LOSS OF SKILLS AND ONSET PATTERNS IN NEURODEVELOPMENTAL DISORDERS: UNDERSTANDING THE NEUROBIOLOGICAL MECHANISMS

February 19, 2016

National Institute of Mental Health
Neuroscience Center
6001 Executive Boulevard
Rockville, Maryland 20852

Conference Call Access:
Phone: (800) 369-2126
Access Code: 2193178

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Morning Agenda

9:00 AM **Welcome & Introductions**

Audrey Thurm, Ph.D.
Staff Scientist, Pediatrics and Developmental Neuroscience Branch (NIMH)

Ann Wagner, Ph.D.
Chief, Neurobehavioral Mechanisms of Mental Disorders Branch
Division of Translational Research (NIMH)

9:10 **Goals of the Workshop**

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Morning Agenda

9:15 **Session 1: Normative Development and Worsening/Declining Onset Patterns in Neurodevelopment**

Lonnie Zwaigenbaum, M.D., Chair
Associate Professor and Director of Autism Research
University of Alberta

9:20 **Regression (NT and NDDs), the Importance of Timing and Cross-Syndrome Comparisons**

Annette Karmiloff-Smith, Ph.D.
Department of Psychological Sciences
University of London

CBCD Centre for Brain and Cognitive Development

Regression (NT & NDDs), the importance of timing and of cross-syndrome comparisons

Annette Karmiloff-Smith
Centre for Brain & Cognitive Development
Birkbeck-University of London

Babylab

Bethesda workshop - Feb.2016

Outline

NT: Examples of regression/loss of skills in neurotypical development

- Behavioural regression vs representational *pro*gression

- Balance – synapse strengthening vs pruning weak connections

NDD – Williams syndrome: lack of specialisation/localisation of neural function

possible lack of pruning?

NDD-ASD: Threshold too high – over-aggressive pruning

Regression due to pruning of strong connections,

Sequence of regressive behaviours hypothesized:

sensory -> motor -> language -> executive function

Early differences: ASD-sibs, controls and other NDDs

Behavioural regression vs representational progression in NT language

Past tense/plural marking in early language development:

T-1 Correct: Papa went/Me caught ball/wet feet

Isolated representations

T-2 Behavioural regression: Papa goed/Me catched ball/wet foots

T-2 Representational progression: overgeneralisation of –ed past tense/-s plural patterns

T-3 Correct as at T-1: Papa went/Me caught ball/wet feet

Representations have now become part of a system of morphological markers

Question we must ask of ASD regression:
Is behaviour *before* regression sustained by same mental reps as behaviour *after* the regression?
Is child *regaining* skills, or are the later underlying skills *different* even if overt behaviour seems the same?

NT loss of skills in infancy: face processing

3-6 month olds: discriminate own-race faces
discriminate other-race faces
discriminate other-species faces

9-10 month-olds: ability lost for other-race/other species due to pruning of lesser-used connections and strengthening of used ones

12 months: if experience given at 9 months with faces of other-race/other species, then ability = retained

Pascalis, Scott, Kelly, Shannon, Nicholson, Coleman & Nelson, 2005
Kelly, Quin, Slater, Lee, Ge & Pascalis 2007
Heron-Delaney, Wirth, & Pascalis, 2011

NT loss of skills in infancy: speech processing

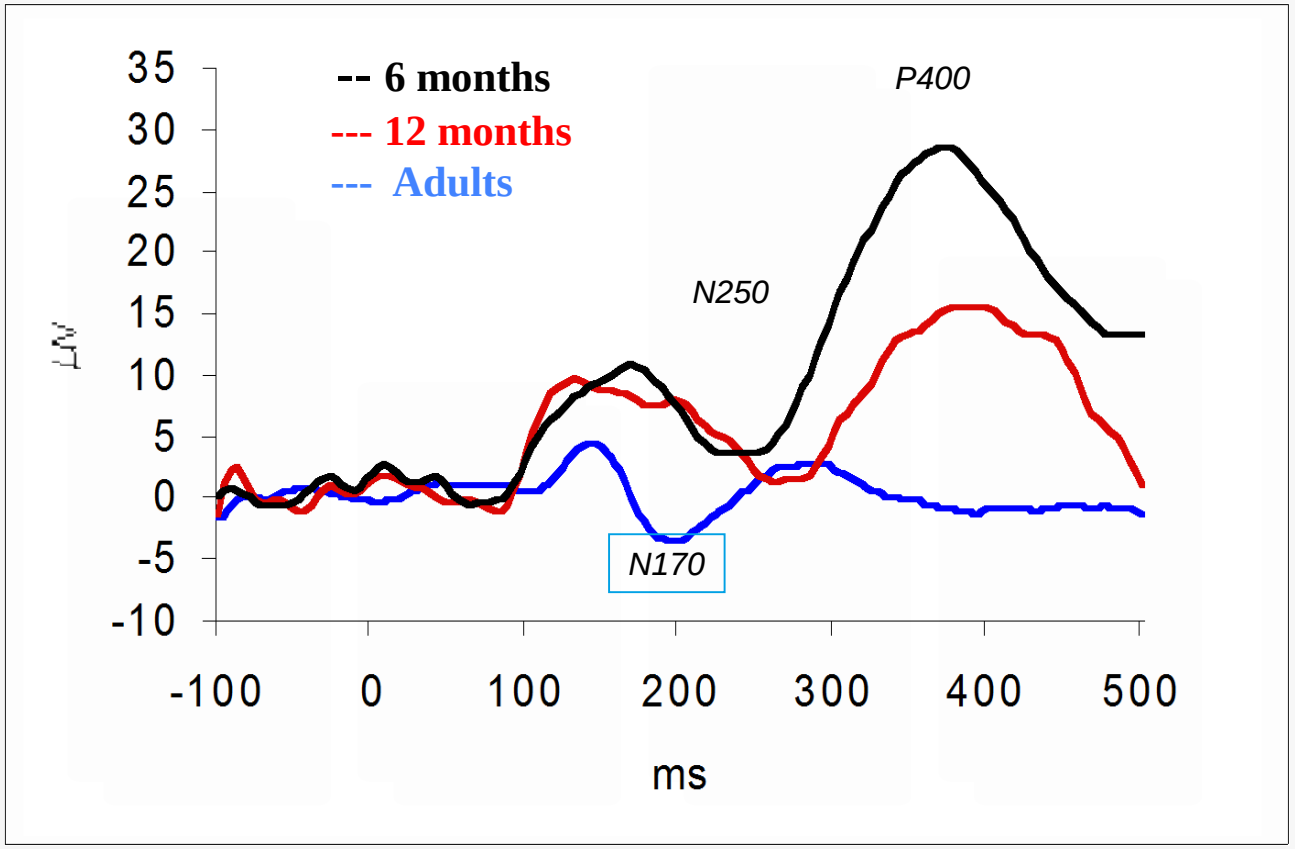
3-6 month olds:	discriminate phonemes in native language discriminate phonemes in non-native languages discriminate other-species' cries??
9-10 month-olds:	ability lost for non-native, due to pruning of lesser-used connections and strengthening of used ones
12 months:	if experience given at 9 months with non-native phonemes, ability retained

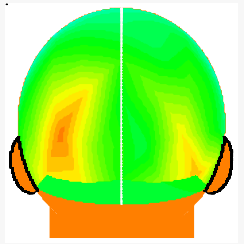
Different groups for face/speech. Single *domain-general* mechanism?
Need to research faces/speech tasks in *same* infants

Face and speech processing undergo perceptual narrowing in NT
Balance: strengthening of used connections + pruning of unused connections
Progressive specialisation and localisation of neural function

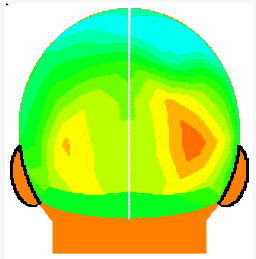
Tees & Werker, 1984
Nazzi, Jusczyk, & Johnson, 2000
Maye, Werker & Gerken, 2002
Best & McRoberts, 2003

Specialization and localisation of brain function are *progressive*

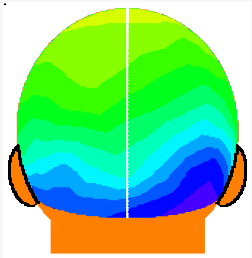




6 months



12 months

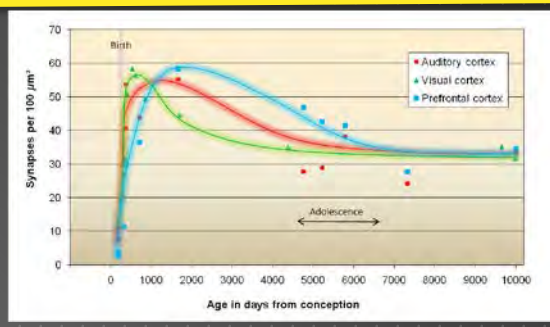


Adults

From de Haan, Pascalis & Johnson, 2002; Halit, de Haan & Johnson, 2005

See, also, Pascalis, deHaan & Nelson, 2002

Hypotheses re NDDs: differences in pruning thresholds and specialisation/localisation of neural function?



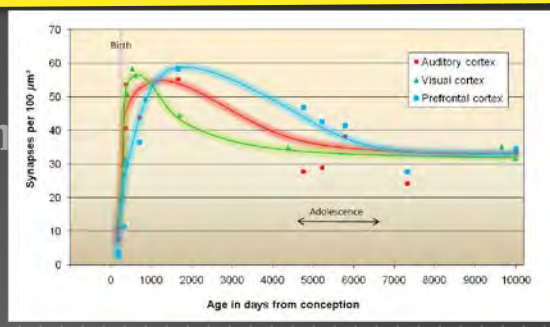
NT – Normal pruning threshold
Balance: strengthening/pruning
Different timing across neural regions
Specialisation = experience-dependent, progressive

WS? Under-pruning – imbalance stengthening/pruning?
Lack of neural specialisation despite behavioural mastery

ASD? Over-pruning – much higher pruning threshold than NT
Regression seen if development underway, camouflaged if development sl
Different timing of pruning across neural regions:
regression: sensori->motor->language->executive function

Huttenlocher & Dabholkar, 1997; Huttenlocher, 2002
Thomas, Knowland, & Karmiloff-Smith, 2011

Hypotheses re NDDs: differences in pruning thresholds and specialisation/localisation of neural function?



NT – Normal pruning heshold
Onset of pruning = timing maturatioanlly constrain
(individual differences?)
Different timing across neural regions
Specialisation = experience-dependent, progressive

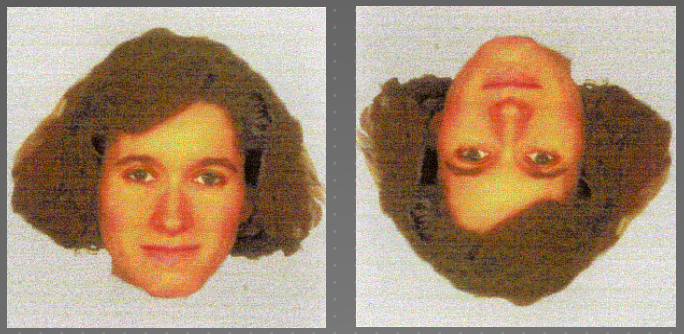
WS? Under-pruning – imbalance strengthening/pruning?
Lack of neural specialisation despite behavioural mastery

ASD? Over-pruning – much higher pruning threshold than NT
Regression seen if development uderway, camouflaged if development slo
Different timing of pruning across neural regions:
regression: sensori->motor->language->executive function

Huttenlocher & Dabholkar, 1997; Huttenlocher, 2002
Thomas, Knowland, & Karmiloff-Smith, 2011

WS face processing:
behavioural scores "in the normal range"

Different teams worldwide:
WS face processing: *'in the normal range'*
on standarised tasks (Benton, Rivermead)



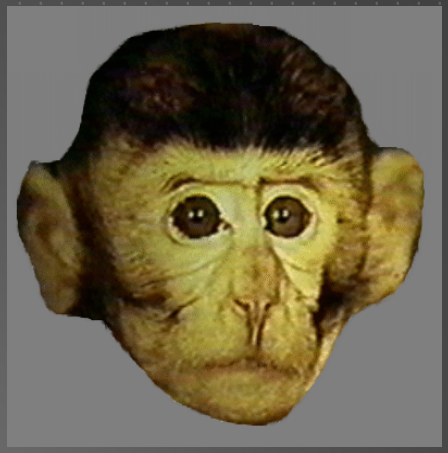
BUT inversion effect (hallmark of *configural* processing)
doesn't emerge developmentally at any age in WS

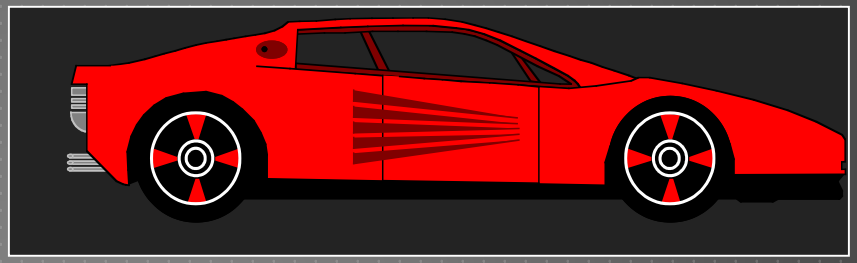
WS brain signature?

Karmiloff-Smith et al., 2004

Temporal neural signatures for face and car processing in WS

WS adolescent in
Geodesic HD-ERP net

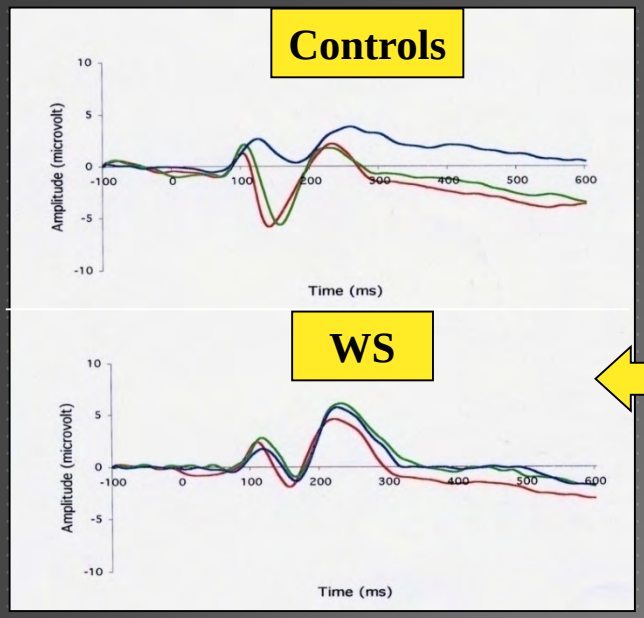




All Ss in “normal range” on standardised face processing tasks

Grice, deHaan, Halit, Johnson, Csibra, & Karmiloff-Smith, 2003

Behavioural scores in normal range… but different *neural* processes

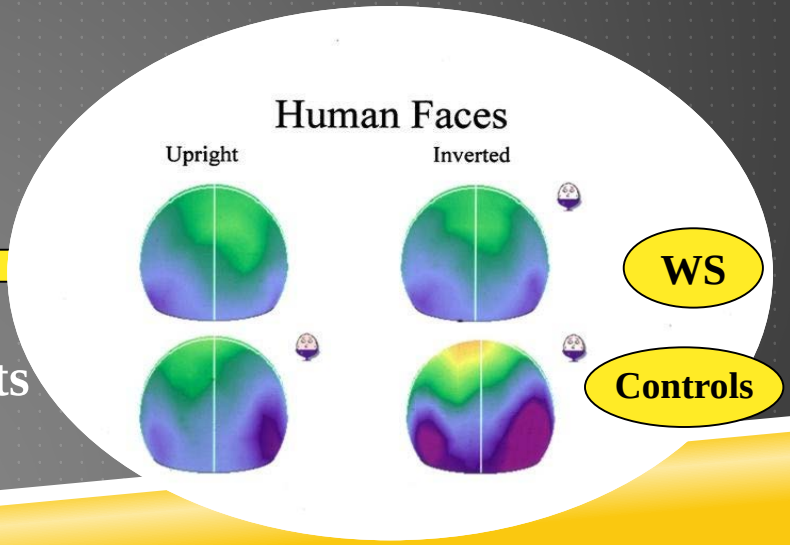


Healthy controls:
Progressive processing restriction of input type

WS: failure to specialise

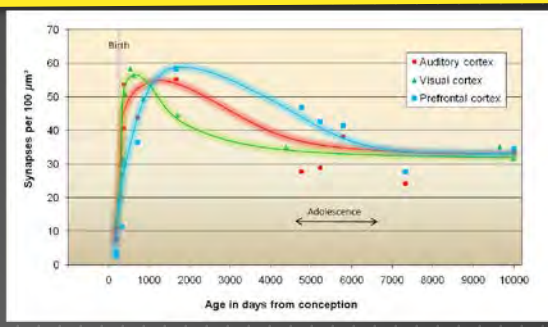
WS: failure to localise

Healthy controls:
Progressive restriction of brain circuits



Featural processing

Hypotheses re NDDs: differences in pruning thresholds and specialisation/localisation of neural function?



NT – Normal pruning heshold
Balance: strengthening/puning
Different timing across neural regions
Specialisation = experience-dependent, progressive

WS? Under-pruning – imbalance strengthening/pruning?
Lack of neural specialisation despite behavioural mastery

ASD? Over-pruning – much higher pruning threshold than NT
Regression seen if development underway, camouflaged if development slow
Different timing of pruning across neural regions:
hypothesis-regression: sensori->motor->language->executive function

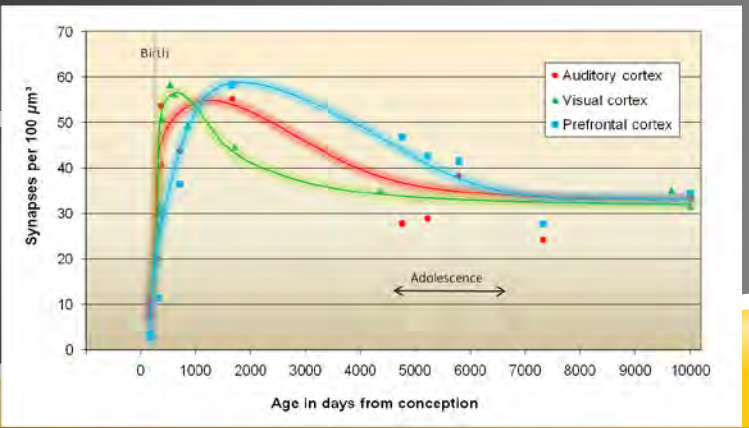
Huttenlocher & Dabholkar, 1997; Huttenlocher, 2002
Thomas, Knowland, & Karmiloff-Smith, 2011

Over-pruning hypothesis – ASD?

Not necessarily rare gene mutation but allelic difference in common gene(s) affecting pruning threshold -> exaggeration of normal developmental pruning process - ASD very high pruning threshold means not not only weak unused connections are pruned, but also stronger ones -> regression

Pruning occurs at different times in different brain areas (Huttenlocher & Dabholkar, 1997)
- Predicts -> first few months, prior to pruning = normal, followed by behavioural symptoms: 1. sensori-motor 2. social/language 3. executive function

Other individual difference factors interact with pruning threshold to create risk, leading to differences in ASD trajectories



Need to consider balance of specialisation vs pruning?
?NT tasks with at risk ASD infants?

Huttenlocher & Dabholkar, 1997; Huttenlocher, 2002
Thomas, Knowland, & Karmiloff-Smith, 2011
Rogers, 2009; Staples & Reid, 2010
Estes, Zwaigenbaum…IBIS group, 2015

Differences in early development
ASD sibs, controls, other NDDs? What is ASD-specific?

- Atypical saccadic eye movements (WS also)
- Shorter fixation times to social and non-social scenes (Wass et al., 2015) (DS also)
- Attention to eyes declines between 2-6 months (Jones & Klin, 2013) (Rett/FXS?)
- Atypical face processing in infants/adults (featural vs configural) (D'Souza et al. 2015) (WS also)
- Follow head shift but not eye gaze shift (Thorup et al., 2016) (WS also)
- Lack of triadic attention (WS also)
- ERPs to dynamic eye gaze differ (Elsabbaghet al., 2012) (NDDs unknown, being tested)
- EEG frontal-occipital hyperconnectivity (Orekhova et al., 2014) (NDDs unknown, being analysed)
- Disengagement problems (Sacrey, Bryson & Zwaigenbaum, 2013) (WS also)
- Lack of attn to/discrimination of speech/pitch stimuli (D'Souza,Karmiloff-Smith, 2016) (FXS also)
- Enhanced visual search (Kaldy, Kraoewr, Carter & Blaser, 2013; Gliga,et al., 2015) (unique)

Similar cross-syndrome exercise re brain differences

More subtle with cross-syndrome than with NT

Concluding thoughts…

NT: initially surplus neurocomputational resources to retain flexible response to environment; then strengthening of used connections/pruning of weak connections to save metabolic

NT: skills not "regained"; change of underlying representations/change of function

ASD? Suffice to have a mutation on a common gene with risk allele regulating pruning threshold
More aggressive, so risk of pruning not only under-used connections but also good ones -> regression.

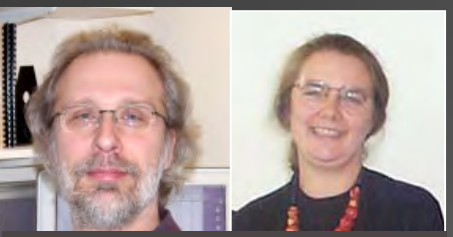
Regression: If behavioural development slow in ASD, then above could happen before behavioural skills emerge and therefore camouflage regression

Regression should first occur in sensori-motor patterns:
Parents likely to notice language loss, but loss of reaching/pincer grip?

If pruning too rapid/aggressive -> lack of flexible response to environment -> Rigidity/repetitive behaviours?

Need to focus on individual differences and subtle cross-syndrome comparisons rather than group data compared to NT controls

Joint work mentioned in talk with past and current Colleagues/Postdocs/Students

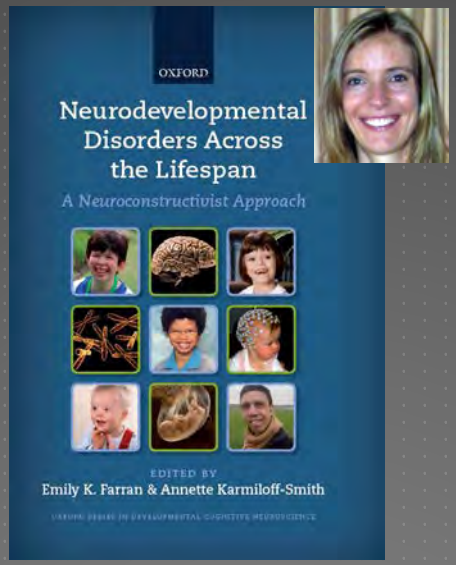


Mark Johnson

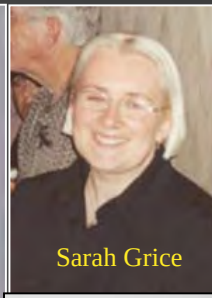
Sarah Jeeves

Gaia Scerif

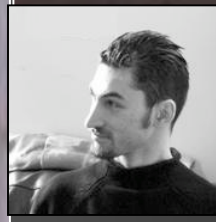
Michelle de Haan



Michael Thomas



Sarah Grice



Dean D'Souza

Victoria Knowland

wellcome trust

MRC Medical Research Council

NATIONAL INSTITUTES OF HEALTH

E·S·R·C ECONOMIC & SOCIAL RESEARCH COUNCIL

AWA

AUTOUR DES WILLIAMS
ASSOCIATION FRANCOPHONE DU SYNDROME DE WILLIAMS & BEUREN

williams SYNDROME FOUNDATION

downsed

DOWNS SYNDROME ASSOCIATION
Reg Charity 1061471

EUROPEAN SCIENCE FOUNDATION

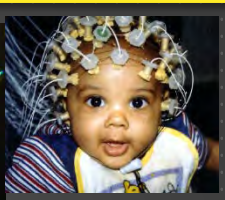
The Fragile X Society

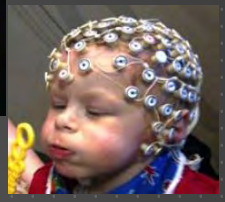
thewaterloofoundation

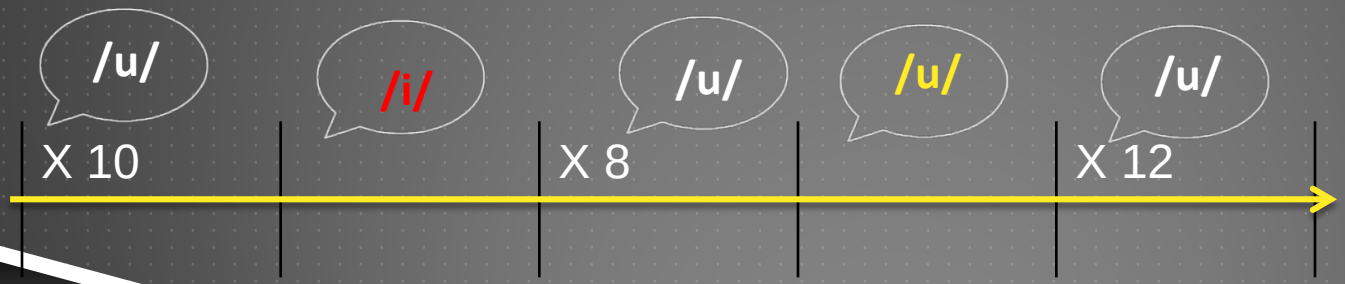
END

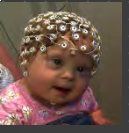
Cross-syndrome comparison
WS/DS/FXS/Sibs (MA-matched on Mullen)

- 70% standards: /u/ low pitch
- 15% speech deviants: /i/ low pitch
- 15% pitch deviants: /u/ high pitch

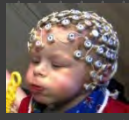


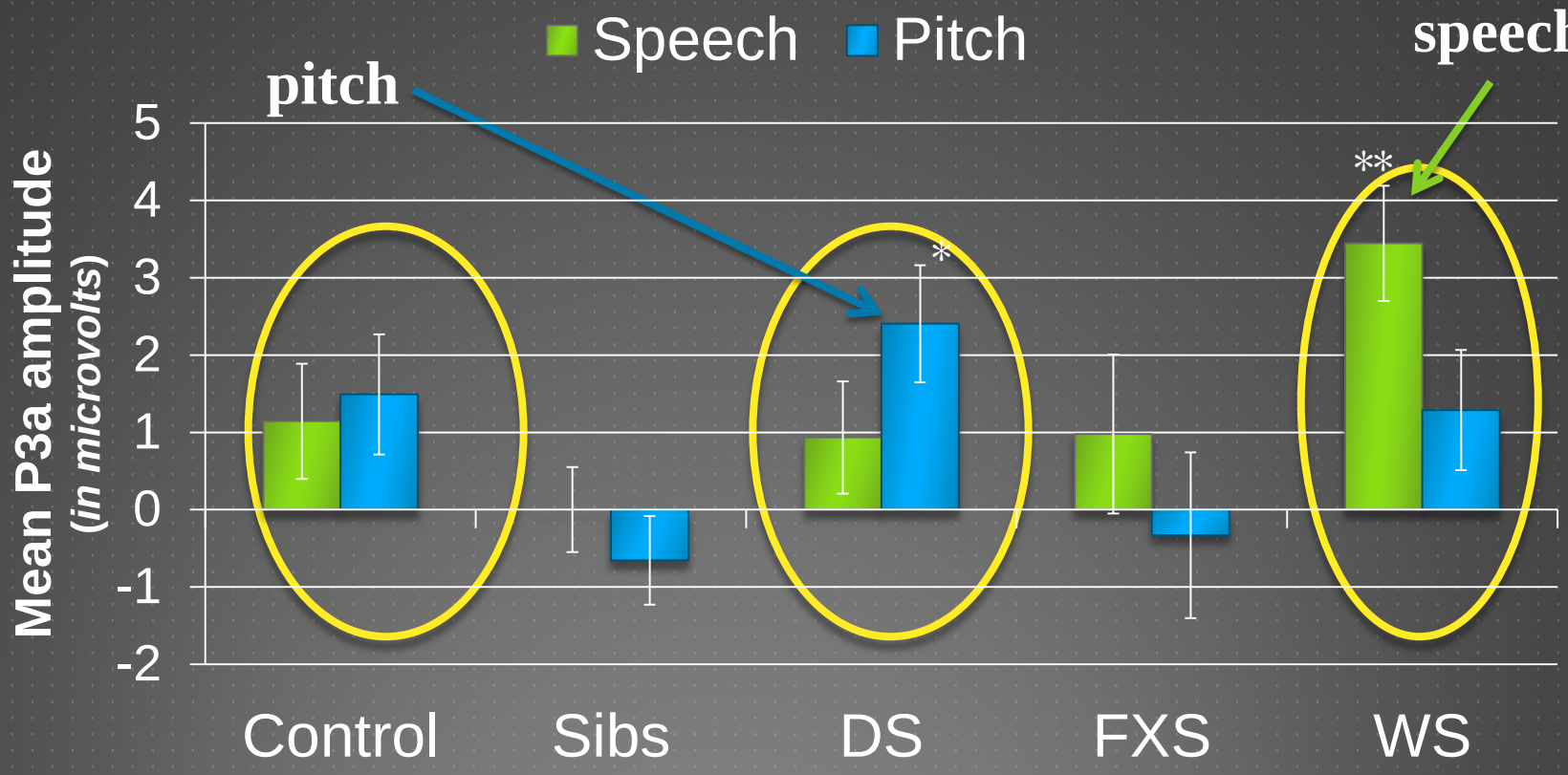






Cross-syndrome neural differences:
P3a (250-350ms-attentive orientation) to pitch/speech

