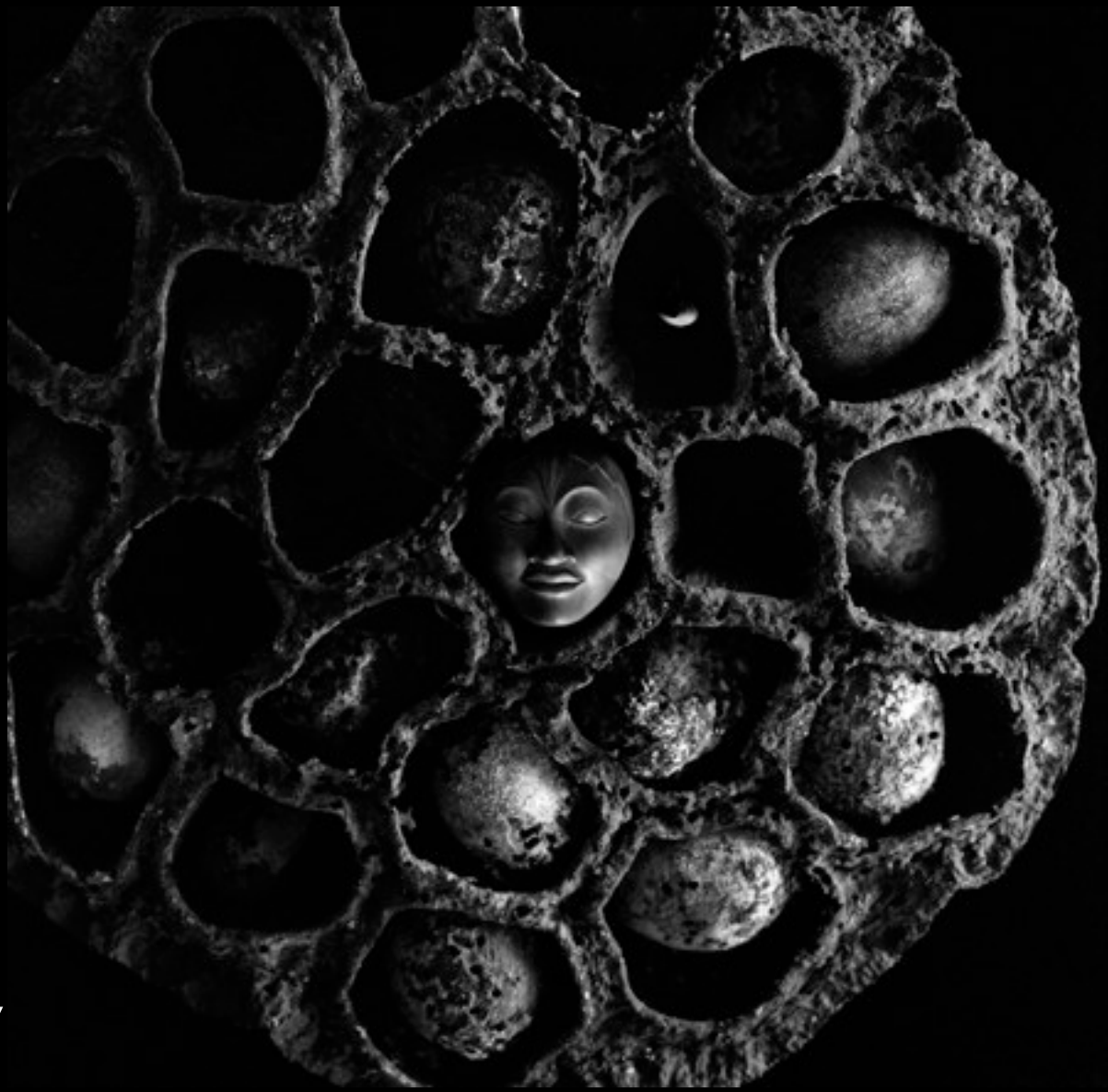
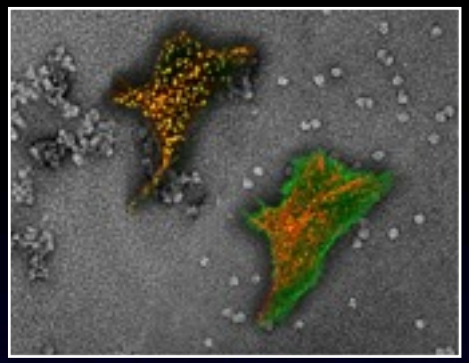
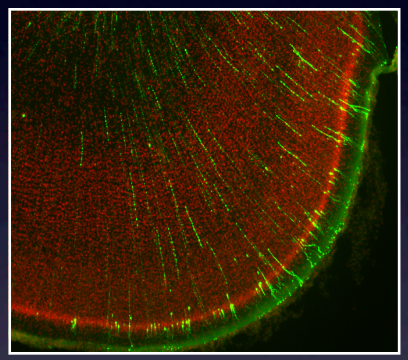
Using stem cells to study to Autism

Ricardo Dolmetsch
Department of Neurobiology
Stanford University



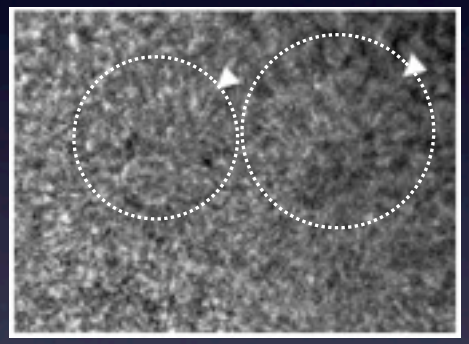
Our Lab





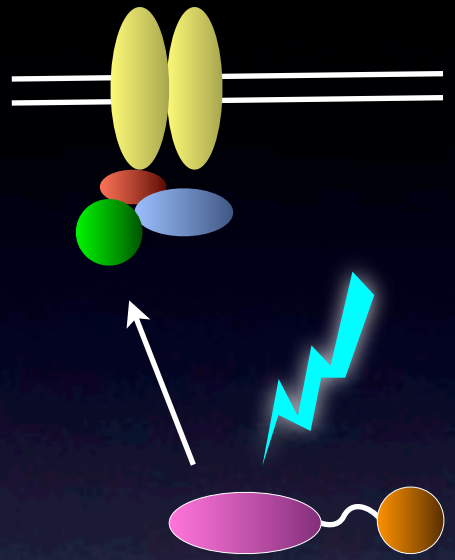
Calcium channel signaling

Science (2010)
Cell 136:876-90 (2009)
PNAS 106 :5495-500 (2009)
Cell 139:380-92 (2009)
Journal of Cell Biology 187:279-94 (2009)
Neuron 55:615-22 (2007)
Cell 127:591-606 (2006)



Autism

Nature (2011)
Nature Medicine (2011)
Nature (in revision)

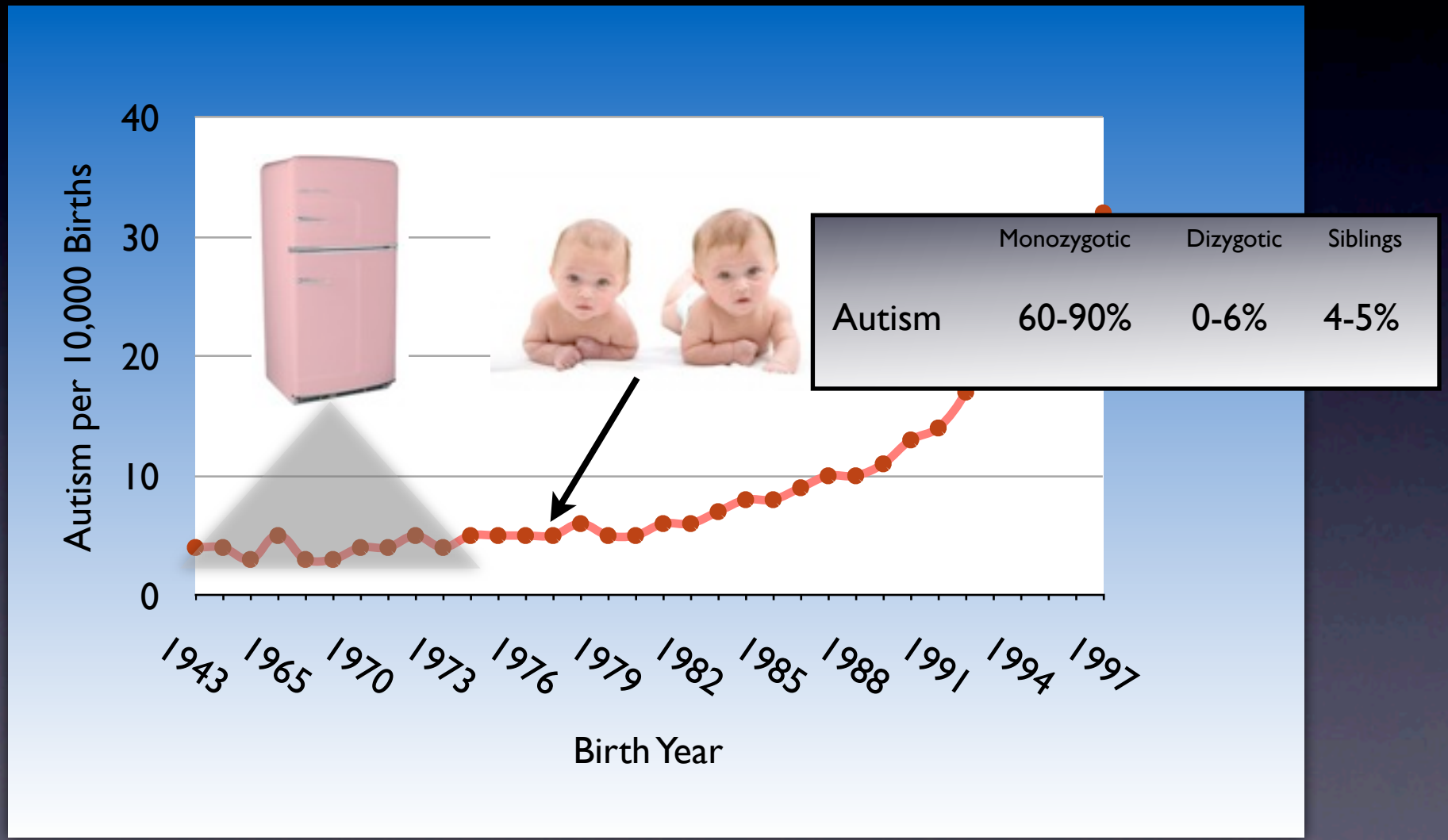


New technologies for studying channel signaling

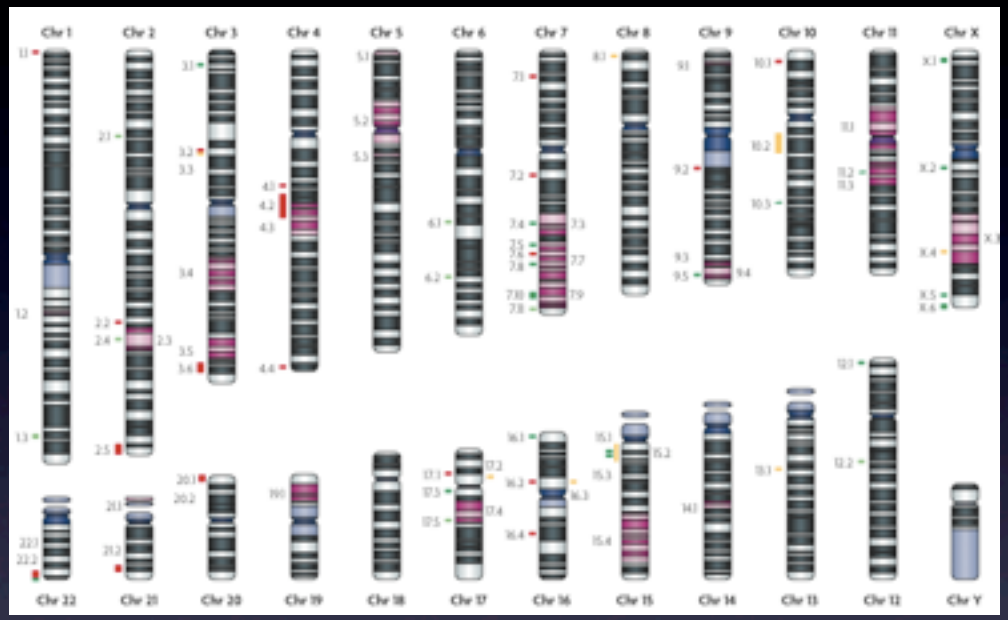
Nature Biotechnology 27:941-5 (2009)

Autism

Prevalence in 2010: 1/100

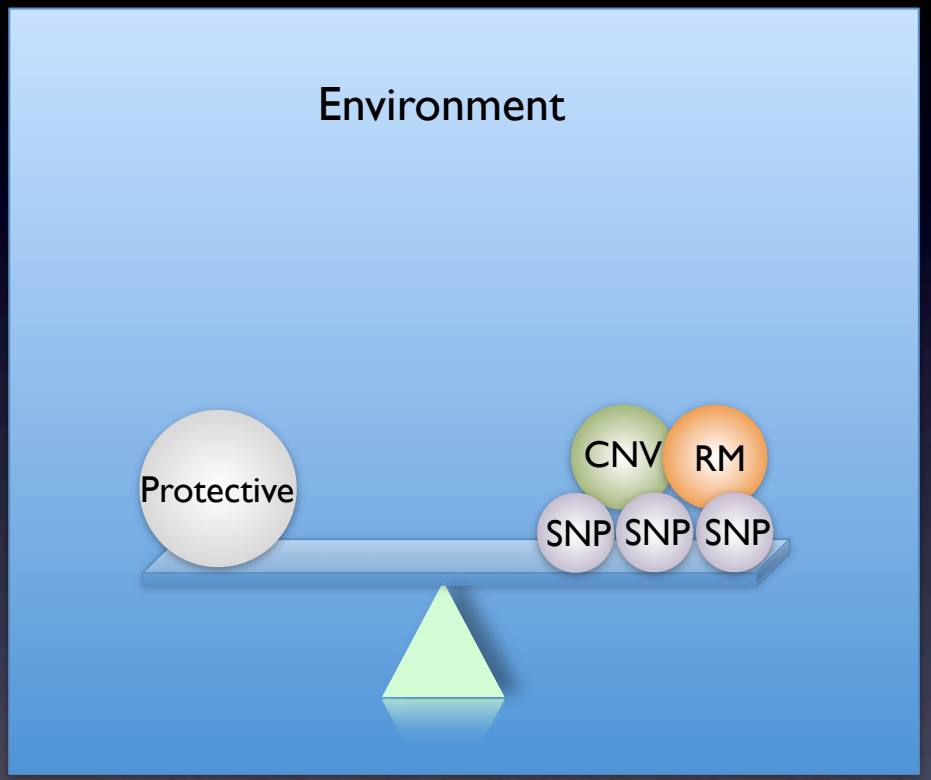


Autism is highly heritable



Geschwind et al.

Model

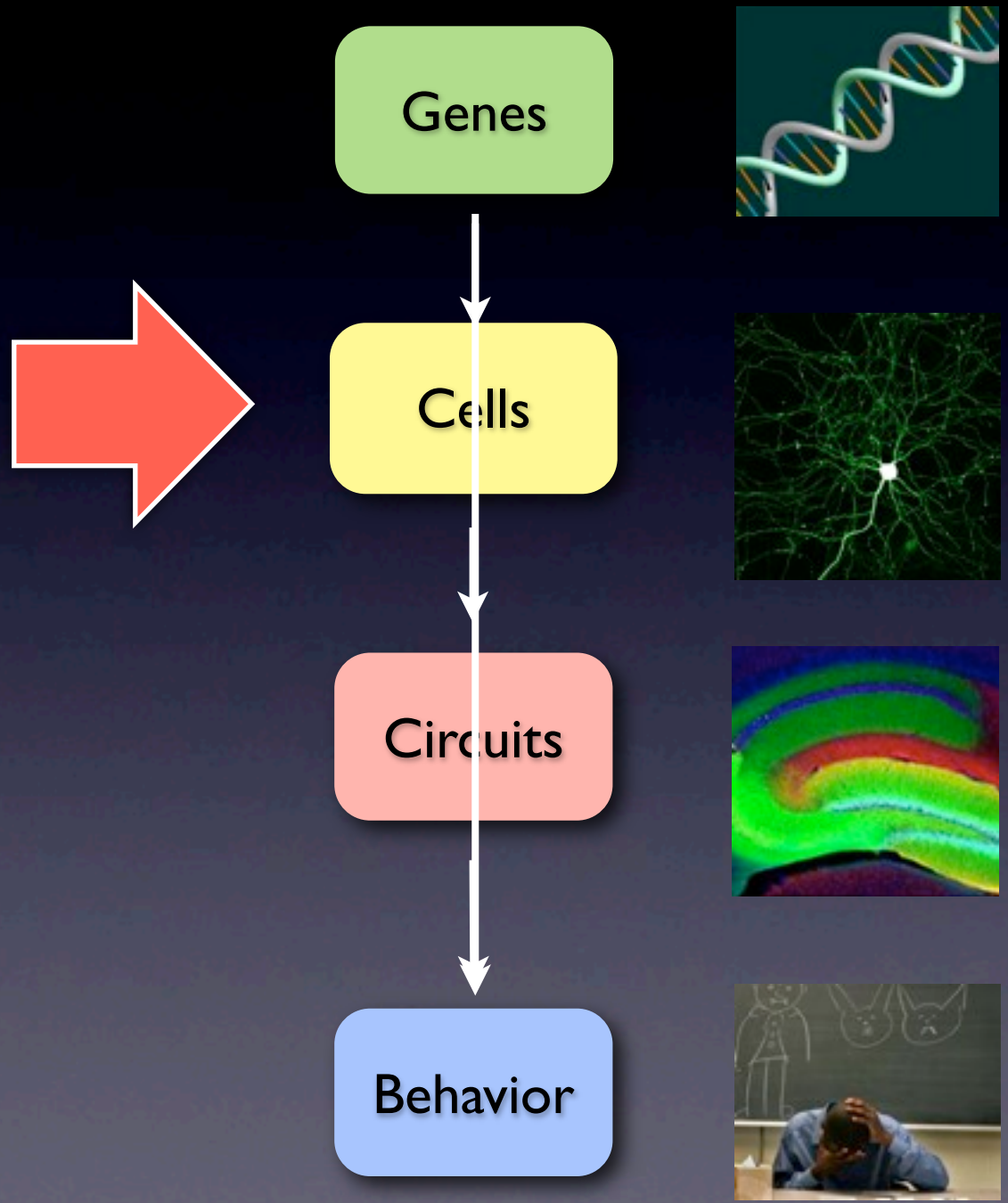


SNP- single nucleotide polymorphisms

RM - rare mutations

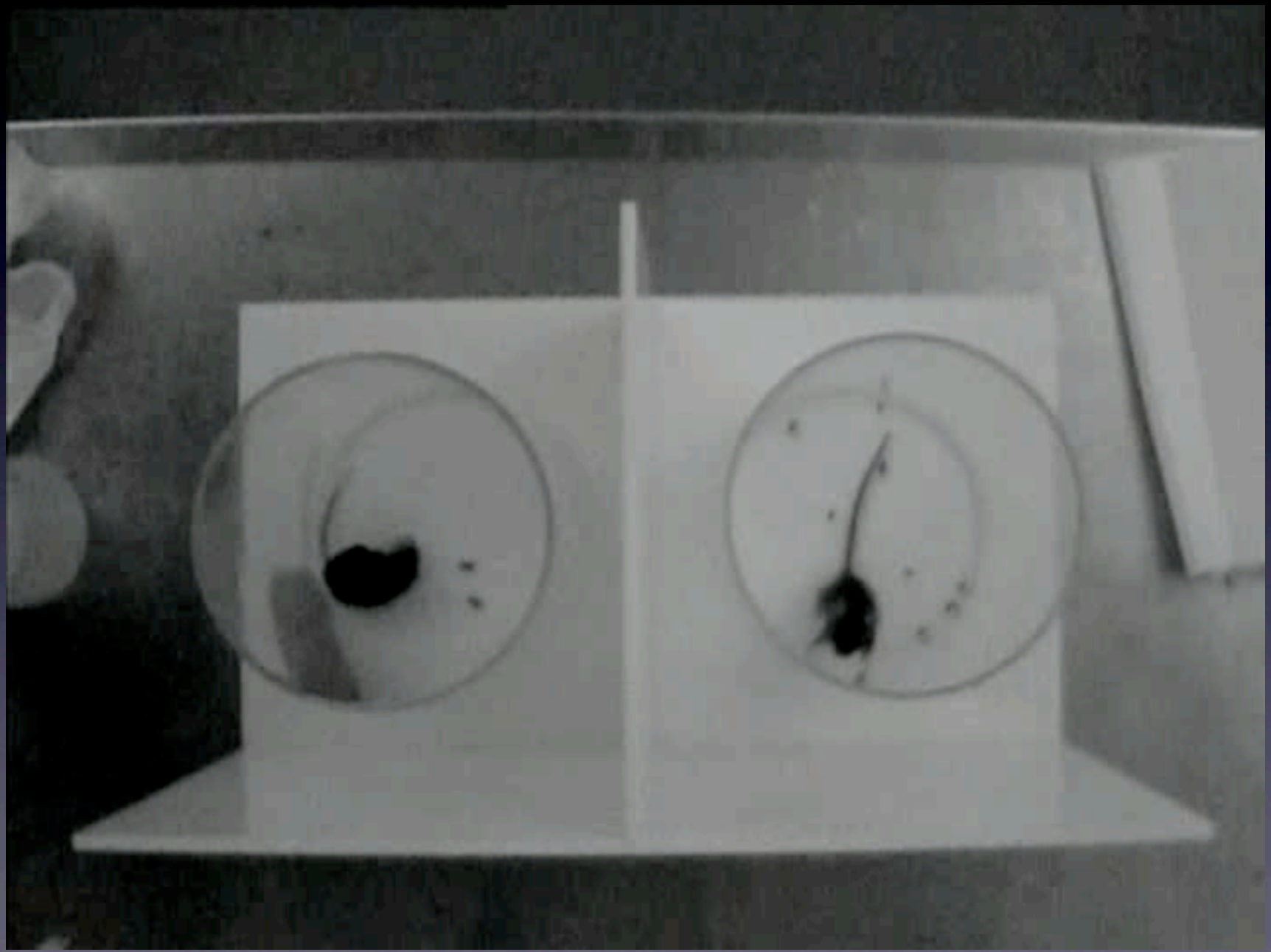
CNV - copy number variations

How do mutations in genes lead to autism?



