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San Francisco

Dr. Pleasure has no conflicts of interest to report



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“Long-lasting behavioral and anatomic consequences of exposure to pathogenic NMDAR antibodies in a mouse model”

Samuel J. Pleasure, MD/PhD

Professor of Neurology

Glenn W. Johnson Jr. Memorial Endowed Professor

Weill Institute of Neuroscience

UCSF

ENCEPHALITIS IN THE 21ST CENTURY: RISE OF AUTOANTIBODIES

- Incidence in Olmsted County, MN 1995-2015:
 - Infectious Encephalitis **0.8/100,000**
 - Autoimmune **1.0/100,000**

Autoantibody associated diseases are increasing in incidence
(probably improved diagnosis)

- Autoimmune 1995-2005 **0.4/100,000**
- Autoimmune 2006-2015 **1.2/100,000**

ETIOLOGIES OF AUTOANTIBODY ASSOCIATED ENCEPHALITIS

Paraneoplastic

May be part of an antitumor response?

Important biomarkers as tumor frequently occult – eg anti-NMDAR and ovarian teratoma or anti-Hu and SCLC

Post-infectious

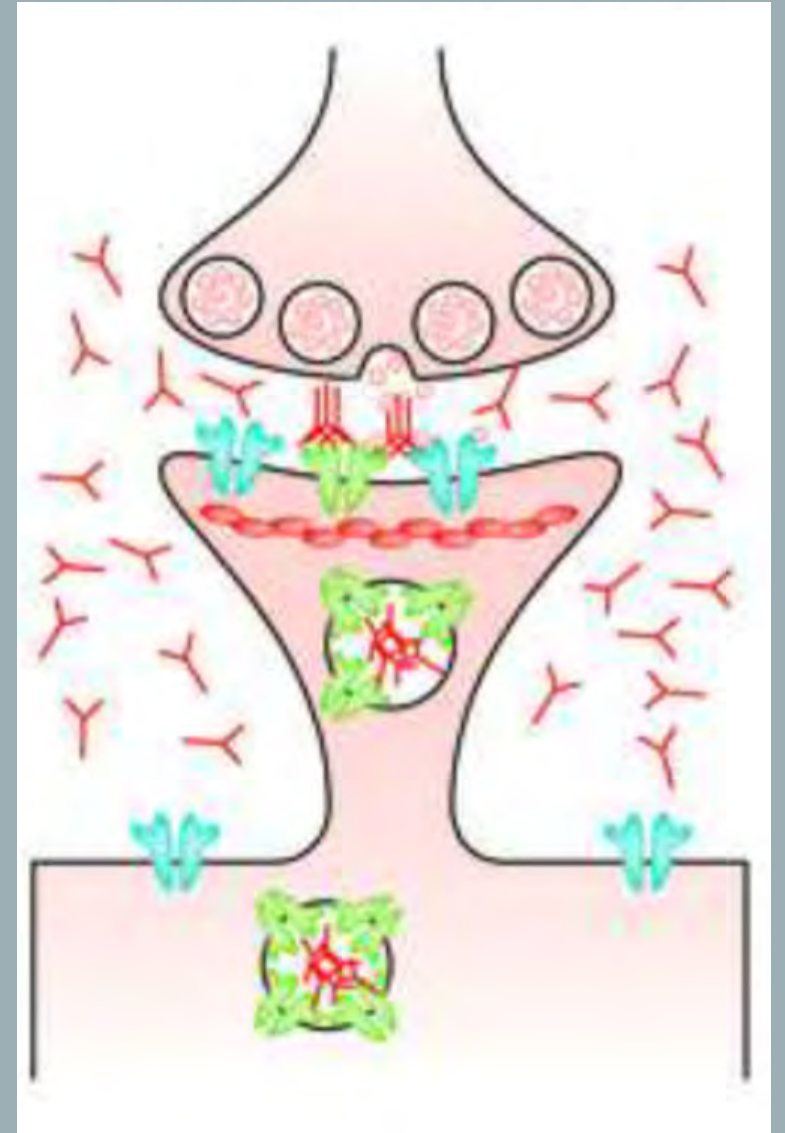
Older examples – GBS

Newer examples – anti-NMDAR and HSV I encephalitis

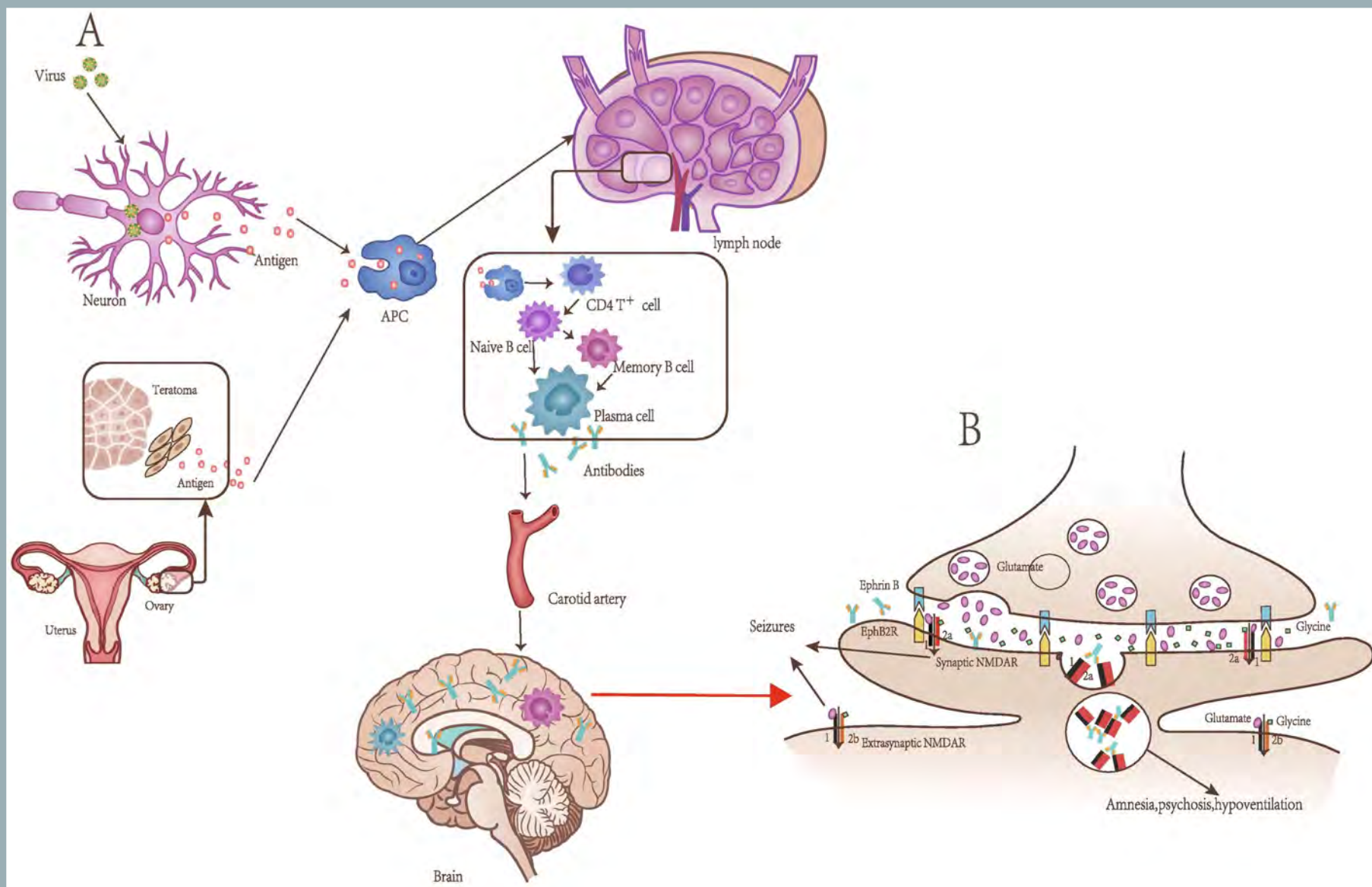
Likely based on molecular mimicry

Idiopathic

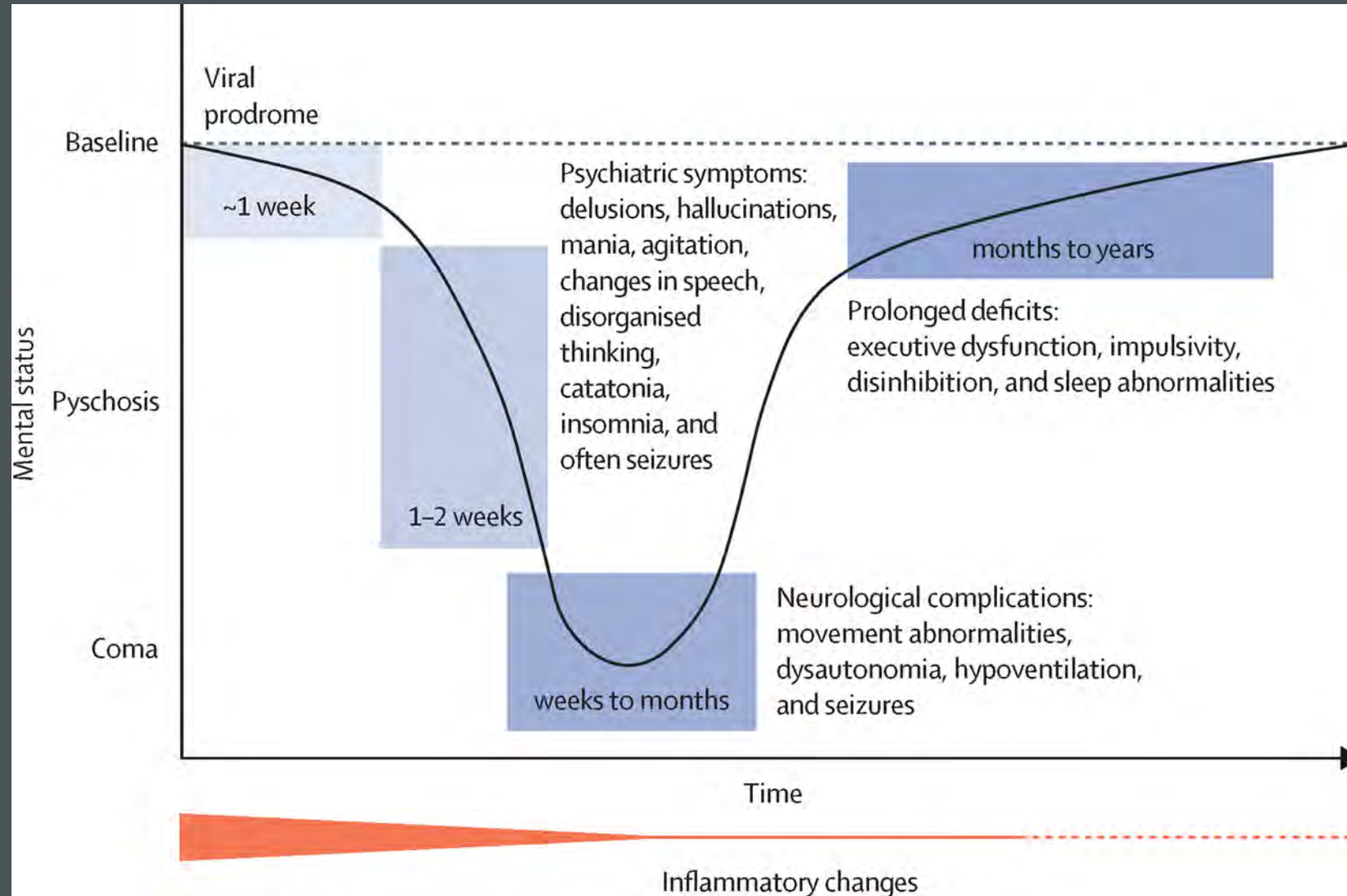
Anti-NMDAR encephalitis as an exemplar



WHAT HAPPENS IN NMDAR AE?