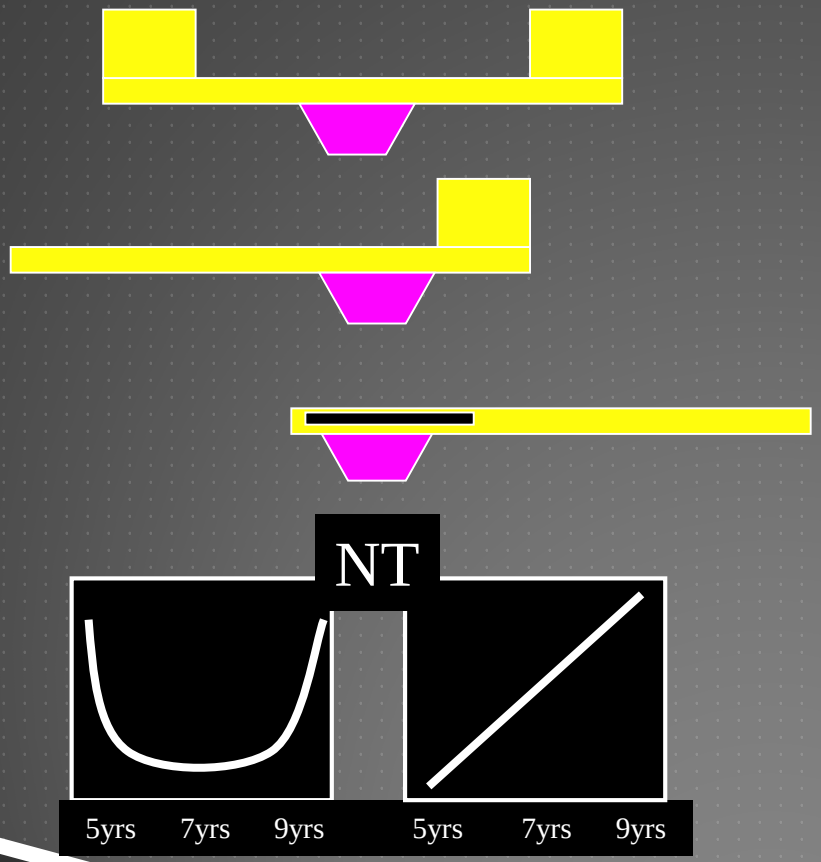




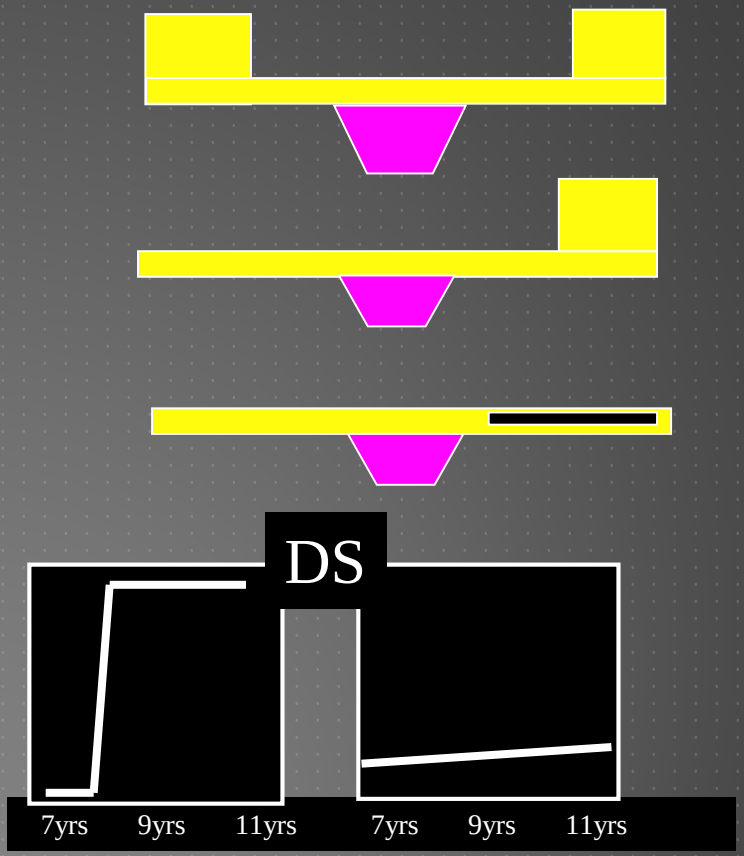
D'Souza, Karmiloff-Smith et al., 2016

Behavioural regression vs representational progression in NT vs Down syndrome

5 and 9 year olds



7 year olds



Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Morning Agenda

9:40 **Regression in Autism Spectrum Disorders**

Kasia Chawarska, Ph.D.
Associate Professor, Child Study Center and Pediatrics
Yale University

10:00 **Early Brain Development in Autism: Neuroimaging Studies of the First Years of Life**

Jason Wolff, Ph.D.
Assistant Professor, Educational Psychology
University of Minnesota

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Morning Agenda

10:20 **Panel Discussion**

Discussant: Lonnie Zwaigenbaum, MD

10:50 **Break**

11:00 **Session 2: How Can We Explore the Biological Basis for Regression Observed in Young Children?**

Jeff Neul, Ph.D., Chair
Chief, Division of Child Neurology
Professor and Vice Chair
Department of Neurosciences
University of California, San Diego

Break

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Morning Agenda – continues

11:05 **Modeling Rett Syndrome in a Dish – Ideas about Disease Regression**

Alysson Muotri, Ph.D.
Associate Professor
UCSD Stem Cell Program, School of Medicine
University of California, San Diego

11:25 **Shaping Brain Circuits by Experience: Uncovering Aberrant Plasticity in Mouse Models of Rett Syndrome**

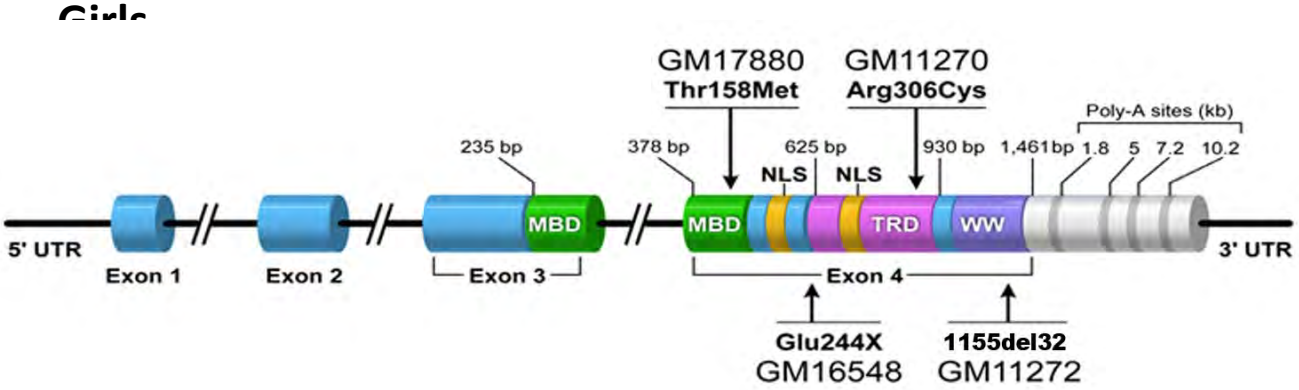
Keerthi Krishnan, Ph.D.
Research Investigator
Department of Neuroscience, Cold Spring Harbor Laboratory

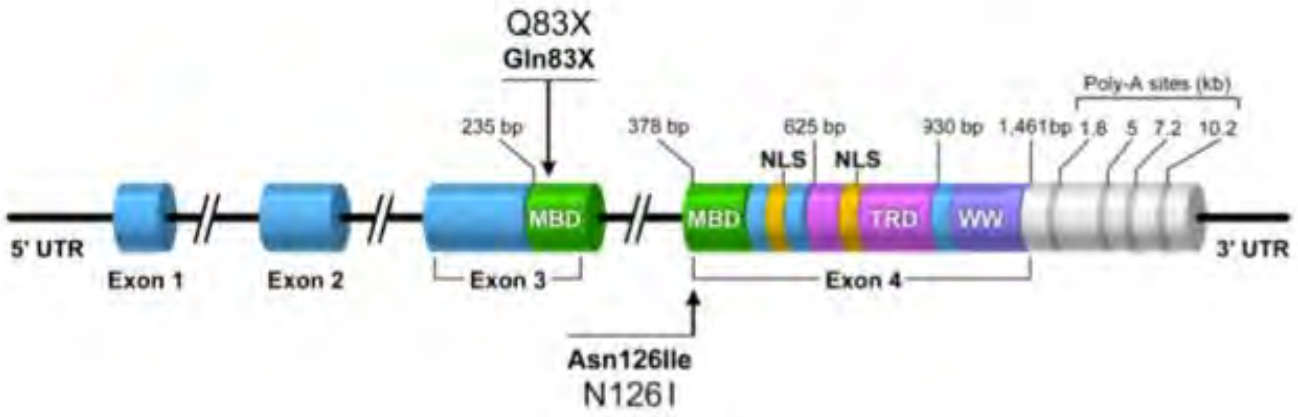
Modeling Rett syndrome with human neurons – insights into regression

Alysson Renato Muotri
University of California San Diego
Dept. Pediatrics/Cellular Molecular Medicine

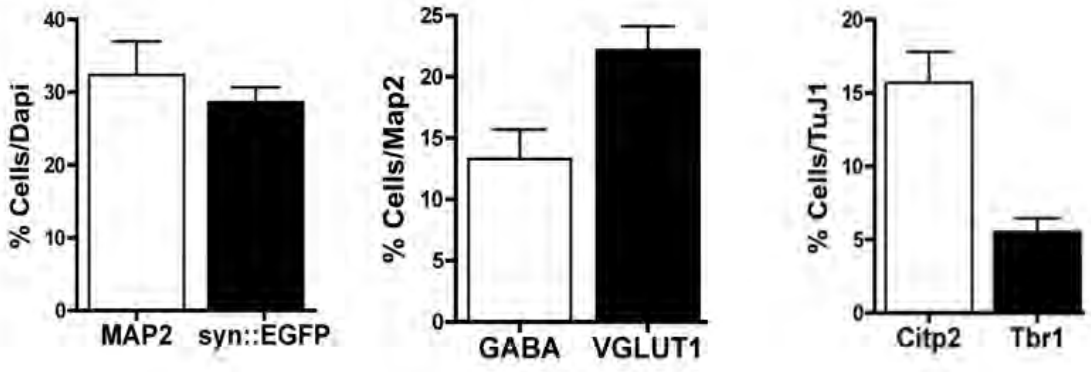
MeCP2 mutants

(X-LINKED GENE)



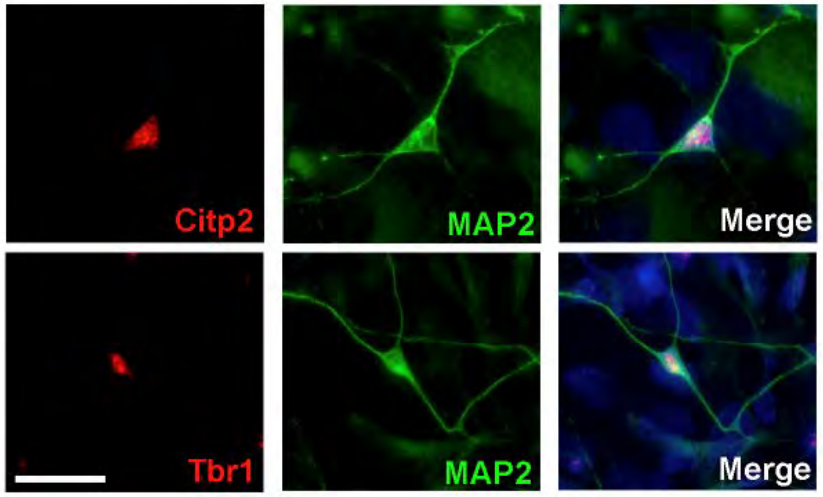


iPSC-derived cortical neurons



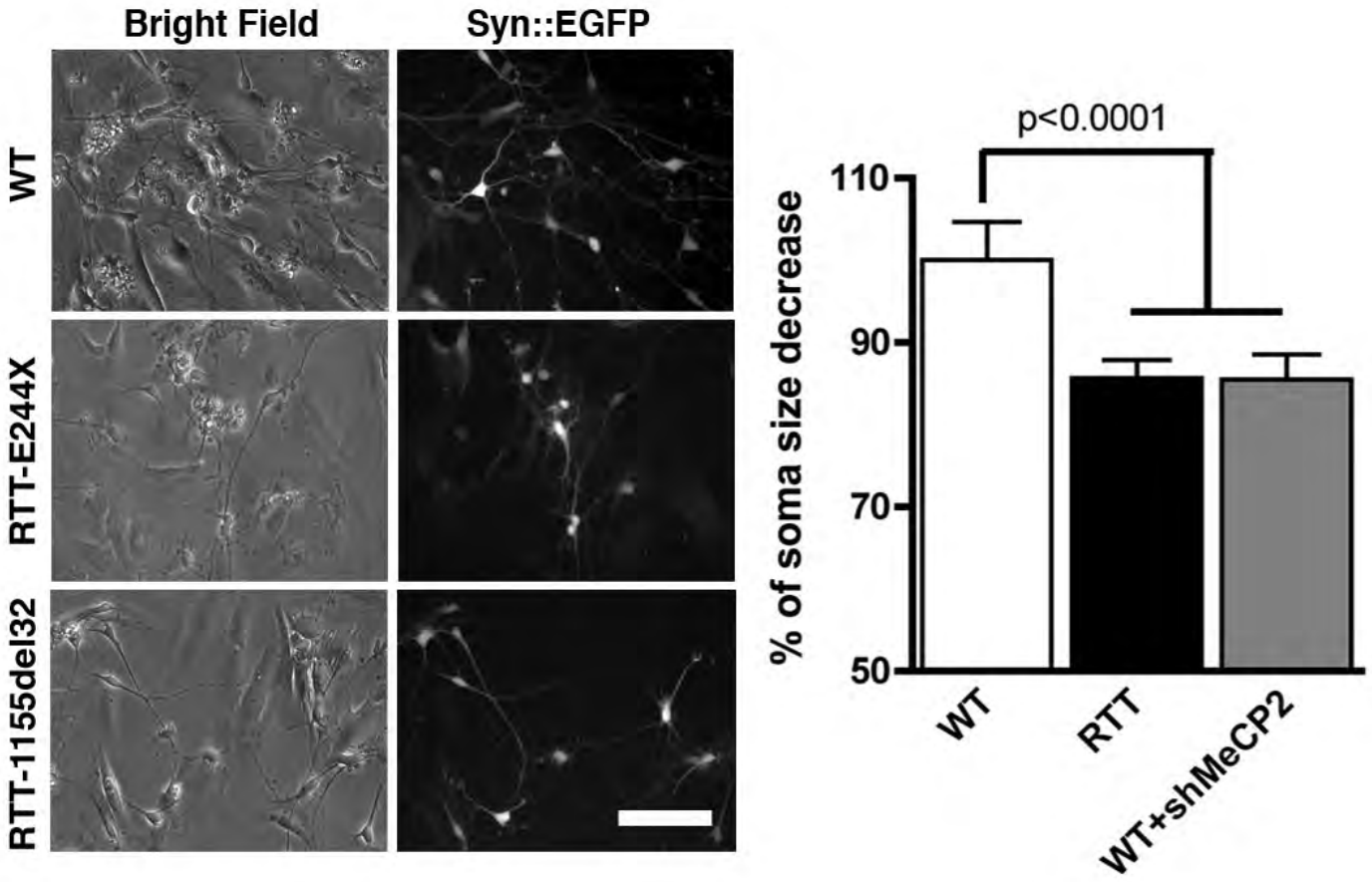
Layers V, VI

Layers I, IV

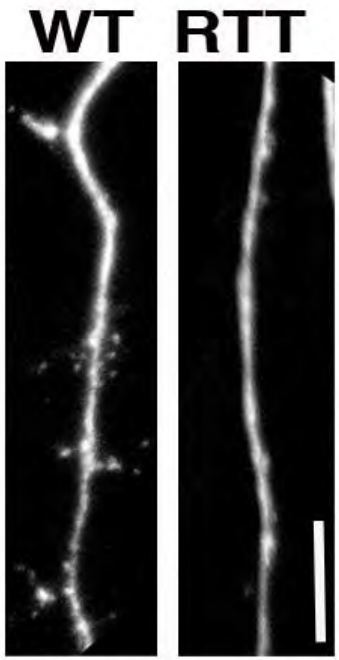


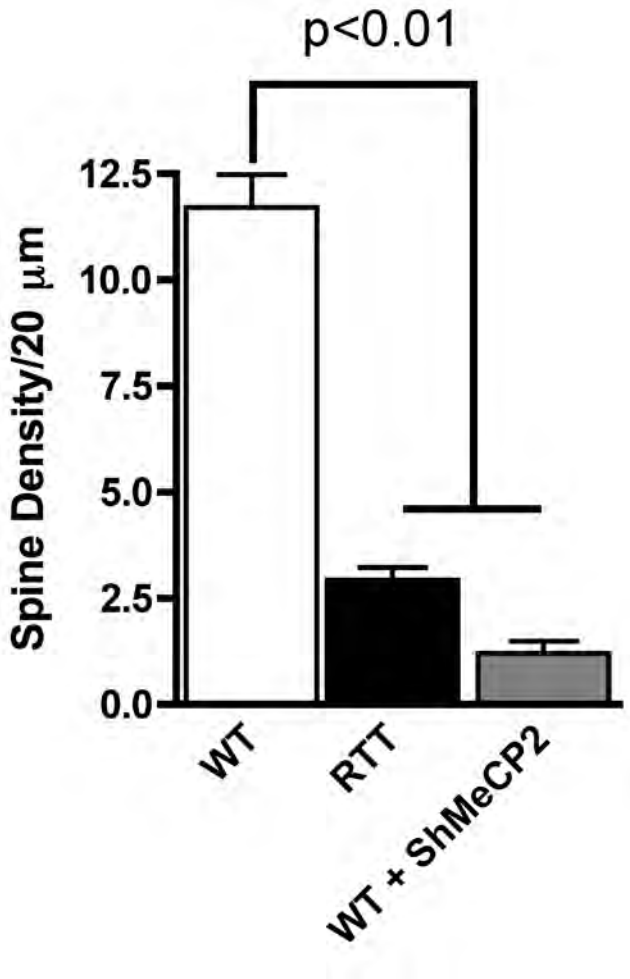
*Expression of Peripherin and En1 (midbrain) were not detected.

RTT neurons have smaller cell neuronal soma

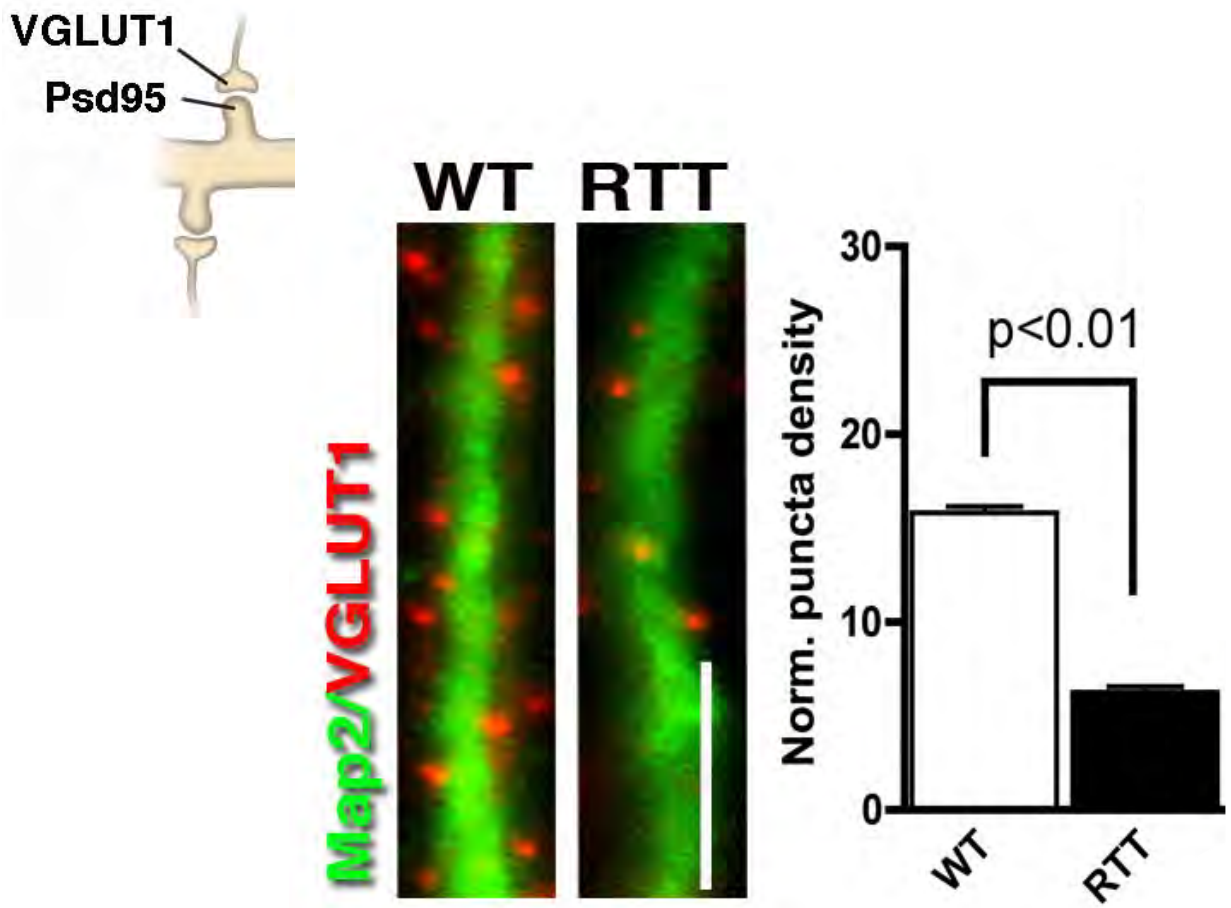


RTT neurons have lower spine density

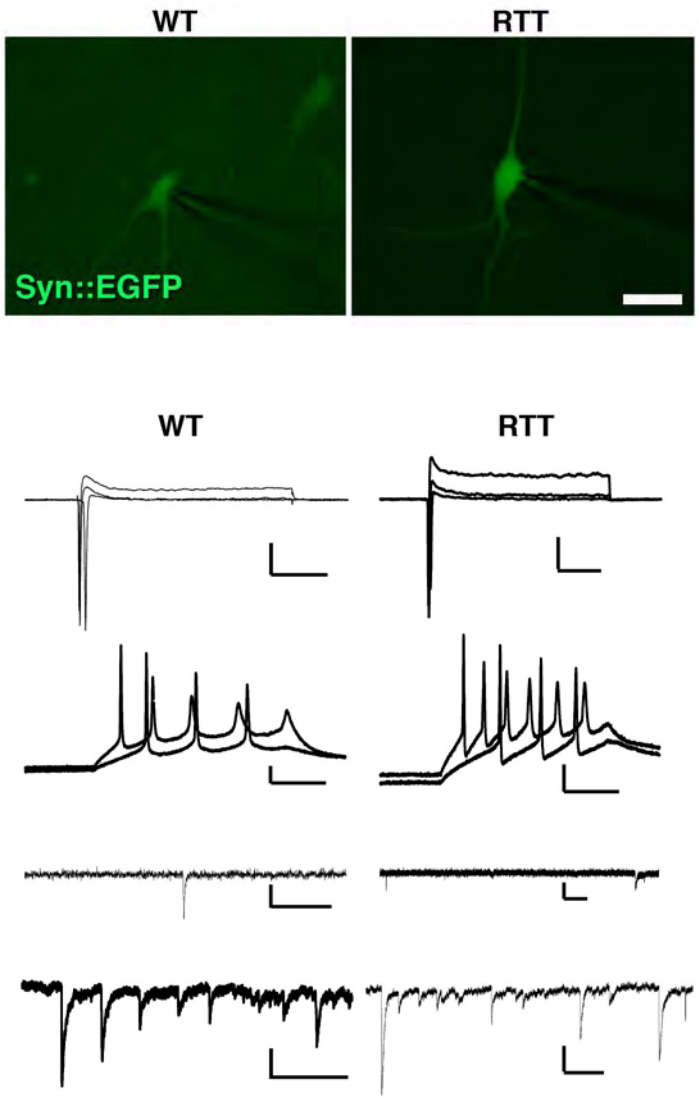


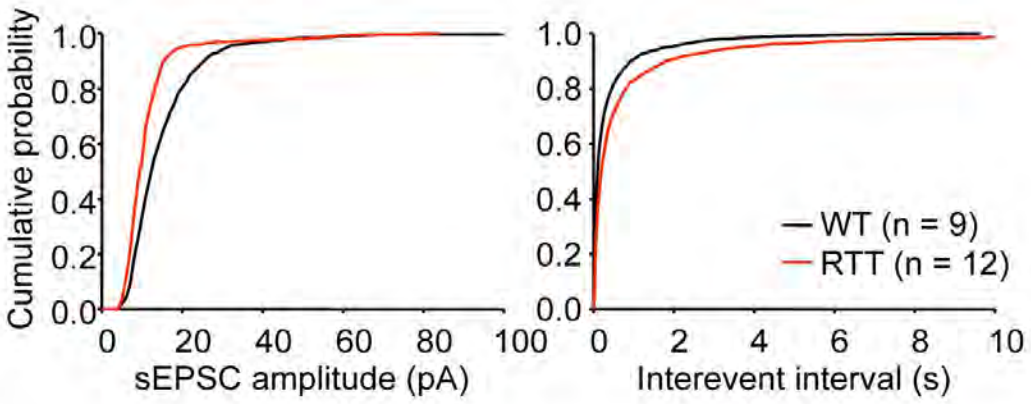


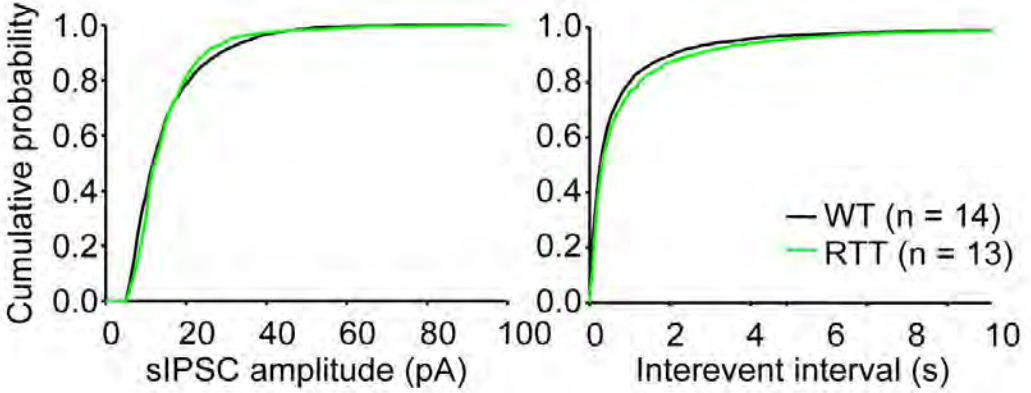
RTT neurons have fewer glutamatergic synapses



