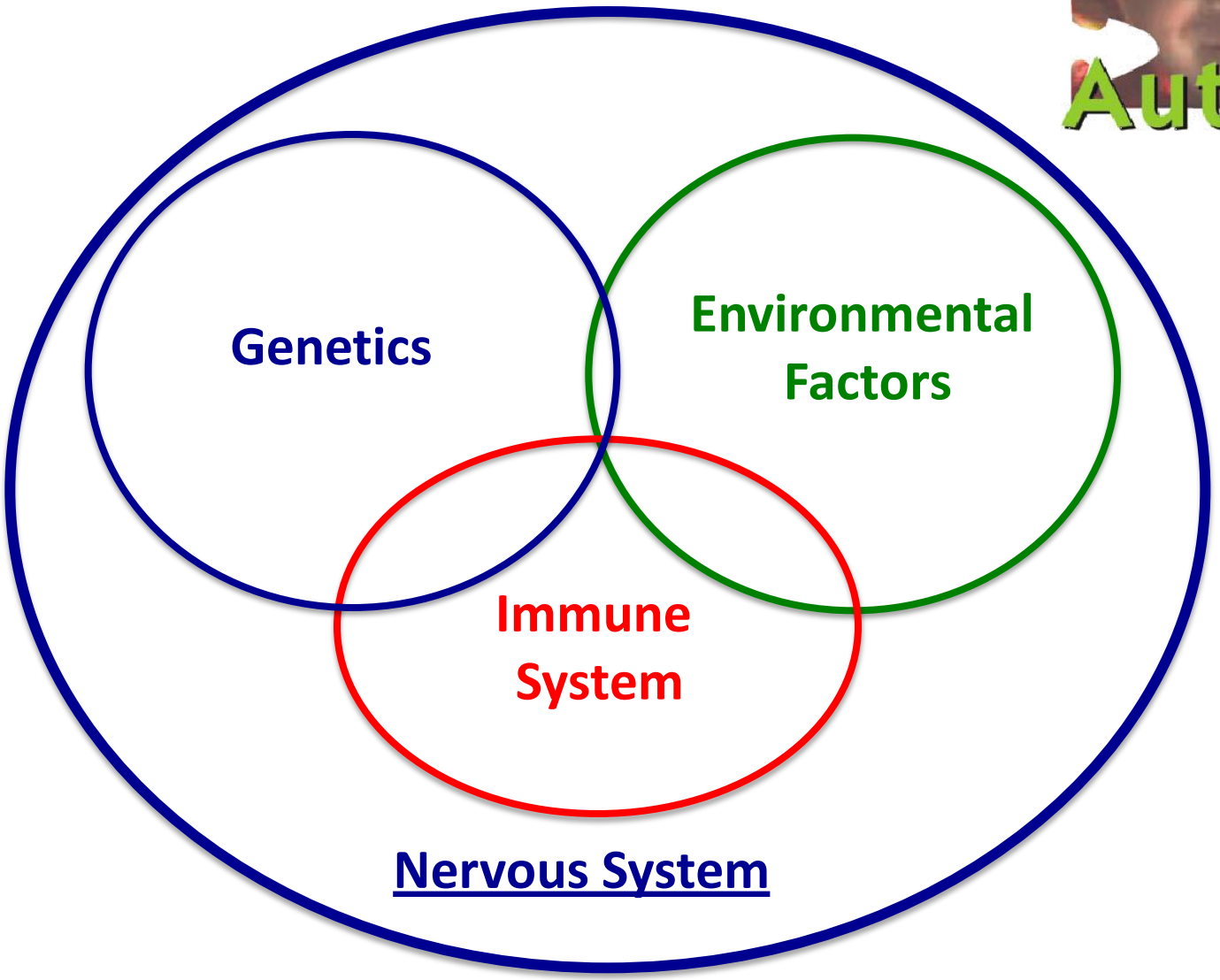
- Various immune system abnormalities have been reported in children with autistic disorders by a number of different laboratories.
- Both enhanced autoimmunity and reduced immune function have been shown.
- Development of ‘autism’ animal models with immune basis

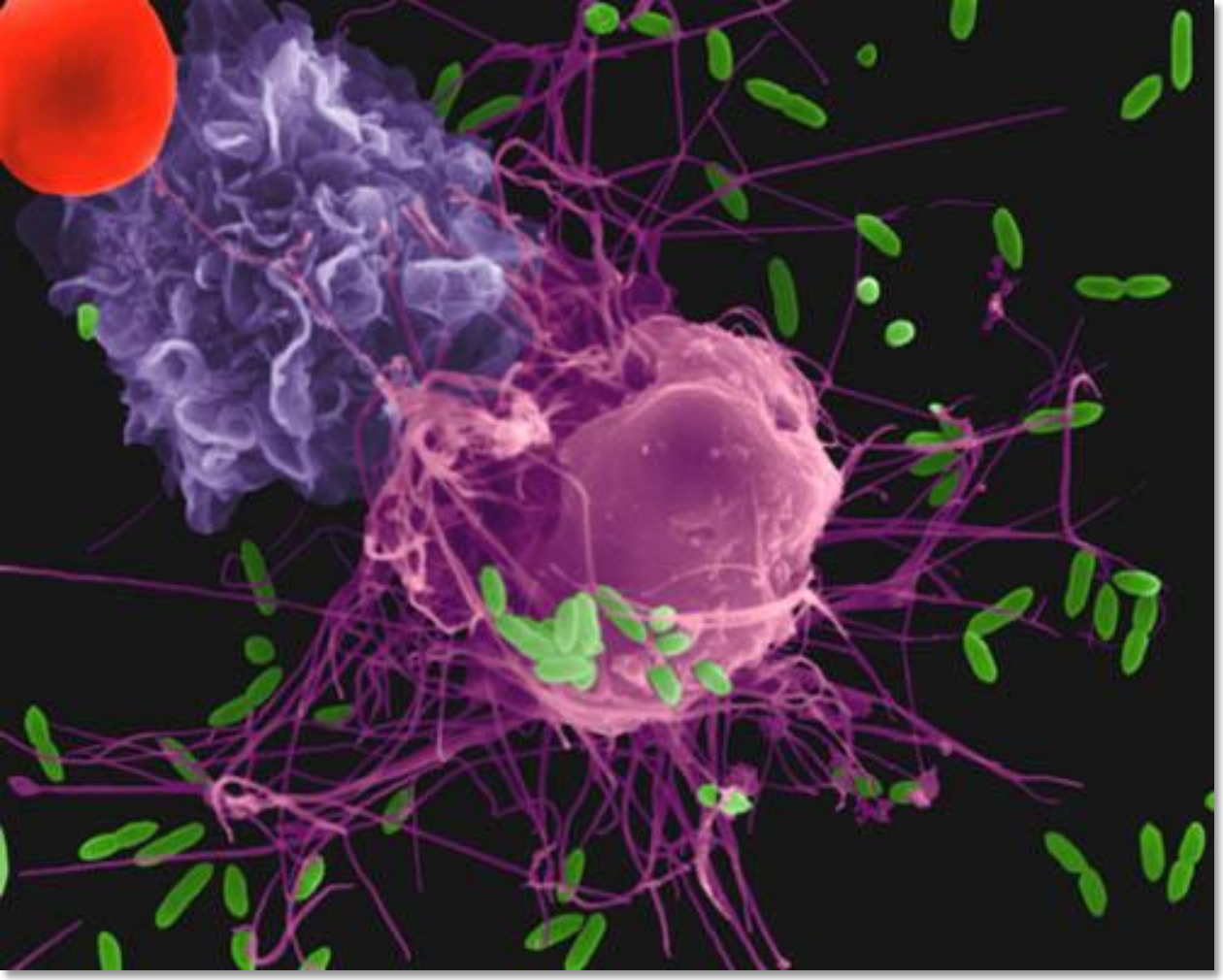
Current evidence for immune dysfunction in autism comes from many avenues



Causes of ASD

- Not well understood



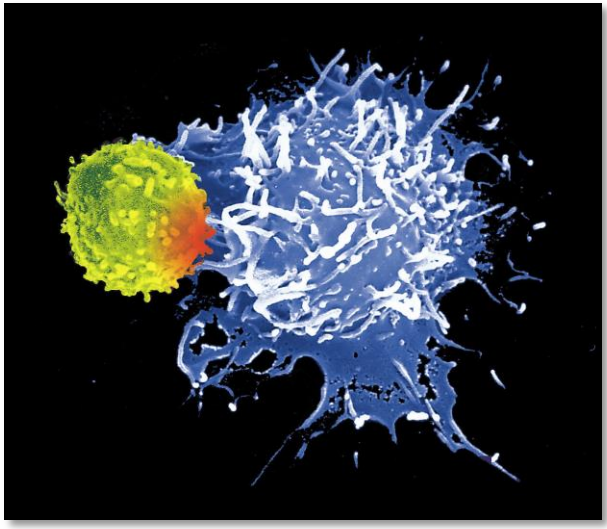


There are two types of immune responses:
Innate and Adaptive

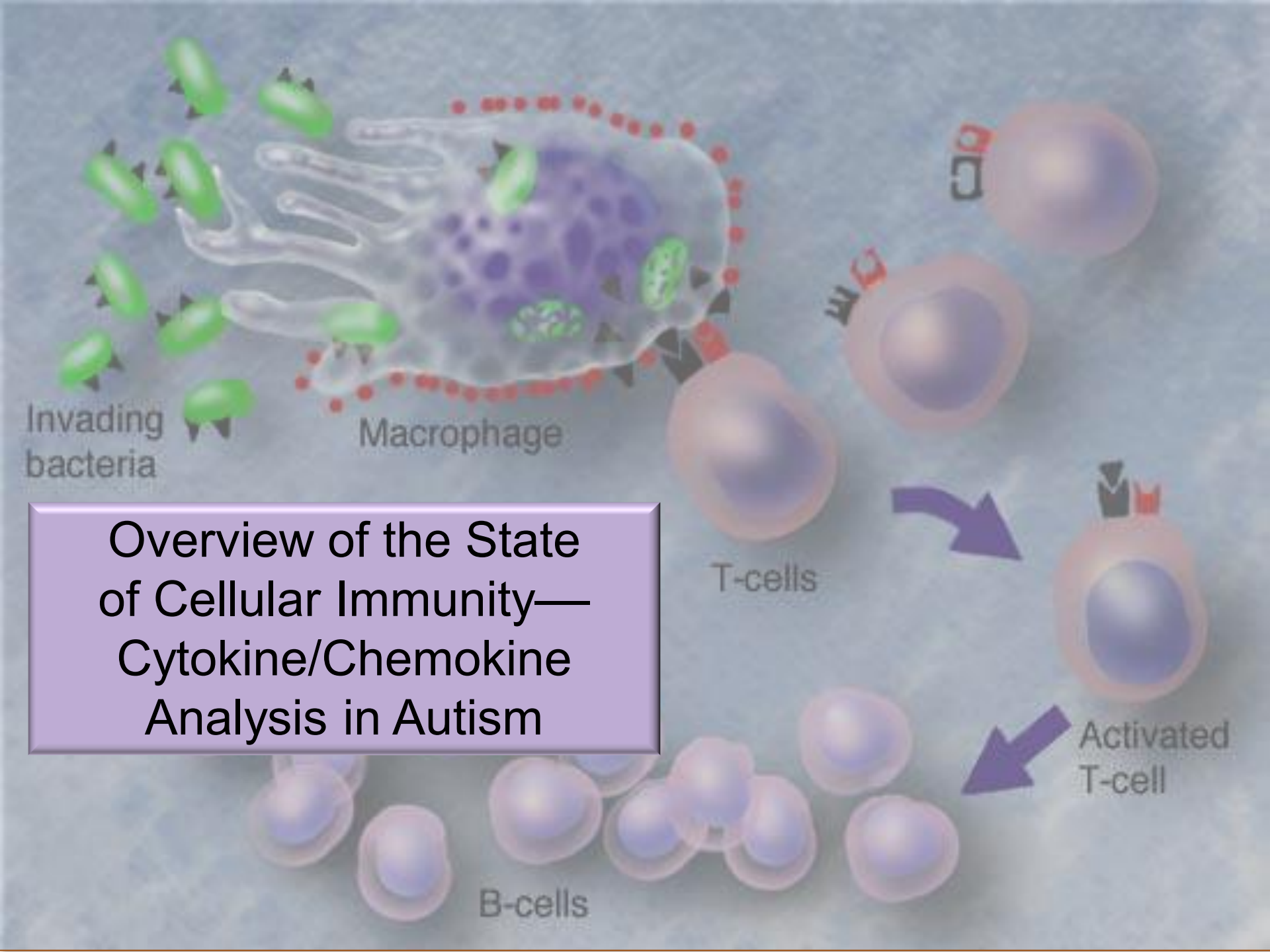
The Innate Immune System

- Innate immunity refers to antigen non-specific defense mechanisms that a host uses immediately or within several hours after exposure to almost any antigen.
- This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

Adaptive Immunity



- Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a particular antigen.
- The response can be long lasting and result in “memory cells”
- There are two major branches of the adaptive immune responses: humoral immunity (antibodies) and cell-mediated immunity.
- The adaptive immune response involves B cells and T cells.

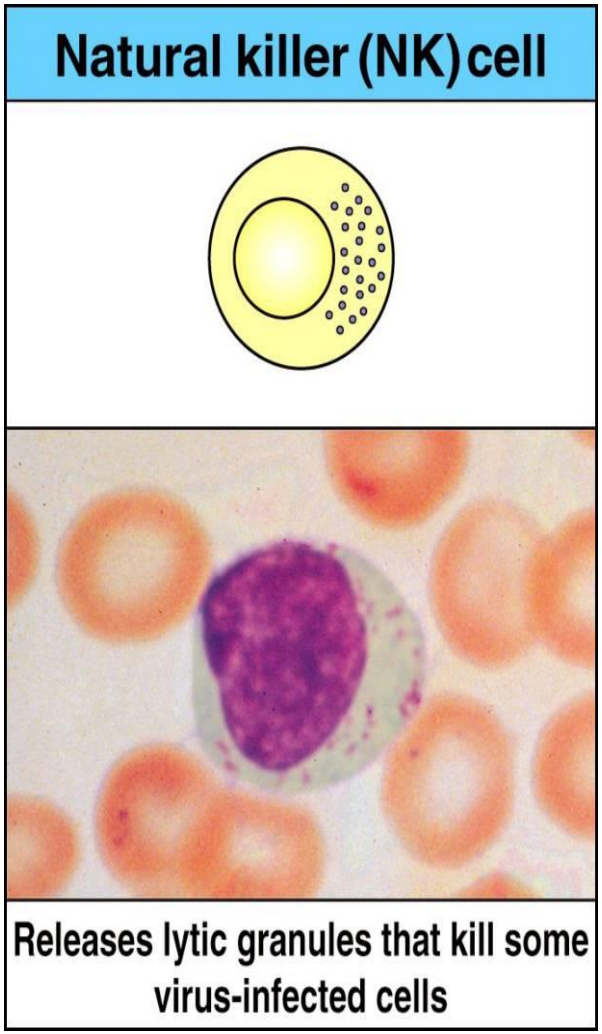


Overview of the State of Cellular Immunity—Cytokine/Chemokine Analysis in Autism

Evolution of Studies on Immune Cells in Autism

- Over two decades ago Warren et al., (1987) described a decrease in NK cell function in children with autism
- More recently, an increase in expression of NK cell associated genes was noted in ASD (Gregg et al., 2008).
- Lower NK cell activity found in about 45% of a subset of children with ASD (Vojdani et al., 2008).
- An imbalance between inhibitory and activating NK cells has been implicated autism (Enstrom et al., 2009; Schleinitz et al., 2010).

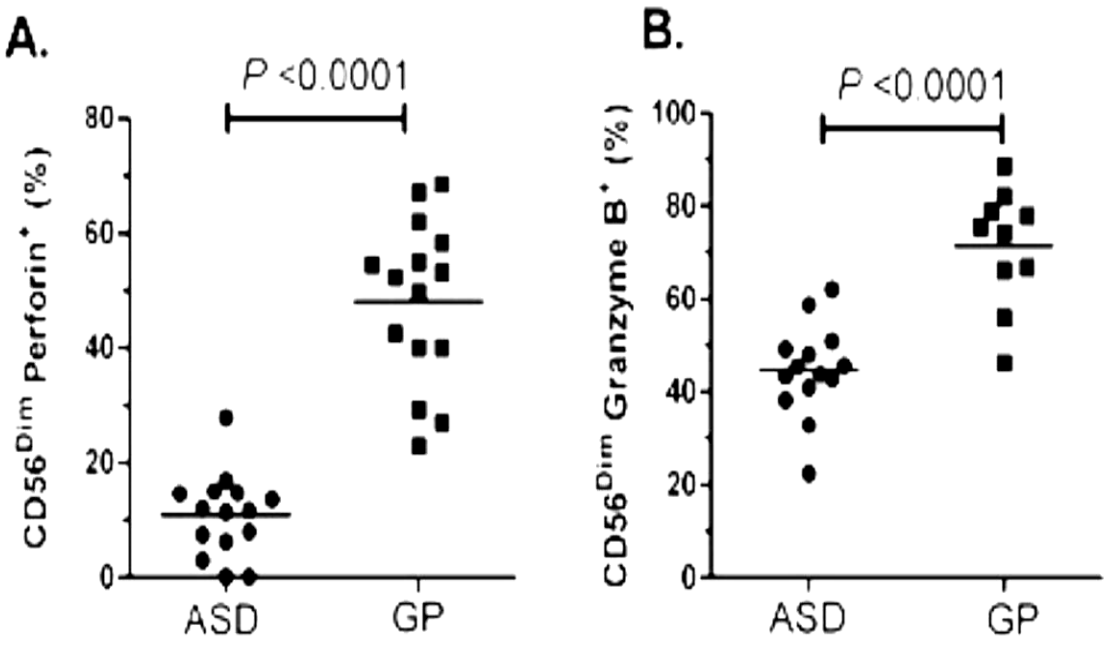
NK Cells



- Large granular non-T and non-B cells that kill virally infected cells and some tumor cells.
- Important in innate immunity to viruses and other intracellular pathogens

NK Cells in Autism

Functionally, following stimulation, children with ASD had a decrease in NK cell cytotoxic activity compared to age-matched controls (Enstrom et al., 2009b).

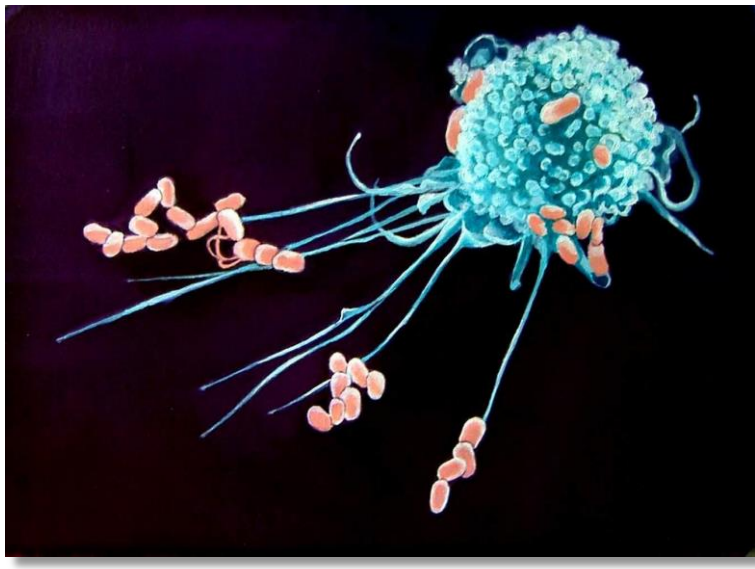


What does this mean?

- There are enough cells there
- Functionally they cannot do their job efficiently
- First line defense for viral infection

Macrophages/Monocytes in Autism

- Significantly higher monocyte count with no difference in the absolute leukocyte counts (Sweeten et al., 2003b).
- TLR-2 activated monocytes had an increase in IL-1b, IL-6 and TNF-a.
- TLR-4 activation gave an increase in IL-1-b.
- TLR-9 activation resulted in a decrease in IL-1b, IL-6, GMCSF and TNF-a (Enstrom et al., 2010).
- Children with ASD have a dysfunction in monocyte signaling that may lead to long-term problems in response to infection.



Macrophages/Monocytes in Autism and GI

- Children with gastrointestinal problems in conjunction with ASD had lower production of the pro-inflammatory cytokines, IL-6, IL-1b, IL-12, IL-23, and the counter-regulatory cytokine IL-10, when monocytes were stimulated (Jyonouchi et al., 2011).

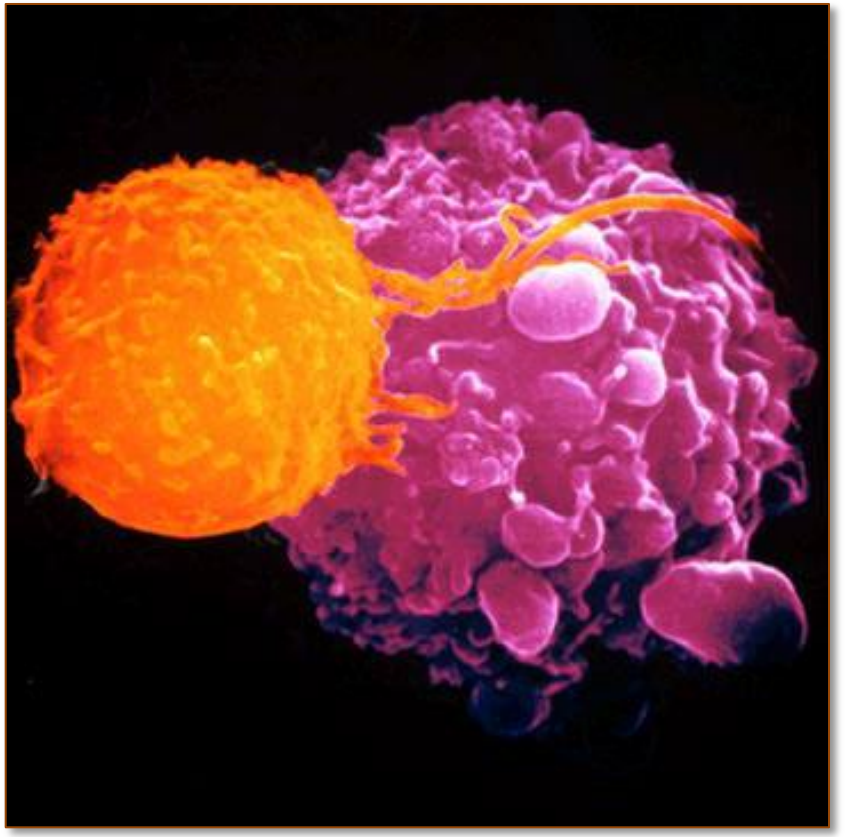
- This impaired signaling was in response to Toll-like receptor agonists for TLR2/6 and TLR 7/8, which are intracellular receptors for ssRNA.

T cell in Autism

- Several studies have indicated abnormalities in T cell immunity in children with autism compared to healthy controls.

- First noted in 1977, lymphocytes cultured from children with autism and challenged with the T cell mitogen PHA had a depressed proliferation compared to controls (Stubbs and Crawford, 1977).

- A similar study of children with autism ages 7-15, cultured PHA-challenged T cells showed a decrease in T helper cells, and a lower suppressor (now called regulatory cells) cell ratio as determined by flow cytometry (Denney et al., 1996).



T cells in Autism - more recently

- PBMCs challenged with PHA or tetanus, showed a significant decrease in the expression of CD3, CD4, and CD8 on T cells (Ashwood et al., 2011).

- CD4+ T lymphocytes from children with autism showed a decreased expression of CD95, the Fas ‘cell death’ receptor.
  - Children with autism might have poor regulation of the cellular immune response (Stranges et al., 2007).

- Lower frequency of Treg cells children with autism compared to controls (Mostafa et al., 2010).
  - a correlation with allergy and family history of autoimmunity.

T cells in Autism - more recently

- When peripheral blood T cells were stimulated, GM-CSF, TNFα, and IL-13 were significantly increased whereas IL-12p40 was decreased in ASD relative to TD controls.

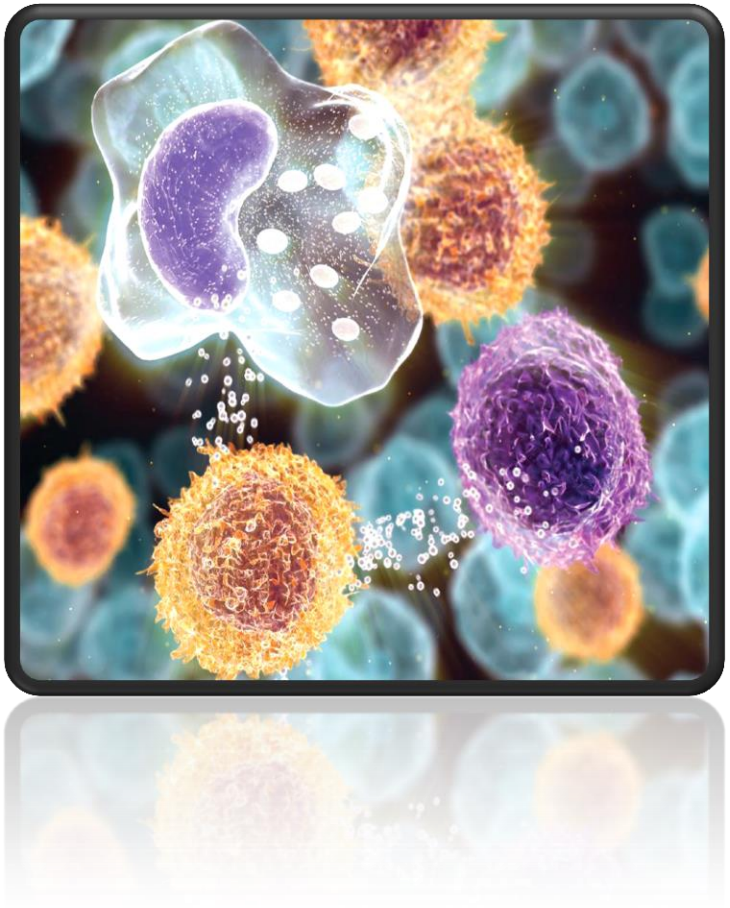
- **Increased pro-inflammatory** or TH1 cytokines were associated with **greater impairments** in core features of ASD as well as aberrant behaviors.

- In contrast, production of GM-CSF and TH2 cytokines were associated with **better cognitive and adaptive function**.

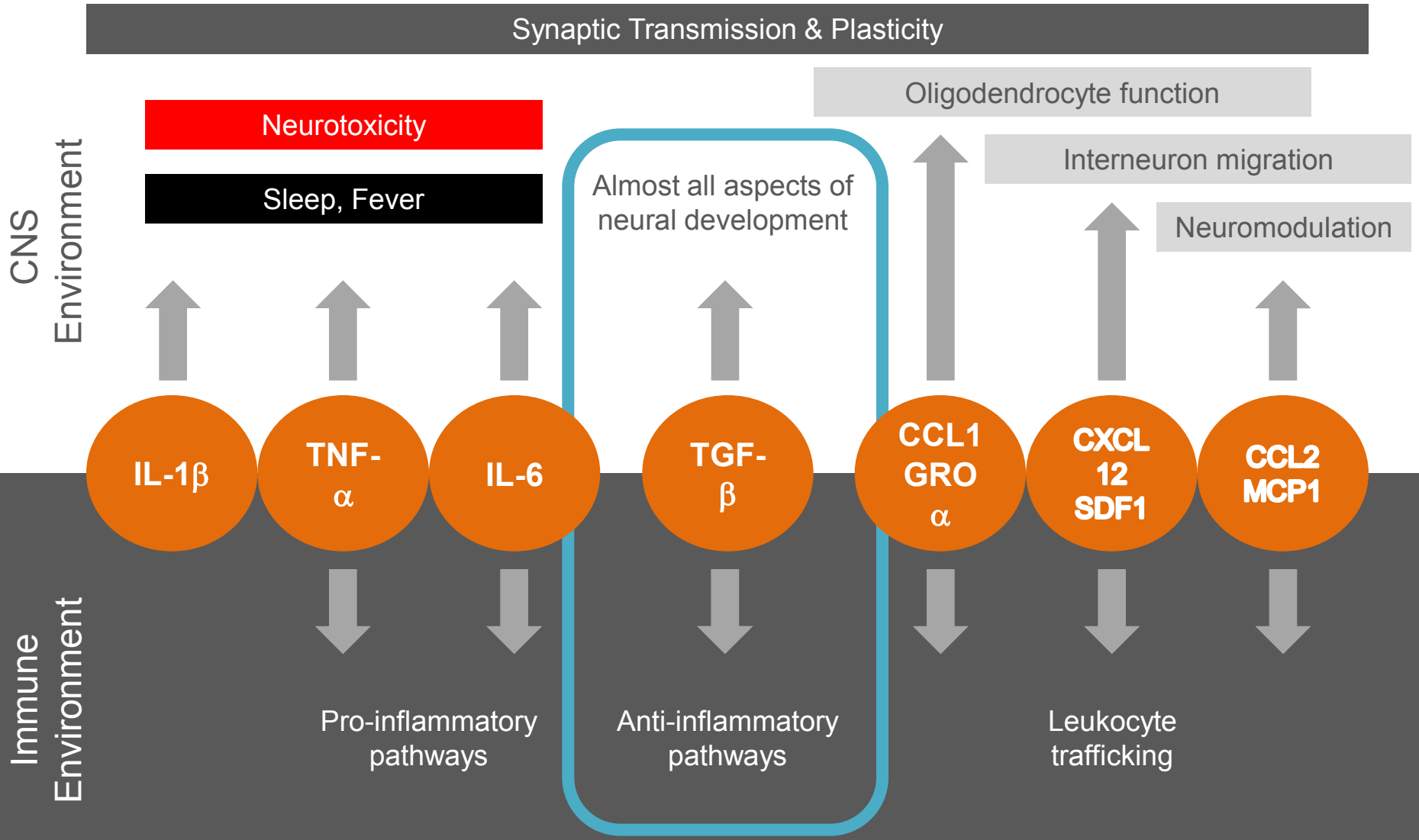
- Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water, J. Brain Behav Immun. 2010 Sep 9. [Epub ahead of print]

Cytokines- Master regulators of the immune system

- The immune response is controlled by mediators known as cytokines that are responsible for cell-cell communication.
- Cells produce cytokines in response to a stimulus. They direct the function of the cell that produces them and the cells nearby if they have appropriate cytokine receptors.
- They are produced by many cell types including including T cells, B cells and macrophages.



Immune-Neuro Interface



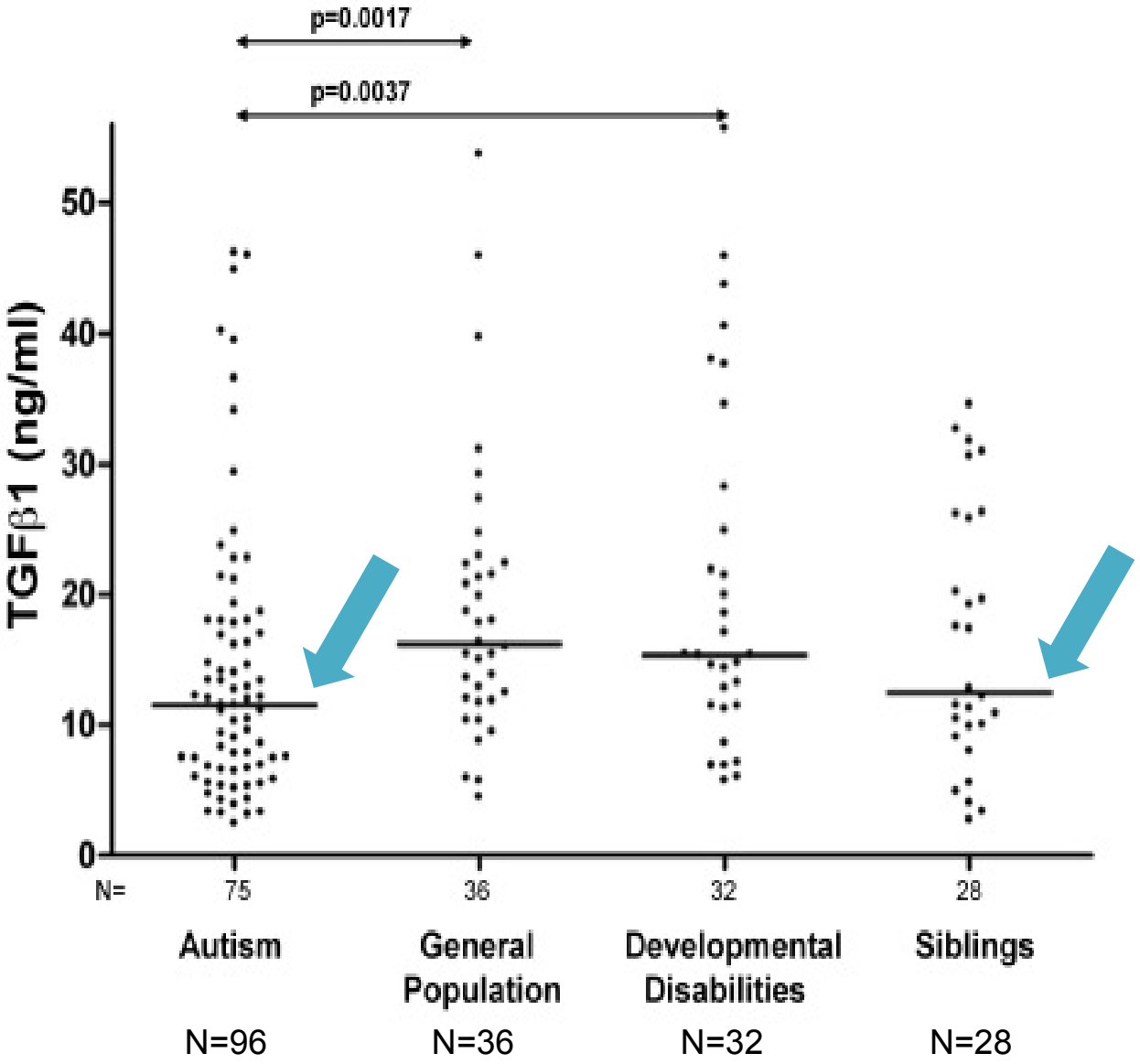
Regulatory T cells/Cytokines

- Significantly lower frequency of CD4(+)CD25(high) regulatory T cells in the blood of 30 AU and 30 age- and sex-matched TD children (Mostafa, 2010). Were not examined for Foxp3, a more definitive marker.

- Children with autism (n=75) had significantly lower plasma TGFβ1 levels compared with typically developing general population controls (n=36) (p=0.0017) (Ashwood, 2008).
  - A significant positive correlation of measures of social interaction and TGFβ1 levels in children with regression (n=42) based on ADOS scores (p=0.0048).

- Decreased serum TGF-β in small groups of ASD subjects compared to matched healthy controls (Okada, 2007).

Plasma TGF-β levels



Cytokines-plasma of children

Study Description	Reference
Elevated levels of IL-1b, IL-6, IL-8 and IL-12p40. Associated with regression	(Ashwood, et al., 2011b)
Increase in chemokine **MCP-1, Rantes** and **Eotaxin** levels in ASD subjects compared to age-matched typically developing controls. An association between increases chemokines levels with aberrant behaviors.	(Ashwood et al., 2011c)
In male ASD subjects, an increase in cytokines **IL-1beta, IL-1RA, IL-5, IL-8, IL-12(p70), IL-13, IL-17** and **GRO-alpha.**	(Suzuki et al., 2011)
Increase in **leptin** levels in ASD subjects compared to age-matched controls.	(Ashwood et al., 2008b)
Increase in **macrophage migration inhibitory factor (MIF)** in ASD subjects compared to age-matched controls.	(Grigorenko et al., 2008)
Decrease in **TGF-beta** in subjects with ASD compared to controls.	(Ashwood et al., 2008a; Okada et al., 2007)
Increase in **IL-12** and **IFN-gamma** in ASD subjects compared to age-matched controls.	(Singh, 1996)

Cytokine/Chemokines- activated cells

Study Description	Reference
In isolated PBMCs stimulated with PHA, increase in **GM-CSF, TNF-alpha** and **IL-13**. A decrease in **IL-12(p40)** in ASD subjects vs. controls.	(Ashwood et al., 2011d)
Stimulation of TLR on monocytes - ASD vs. to age-matched controls. Increase in **IL-1beta, IL-6, TNF-alpha**, with stimulation of TLR2. Increase in **IL-1beta**, with stimulation of TLR4. Decrease in **IL-1beta, IL-6, GMCSF, TNF-alpha** with TLR9.	(Enstrom et al., 2010)
Increase in **IFN-gamma** in NK cells from subjects with ASD.	(Enstrom et al., 2009b)
Increase production of cytokines from Th1 and Th2 cytokines in ASD subjects vs age-matched controls.	(Molloy et al., 2006)
Increase in **IL-12** and **TNF-alpha** in ASD subject with GI symptoms.	(Jyonouchi et al., 2005)
Increase in **IFN-gamma** and **TNF-alpha** in isolated PBMCs from ASD subjects compared to age-matched controls stimulated with LPS.	(Jyonouchi et al., 2002)
Unstimulated whole blood from ASD vs. age-matched controls – increase in **IFN-gamma** and **IL-1RA** with -higher **IL-6** and **TNF-alpha.**	(Croonenberghs et al., 2002)
Unstimulated PBMC- ASD subjects: higher levels of **TNF-alpha, IL-1beta,** and **IL-6** vs. controls. PBMCs stimulated with LPS, PHA and tetanus produced increase levels of **IL-12** and **IL-1beta**.	(Jyonouchi et al., 2002)

Brain, Behavior, and Immunity 25:40-45 (2011)

Contents lists available at ScienceDirect

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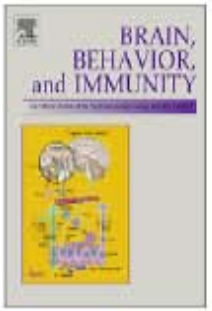


Short Communication

Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome

Paul Ashwood [a,f,*], Paula Krakowiak [b], Irva Hertz-Picciotto [b,f], Robin Hansen [c,f], Isaac Pessah [d,f], Judy Van de Water [e,f]

Plasma levels of IL-6, IL-8,IL-1β and IL-12p40 are significantly higher in the ASD (n=97) group when compared to TD (n=87) and DD (n=39) controls.

Onset status – relationship with cytokines

Cytokine (pg/ml)	Typically Developing (n=87)	Early Onset (n=53)	Regression (n=40)
IL-1β	62.8	61	144.3*‡
IL-2	8	17.7	19.3
IL-4	36.7	28.8	39.4
IL-5	9.8	9.2	11.5
IL-6	11.8	15.1	32.6*
IL-8	3.9	6.8*	14.5*
IL-10	16.4	7.5	15.6
IL-12 p40	171.7	192.3*	198.6*
IL-13	20.9	14.1	29.4
GM-CSF	54.2	51.3	101.2*‡
IFNγ	62.8	51.2	94.1
TNFα	63.9	56.2	111.1*

Raw data Plasma - all AU, ages 2-4 yrs

Cytokine	AUT Age 4 yrs	Age 3 yrs	AUT	AUT	TYP	TYP
IFN gamma	5.27	7.52	212.8	1.89	78.18	18.2
IL-2	OOR <	OOR <	49.05	8.25	18.9	8.76
IL-4	190	498.49	80.64	8.43	855.6	8.9
IL-6	14.6	19.24	114.33	2.88	327.6	2.31
IL-7	173.4	6.7	946.5	OOR <	926.2	23.62
IL-8	39.4	80.42	77.22	4.43	111.4	11.85
IL-10	70.2	129.56	32.89	4.79	307.3	2.22
IL-12 (p70)	1.4	*0.99	10.69	6.66	37.4	5.74
IL-13	16.6	22.61	45.67	1.88	128.5	1.15
MCP-1	286.5	275.41	247.2	300.9	313.2	355.8
Eotaxin	84.7	126.7	63.2	120.1	146.3	157.1
GM-CSF	835	1329.72	497.9	363.4	1888.7	480.4
IL-1a	119	153.42	434.7	12.14	716.2	35.68
IL-1B	15.4	25.96	15.13	2.36	132.15	7.2
IL-12 (p40)	401	488.02	268.2	46.95	1777.1	42.78
IL-17	0.95	1.97	194.6	4.19	28.6	16.01
IP-10	86.4	82.1	122.6	113.03	167.1	139.7
MIP-1a	62.1	101.56	519.1	4.21	320.2	67.89
TNFa	3.2	3.09	3.96	14.46	9.2	3.2
IL-1ra	142	289.7	127.2	60.39	898.4	85.66
MIP-1B	59.7	64.73	724.5	33.17	235.1	52.67

Immune Dysregulation

Dysregulation of the immune system can lead to autoimmunity, for which autoantibodies are one hallmark feature

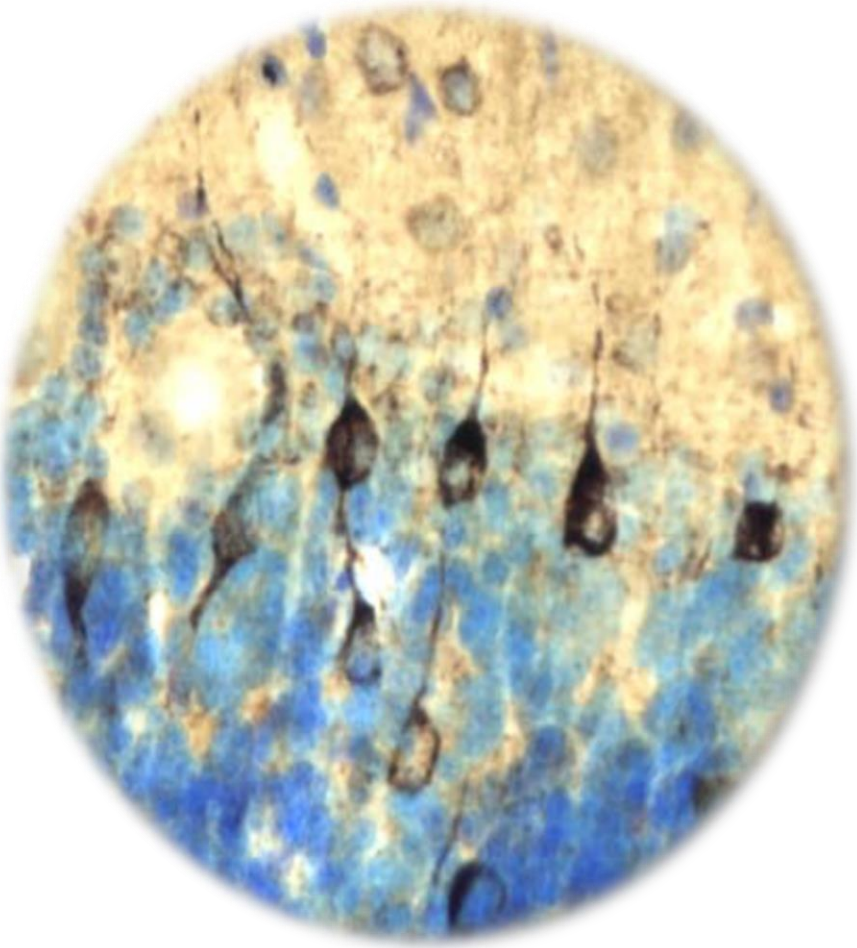
Autoantibodies
when the immune system gets it wrong

Autoantibodies in Children

- Several investigators have proposed an autoimmune-based etiology for a subset of children with autism.