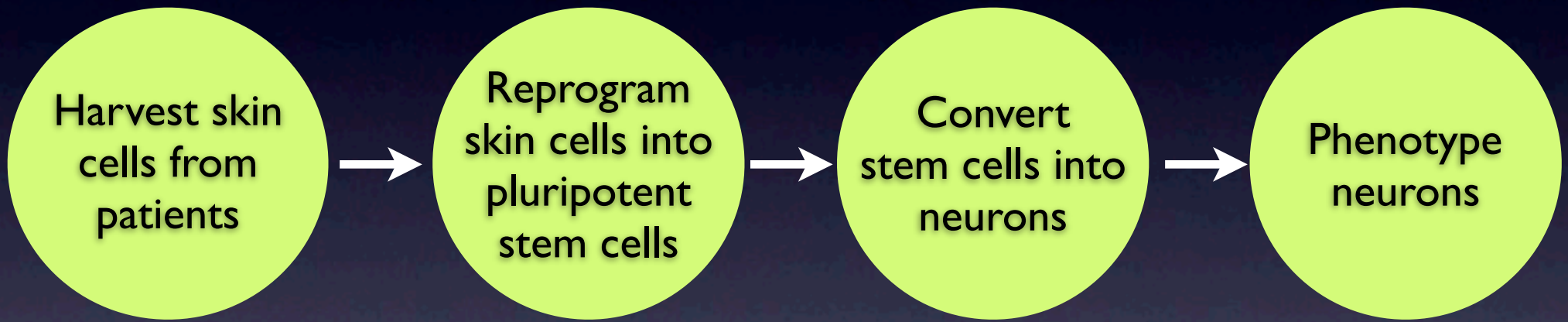
You make a mouse

16p11.2 deletion mouse



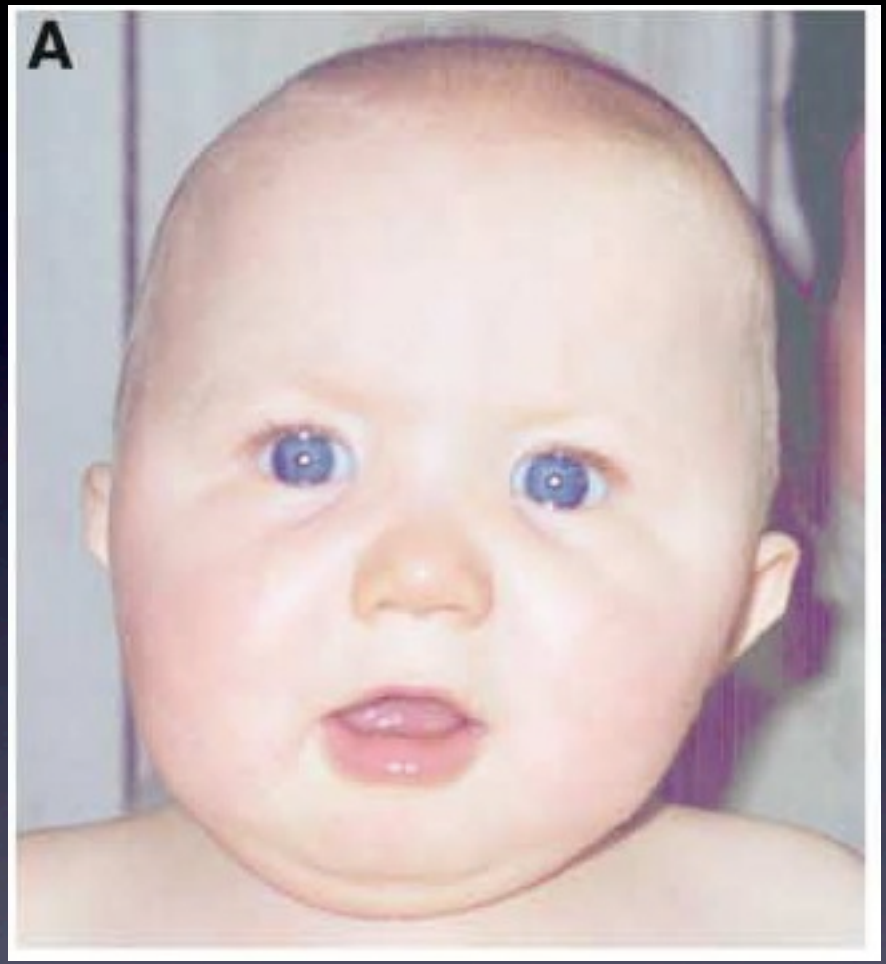
Autism involves many genes and it is hard to replicate a human genetic background in a mouse

And...
mice are not humans

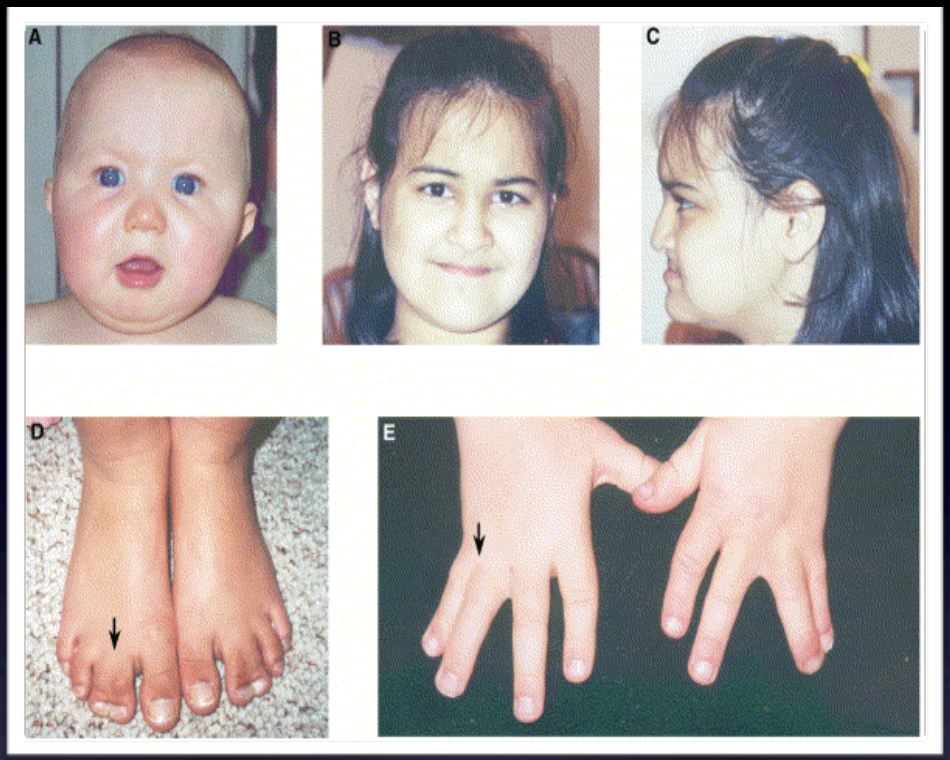

Harvest skin cells from patients
Reprogram skin cells into pluripotent stem cells
Convert stem cells into neurons
Phenotype neurons

Which patients ?

- Timothy Syndrome
- Ch16p11.2 Deletion Syndrome
- Phelan McDermid Syndrome
- Di-George Syndrome
- Dravet Syndrome



Timothy Syndrome

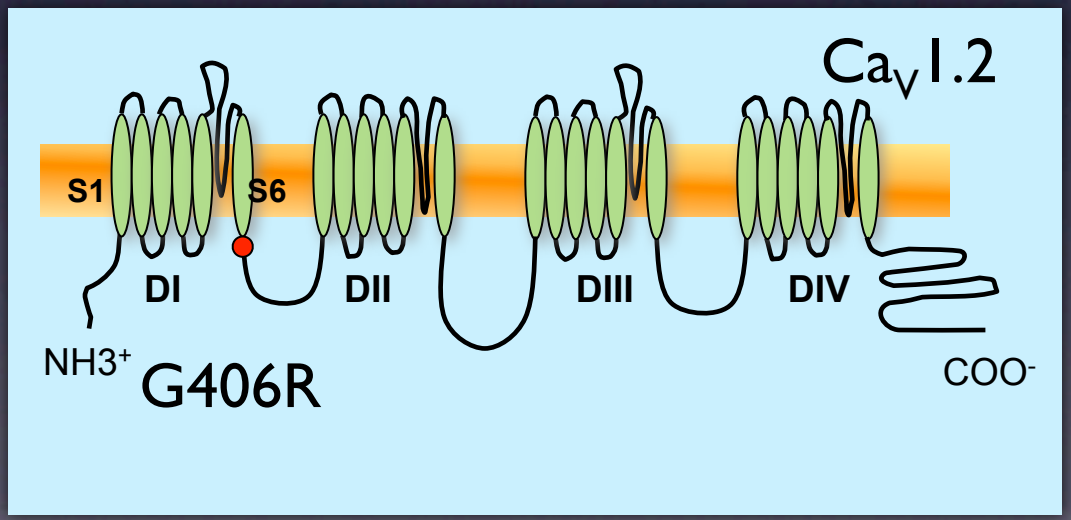


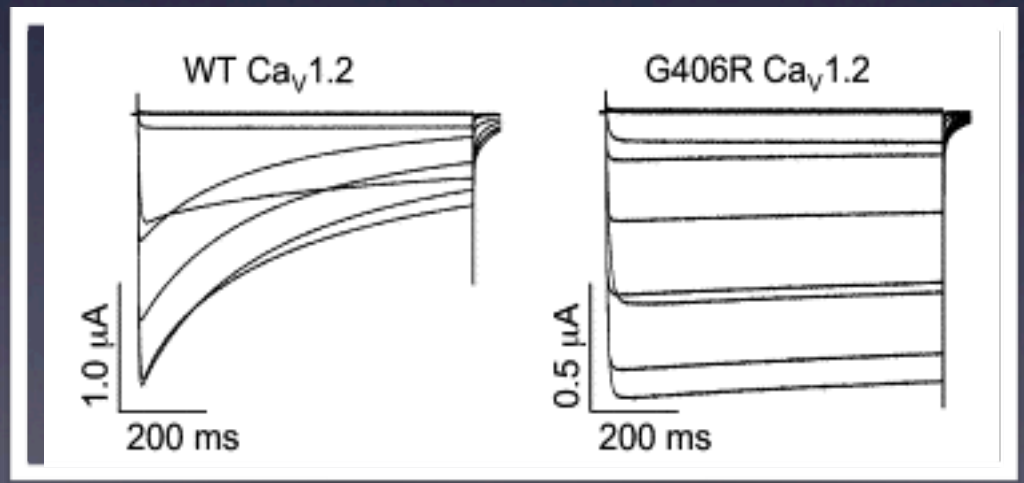
Cutaneous Syndactyly

Hypoglycemia

Autism

Cardiac Arrhythmia





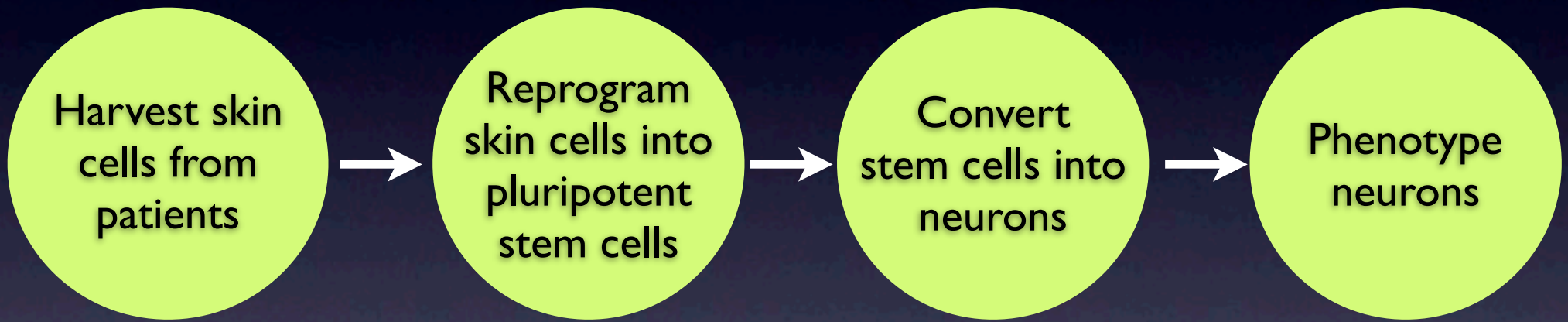
Splawski et al., 2004

L-type calcium channels in autism, bipolar disorder and schizophrenia

Splawski et al. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell (2004) vol. 119 (1) pp. 19-31

Ferreira et al. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet (2008) pp. 3

Nyegaard et al. CACNA1C (rs1006737) is associated with schizophrenia. Molecular Psychiatry (2010) vol. 15 (2) pp. 119-121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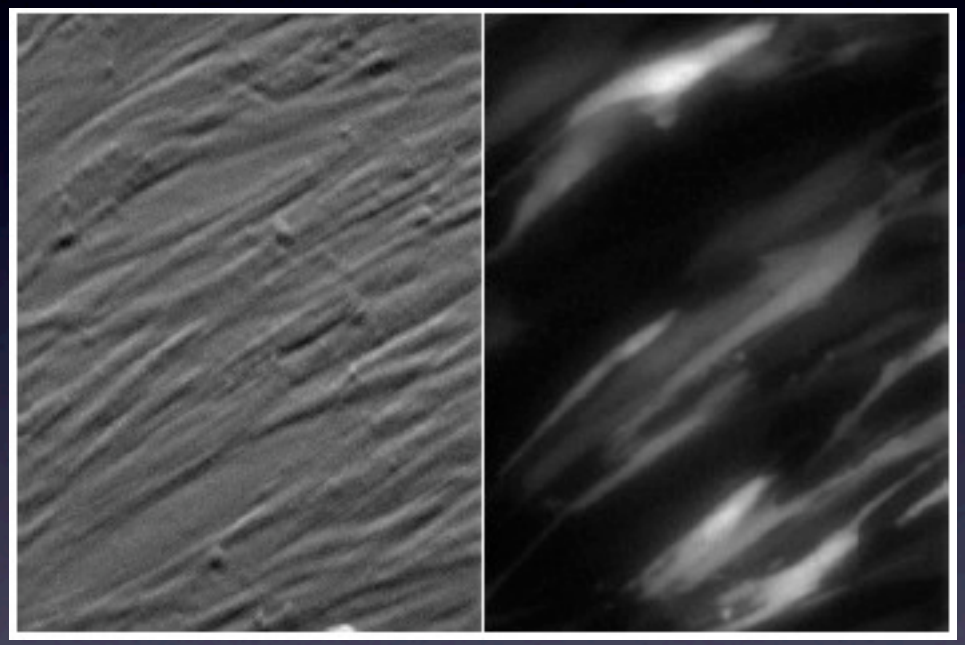
Making stem cells from the skin cells of ASD patients

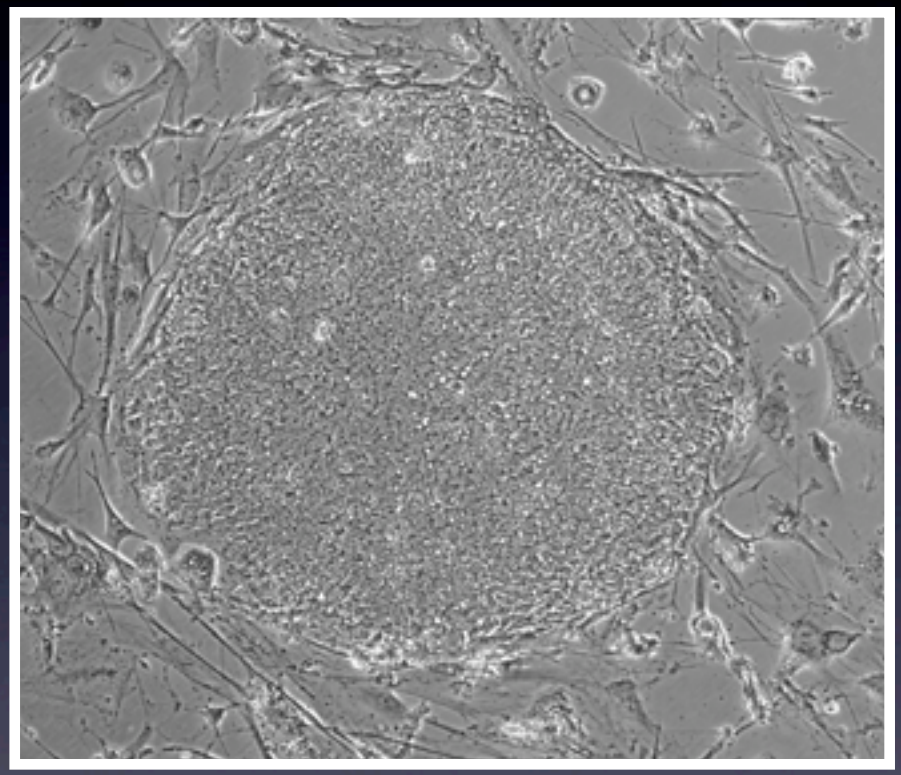


+

Oct3/4

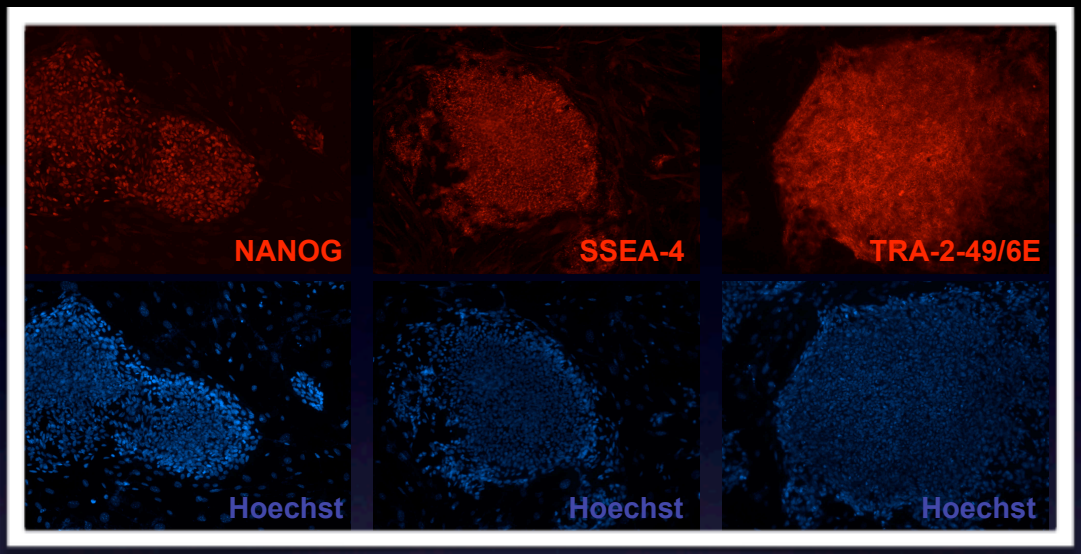
Sox-2

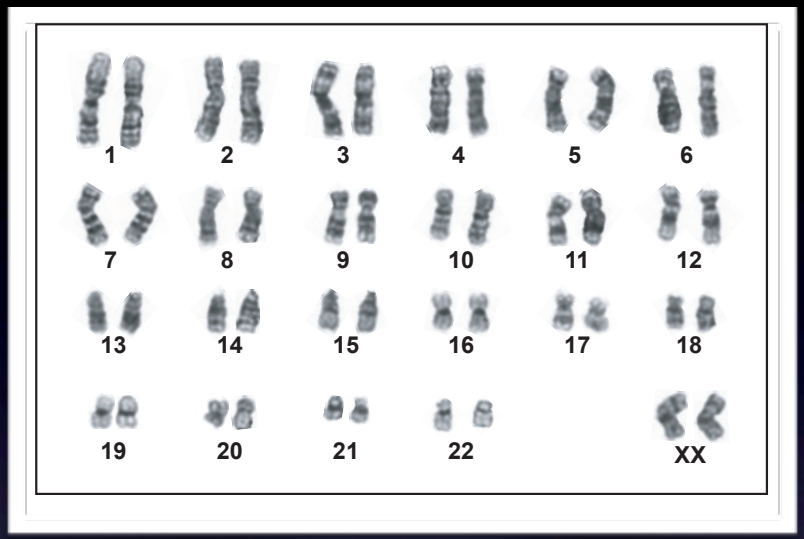
Klf-4

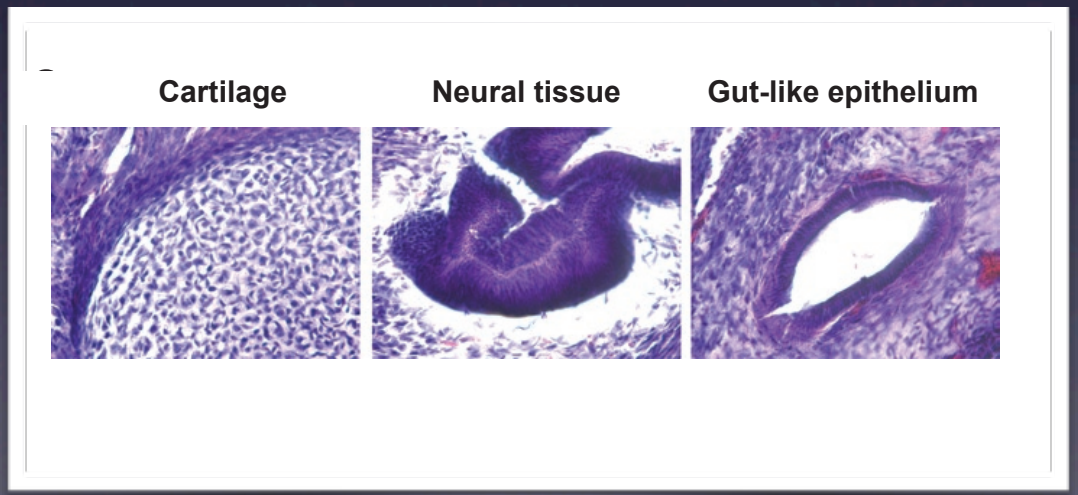


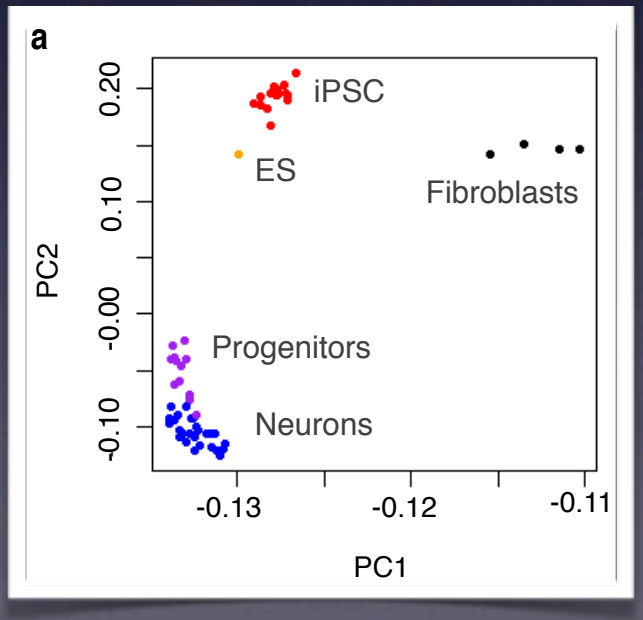
Kazutoshi Takahashi.... Shinya Yamanaka Cell 2007