Decrease frequency of spontaneous postsynaptic currents in RTT neurons

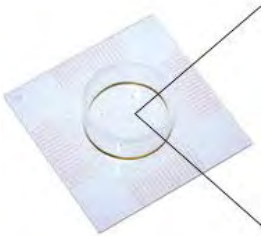


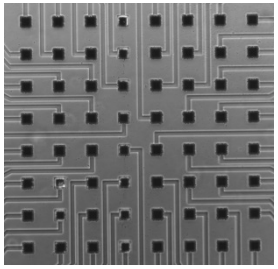


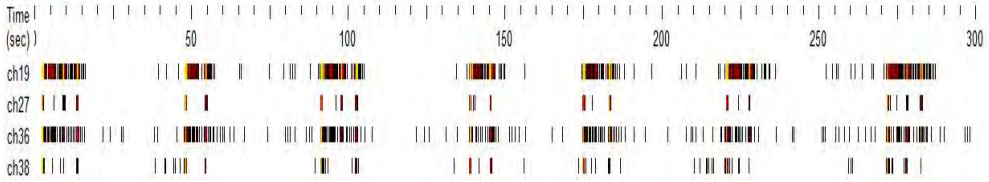


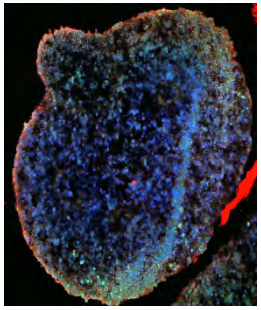
Gong Chen

RTT neuronal networks are not synchronized

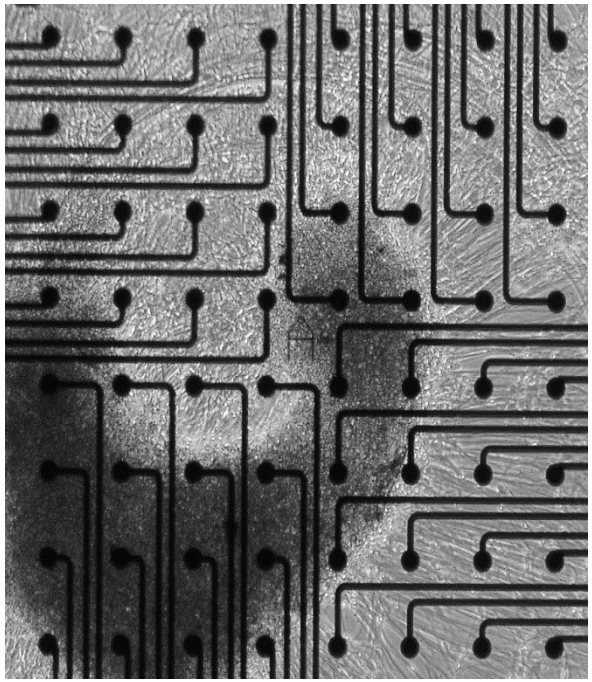


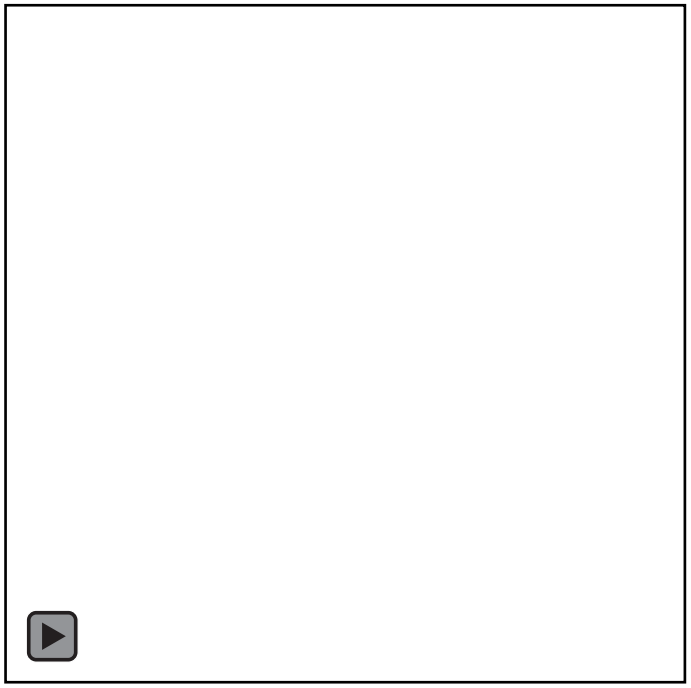






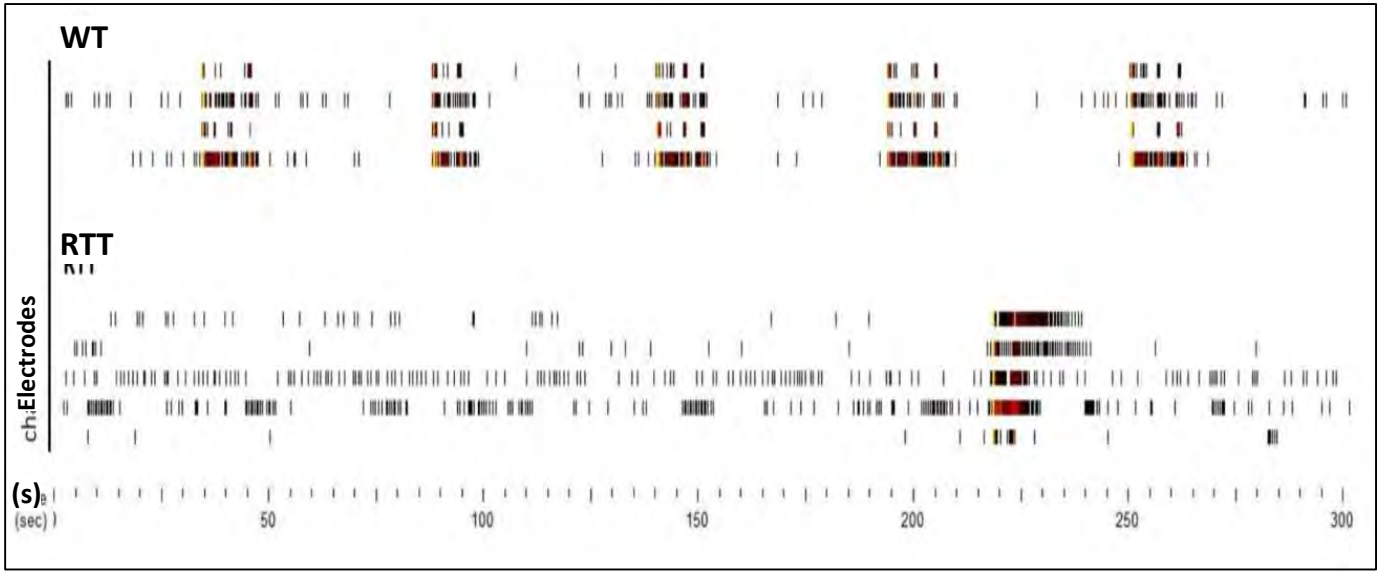
3D corticoids

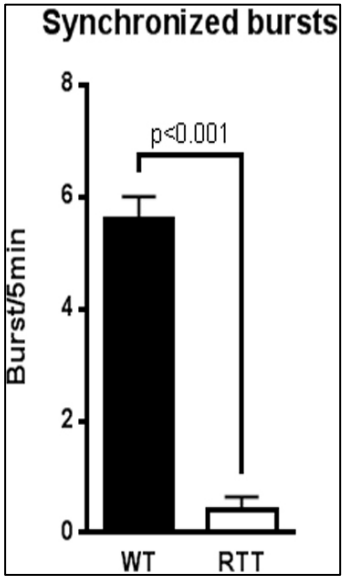




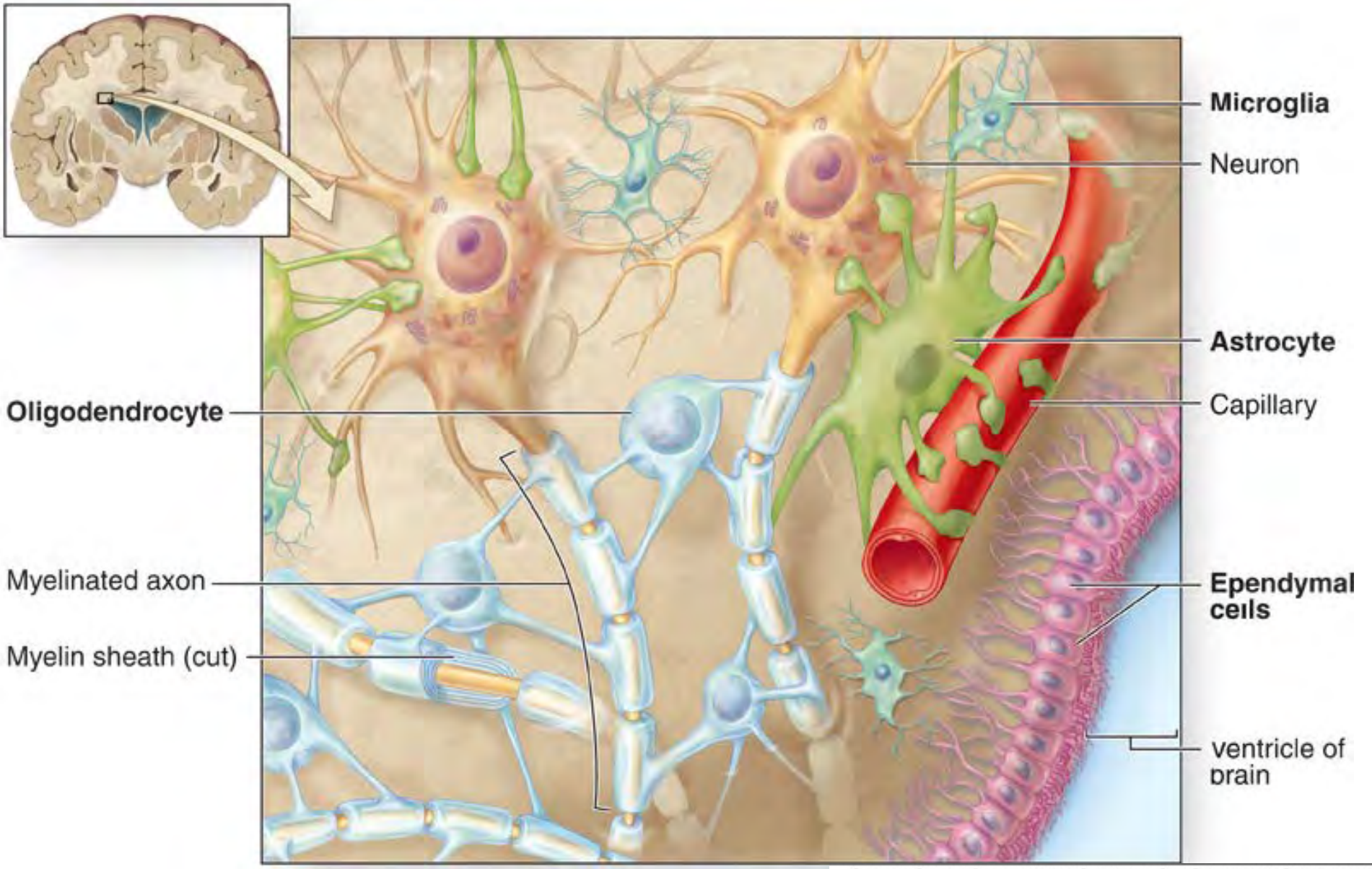
Activity Map

RTT neuronal networks are not synchronized

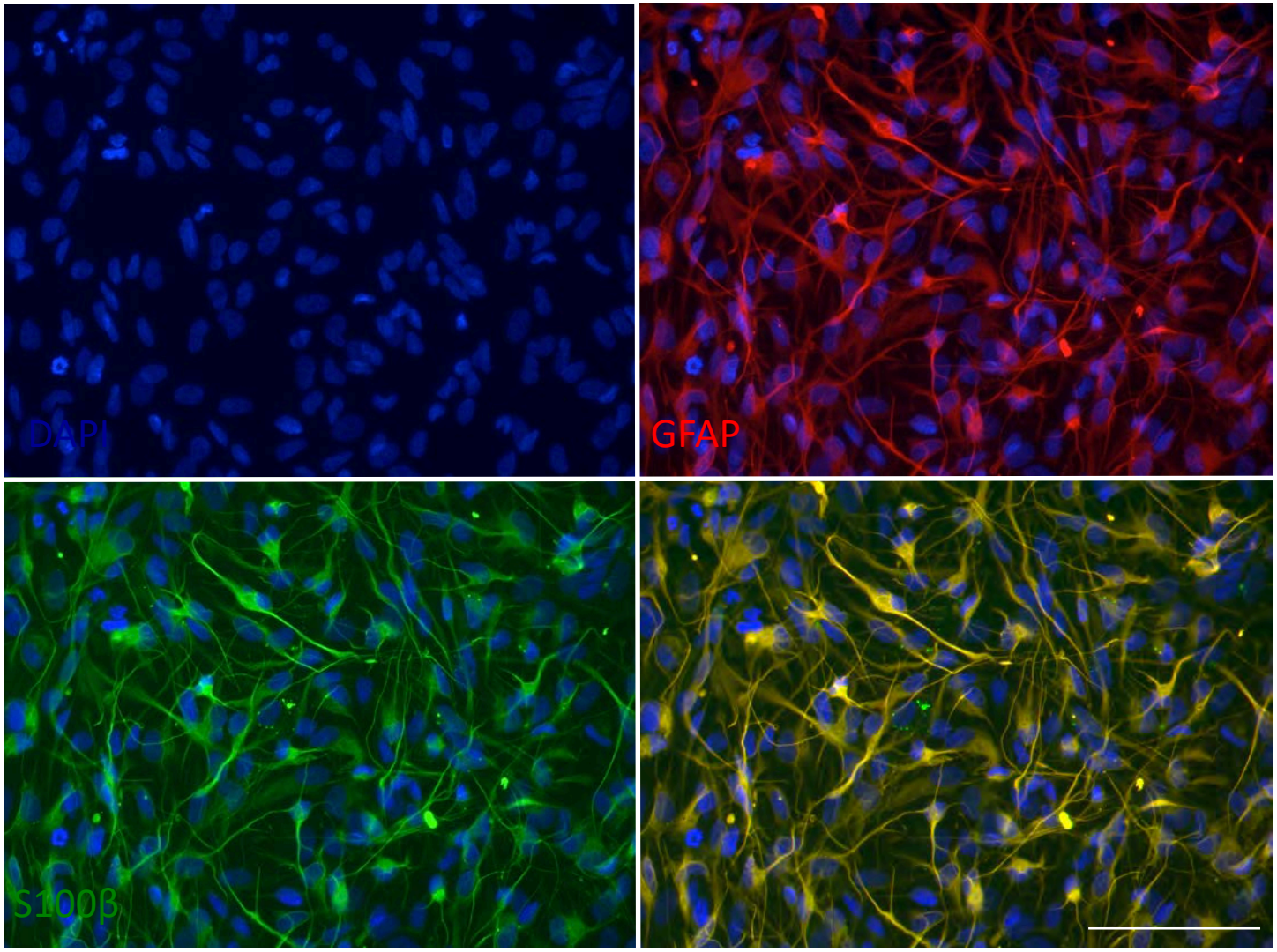




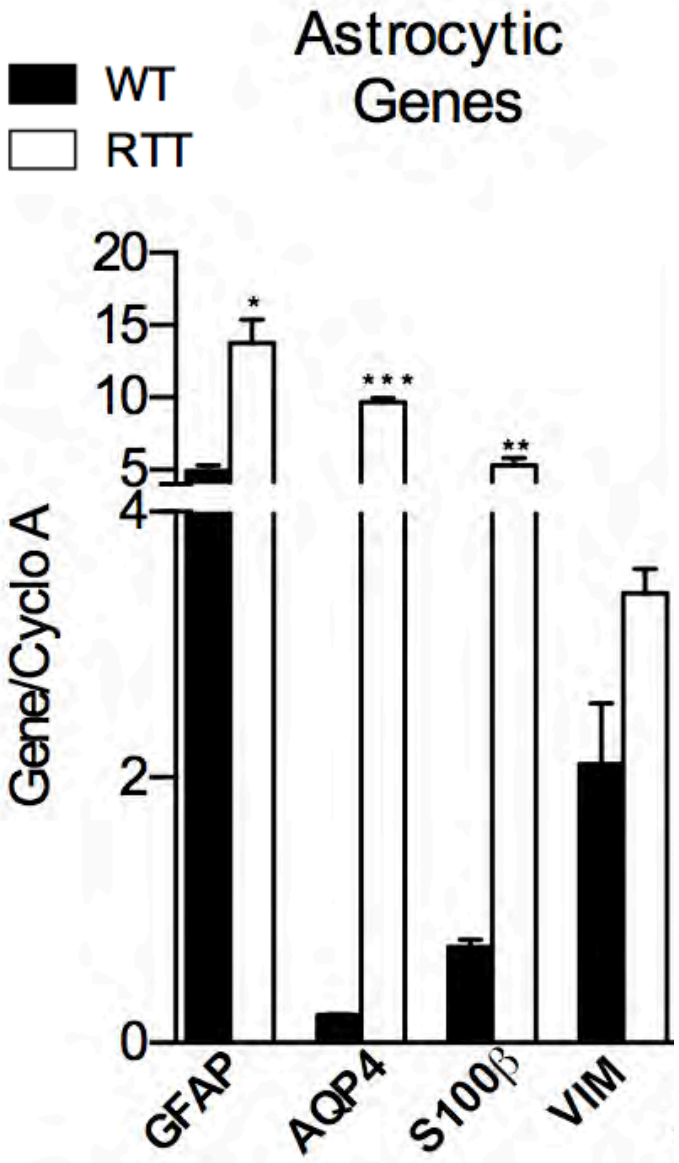
GLIA



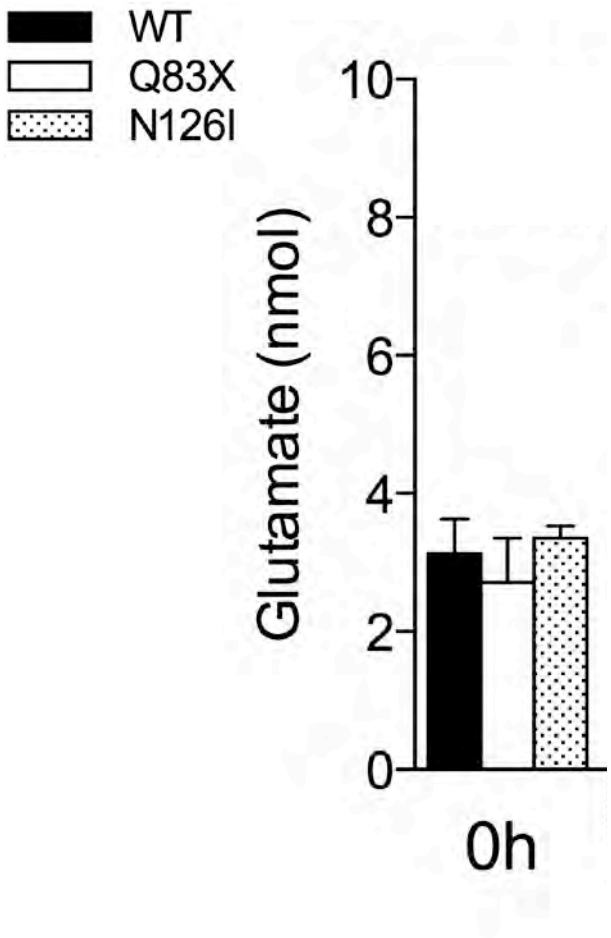
Muotri lab iPSC astrocyte protocol (30 days, no growth factors)



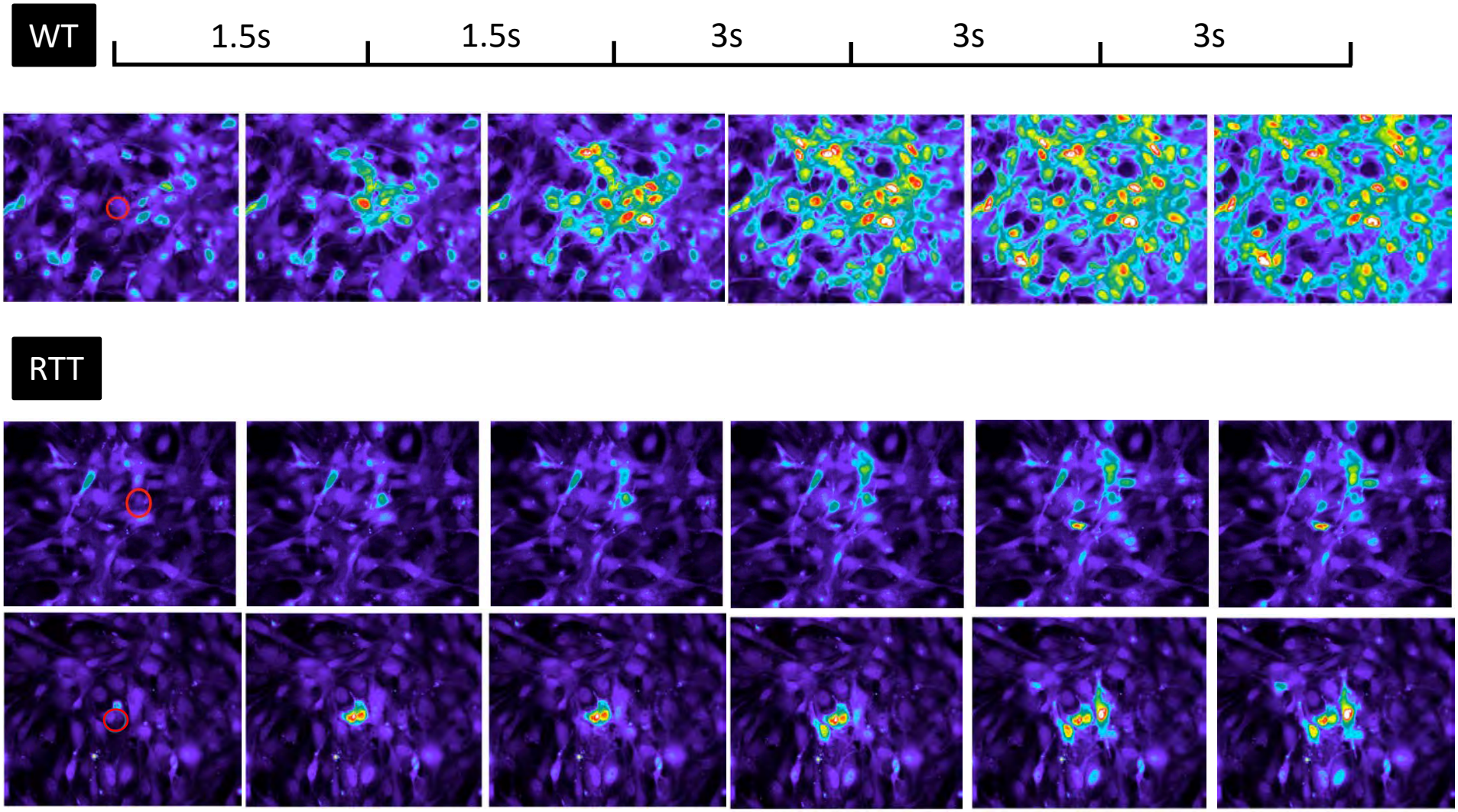
RTT astrocyte altered gene expression



RTT astrocytes have slower glutamate clearance

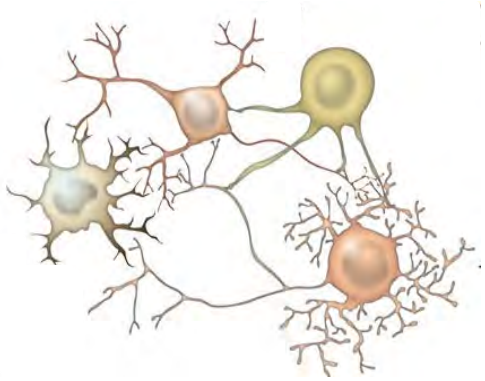


RTT astrocytes have impaired calcium waves



Hang Yao, PhD

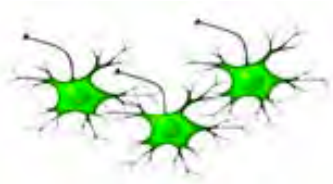
Effect of RTT astrocytes on human neurons