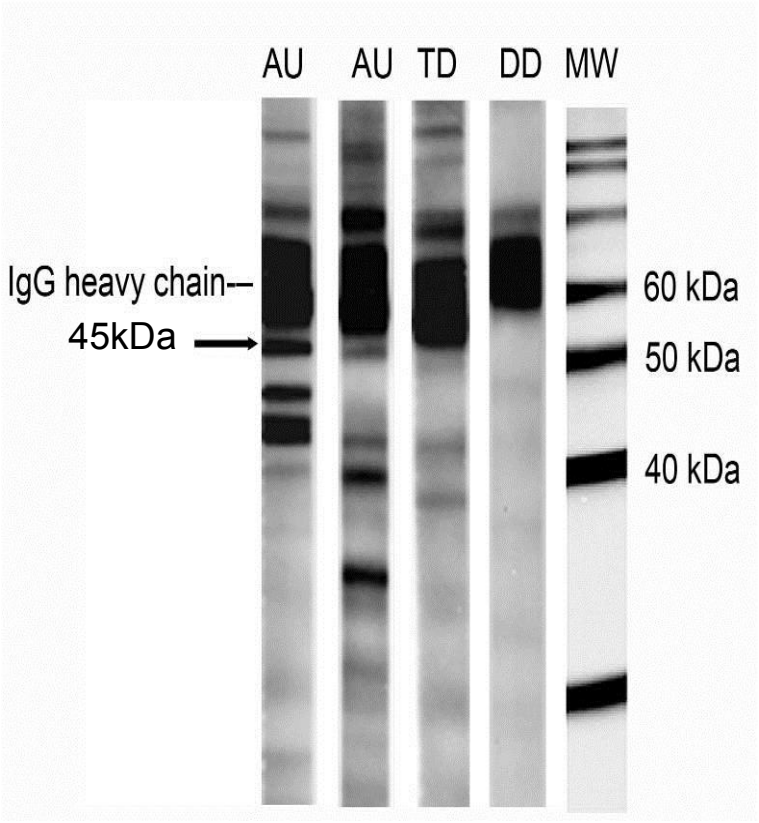
- The autoantibodies are directed against various brain components including:
  - serotonin receptors, heat shock proteins, glial filament proteins and myelin basic protein, as well as other proteins with significant neurological relevance (Connolly et al., 2006; Connolly et al., 1999; Goines et al., 2011a; Wills et al., 2007; Wills et al., 2009; Wills et al., 2011).

Autoantibodies from children with ASD



Immunohistochemical and Western blot analysis of autoantibody localization in cerebellum of Rhesus monkeys (Wills, 2009).

Western blot - Human cerebellum



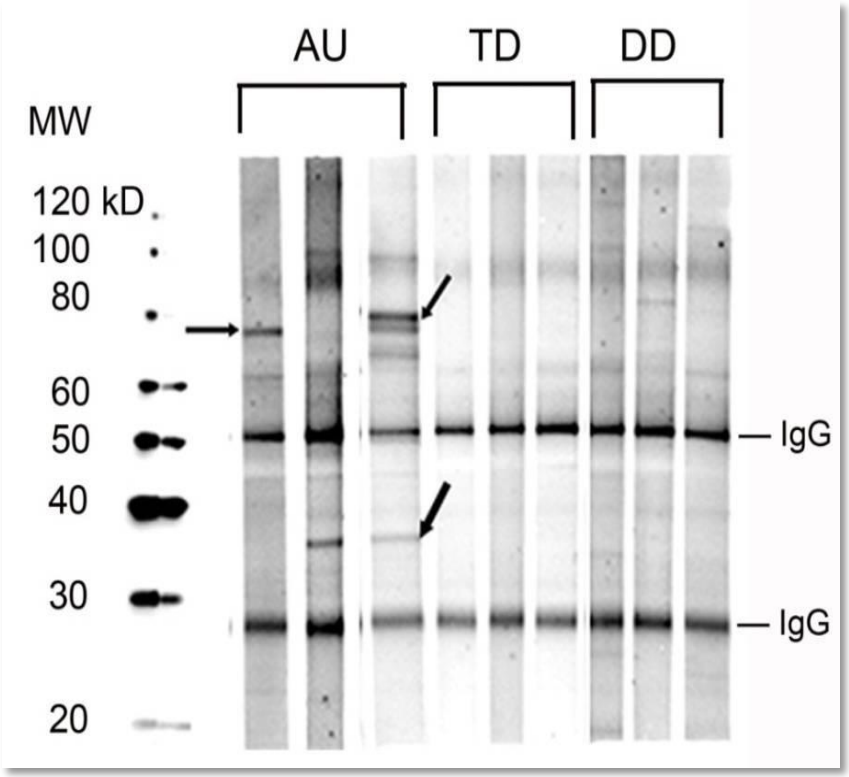
- The presence of the ~45 kDa band corresponds to Golgi staining by IHC (p=0.04).
- Children with these antibodies had lower adaptive and cognitive function
- Increased aberrant behaviors when compared to children without these antibodies

Goines, et al. BBI, 2011

Autoantibody Specificity

	Child Autoantibody Targets in Cerebellum				P values			
Band	ASD n=70	AU n=207	AU/ASD n=277	TD n=189	ASD vs. TD	AU vs. TD	AU/ASD vs. TD	AU vs. ASD
45	5 (7.1%)	2(9.7%)	25 (9%)	7 (3.6%)	NS	0.017	0.025	NS
62	12(16%)	17(8.2%)	29 (10%)	16 (8.2%)	0.043	NS	NS	0.043
45 + 62	0 (0%)	6 (3%)	6 (2%)	0 (0%)	NS	0.03	0.05	0.34

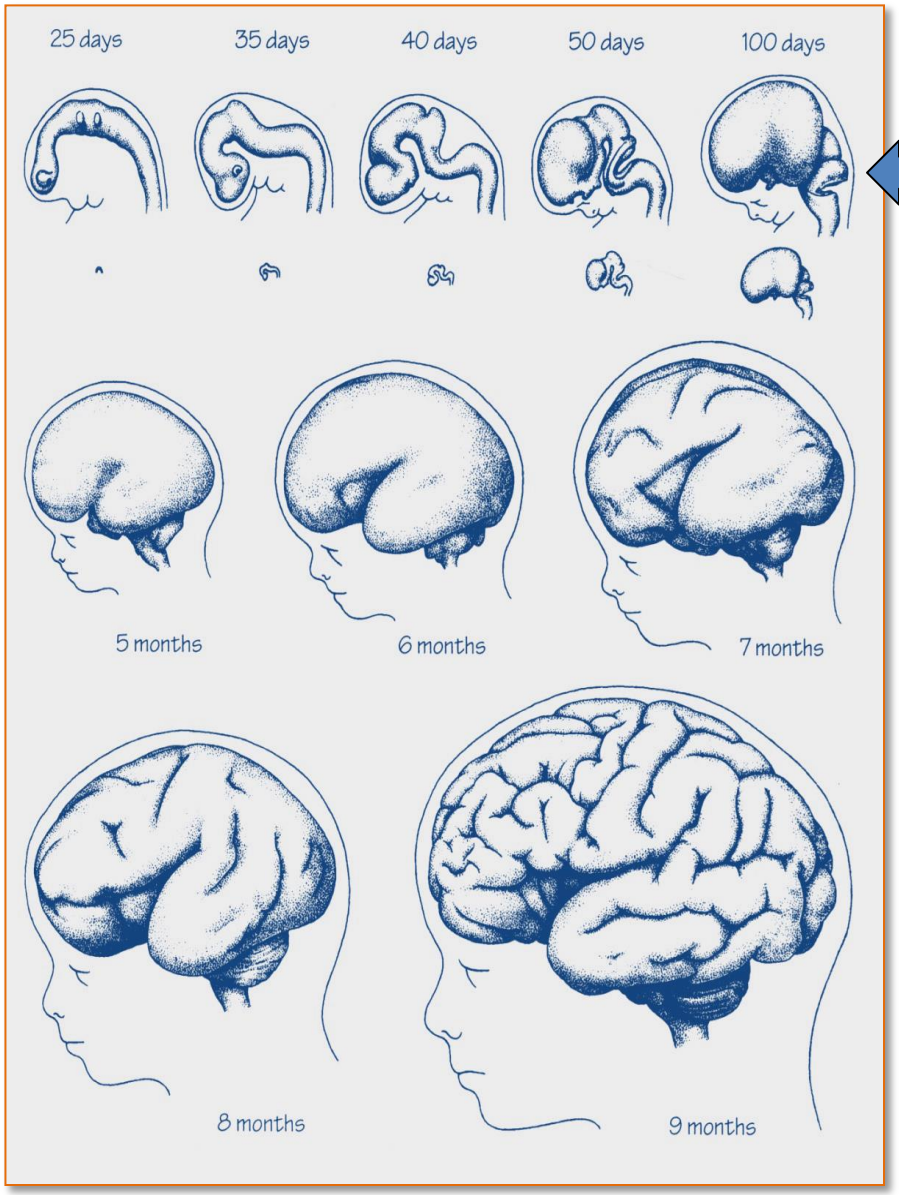
Maternal antibodies to fetal brain proteins

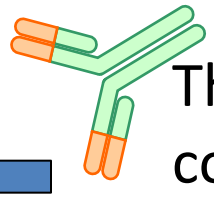


- Demonstrated the presence of anti-fetal brain autoantibodies in maternal circulation (Braunschweig, 2007, Singer, 2008, Braunschweig, 2011).

- These antibodies are highly specific for autism, and have demonstrated pathology in animal models (Martin, 2008, Singer, 2009 and Braunschweig, 2012).

Prenatal Growth of the Human Brain





The human brain consists of approximately 100 billion neurons (which is as many cells as there are stars in the Milky Way).

During the last trimester, neurons form at a rate of around 580,000 per minute.

After Cowan 1979

Maternal Anti-Brain Antibodies and ASD:

The studies behind the novel immune biomarker for autism risk

Epidemiology
Large population studies to identify potential risk factors for ASD

+

Immunology
Some mothers who have children with autism produce anti-brain anti-bodies

Is prenatal exposure to maternal anti-brain antibodies associated with specific subtypes of ASD?

Is prenatal exposure associated with abnormal brain growth?

Why do some women produce these anti-brain antibodies?

How might the antibodies impact fetal brain development?

Does prenatal exposure <u>cause</u> changes in brain and behavior relevant to ASD?

ASD Behavior

Antibodies have been associated with regression and language deficits, and stereotypic behavior

MRI

Enlarged brain volume in male children prenatally exposed the antibodies

Genetics

MET genetic variant associated with production of the anti-brain antibodies

Basic Science

Efforts are underway to verify the antigenic targets of the antibodies

Brain Tissue Studies

While there are no known postmortem cases, animal models provide an opportunity to explore brain pathology

Animal Models

Animal models show behavioral change following prenatal exposure to the antibodies; monkey model has also reported increased brain volume

Translational Potential:

Identify children with this sub phenotype and develop tailored behavioral treatment

Screen women at risk and develop preventative strategies

Establish the pathophysiology associated with these antibodies and develop therapeutic interventions

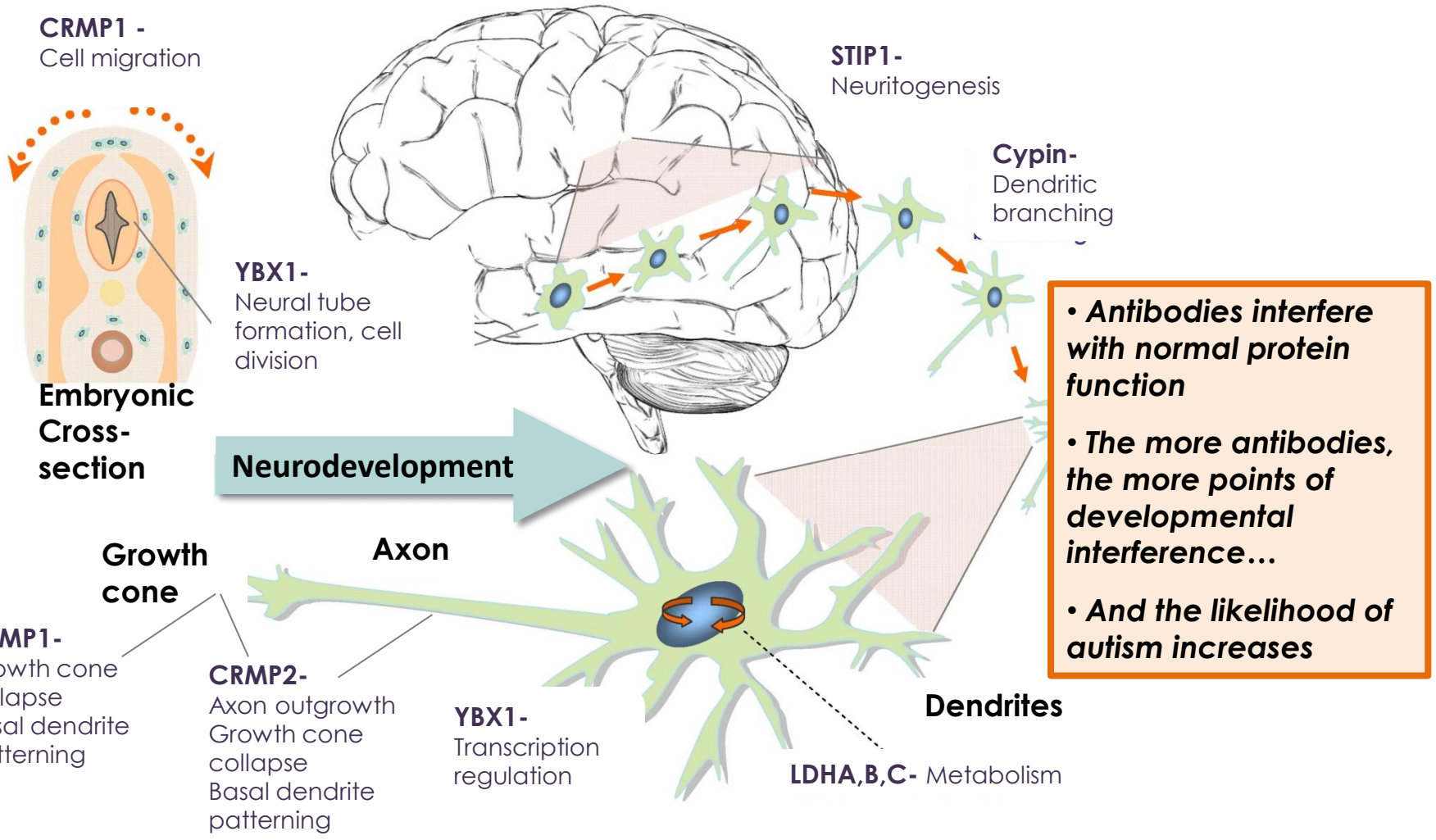
Autism Risk and Severity Correlates with Increasing MAR Antibody (Ab) Types and Numbers

MAR Antibody Presence in Maternal Blood			
MAR Ab Groupings	**Incidence in Autism**	**Incidence in Normal Pop.**	**Clinical Utility/ Implications**
Only one Ab	**89%**	**70%**	**not clinically significant**
Significant Ab doublets	**70%**	**27%**	**3X AU risk; counsel caution and early intervention**
Specific Ab doublets	**4%**	**0%**	**mothers w/specific combinations have 99%+ risk; counsel early diagnosis and intervention**
Specific Ab triplets	**19%**	**0.6%***	
All specific MAR combinations	**23%**	**0.6%**	

***1 Typically Developing (TD) child with score abnormally high score of 22 on ABC subscale for hyperactivity**

Braunschweig et al, Nature Translational Psychiatry, 2013

We Identified 8 MAR Autoantibodies That Bind to Protein Targets* Critical to Normal Brain Development



MAR Patterns Correlate with ASD Behaviors

P-values for Significant Behavior Correlations (P<.05)

Antibody Status	Irritability	Lethargy	Stereotypy	Hyperactivity	Moods
Any LDH (n=63)	n.s.	n.s.	0.024	n.s.	n.s.
Any Cypin (n=24)	n.s.	0.006	n.s.	n.s.	n.s.
LDH + Cypin (n=4)	n.s.	0.041	n.s	n.s.	n.s.
LDH + STIP1 (n=36)	n.s.	n.s.	0.015	n.s.	0.062
LDH + CRMP1 (n=21)	n.s.	n.s.	0.028	0.058	0.047
LDH + STIP1 + CRMP1 (n=12)	n.s.	n.s.	0.007	0.057	0.061
LDH + STIP1 + CRMP1 or LDH + Cypin (n=15)	n.s.	n.s.	0.013	n.s.	n.s.

- *Presence of LDH antibodies appear to contribute heavily to stereotypic behavior, a core feature of ASD*
- *Antibodies to LDH in combination with STIP1 and CRMP1 are highly significantly associated with stereotypic behavior*
- *Antibodies to Cypin alone is highly significantly associated with lethargic behavior*

Brain, Behavior, and Immunity xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

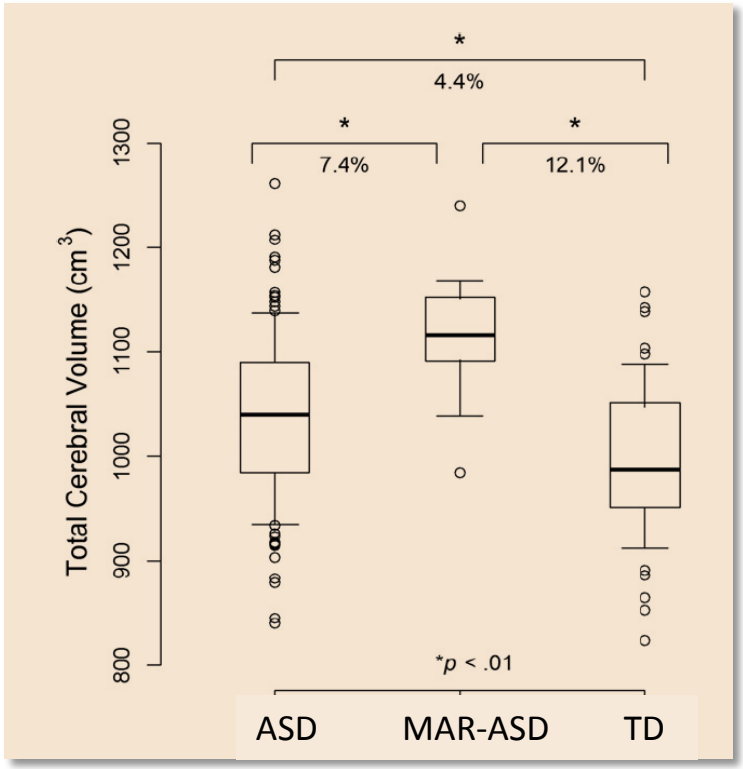
journal homepage: www.elsevier.com/locate/ybrbi

Short Communication

Maternal autoantibodies are associated with abnormal brain enlargement in a subgroup of children with autism spectrum disorder

Christine Wu Nordahl [a,b,*], Daniel Braunschweig [c], Ana-Maria Iosif [d], Aaron Lee [b], Sally Rogers [a,b], Paul Ashwood [a,c], David G. Amaral [a,b], Judy Van de Water [a,c]

- Studied 181 2-4 YO male children (131 ASD, 50 typically developing (TD) controls) and evaluated total brain volume using structural magnetic resonance imaging (MRI).
- The ASD MAR group exhibited a **more extreme 12.1% abnormal brain enlargement** relative to TD controls.
- The remaining ASD children had a smaller 4.4% abnormal brain enlargement relative to TD controls.
- Lobar and tissue type analyses revealed that the frontal lobe is selectively enlarged
- **MAR autoantibodies may impact brain development leading to abnormal enlargement**.

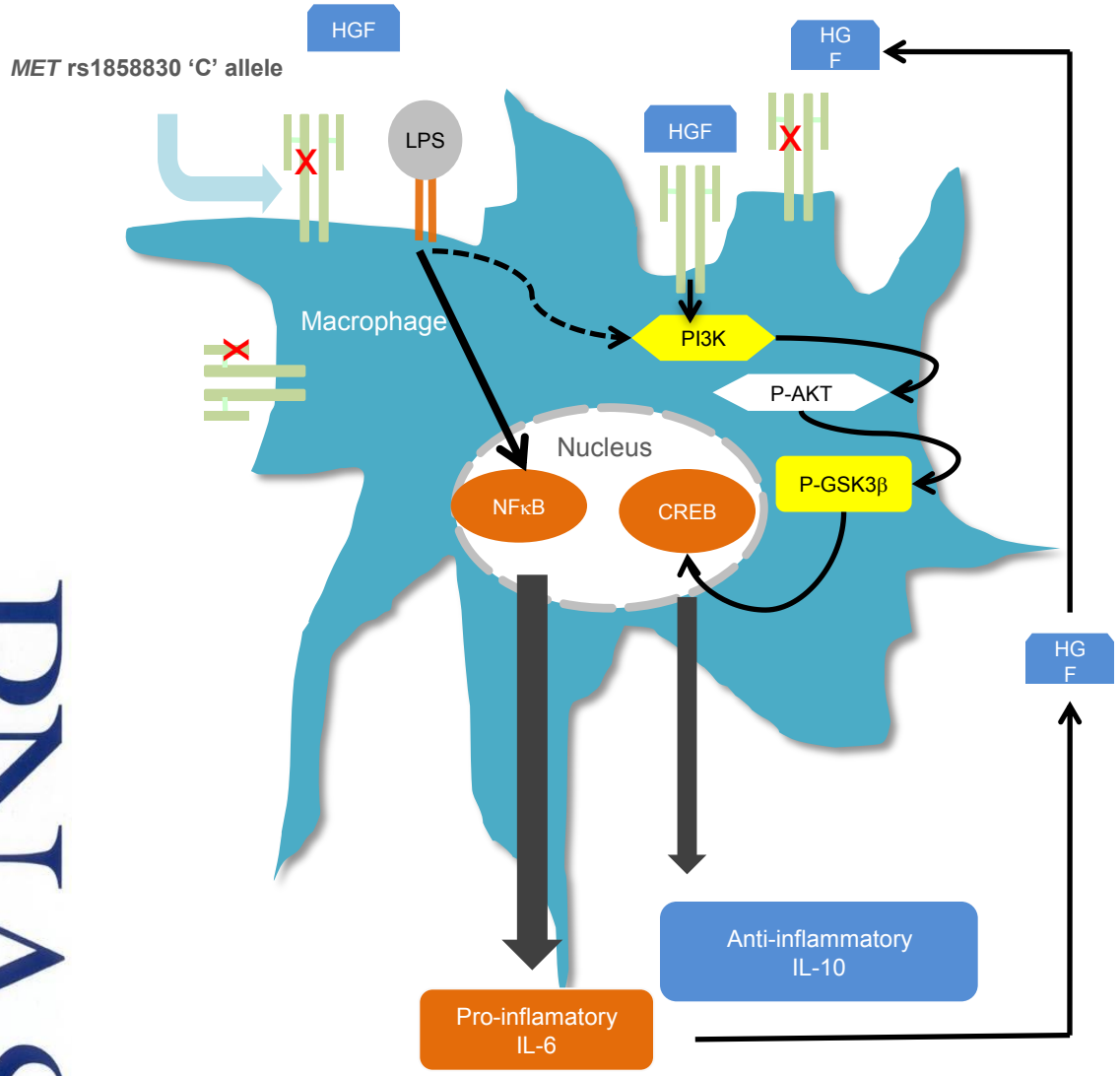


What is the susceptibility factor
for the production of these antibodies?

Where genetic susceptibility and
immune function converge…..

A genetic variant that disrupts the MET transcription is associated with autism

Daniel B. Campbell *, James S. Sutcliffe †‡, Philip J. Ebert *, Roberto Militerni §, Carmela Bravaccio §, Simona Trillo ¶, Maurizio Elia ||, Cindy Schneider **, Raun Melmed ††, Roberto Sacco ‡‡§§, Antonio M. Persico ‡‡§§, and Pat Levitt *‡¶¶



The MET receptor tyrosine kinase is a key negative regulator of immune responsiveness, controlling the degree of activation of antigen presenting cells (APCs; e.g., dendritic cells, monocytes, and B cells).

The 'C' allele with polymorphism rs1858830 increases relative risk for ASD approximately 2.25-fold in children.

What is the relationship between MET and anti-fetal brain antibodies?

37/73 kDa bands	Diagnosis Groups	MET Genotype			Allelic Chi-square p-value
		C/C	C/G	G/G	
Positive	(All have ASD) n=19	11 (58%)	7 (37%)	1 (5%)	Reference
Negative	ASD + TD (n=346)	101 (29%)	154 (45%)	91 (26%)	0.003
	ASD Only (n=183)	51 (28%)	79 (43%)	53 (29%)	0.002

- We have found a higher incidence of the MET ‘C’ allele in the blood of mothers who have antibodies to fetal brain proteins (Heuer et al, 2011).
- This allele confers a functional reduction in the receptor MET production.

The *MET* promoter variant is a possible susceptibility factor

- The functional MET promoter variant alters expression of the MET receptor in immune cells.
  - This may predispose to the development of antibodies to fetal brain proteins in some mothers whose children develop autism.

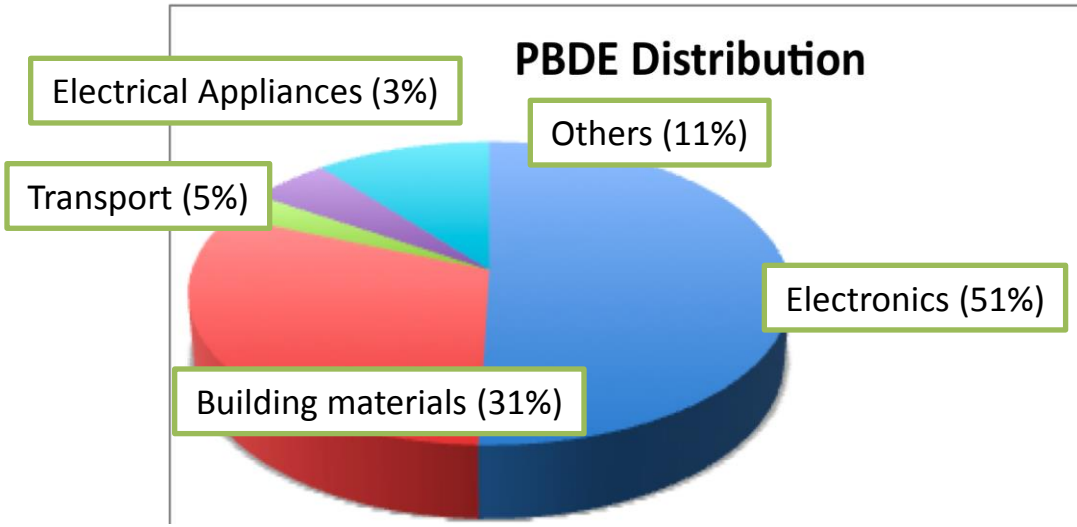
- Further, this genetic susceptibility may lead to loss of immune regulation during gestation, which may, even in the absence of autoantibody production, have an effect on neurodevelopment.

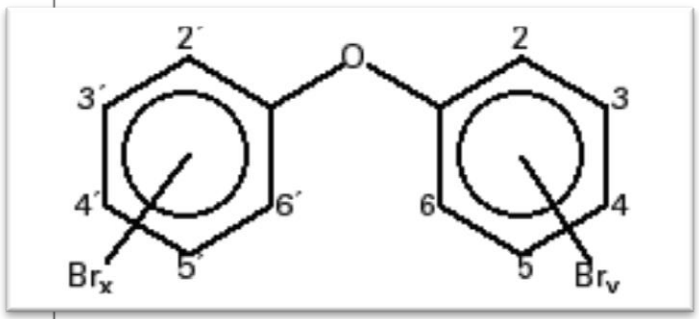
IMMUNE DYSREGULATION REPORTED IN AUTISM

What is the role of the environment in the immune dysregulation noted in ASD?

Polybrominated Diphenyl Ethers (PBDEs)

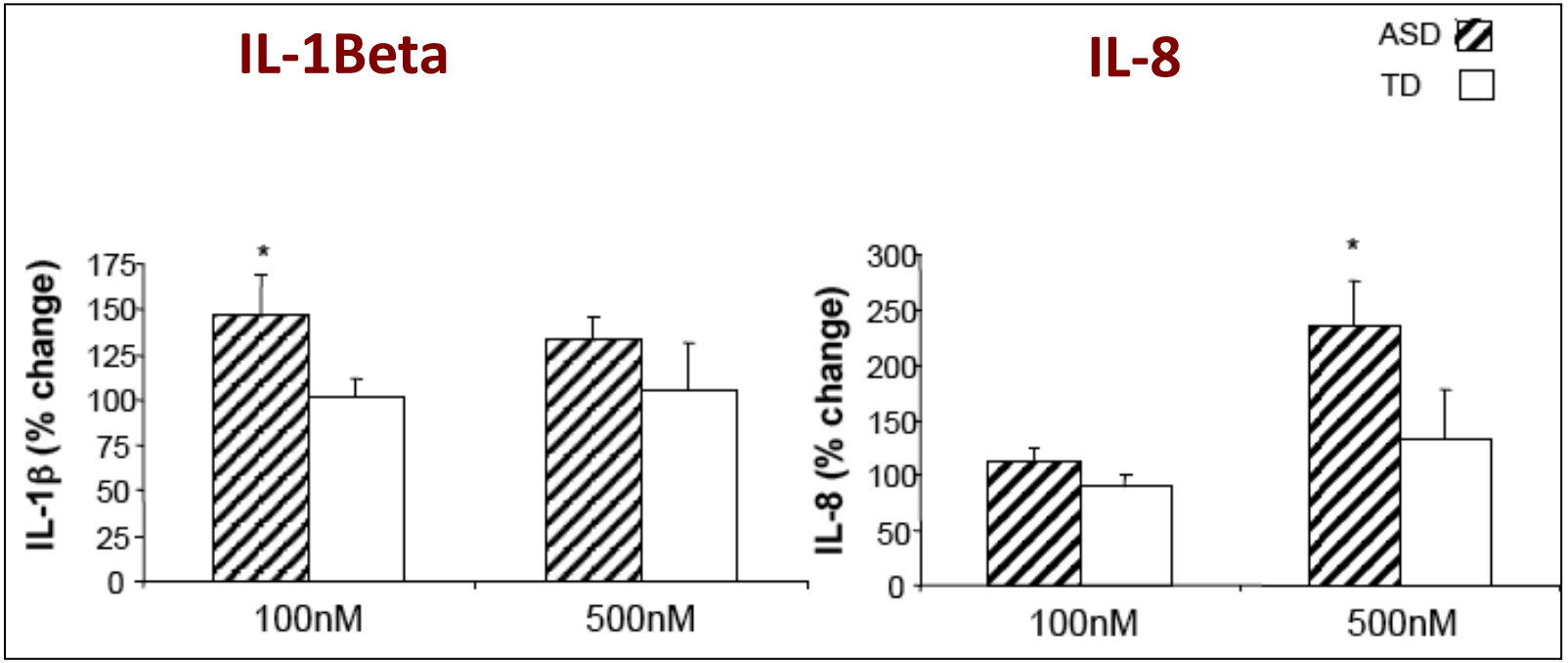
- Persistant Organic Pollunts (POPs)
- Flame-retardants
  - Textile, building and manufacture of electronic appliances
- Widely dispersed in global environment
- May interfere with normal immune and or neurological development (Lawler et al., 2004)
- Varies routes of exposure





Dingemans et al., 2011 *Environmental Health Perspectives Supplements*

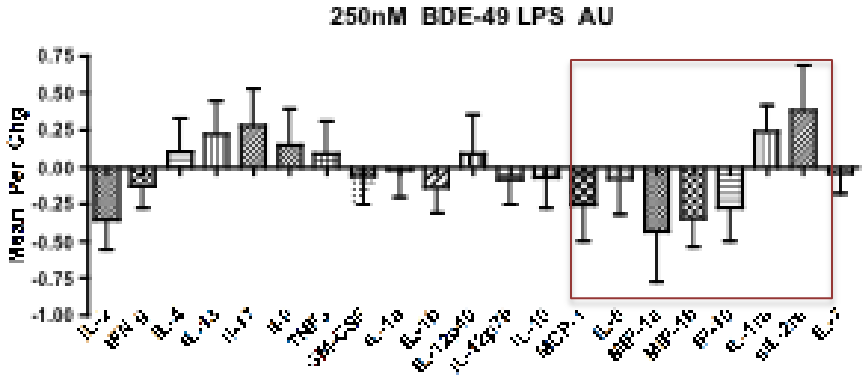
Early study: *In vitro* Effects of BDE-47 on Innate Immune Response in Children with Autism Spectrum Disorders (ASD)

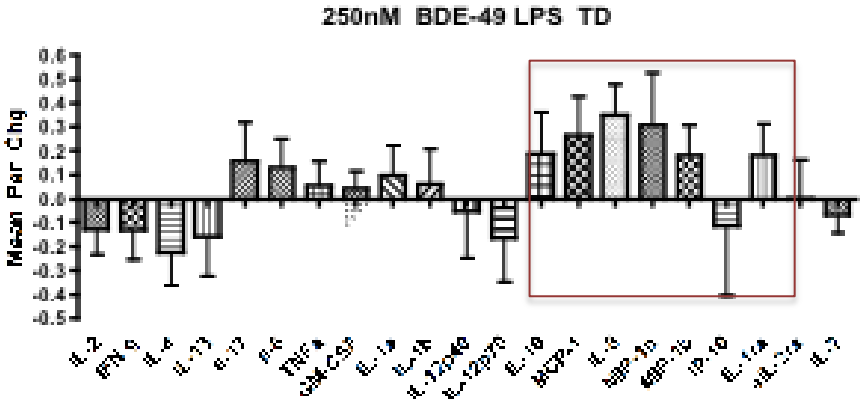


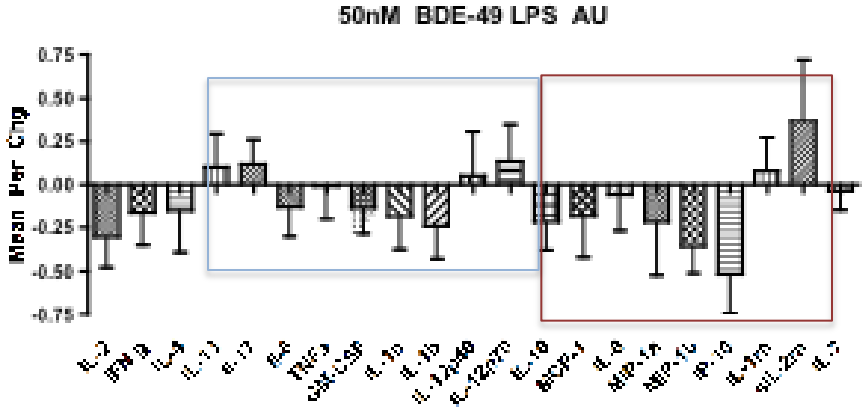
- Increased production of inflammatory cytokines in ASD compared to age matched typically developing (TD) controls
- Children with ASD have differential immune sensitivity to some environmental toxicants
- Do children with ASD have a genetic susceptibility to PBDE effects?

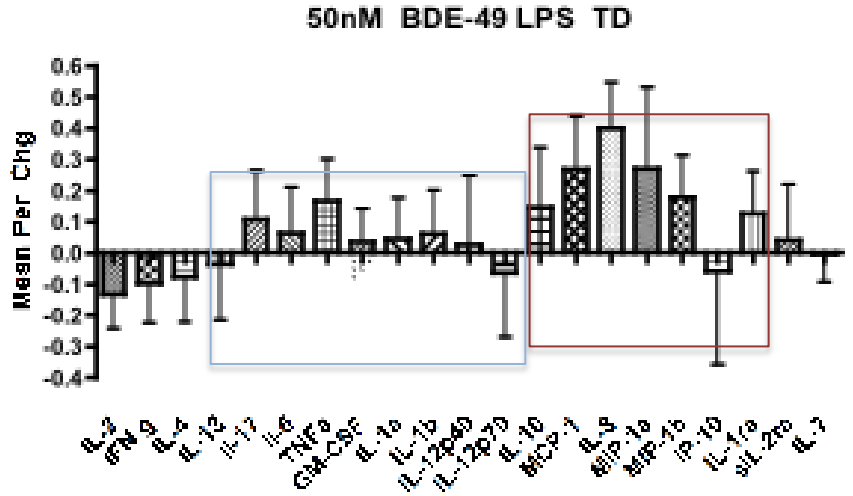
Ashwood et al., 2009 Brain Behav Imm

LPS Challenged BDE-49 exposed AU and TD children:





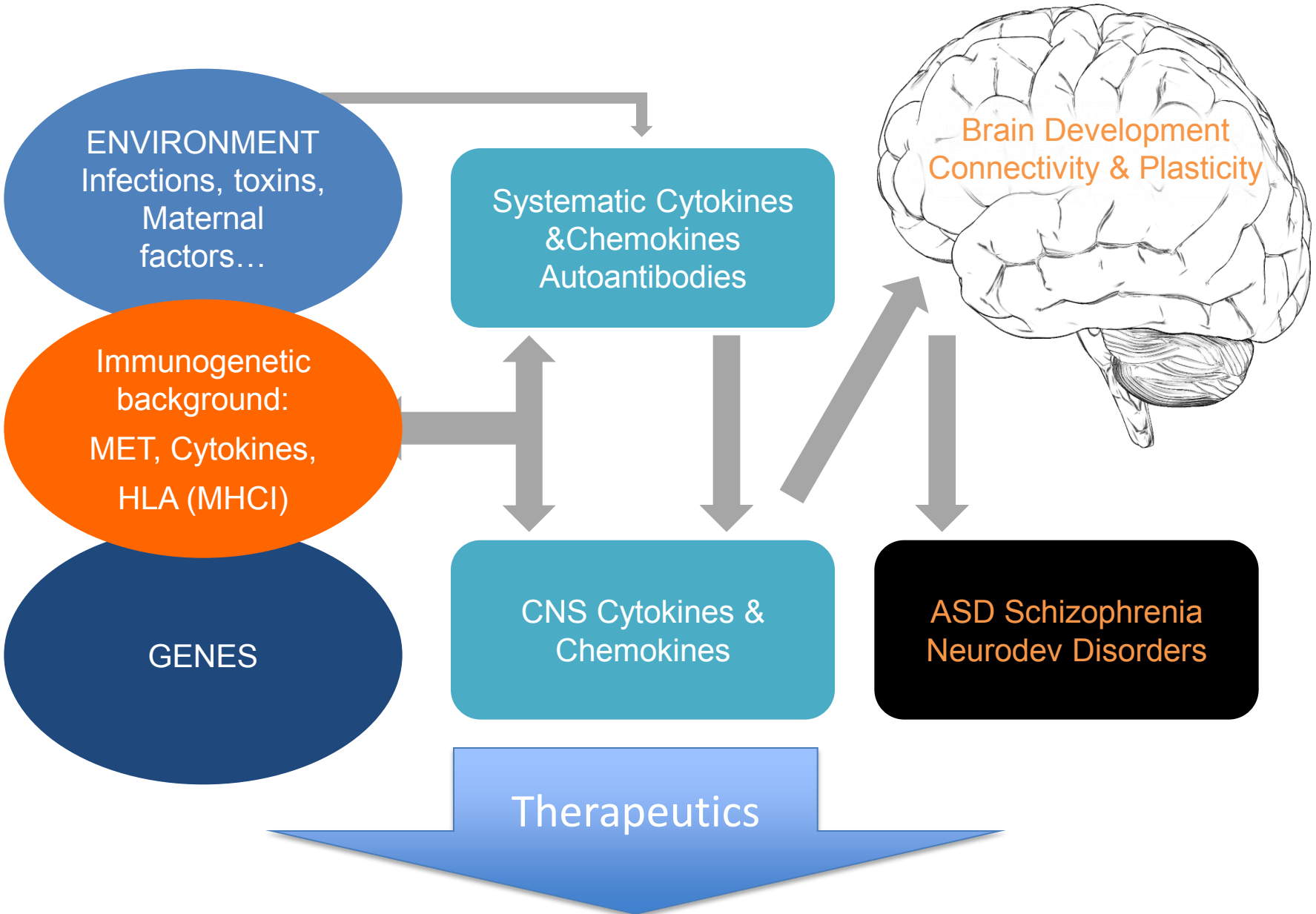




Conclusions

- We see changes in immune function in several branches of the immune system including altered antibody production, altered NK cell function, autoantibodies, and differential cellular responses to various stimuli.
  - Maternal autoantibodies are specific for ASD
- Plasma cytokines can be informative
  - Fairly stable over time with multiple samples
- Very variable from subject to subject both ASD and controls
- See several profiles within autism
  - Elevated inflammatory
  - Reduced profile
  - Normal profile
  - What appears to be a somewhat ASD-specific profile
  - Onset status driven

Immune & CNS systems interactions in disease



The UC Davis Team

Dr. Judy Van de Water

Dr. Paul Ashwood

Dr. David Amaral

Dr. Christine Wu Nordahl

Dr. Melissa Bauman

Dr. Daniel Braunschweig

Robert Boyce

Elizabeth Fox

Lauren Matelski

Marjannie Eloi

Lori Haapanen

UCD Center for Children's Environmental Health and C.H.A.R.G.E

Dr. Isaac Pessah

Dr. Irva Hertz-Picciotto

Dr. Robin Hansen

Melissa Rose

This work was supported by grants NIEHS 1 P01 ES11269-01, the U.S. Environmental Protection Agency (U.S. EPA) through the Science to Achieve Results (STAR) program (Grant R829388), Autism Speaks, the JBJ Foundation, and the UC Davis M.I.N.D. Institute.