Clinical Course of NMDAR AE

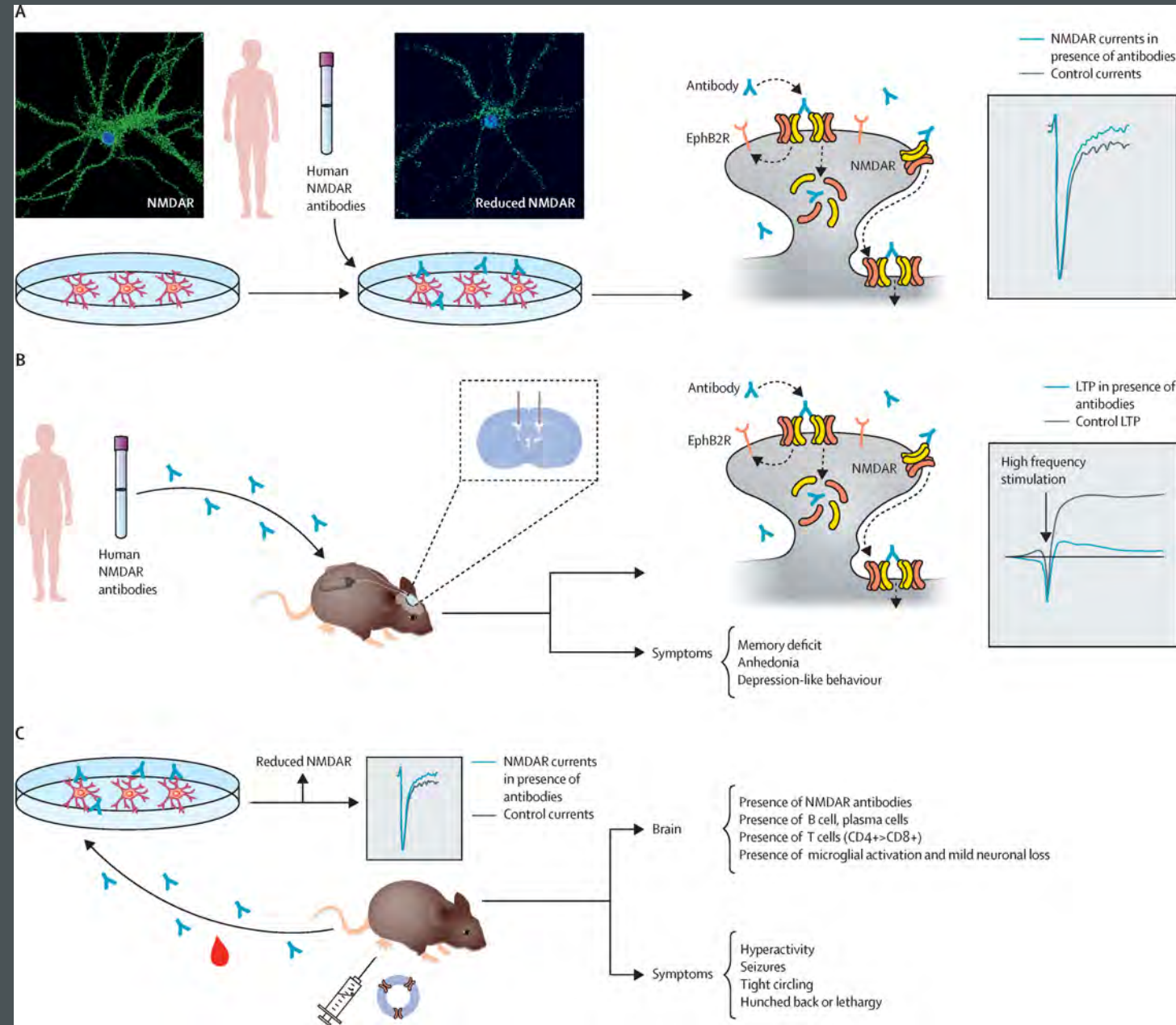


Investigational studies in understanding the pathophysiology of NMDAR AE

A. Identification of antibodies in CSF from patients that bind to receptors and which alter channel dynamics by several potential mechanisms

B. Transferring these antibodies into mice leads to similar effects and causes adult memory problems as long as the antibodies are infused.

C. Immunizing mice causes the development of a spectrum of behavioral problems and inflammation in the mouse brain related to antibodies.



Critical Question

Why do patients have long term deficits even after the disorder is treated and they no longer have abnormal antibodies at high levels?

Possibilities

- a) Chronic inflammation leads to brain injury
- b) Seizures and critical illness leads to brain injury
- c) Direct effects of the antibodies on circuits leading to long term alterations.

We decided to turn to the question of direct long-term effects of antibodies in the disease to determine whether the more severe long-term sequelae in children with NMDAR AE are due to direct effects of antibodies in developing circuits.

To do this we had to develop a new model.