Mixed cell population
4 weeks of neuronal induction

Magnetic sorting

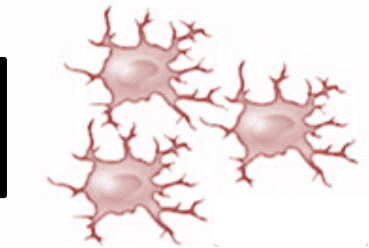
CD44 -
CD184-



WT
RTT

Neuron enriched population

WT
RTT

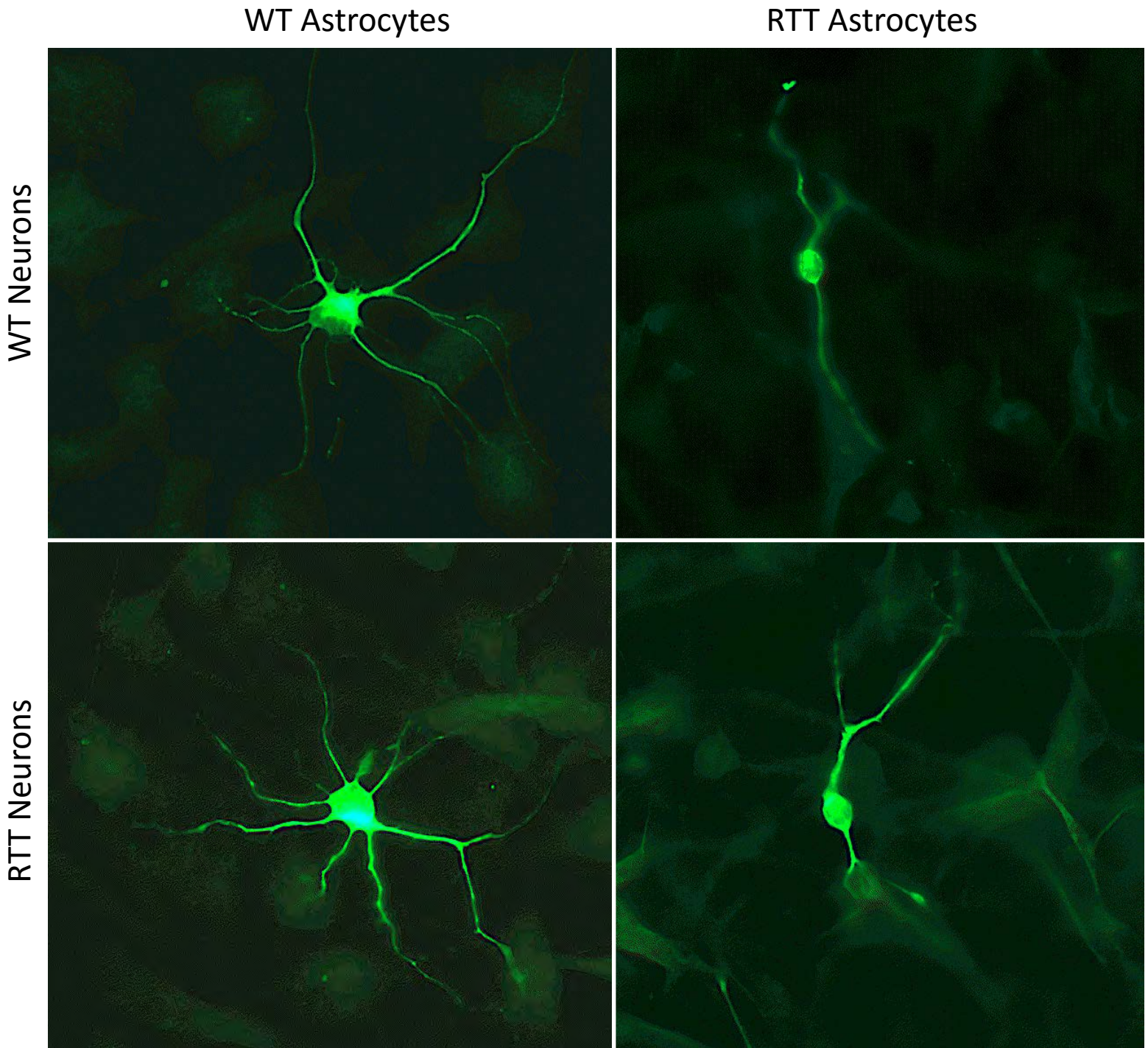


Astrocytes

Plated on coverslips

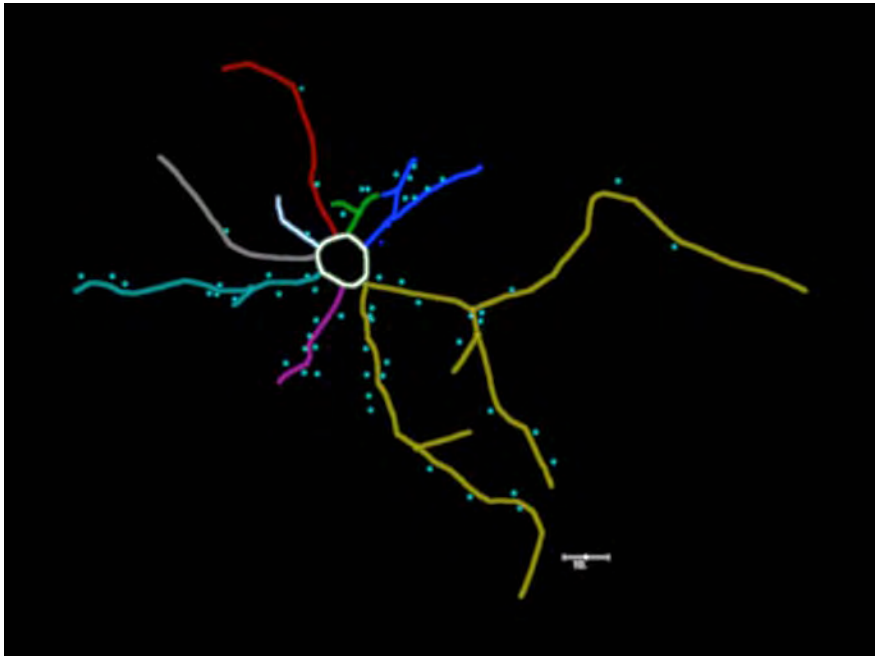
2w

Fix
&
Stain

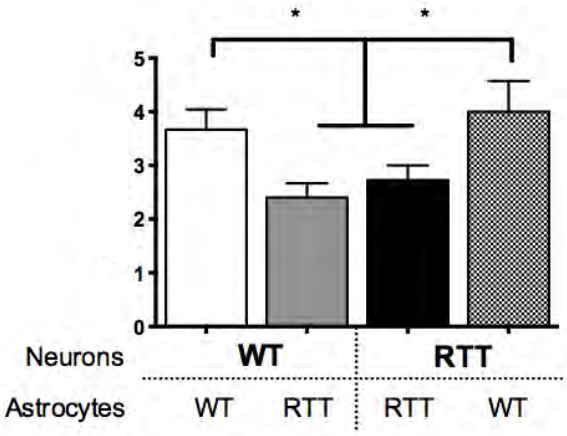

WT Astrocytes
RTT Astrocytes
WT Neurons
RTT Neurons

RTT neuronal rescue by astrocytes

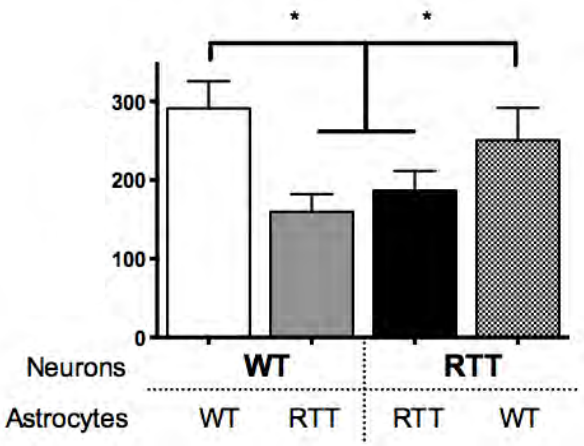
Branka



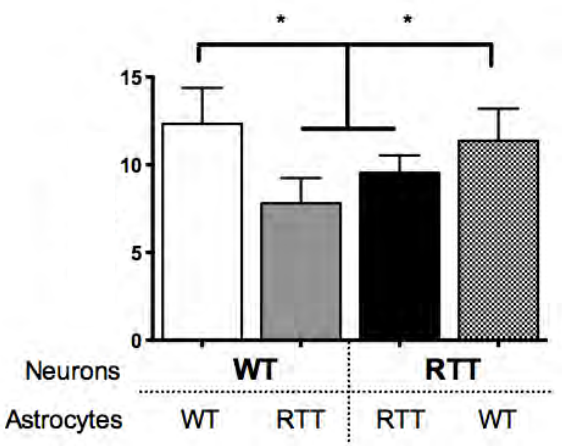
Dendrites



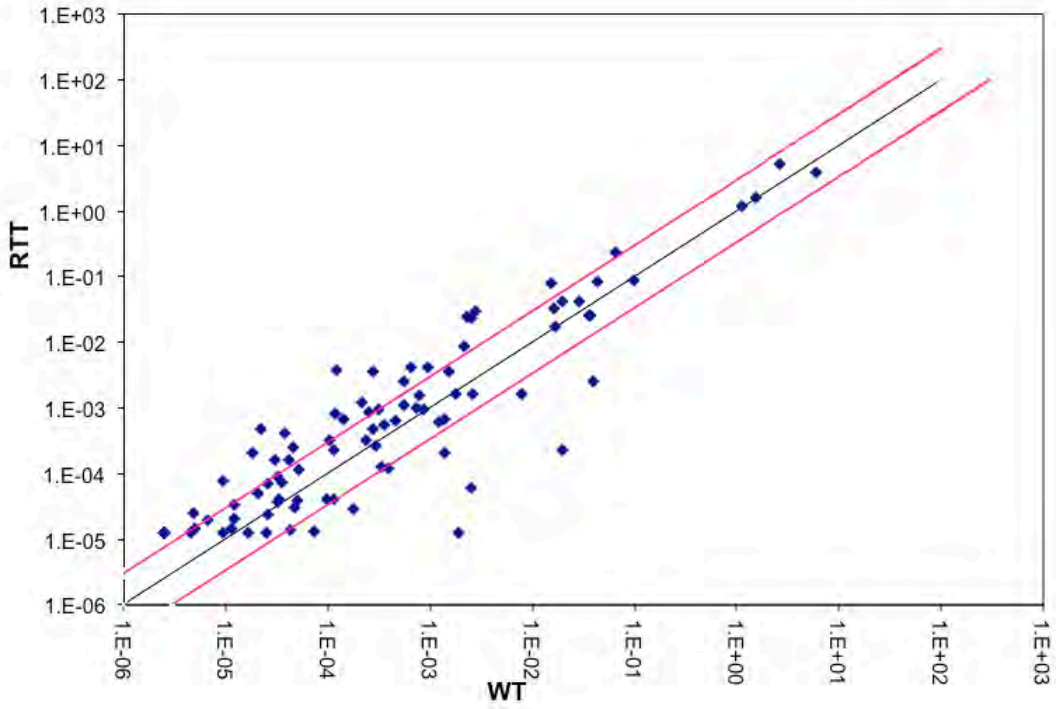
Neuronal Length



Segments

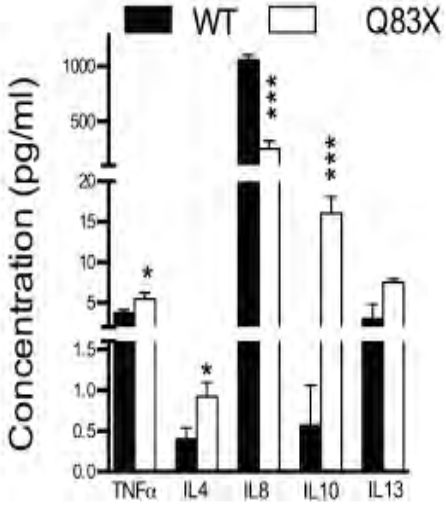


RTT astrocytes aberrant cytokines

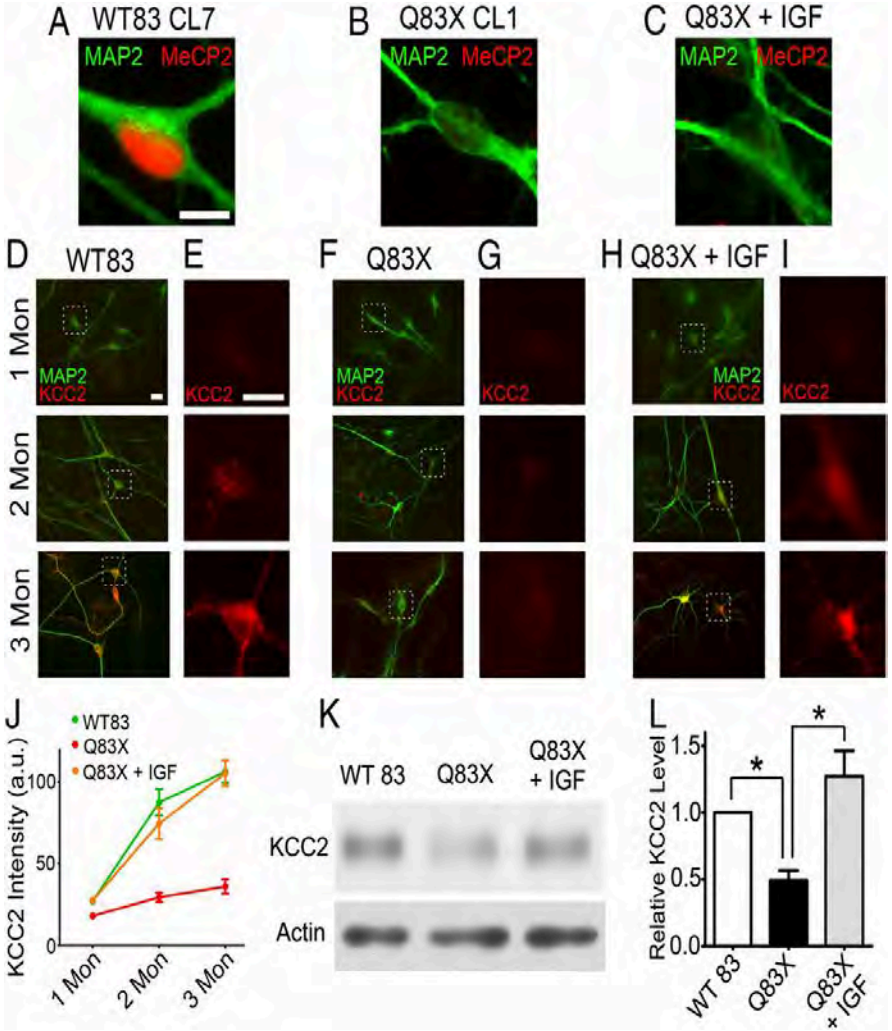


Up-regulated	
Symbol	Fold dif.
BMP5	4.68
CD40LG	5.36
CSF2	2.29
CSF3	3.89
IFNA4	2.97
IL13	2.69
IL15	6.58
IL23A	2.98
IL3	2.97
IL4	5.42
IL5	5.62
INHBA	5.34
LIF	9.24
TGFB1	1.89
TGFB2	3.55
TGFB3	10.64
TNFSF12	3.98
TNFSF13B	3.39
TNFSF8	5.18
TXLNA	2.15

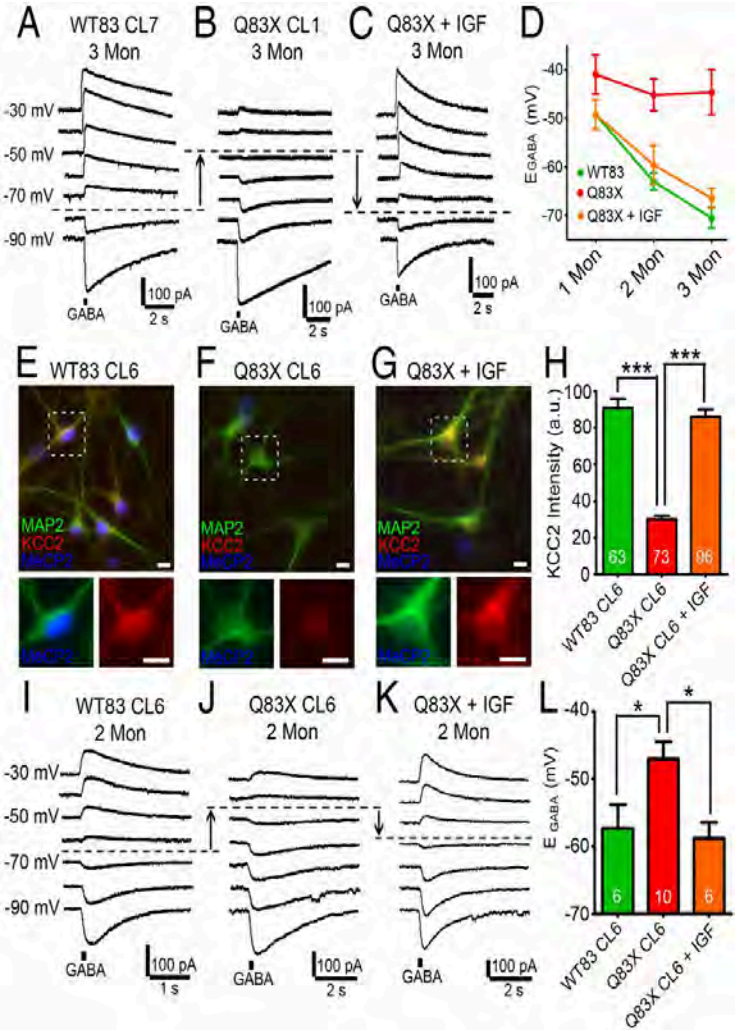
Down-regulated	
Symbol	Fold dif.
BMP2	0.15
BMP3	0.01
BMP4	0.5
CD70	0.06
IL10	0.17
IL17B	0.32
IL18	0.2



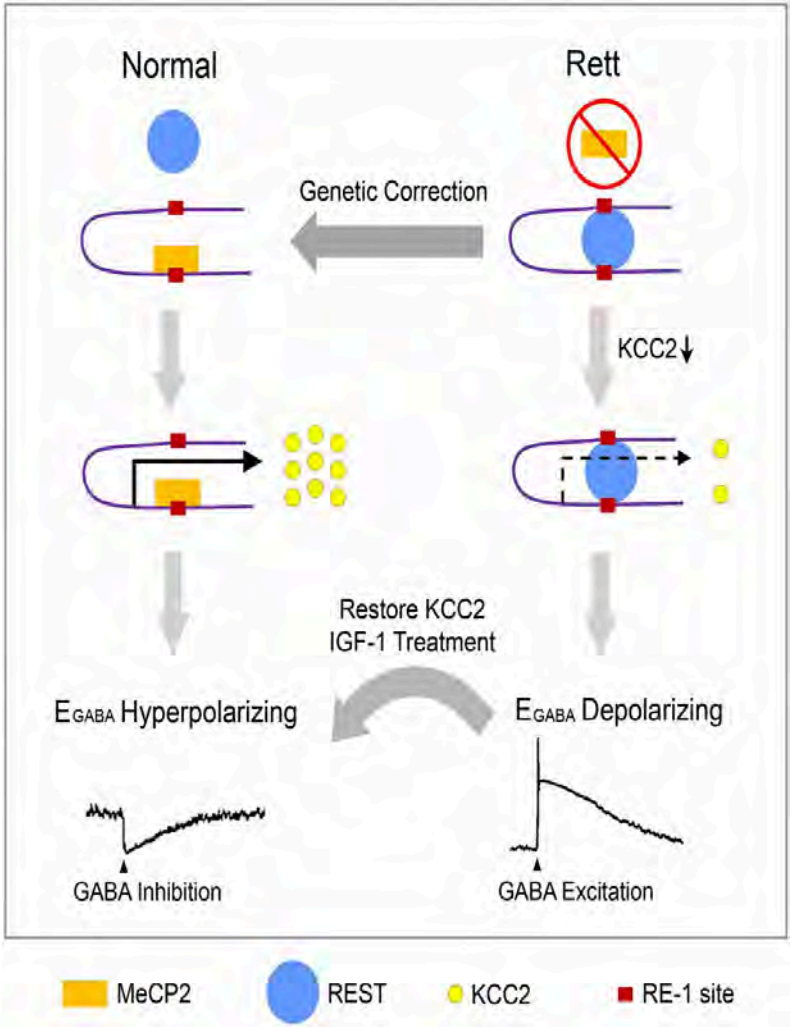
RTT neurons show deficits in KCC2 expression



RTT neurons show deficits in GABA functional switch



A model depicting the molecular mechanisms underlying KCC2 deficiency in RTT neurons



Xin Tang et al. PNAS 2016;113:751-756

Take Home Messages:

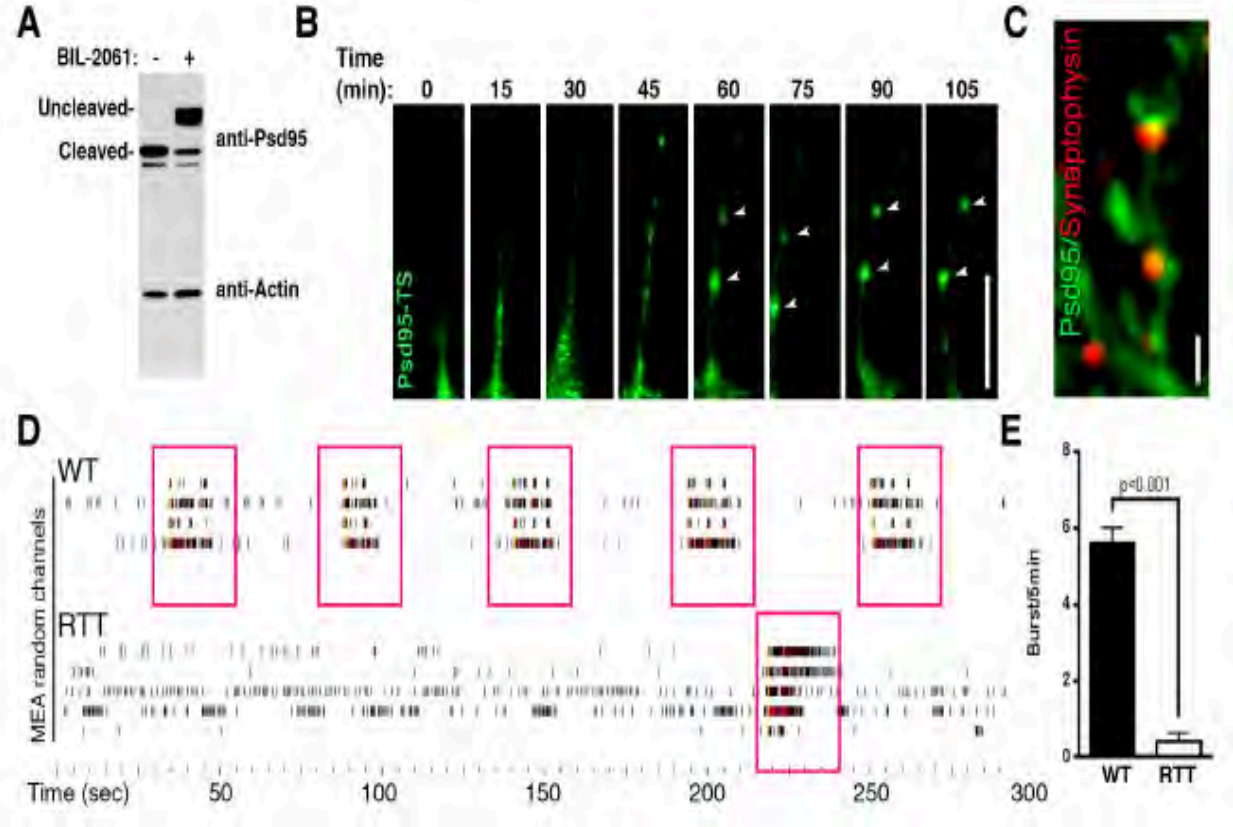
-Loss of MeCP2 function is involved in glutamatergic synapses formation, neuronal morphology and defective network formation.

-RTT astrocytes have impaired metabolism and display an inflammatory cytokine signature. RTT neurons can be rescued by healthy astrocytes.

-RTT neurons are defective in KCC2 expression, resulting in a delayed GABA functional switch that might contribute to the late disease onset.

Ideas about “Regression”

- Use a Psd95-TS/MEA to distinguish between developmental failure or loss of synapses over time.



Ideas about “Regression”

- Study the RTT astrocyte-derived cytokine dynamics over time and the impact on neuronal networks (synaptogenesis/MEA)
- To restore KCC2 expression in the symptomatic RTT mouse model
- To explore a potential pruning defect in RTT neurons with microglia co-culture.

THE MUOTRI LAB

muotri@ucsd.edu
http://muotri.ucsd.edu

NIH Director's New Innovator Award Program
R01 NIMH
CIRM Early Translational Award
IRSF Foundation

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Morning Agenda – continues

11:05 **Modeling Rett Syndrome in a Dish – Ideas about Disease Regression**

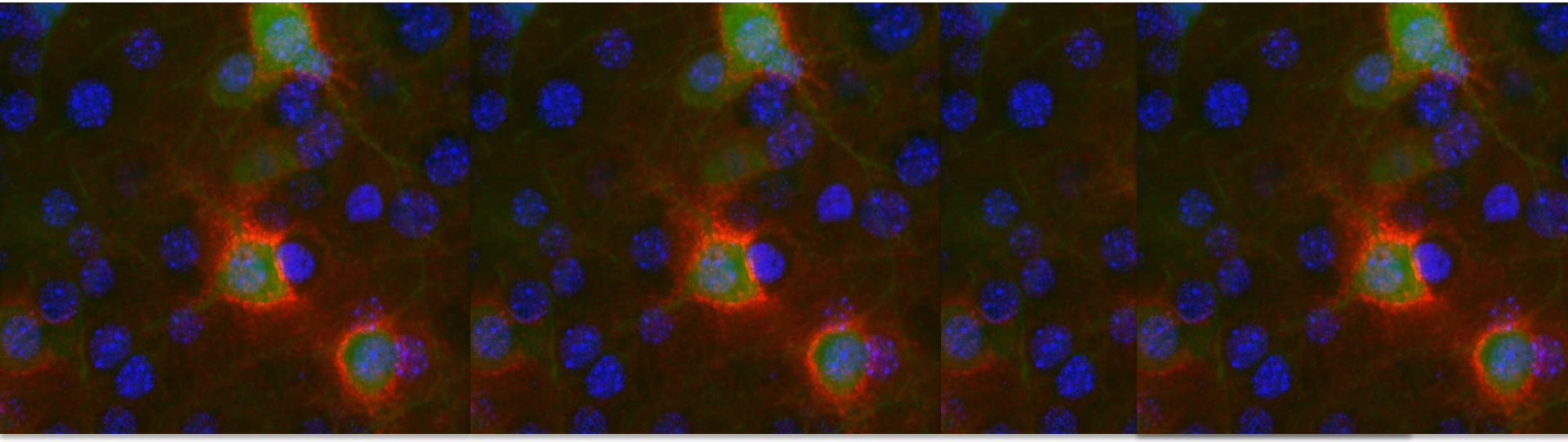
Alysson Muotri, Ph.D.
Associate Professor
UCSD Stem Cell Program, School of Medicine
University of California, San Diego

11:25 **Shaping Brain Circuits by Experience: Uncovering Aberrant Plasticity in Mouse Models of Rett Syndrome**

Keerthi Krishnan, Ph.D.
Research Investigator
Department of Neuroscience, Cold Spring Harbor Laboratory

Shaping neural circuits by experience: Uncovering aberrant plasticity in mouse models of Rett Syndrome

Dr. Keerthi Krishnan
Cold Spring Harbor Laboratory



MeCP2 mutations cause Rett Syndrome

Methyl-CpG-bindi

- Binds to DNA

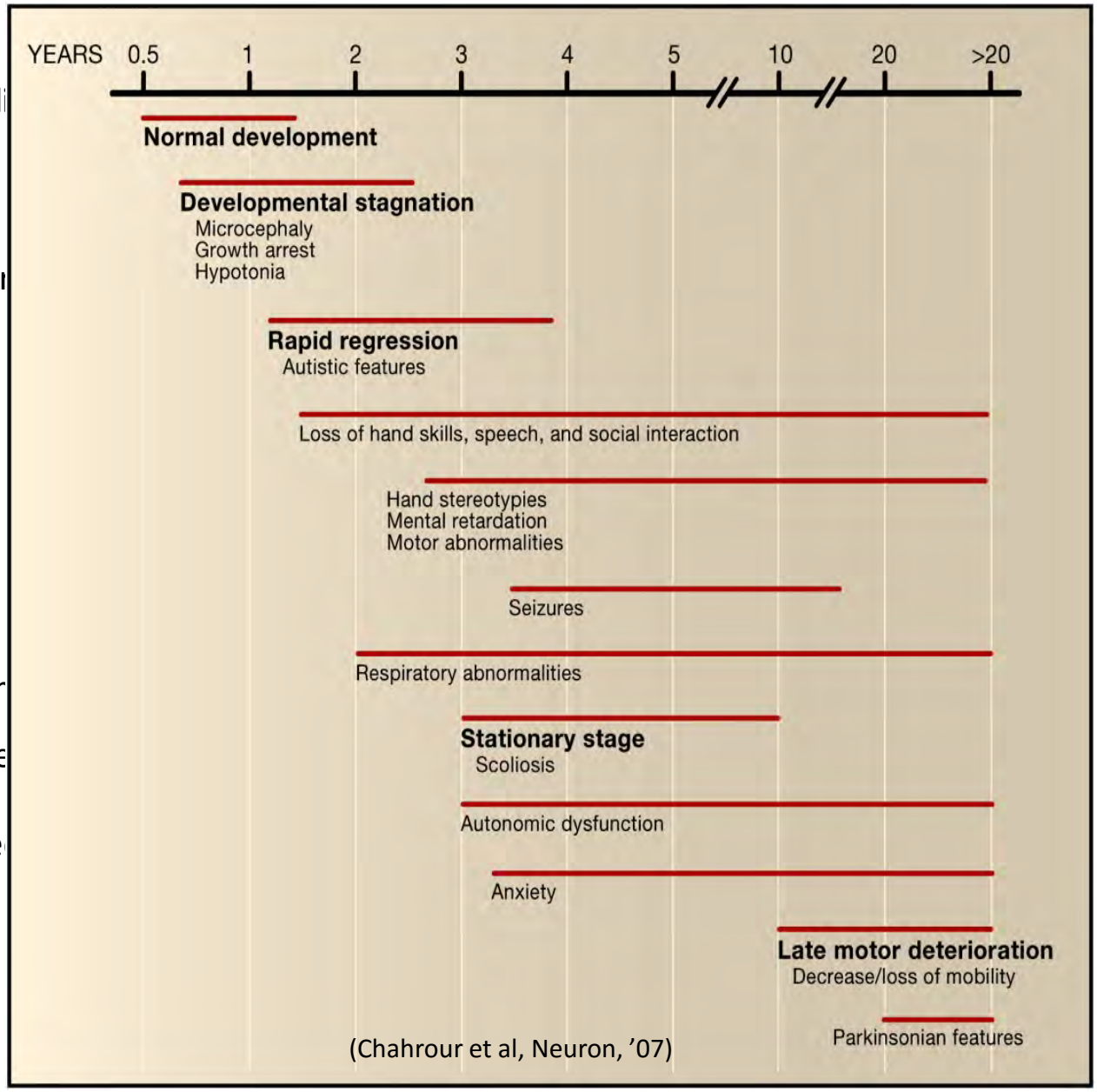
- chromatin and tr

Rett Syndrome

-May result from

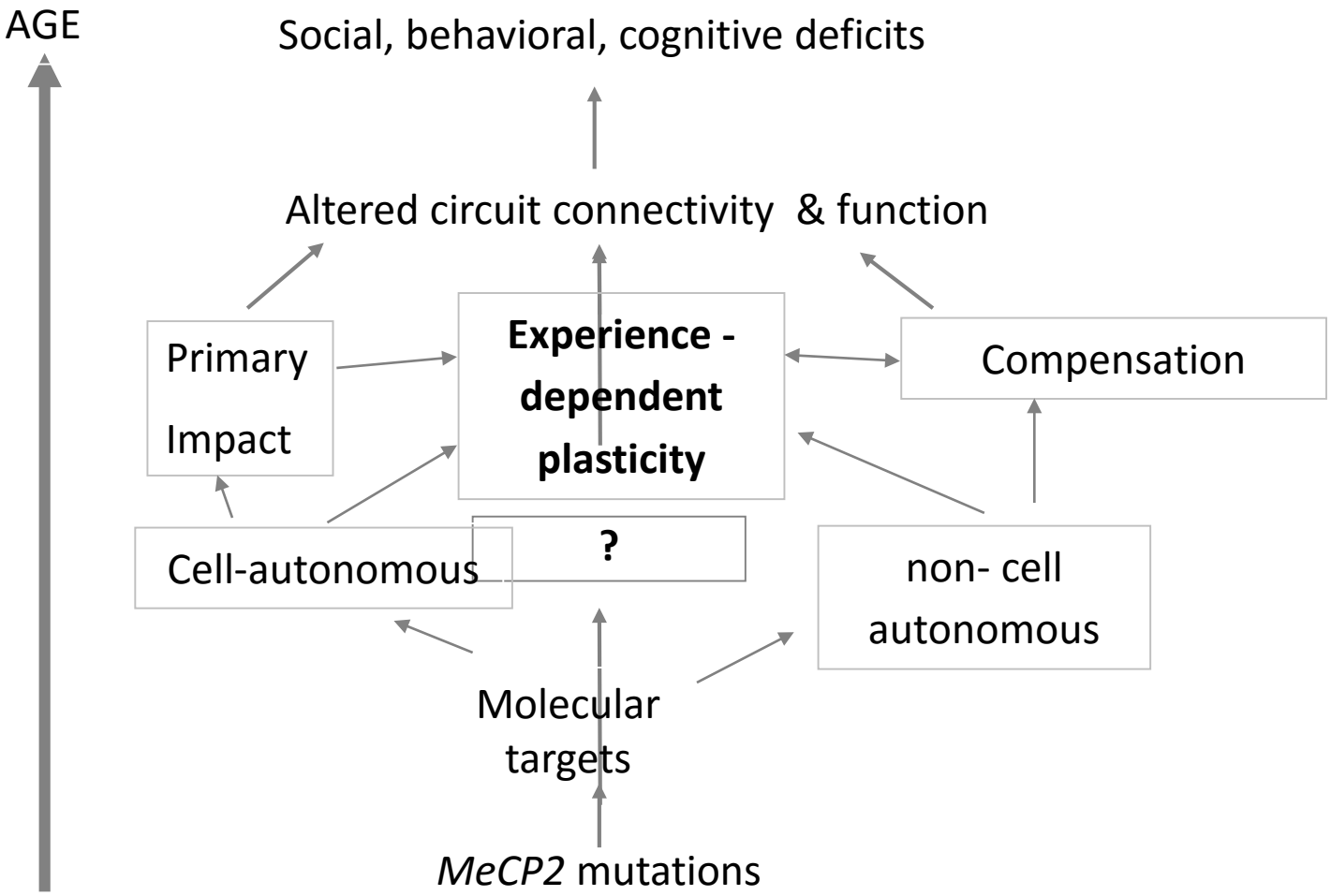
experience-depe

- pathogenic me

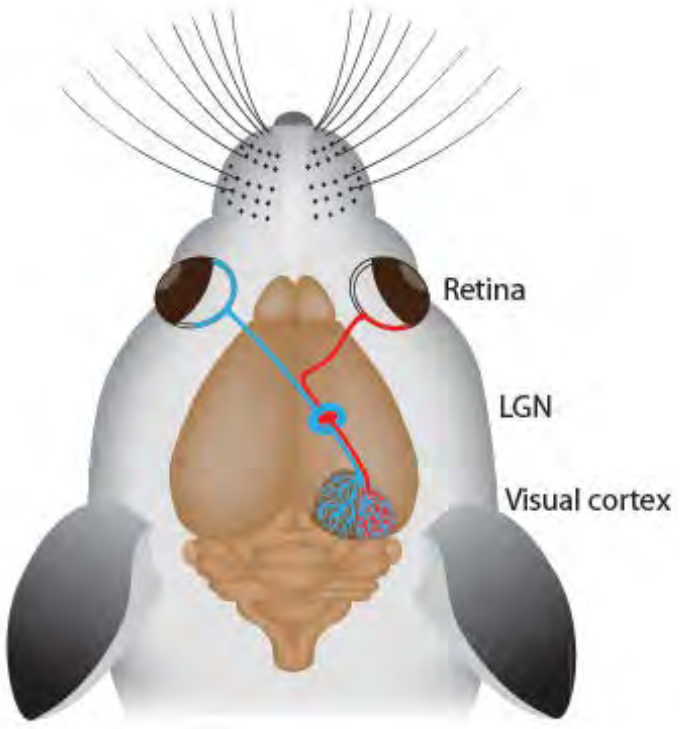


(Chahrour et al, Neuron, '07)