CCEH Center for Children's Environmental Health

Polychlorinated Biphenyls (PCBs): Environmental Risk Factors for ASD?

Pamela Lein, Ph.D.
Department of Molecular Biosciences
Center for Children's Environmental Health
UC Davis School of Veterinary Medicine

What is the evidence that environmental factors contribute to ASD risk?

1. Rapid increase in ASD prevalence
2. Genetic studies
3. Clinical heterogeneity of ASD
4. Systemic and CNS pathophysiology

- Oxidative stress
- Immune dysfunction (including neuroinflammation)
- Mitochondrial dysfunction

These pathophysiological outcomes known to be exacerbated by environmental factors

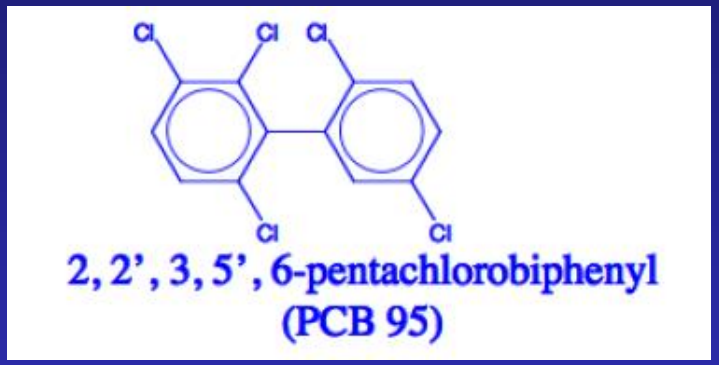
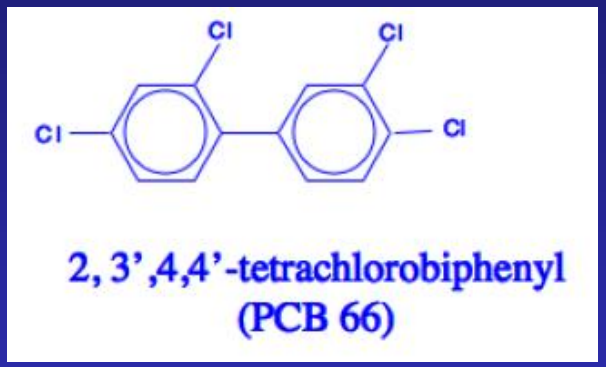
air pollution, organophosphorus pesticides, heavy metals

PCB Developmental Neurotoxicity

- **Human epidemiological data suggest a negative association between developmental exposure to environmental PCBs and cognitive function in infancy or childhood**
  - **Decreased IQ, impaired learning and memory, attentional deficits, lowered reading comprehension, psychomotor problems**

- **Comparable cognitive and behavioral deficits observed in primate and rodent models following developmental PCB exposures**
  - **Developmental neurotoxic effects of PCBs have been observed at relatively low exposure levels corresponding to between 1 and 10x the background levels observed in humans**

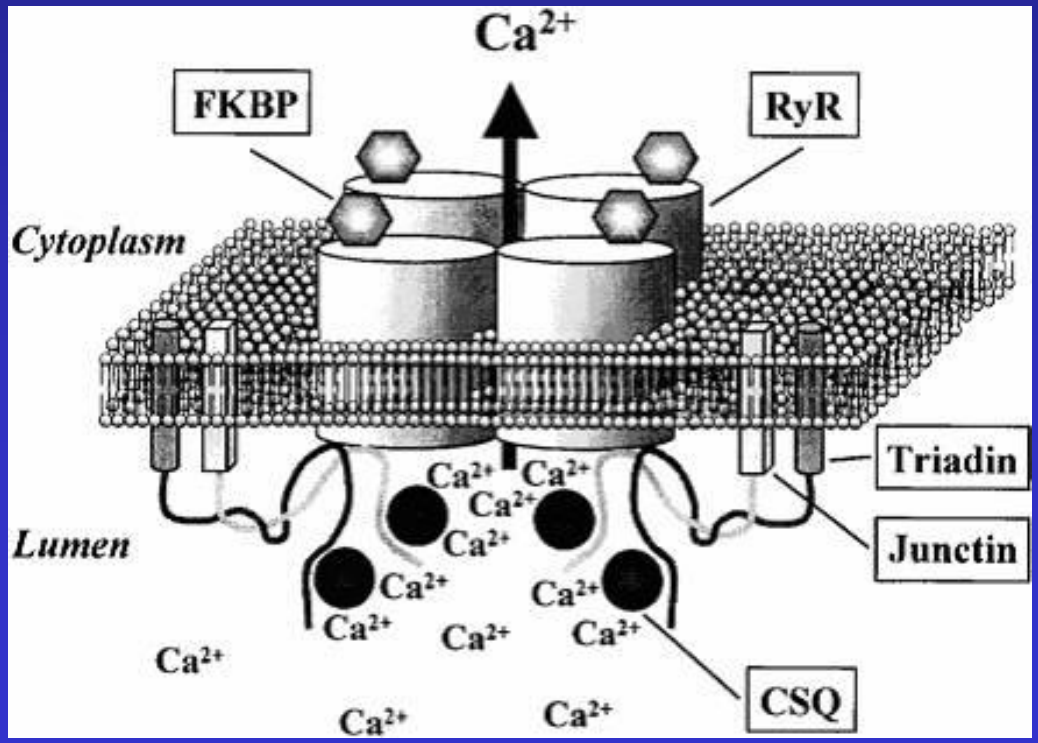
PCB developmental neurotoxicity mediated primarily by non-dioxin-like PCB congeners

	Non-dioxin-like congeners	Dioxin-like congeners
	 2, 2', 3, 5', 6-pentachlorobiphenyl (PCB 95)	 2, 3',4,4'-tetrachlorobiphenyl (PCB 66)
Developmental Neurotoxicity	+++	+/-
Carcinogenic	+/-	+++
Arylhydrocarbon Receptor (AhR)	**Low to no affinity**	**High affinity**

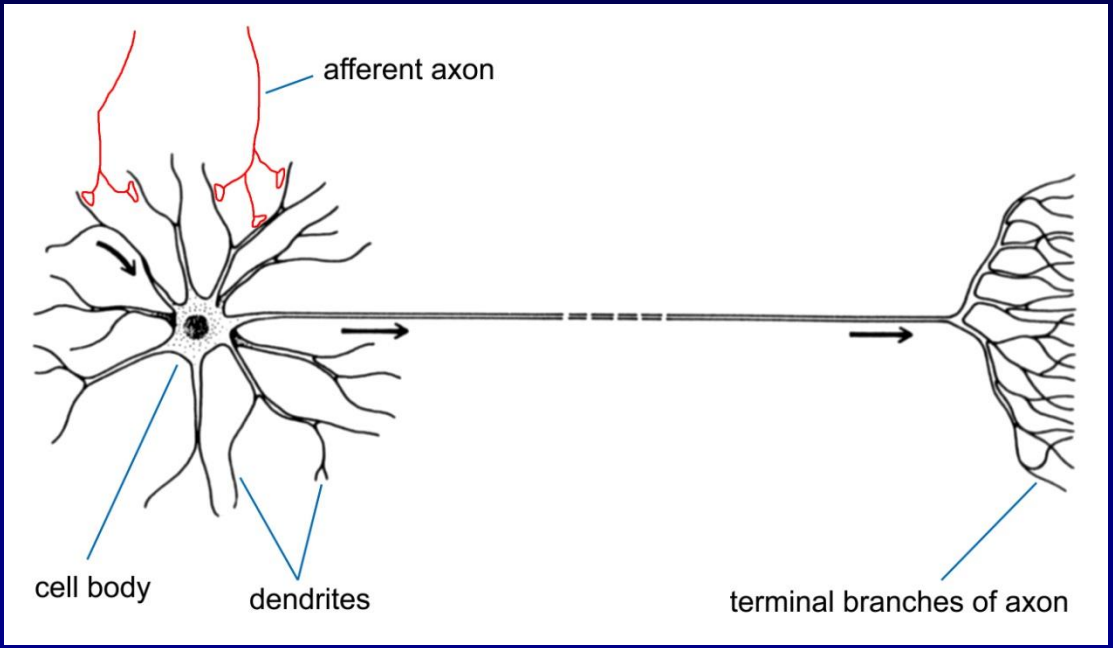
Cell and molecular mechanism(s) of PCB developmental neurotoxicity unknown

- Decreased dopamine content
- Interference with thyroid hormone signaling
- Increased levels of intracellular calcium Ca^{2+}

  - **Sensitization of the ryanodine receptor (RyR)**



Does developmental exposure to non-dioxin-like PCBs alter dendritic growth?

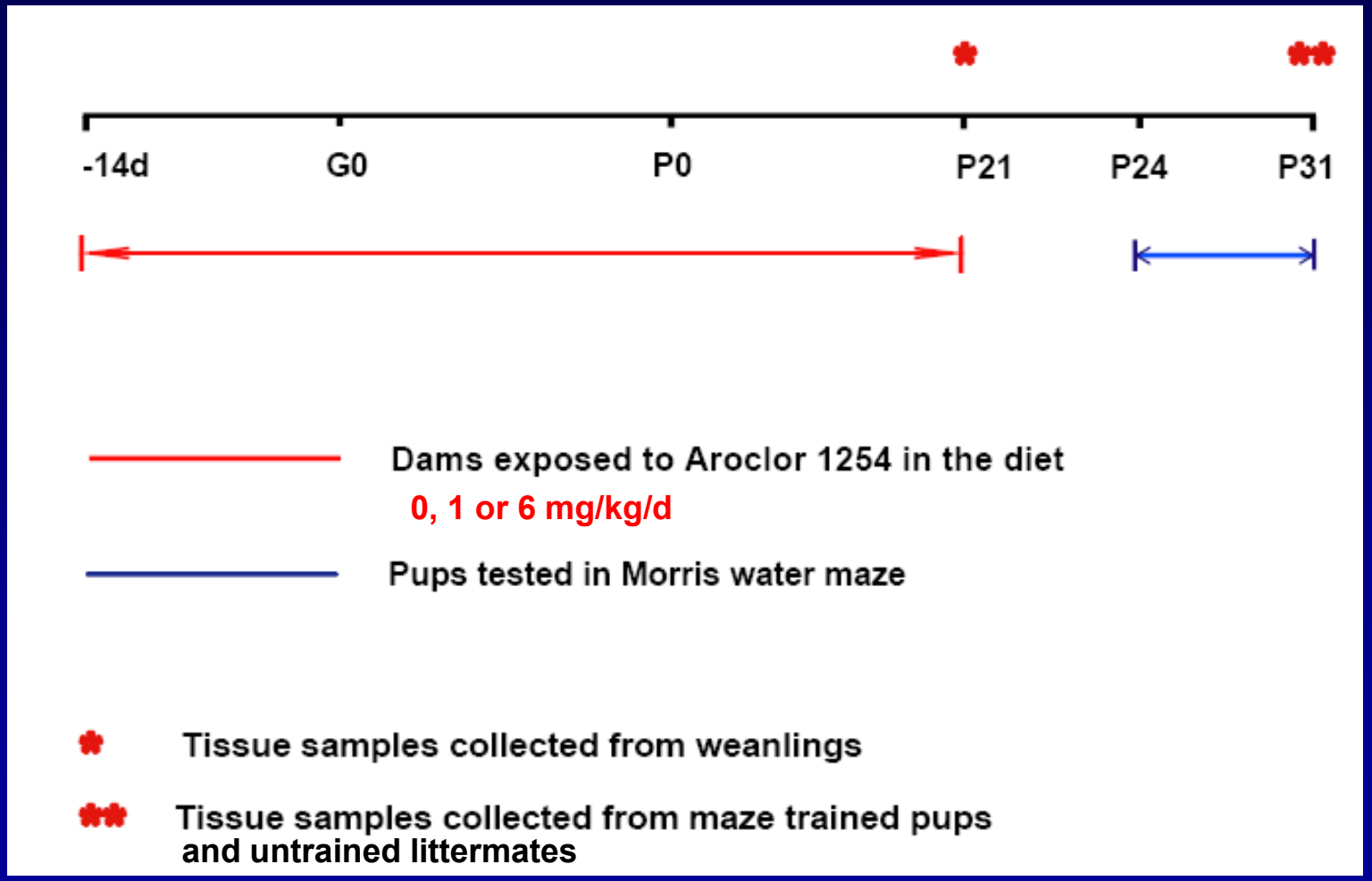


- **Dendritic branching patterns influence the number, types and distribution of synaptic inputs**
- **Structural plasticity of dendrites is thought to be the cellular substrate of learning and memory**
- **Altered patterns of dendritic growth and plasticity are associated with ASD and other neurodevelopmental disorders**

Hypothesis:

Developmental exposures to non-dioxin-like PCBs cause behavioral deficits via altered pattern of dendritic growth and plasticity

Experimental Design of *In Vivo* Studies Using Aroclor 1254



Environmental risk factors for ASD

- Rubella infection during the first trimester of pregnancy
- *In utero* exposure to thalidomide or valproic acid
- Paternal age
- Environmental chemicals (?)

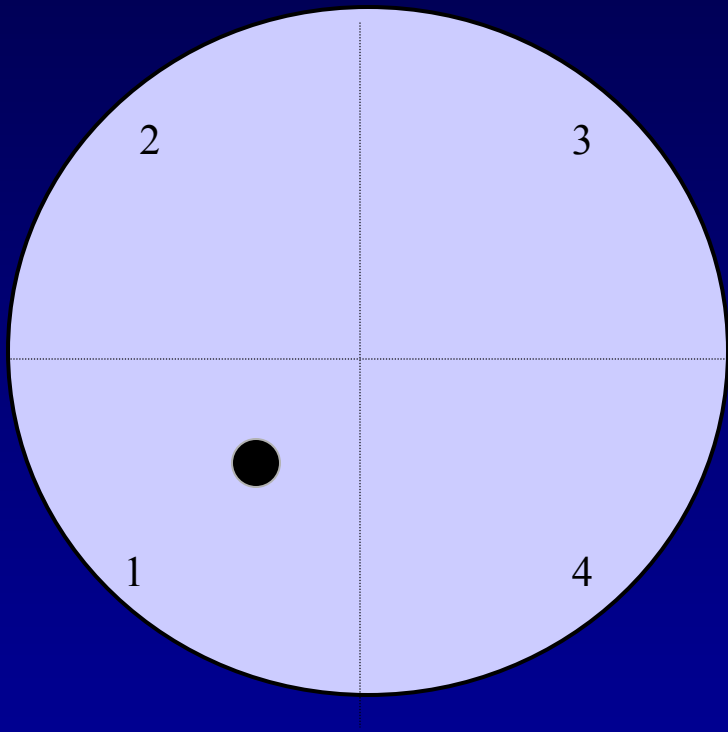
However, efforts to identify specific environmental risk factors for ASD have produced a number of candidates but few definitive hits

- Heavy metals (lead, methylmercury)
- Pesticides
  - Organophosphorus pesticides (OPs), e.g., chlorpyrifos, diazinon
  - Organochlorine pesticides (OCs), e.g., DDT, dieldrin, lindane
- Persistent organic pollutants (POPs)
  - Polychlorinated biphenyls (PCBs)
  - Polybrominated diphenyl ethers (PBDEs)
  - Polycyclic aromatic hydrocarbons (PAHs)

Morris Water Maze

Spatial Test:

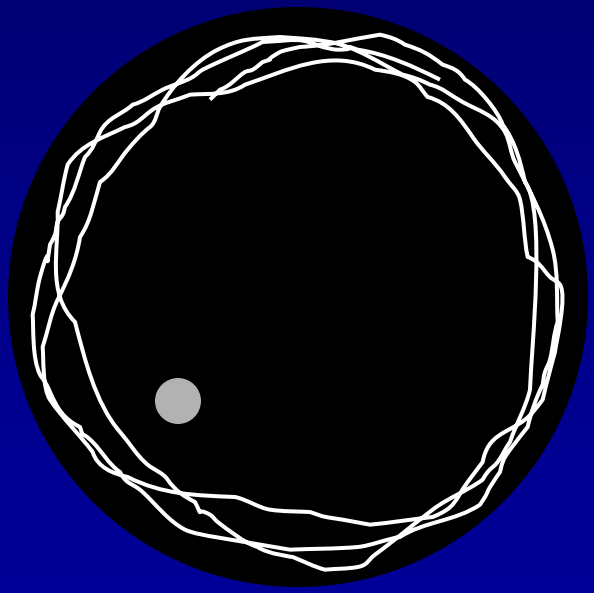
1. subject is placed on the platform for 20 sec
2. subject is placed in start quadrant
3. subject is allowed to swim for 45 sec or until the platform is found
4. subject is placed on the platform for 20 sec before being removed from pool



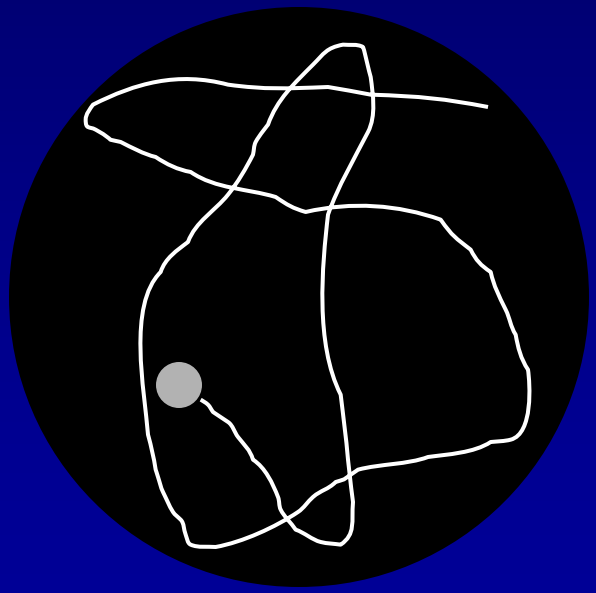
Average time to find the platform **(escape latency)** provides a measure of learning over successive trials

Morris Water Maze

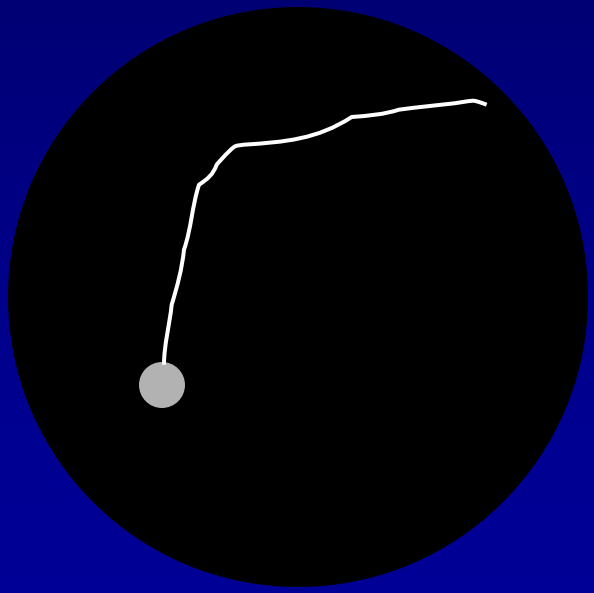
- In early trials subjects tend to swim around perimeter
- After several trials animals use searching behavior
- Once task is learned, subjects swim directly to platform



thigmotaxis

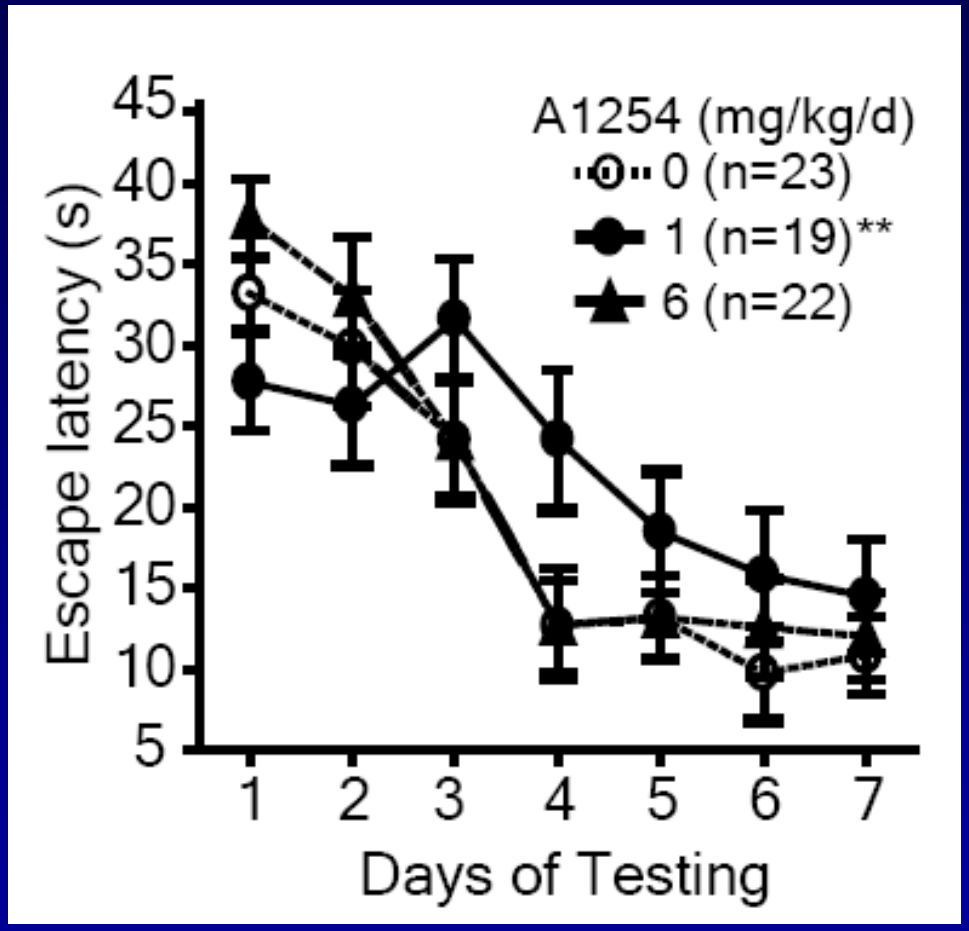


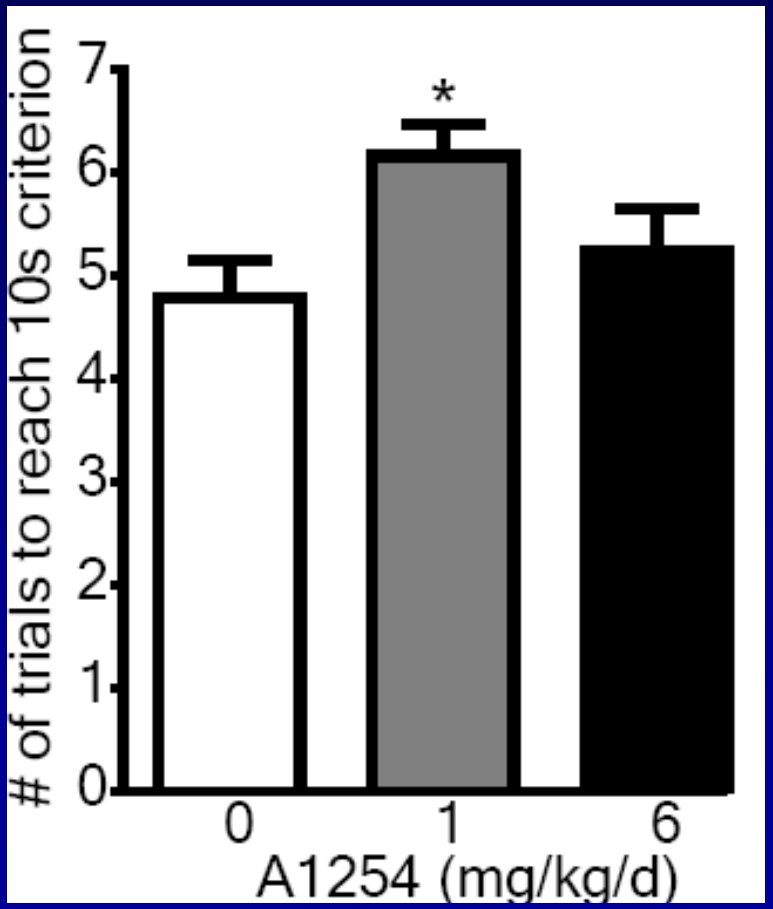
searching



learned

Developmental Aroclor 1254 exposure causes deficits in spatial learning





Yang et al., 2009, *Environ Health Perspect.* 117:426-435.

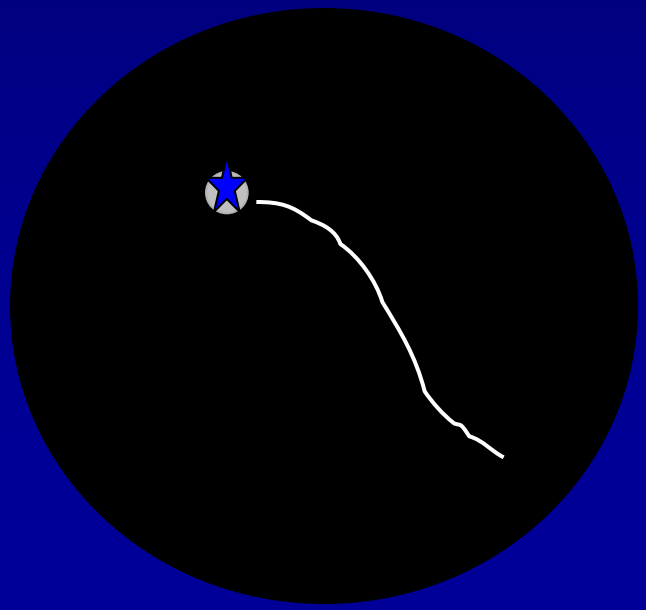
Morris Water Maze

Probe Test

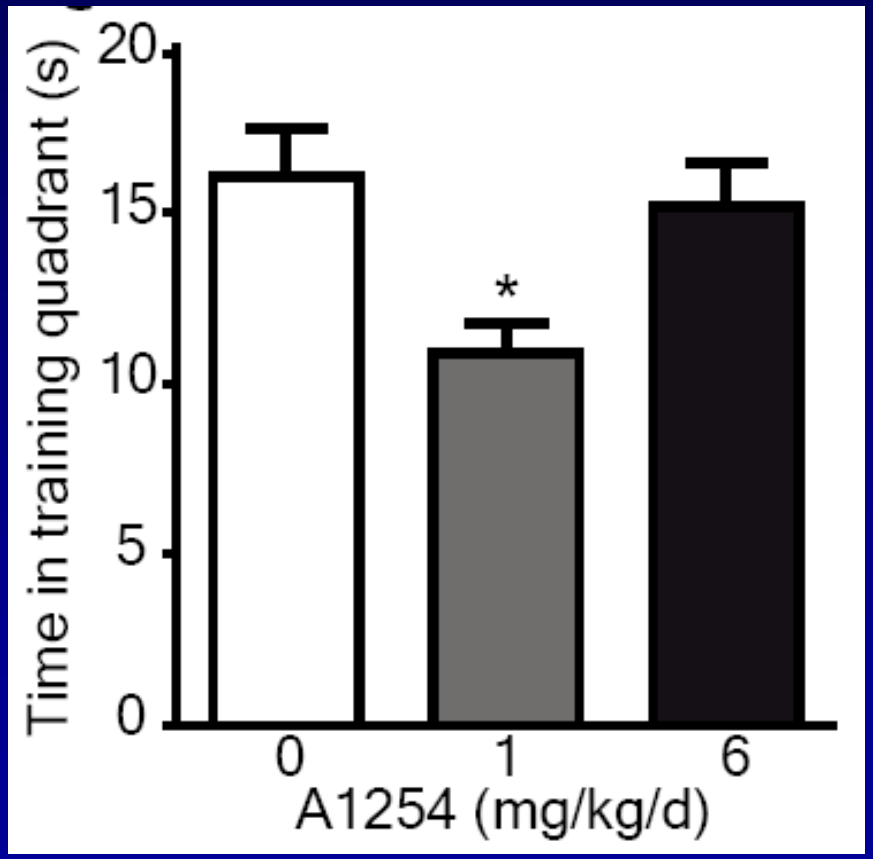
1. platform removed from pool
2. subject placed in a start quadrant
3. subject allowed to swim for 45 sec
4. time spent in platform quadrant is used as a measure of learning/memory

Cue Test (motivation/vision/motor function)

1. platform moved and marked with a flag
2. subject placed in a start quadrant
3. subject allowed to swim for 45 sec
4. time to platform gives measure of motor function, motivation and visual function



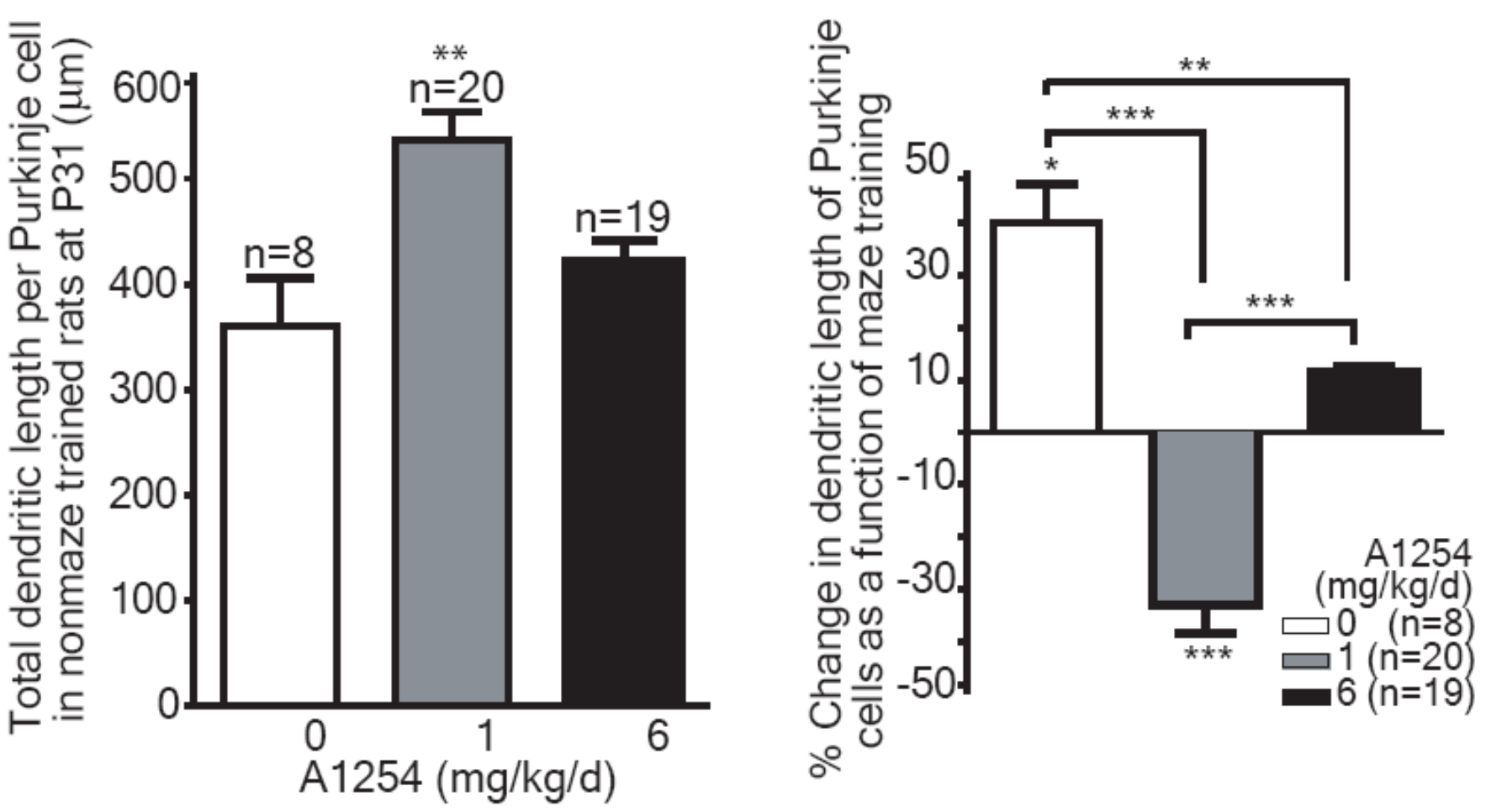
Developmental Aroclor 1254 exposure causes deficits in spatial memory



A1254 (mg/kg/d)	Visual cue test	
	Escape latency (s)	speed (cm/s)
0	7.7±1.2	21.0±0.1
1	8.3±2.5	20.7±0.2
6	8.2±2.0	20.2±0.4

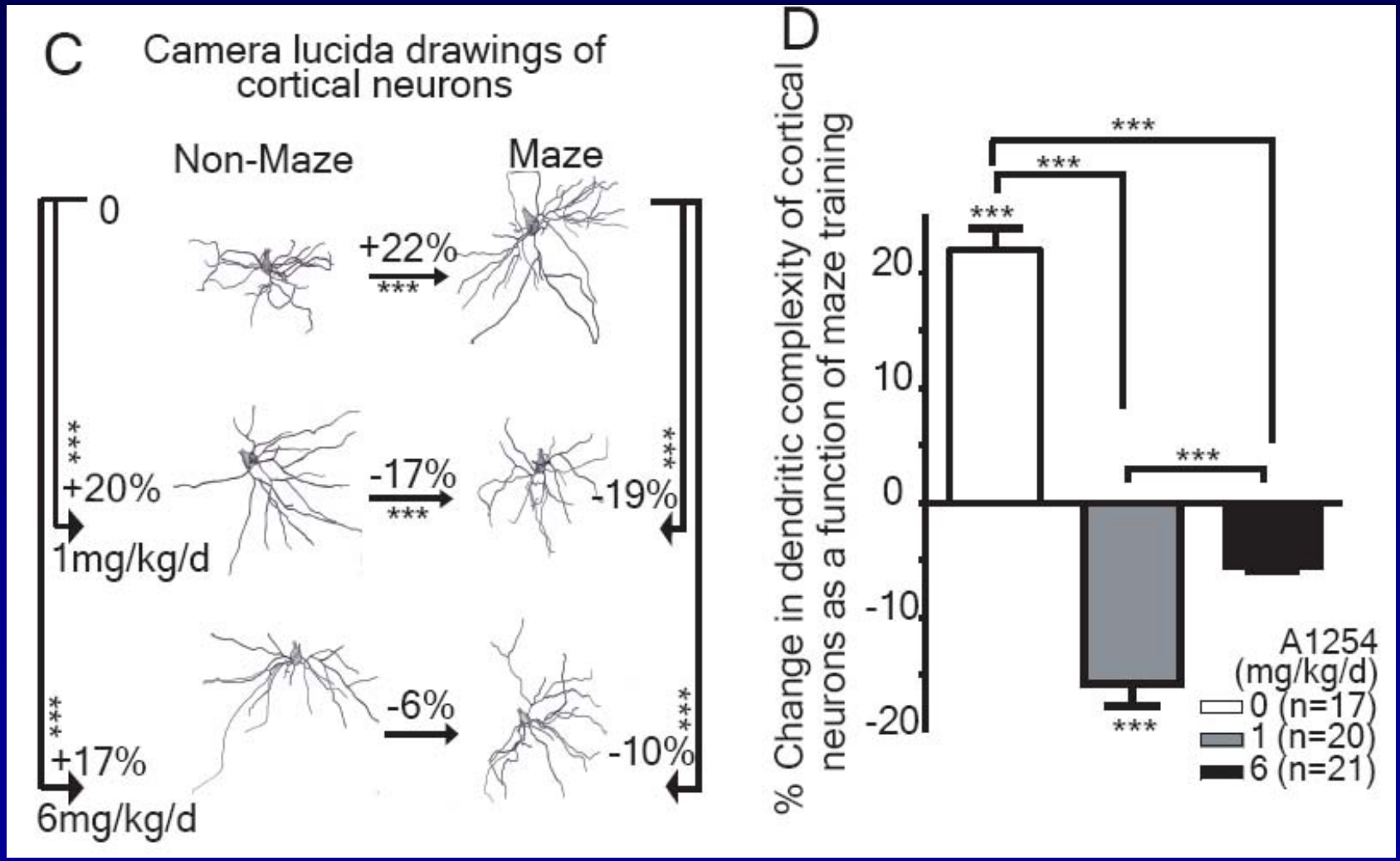
Yang et al., 2009, *Environ Health Perspect.* 117:426-435.

Developmental Aroclor 1254 exposure alters dendritic growth in cerebellar Purkinje cells



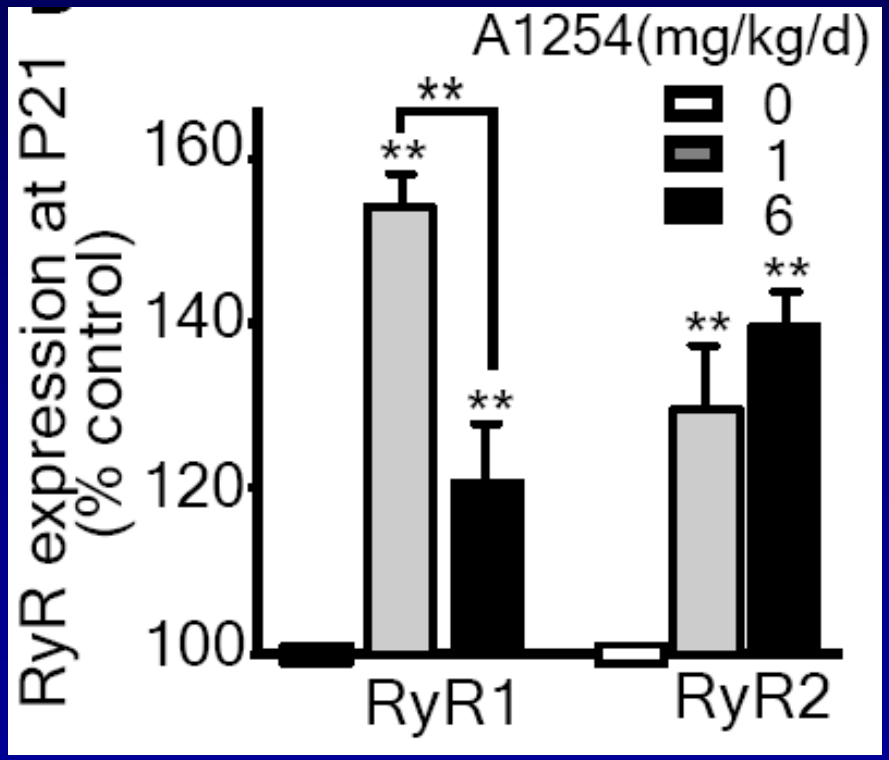
Yang et al., 2009, *Environ Health Perspect.* 117:426-435.

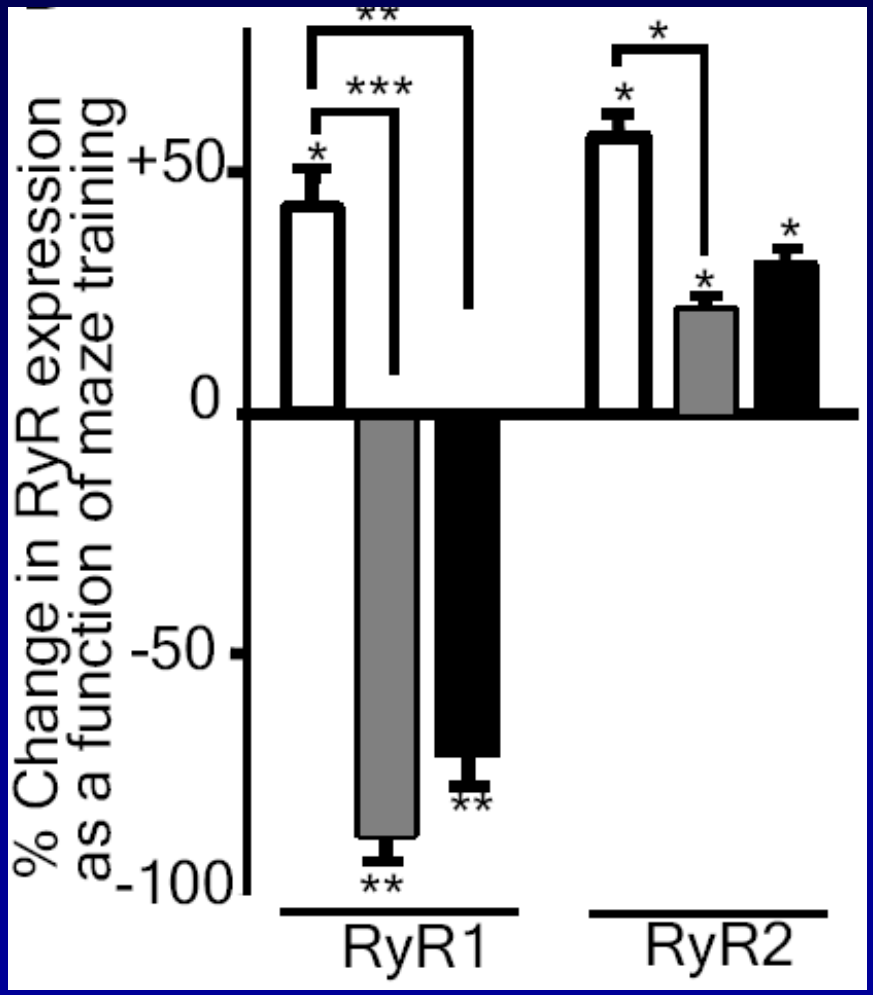
Developmental Aroclor 1254 exposure alters dendritic growth in cortical pyramidal neurons



Yang et al., 2009, *Environ Health Perspect.* 117:426-435.