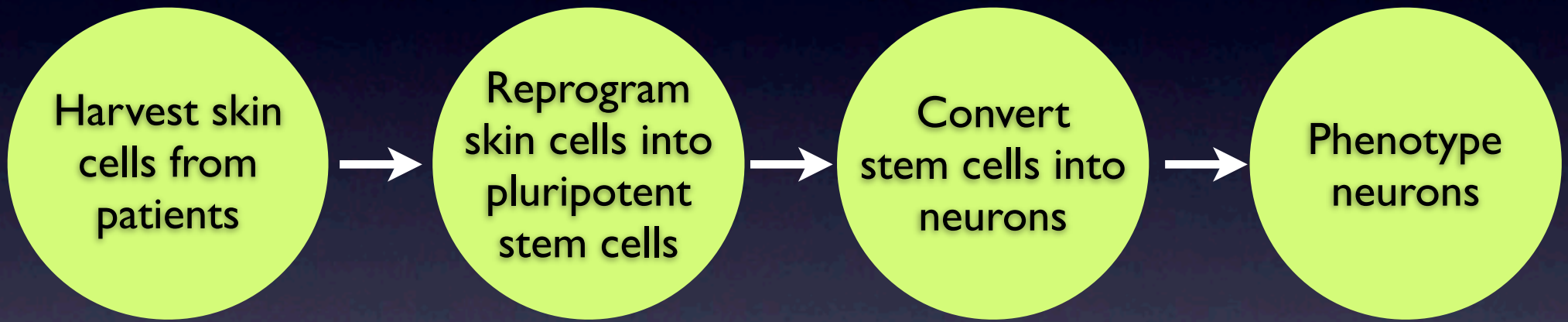
Characterization of iPS cell lines










Harvest skin cells from patients
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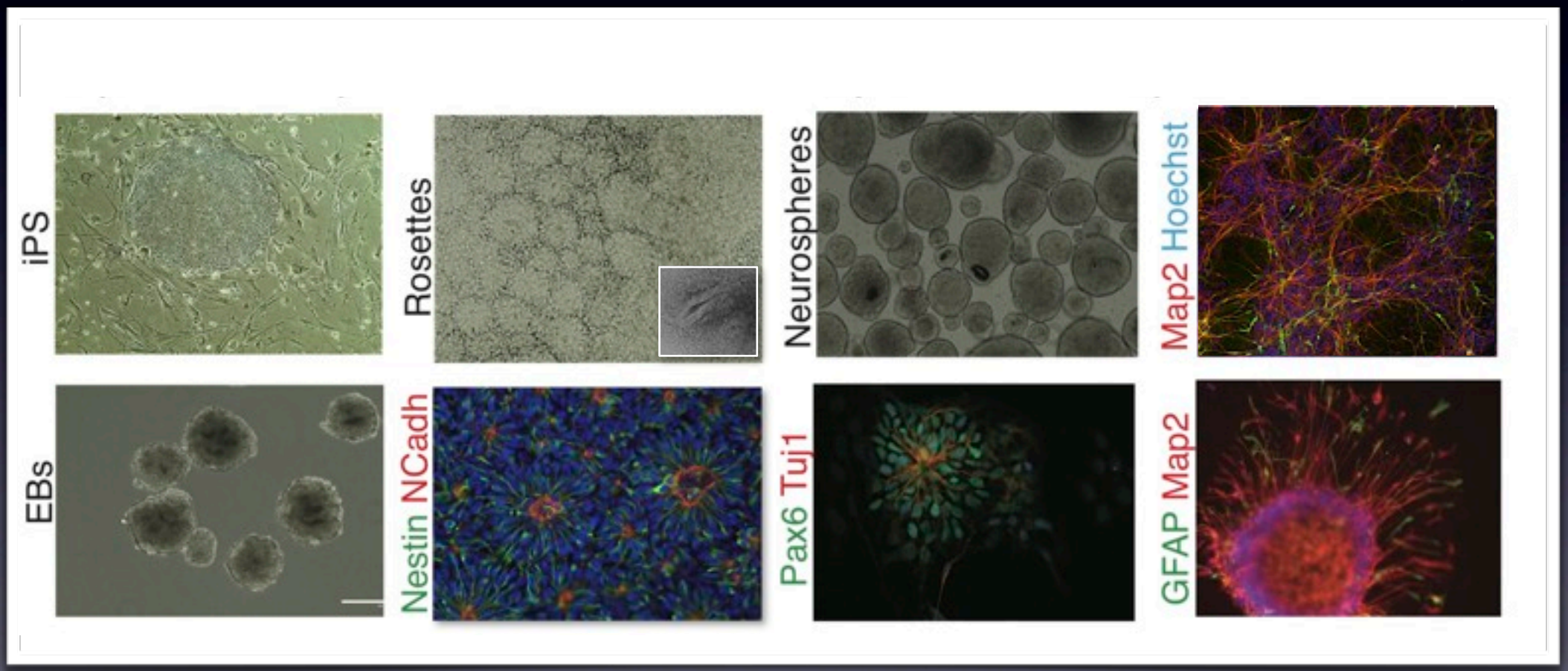
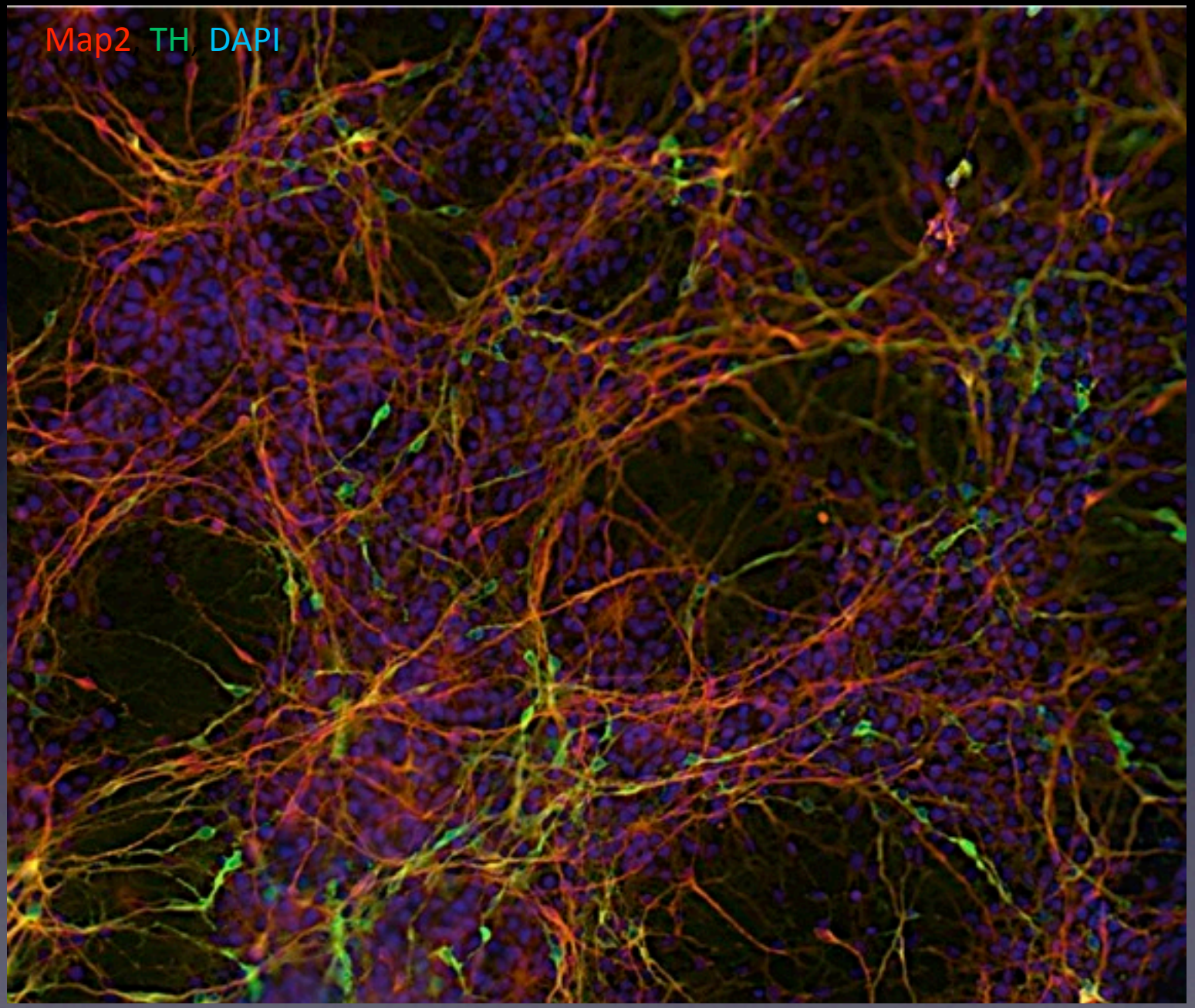
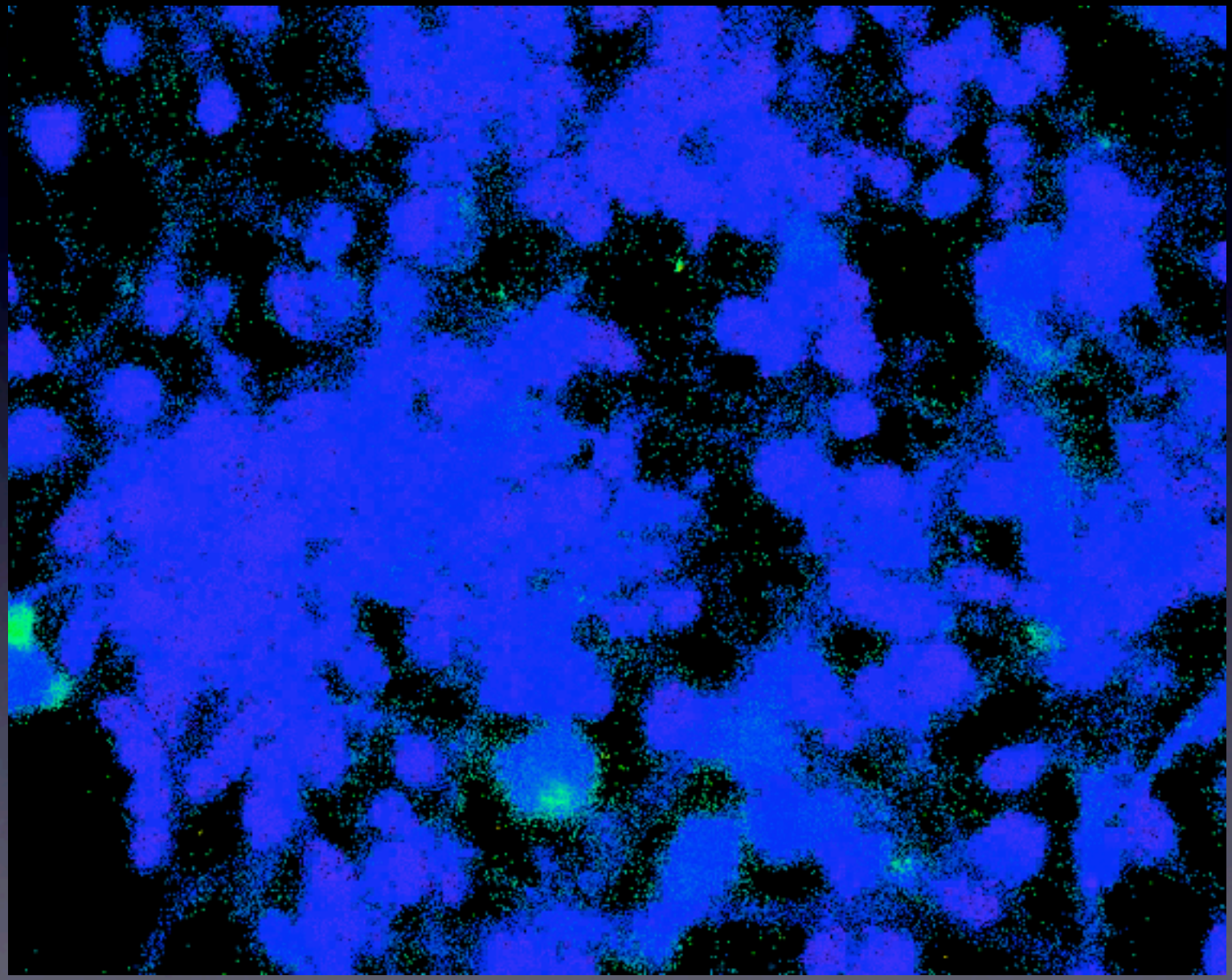
Recapitulating human neural development *in vitro*