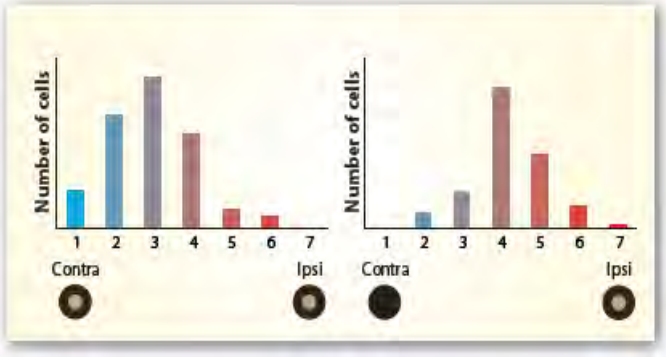
Challenges in determining the pathogenesis of Rett Syndrome (RTT)



Critical period of plasticity and experience-dependent wiring of neural circuits in primary visual cortex (V1)

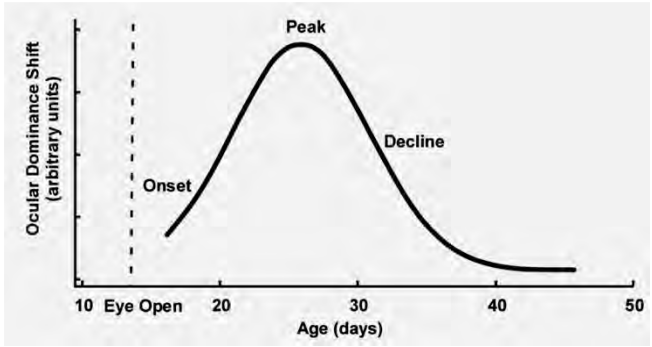


Ocular dominance shift



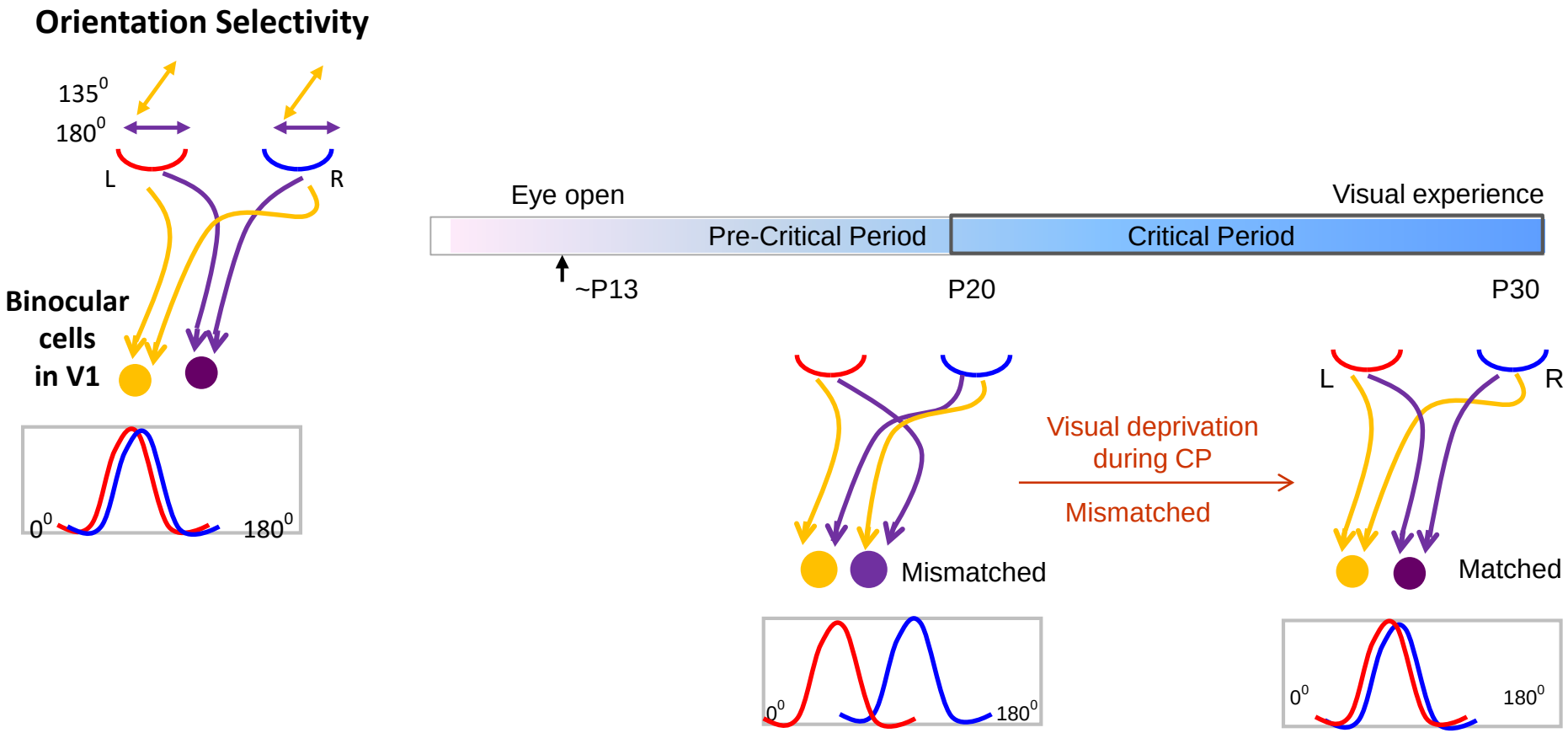
Hubener 2012

Critical period of plasticity



Gordon & Stryker 1996

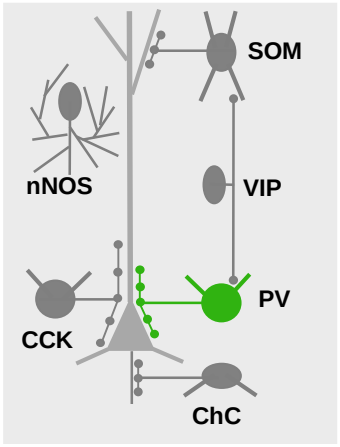
Critical period of plasticity and experience-dependent wiring of neural circuits in primary visual cortex (V1)

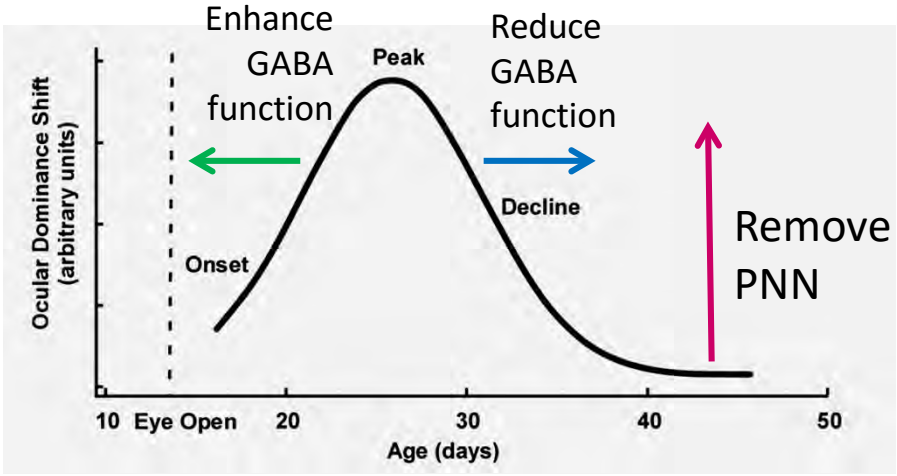


Critical period allows visual experience to drive the matching of orientation tuning onto binocular V1 cells

Wang et al., Neuron, 2010

Maturation of GABA inhibition and Parvalbumin (PV) interneurons regulate the timing of critical period

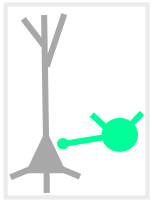


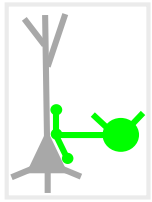


Hensch et al., 1998
Huang et al ., 1999
Fagiolini et al ., 2004
Pizzorusso et al, 2002

Onset **Closure**

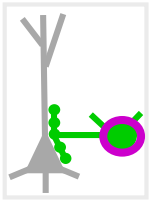
Eye open — Pre-Critical Period — Critical Period





Experience-dependent maturation

GAD67 expression

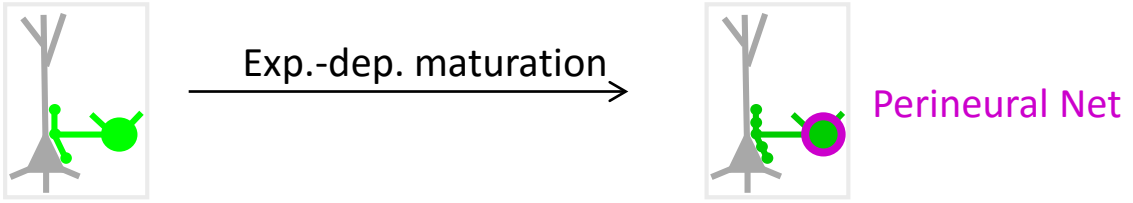


Perineuronal Net (PNNs)

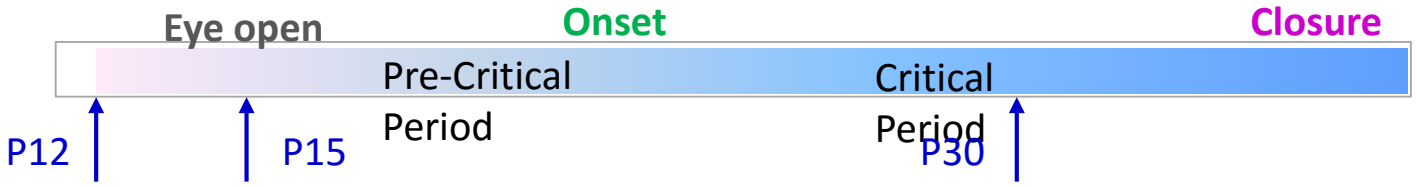
PNNs - Extracellular matrix proteins that inhibit axon growth and structural plasticity

Experience-dependent wiring of V1 circuits: knowledge of developmental trajectory allows better tracking of its alterations

- Maturation of GABA circuits



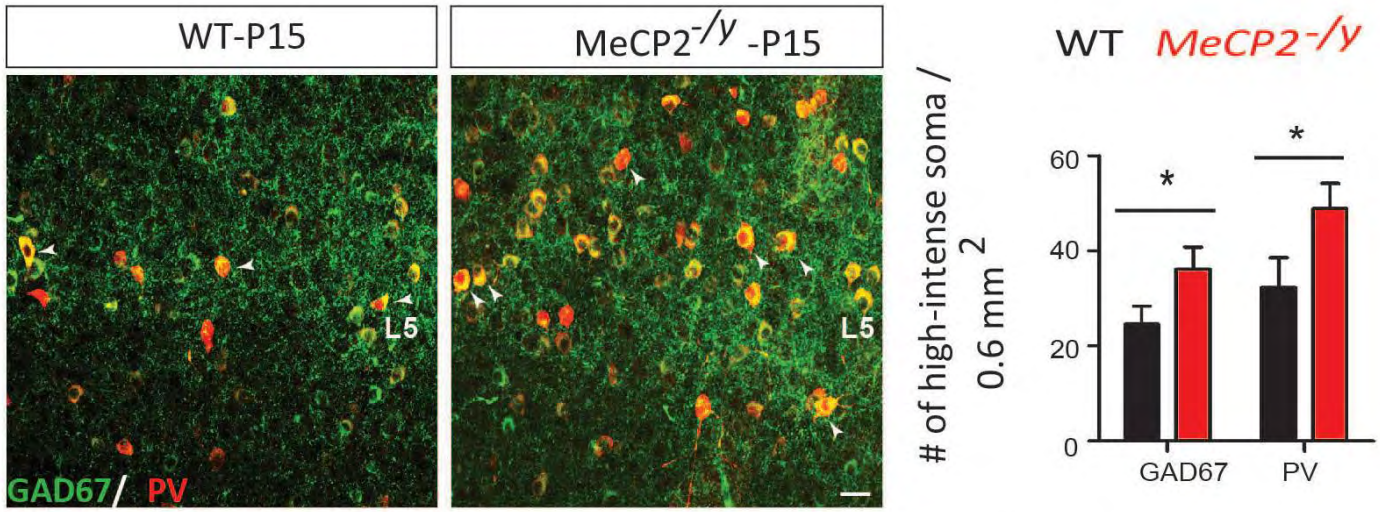
- Timing of Critical period



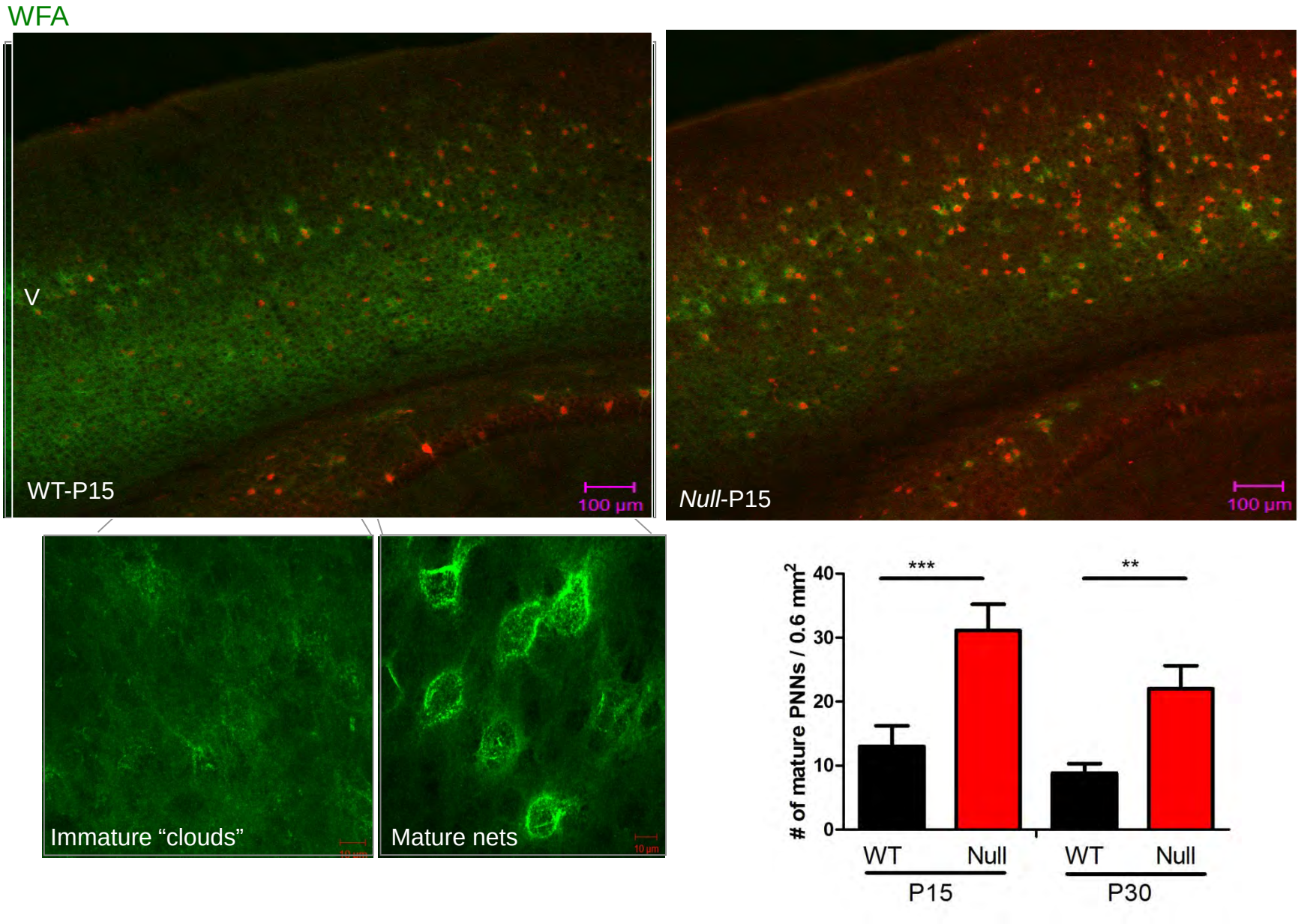
- Development of Visual function

What is the impact of MeCP2 deletion in germ-line male mice (MeCP2$^{-/y}$) ?

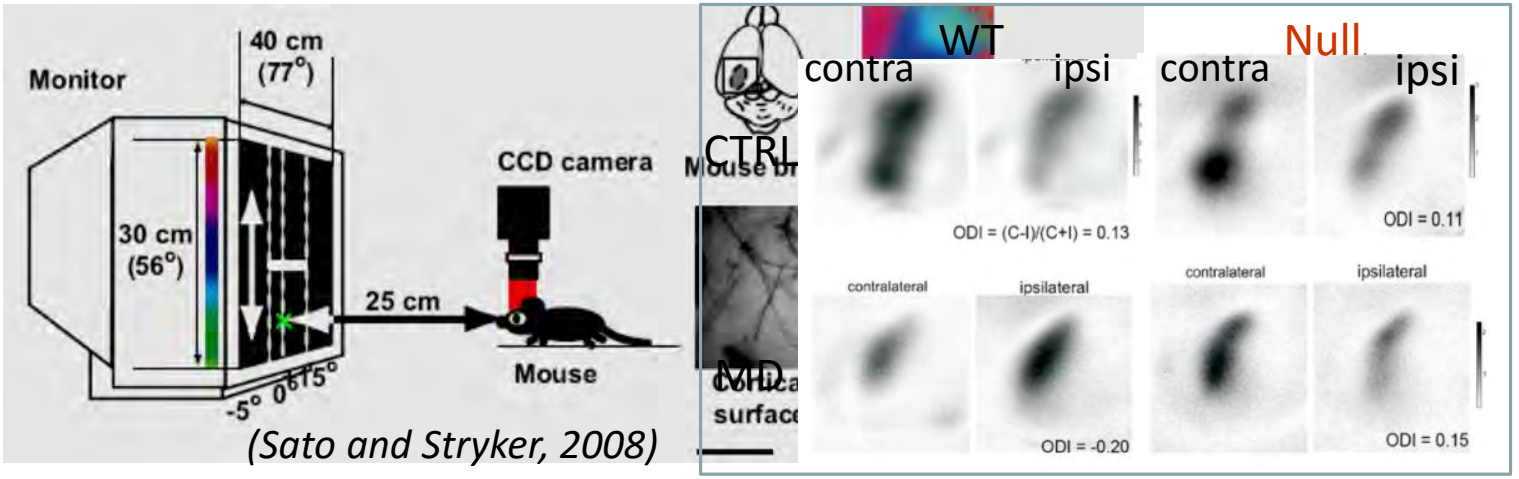
Precocious increase in key components of GABA transmission in Null V1



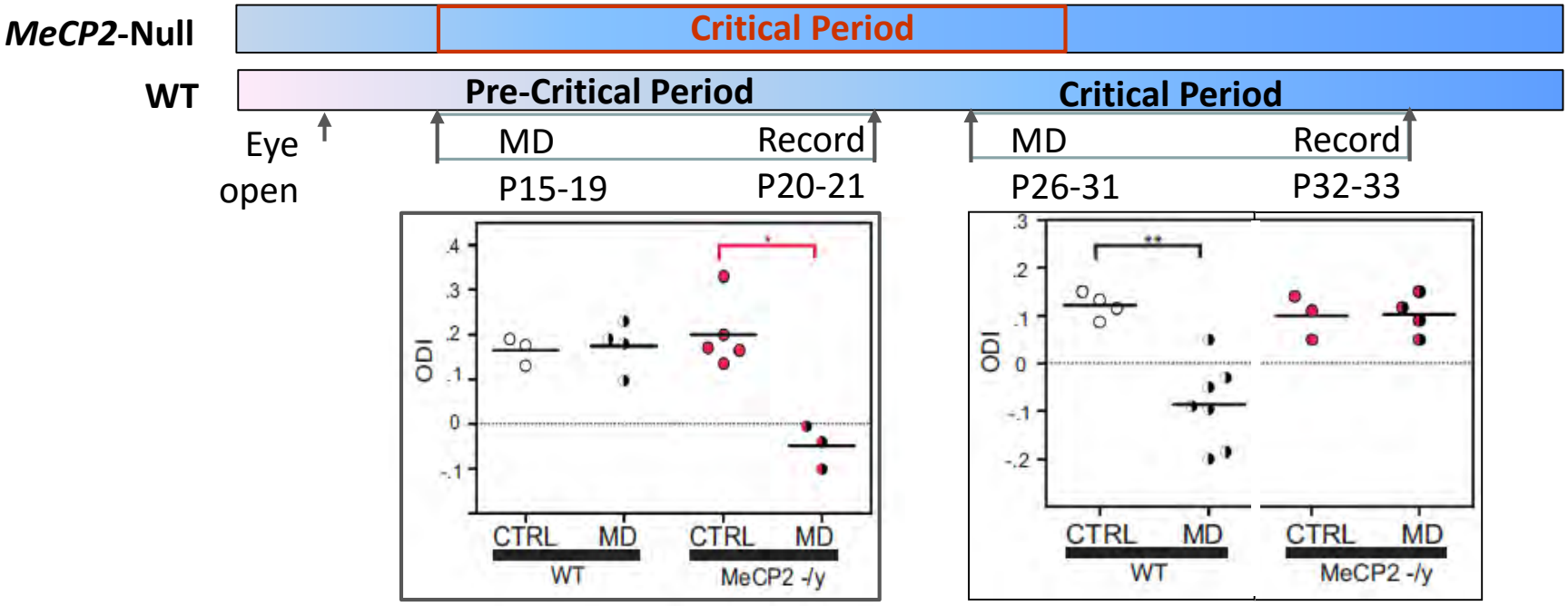
PNN formation on PV^{+} neurons is accelerated in Null cortex



Precocious critical period of plasticity in Null V1

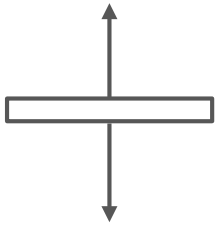


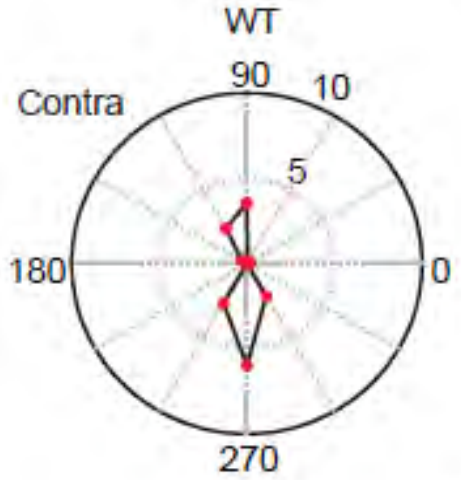
(Sato and Stryker, 2008)

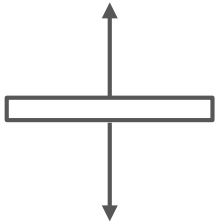


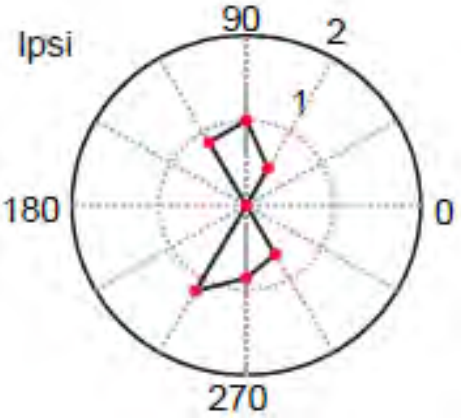
Collaboration with Bor-shuen Wang, Jianhua Cang lab, Northwestern University

Orientation tuning of inputs from two eyes onto individual binocular cells remains mismatched in Null V1