Developmental PCB exposure alters RyR expression (data collected by Kyungho Kim, Pessah lab)





Yang et al., 2009, *Environ Health Perspect.* 117:426-435.

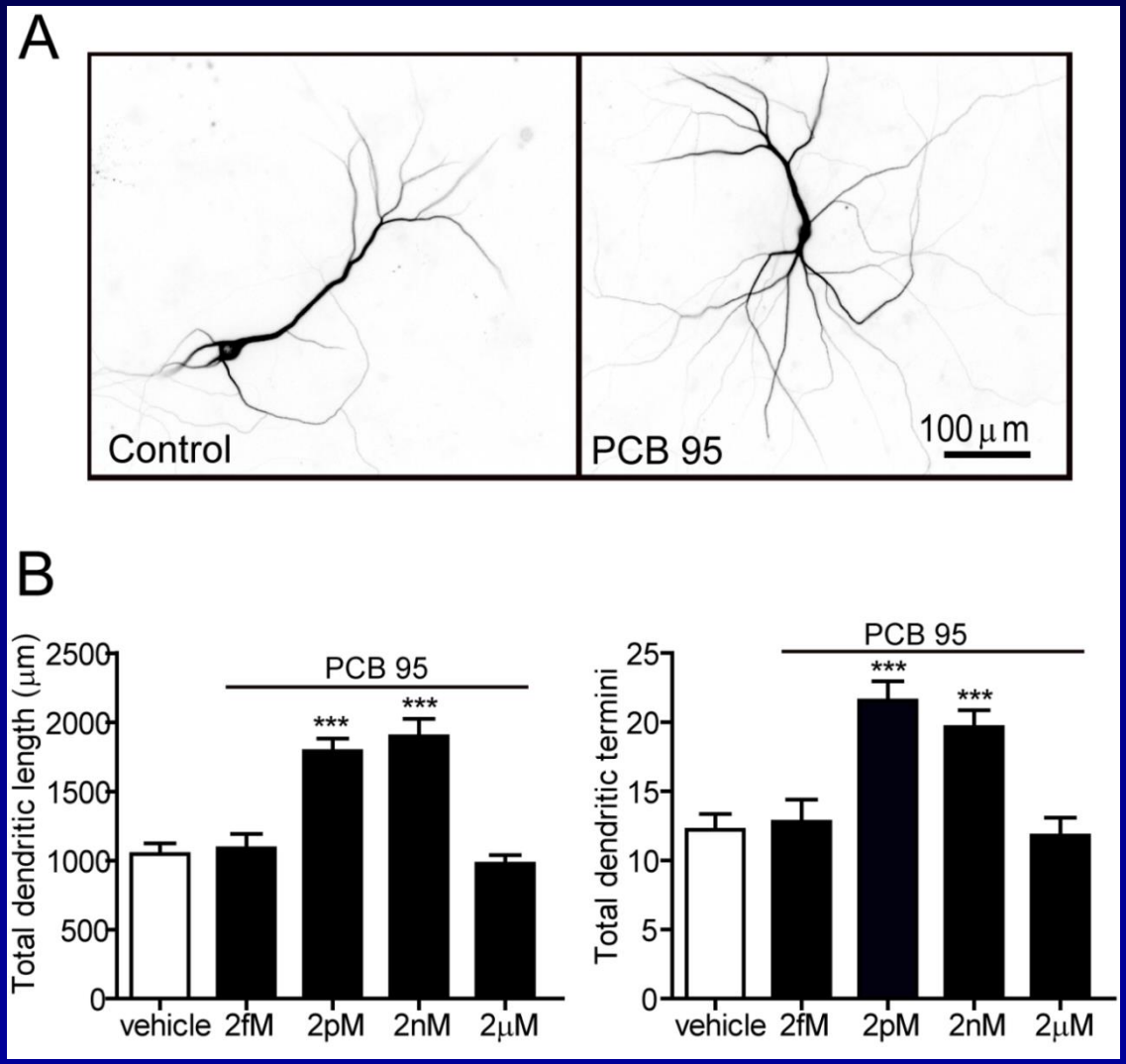
Conclusions from this study:

- **Developmental PCB exposure enhanced basal dendritic growth but decreased experience-dependent dendritic plasticity**
- **Effects of PCBs on dendritic arborization correlated with altered RyR expression**

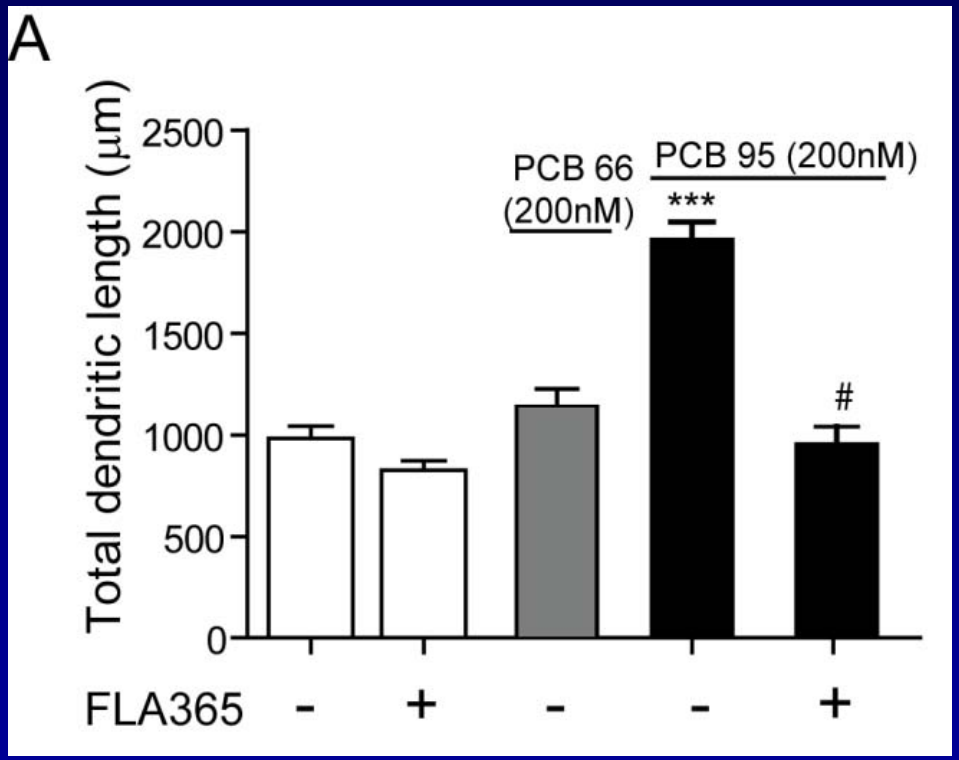
Hypothesis:

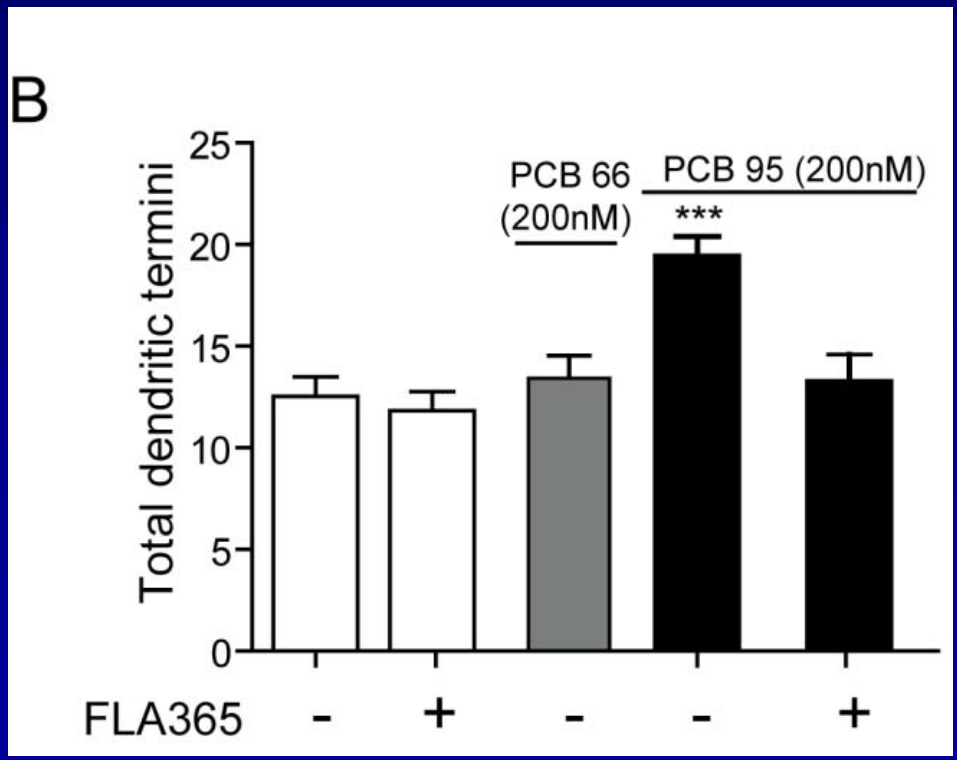
Non-coplanar PCBs disrupt neuronal connectivity via RyR-mediated mechanisms that modulate Ca^{2+}-dependent signaling pathways linked to activity-dependent dendritic growth and plasticity.

PCB 95 alters dendritic growth in primary cultures of hippocampal neurons

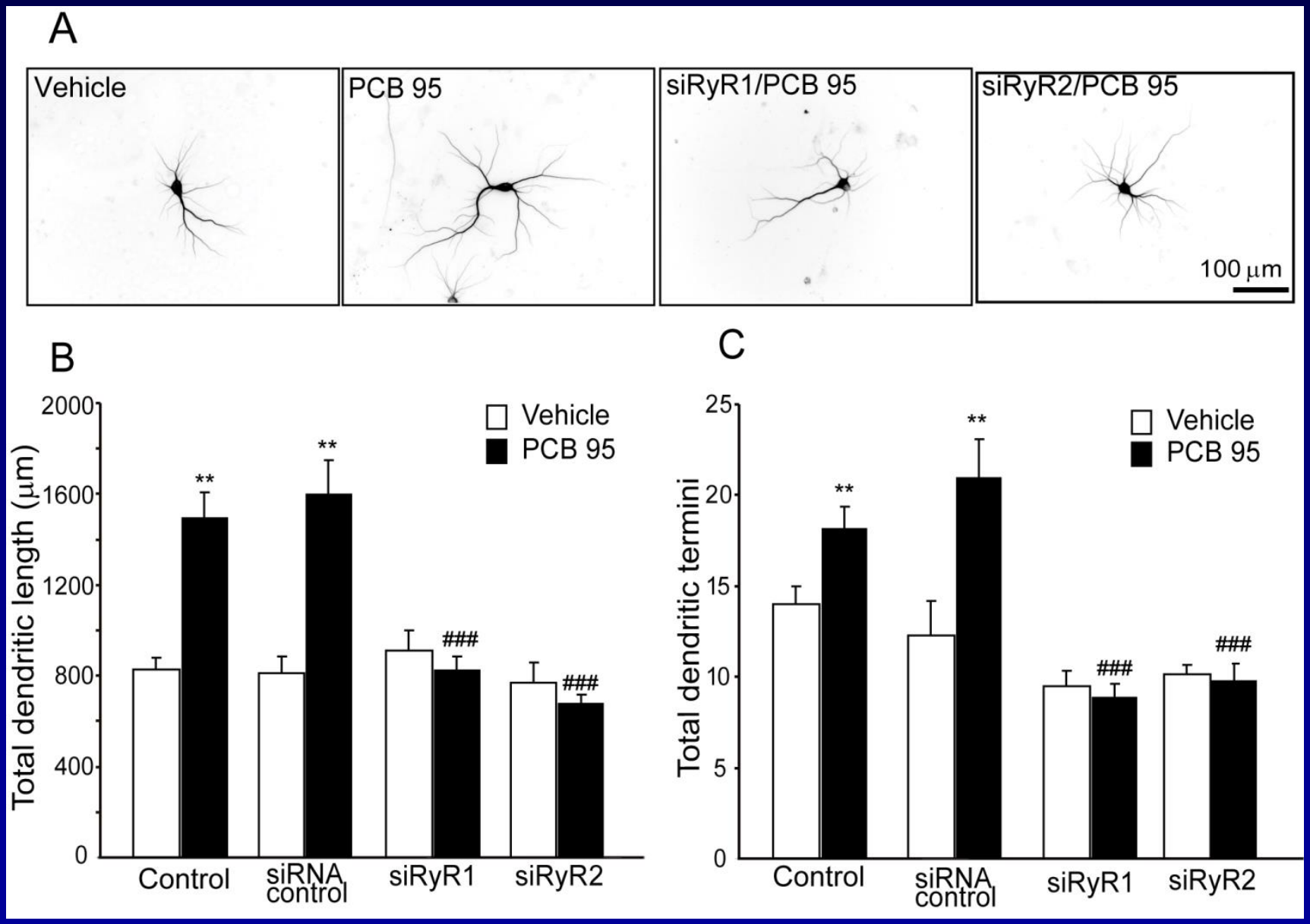


SAR and pharmacological RyR blockade suggest dendrite-promoting activity of PCBs is RyR-dependent

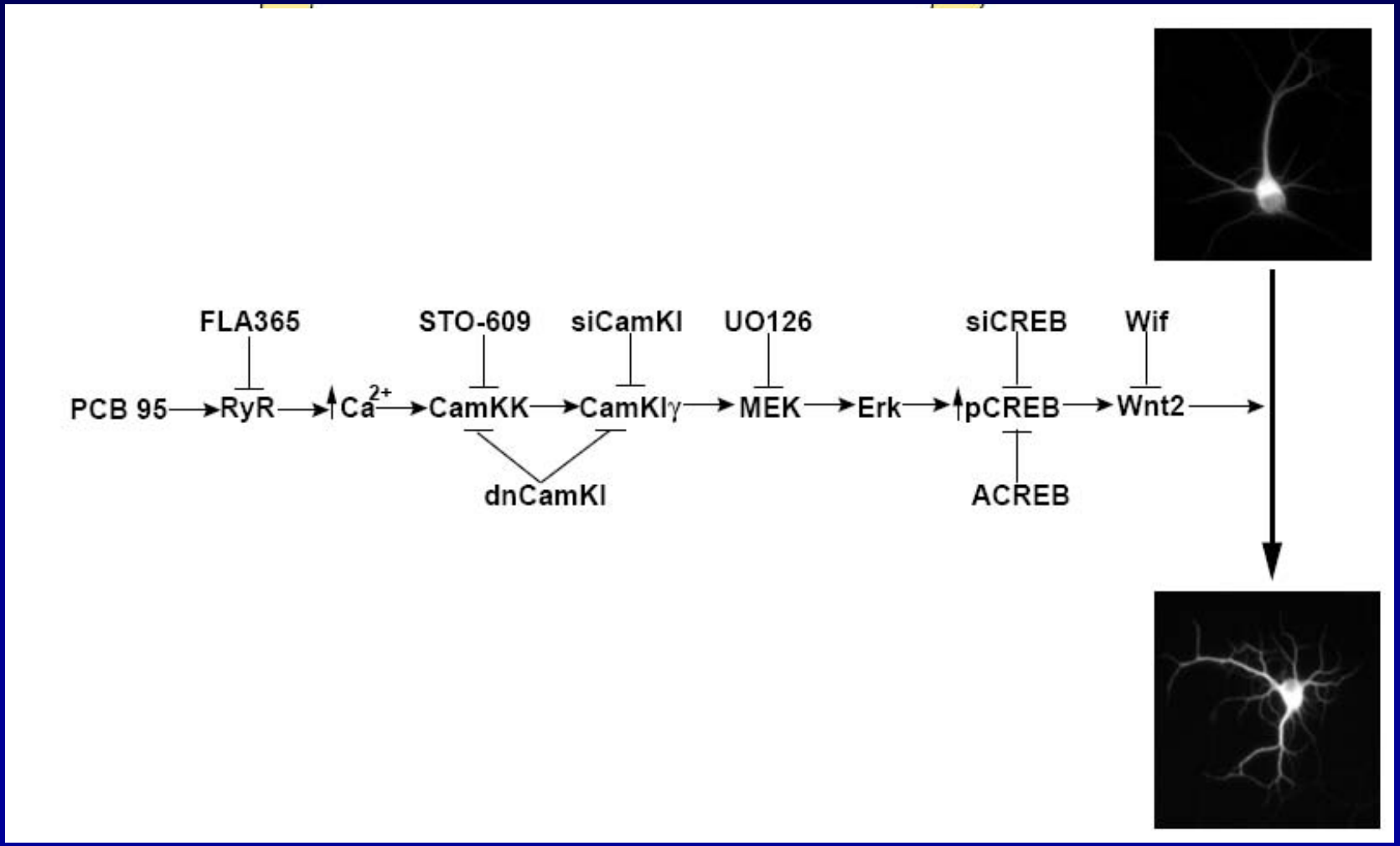




RyR activity required for PCB effects on dendrites

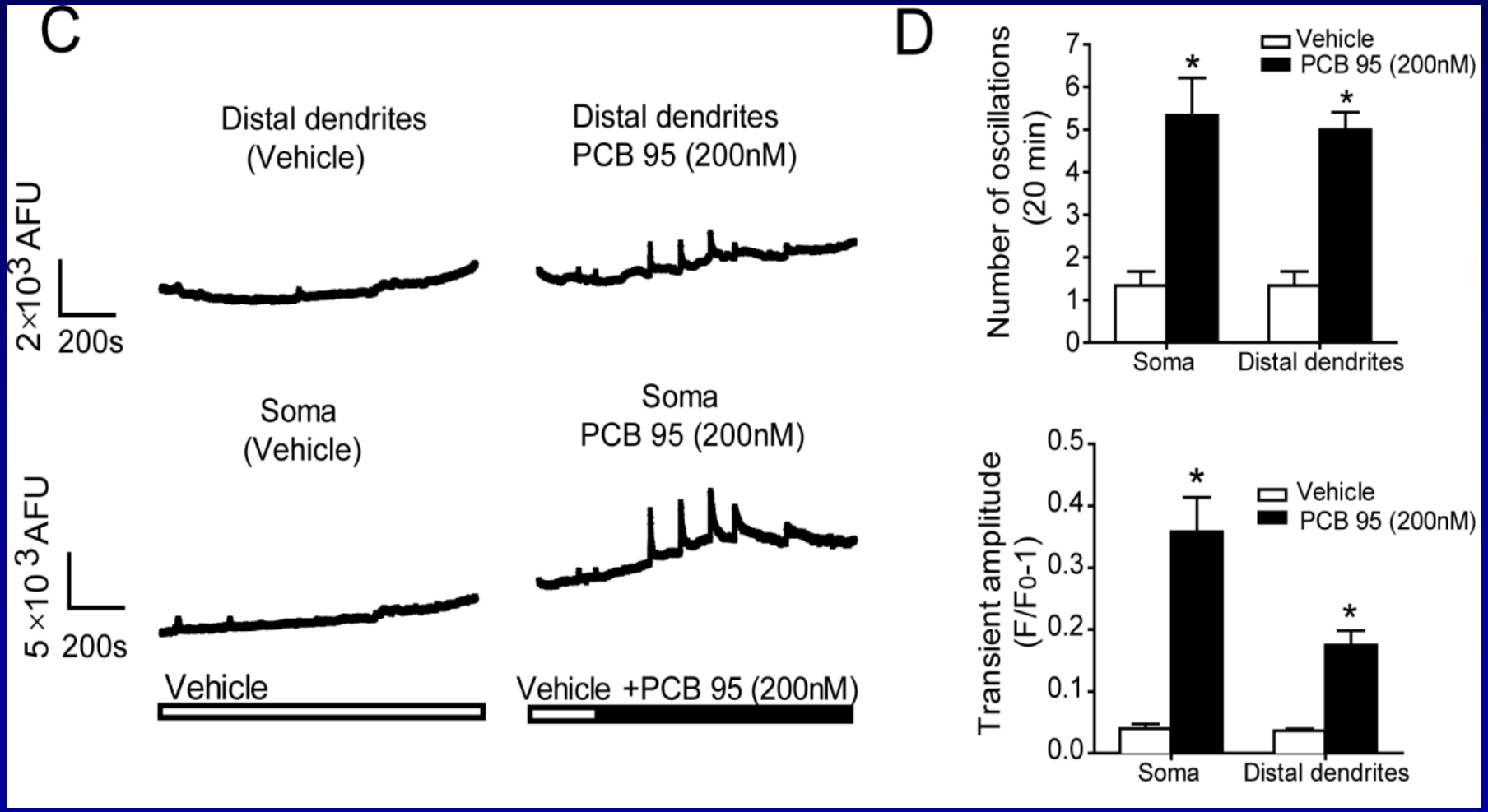


Experimental approaches for investigating Ca^{2+}-dependent signaling pathways in PCB-induced dendritic growth

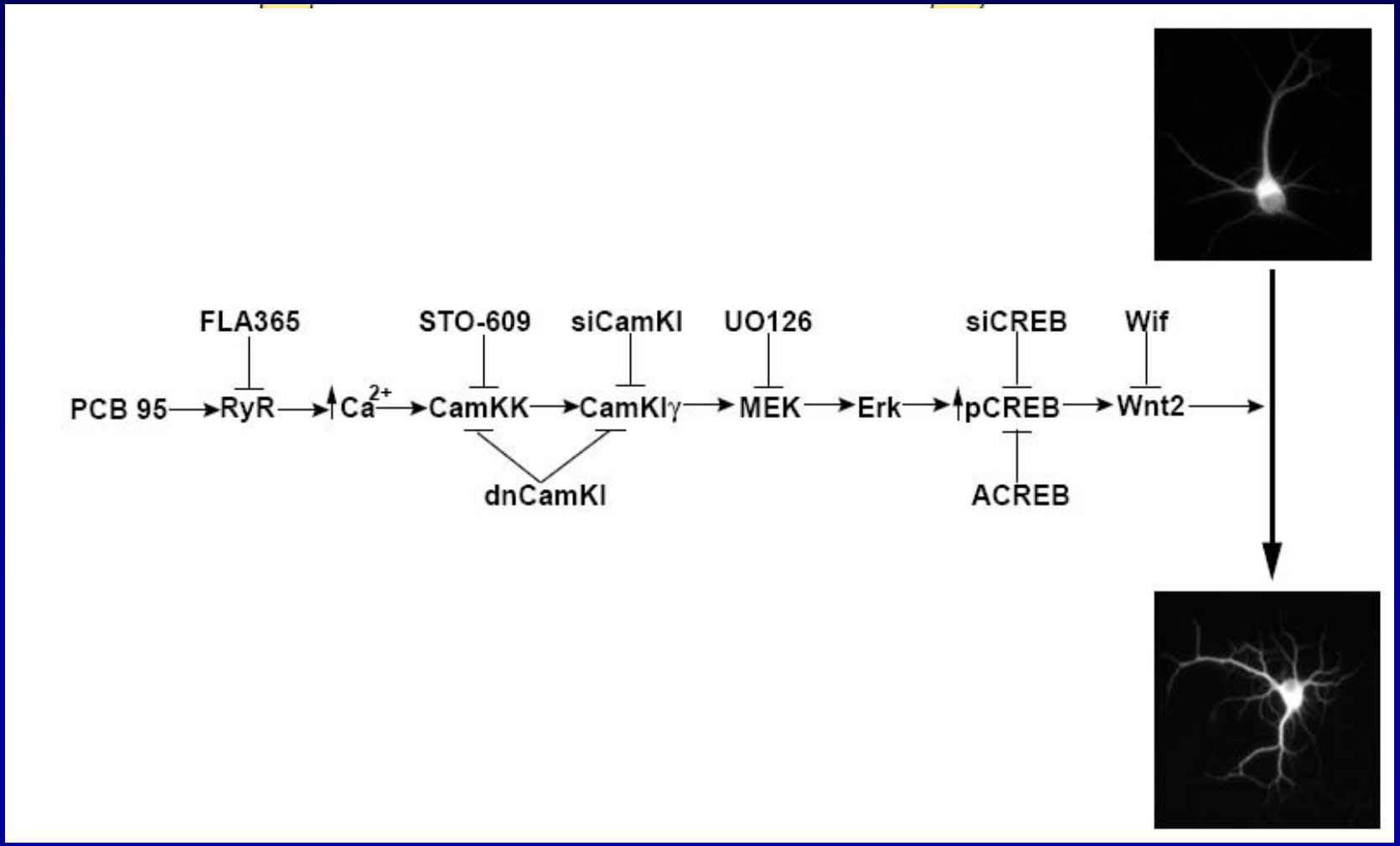


PCB 95 increases Ca^{2+} in primary cultured hippocampal neurons

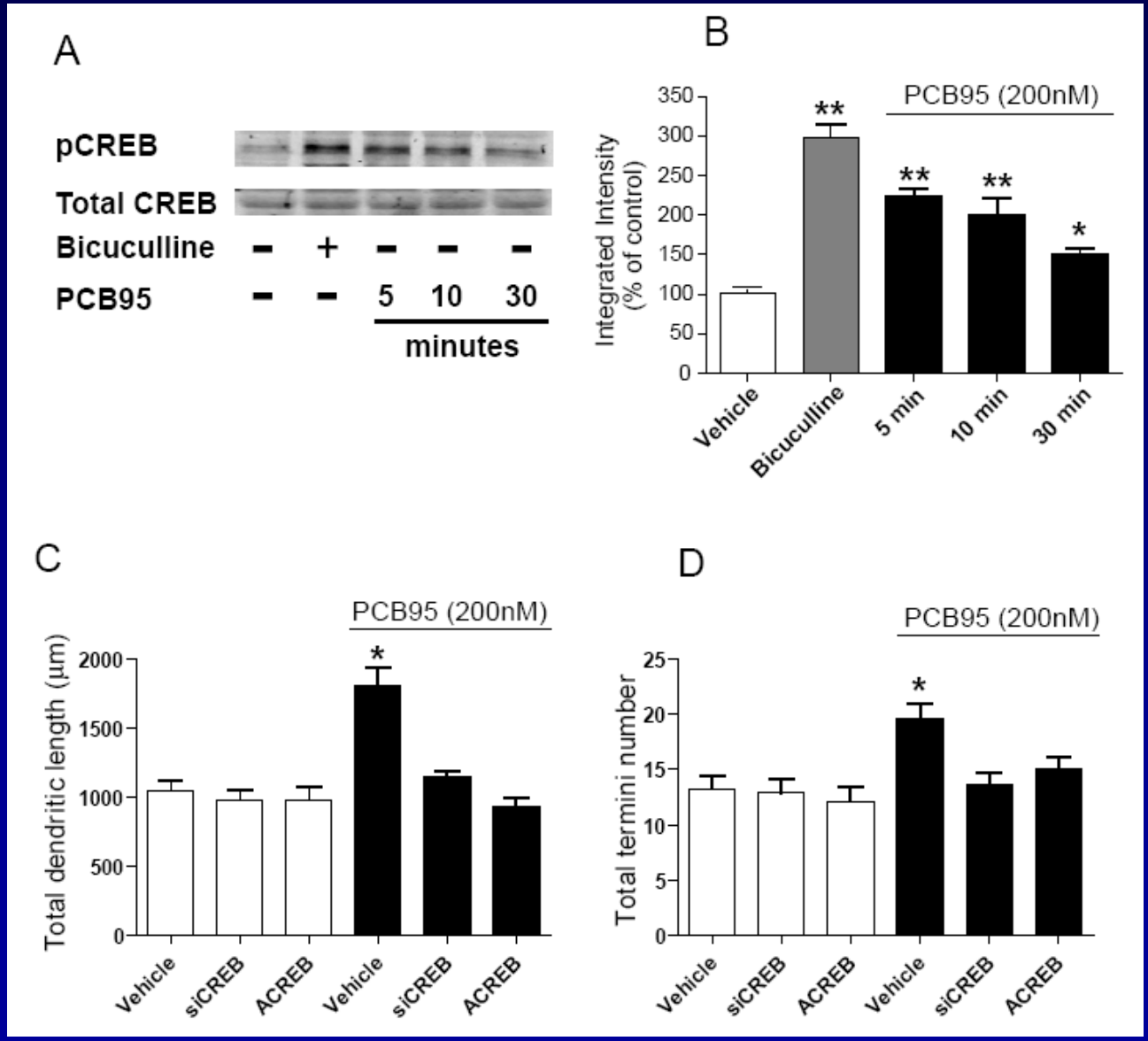
(data collected by Diptiman Bose, Pessah lab)



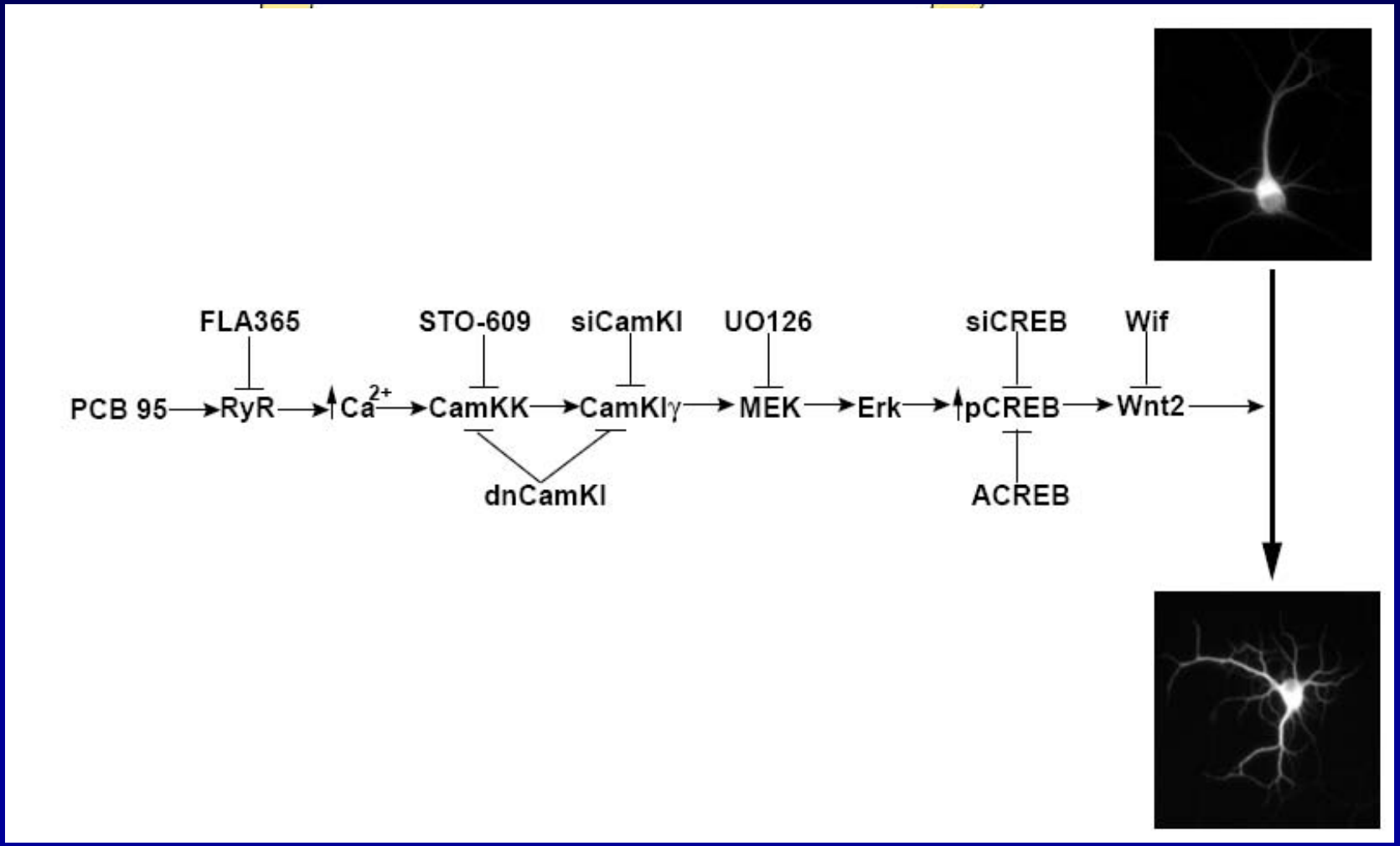
Experimental approaches for investigating Ca^{2+}-dependent signaling pathways in PCB-induced dendritic growth



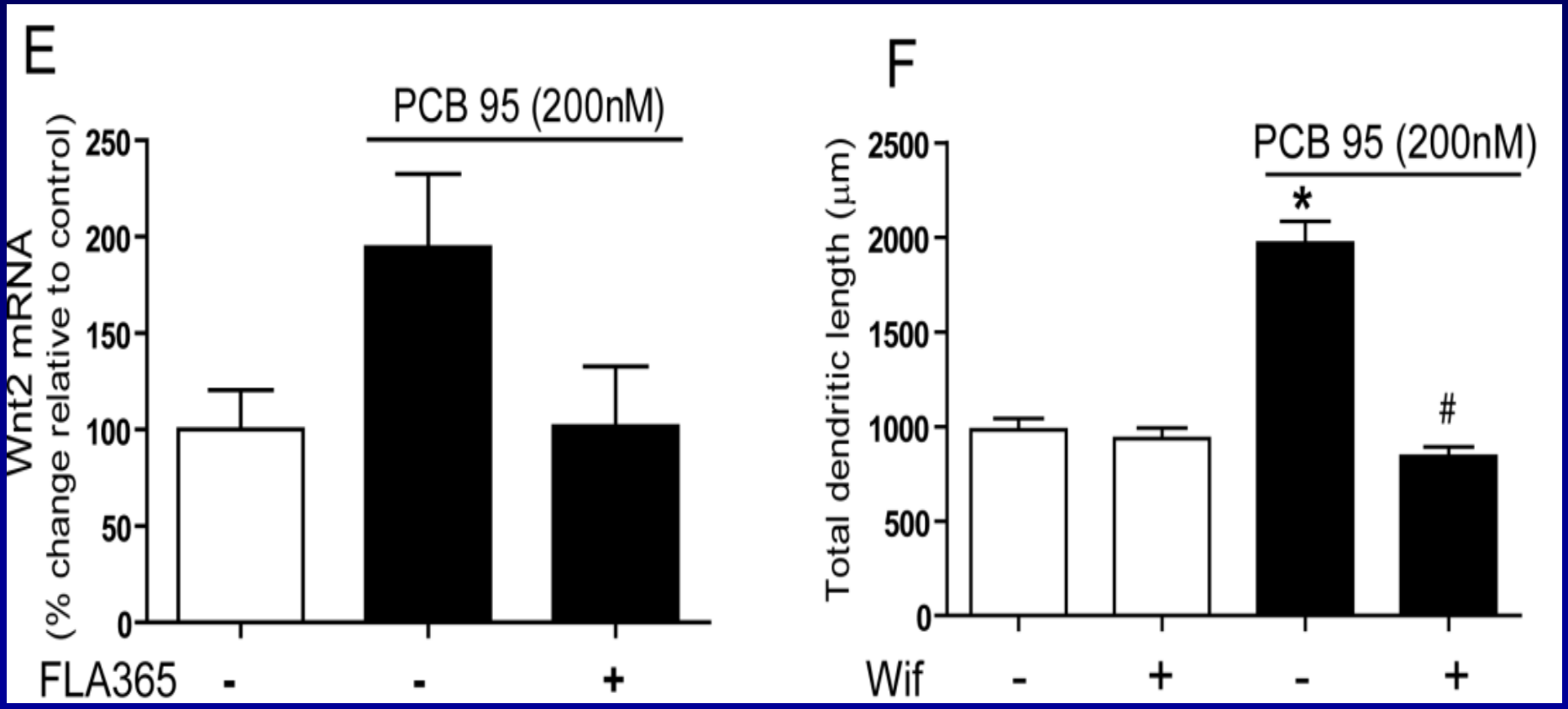
PCB-induced dendritic growth requires CREB activation



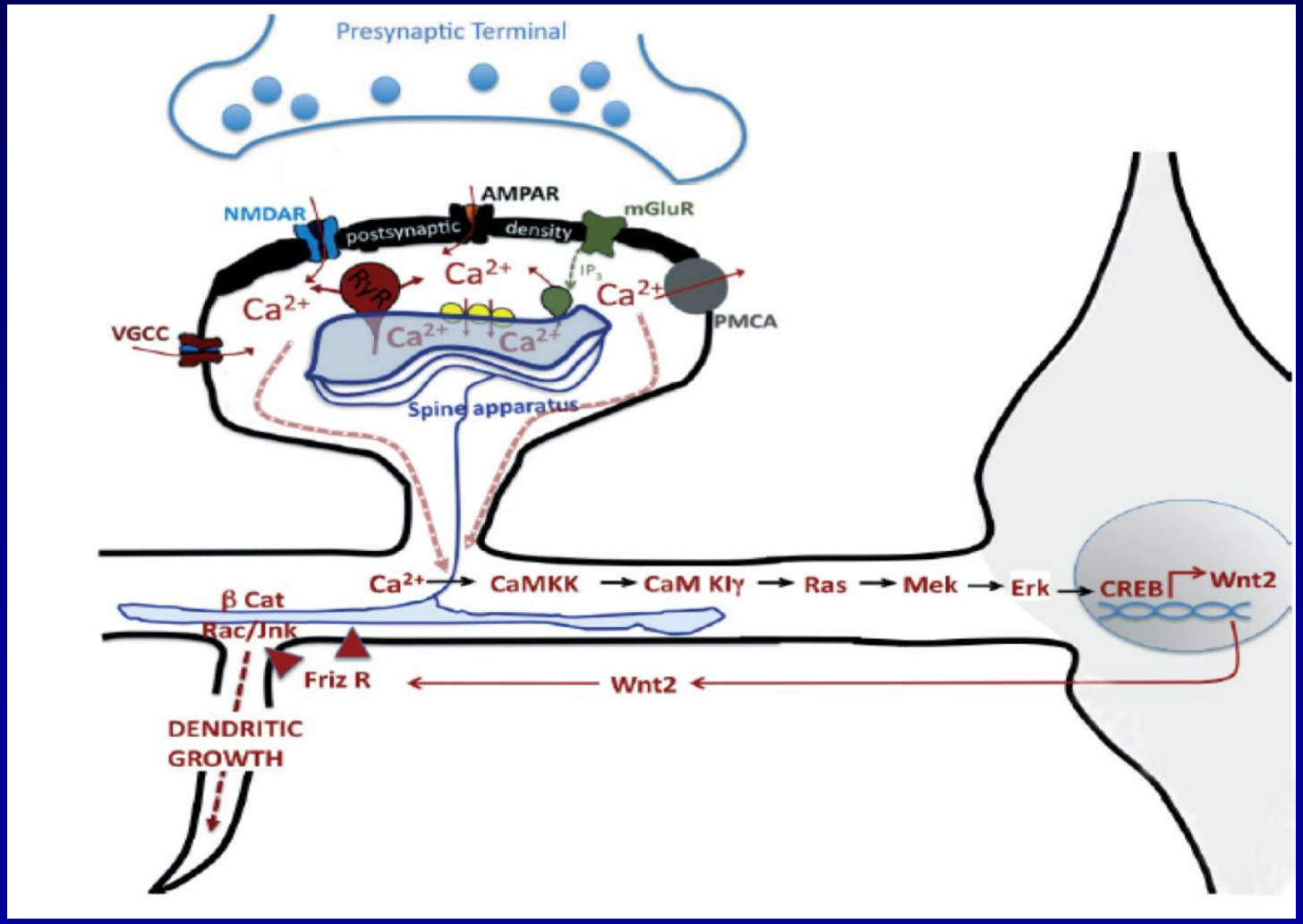
Experimental approaches for investigating Ca^{2+}-dependent signaling pathways in PCB-induced dendritic growth



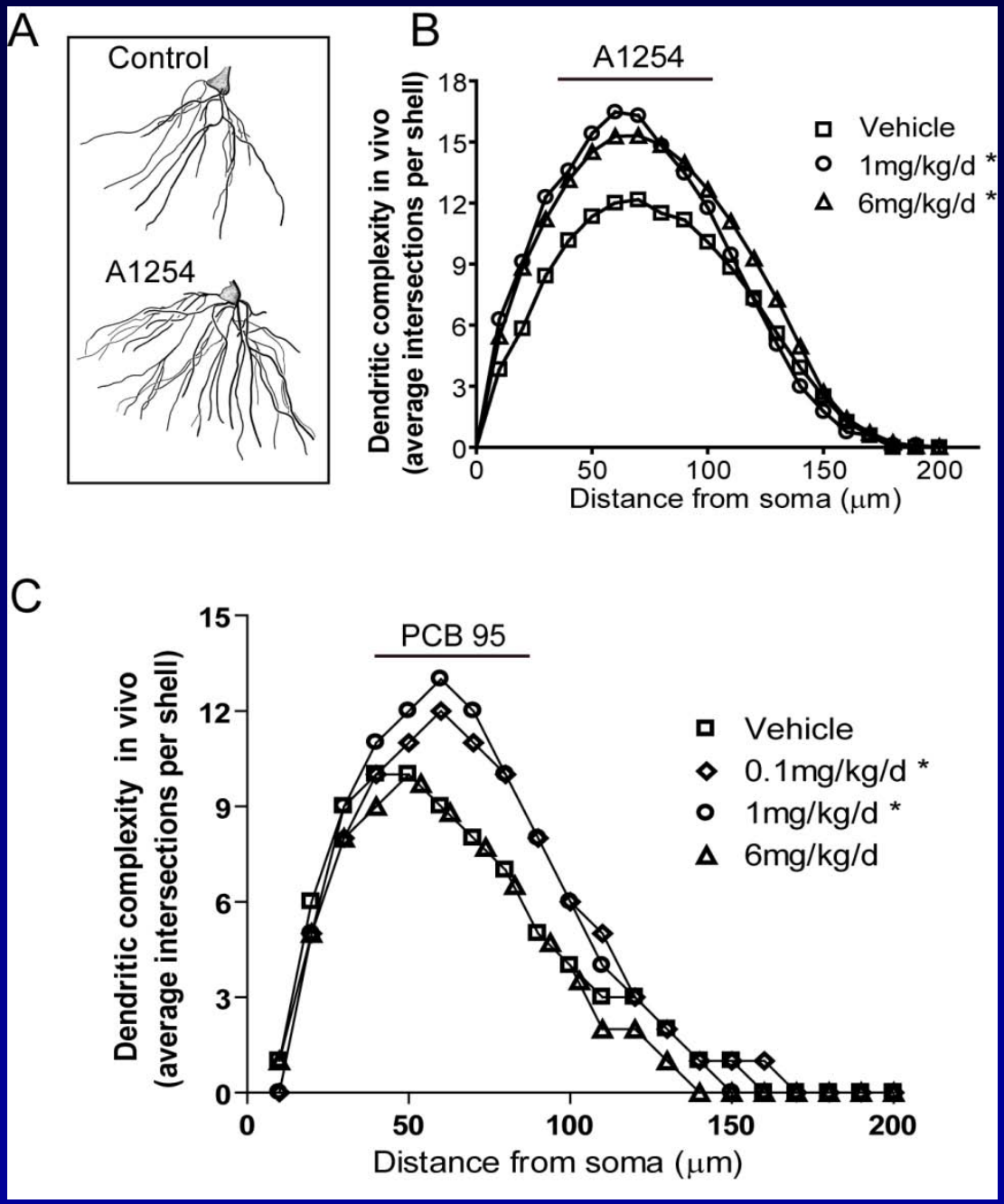
PCB-induced dendritic growth requires Wnt signaling



Non-dioxin-like PCBs hijack the signaling pathway that controls normal activity-dependent dendritic growth



Exposure of rat pups to PCBs in the maternal diet throughout gestation and lactation interferes with normal patterns of dendritic growth in the hippocampus of weanling rats



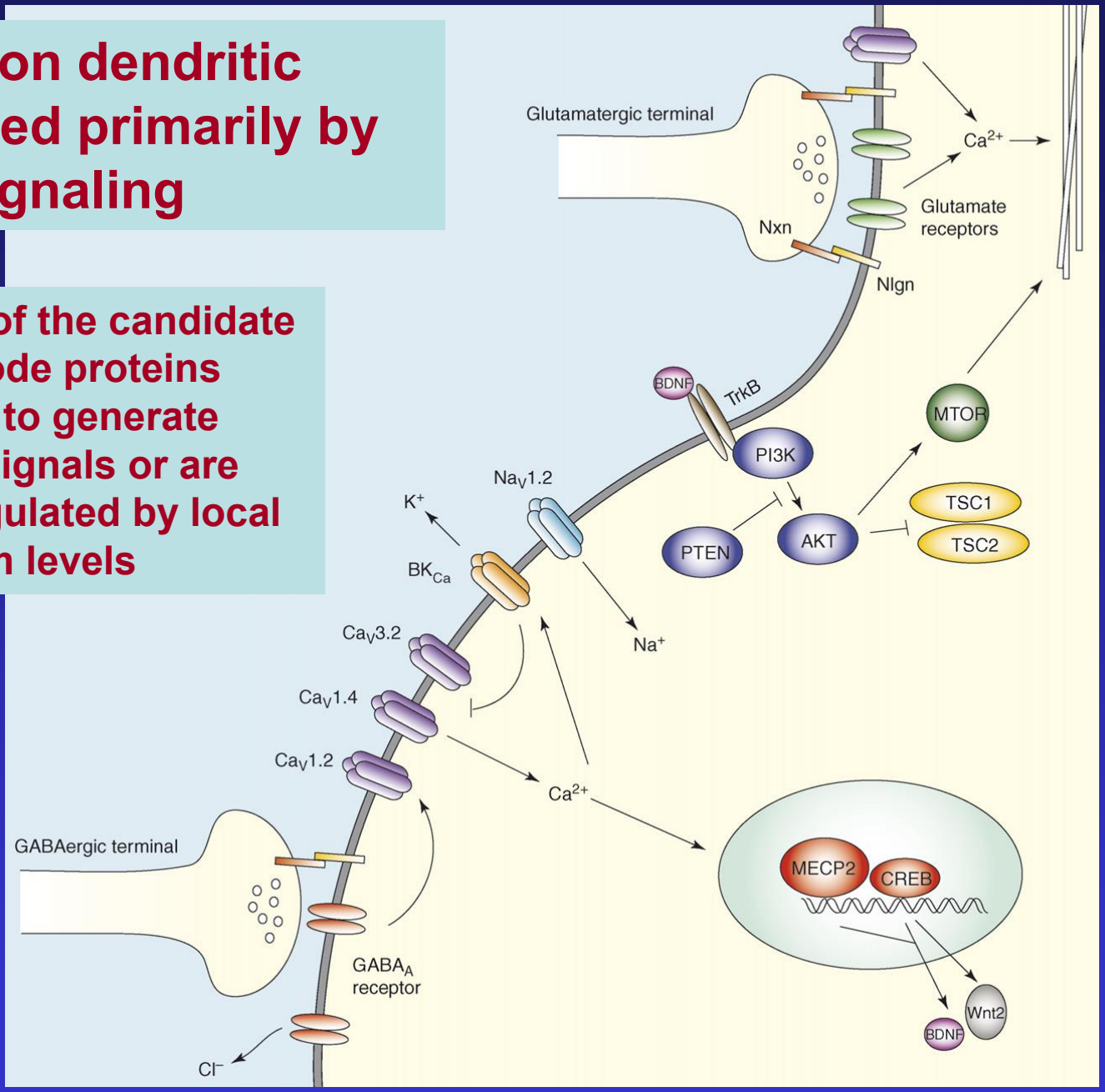
Are these findings relevant to ASD?