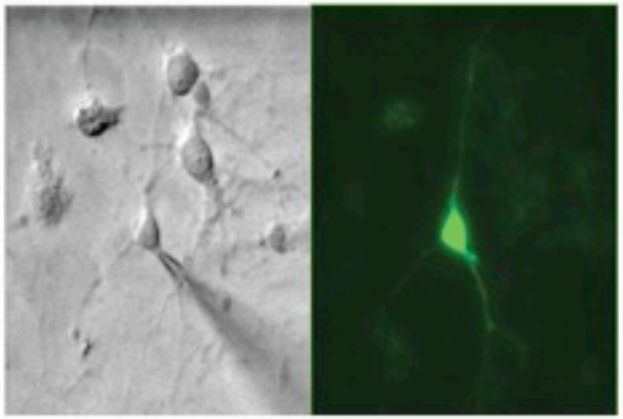
iPSC -derived neurons

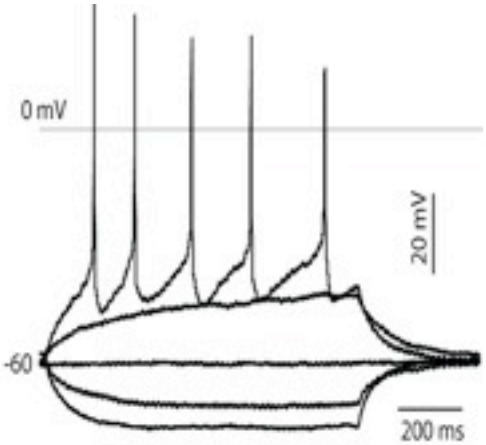


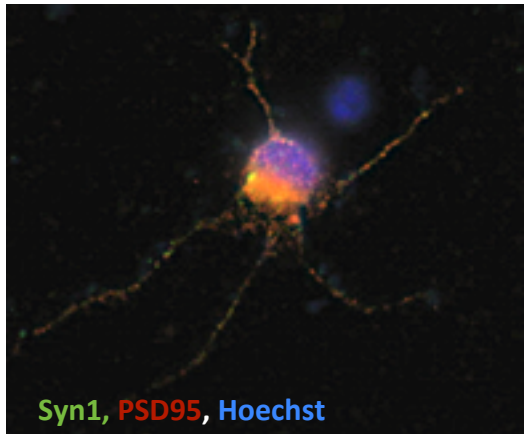
Neurons have calcium signals

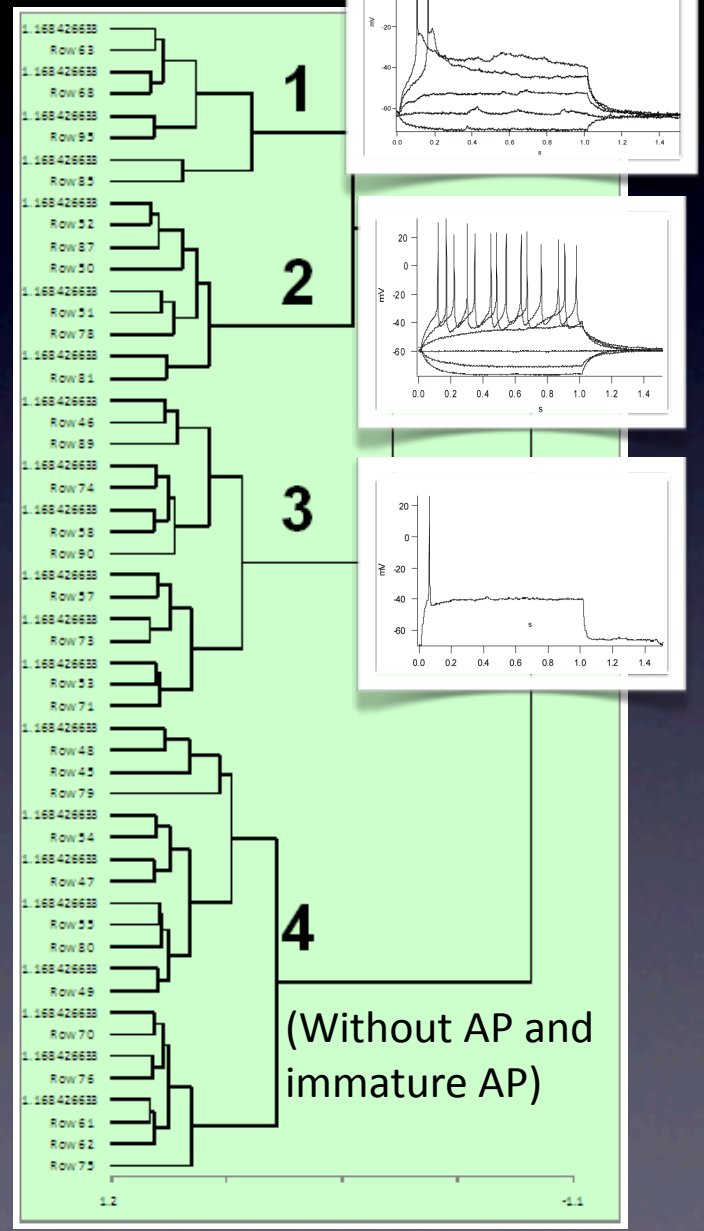


Neurons fire action potentials and form synapses

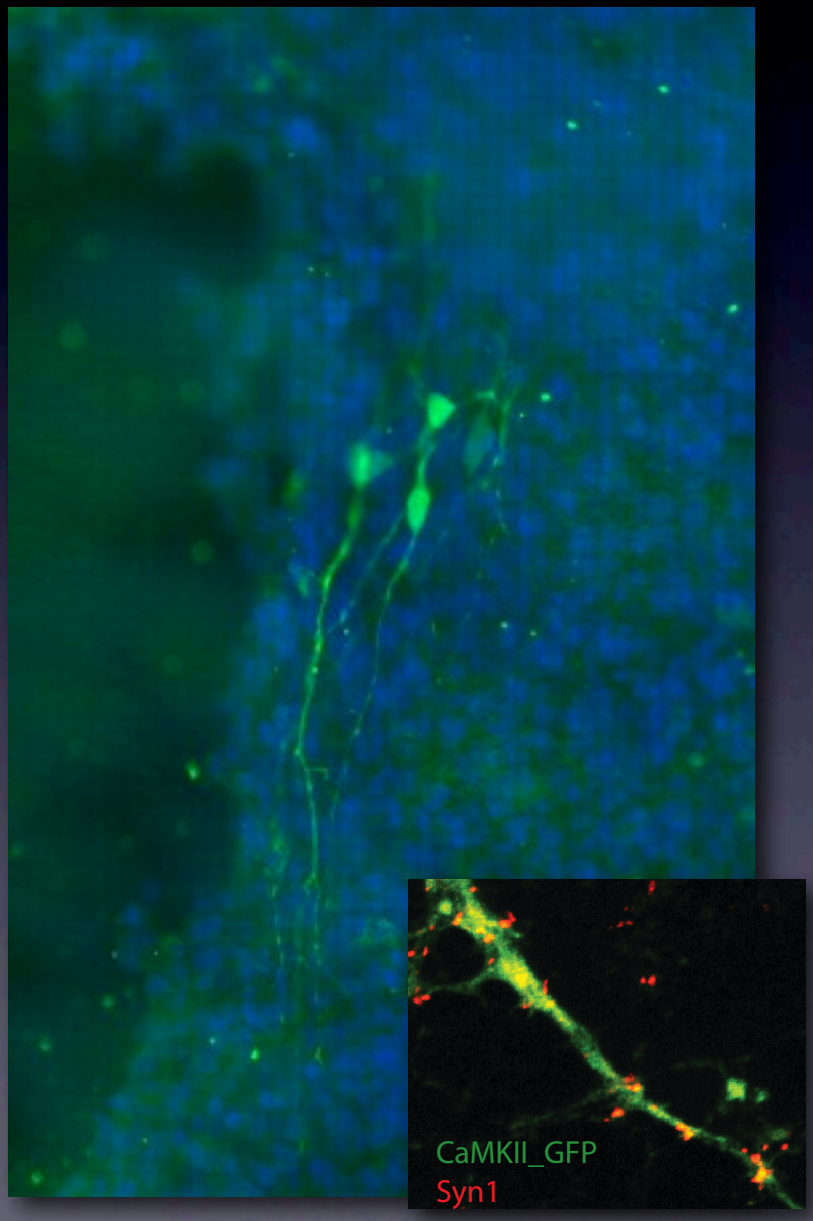


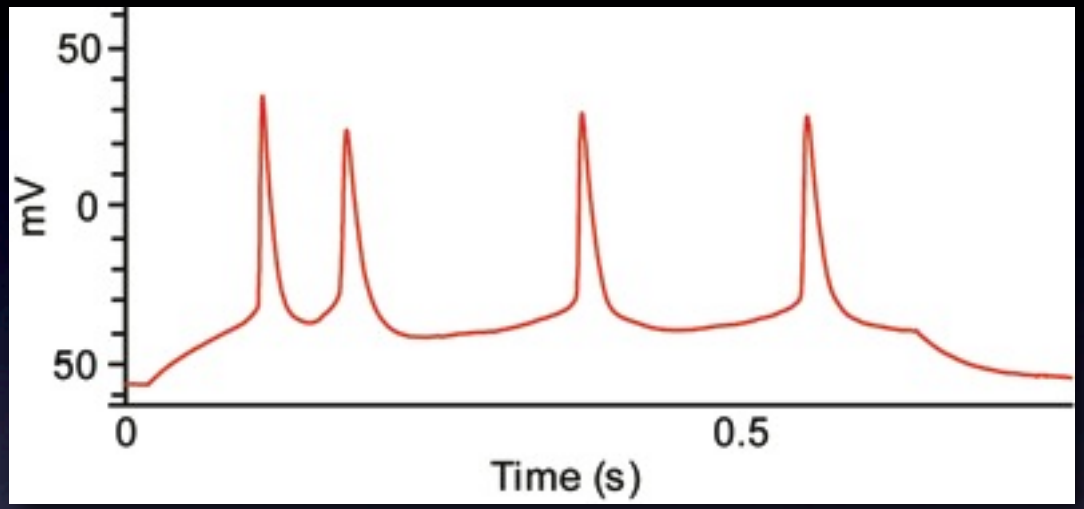


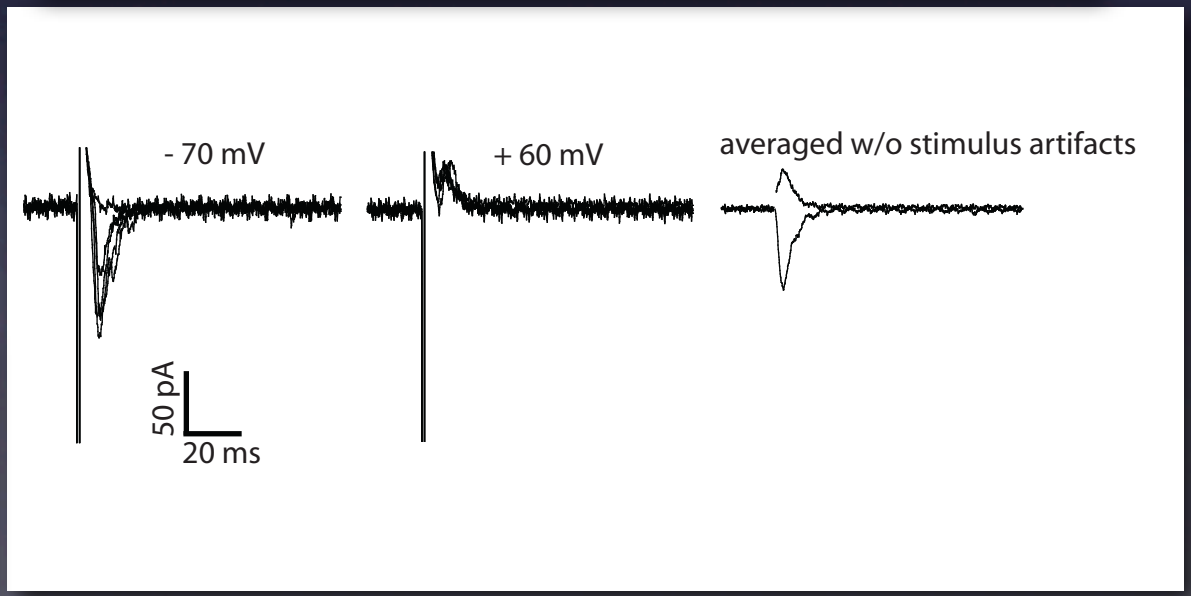




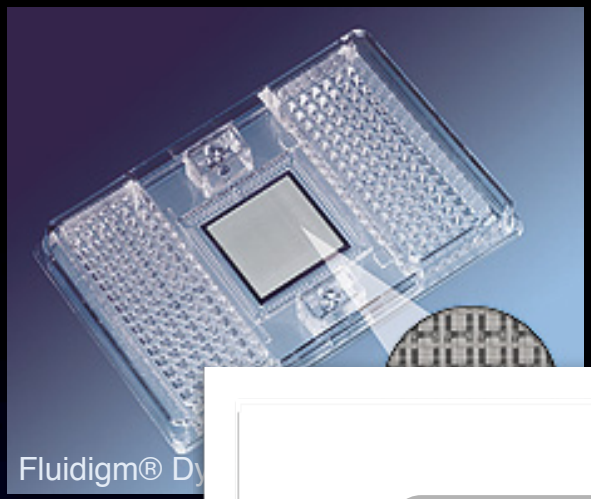
Human iPS derived neurons in a mouse

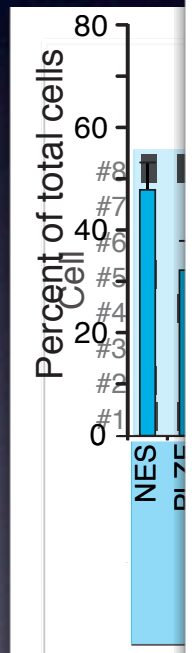


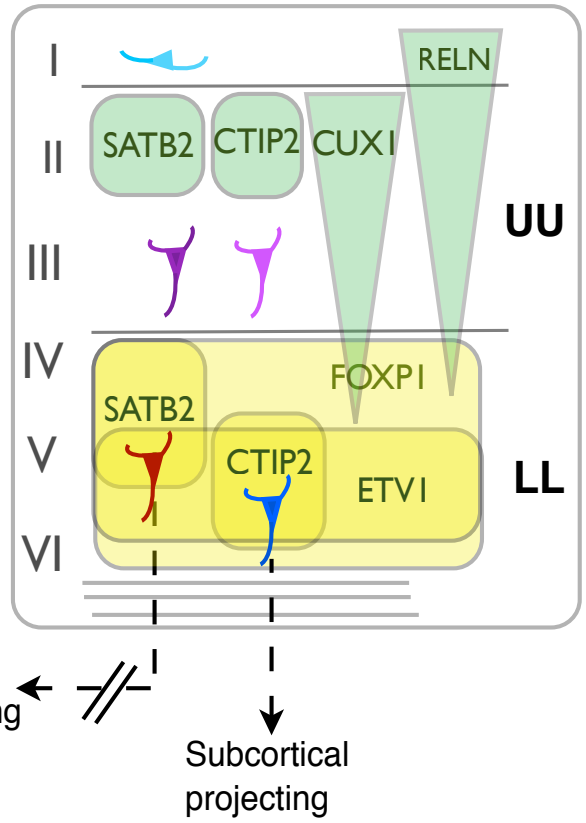


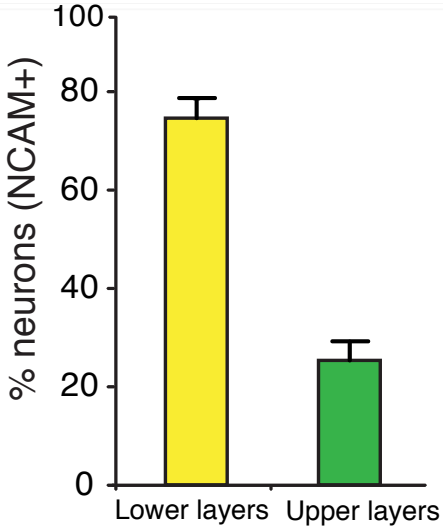


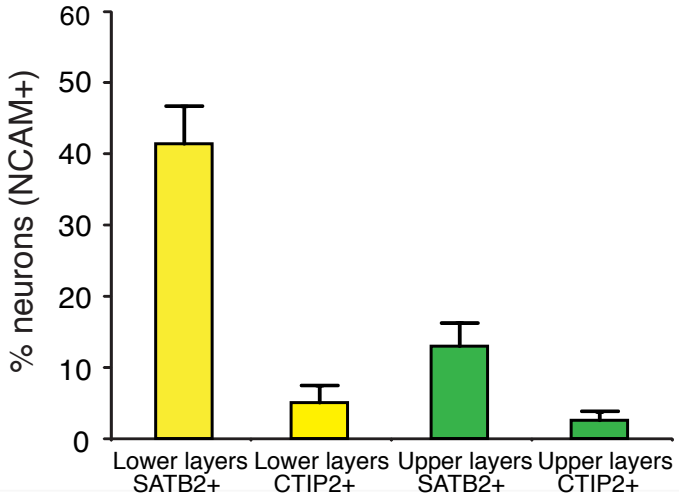
IPSC derived neuronal cell types

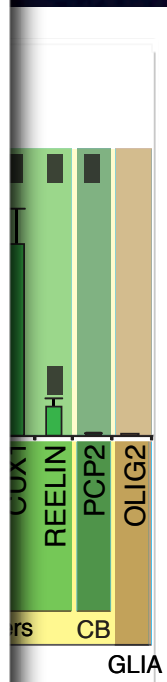


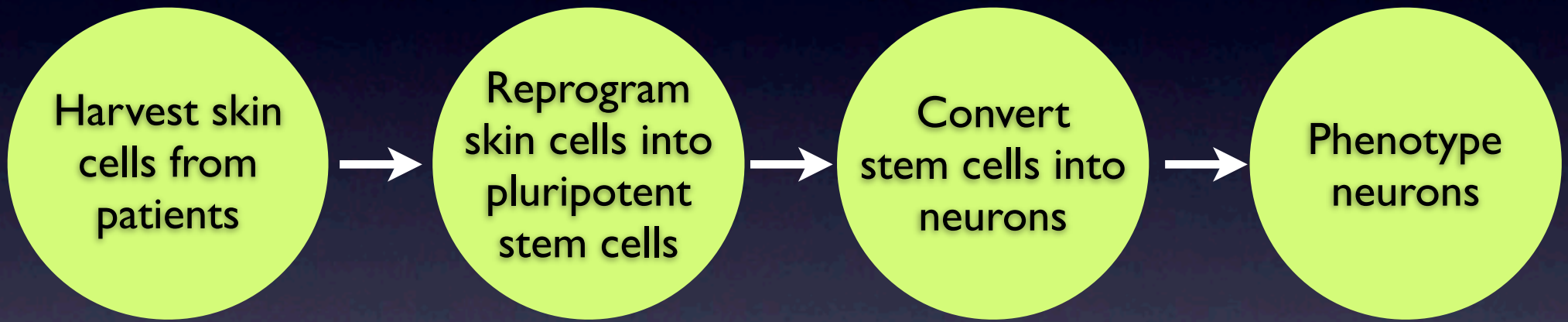








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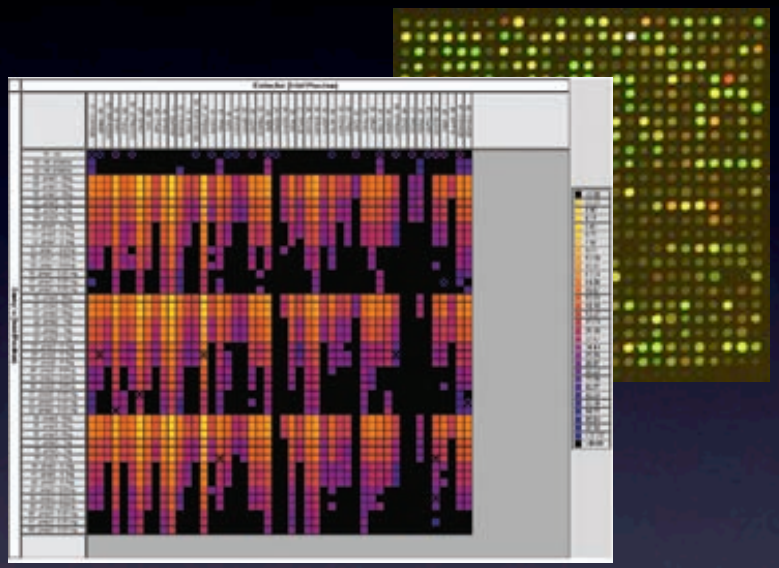
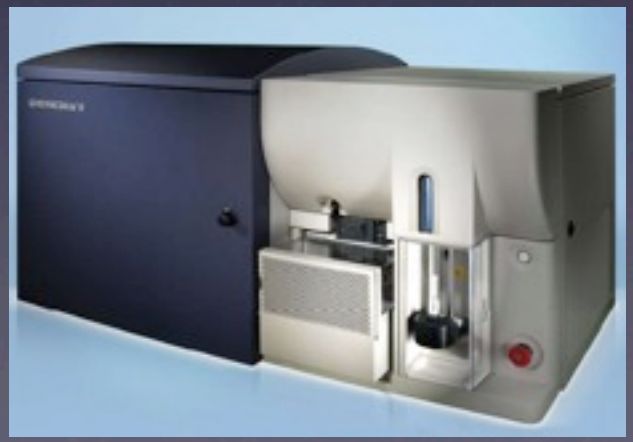
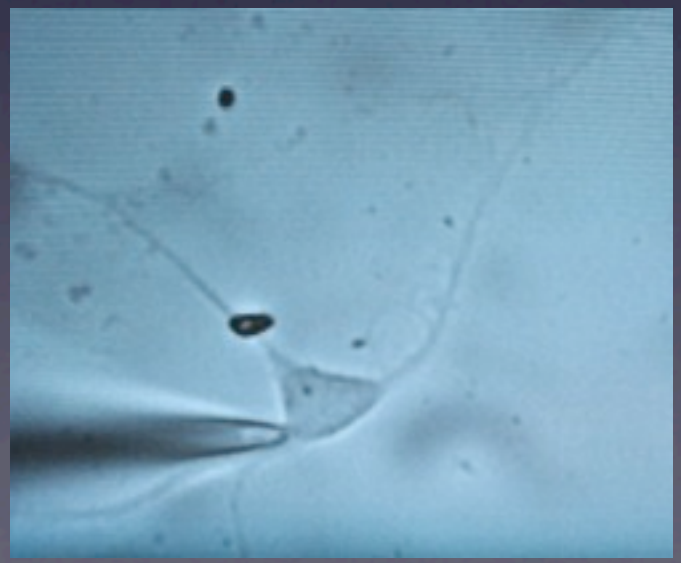
Will we see a cellular phenotype?