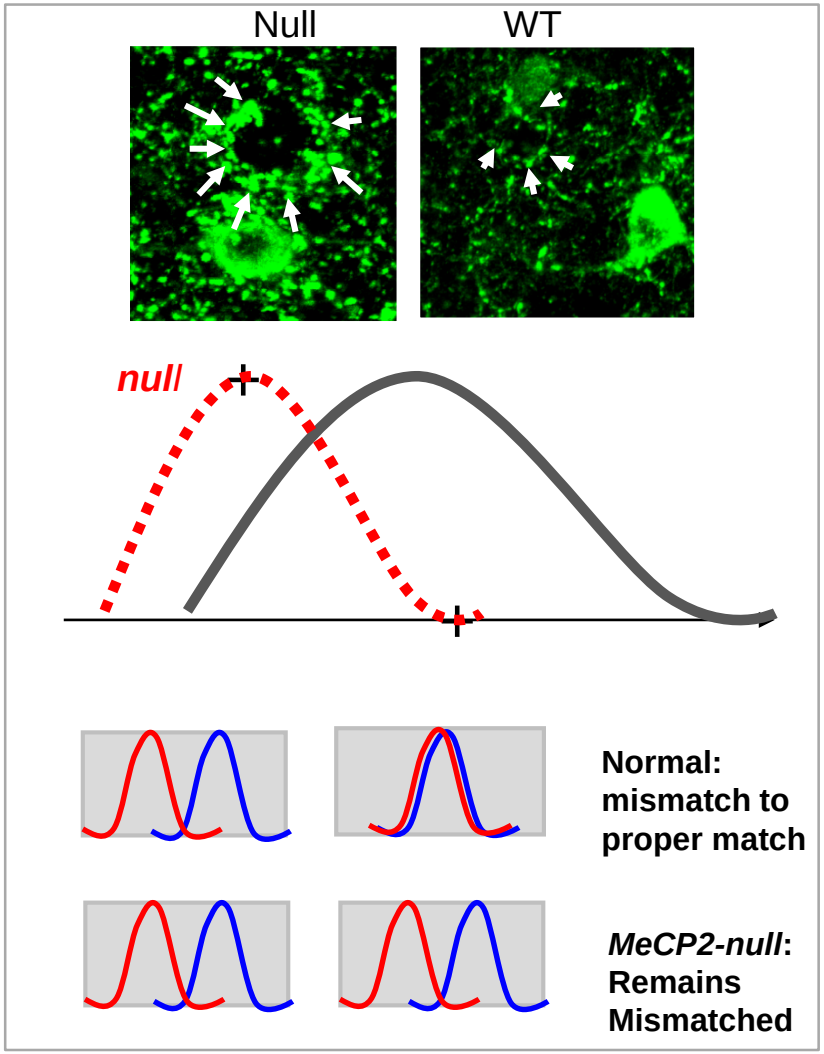




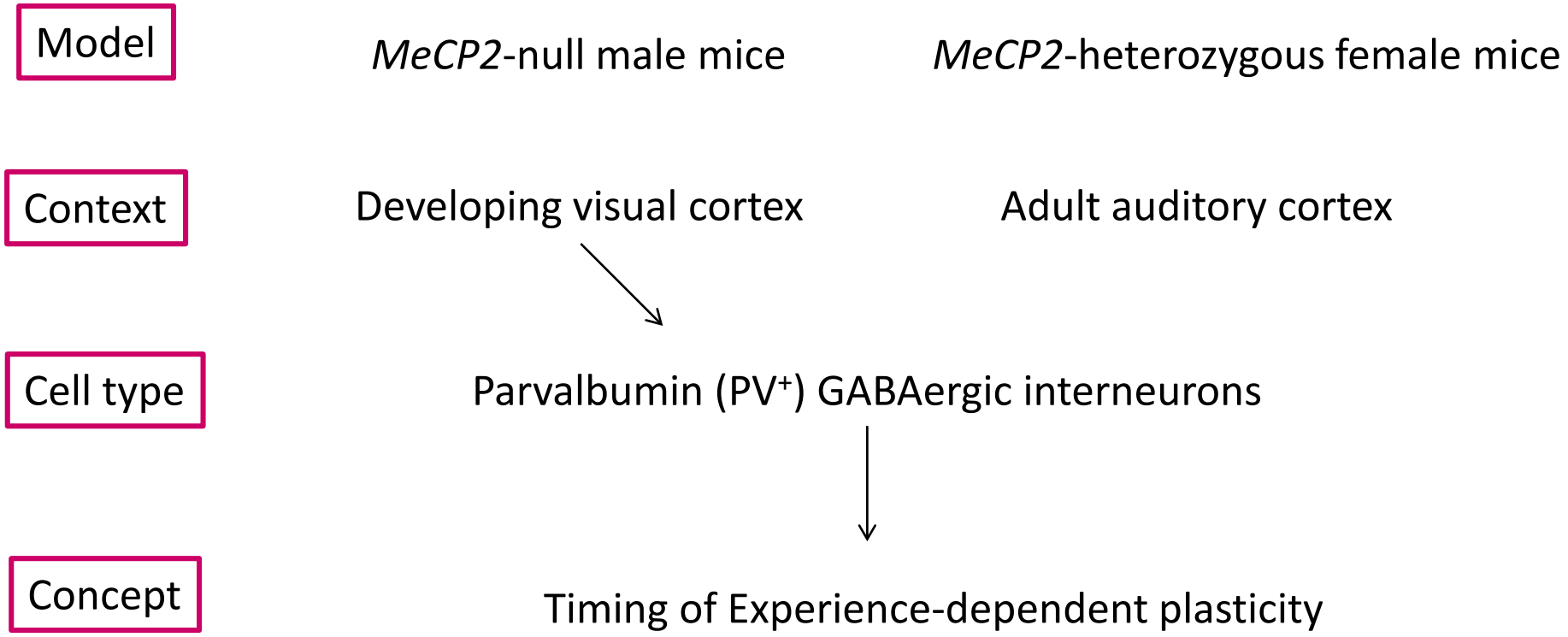
MECP2 regulates timing of critical period plasticity

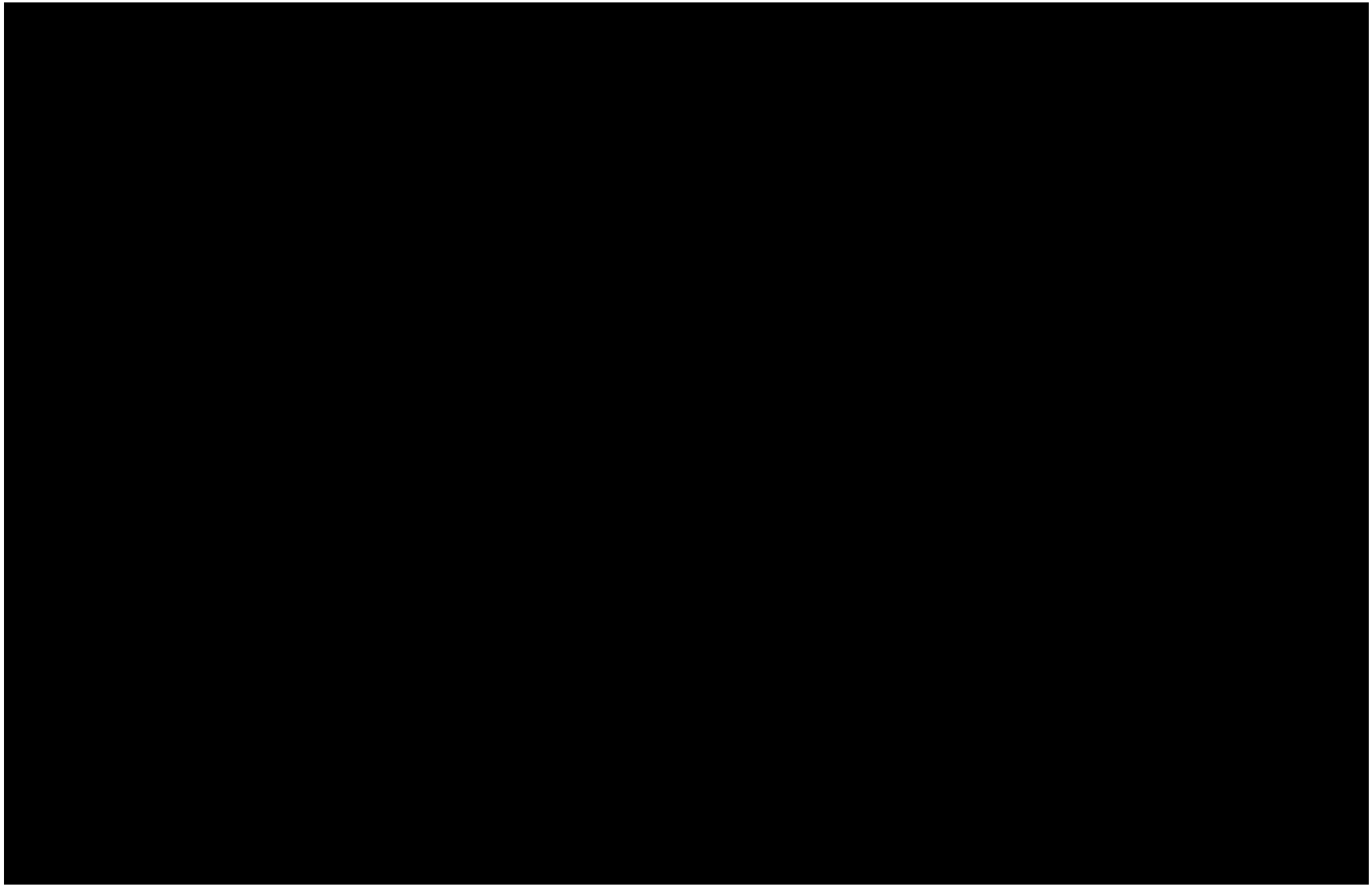
In Visual cortex of *MeCP2-null*:

- Precocious increase in key components of GABA transmission, mainly in PV+ neurons
- Precocious onset and termination of critical period
- Aberrant visual function, measured by binocular matching of orientation tuning of inputs
- Reducing GAD67 levels rescues onset of the precocious of critical period plasticity

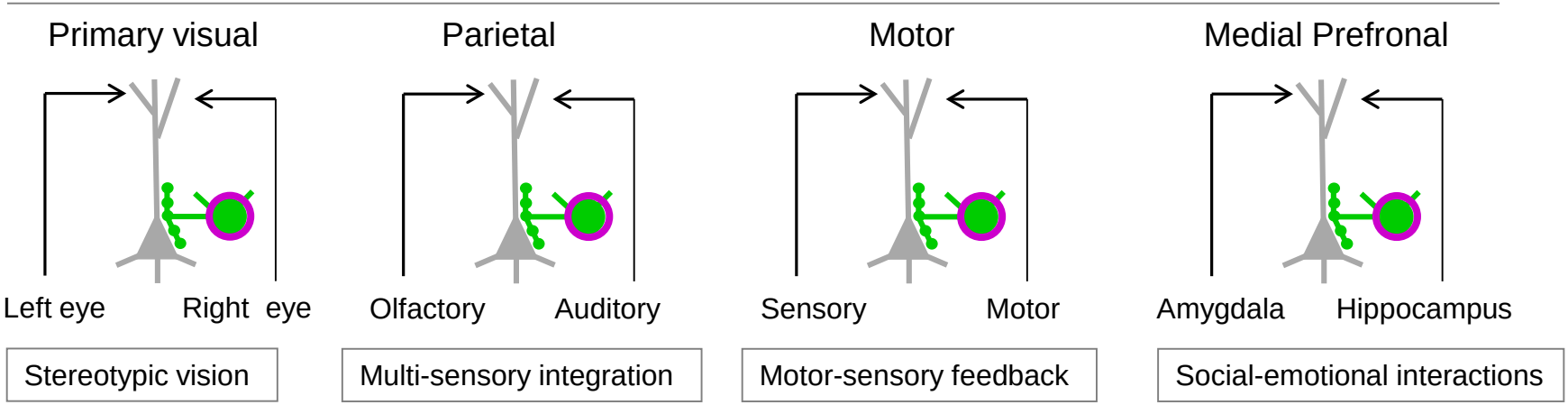


Krishnan, Wang et al, 2015


Model
MeCP2-null male mice
MeCP2-heterozygous female mice
Context
Developing visual cortex
Adult auditory cortex
Cell type
Parvalbumin (PV+) GABAergic interneurons
Concept
Timing of Experience-dependent plasticity



Speculation: Role of MECP2 in regulating timing of experience-dependent plasticity may apply to other brain systems



Sensory processing in RTT and ASD patients is affected

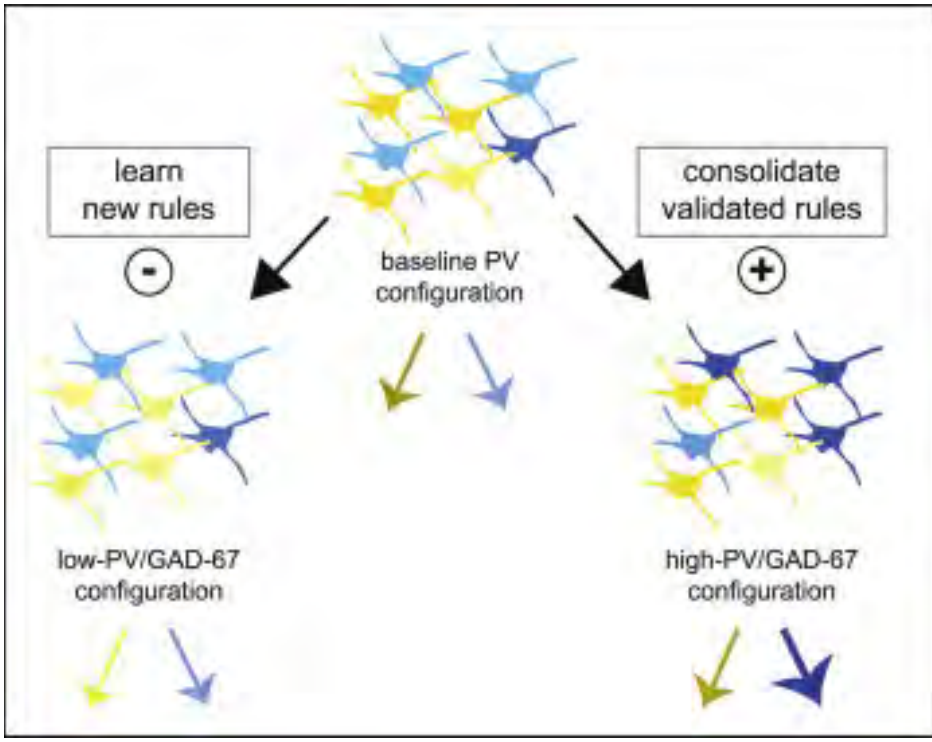
Speculation

In RTT and ASD,

- specific cells and networks involved in consolidating skills might be prematurely closed.

- Similar mechanisms involving PV^{+} network (PV/GAD67/PNNs) might be involved in regression

- These markers can be used to study and perhaps “rescue” regression phenotype (once identified) in animal models.



Caroni, 2015

Acknowledgements

Josh Huang
Bor-Shuen Wang
Jiangteng Lu

Stephen Shea
Billy Lau

Collaborators at other institutions
Jianhua Cang, NWU
Arianna Maffei, SUNY

IRSF INTERNATIONAL RETT SYNDROME FOUNDATION

SFARI SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE

BRAIN & BEHAVIOR RESEARCH FOUNDATION
Awarding NARSAD Grants

NIH National Institute of Mental Health

WHITEHALL FOUNDATION
75th ANNIVERSARY 1937 - 2012

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Morning Agenda – continues

11:45 **Using the Visual System as a Means to Quantitatively Evaluate Cortical Function and Cognitive performance in Rett Syndromes**

Charles Nelson, Ph.D.
Professor of Pediatrics and Neuroscience
Harvard Medical School, Boston Children's Hospital

12:05 **Panel Discussion**

Discussant: Elliott Sherr, M.D., Ph.D., Professor of Neurology, Pediatrics, University of California, San Francisco

12:40 PM **Lunch**

Using the visual system as a means to quantitatively evaluate cortical function and cognitive performance in Rett syndrome

Charles A. Nelson
Professor of Pediatrics and Neuroscience
Professor of Psychology in Psychiatry
Harvard Medical School
Richard David Scott Chair in Pediatric Developmental Medicine Research
Boston Children's Hospital

Talk presented at NIH meeting on *Loss of skills and onset patterns in neurodevelopmental disorders: Understanding the neurobiological mechanisms*, 19 February 2016

Rett Syndrome Marked by Developmental Regression

ABOUT RETT SYNDROME

- X-linked, spontaneous mutation of MeCP2
- < 1% cases inherited
- Primarily affects females
- Prevalence: ~1/10,000 females
- Classified by toddlerhood regression, loss of purposeful hand use, loss of acquired speech, gait abnormalities, and stereotypies.

Source: Rett, 1966; Hagberg, 1983; NINDS, 2015.

Nelson Lab Rett Syndrome Studies

Specific Aims

1. to **quantitatively evaluate cortical function** in girls with RTT using electroencephalography (EEG), event-related potentials (ERP) and visual evoked potentials (VEP).
2. to monitor and **measure neurological signs of response to pharmacological treatment** through changes in VEPs, and resting state EEG over treatment course. *This work being done in parallel with work in mouse (Michela Fagiolini)*
3. to **develop a cognitive assessment that circumvents confounds of impairment in motor function and expressive language** when assessing domains of receptive language and visual reception.

Gaps of knowledge in Rett syndrome research

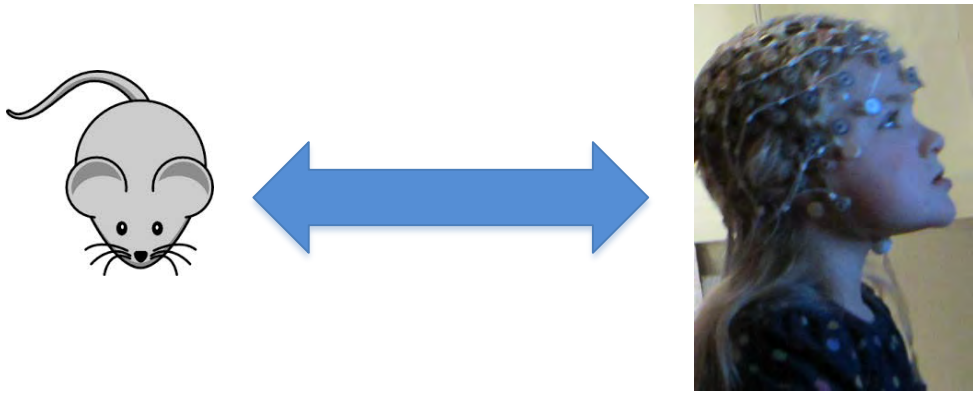
– **Cortical function**

Part I – Evaluating cortical function with visual evoked potentials (VEPs)

– **Cognitive function**

Part 2 – Evaluating cognitive function with a developmental behavioral assessment (MSEL) and eye-tracking

VEP as a translational biomarker in RTT



- Reflects the summation of cortical response to a visual stimulus
- Robust signal with distinct, quantifiable components
- Matures within the first year of life
- Passive task not dependent on attention
- Non-invasive, quick, and cost-effective
- *Can use the knowledge gained in mouse models of RTT to better understand the cellular and circuit impairments in RTT patients and inform treatments*

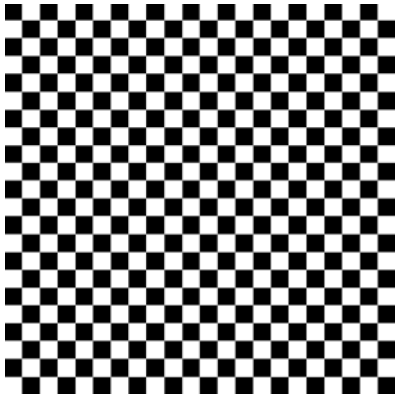
Population for VEP study

RTT subjects were recruited through the Natural History Study or the Rett Syndrome Program at Boston Children's Hospital (BCH)

20 typically developing girls recruited as controls

	Control	RTT
Number of subjects	20	34
Mean age in months	57	56
Age range	24-112	22-103

Pattern-reversal VEP paradigm

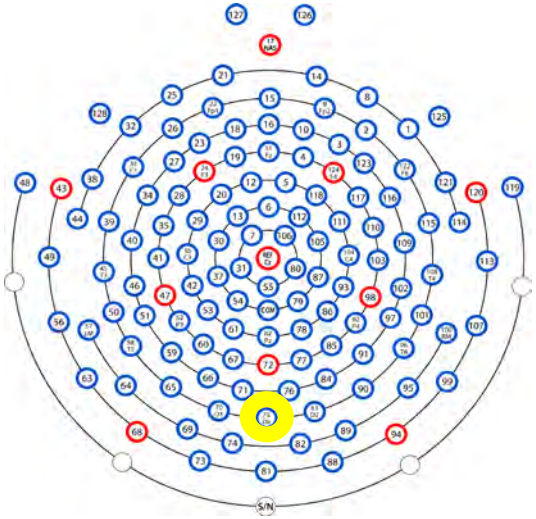


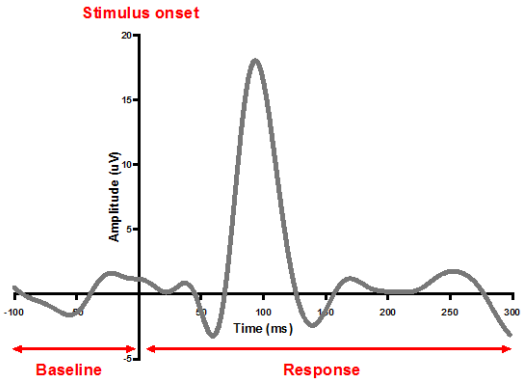
Stimulus

Eye-gaze contingent

Data collection

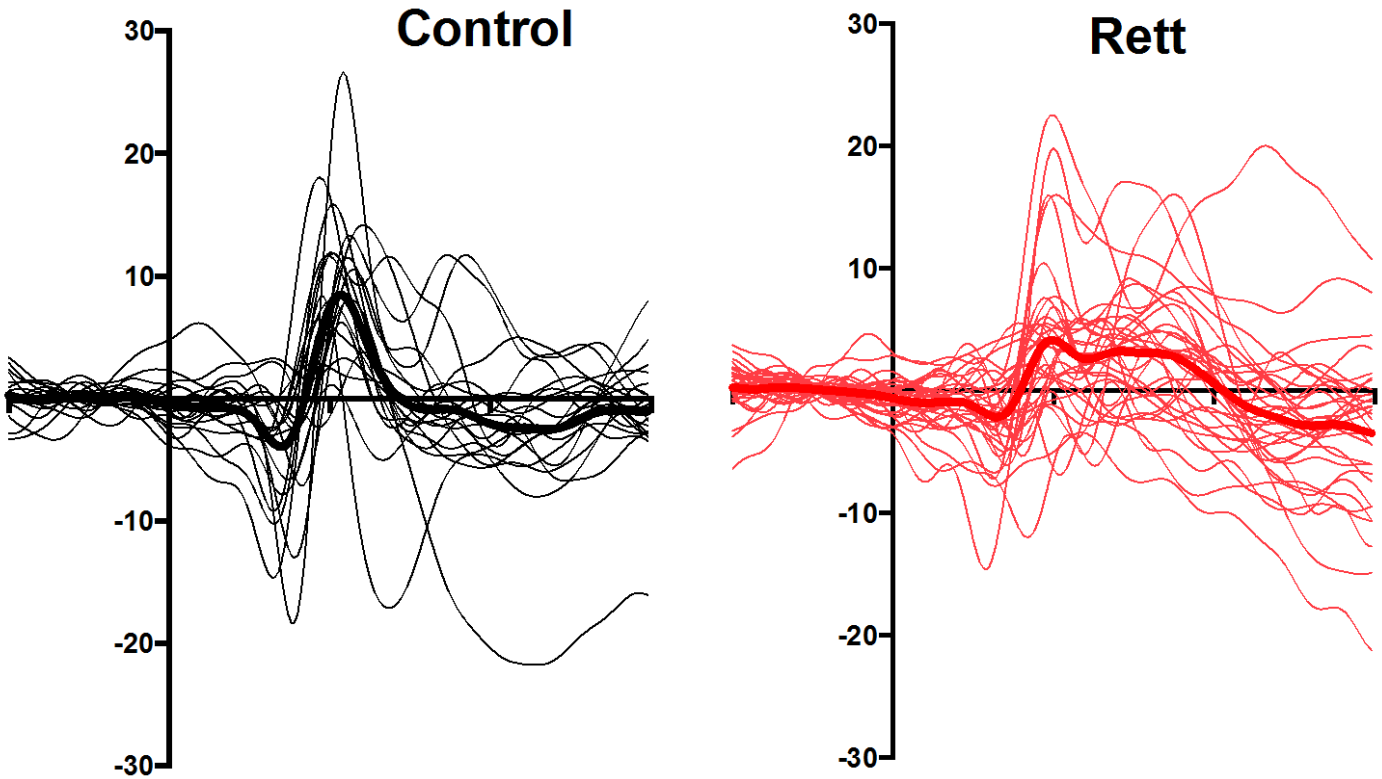
128-channel EEG net



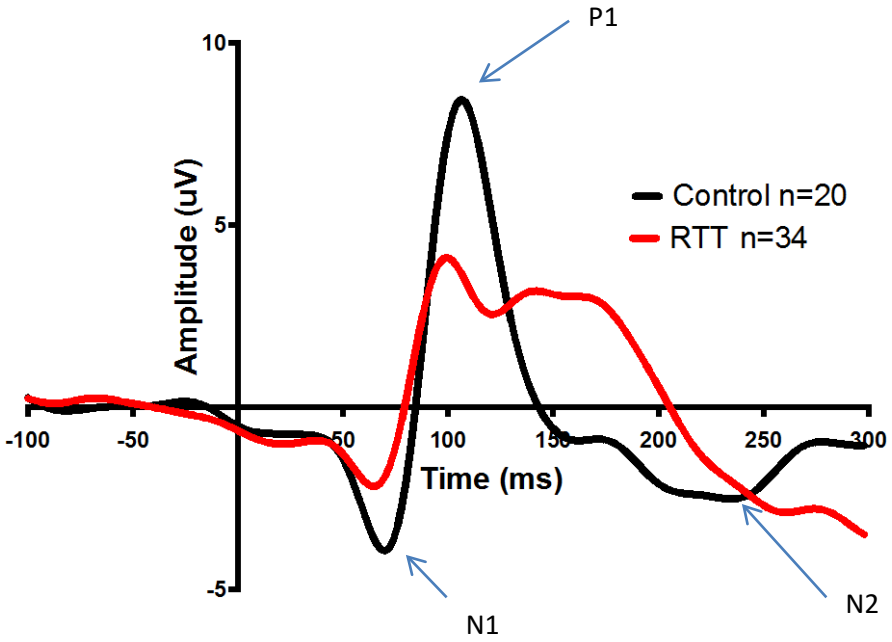


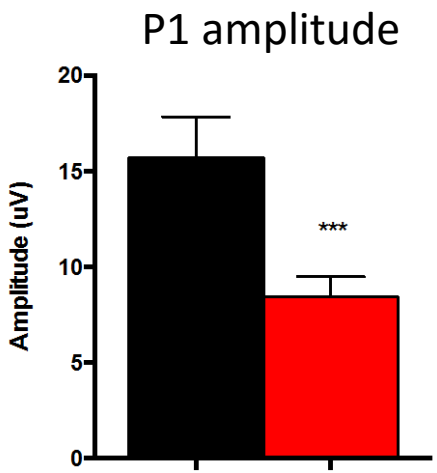
VEP waveform is abnormal in children with RTT

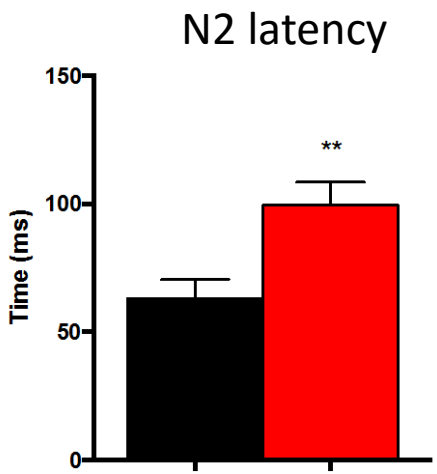
(displayed are individual subject averages)



Group differences: Control vs. Rett

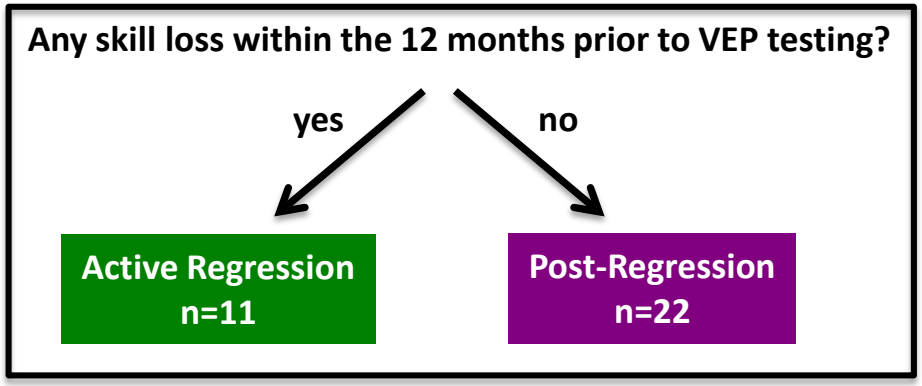


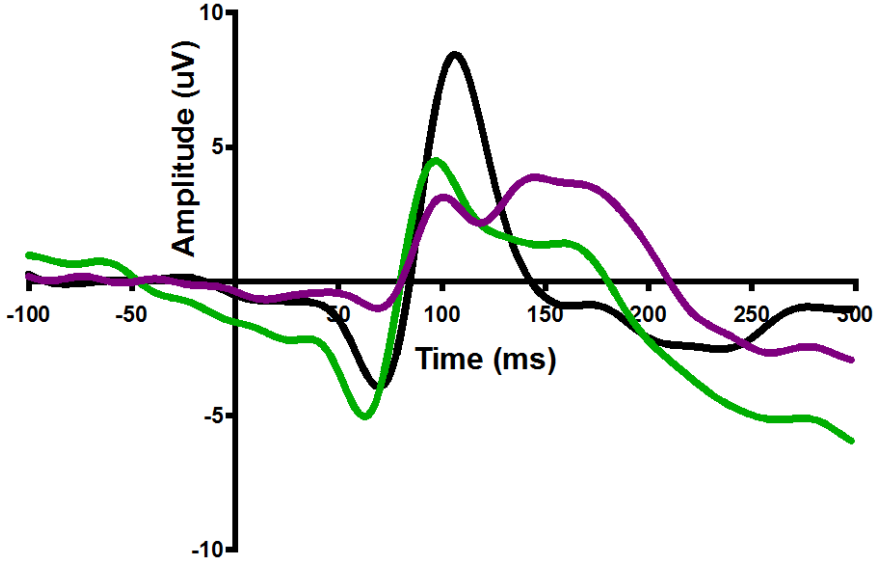


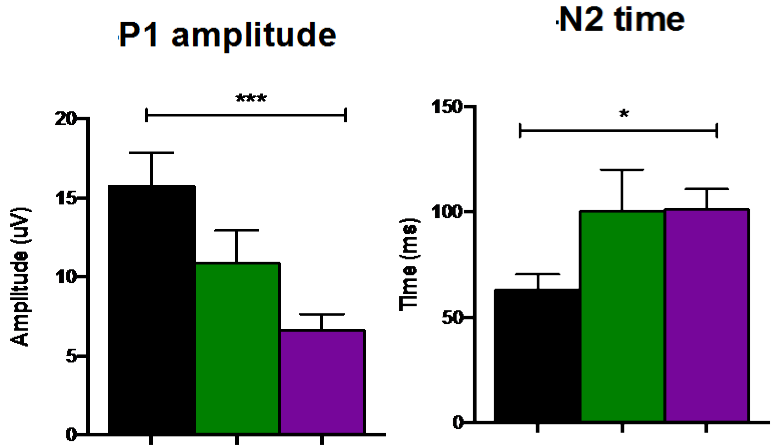


P1 amplitude is diminished and N2 time is increased in RTT

How does the VEP change with developmental progression in RTT?