- **Increased dendritic arborization and altered plasticity are associated with ASD**

- **Experimental evidence suggests that developmental PCB exposure causes effects that mimic some aspects of ASD**
  - **Developmental exposure to PCB 95 causes an imbalance between excitation and inhibition in the auditory cortex of weanling rats (Kenet et al., 2007)**
  - **Perinatal exposure to a mixture of PCB 47 and 77 alters social behaviors in rats (Joulous-Jamshidi et al. 2010)**

Effects of activity on dendritic growth are mediated primarily by Ca^{2+}-dependent signaling

A significant number of the candidate genes for autism encode proteins whose primary role is to generate intracellular calcium signals or are themselves tightly regulated by local fluctuations in calcium levels



Krey and Dolmetsch (2007) Current Opinion in Neurobiology 17:112–119

How might non-dioxin-like PCBs influence ASD susceptibility?

environmental exposures *X* **genetic susceptibility** *X* **timing**

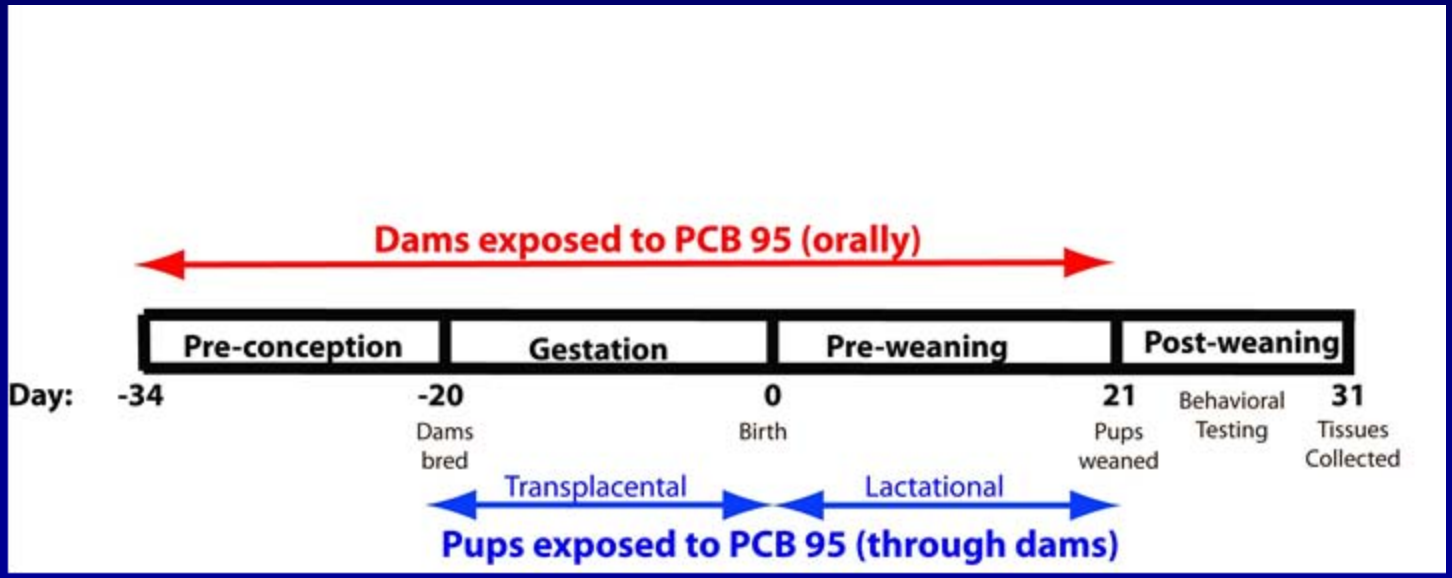
non-dioxin-like PCBs **heritable defects in Ca^{2+} signaling**

↓

risk, severity and treatment outcome

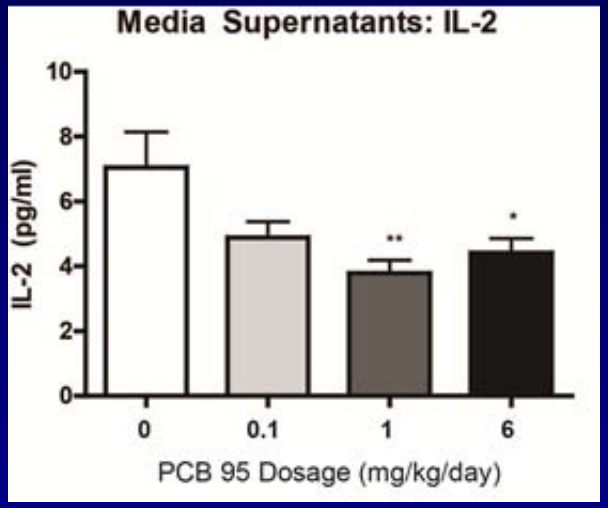
One fundamental way by which heritable genetic vulnerabilities could amplify adverse effects triggered by environmental exposures is if both factors (genes and environment) converge to dysregulate the same signaling system at critical times of development.

PCBs may also influence neurodevelopment via effects on the immune system

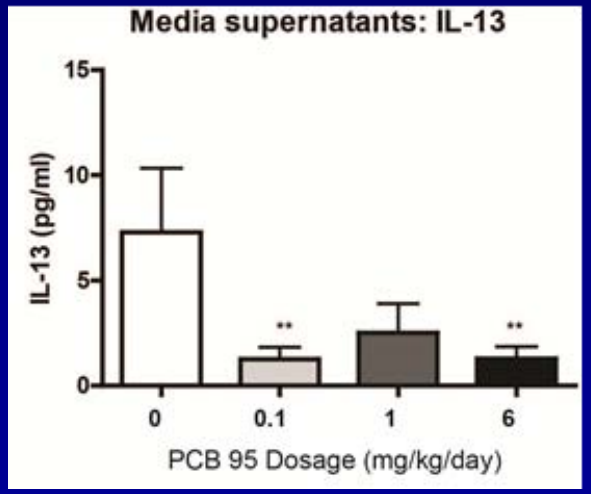


PCB 95 exposure decreases baseline immune activity

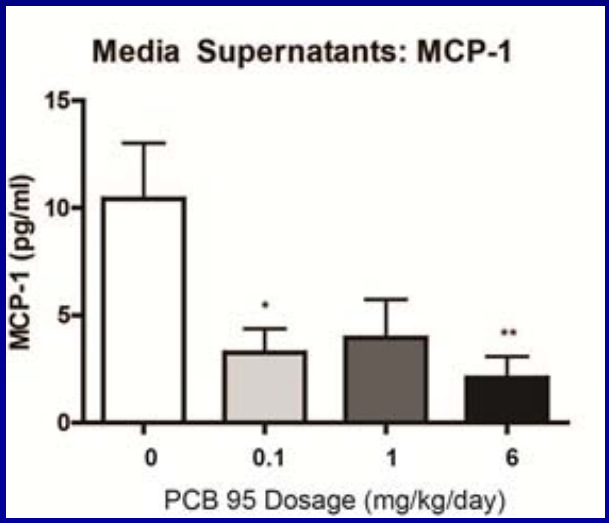
T cell growth factor



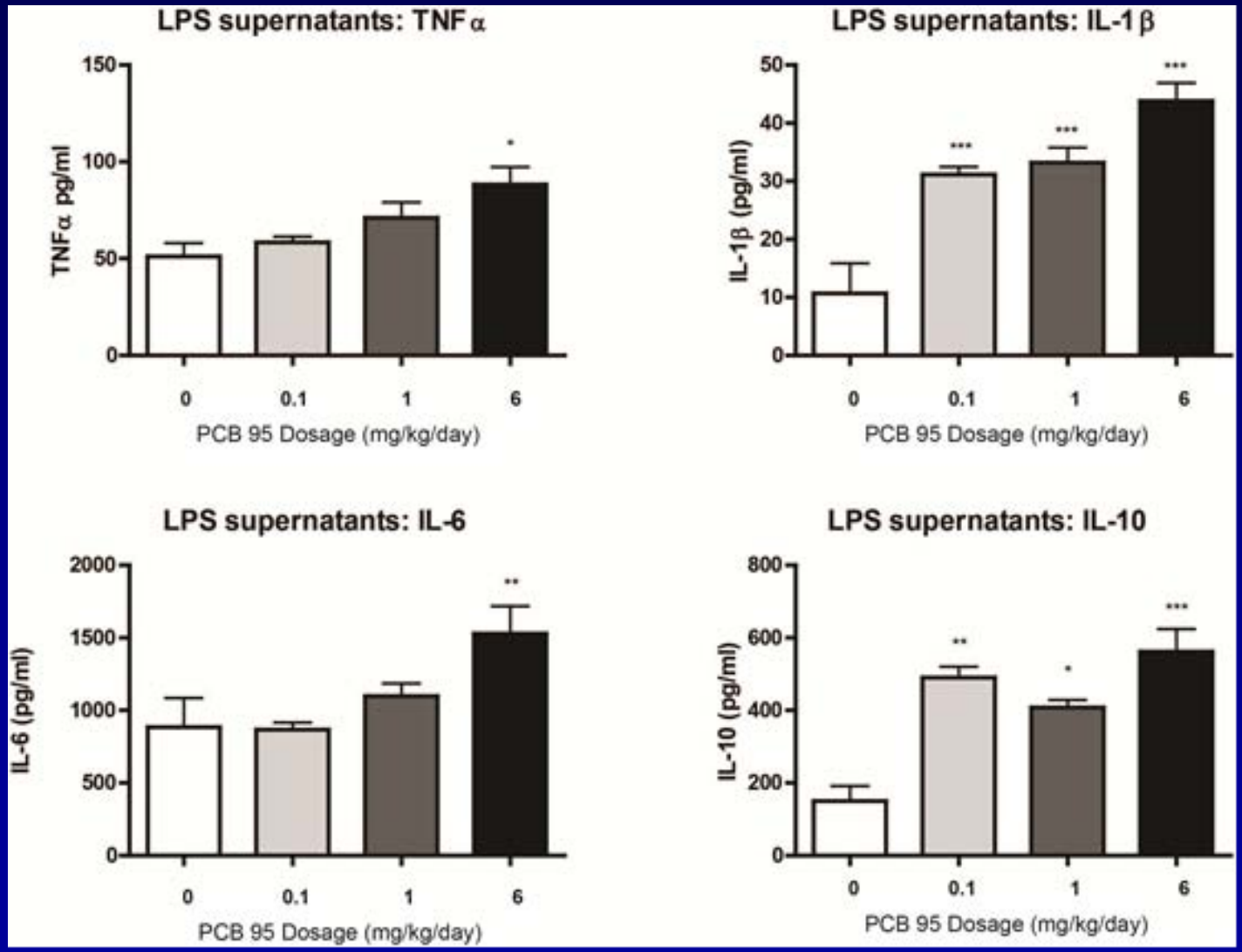
T cell cytokine



Chemokine



PCB 95 exposure increases innate immune activity



Do PCB effects on immune activity contribute to PCB effects on neurodevelopment?

Relevance to ASD?

Acknowledgements

Oksana Lockridge, University of Nebraska

Isaac Pessah, UC Davis

Gary Wayman, Washington State University

Lein Laboratory

Angela Howard

Dongren Yang

Donald Bruun

Christopher Barnhart

Funding Sources

CROET, OHSU

NIH

USEPA

M.I.N.D. Institute, UC Davis


FRAGILE X SYNDROME
TARGETED TREATMENTS AND AUTISM

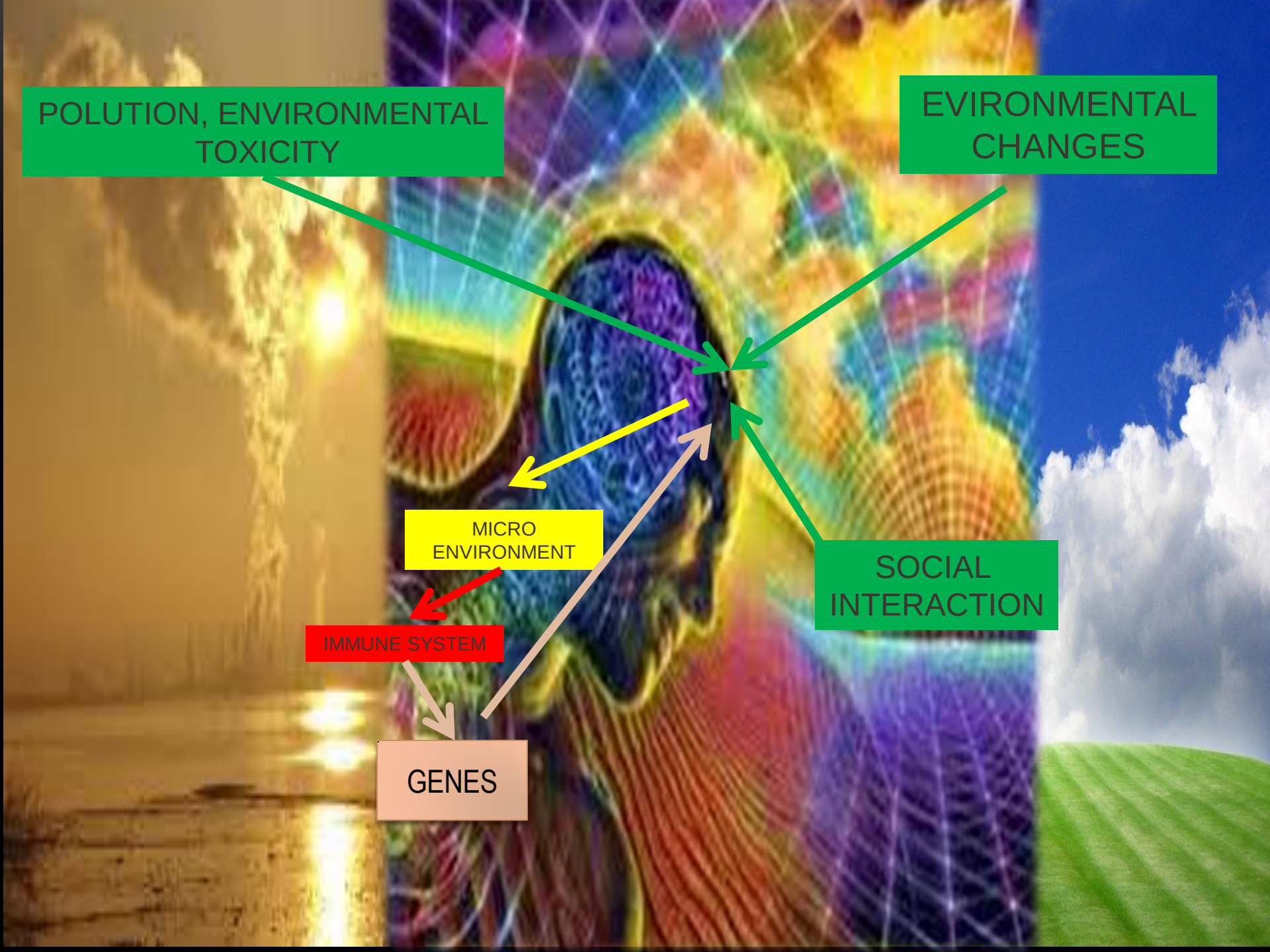
Reymundo Lozano MD

Fragile X Research

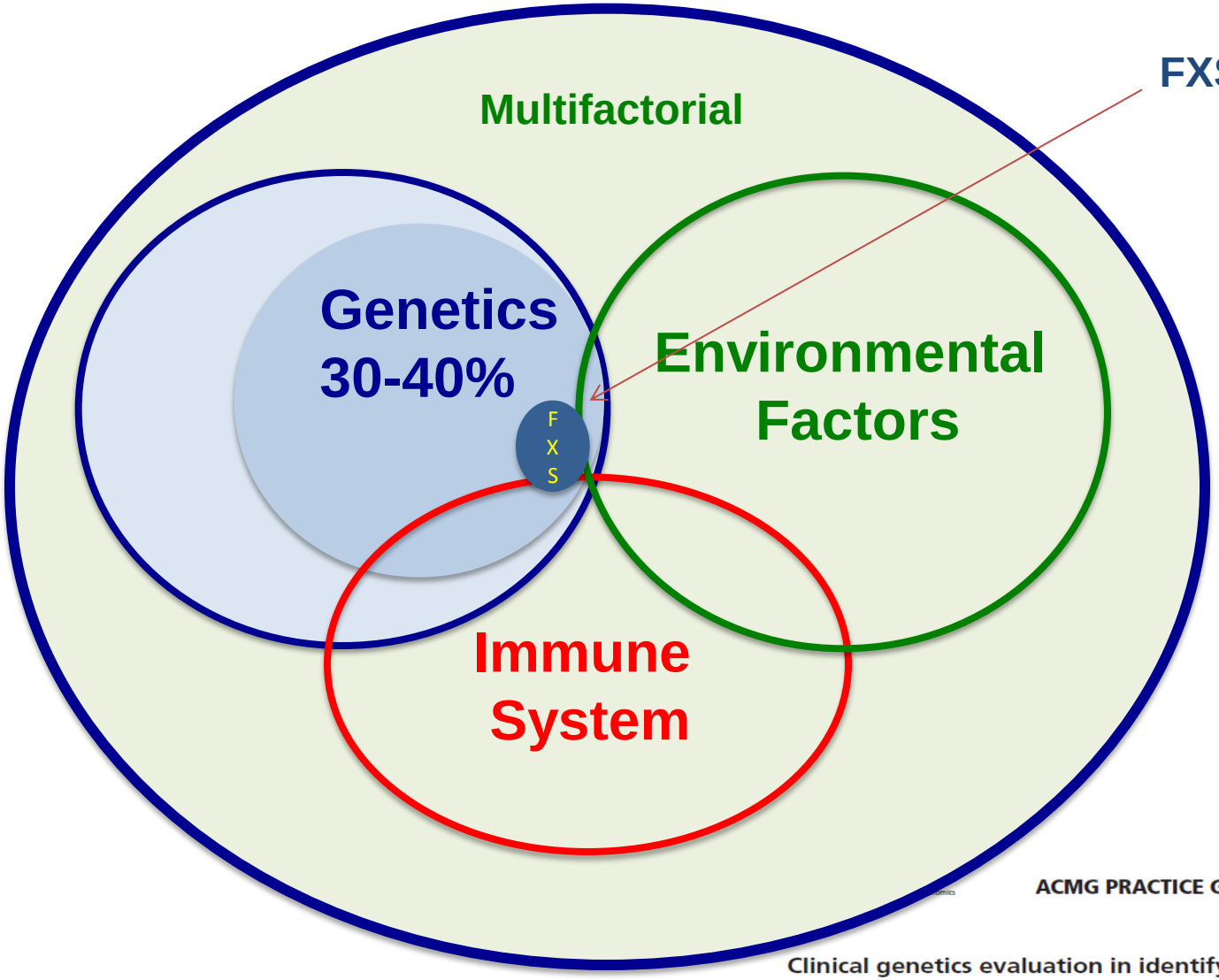
MIND Institute

University of California at Davis Medical Center

Genes and Autism. 06/01/13


POLUTION, ENVIRONMENTAL TOXICITY
EVIRONMENTAL CHANGES
MICRO ENVIRONMENT
SOCIAL INTERACTION
IMMUNE SYSTEM
GENES

Causes of ASD and Fragile X Syndrome

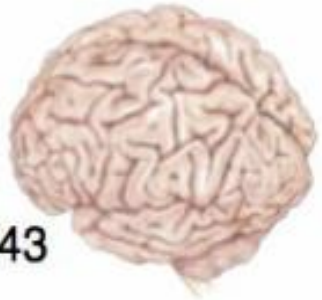


ACMG PRACTICE GUIDELINES | Genetics inMedicine

Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions

G. Bradley Schaefer, MD[1] and Nancy J. Mendelsohn, MD[2]; for the Professional Practice and Guidelines Committee

A new paradigm for psychopharm drug development in fragile X syndrome



1943

Martin-Bell (fragile X) syndrome

FMR1 gene silenced by CGG repeat

1991

Fmr1 knockout mouse

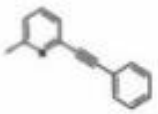
1994

Basic neurobiology

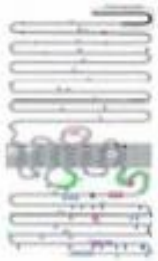
Excessive mGluR5 function

Rescue of *Fmr1* KO by reducing mGluR5

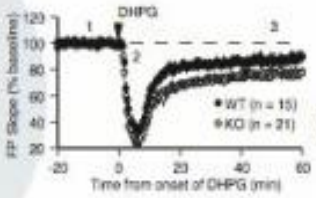
mGluR5 NAMs in phase 2 clinical trails



2010



2007



2002

A NEW AGE
TARGETED TREATMENTS

- Advances in the last 3 years or so have ushered in a new age of targeted treatments to reverse the neurobiological abnormalities for neurodevelopmental disorders

- Fragile X syndrome: mGluR5 antagonists, GABA agonists, minocycline, Arbaclofen

- Autism has similarities in GABA and glutamate imbalances, common pathways ie mTOR, miRNA dysregulation, mitochondrial abnormalities, oxidative stress, synaptic plasticity deficits and environmental toxicity

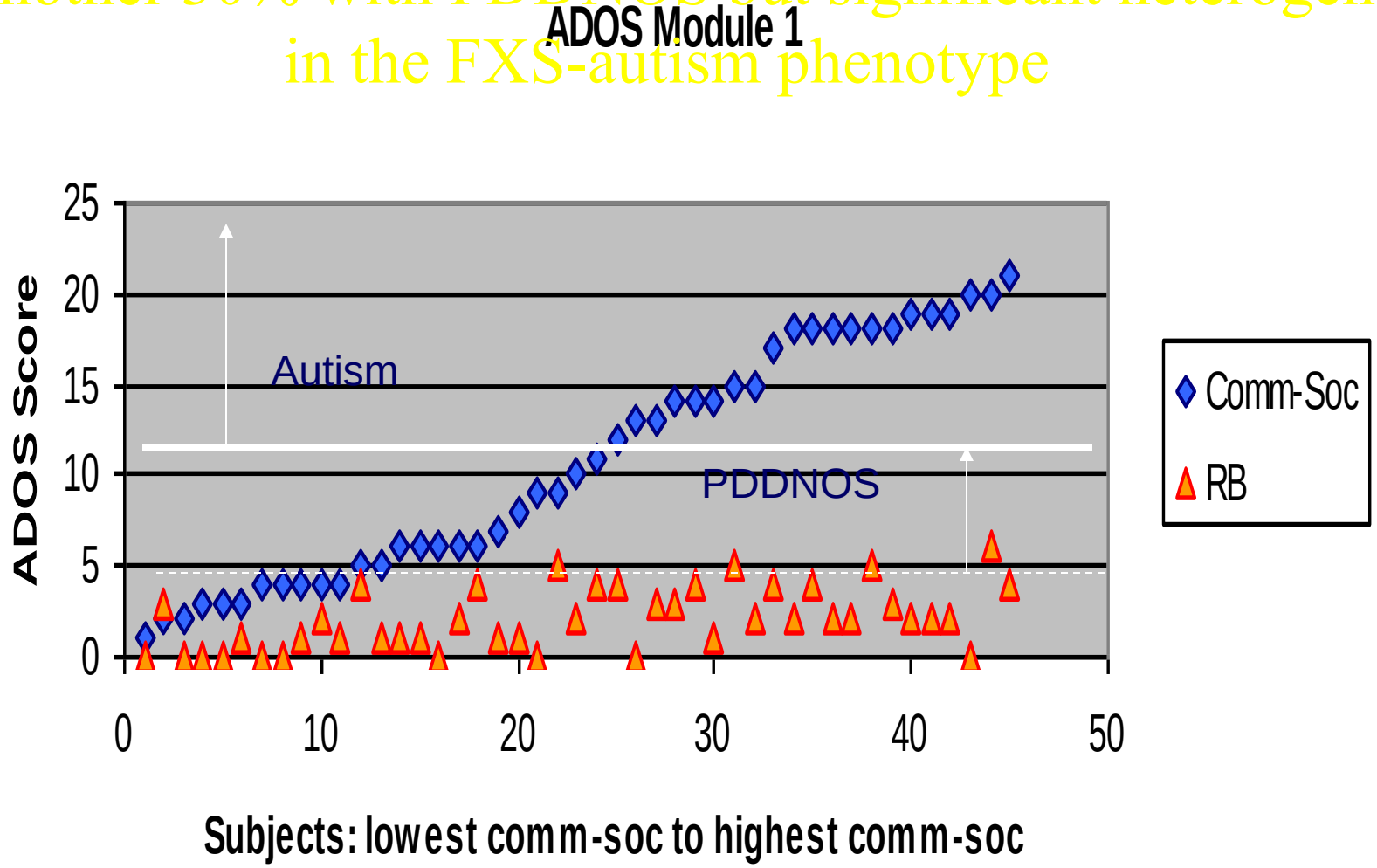
Targeted treatment research in other conditions

Condition	Animal model / Drug target	Drug / Effect in animal	Human trial
NF	*Nf1* mouse / increased RAS/ERK signaling	Statins / improved attention and spatial cognition	Possible improvement in spatial skills
Rett syndrome	*Mecp2* mouse / reduced BDNF signaling	IGF-1 fragment / rescue of lethality, neuropathology, autonomic abnormalities	Recruiting
Down syndrome	Ts65Dn mouse / excessive inhibitory neurotransmission	GABA-A negative modulators / improved cognition	Recruiting
Tuberous sclerosis (TSC2)	TSC2 mouse / elevated mTOR signaling	Rapamycin / improved spatial learning & contextual discrimination	Recruiting

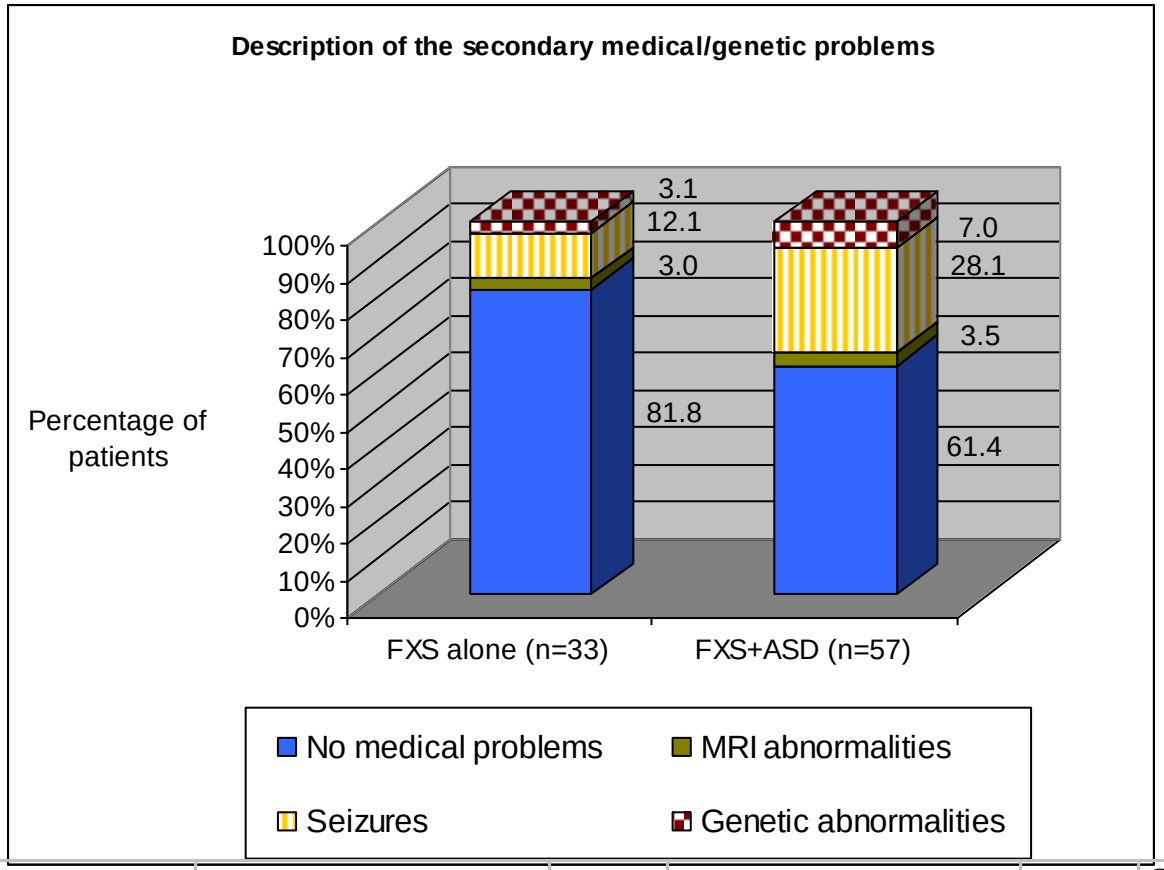
FRAGILE X AS A MODEL OF AUTISM

- Many children with FXS have autism (30%) or PDDNOS (30%) and many children with autism (2-6%) have fragile X
- Both disorders have big heads and rapid brain growth early in childhood
- FXS has problems with hyperarousal and anxiety so it models this subtype of autism
- Both disorders have problems with facial processing ie avoiding looking at the eyes which overactivates the amygdala
- Those with FXS and autism have lower IQ than FXS alone.
- FMRP regulates the translation of many genes associated with autism- latest estimate 30 % to almost 50% of autism genes (Darnell 2011 Cell; Iossifov et al 2012 Neuron)

Communication and Social Deficits are continuous in boys with FXS: 30% with autism and another 30% with PDDNOS but significant heterogeneity in the FXS-autism phenotype



Harris et al 2008 AJIDD



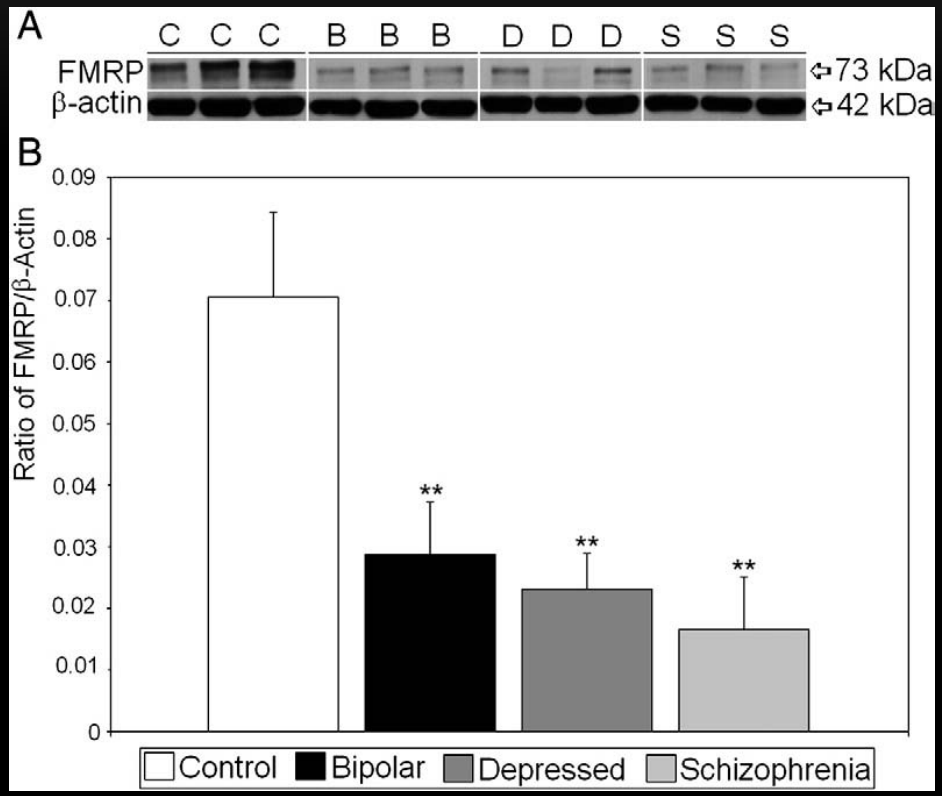
	FXS Alone (n = 33)			FXS+ ASD (n = 57)			Chi-Square Test (p-value)
	n	percentage		n	percentage		
Full mutation	19	57.6		37	64.9		.14
Mosaic	14	42.4		20	35.1		.18
No medical problems	27	81.8		35	61.4		.04
Seizures	4	12.1		16	28.1		.15
MRI Abnormalities	1	3.0		2	3.5		.90
Genetic Abnormalities	1	3.1		4	7.0		.43
Total Medical Problems	6	18.2		22	38.6		.04

Garcia-Nonell et al 2007

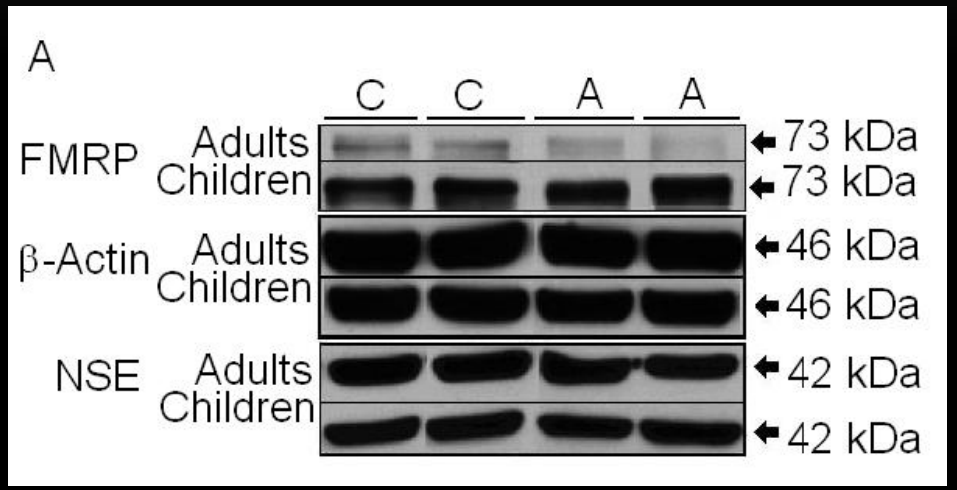
FMRP has many functions and its absence causes dysregulation of several systems known to be associated with autism

- Transporter of mRNAs to the synapse
- Controls (usually suppression of) translation of many mRNAs related to synaptic plasticity
- Absence of FMRP causes increased protein production throughout the brain
- Up regulation of mGluR5 pathways leading to LTD
- Down regulation of $GABA_A$ receptors
- Dysregulation of dopamine pathways
- Enhanced APP production
- Increased oxidative stress damage to neurons