Unbiased

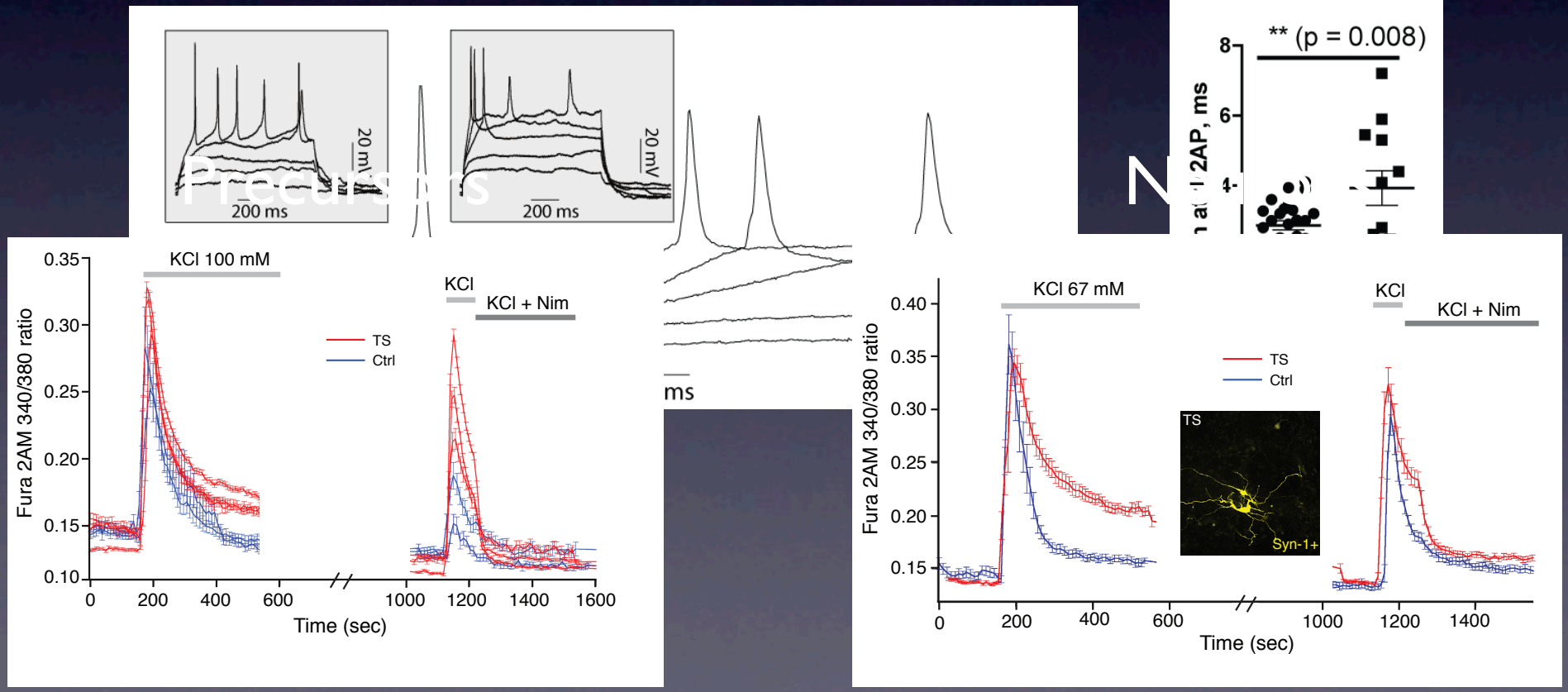




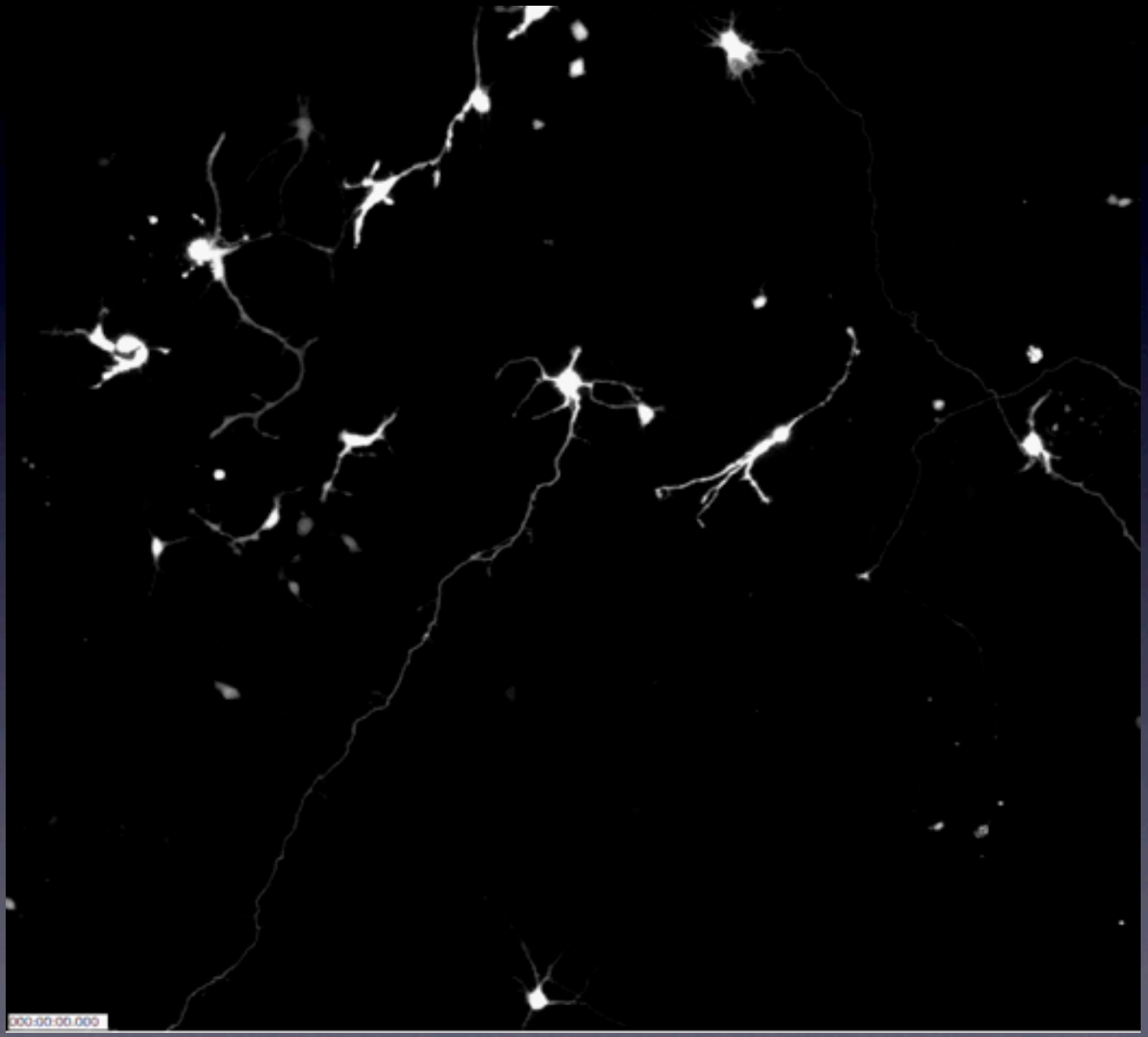
Hypothesis Driven



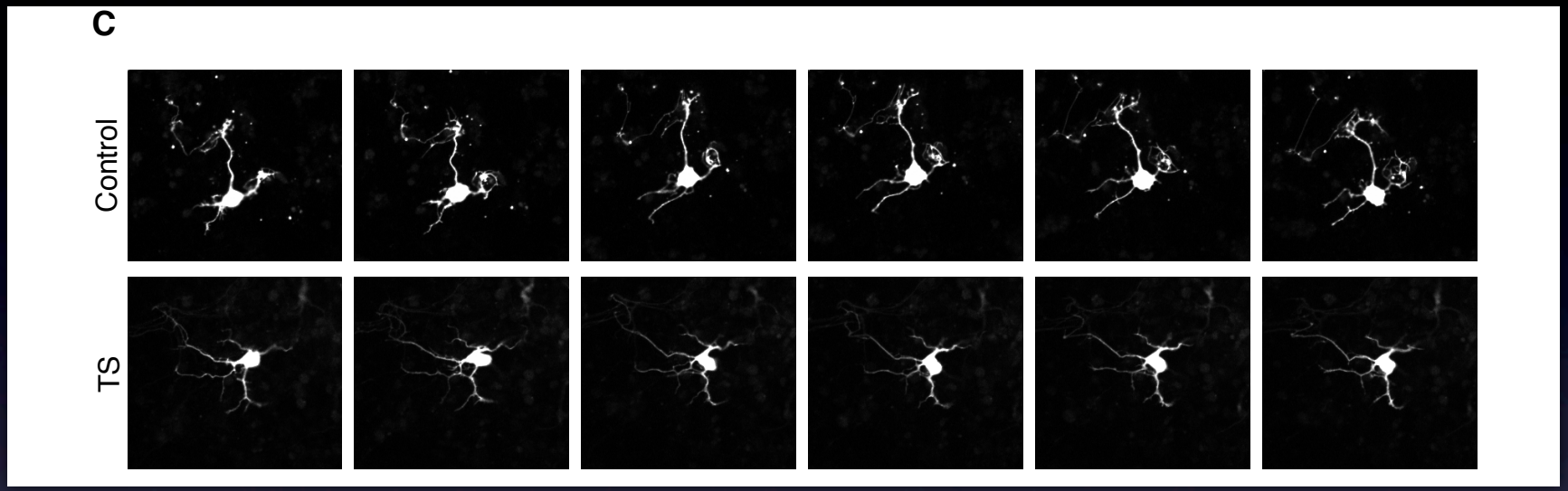
Calcium signaling defects in TS neurons

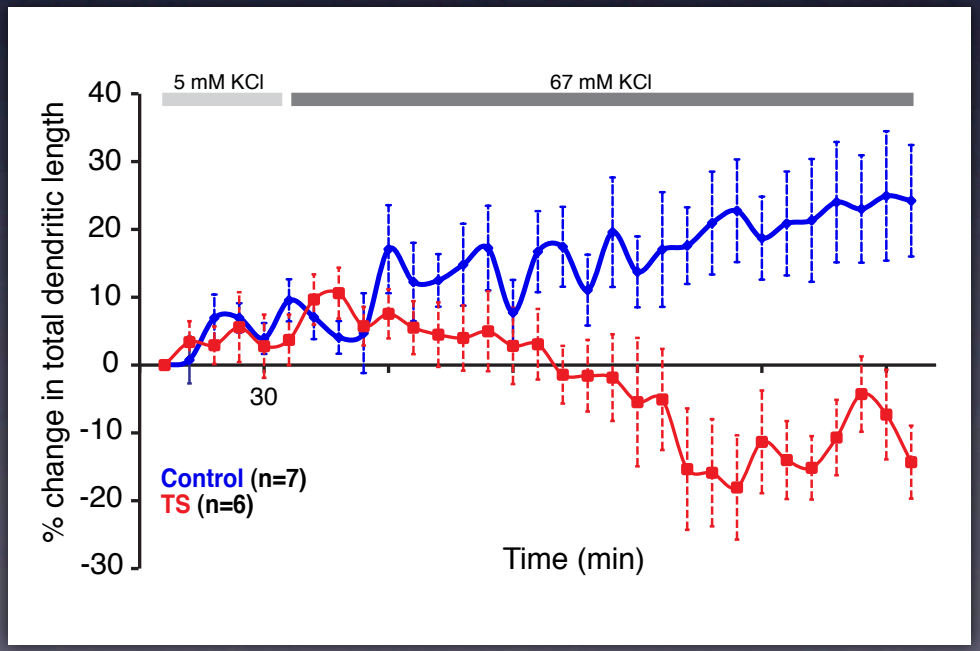


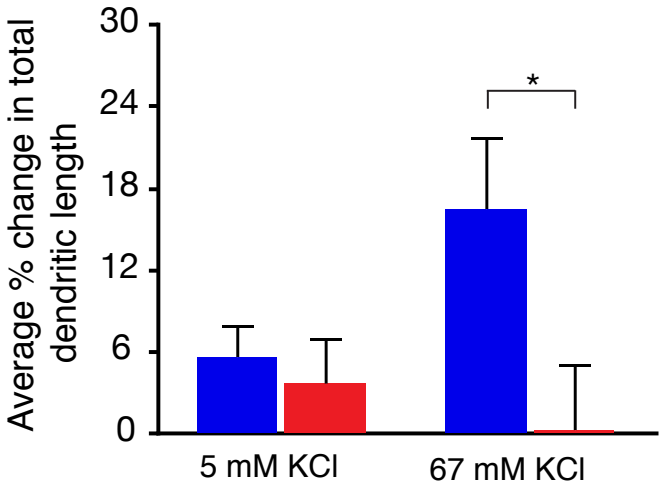
TS channels cause dendritic retraction *in vitro*



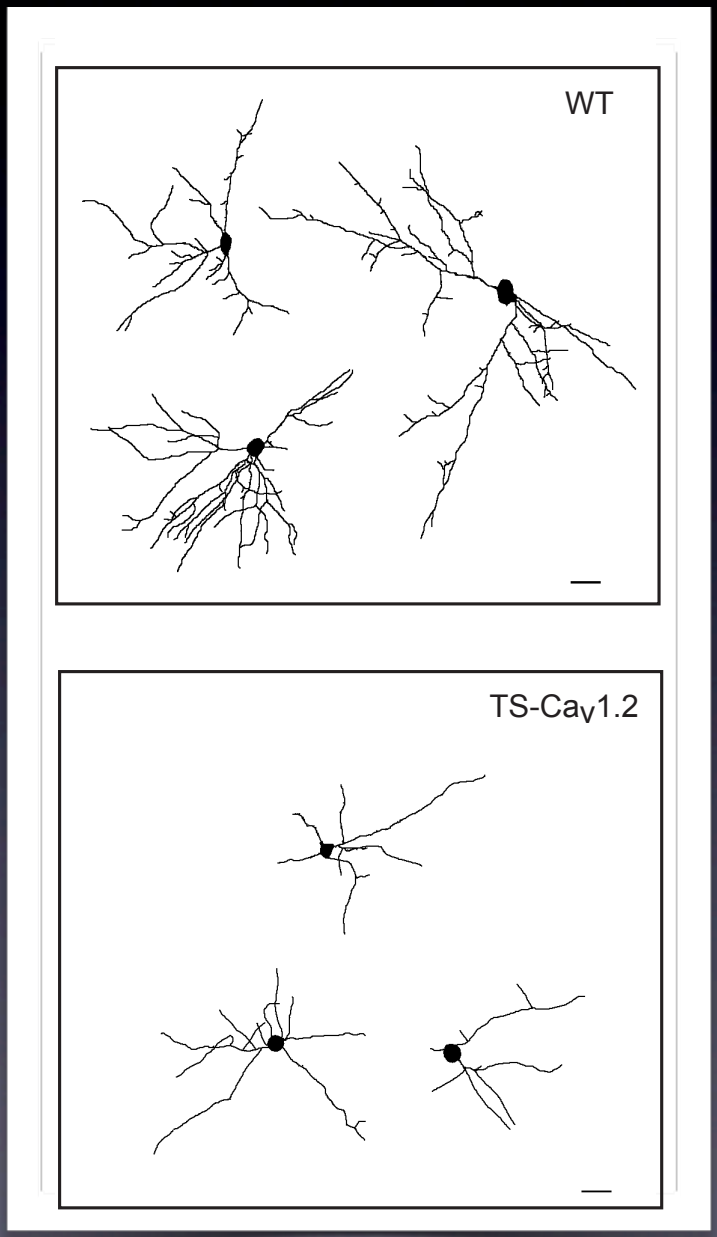
Dendritic retraction in human neurons from TS patients

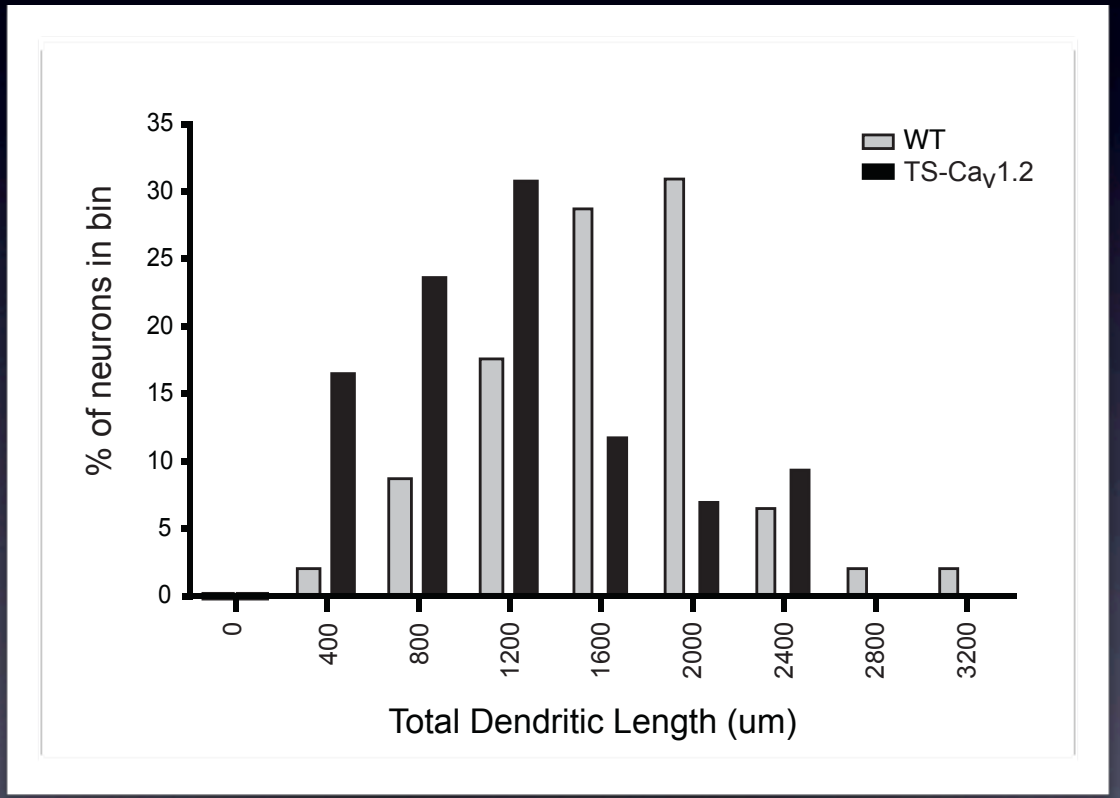




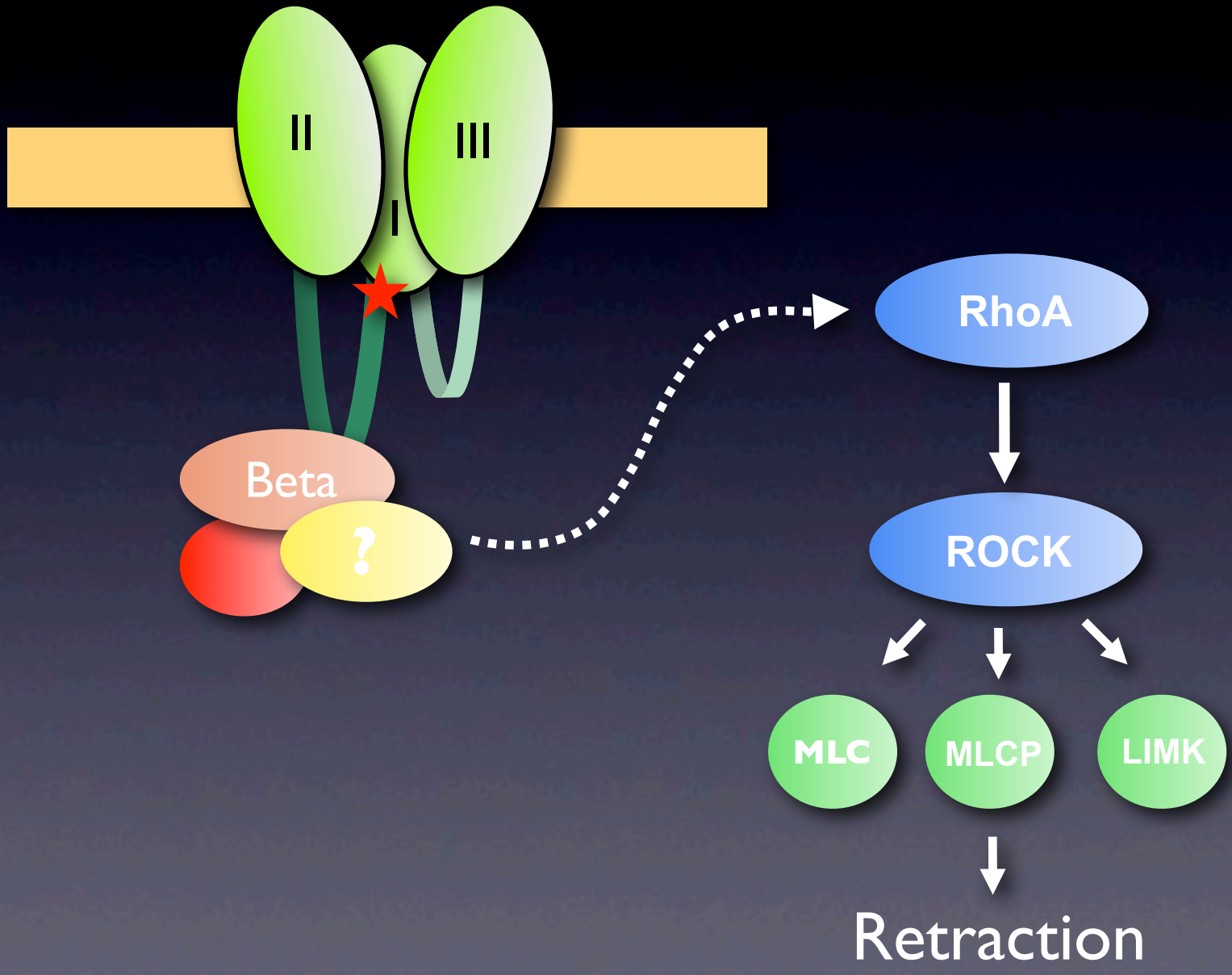


Layer 5 pyramidal neurons in the TS mouse have short dendritic arbors

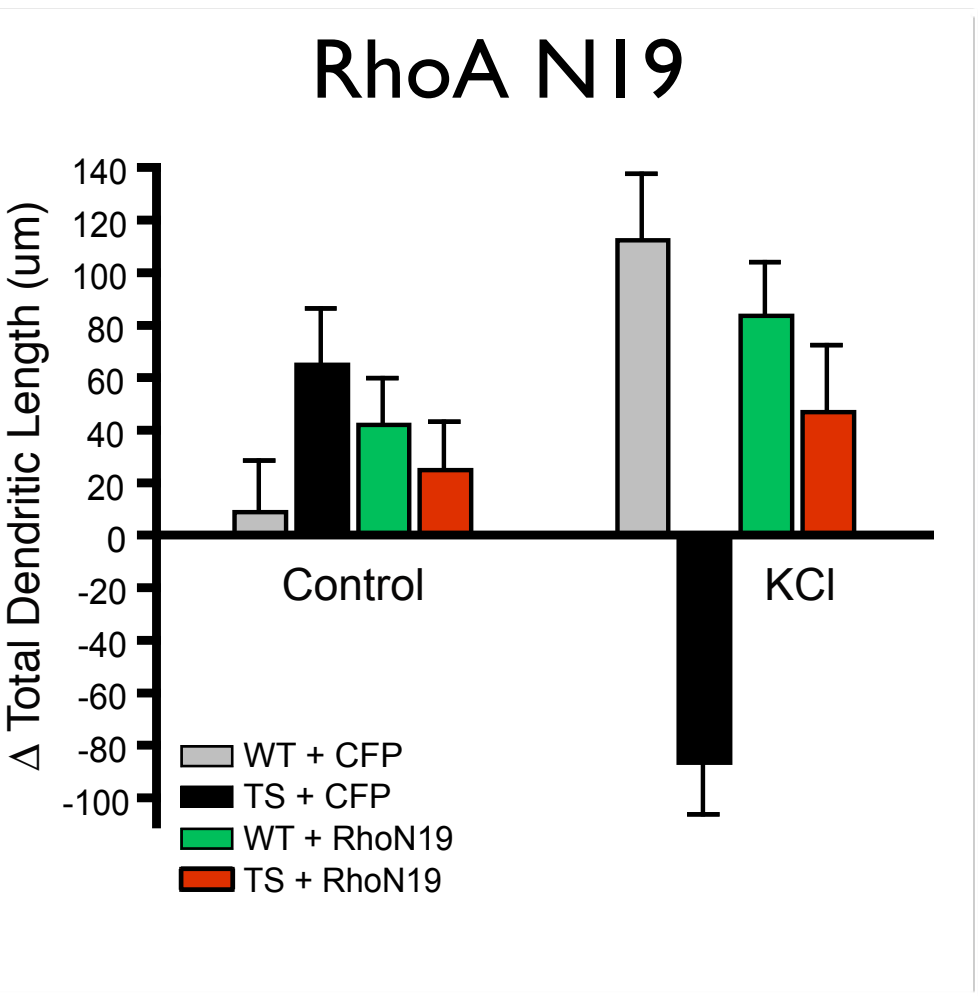


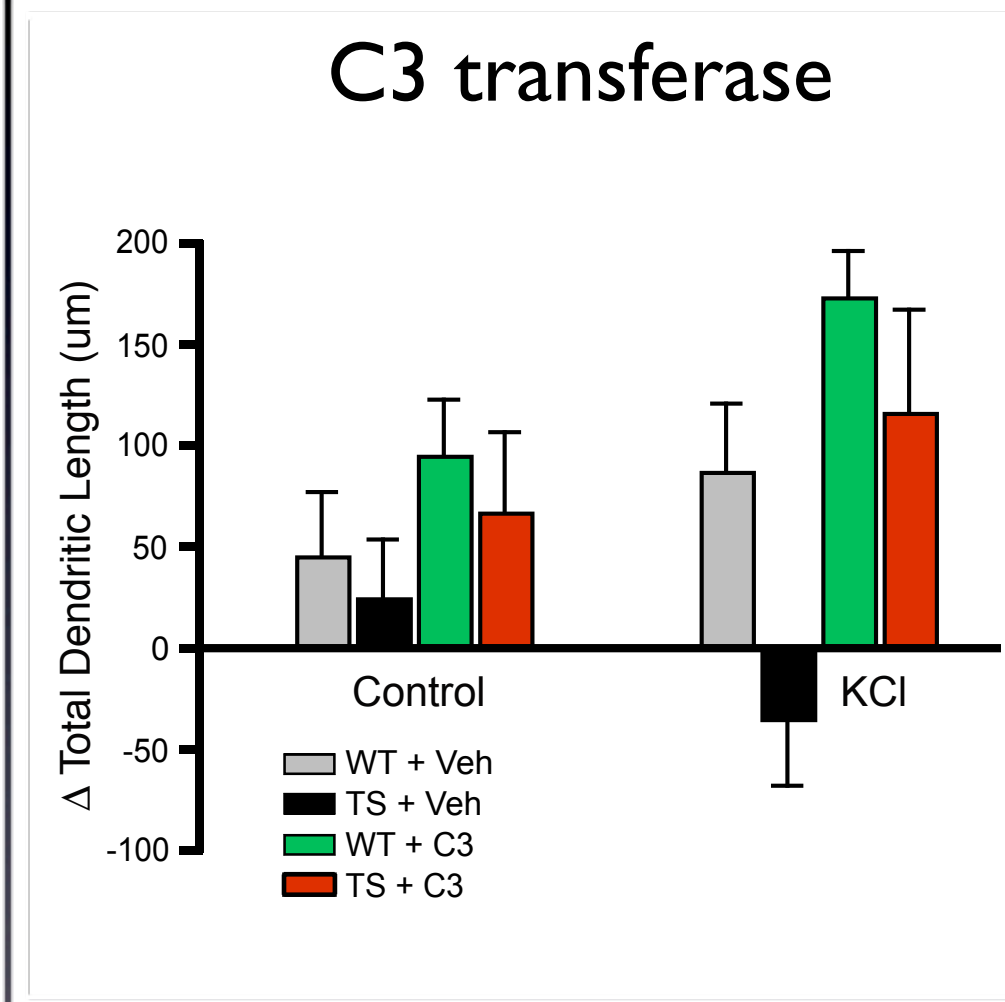


How does TS-$Ca_V1.2$ cause dendrite retraction?