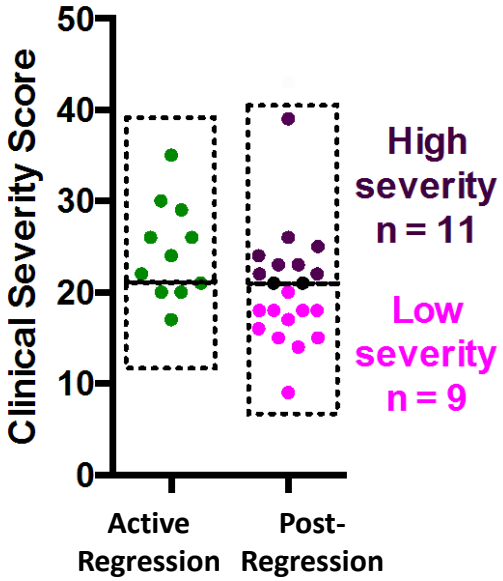


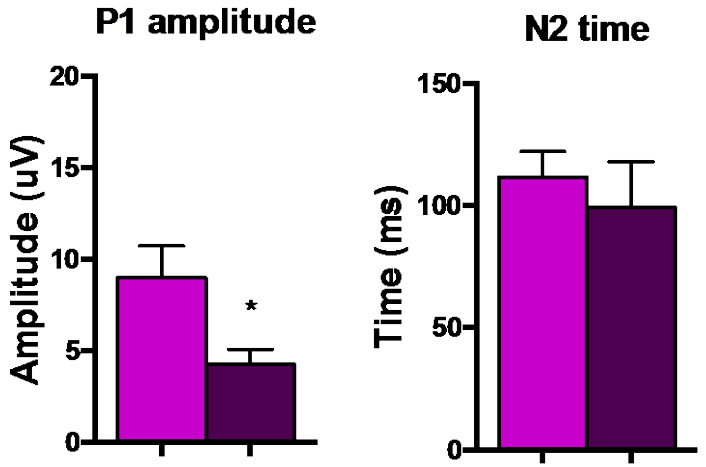


- **P1 amplitude diminishes with progression of the disorder**
- **Longer N2 time is a consistent feature of RTT throughout the stages**

Is the VEP sensitive to clinical severity?

Clinical severity score
Age of onset of regression
Head Growth
Sitting
Crawling
Ambulation
Non-verbal comm.
Language
Respiration
Seizures
Hand use
Feeding
Onset of stereotypies
Somatic growth
Autonomic dysfunction
Scoliosis



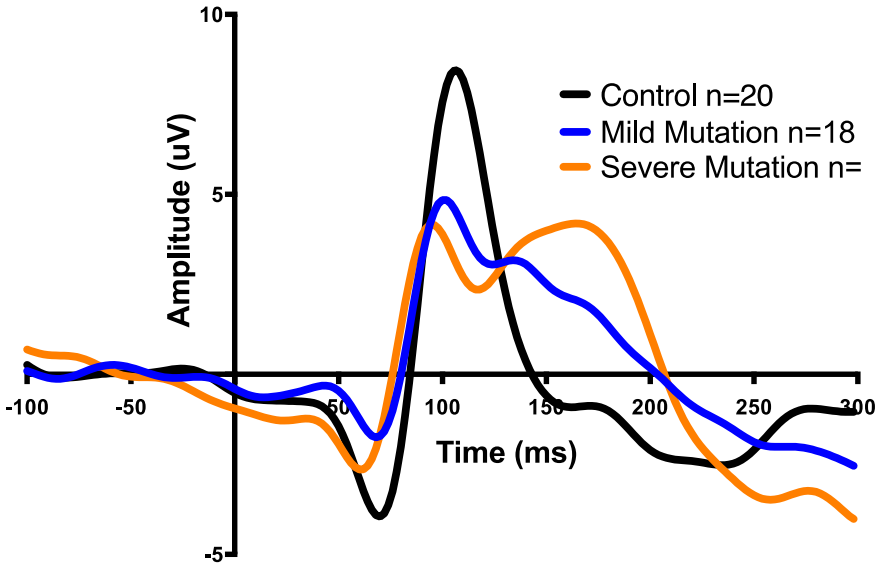


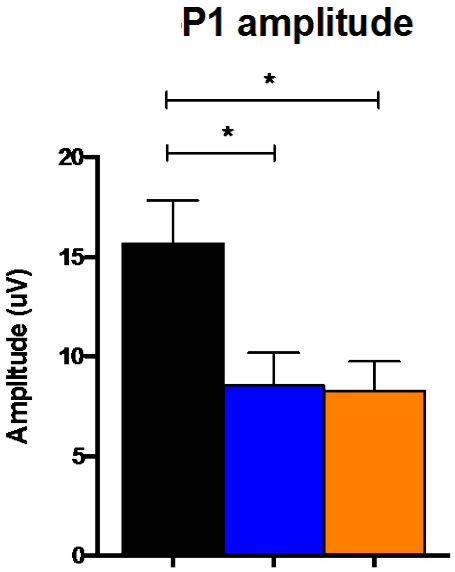
P1 amplitude is an index of clinical severity during the Post-Regression stage

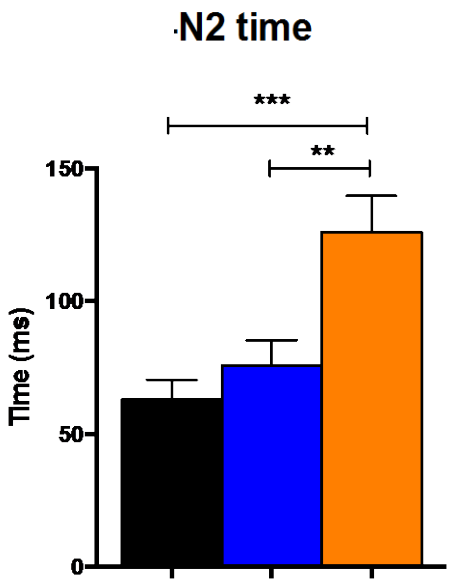
Is the VEP sensitive to MeCP2 mutation type?

Mild
R133C (n=2)
R294X (n=1)
R306C (n=4)
T158M (n=3)
C-terminal trunc (n=5)
Other deletions (n=3)

Severe
R168X (n=9)
R255X (n=3)
R270X (n=1)
Large deletions (n=2)

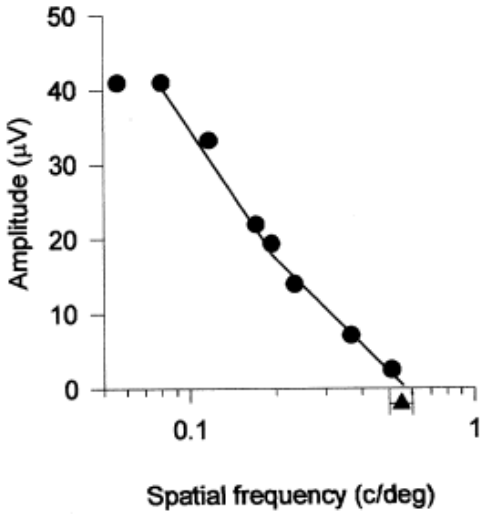


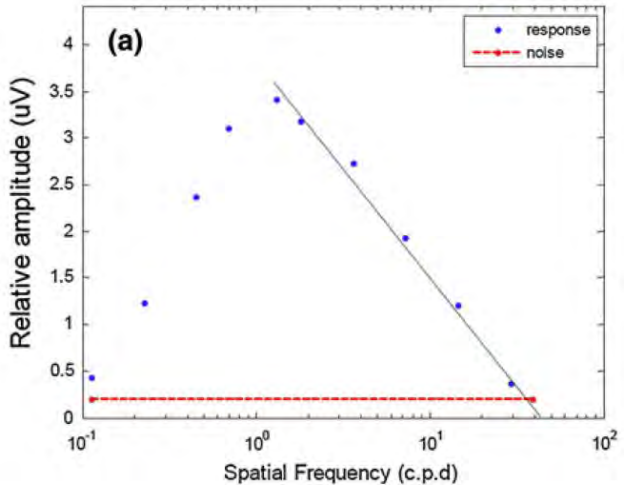




MeCP2 mutation severity selectively impacts N2 time and not P1 amplitude

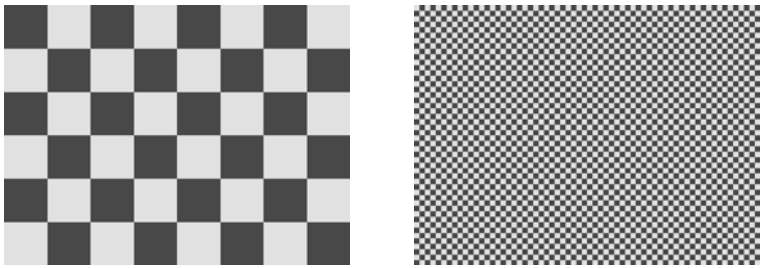
VEPs can be used to measure spatial resolution (acuity)





Low **High**

Spatial Frequency



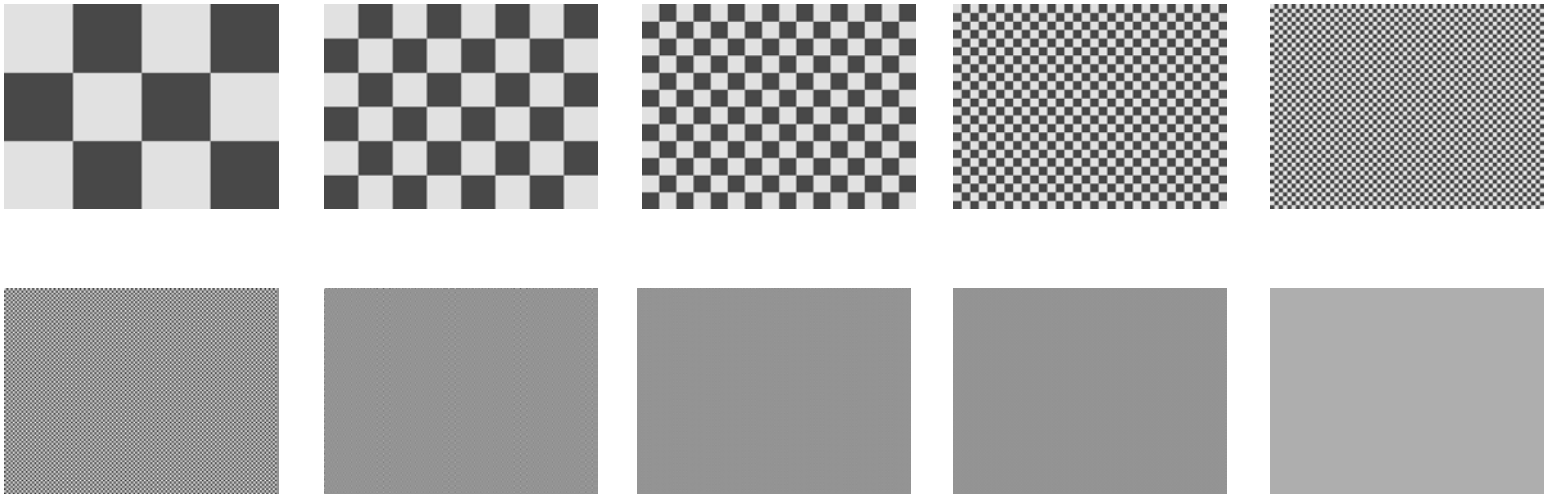
Porciatti et al., *Vision Research*, 1999

Iyer et al., *Doc Oph*thalmol, 2013

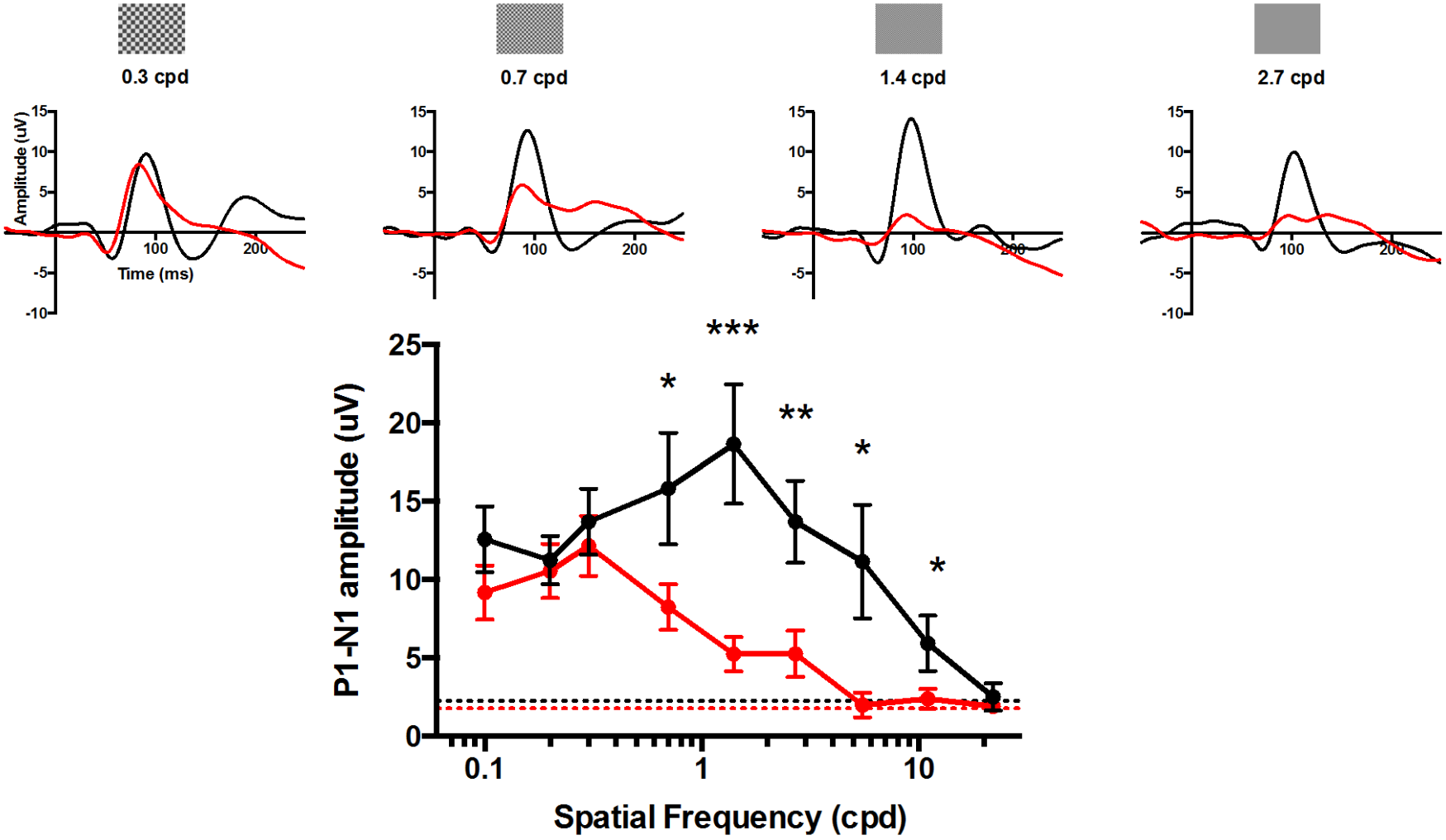
Testing acuity in Rett patients using VEP

Modifications:

- lower contrast (83%) to avoid eye strain
- faster frequency (4 Hz) to fit in more trials
- 50 trials instead of 100 to reduce total time
- varied spatial frequency



Spatial frequency tuning and acuity



Summary

- The Fagiolini lab found that MeCP2 knock-out mice displayed reduced behavioral and VEP acuity
- This inspired recording VEPs in humans with RTT in the Nelson lab
- We identified quantifiable alterations in waveform morphology that reflect cortical processing deficits
- These alterations were differentially impacted by disease stage and mutation type, indicating that VEP may be used as a biomarker
- We identified a functional impact on spatial resolution (acuity) in the girls that directly supports results in the mouse model

Summary: Part 1

- Intracortical processing of sensory stimuli is impaired in RTT
- Reduction in P1 amplitude worsens with progression of the disorder and is an index of clinical severity
  - Weak or asynchronous excitation
  - Local hypoconnectivity
- Prolonged N2 time is a consistent feature of Rett throughout the progression of the disorder but *does* reflect mutation type
  - Impaired intracortical signaling, ineffective inhibition, demyelination
- The VEP provides a quantitative unbiased biomarker for cortical function

Gaps of knowledge in Rett syndrome research

– **Cortical function**

Part I – Evaluating cortical function with visual evoked potentials

– **Cognitive function**

Part 2 – Evaluating cognitive function with a developmental behavioral assessment (MSEL) and eye-tracking

Part 2: Cognitive functioning

- We need cognitive assessments to
  - provide a functional correlate for research measures
  - provide an outcome measure for interventions or treatments
  - better understand needs and improve quality of life

- Current evaluations underestimate the cognitive abilities of children with RTT

Goal:

To assess cognitive skills while minimizing confounds from fine motor and expressive language deficits

Method:

Adapt the conventional administration of the MSEL for girls with RTT (n=36, mean age is 58 months, range is 22-123 months) for use with eye tracker

Mullen Scales of Early Learning (MSEL)

- 5 domains or "scales"
  - Gross Motor
  - Fine Motor
  - Expressive Language
  - Receptive Language
  - Visual Reception

- Play-based, interactive assessment
- For use from birth to 6 years old

- Output:
  - Raw score
  - Equivalent age
  - Descriptive category
  - Developmental quotient

Limitation for RTT: basic verbal and/or motor skills needed for most items

Incorporating eye tracking technology into the Adapted MSEL

- Girls use eye gaze to "greet, point, request, and refuse"
- Previous pilot studies have suggested that eye gaze tracking can be an effective method for assessing some aspects of cognition*
- Our lab has expertise with eye tracking systems

Pilot Study:

<u>Experimental Design</u>

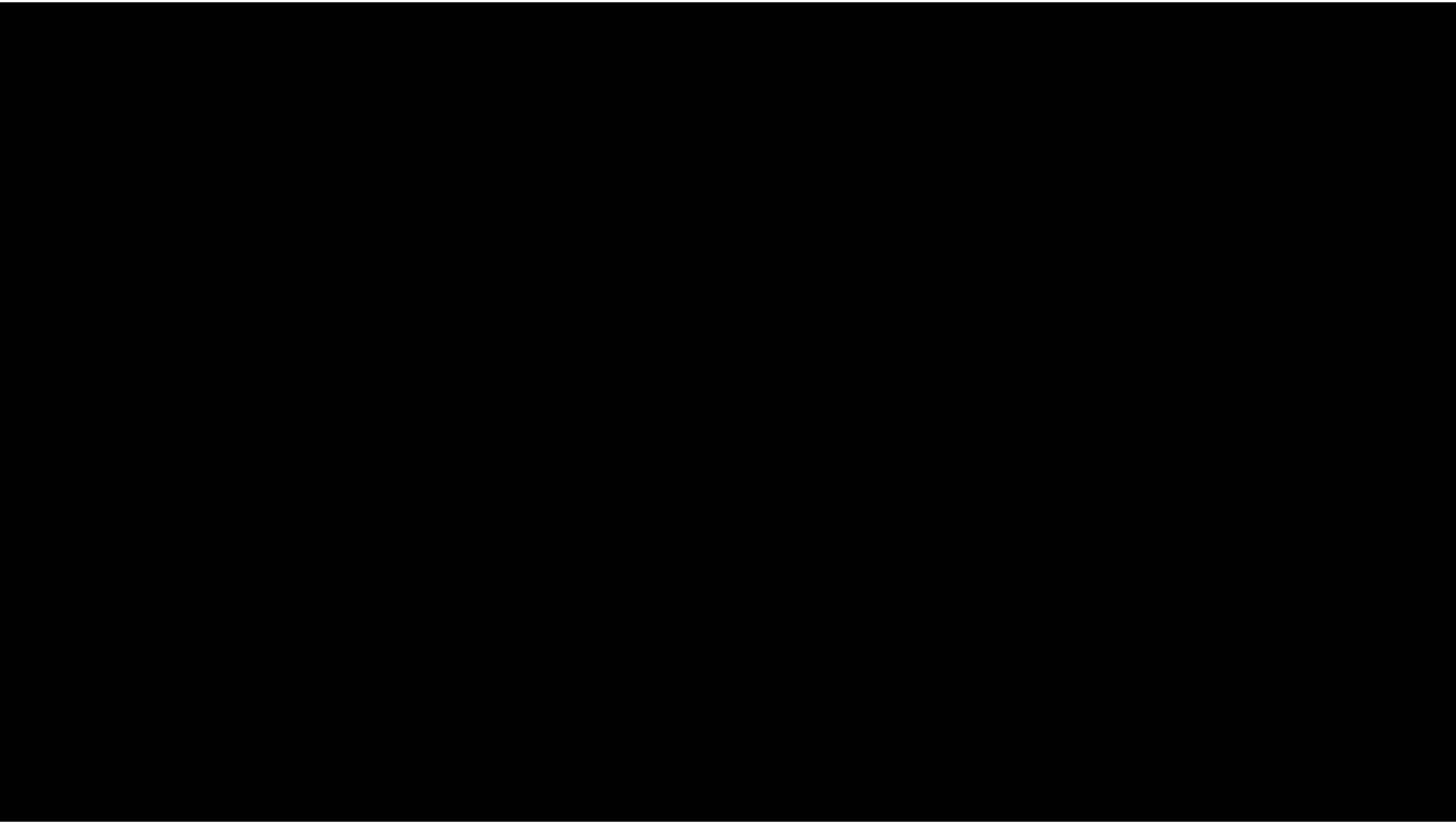
Translate Visual Reception and some Receptive Language MSEL items into PowerPoint slides presented on a Tobii® eye tracker monitor

<u>Subjects</u>

12 girls with RTT, 2-4 years old

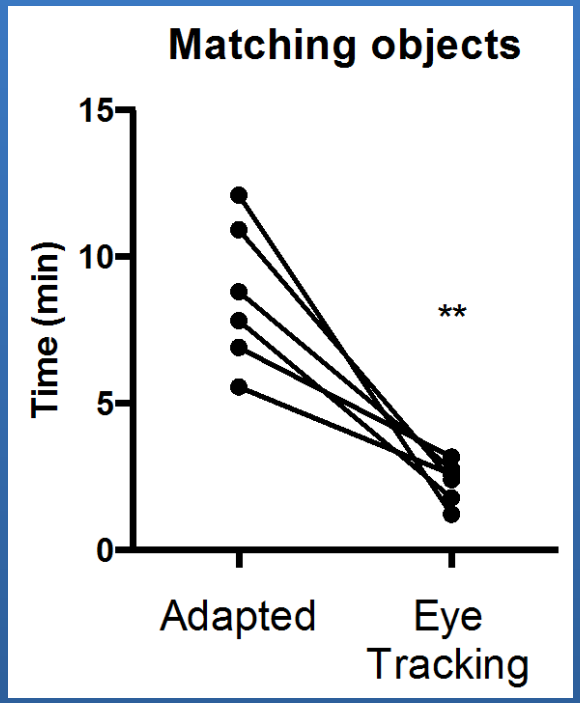
Administered both Adapted and Eye tracking MSEL to same individual

*von Tetzchner *et al.*, 1996; Baptista *et al.*, 2006; Djukic *et al.*, 2012; Rose *et al.*, 2013

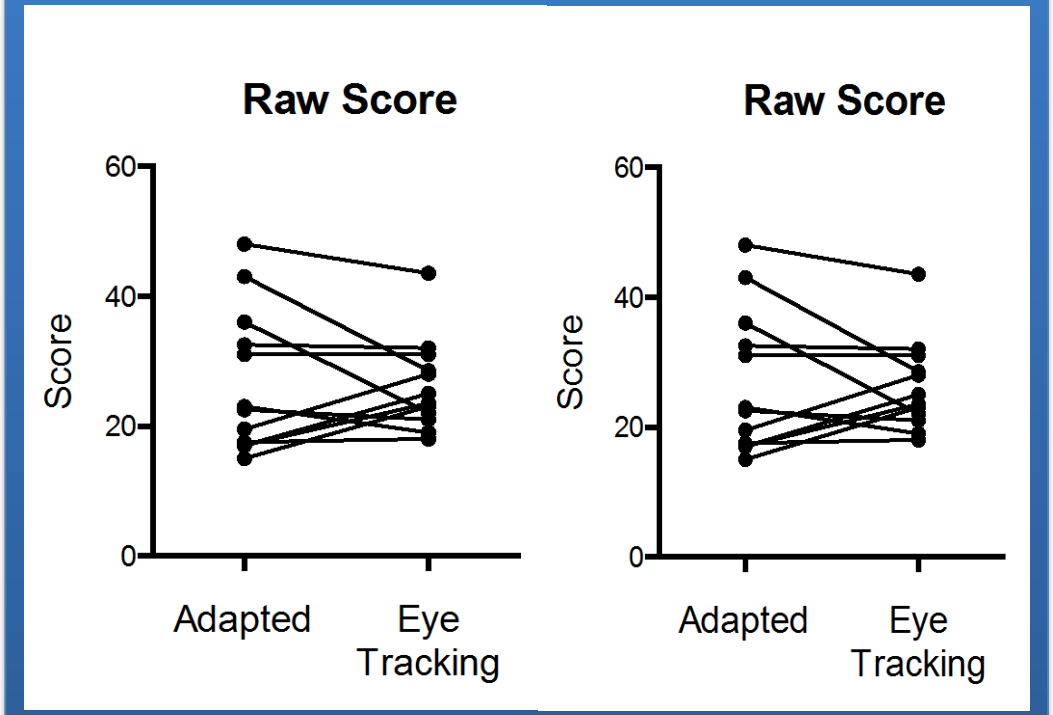


Adapted vs. Eye Tracking MSEL outcomes

Less time to administer items on the Eye Tracking MSEL



Similar outcomes on both paradigms for Visual Reception



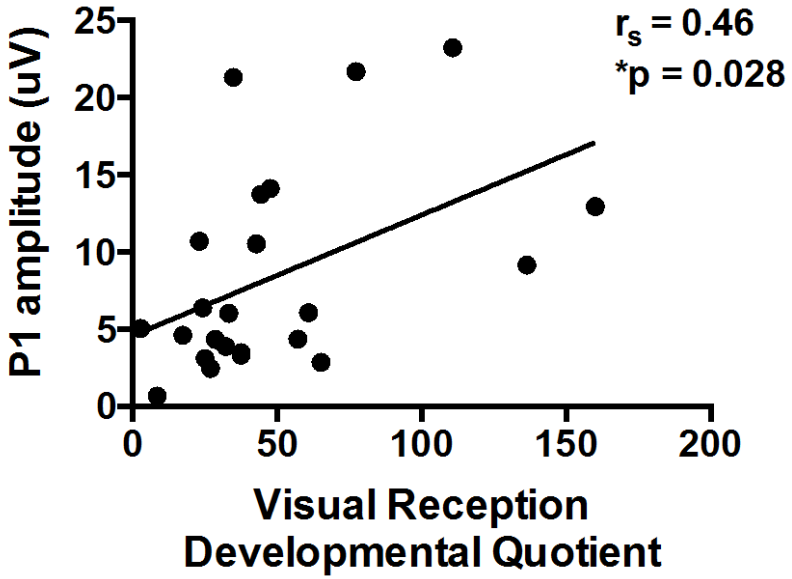
Summary: Part 2

- Cognitive impairment is a significant feature of RTT
- HOWEVER, some do surprising well, indicating some “hidden abilities” that might not be detected by standard assessments
- Impacts how parents interact and communicate with their child
- Eye gaze represents an important avenue for cognitive assessment in RTT and other disorders with fine motor or expressive language limitations

Does the VEP reflect cognitive function?