LOWERED BRAIN FMRP LEVELS IN PSYCHIATRIC DISORDERS

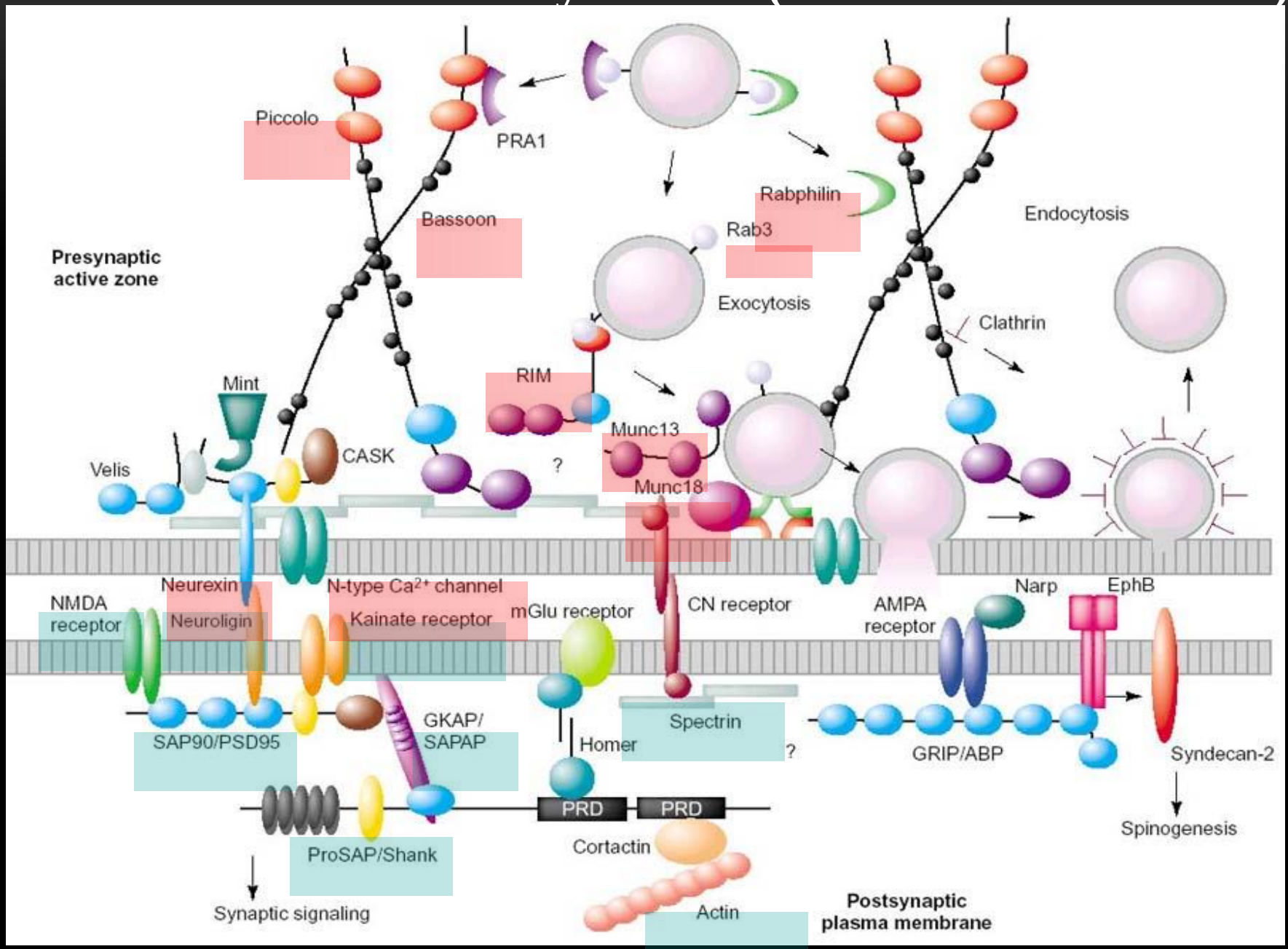


FMRP lower in adult autism brains

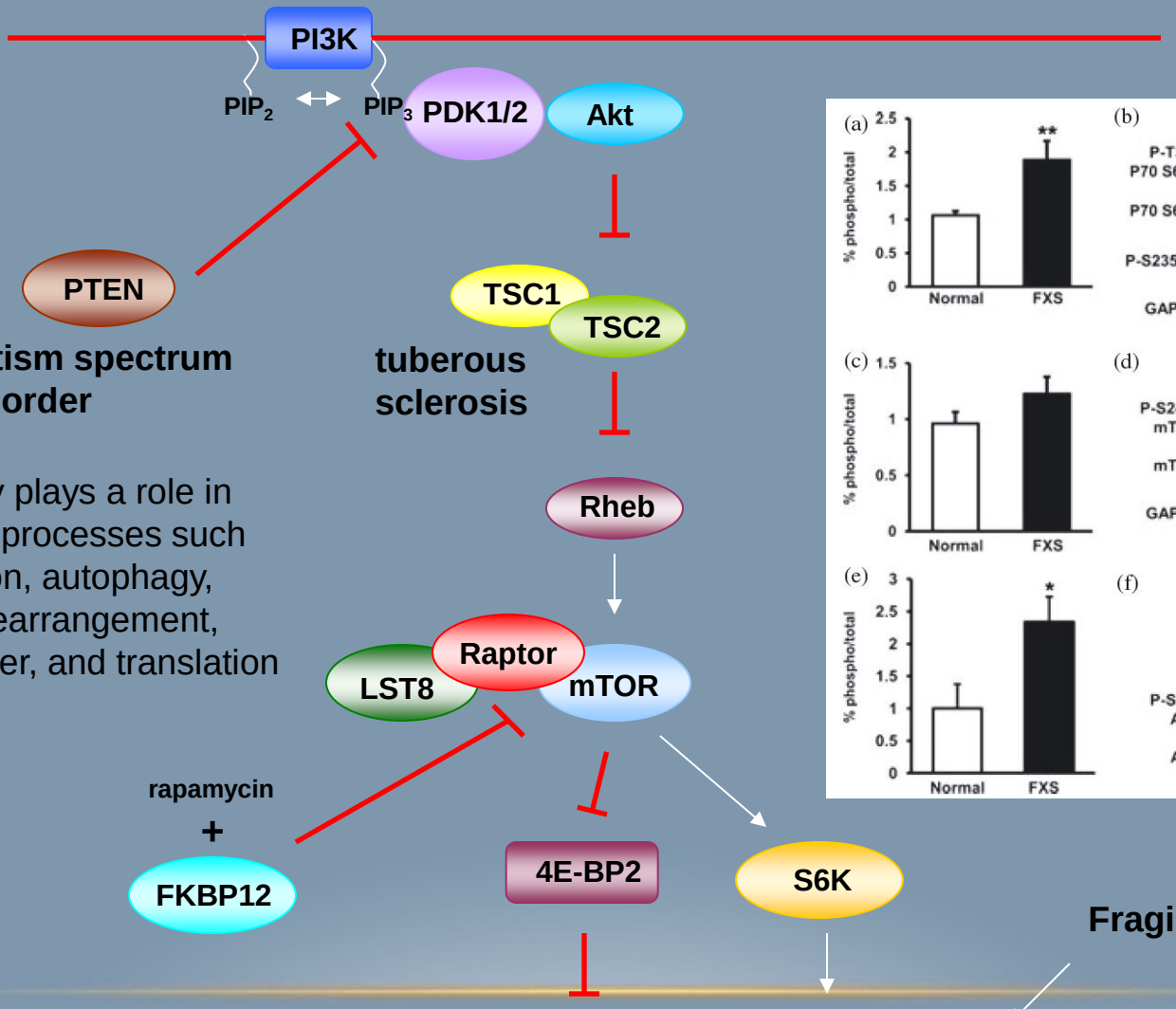


Fatemi et al schizophrenia research 2010

Proteins Controlled by FMRP (Darnell et al 2011 Cell)



Regulation of mTOR Signaling



mTOR activity plays a role in many cellular processes such as transcription, autophagy, cytoskeletal rearrangement, protein turnover, and translation initiation

Hoeffer et al., 2012

Fragile X Syndrome

Fragile site

- 1 in 3,600
- Leading inherited of ID leading
- Single gene associated with autism
- 2-6% with autism have FXS
- Anxiety disorders, mood instability. ..

FRAGILE X SYNDROME AND THE EXPRESSION OF THE FMR1 GENE

FXS is the most common form of intellectual disabilities and the leading known heritable form of autism

. . . is caused by a large CGG-repeat expansion in a non-coding portion of the FMR1 gene

Fragile site
Xq27.3

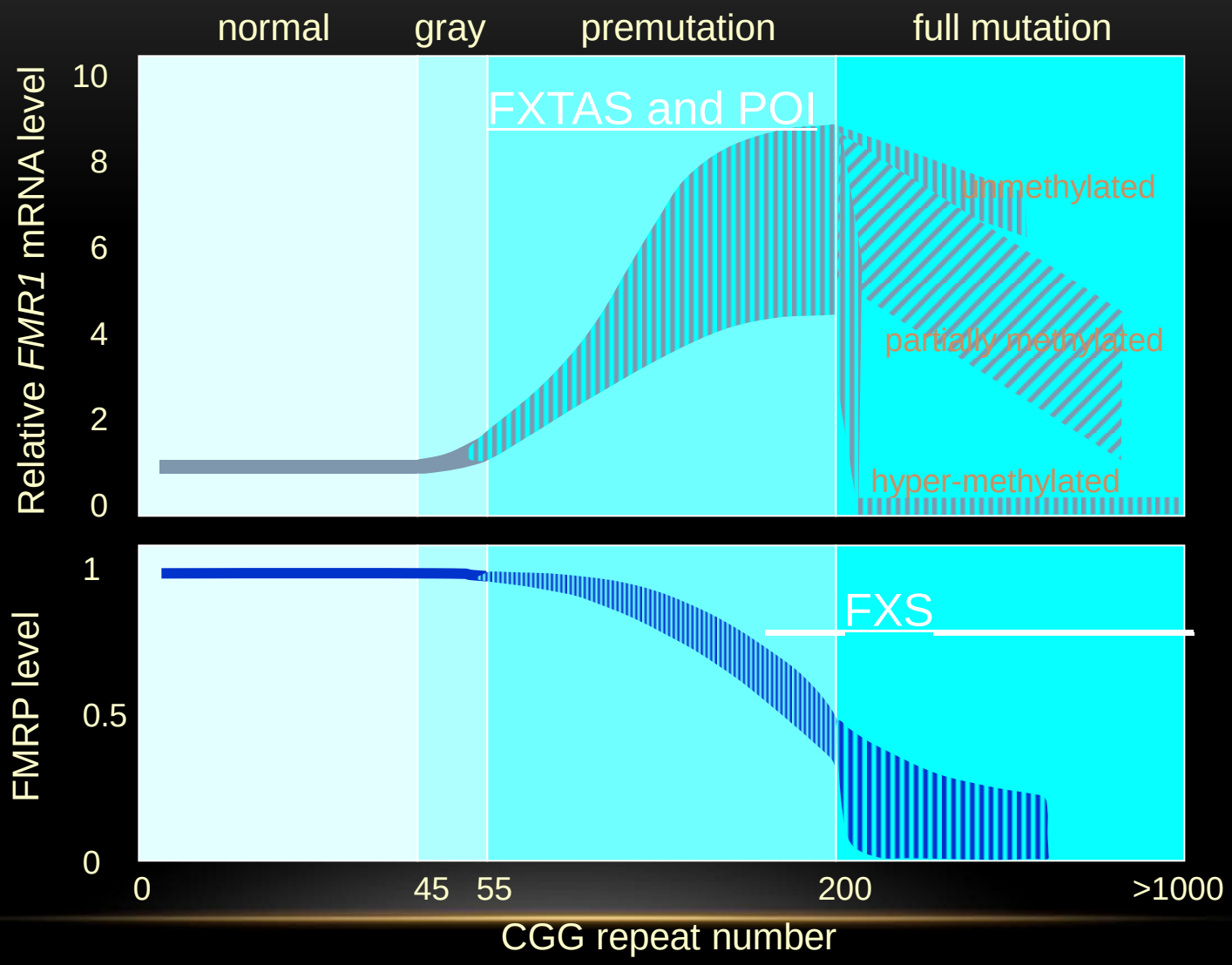
	Typical	Premutation	Full
DNA	(CGG) < 45	(CGG) 55 - 200	(CGG) > 200
mRNA		Gray/intermediate alleles 45-54 CGG	
FMRP	Normal		
Clinical		Primary ovarian insufficiency (FXPOI) Tremor/ataxia syndrome (FXTAS)	Fragile X syndrome

1/130-250 females

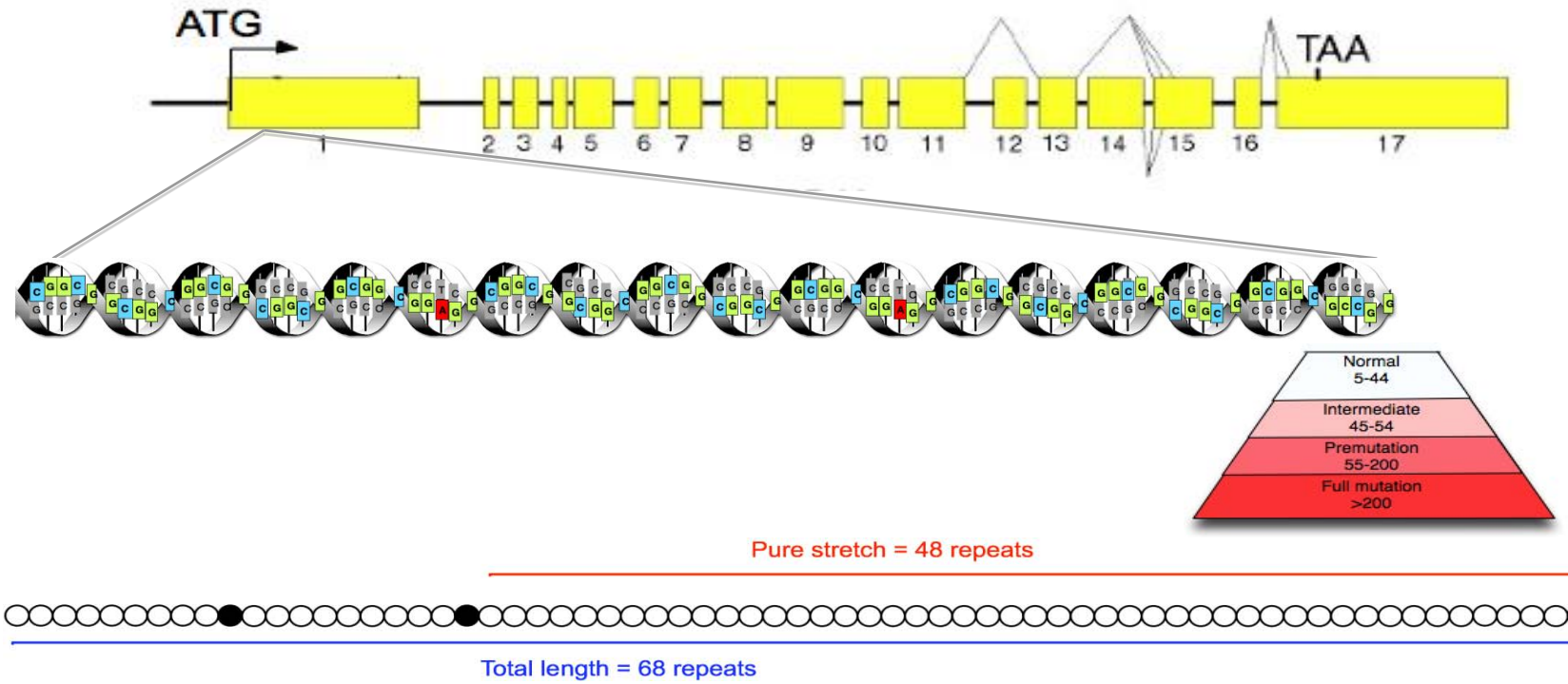
1/250-810 males

1/3600-4000

Expression of the *FMR1* gene



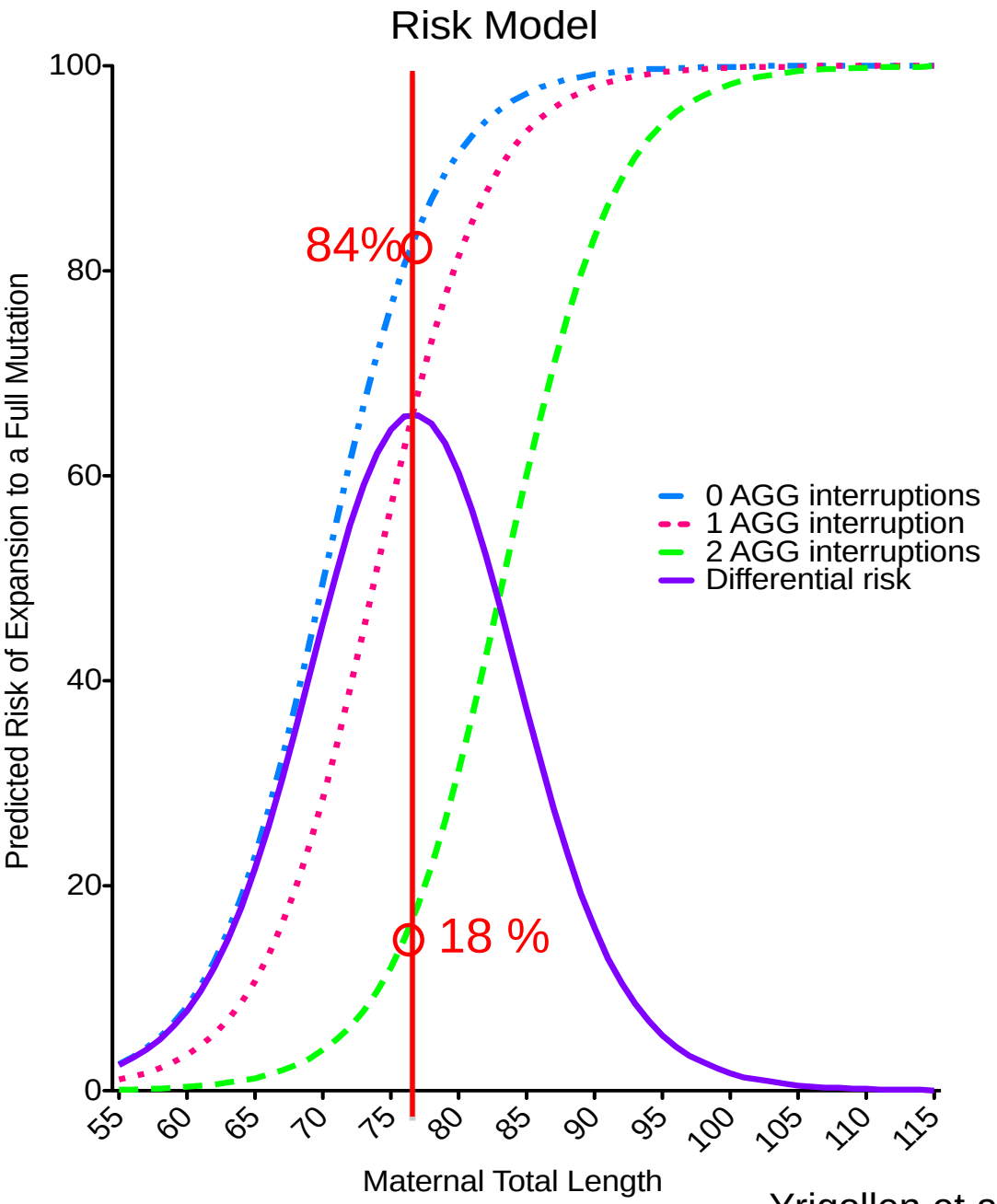
The *FMR1* Gene



AGG interruptions are normally present in normal CGG repeats. 0, 1, 2, or 3 interruptions are common.

They typically occur around 9-10 CGG repeats.

(Eichler et al. 1994; 1996)

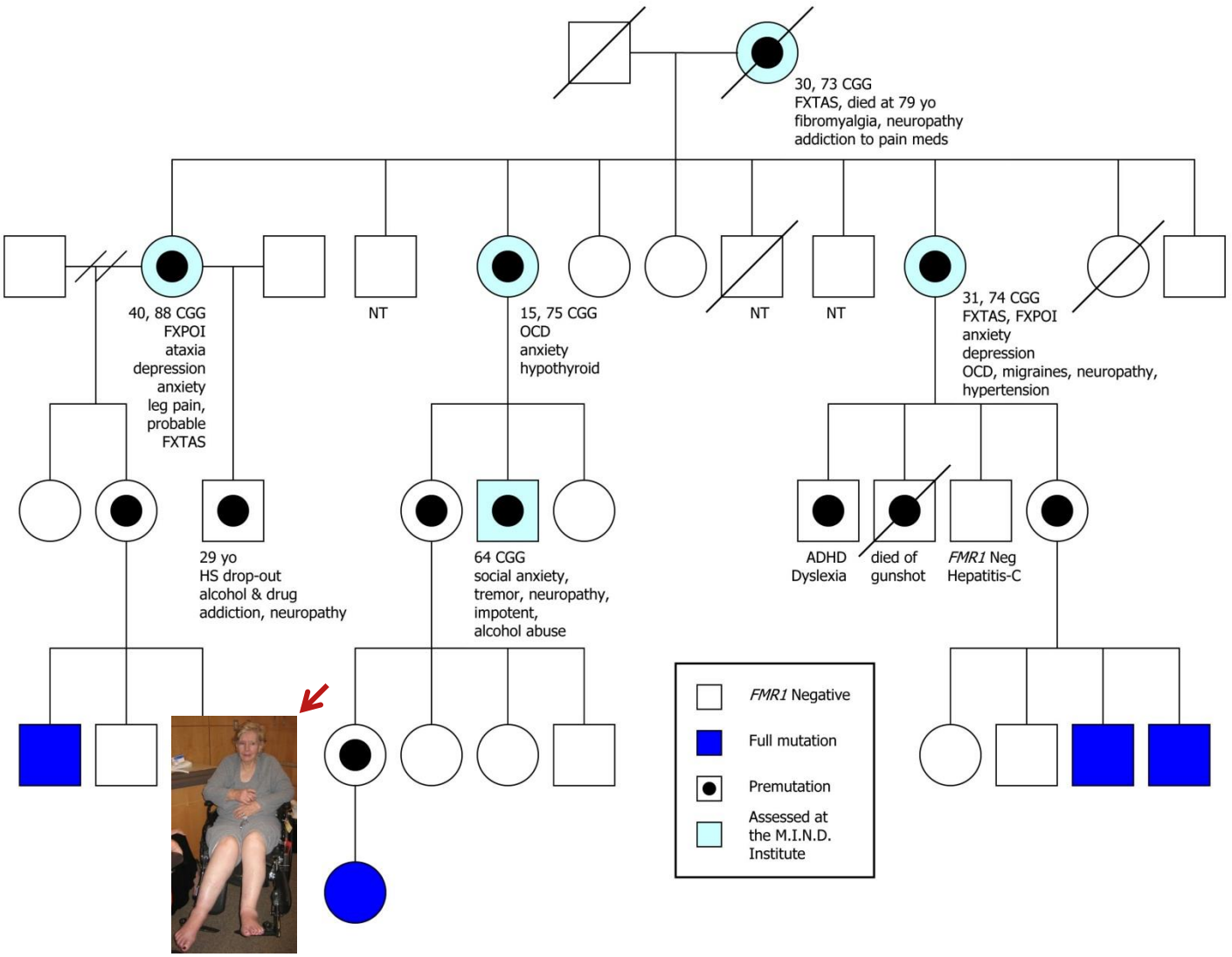


Yrigollen et al., 2012

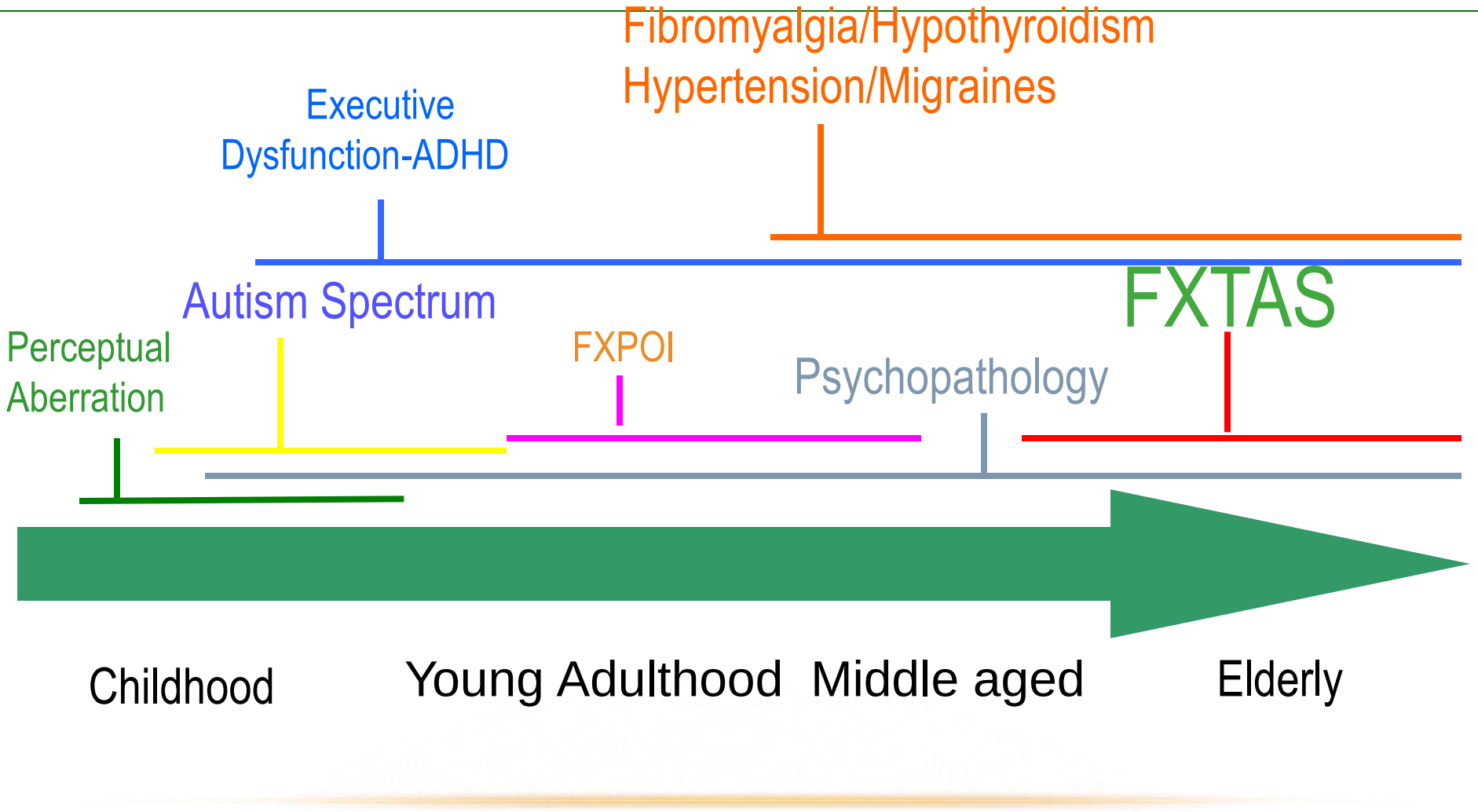





FRAGILE X SYNDROME AND RELATED DISORDERS



PREMUTATION INVOLVEMENT ACROSS THE LIFESPAN



Spectrum of Premutation Involvement

Background gene effects

Environmental effects

Cellular dysregulation

Up-regulation of **heatshock** proteins

Kinase activation

FMR1 CGG-repeat toxic RNA "trigger"

Sequestration of **DROSHA/DGCR8**

miRNA dysregulation

Mitochondrial dysfunction

Inclusion formation

Neuropathology

Neurodevelopmental problems
- Social anxiety → ASD
- ADHD; Cognitive deficits
- Seizures

Psychiatric involvement
- Anxiety
- Stress
- Depression

Endocrine dysfunction
- FXPOI

Immune dysregulation
- Hypothyroidism
- Fibromyalgia; chronic fatigue
- Lupus- MS features

Neurological problems
- Neuropathy
- Migraine
- Memory problems, foggy thinking
- Hypertension , erectile dysfunction

FXTAS
- tremor, ataxia, Parkinsonism
- autonomic dysfunction, EF deficits, memory and cognitive decline

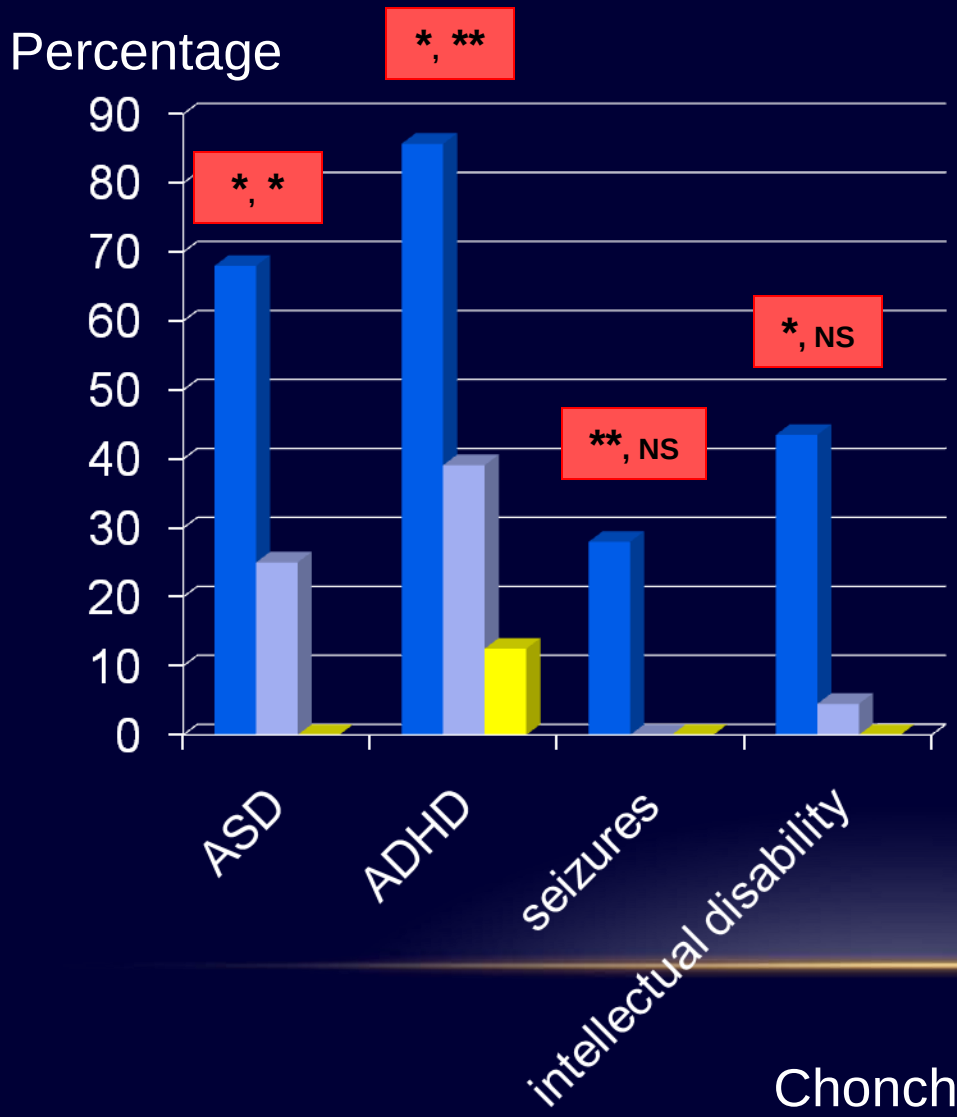
Boys with the premutation are at high risk for ADHD and autism or ASD: A developmental form of RNA toxicity?

- ADHD (CGI≥15 and DSM-IV)
  - 93% (13/14) of probands
  - 38% (6/13) of nonprobands
  - 13% (2/16) of controls
- ASD (DSM-IV and ADOS/ADI)
  - 73% (11/14) of probands*
    - 29% (4/14) Full autism
    - 50% (7/14) PDDNOS
  - 8% (1/13) of nonprobands
    - 8% (1/13) Full autism
  - None of controls

Two brothers with the *FMR1* premutation ages 6 and 7. Boy on right presented as proband with autism and ADHD and his brother has anxiety and ADHD.

Farzin et al, 2006 J Dev Beh Pediatrics

A NEW COHORT OF PREMUTATION BOYS COMPARED TO CONTROLS



Most of the patients with seizures developed ASD

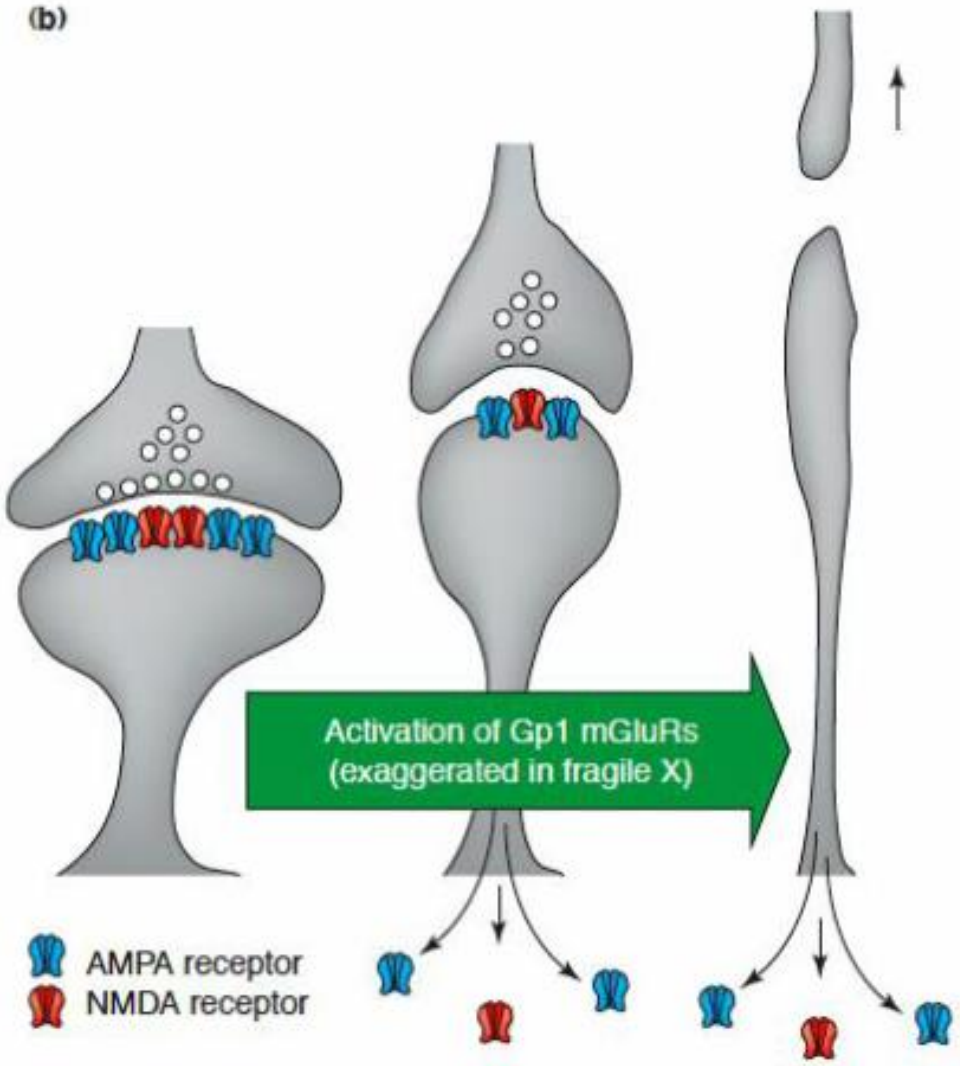
= $p < 0.01$
****** = $p < 0.05$***

- probands (N = 25)
- nonprobands (N = 24)
- controls (N = 31)

probands vs. nonprobands,
nonprobands vs. controls

Chonchiaya et al 2011 Human Genetics

Abnormal synaptic plasticity in *FMR1* KO mice

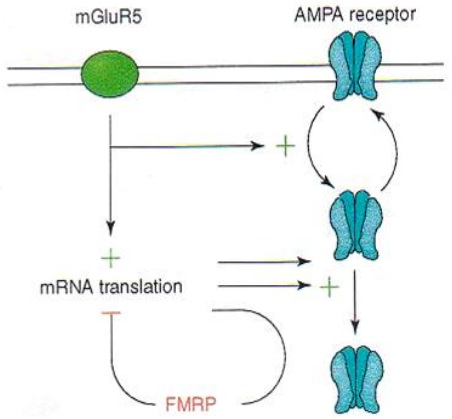


TRENDS in Neurosciences

Bear et al., 2004

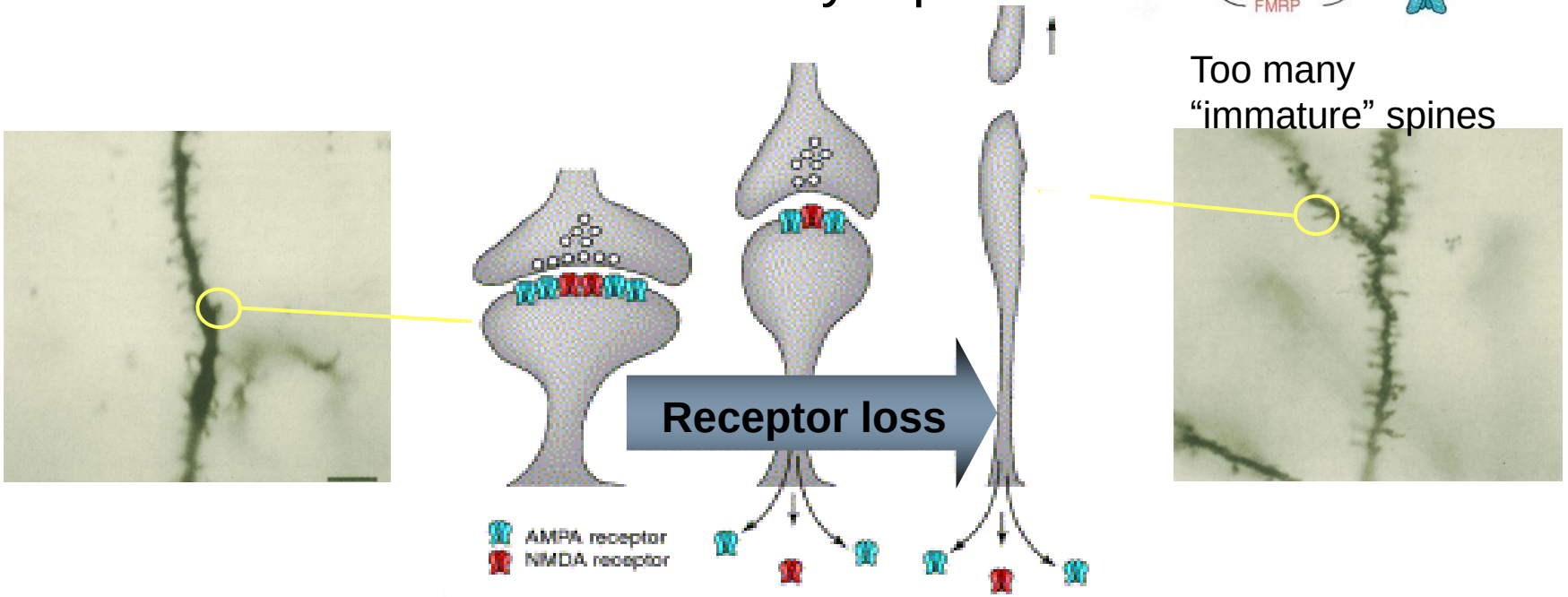
A Model for FMRP function

The mGluR hypothesis



Gradual loss of synapses

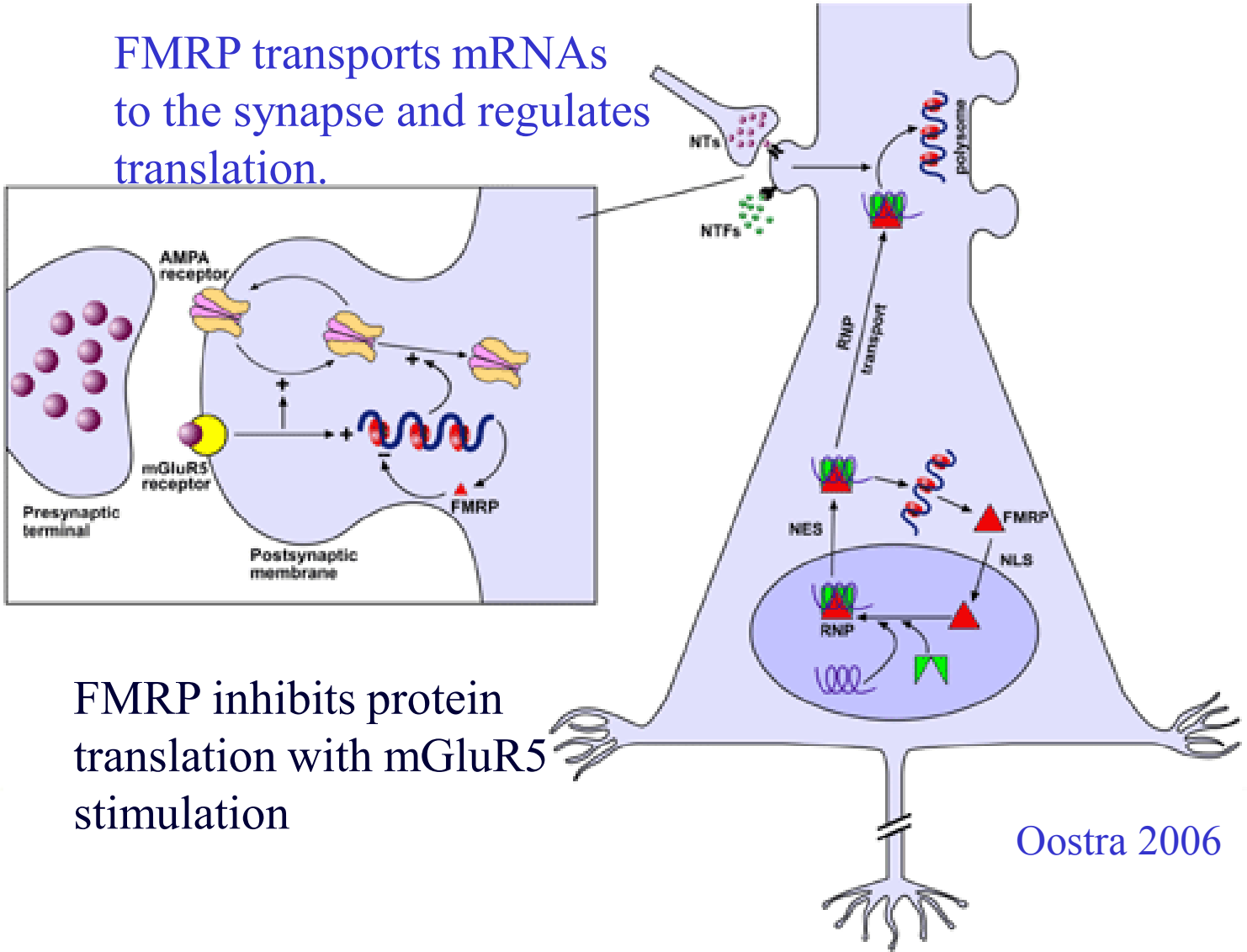
Too many "immature" spines



mGluR mediated signaling is directly coupled to the regulation of translation initiation in neurons.