A

CSF

B cell

Single cell Sorting

Mature B cells

mRNA extraction

cDNA

PCR: V(D)J

Sequencing

Cloning & Antibody purification

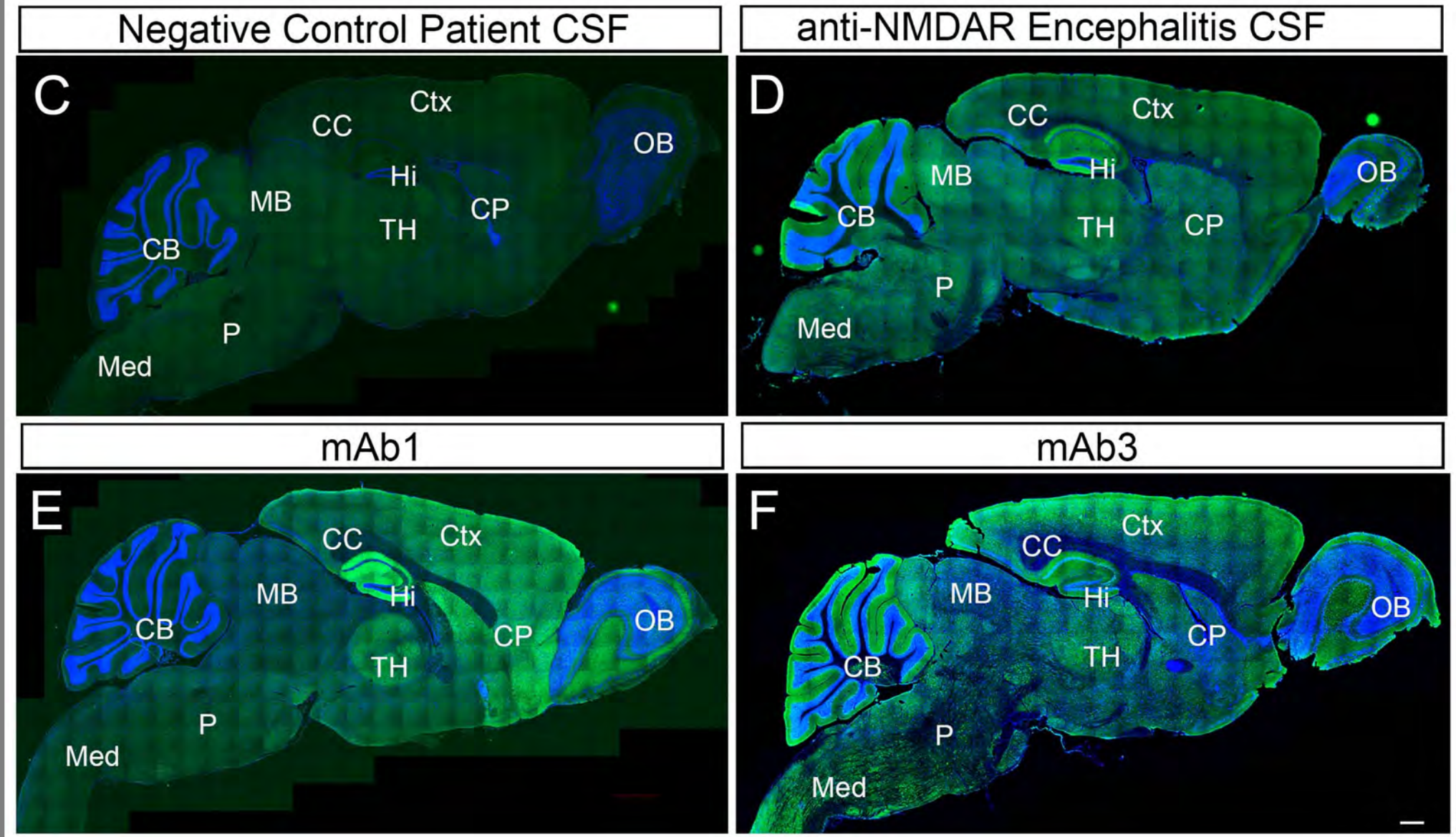
HEK293

The diagram illustrates the workflow for generating a B cell antibody library. It begins with the isolation of B cells from CSF (Cerebrospinal Fluid). These cells are then sorted using single-cell sorting technology. The sorted mature B cells are then used for mRNA extraction, followed by cDNA synthesis. The cDNA is then amplified using PCR, specifically targeting the V(D)J regions. The resulting PCR products are then sequenced. Finally, the sequences are used for cloning and antibody purification, which involves transfecting HEK293 cells and purifying the resulting antibodies.