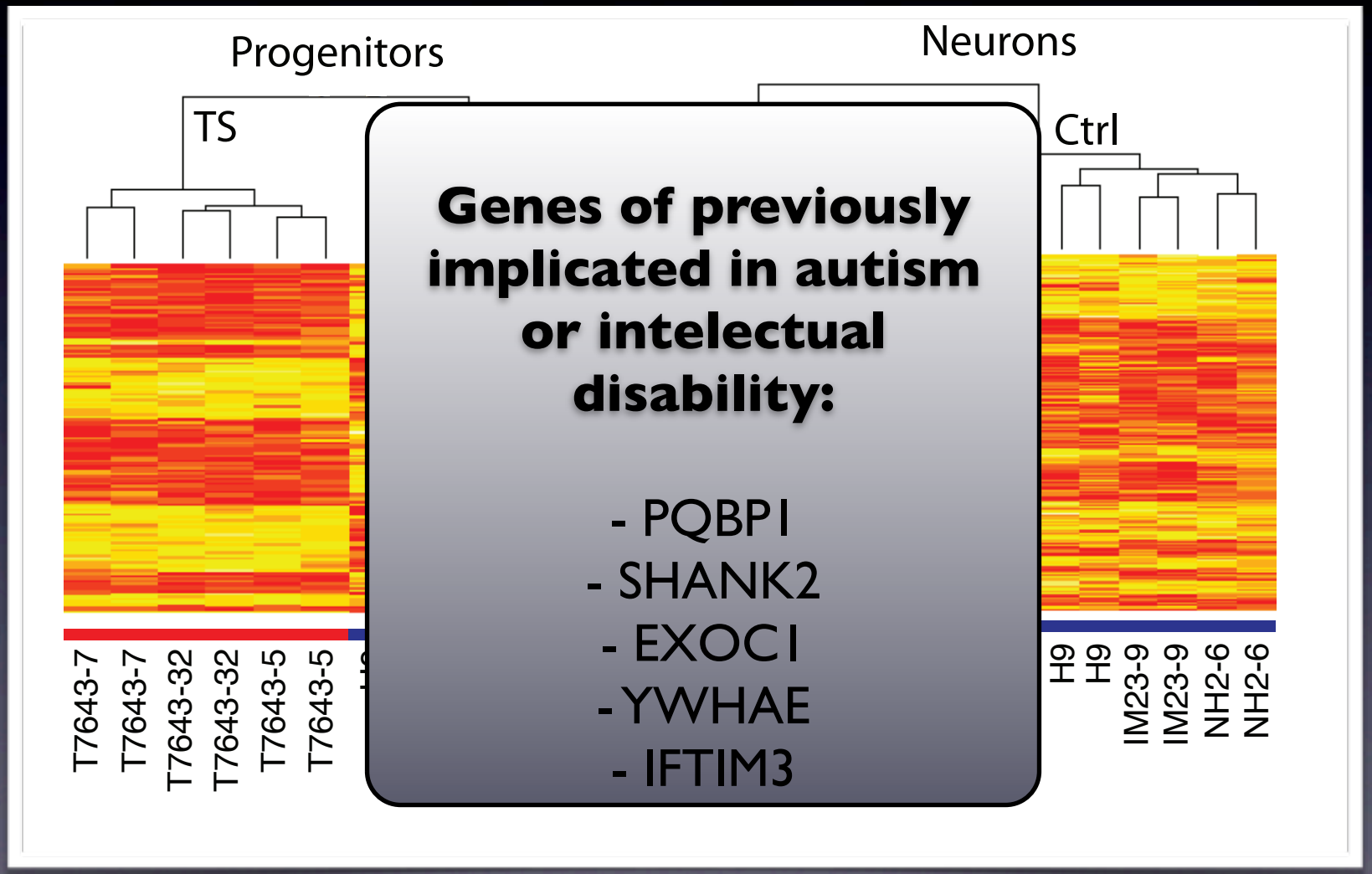


Inhibition of RhoA prevents TS-induced retraction



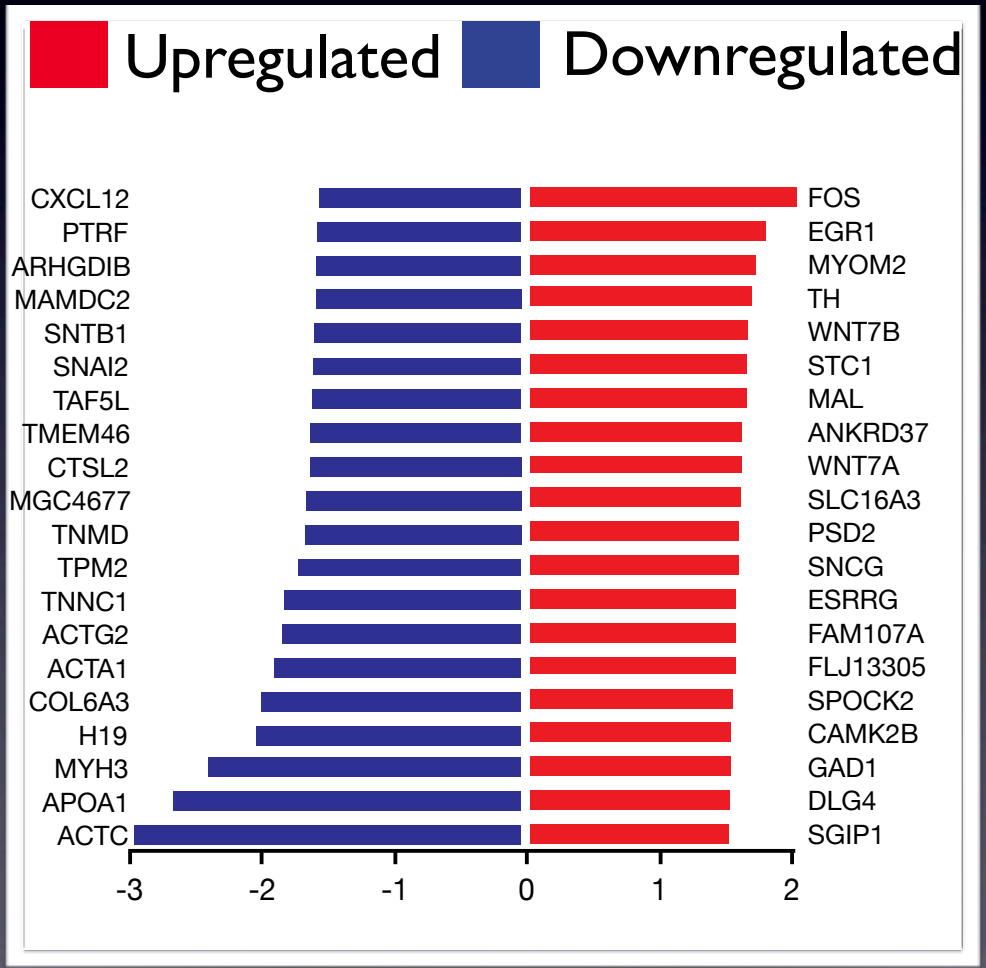


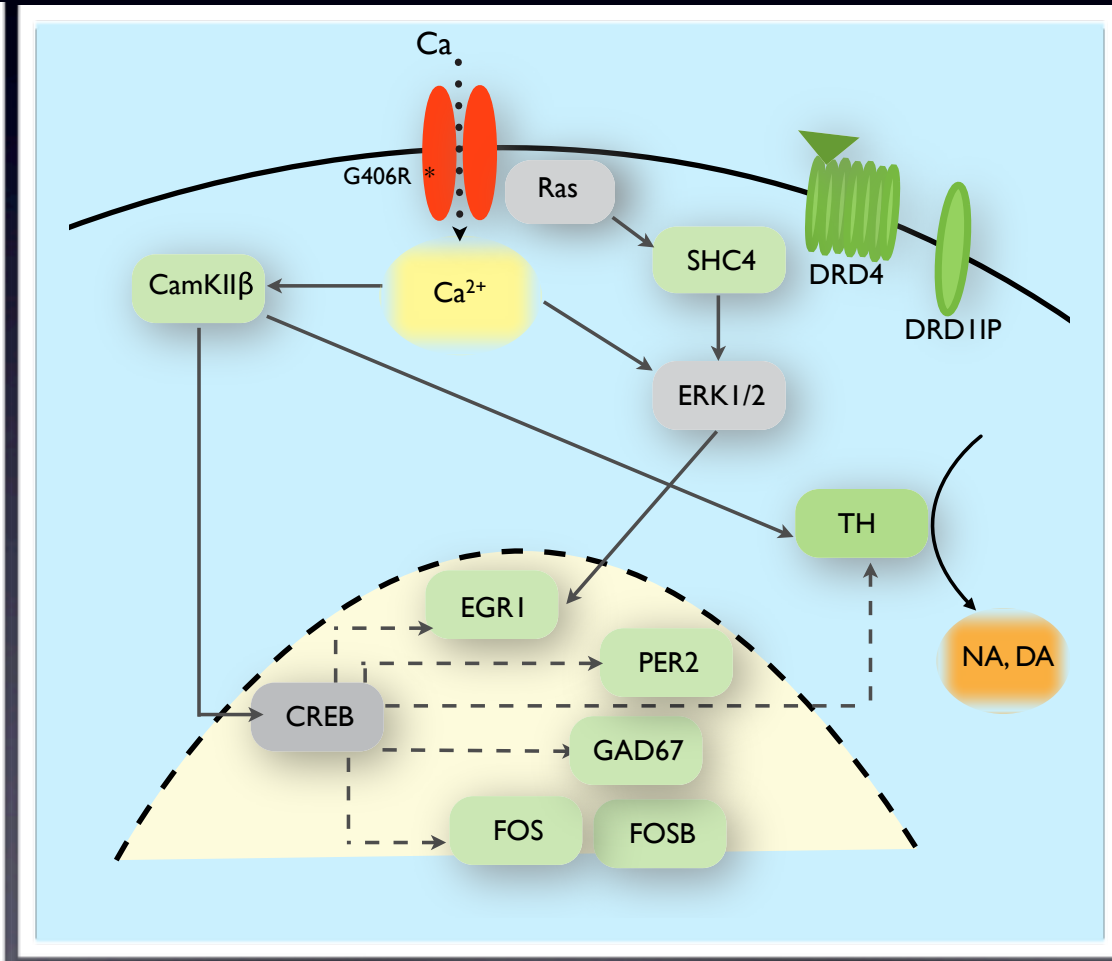
Are there differences in gene expression in TS neurons?



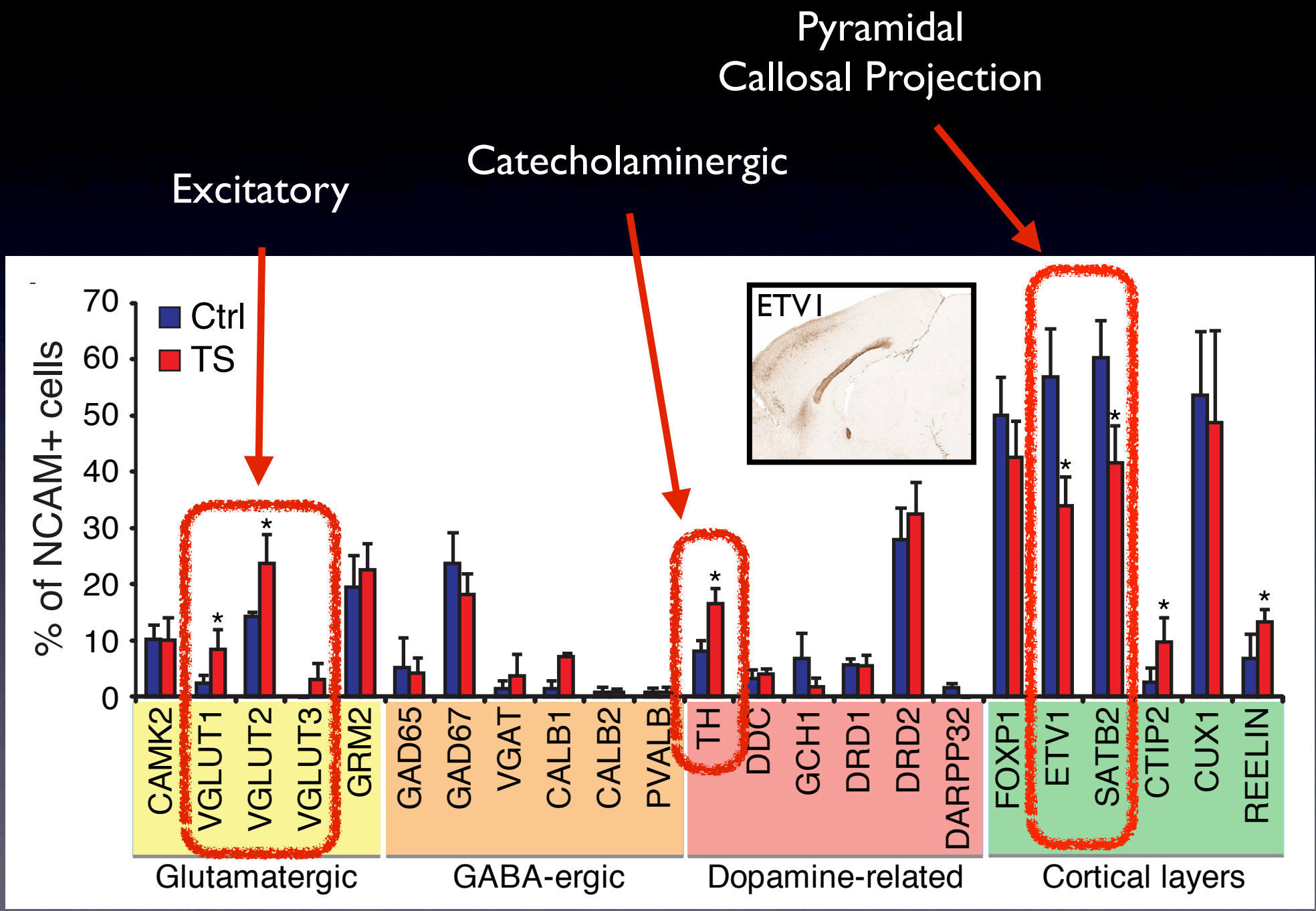
Irina Voineagu and Dan Geschwind

Genes differentially regulated in TS neurons upon stimulation



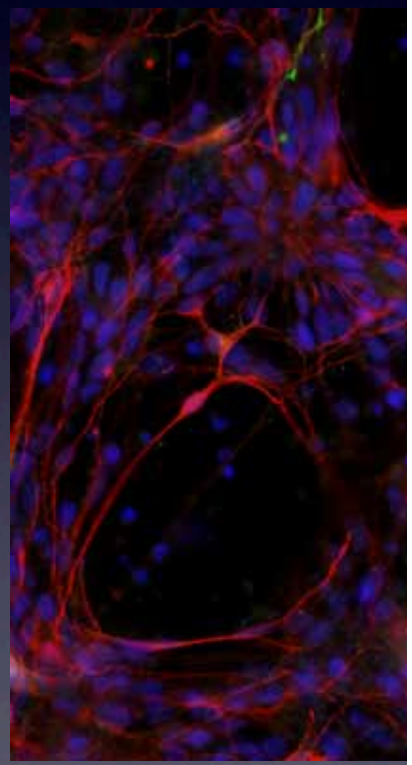


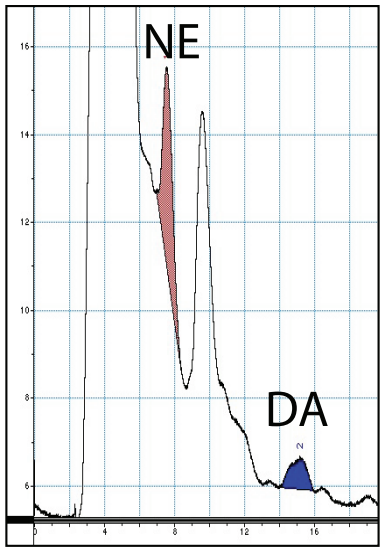
Altered Cell Fate in TS

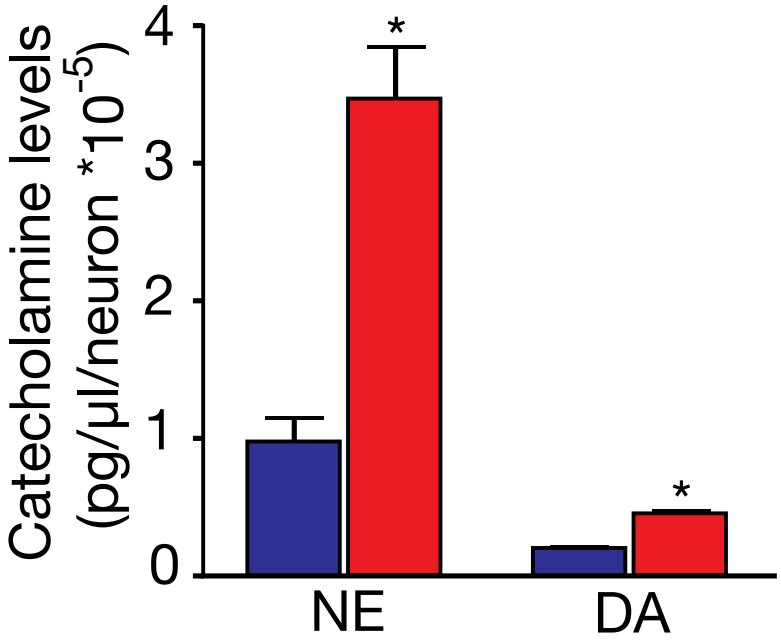


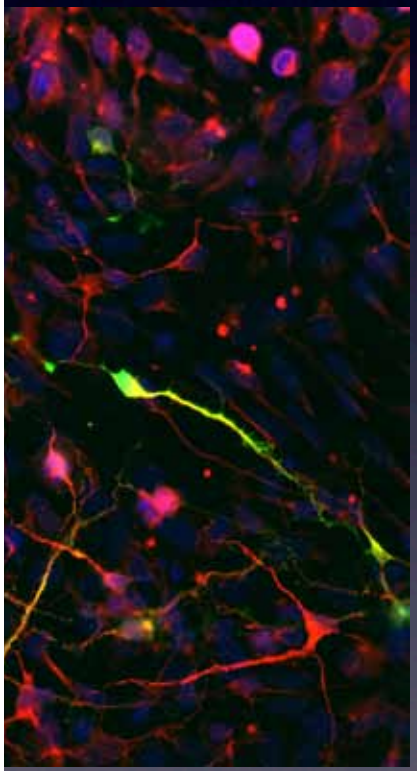
Increased numbers of TH positive neurons in TS patients

Control



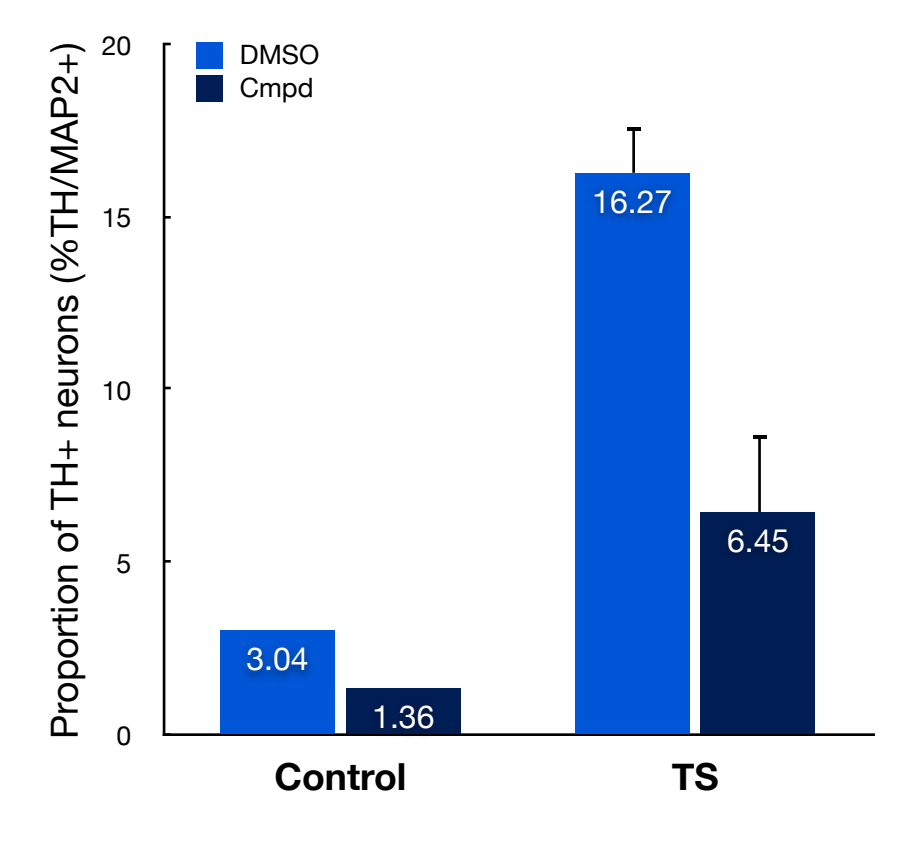






Pharmacological reversal of neuronal phenotypes

DMSO: 11312 cells/1619 neurons
CMPD: 11339 cells /1873 neurons



J Autism Dev Disord. 1987 Sep;17(3):439-46.