VEP P1 amplitude positively correlates with visual reception skills on the Adapted MSEL

We have both VEP and Adapted MSEL data from 23 girls



Overall summary and future directions

Summary

1) The VEP provides a promising biomarker of cortical function

2) Adaptation of the MSEL improves assessment of cognition in RTT

Future Directions

- Complete eye tracker version for Receptive Language domain
- Continue to further adapt items for RTT
- Incorporate both VEP and eye tracking MSEL into clinical trials
- Further ground human VEP work in animal models

THE END

- charles_nelson@harvard.edu

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Lunch

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Afternoon Agenda

1:40 **Session 3: Moving Forward with Linking Biology to Clinical Observation**

Elizabeth Powell, Ph.D., Chair
Associate Professor
Department of Anatomy and Neurobiology, University of Maryland

1:45 **Modeling the Social Brain: Developing Preclinical Assays of Symptom Onset Patterns**

Jill Silverman, Ph.D.
Assistant Professor
MIND Institute and Department of Psychiatry and Behavioral Sciences, University of California Davis School of Medicine

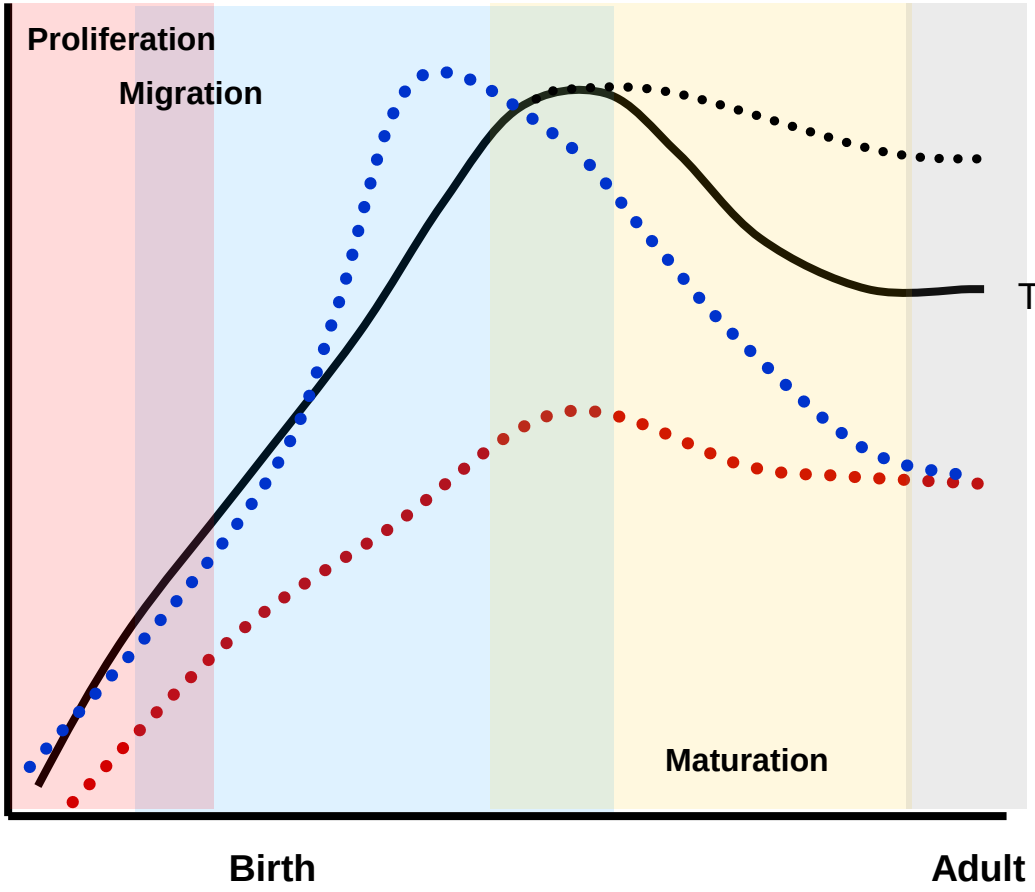
Moving forward with linking biology to clinical observation

Elizabeth Powell, PhD

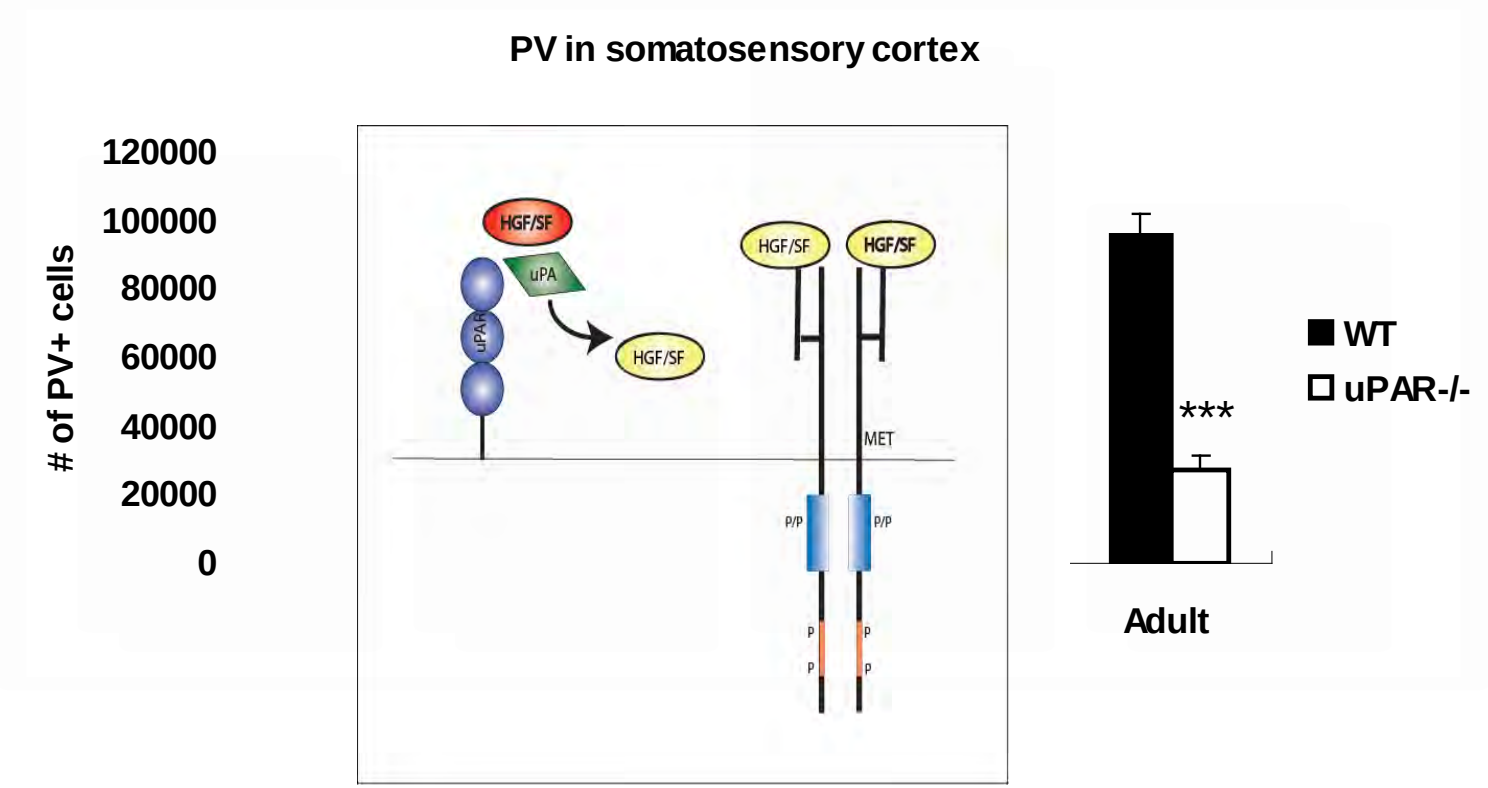
Session 3

What are the underlying parameters that regulate onset patterns?

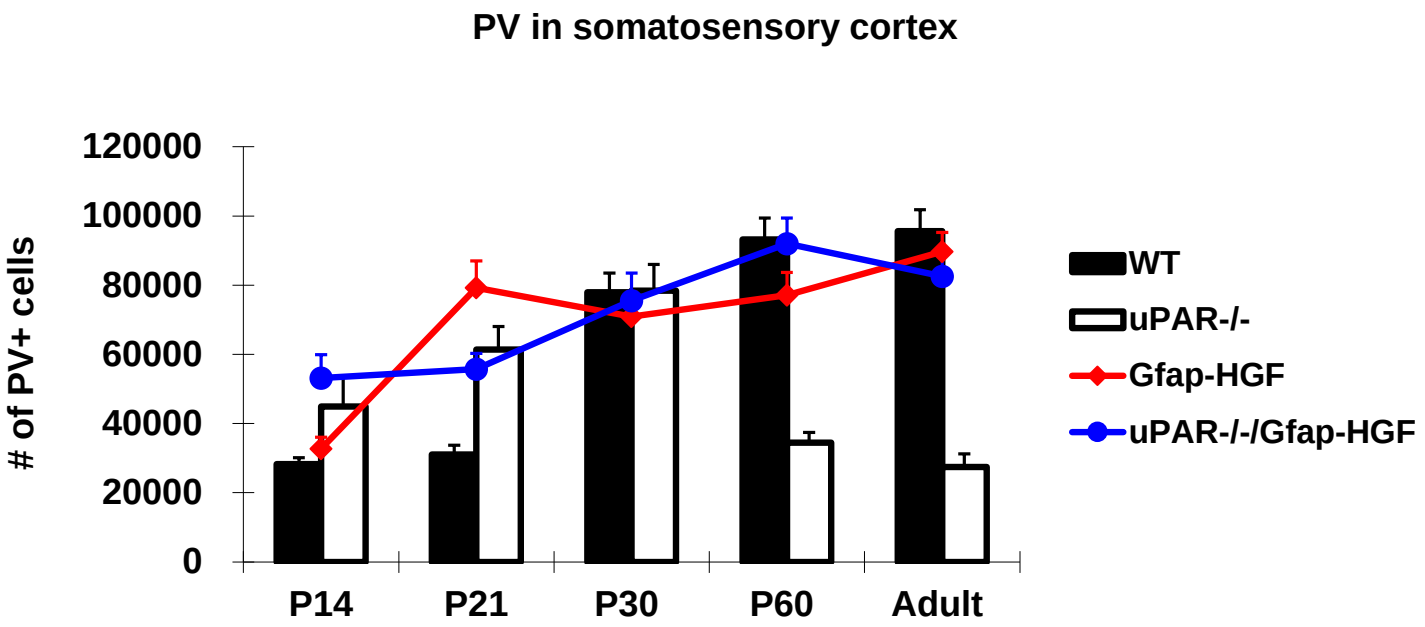
Skill,
Cell number.
Biomarker



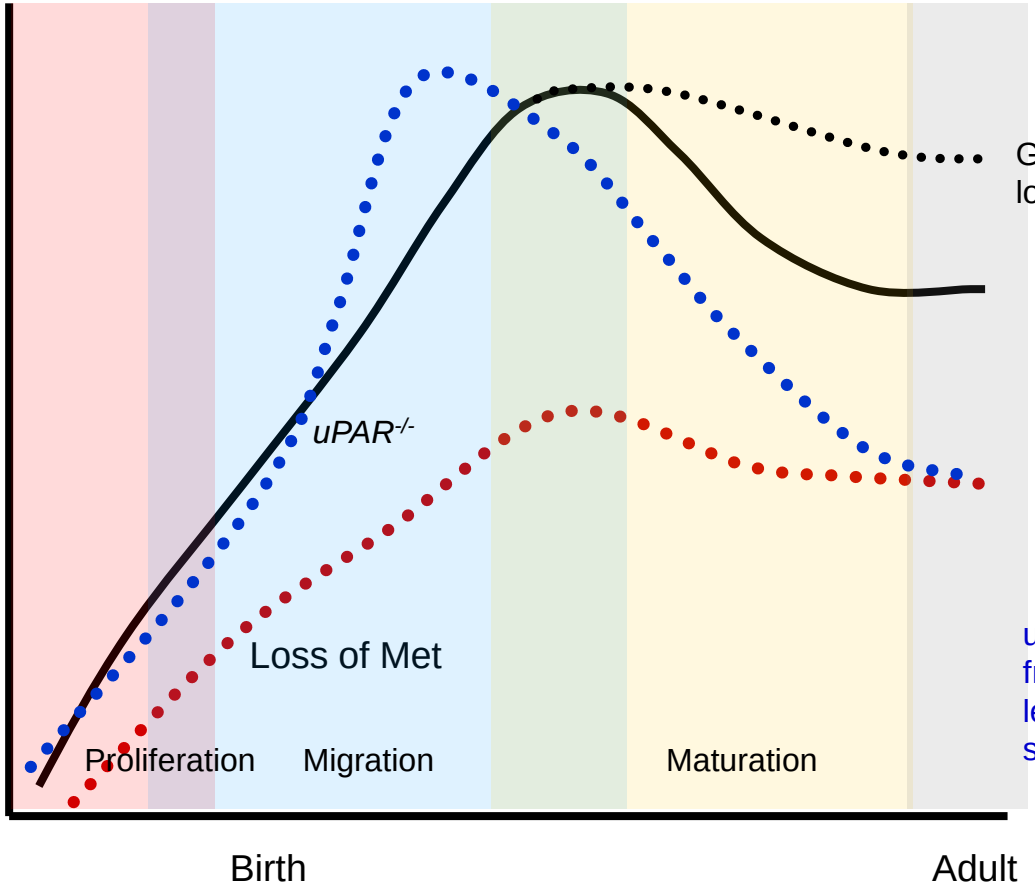
History may be important



More ligand (HGF) restored the adult interneurons…



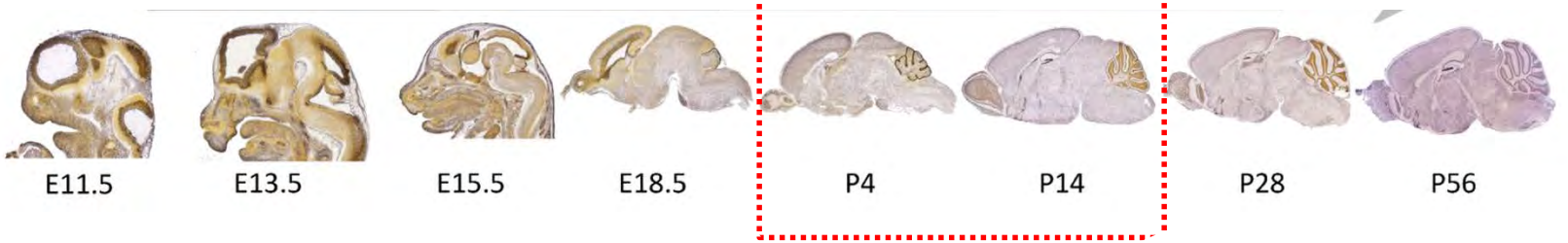
But…only some behavior measures were improved and some new deficits were found

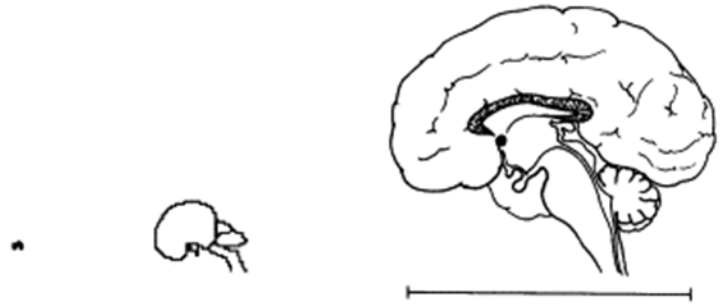


Gfap-HGF - Impaired frontal lobe function

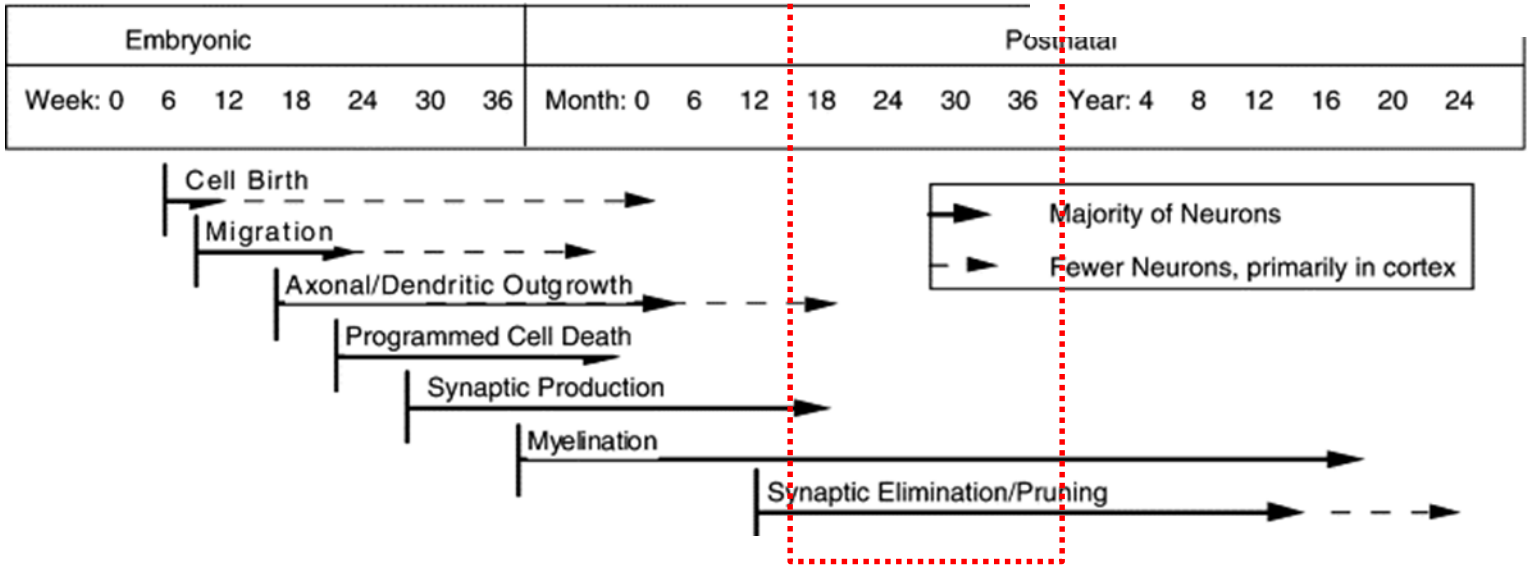
Cortex: (Met-Emx1) - Epilepsy, Dyskinesia,
Basal Ganglia (Met-Dlx5/6) -Impaired cued learning

uPAR null- Epilepsy, Impaired frontal lobe function, Impaired learning, anxiety, impaired social interaction





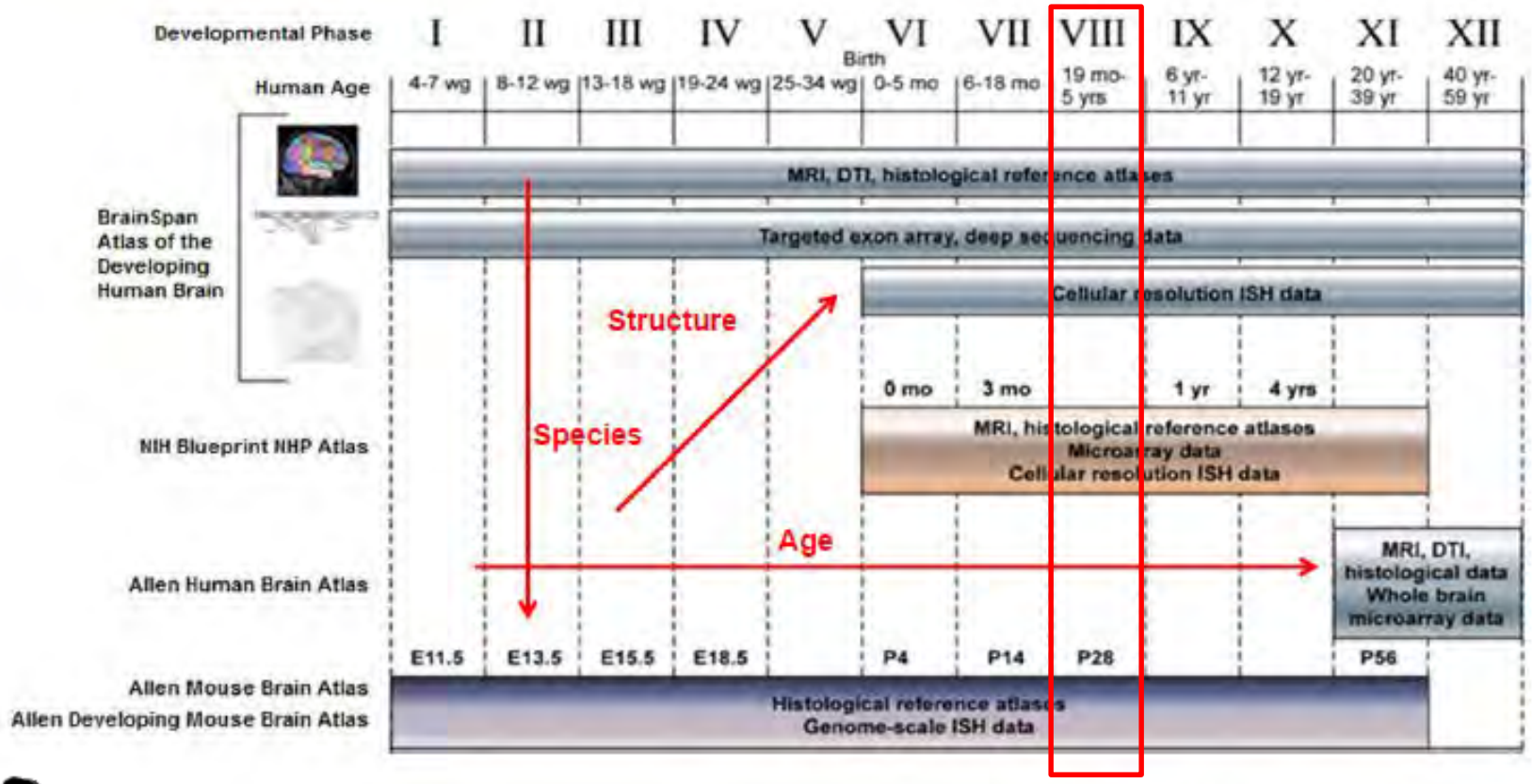
Behavior – Jill Silverman
Anatomy/imaging – Jason Lerch



Neurosci; Biobehav Rev 27:3-18 (2003)

Other models and tools? Can we fill in the gaps?

Genetics - Eric Morrow
Ferret development – Sharon Juliano
Primate social behavior – Karen Parker



NIH Toolbox®
For the Assessment of Neurological and Behavioral Function

ALLEN INSTITUTE for BRAIN SCIENCE

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Afternoon Agenda

2:05 **Mouse Brain Imaging**

Jason Lerch, Ph.D.
Senior Scientist, Associate Professor in Medical Biophysics
University of Toronto

2:25 **Panel Discussion**

Discussant: Eric Morrow, M.D., Ph.D., Associate Professor of Biology and Psychiatry, Brown University

Discussant: Sharon Juliano, Ph.D., Professor, Neuroscience and Molecular Biology, Uniformed Services University of the Health Sciences

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Afternoon Agenda - continues

2:25 **Mouse Brain Imaging**

Panel Discussion - Continued

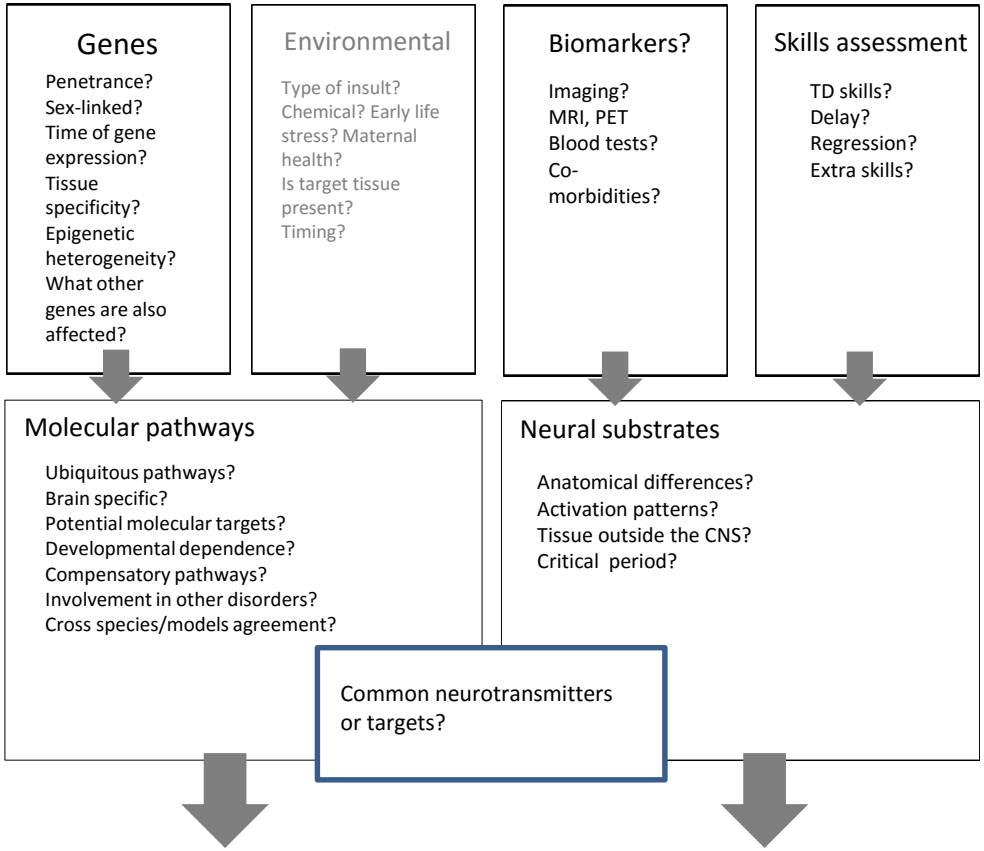
Discussant: Karen Parker, Ph.D., Associate Professor, Department of Psychiatry and Behavioral Sciences, Stanford University

3:10 **Break**

3:20 **Session 4: Bringing it all Together and Back to ASD**

Panel Discussion
(Session Chairs and Presenters)

Session 4



Actions

Timing of treatment?
Single or multiple treatments?
Expected outcome: short term, long term?
Specificity? Off–target issues?
Risks?
Preclinical validity?
Cross species (models) applicability?

Break

Loss of Skills and Onset Patterns in Neurodevelopmental Disorders: Understanding The Neurobiological Mechanisms

Afternoon Agenda - continues

4:30 **Next steps and Closing Remarks**

Audrey Thurm, Ph.D.
Staff Scientist, Pediatrics and Developmental Neuroscience Branch (NIMH)

Ann Wagner, Ph.D.
Chief, Neurobehavioral Mechanisms of Mental Disorders Branch
Division of Translational Research (NIMH)

5:00 **Adjournment**