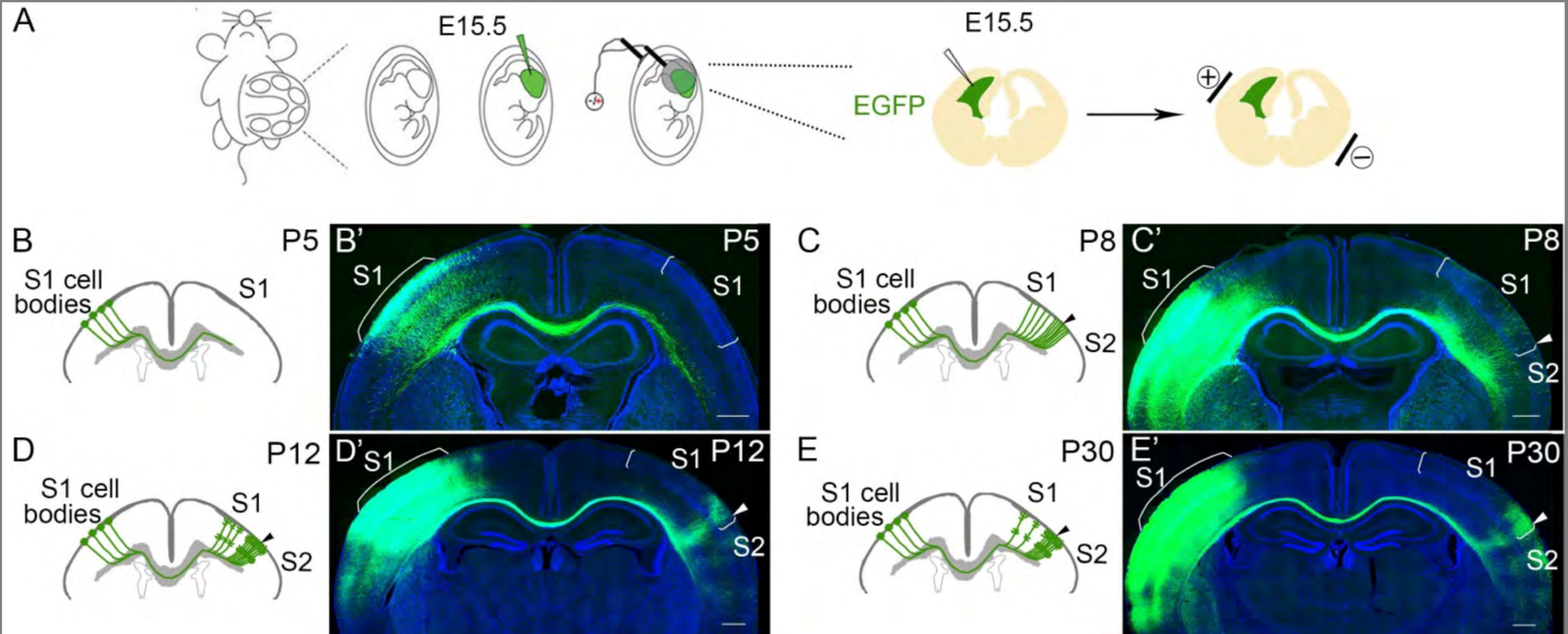
B

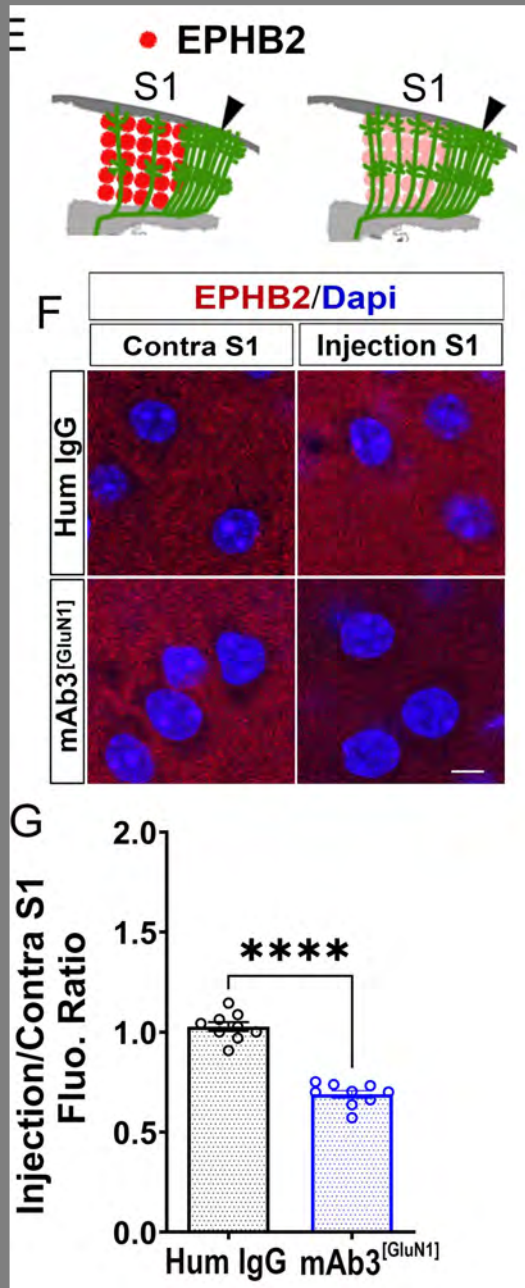
	Input	Negative Control		anti-NMDAR Encephalitis				← Anti-GluN1
		IgG	CSF	CSF	mAb #1	mAb #2	mAb #3	
260 — green	[band]							
160 — red	[band]							
125 — yellow	[band]			[band]	[band]		[band]	
90 — blue	[band]							