Open trial effects of beta-blockers on speech and social behaviors in 8 autistic adults.

Ratey JJ, Bemporad J, Sorgi P, Bick P, Polakoff S, O'Driscoll G, Mikkelsen E.

Source

Harvard Medical School, Massachusetts Mental Health Center.

Neurocase. 2008;14(4):378-83.

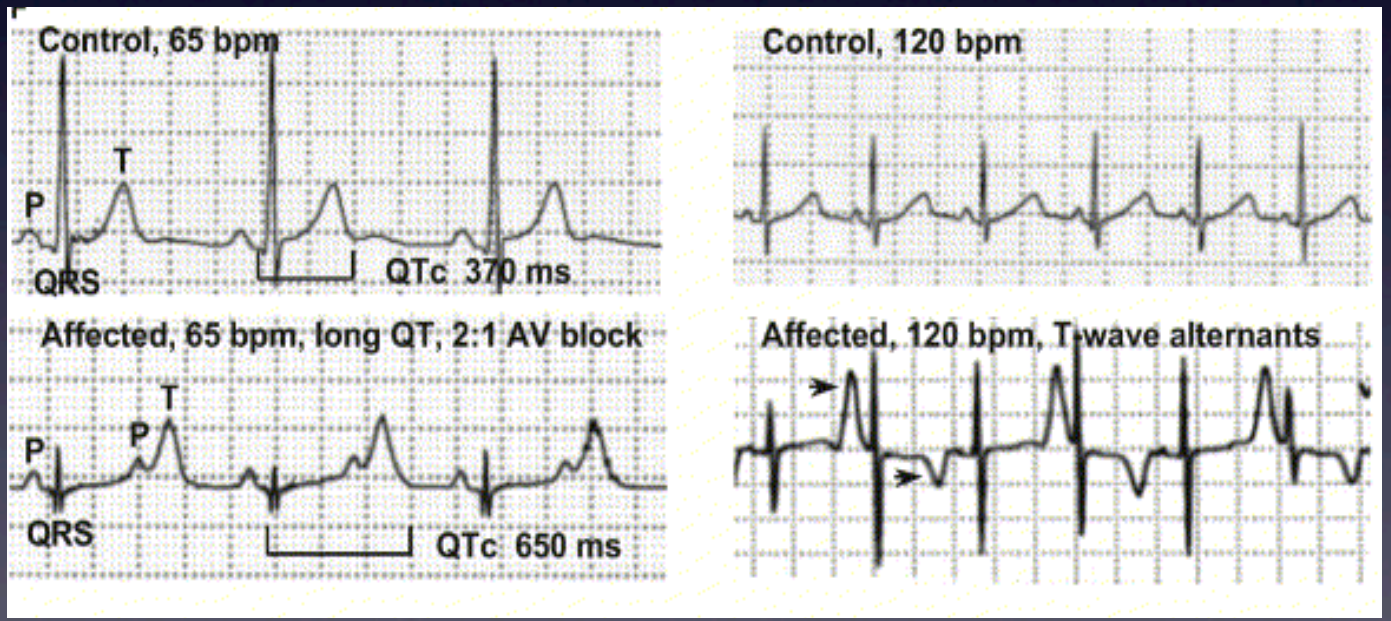
Effect of propranolol on verbal problem solving in autism spectrum disorder.

Beversdorf DQ, Carpenter AL, Miller RF, Cios JS, Hillier A.

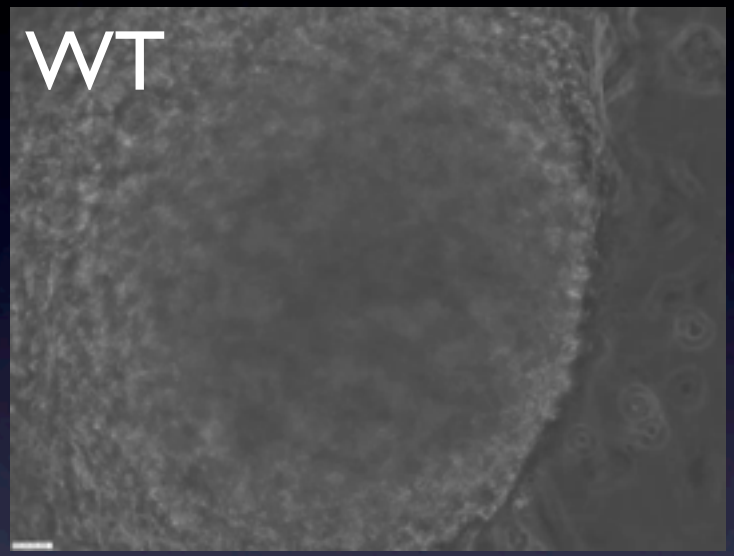
Source

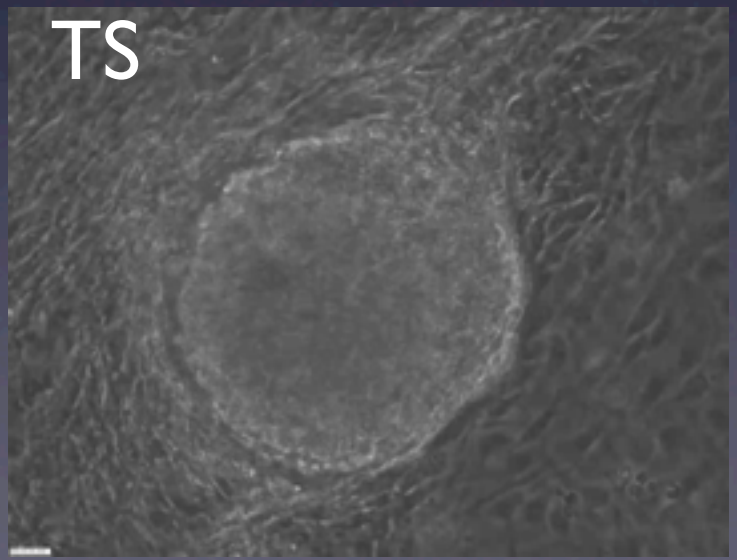
Department of Radiology, Thompson Center, University of Missouri, Columbia, MO 65211, USA. beversdorfd@helath.missouri.edu

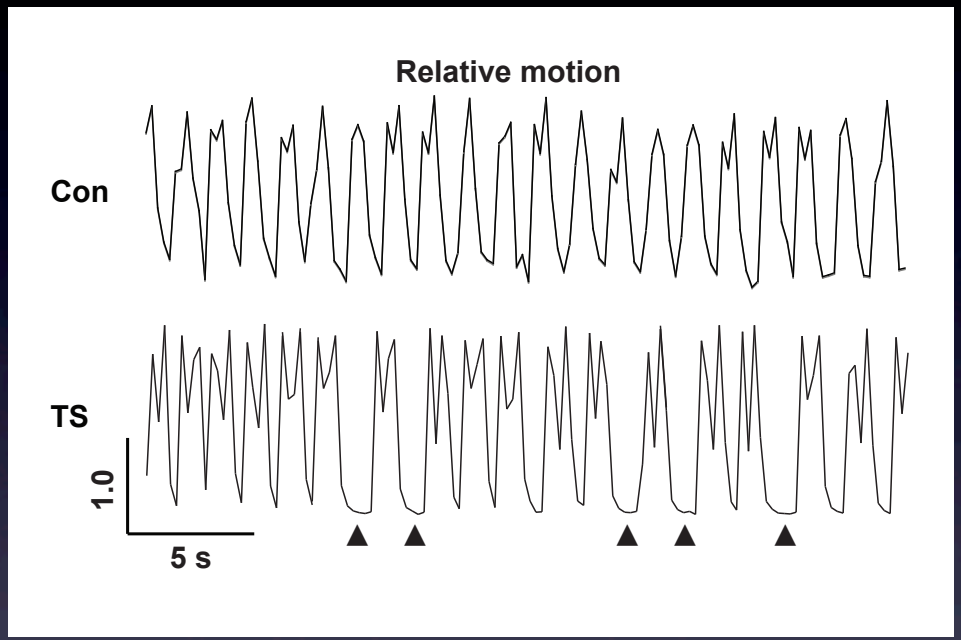
How about cardiac arrhythmia?

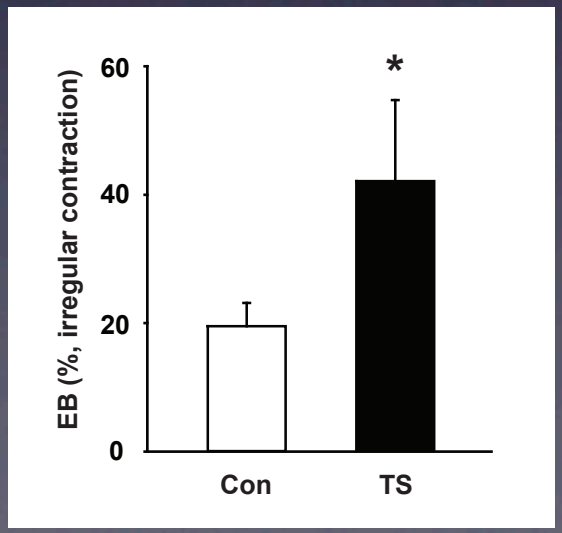


Cardiomyocytes from TS patients are Arrhythmic

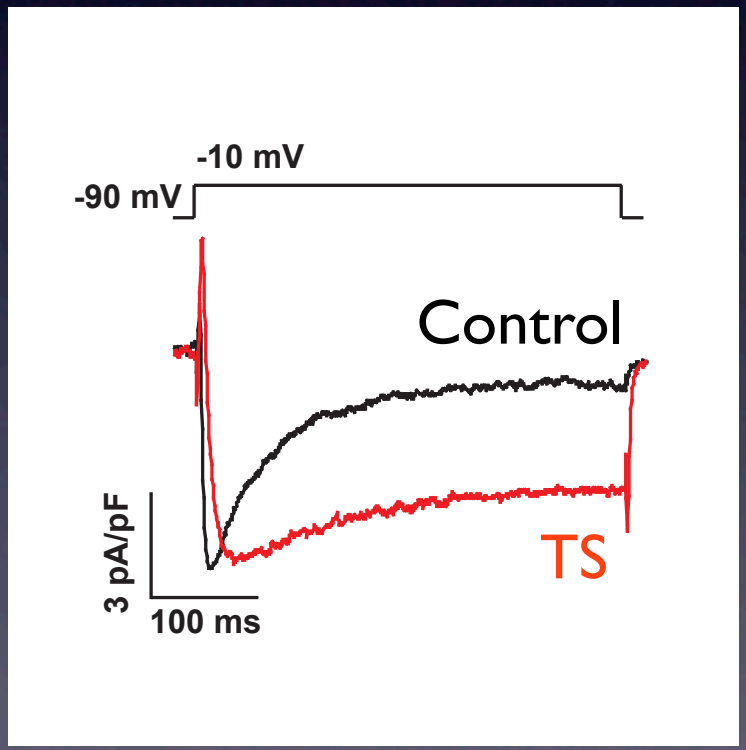


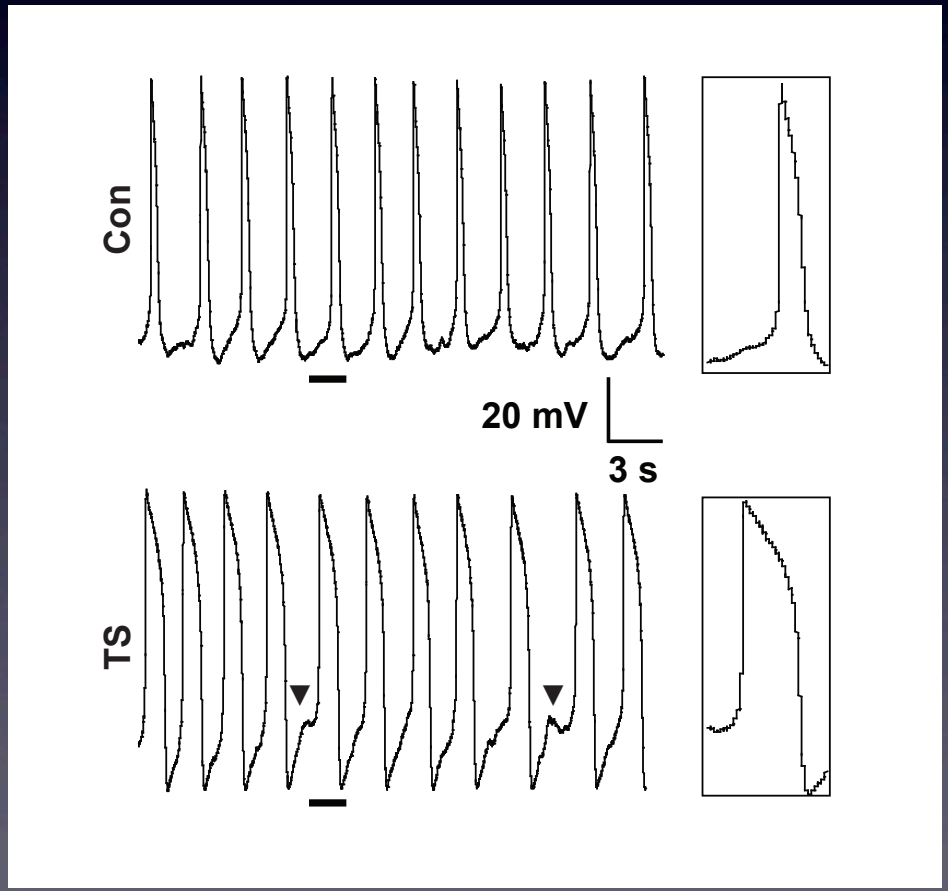




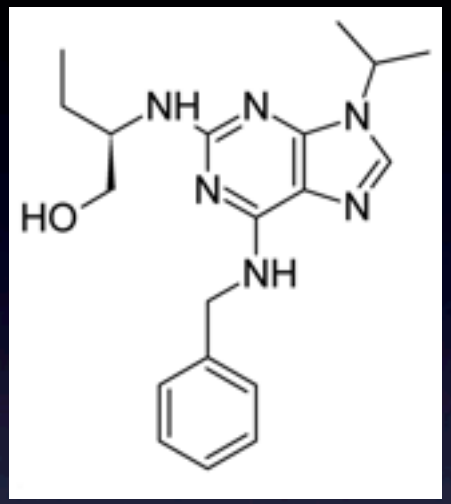


Electrical defects in TS cardiomyocytes

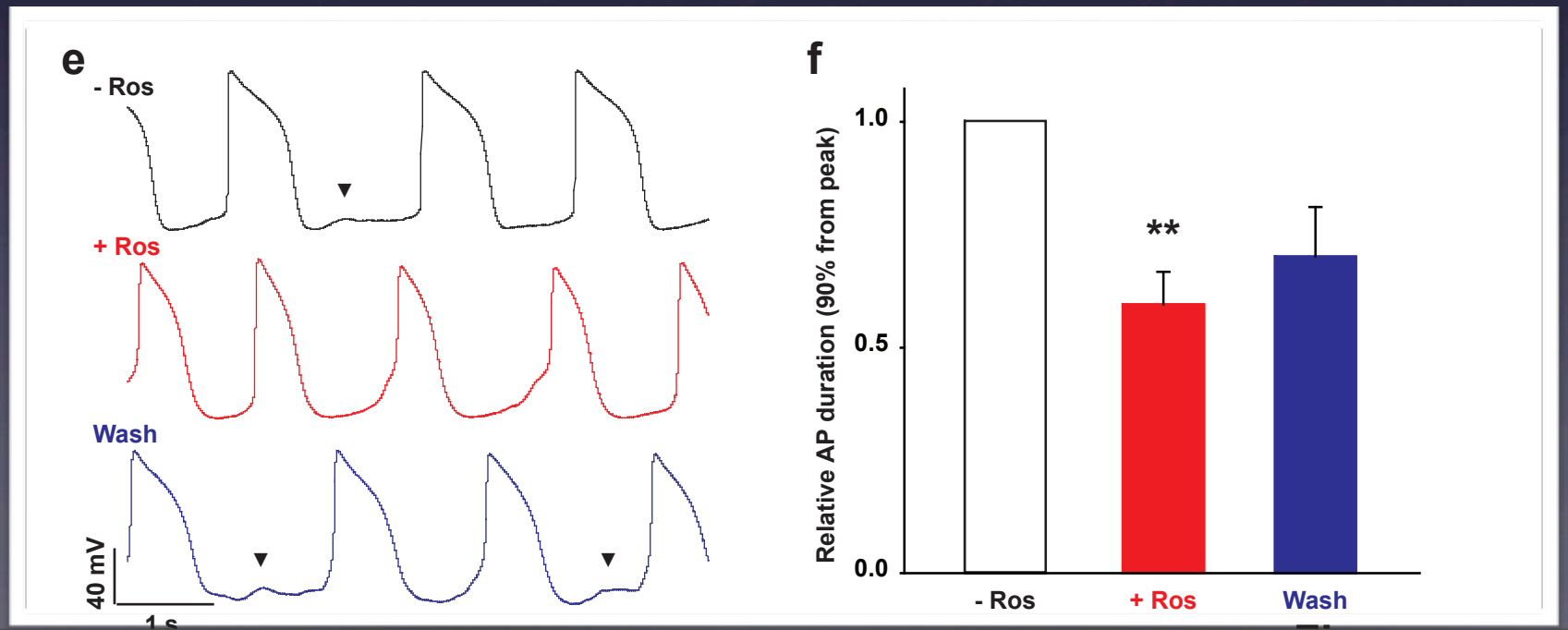




Small molecule reverses the phenotype of TS cells

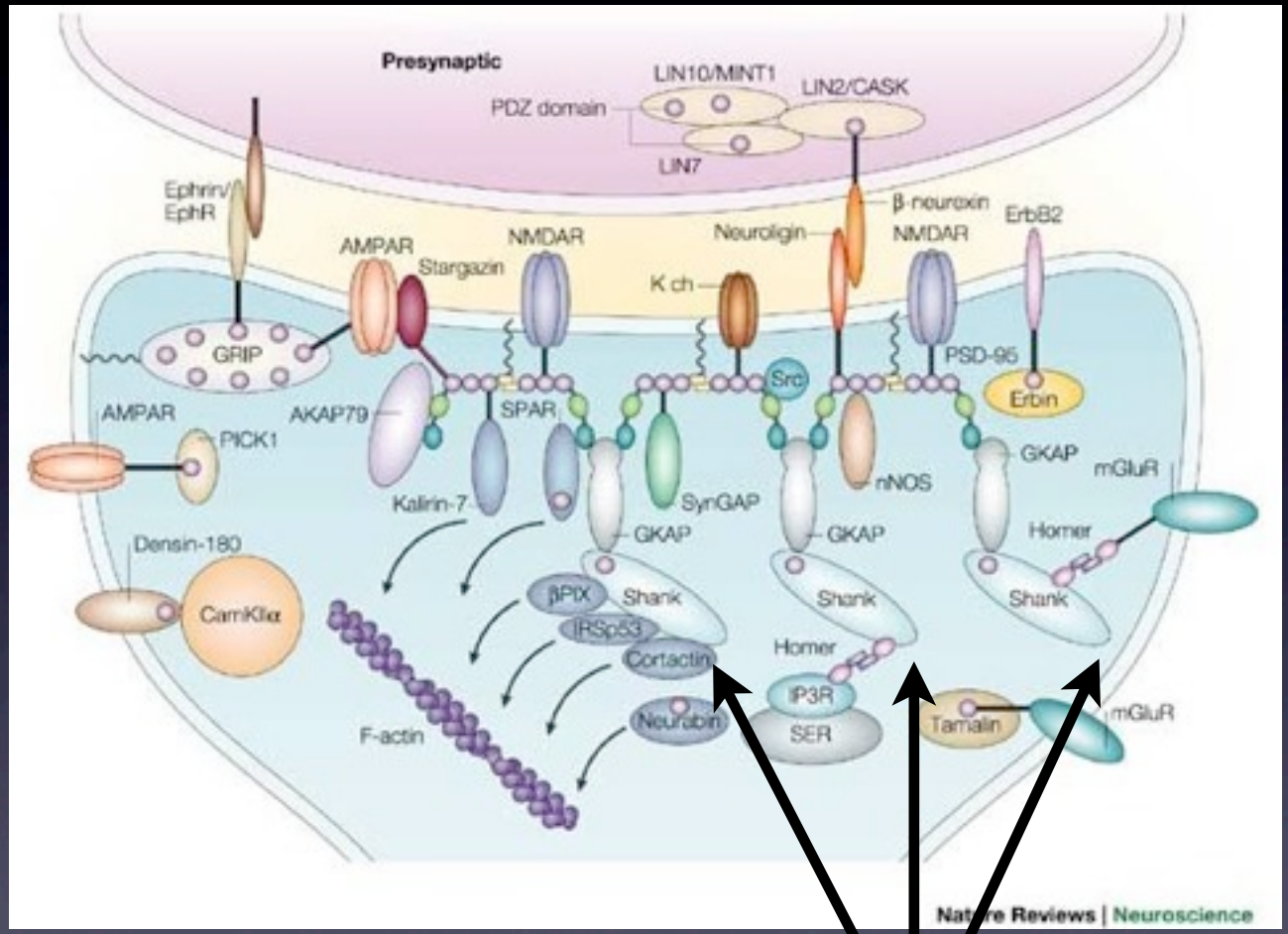


Yarotskyy et al. The Timothy syndrome mutation of cardiac CaV1.2 (L-type) channels: multiple altered gating mechanisms and pharmacological restoration of inactivation. J Physiol (Lond) (2009) vol. 587 (Pt 3) pp. 551-65