The Role of FMRP: binds and transports mRNAs And regulates translation usually through inhibition

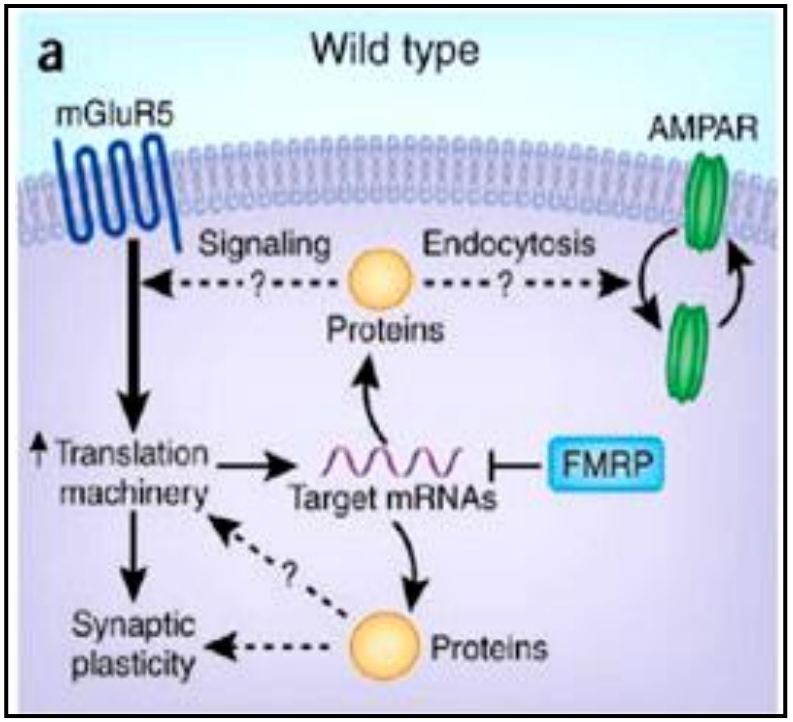
FMRP transports mRNAs to the synapse and regulates translation.

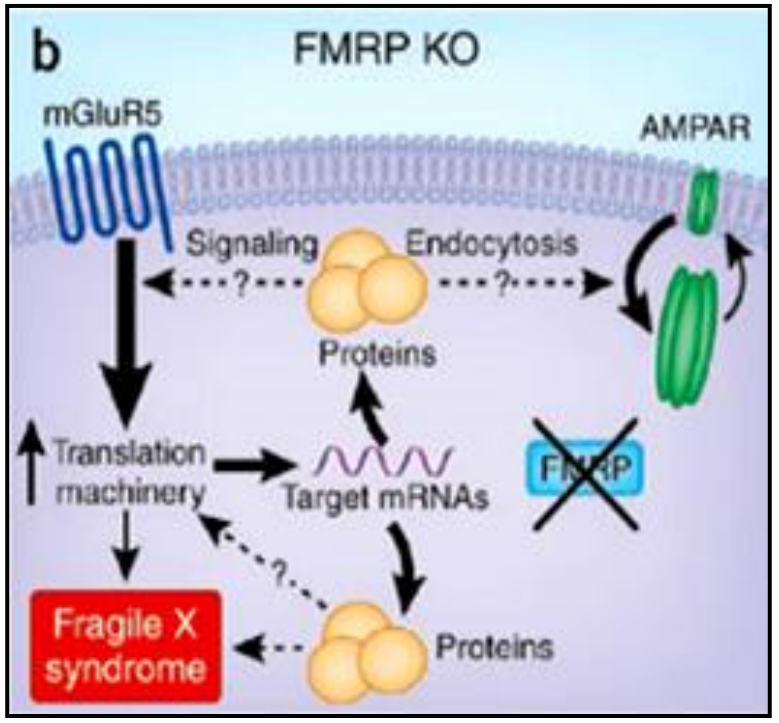


FMRP inhibits protein translation with mGluR5 stimulation

Oostra 2006

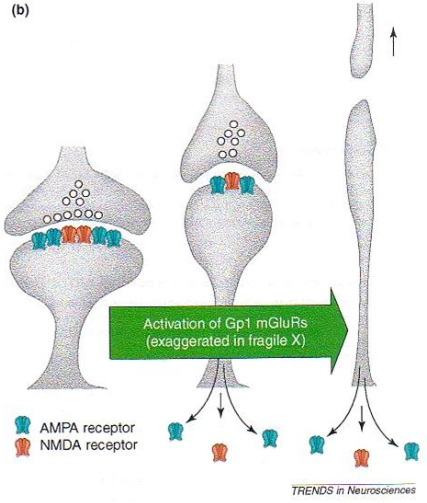
Dramatic Up-regulation of Proteins in the CNS without FMRP

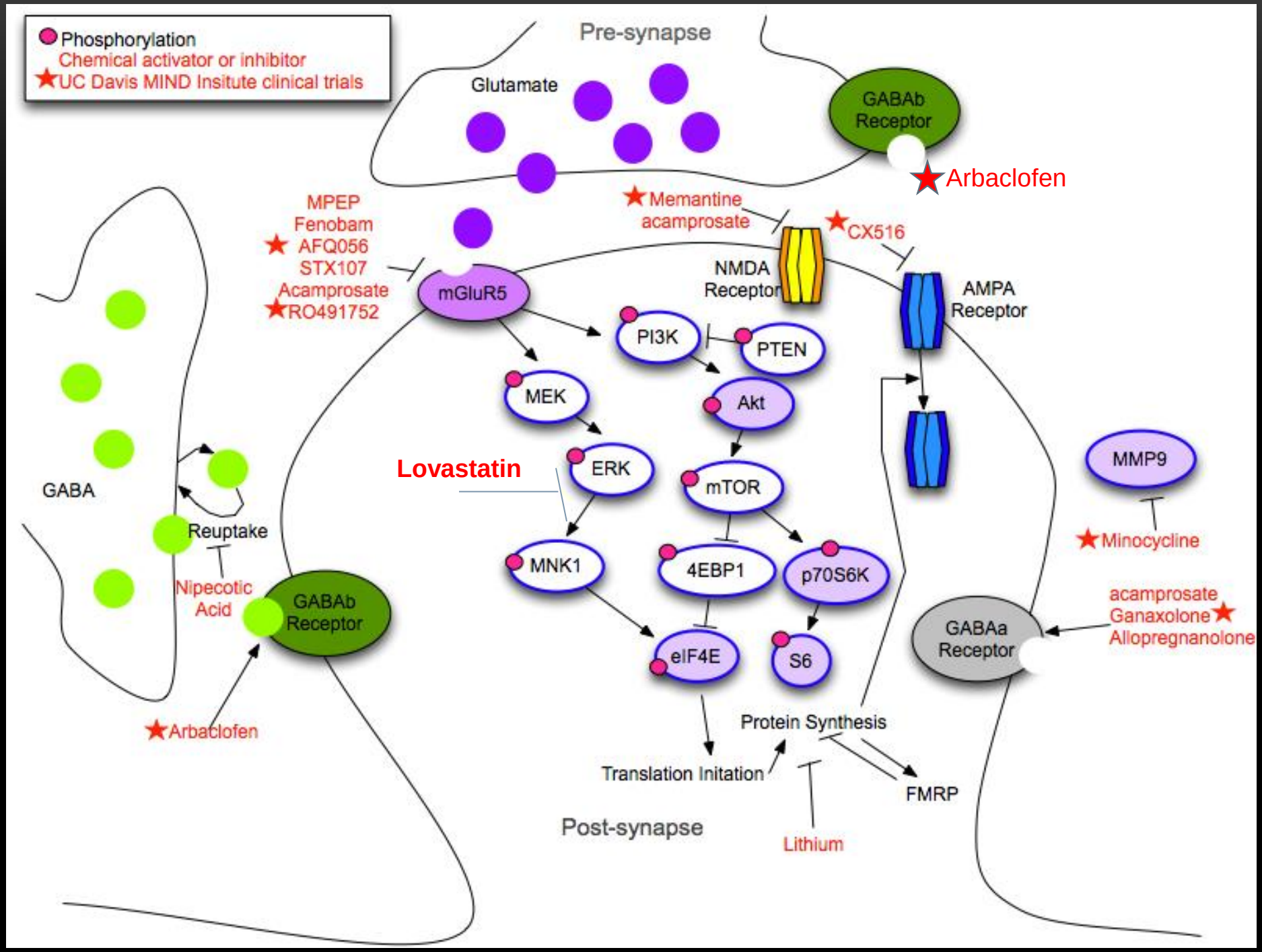




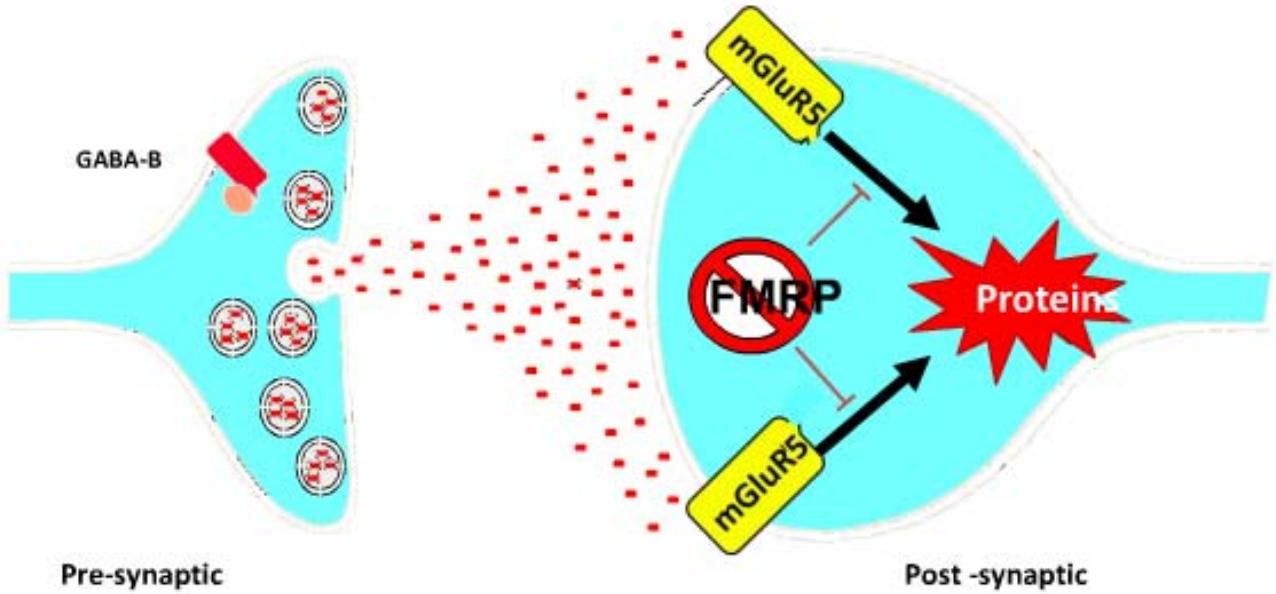
Bassell and Gross 2008

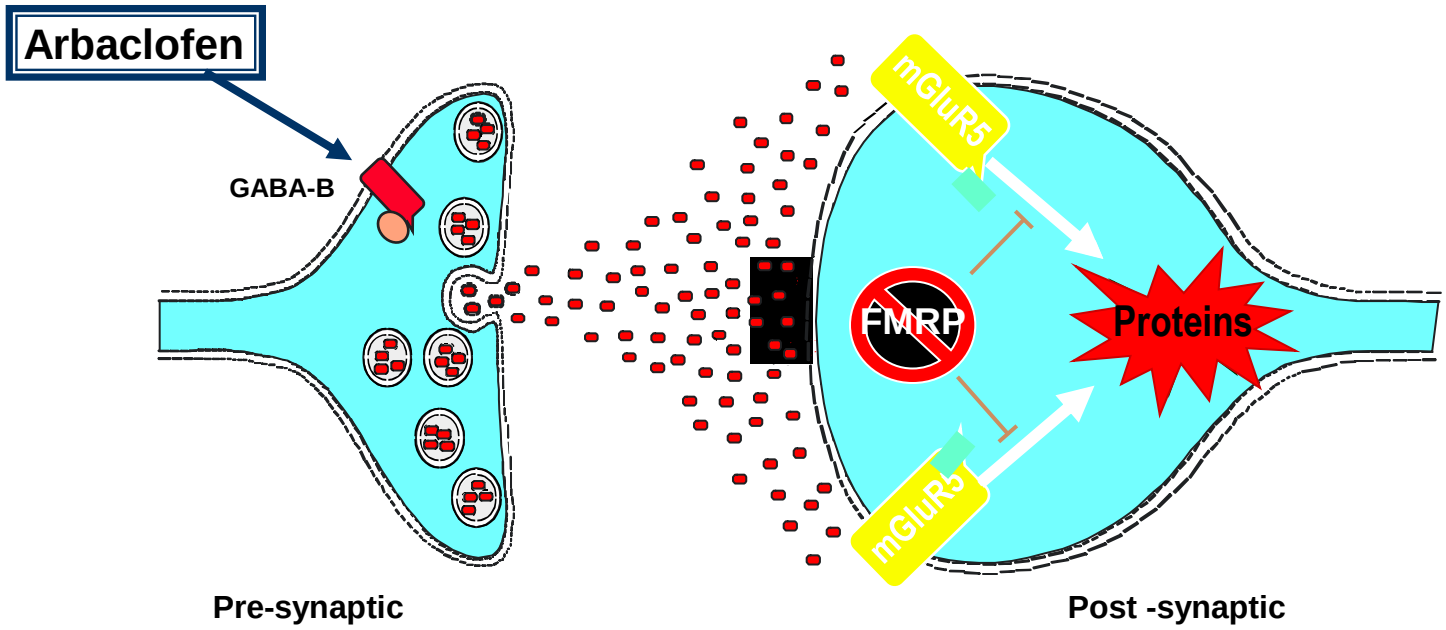
mGluR theory of FXS
Bear et al 2004



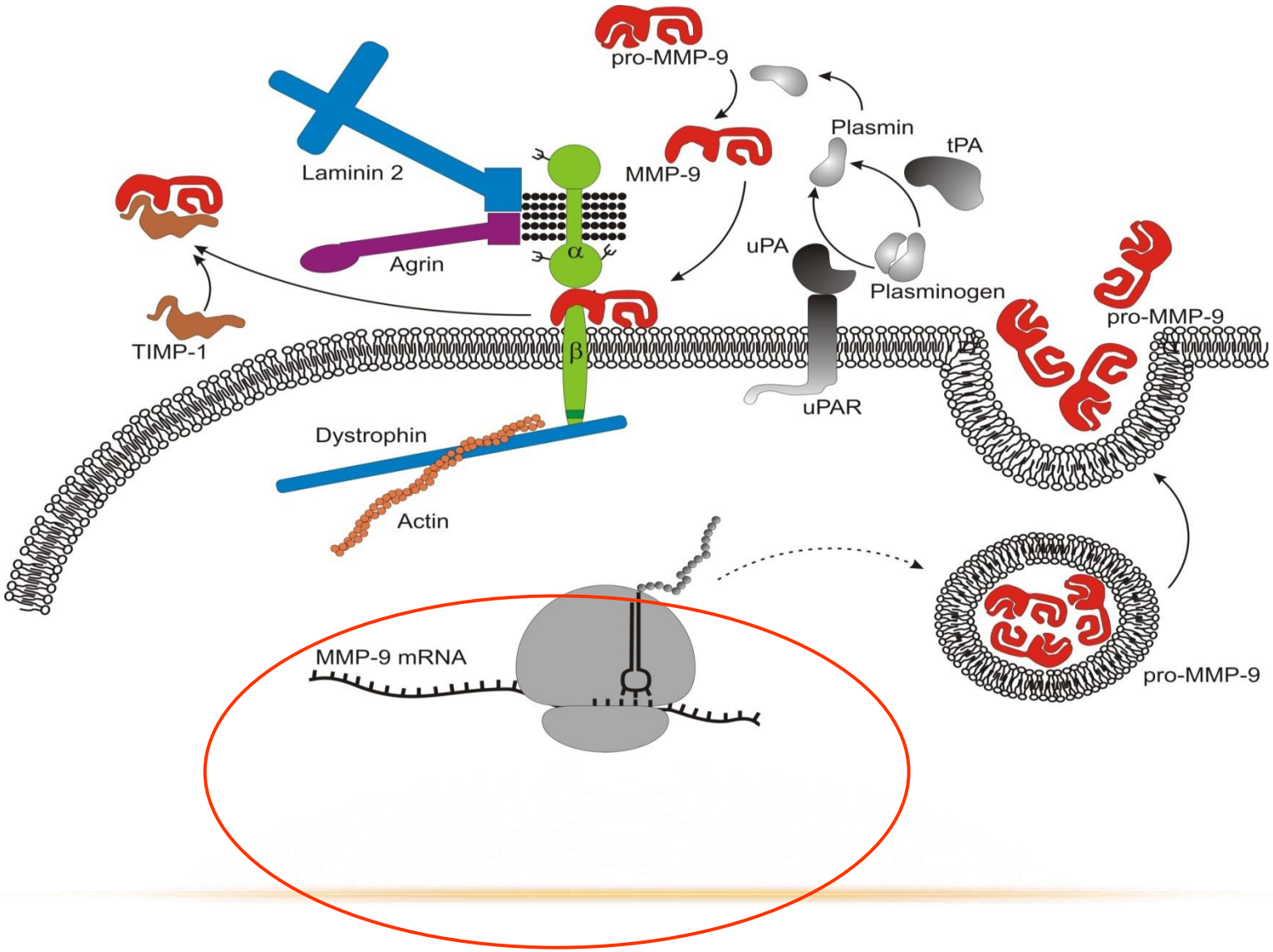

Phosphorylation
Chemical activator or inhibitor
UC Davis MIND Insitute clinical trials
Pre-synapse
Glutamate
GABAb Receptor
Arbaclofen
MPEP
Fenobam
AFQ056
STX107
Acamprosate
RO491752
Memantine
acamprosate
CX516
NMDA Receptor
AMPA Receptor
mGluR5
PI3K
PTEN
MEK
Akt
ERK
mTOR
Lovastatin
MMP9
Minocycline
GABA
Reuptake
Nipecotic Acid
GABAb Receptor
MNK1
4EBP1
p70S6K
eIF4E
S6
GABAa Receptor
acamprosate
Ganaxolone
Allopregnanolone
Arbaclofen
Protein Synthesis
Translation Initation
FMRP
Post-synapse
Lithium

A therapeutic approach for FXS

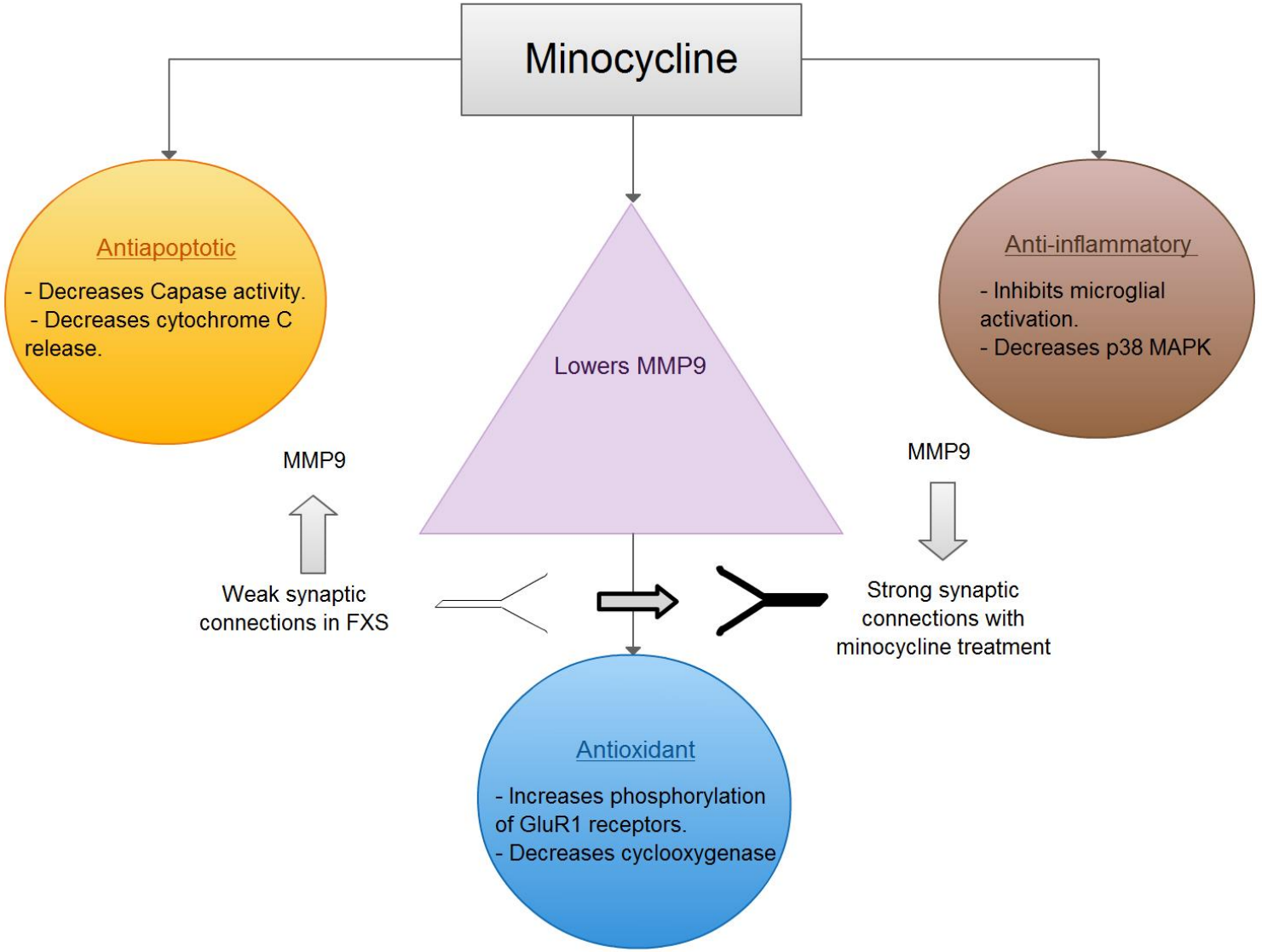



Arbaclofen
GABA-B
mGluR5
FMRP
Proteins
mGluR5
Pre-synaptic
Post -synaptic

MMP-9 synthesis, release and activation



Minocycline Mechanisms for Neuroprotection



mGluR5 antagonists for FXS

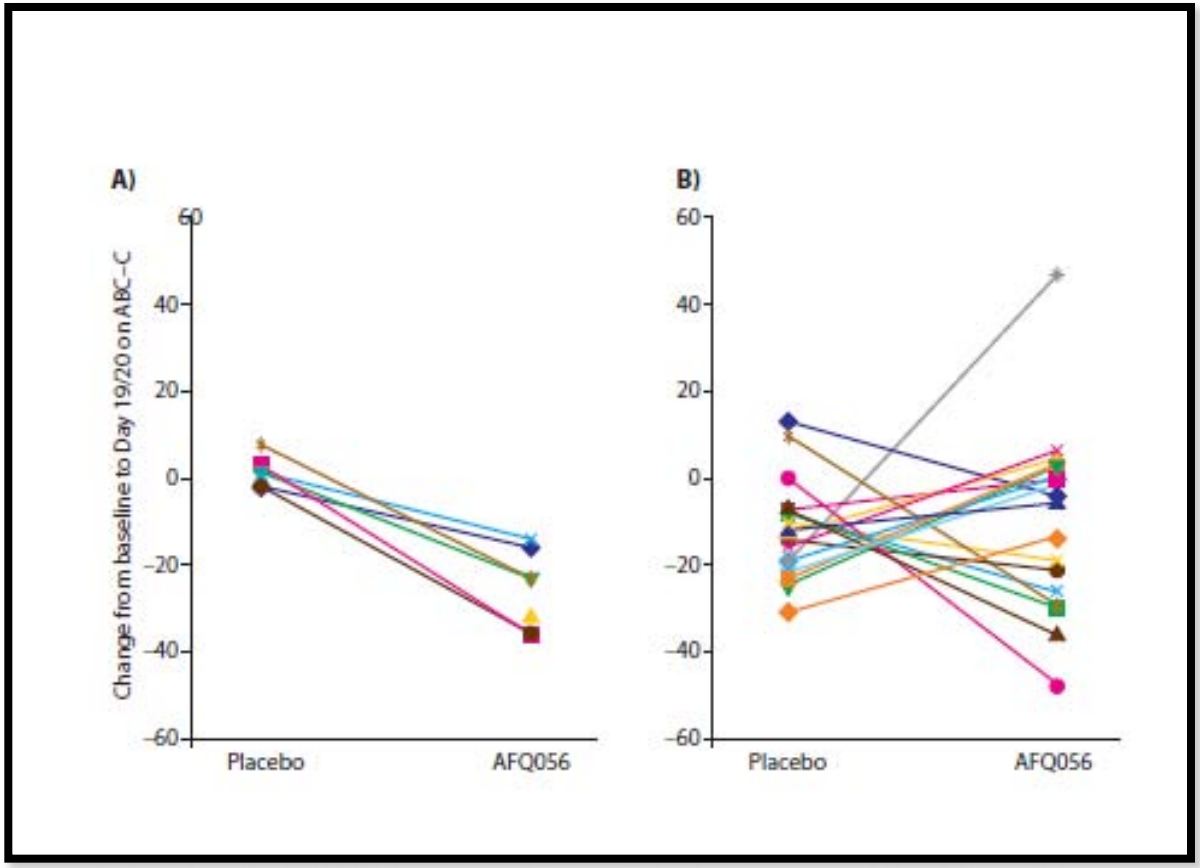
- Fenobam : improvement in PPI and behavior in single dose with 12 adults with FXS at MIND and Rush (Berry-Kravis et al 2009 JMG)
- Roche mGluR5 antagonist RO4917523 currently in controlled trials at multiple centers including MIND (16yo and older with FXS). Initiated 5-12 childhood PK 3 mo studies
- Novartis AFQ056 European trial (Jacquemont et al 2011 Sci Trans Med). Current controlled trials and open label in 12 through adultshood. To initiate childhood PK studies.
- STX 107 an mGluR5 antagonist licensed by Seaside Therapeutics.

Study Measures

- Baseline:
  - Cognitive Assessment: Stanford Binet V, WISC IV, Leiter-R or Mullen Scales of Early Learning
  - Autism Assessment: Autism Diagnostic Observation Schedule (ADOS), DSMIV Criteria for Autism Checklist
- Primary Outcome Measures
  - Clinical Global Impression Scale-Improvement (CGI-I)
  - Visual Analogue Scale for Severity of Behavior (VAS)
- Secondary Outcome Measures
  - Vineland Adaptive Behavior Scale-II (VABS-II)
  - Aberrant Behavior Checklist- Community Edition (ABC-C)
  - Expressive Vocabulary Test-II (EVT-II)

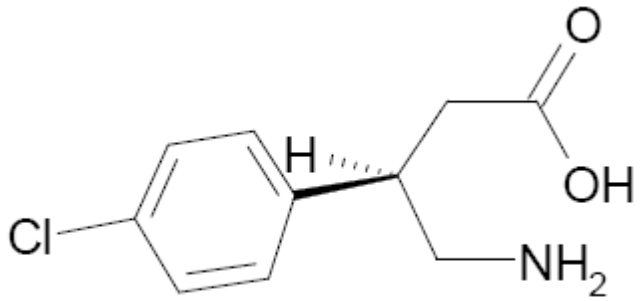
ONLY THE FULL MUTATIONS FULLY METHYLATED RESPONDED TO AFQ056

JACQUEMONT ET AL 2011 SCI TRANS MED

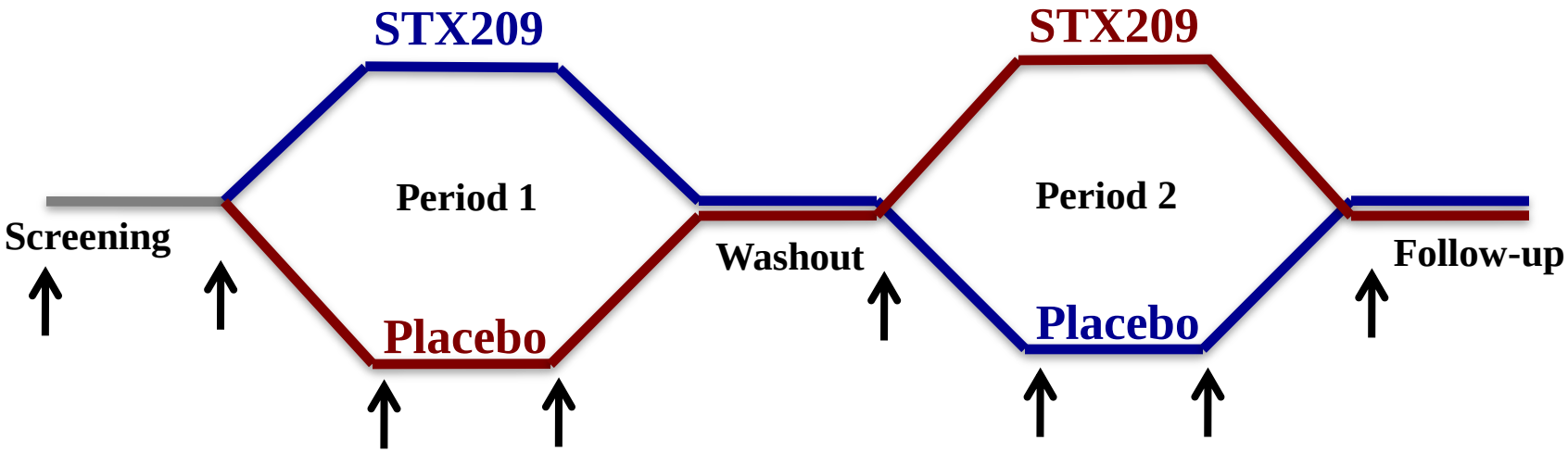


R-BACLOFEN= ARBACLOFEN: STX209

- Baclofen is racemic



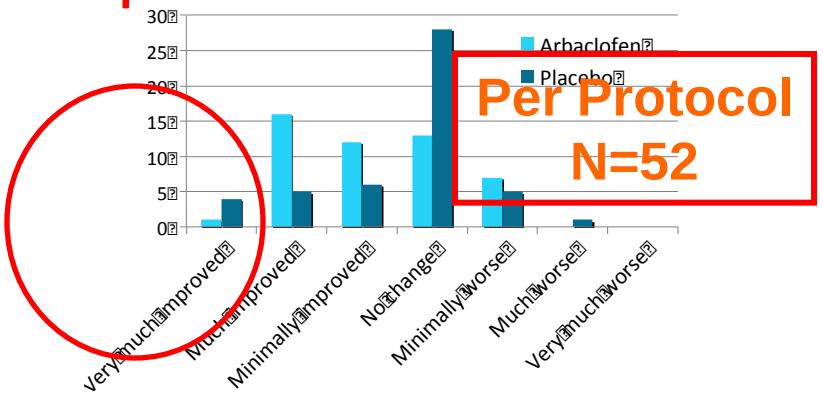
- Both isomers are selective GABA-B agonists
  - GABA-B: **R:S** potency ratio **15:1**
  - *in vivo:* **R:S** potency ratio **10-100:1**
- R-Baclofen kinetics comparable when given alone or as part of racemic mixture (with S-baclofen)
- R-Baclofen is more potent in blocking presynaptic release of glutamate and therefore may be helpful in FXS and perhaps in autism



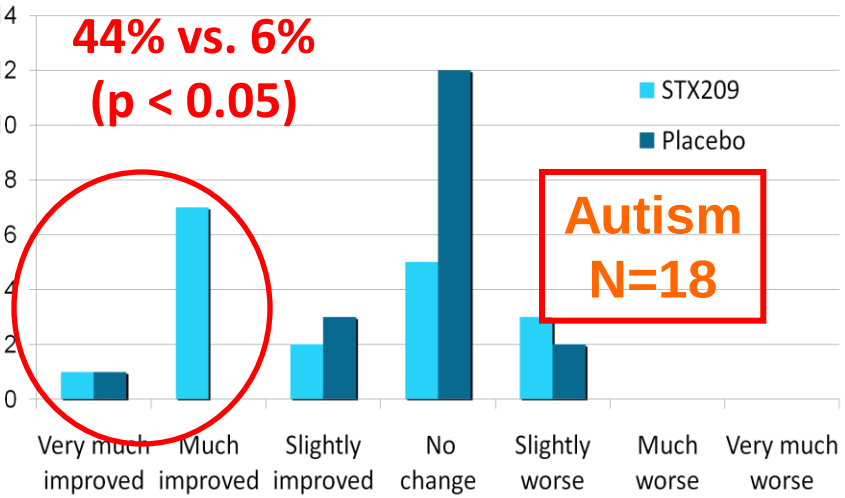
- Double-blind, randomized, placebo-controlled, 2-period crossover
- Endpoints
  - Global: CGI-I; CGI-S; blinded treatment preference
  - Focused: Aberrant Behavior Checklist - Irritability (ABC-I) scale; ABC-Total & other subscales; Vineland Adaptive Behavior Scale; Visual Analog Scale of top 3 problem behaviors; other
- Down titration after completion of 4 week period
- Ages 6 to 40 years and maximum dose was 10 mg tid
- Published now Berry-Kravis et al 2012 Science Translational Medicine

CGI-I (IMPROVEMENT) RESULTS IN ARBACLOFEN (STX209) TRIAL

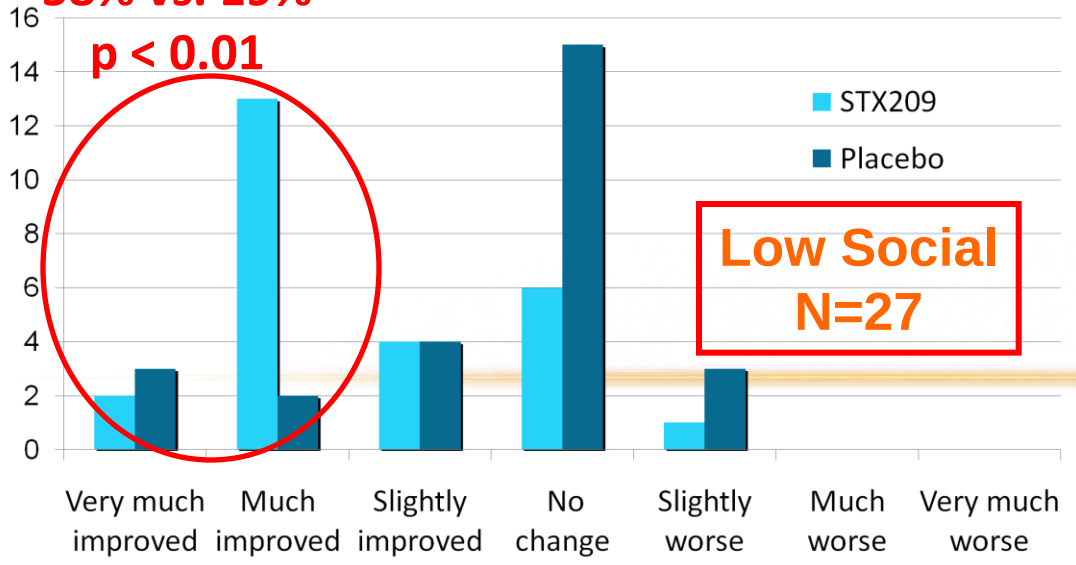
"Responders"
35% vs. 18%
p = 0.11



44% vs. 6%
(p < 0.05)

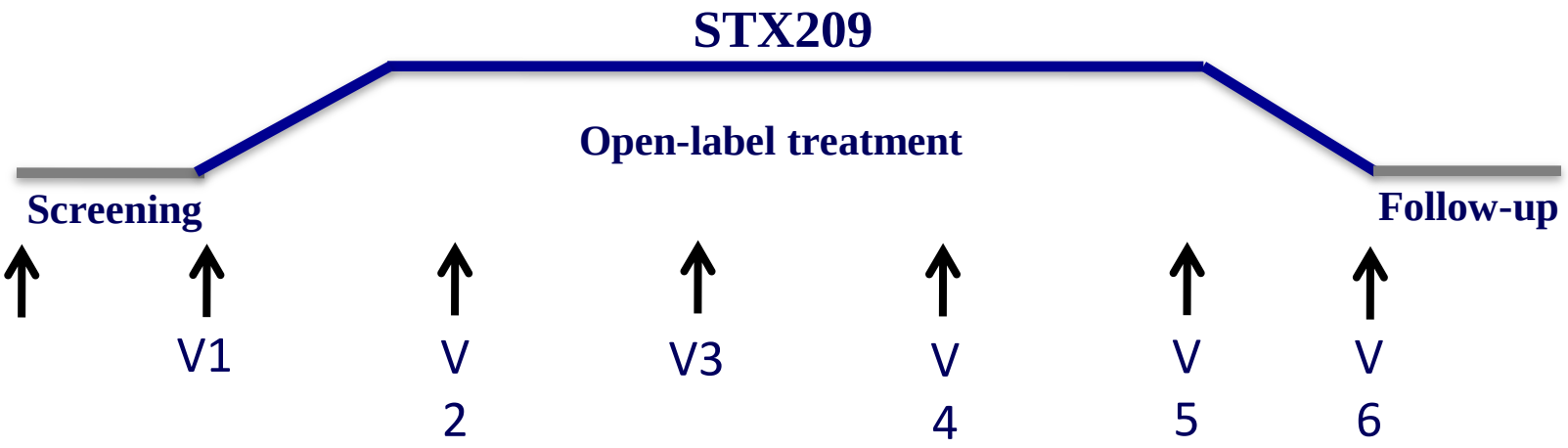


58% vs. 19%
p < 0.01



Berry-Kravis et al 2012

ARBACLOFEN FOR ASD



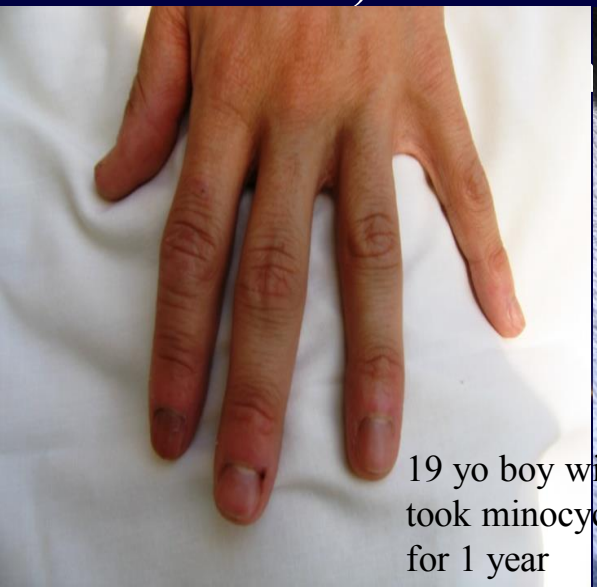
- Autistic Disorder or PDD-NOS
- ABC-Irritability ≥ 16 at baseline
- n = 32; age 6 – 17 years
- Concomitant meds: ≤ 2 psychoactives; no antipsychotics
- Treatment period: 8 weeks

ARBACLOFEN OPEN LABEL EFFICACY IN ASD

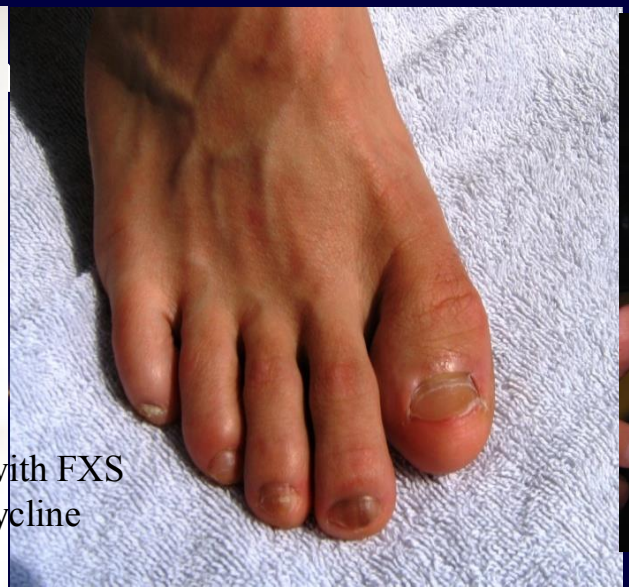
	Baseline (mean ± SD)	Week 8 (mean ± SD)	p-value
ABC-Irritability	27.0 ± 7.6	17.7 ± 10.4	< 0.001
ABC-Social Withdrawal	17.3 ± 8.2	12.6 ± 9.3	= 0.001
ABC-Total	90.3 ± 29.4	64.0 ± 35.0	< 0.001
CGI-I	–	2.5 ± 0.9	< 0.05
CGI-S	5.1 ± 0.9	4.4 ± 1.2	< 0.001
ADHD-IV Rating Scale	34.2 ± 11.4	26.1 ± 13.0	< 0.001
CY-BOCS	14.8 ± 4.1	11.6 ± 5.0	< 0.001
CASI-Anxiety	20.4 ± 10.6	16.5 ± 13.8	< 0.001
Social Responsiveness	117.0 ± 33.8	103.0 ± 29.6	< 0.05
Vineland-Communication	61.4 ± 10.5	65.4 ± 9.5	< 0.01