Human monoclonal antibodies to NMDAR stain mouse brain

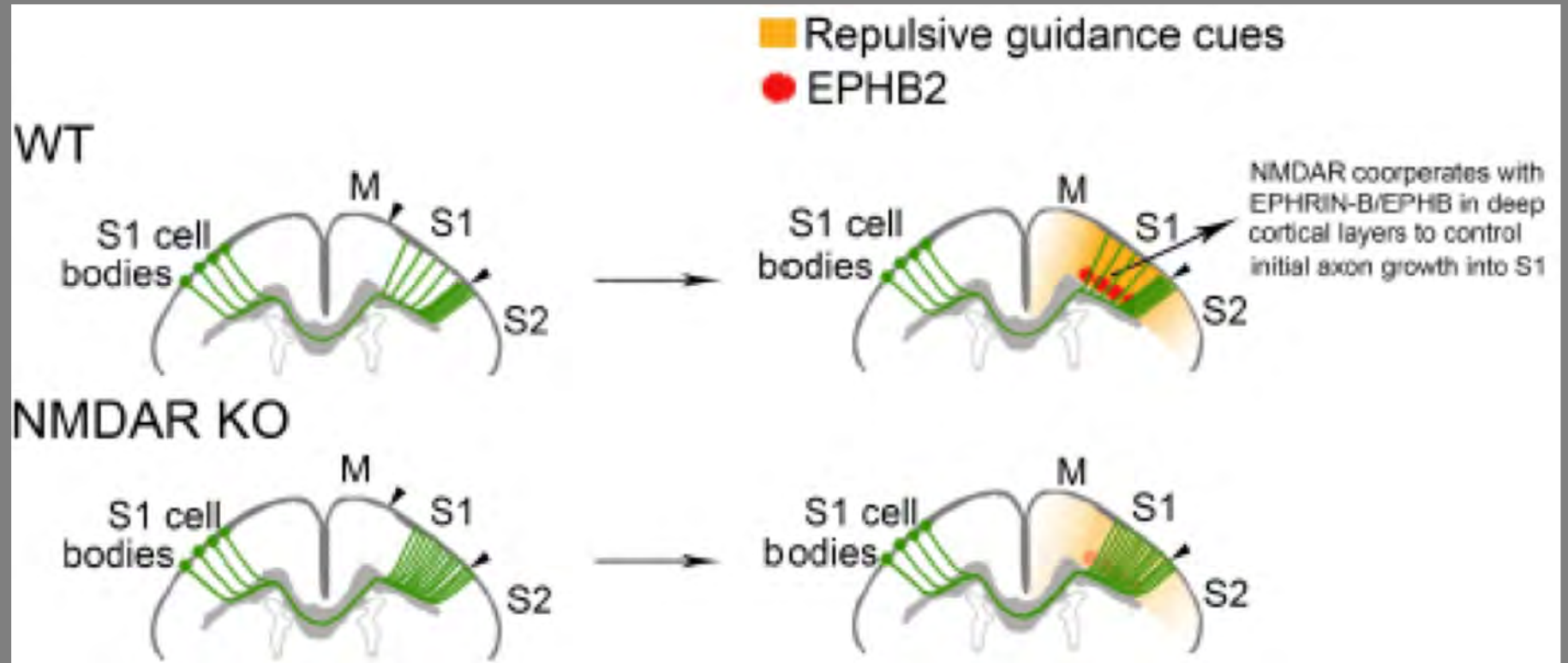


Our Model: A developing cortical circuit required