Phelan McDermid Syndrome

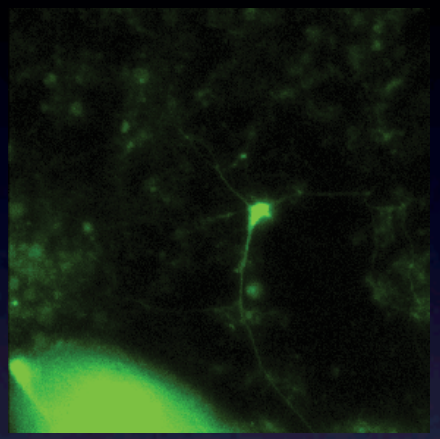
https://pmsiregistry.patientcrossroads.org/

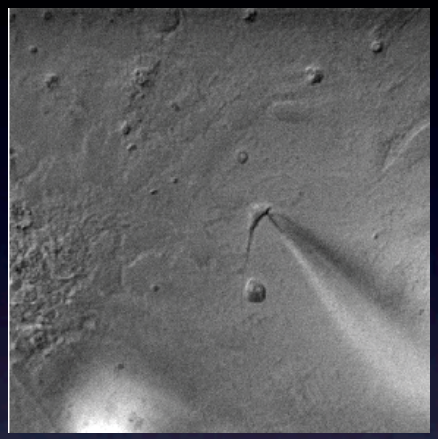


- Neonatal hypotonia
- Global developmental delay
- Absent or severely delayed speech

Shank-3

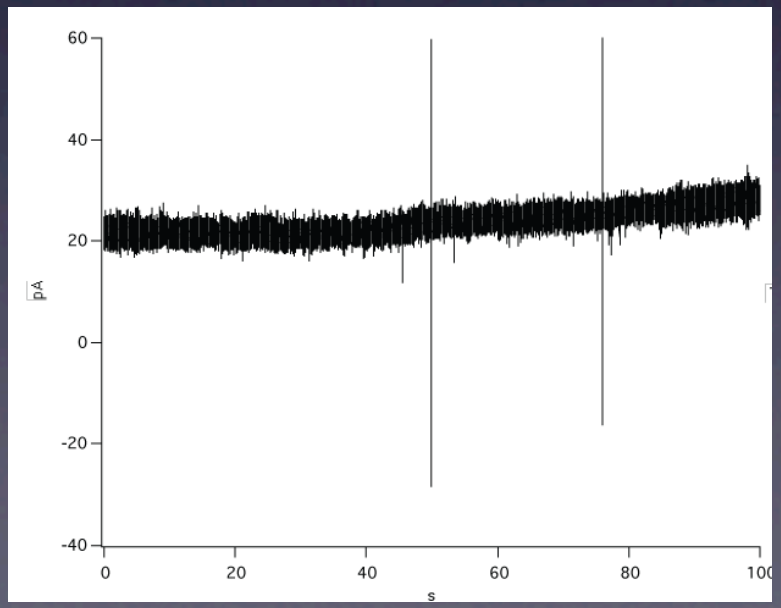
Increased spontaneous action potential firing in PM Syndrome neurons



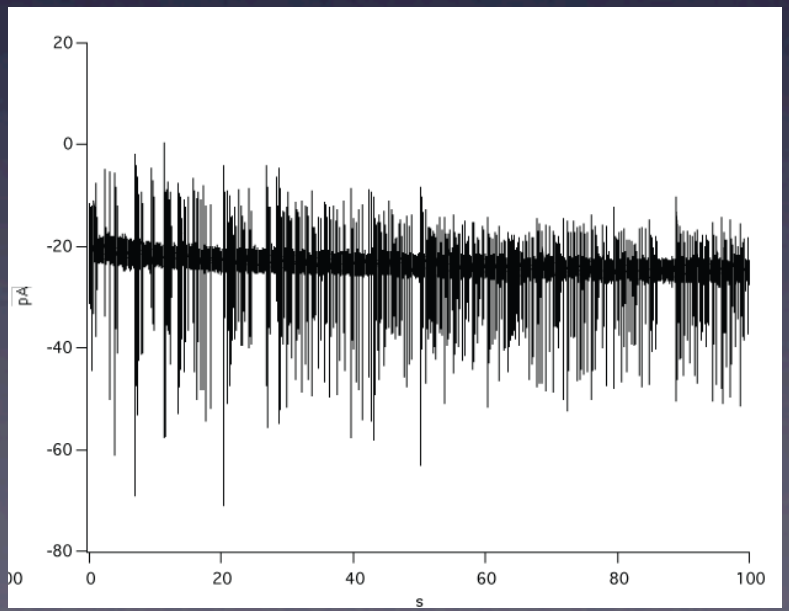


Cell attached mode

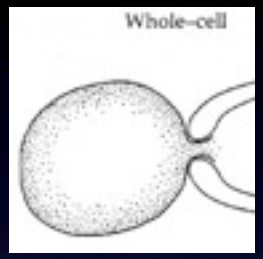
Control



PMS

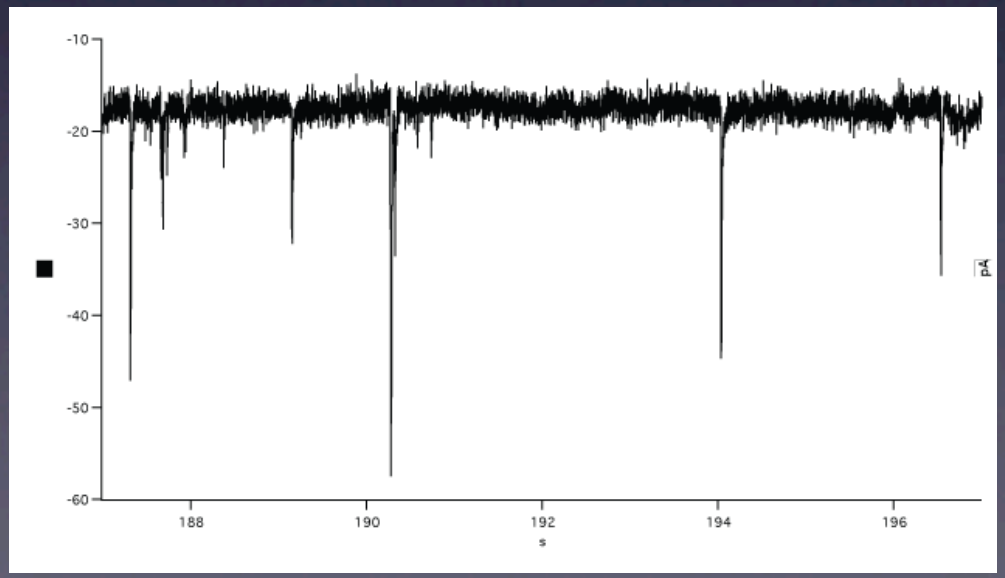


Decreased mini amplitude and frequency in 22q13 neurons

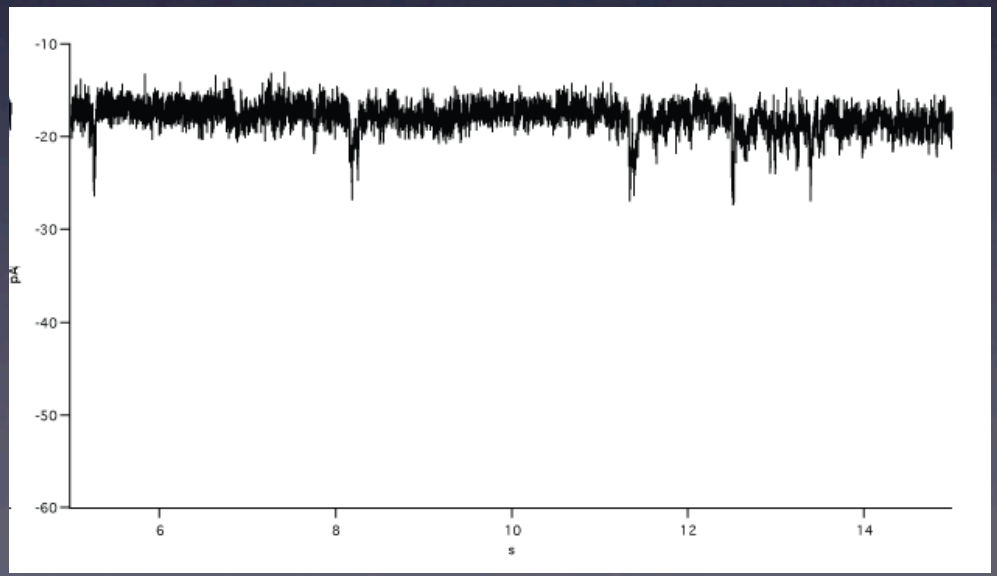


Whole cell mode

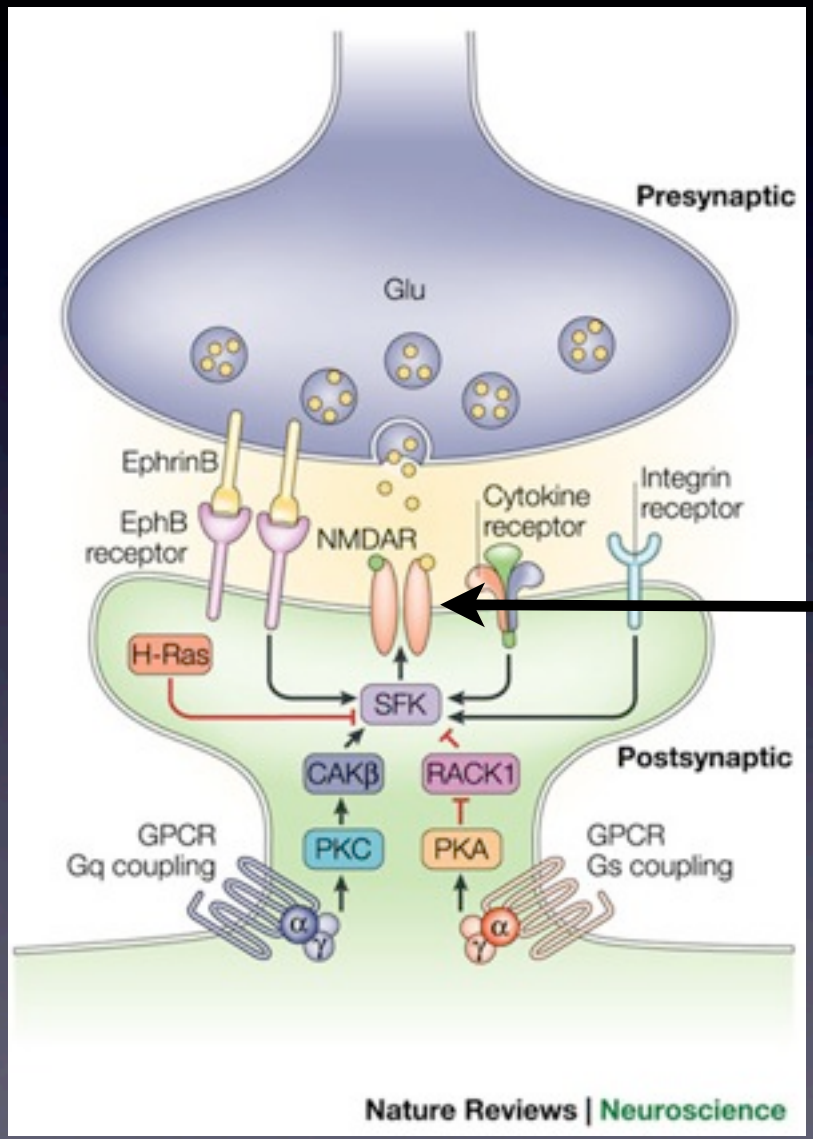
Control

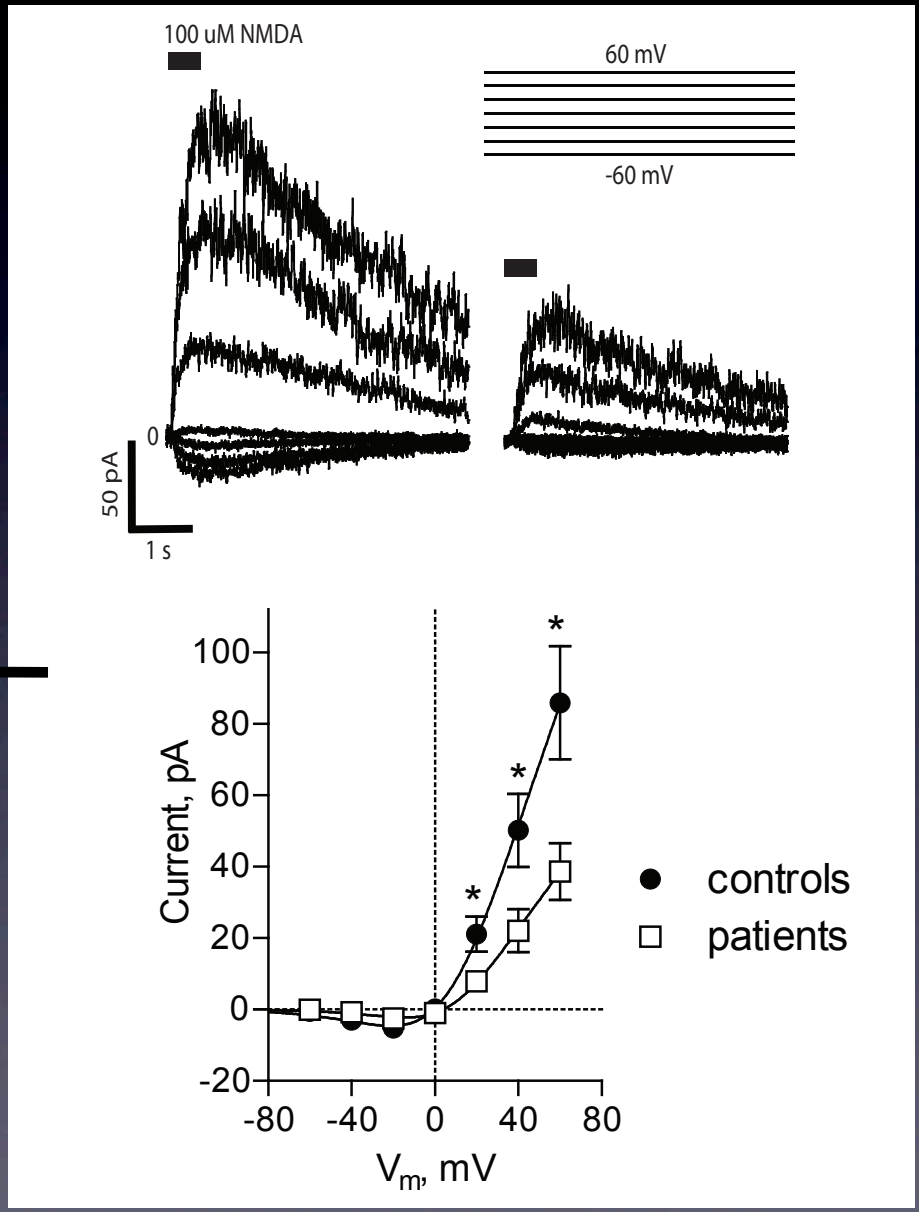


22q13

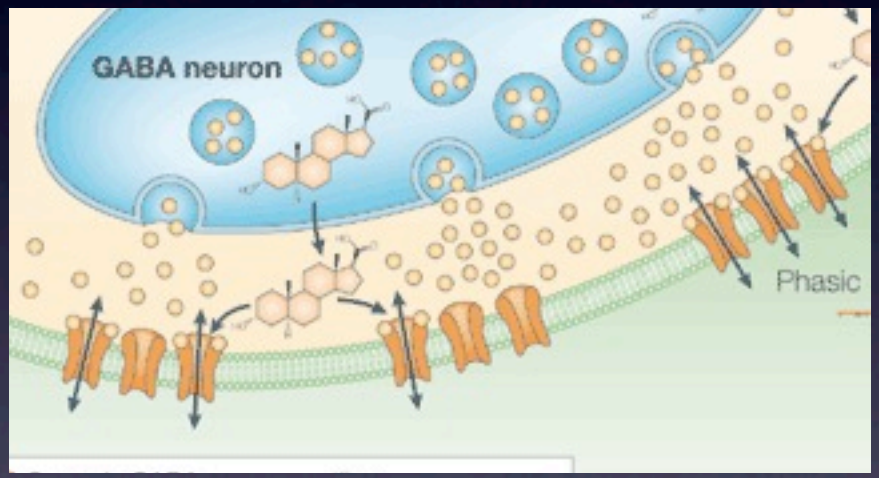


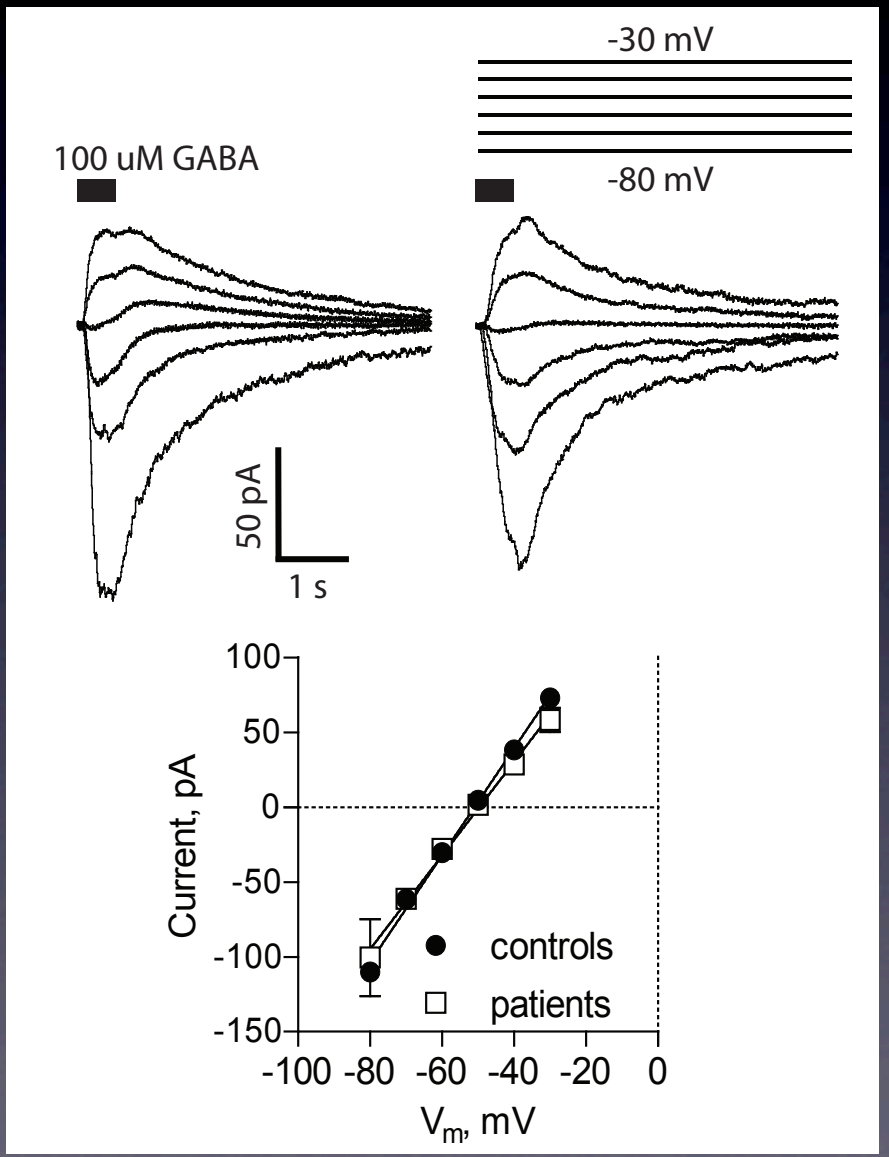
NMDA receptor currents are decreased in neurons from PM patients





GABA currents are unaffected in PMS neurons





The glass is half full

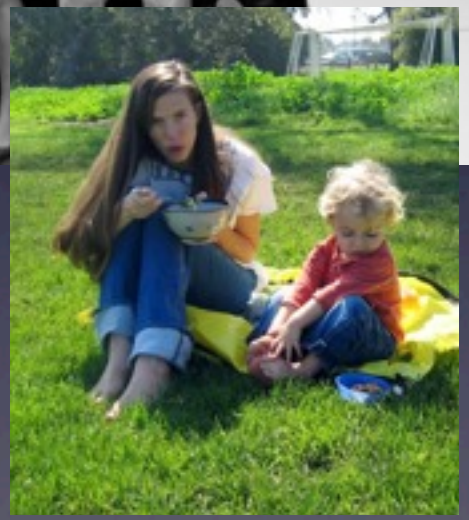
- We can make human neurons from kids with some kinds of autism
- We can identify defects in these cells
- We can find drugs that improve some of these defects

Wrong half of the glass

- Human neurons are not mature
- We don't know if the phenotypes that we observe *in vitro* cause the disease
- It will take time for these findings to be validated and translated to the clinic

Alex Shchlegovitov
Thomas Portmann
Masayuki Yazawa
Anna Krawisz
Jocelyn Krey
Sergiu Pasca
Anca Pasca
Brian Hsue
Yishan Sun
Rong Mao
Karen Chan
Susanna Wen
Chan Young Park
Georgia Panagiotakos
Masoud Sandhaghiani

The ASD Families



Irina Voineagu, and Dan Geschwind, UCLA
Joachim Hallmayer and Jon Bernstein, Stanford
Judy Rapoport NIMH

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