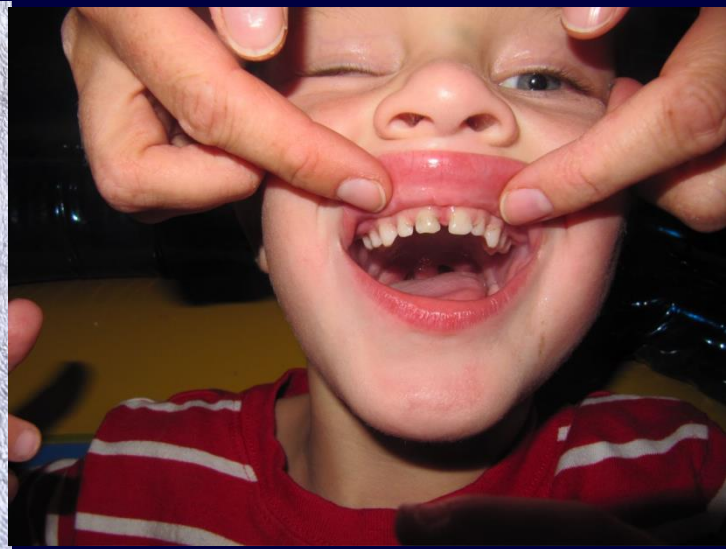
MINOCYCLINE STUDIES IN FXS OR AUTISM

- Bilousova et al 2009 demonstrated that minocycline lowers MMP9 levels in FXS and improved behavior and cognition in the FX mouse
- Agustini Utari MD surveyed 50 families whose child was Tx with minocycline for >2wks and found 70% positive response especially in language and limited side effects (Utari et al 2010 AJIDD).
- Positive open trial in FXS in Toronto with age ≥ 13 years (Paribello et al 2010)



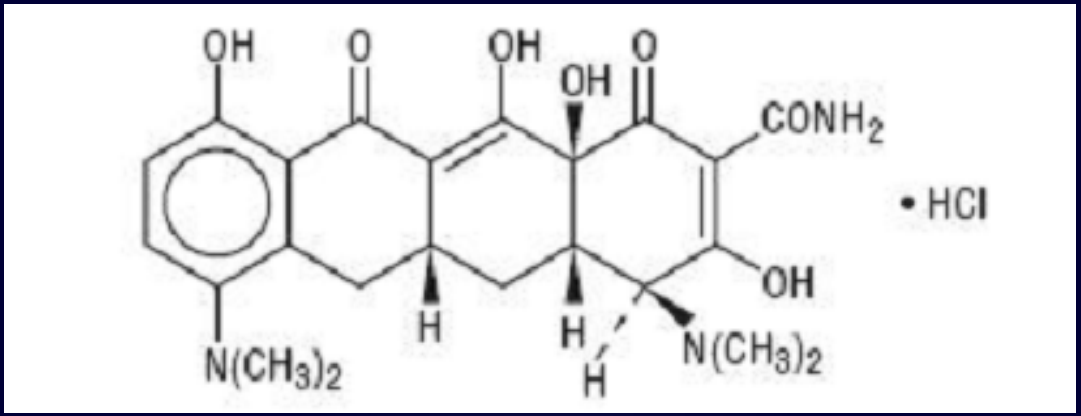
19 yo boy with FXS took minocycline for 1 year





MINOCYCLINE HYDROCHLORIDE

- Semisynthetic tetracycline derivative
- Commonly used in treatment of acne vulgaris
- Found to have neuroprotective effects
- Investigated in Huntington’ s Disease, ALS multiple sclerosis



STUDY DESIGN

- Randomized
- Double blind Placebo controlled trial 3.5-16y
- Crossover : 3 months for each arm
- Voluntary recruitment from UC Davis MIND Institute Fragile X Research and Treatment Center 66 entered 48 completed www.clinicaltrials.gov

Minocycline Dosing

Weight	Minocycline Daily Dose
<25kg	25mg
25-45kg	50mg
>45kg	100mg

CONTROLLED TRIAL OF MINOCYCLINE

Visit 1

Cognitive Assessment
Autism Assessment
VABS-II
ABC-C
CGI
EVT-II
VAS
Hx & PE
Con Meds

Visit 2

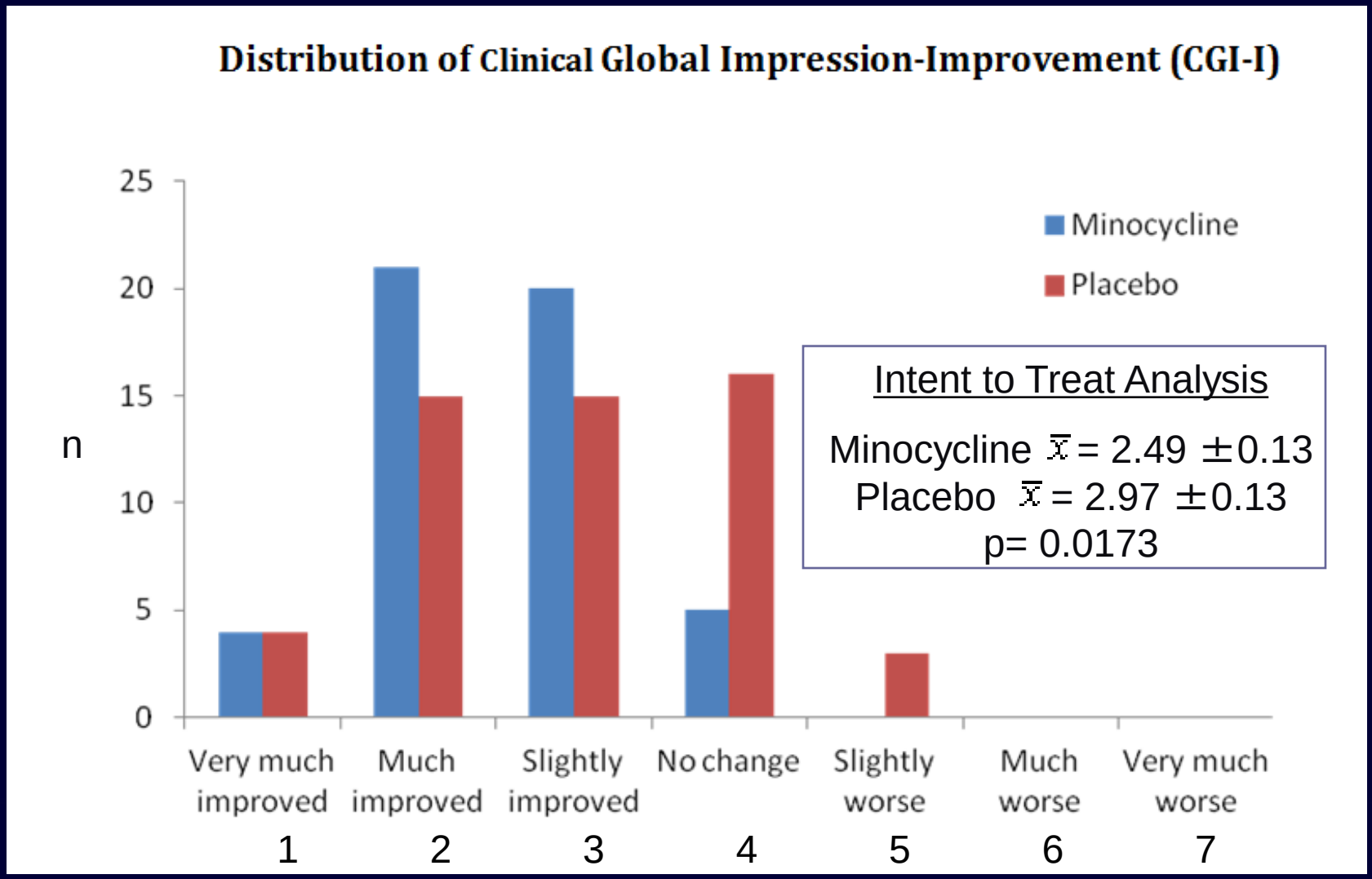
VABS-II
ABC-C
CGI
EVT-II
VAS
Hx & PE
Con Meds
AEs

Visit 3

VABS-II
ABC-C
CGI
EVT-II
VAS
Hx & PE
Con Meds
AEs

Study Period: January 2010-December 2011

CONTROLLED CROSS-OVER DOUBLE-BLIND TRIAL OF MINOCYCLINE, SIGNIFICANT IMPROVEMENT ON CGI



RESULTS: VISUAL ANALOGUE SCALE

	Minocycline		Placebo		P Value
VAS Behavior Category	Mean	SE	Mean	SE	
Aggression/ADHD (n=46)	4.47	0.35	4.25	0.32	0.5355
Anxiety/Mood (n=26)	5.26	0.46	4.05	0.46	0.0488
Language/Cognition (n=38)	4.99	0.36	4.70	0.34	0.5345
Other (n=11)	6.02	0.58	3.45	0.34	0.0175

Significant change in VAS for Anxiety/Mood and for “other” category including diverse problems such as toilet training and social interactions

No influence of FSIQ & ADOS total score on response to minocycline

ADVERSE EVENTS

78% of participants reported AE; 49% on minocycline, 51% on placebo

	Minocycline		Placebo	
Category	**Count**	**%**	**Count**	**%**
Diarrhea/Loose Stools	15	21.13	15	20.55
GI Upset/Vomiting/Loss of Appetite	9	12.68	15	20.55
Dizziness/Unsteadiness	0	0	1	1.37
Headaches	4	5.63	5	6.85
Drowsiness	2	2.82	3	4.11
Skin Rash/Itching/Swelling	12	16.9	7	9.59
Fever/chills/URI symptoms/Sore Throat	6	8.45	11	15.07
Blue-grey/grey hue to teeth or other tissues	3	4.23	1	1.37
Dark colored urine/changes in urination	1	1.41	2	2.74
Sunburn/sun sensitivity	4	5.63	1	1.37
Other	15	21.13	12	16.44

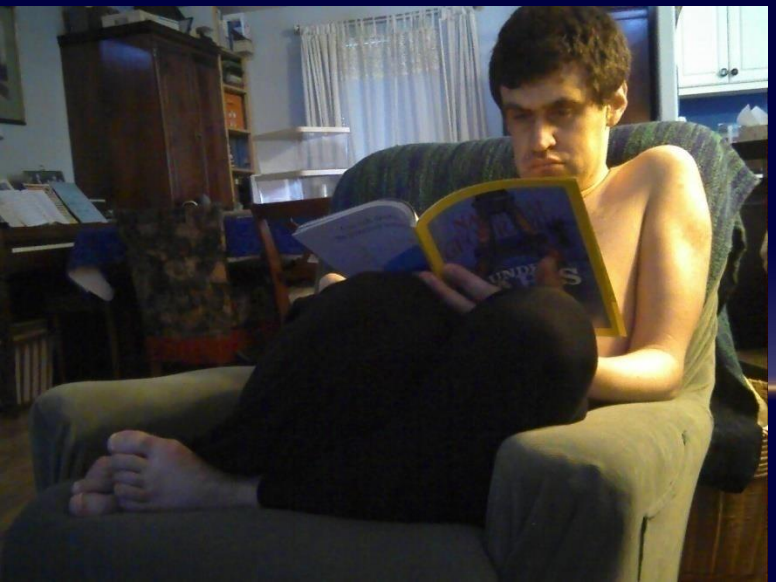
No difference in AEs on minocycline vs placebo

p=0.551

Sanchez 2004

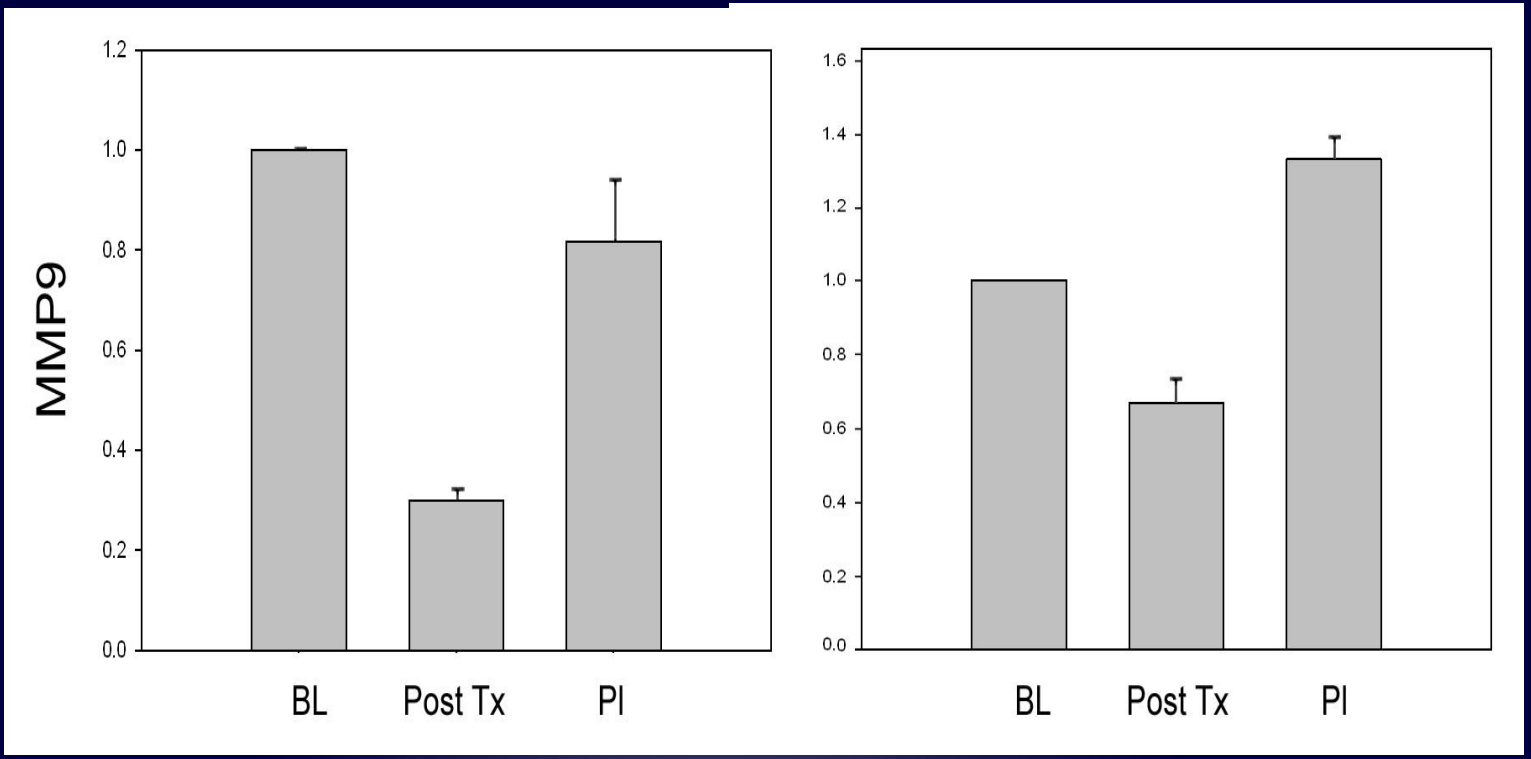
Severe involvement from FXS
Autistic , non verbal, aggressive, would not tolerate clothes
could not go outside

After 2 years on minocycline



He can talk and dress
He drinks from a cup
He walks with his social worker
Aggression is gone
He can come to clinic
Looks at magazines and TV

ONLY 2 INDIVIDUALS HAD MMP9 LEVELS DONE IN EACH PHASE OF STUDY AND BOTH WERE RESPONDERS TO MINOCYCLINE



Tassone et al unpublished

MINOCYCLINE IN ANGELMAN SYNDROME

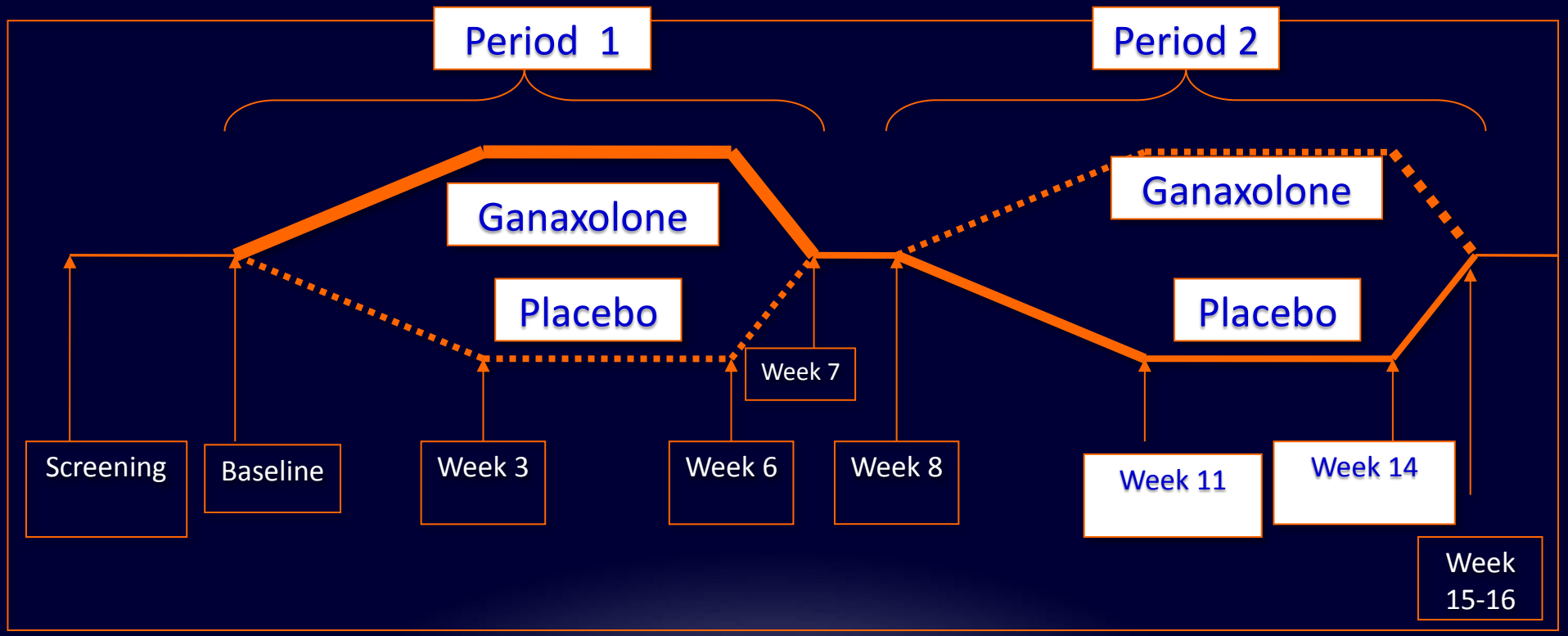
- Ed Weeber carried out trials of minocycline in AS mouse model with positive effects and is carrying out a controlled trial of minocycline in AS children and has preliminary positive results

- Clinical use of minocycline in AS at the MIND has shown improvements in language and motor abilities in 5 children with AS.

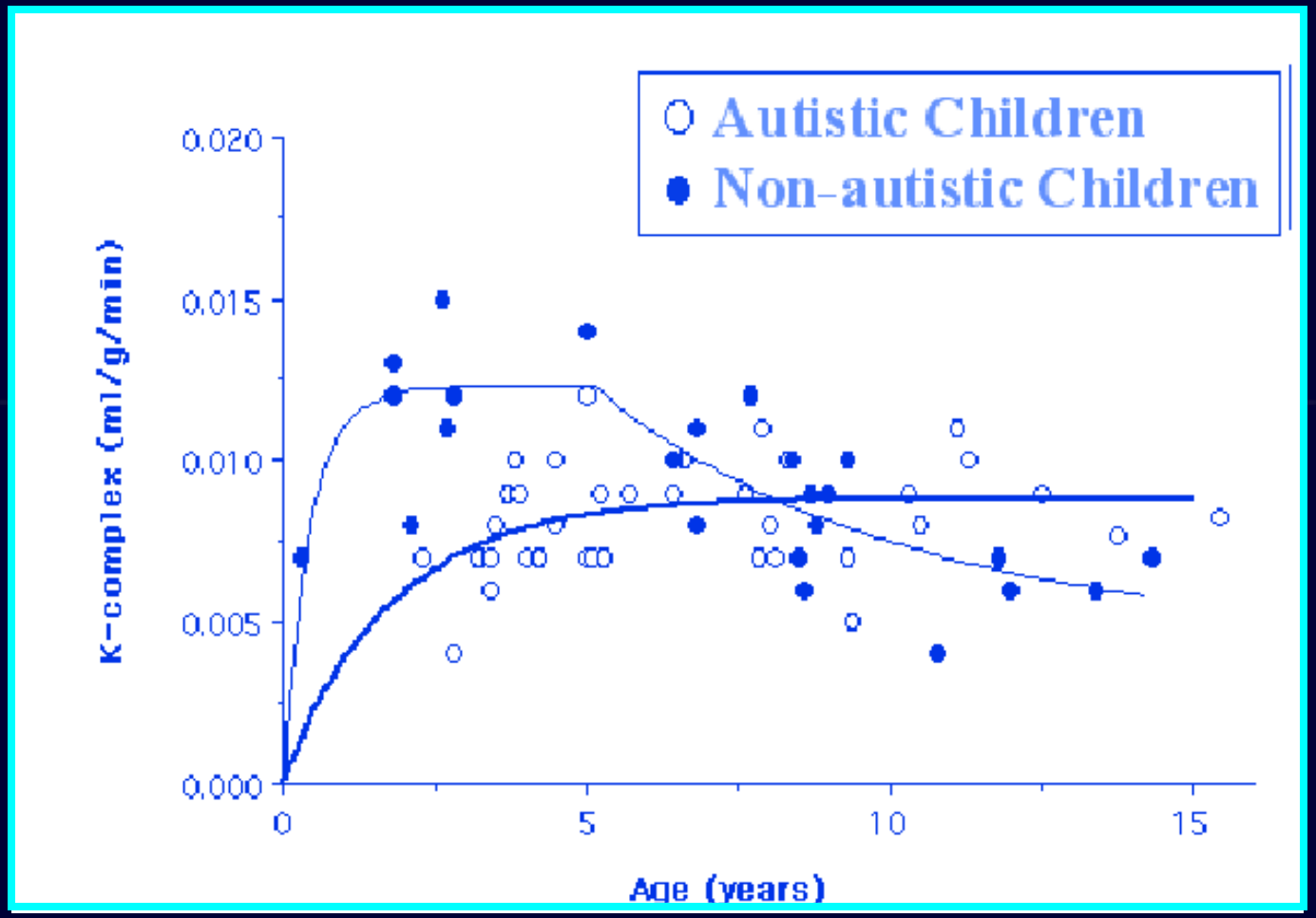
$GABA_A$ RECEPTOR EXPRESSION IS DOWN IN FXS

- $GABA_A$ expression is down regulated in the KO mouse (D' Hulst et al 2007; Kooy et al 2005)
- $GABA_A$ agonists: Ganaxolone
  - Investigational medication with efficacy in infantile spasms and other types of epilepsy: A controlled trial in children with FXS (6 -18y) funded by DOD is in progress at the MIND Institute; Marinus to supply ganaxolone
  - Targeting improvement in anxiety, behavior and seizure frequency

GANAXOLONE TREATMENT TIMELINE DOUBLE-BLIND CROSSOVER CONTROLLED TRIAL



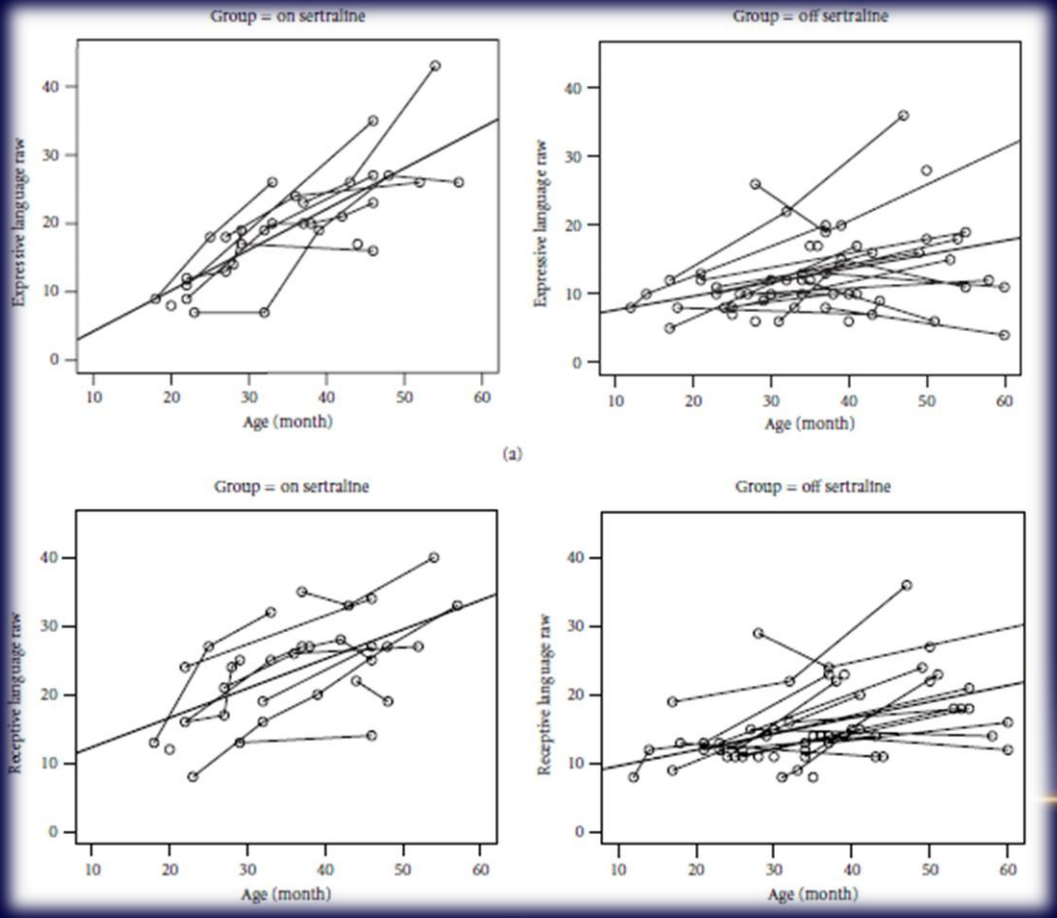
In autism serotonin synthesis is reduced frontally.
This may be true for FXS
since clinically they respond to early sertraline Tx



Chugani et al., 1999

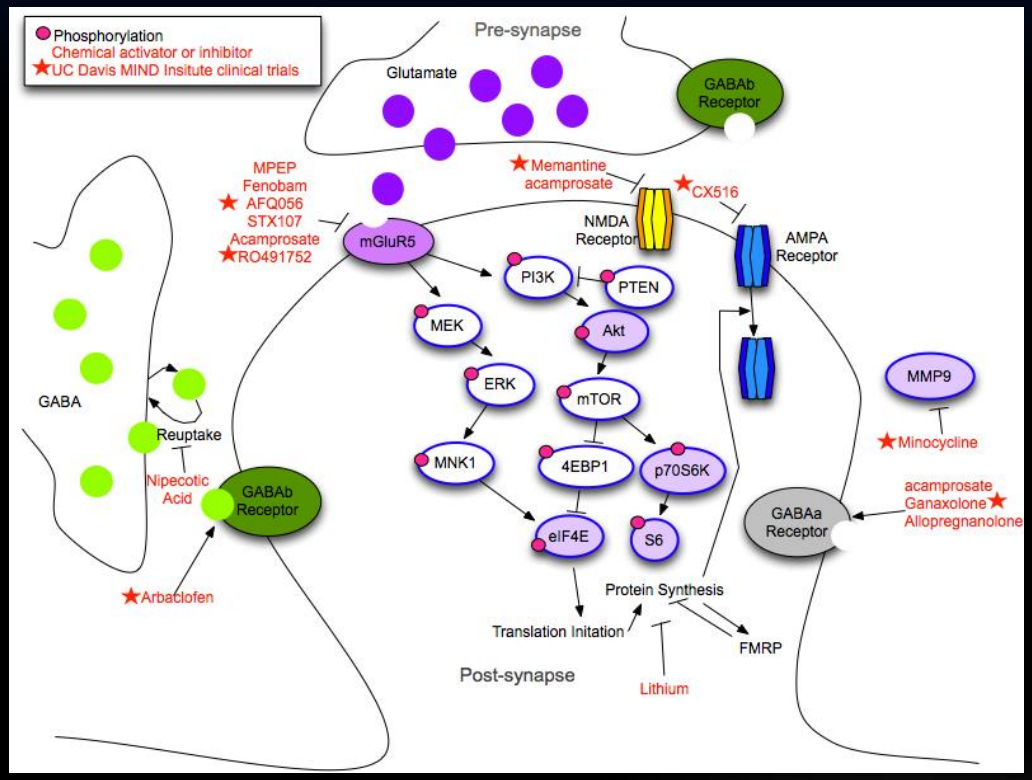
SERTRALINE TREATMENT IN EARLY CHILDHOOD IN FXS

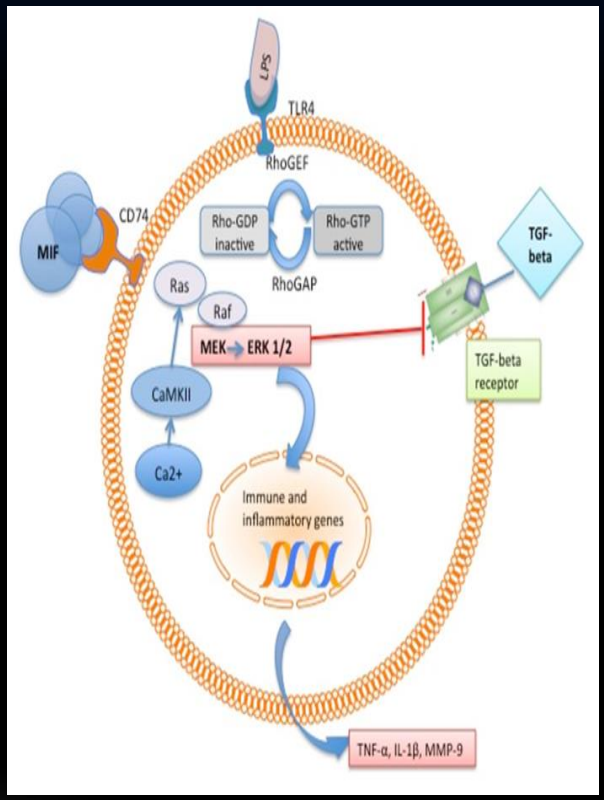
A RETROSPECTIVE STUDY OF 45 CHILDREN FOLLOWED 12 TO 50 MONTHS AND 11 TREATED WITH SERTRALINE: SIGNIFICANT DIFFERENCES IN EXPRESSIVE AND RECEPTIVE LANGUAGE IN TX VS NON TREATED (P=0.0001 AND P=0.0071 RESPECTIVELY)



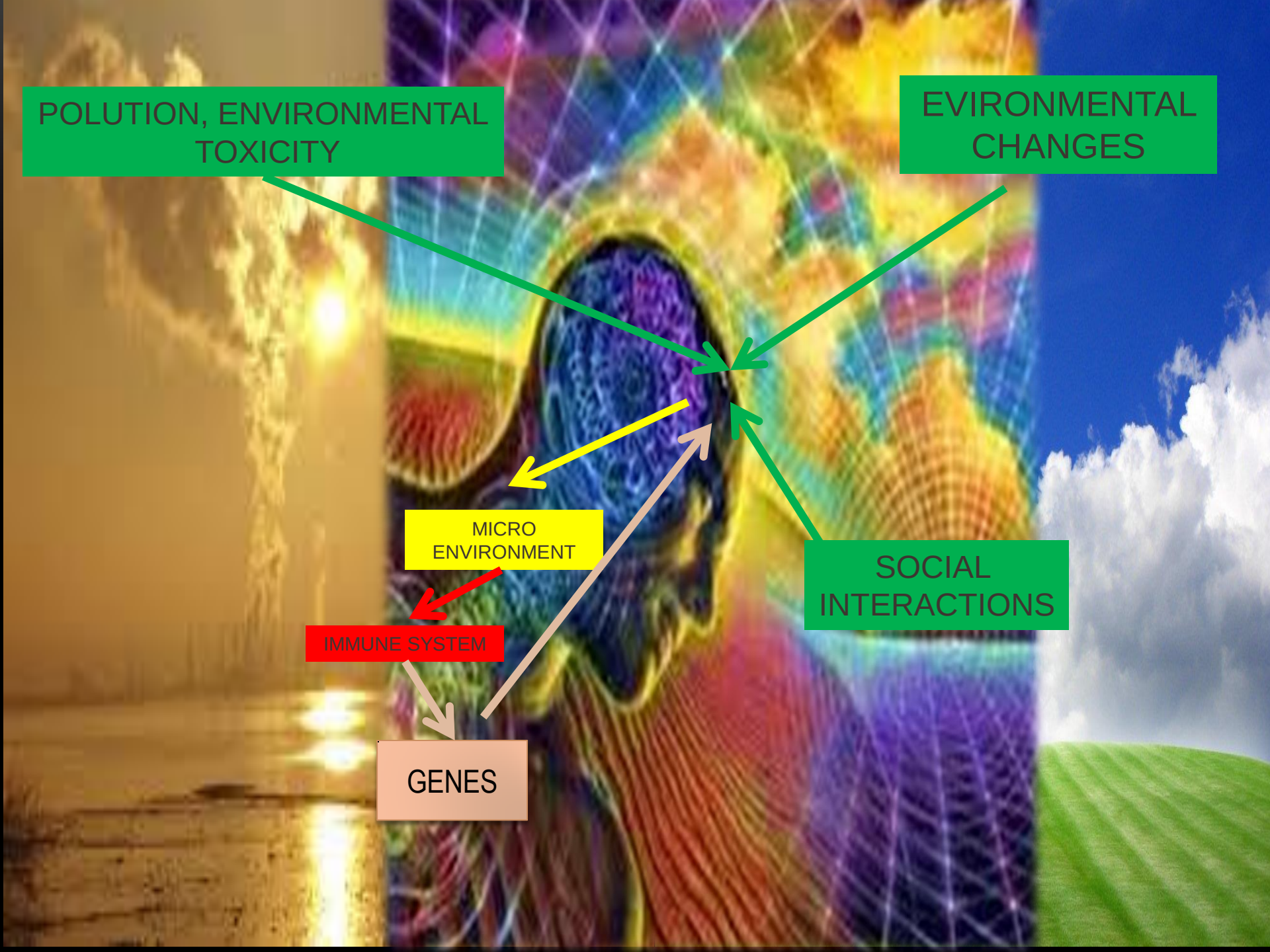
Winarni et al 2012 Autism Treatment and Research

LOVASTATIN AND LANGUAGE INTERVENTION

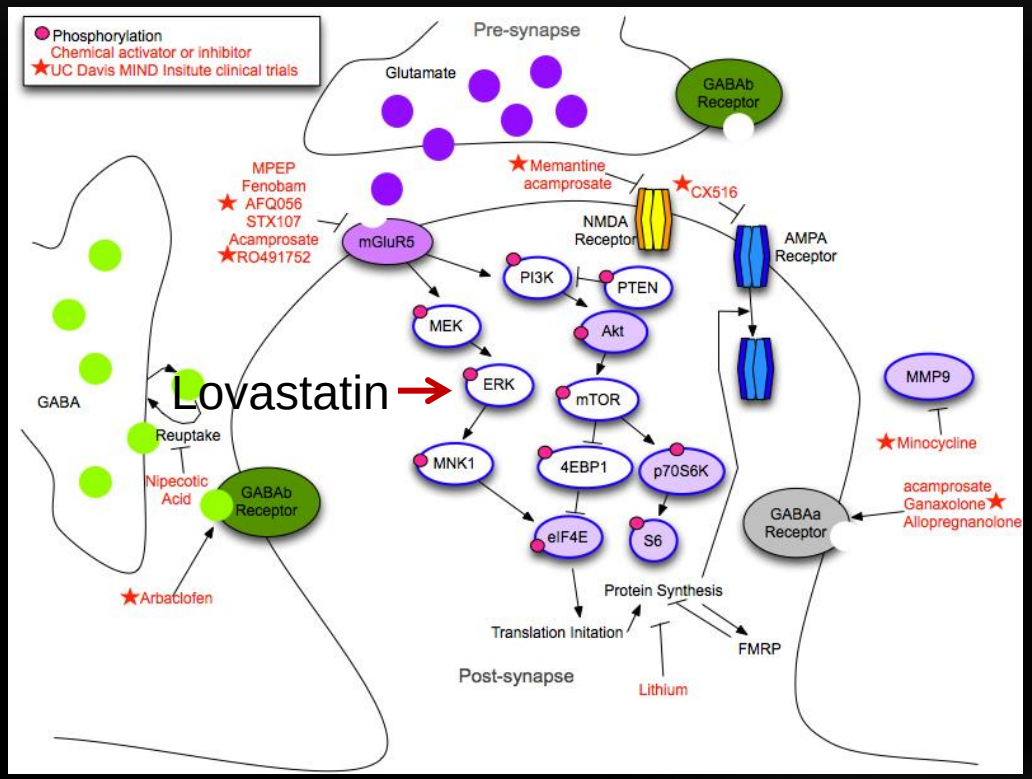


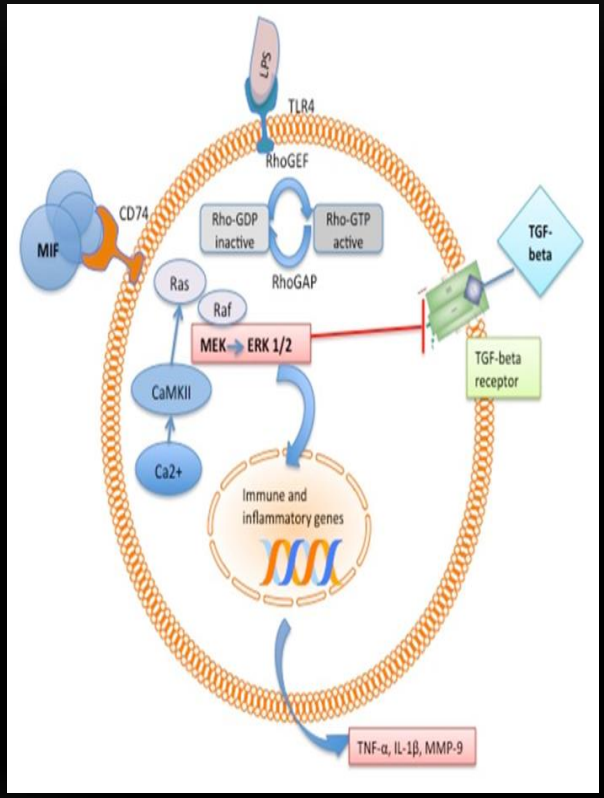


- Relationship between MIF, MMP-9, Ca2+ signaling, and the MEK/ERK pathway in inflammation.


POLUTION, ENVIRONMENTAL TOXICITY
EVIRONMENTAL CHANGES
MICRO ENVIRONMENT
SOCIAL INTERACTIONS
IMMUNE SYSTEM
GENES

LOVASTATIN AND LANGUAGE INTERVENTION





- Relationship between MIF, MMP-9, Ca2+ signaling, and the MEK/ERK pathway in inflammation.

TARGETED TREATMENTS MUST BE COMBINED WITH INNOVATIVE EDUCATIONAL PROGRAMS

- If synaptic connections are improved with targeted treatment we must enhance these connections with educational interventions

- Combine treatment trials with educational interventions, computer programs such as CogMed, AT devices, iPAD apps.

FX tracking game

iPAD apps

Co-Writer and write out loud

WHY RESEARCH ABOUT NEW TECHNOLOGIES IN DEVELOPMENTAL DISORDERS?

APPS **iPAD** **AAT** **TABLET** **FAMILY**
SCHOOL **THERAPY**

There **is little empirical research** on the key factors that promote or hinder:

- language improvement
- social communication progress and
- learning acquisition

in children with ASD and FXS or both, by using **an iPad®-centered intervention approach.**

PI: María Díez-Juan, M.A. Clinical Psychologist. ARTP Research Scholar.
MIND Institute. UC Davis

MIND APPs Objective

We aim to demonstrate the efficacy of the iPad®-centered intervention on *social communication, language development and academic gains* in children with *FXS & ASD.*

Methods

- iPad-centered intervention by collecting data on young children (2 to 5 years) and children (6 to 12 years) with ASD and/or FXS - 6 weeks.
- randomized clinical trial (RTC)
- 30 enrolled subjects
- Crossover

- We will use 

application as a platform to coordinate the whole plan and guidelines. Also to track data through parents' reports.

HOW WILL CLINICAL PRACTICE CHANGE

- All individuals would be treated – need clinic resources to accommodate management, patient education and monitoring – FXCRC
- Early diagnosis and treatment imperative – newborn screening
- Dosing may be tricky, combinations with different pathway targets may work best – need practitioners with FXS experience to assess response
- Likely stepwise improvement in treatments – need ongoing clinical trials network (FXCRC) to keep building best treatment protocols (like cancer tx model)

Collaborators

M.I.N.D. Institute Clinical Trials

Yingratana McLennan
Norman Brule
Mary Jae Leigh
Kushma Govindappa
Gayatri Mahajdan
Michele Ono
Emma Hare
Alyssa Hamlin
Lindsey Partington
Kindra Root
Tasleem Chechi
Jonathan Polussa
Maria Diez-Juan

Dept. Radiology
James Brunberg

Dept. Pathology
Claudia Greco

NTRI Researchers
Isaac Pessah
Rob Williamson
Rob Berman

Dept. Neurology
Lin Zhang
John Olichney
Ricardo Maselli
Grace Fenton-Farrell

UC Davis School of Medicine

Dept. Biochem & Molec. Medicine
Paul Hagerman Flora Tassone
Chris Iwahashi
Anna Ludwig
Dolores Garcia-Arocena
Greg Mayeur Chris Raske

Dept. Biostatistics
Danh Nguyen

University of Washington and UC Davis Fragile X Research Center NICHD Funded
Charles Laird Mike Guralnick Gwen Glew

University of Colorado Health Sciences Center (Denver)
Nicole Tartaglia Maureen Leehey James Grigsby ; Karen Riley at DU

RUSH-Presbyterian-St. Luke's Medical Center (Chicago)
Elizabeth Berry-Kravis Deb Hall Christopher Goetz

Waisman Center-University of Wisconson
Len Abbeduto has come to the MIND

Latrobe University, Melbourne Australia
Danuta Loesch Richard Huggins

Support: NICHD, NINDS, NIA, NFXF, CDC, NFXF
Neuropharm, Roche, Novartis, Seaside Therapeutics