for motor coordination

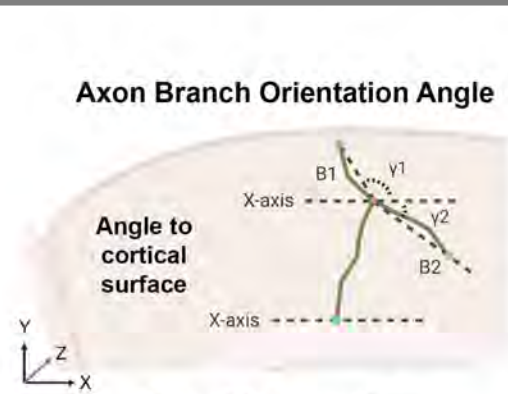
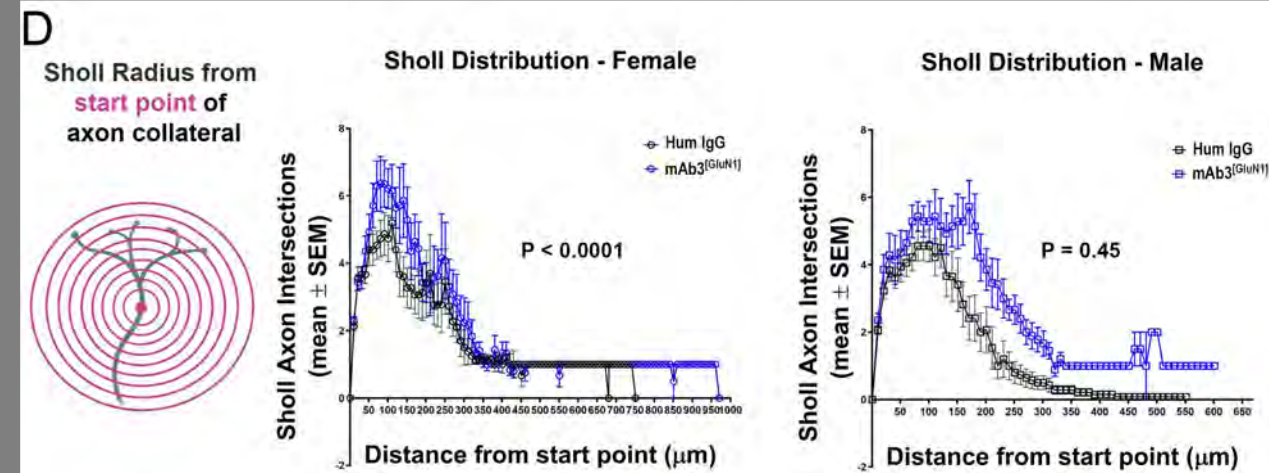
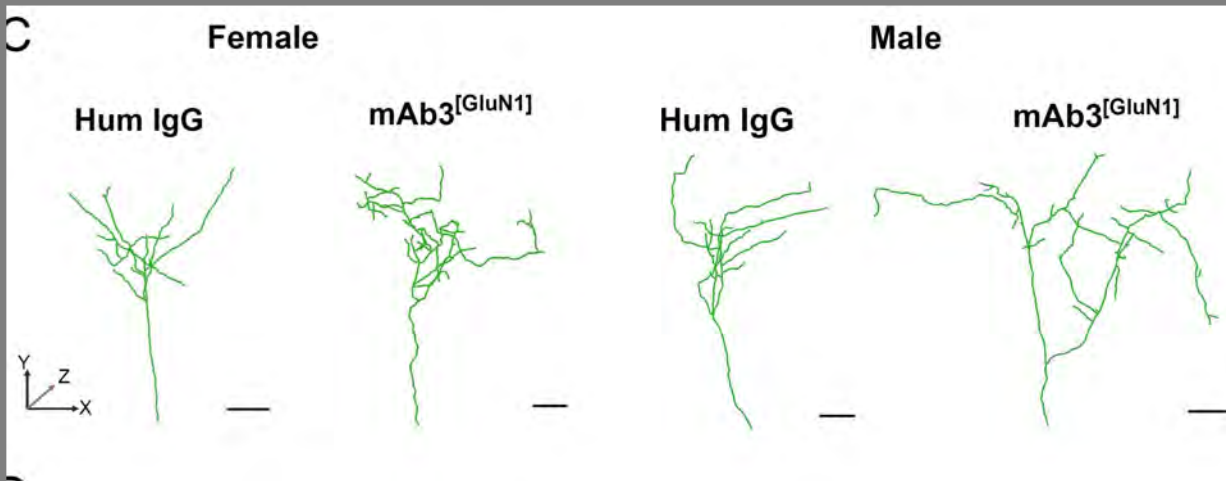




The human mAb against NMDAR phenocopies what we previously demonstrated in the mouse mutants for the receptor – ie collaboration with EphB2 as an axon guidance receptor.



Now, what we did is prepared these mice and waited until they were 4 months old and studied the anatomy of the circuit

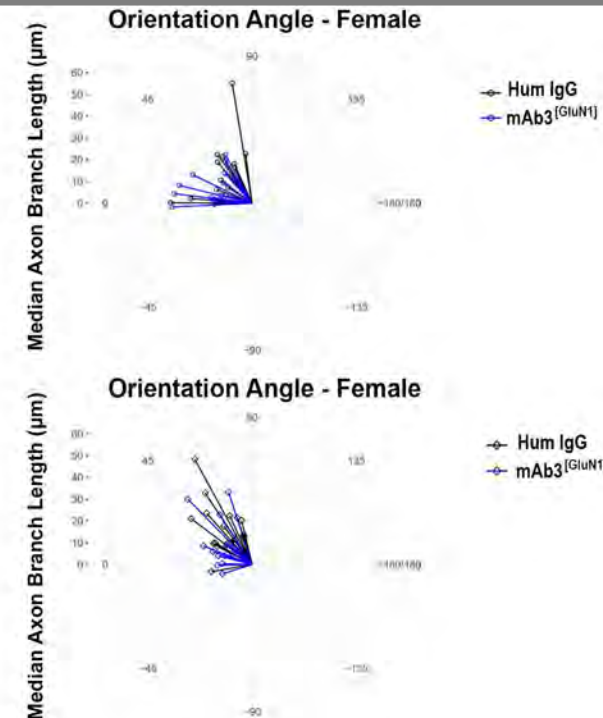
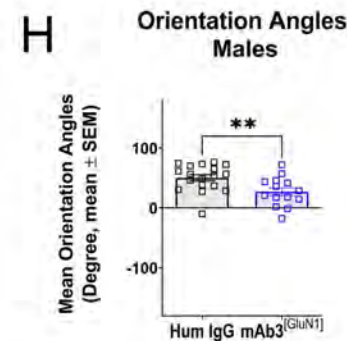
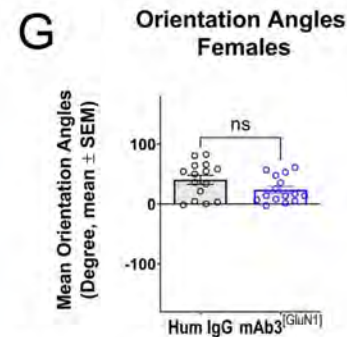


Branching towards to Cortical Surface:

$$0 < Y1 < 180$$

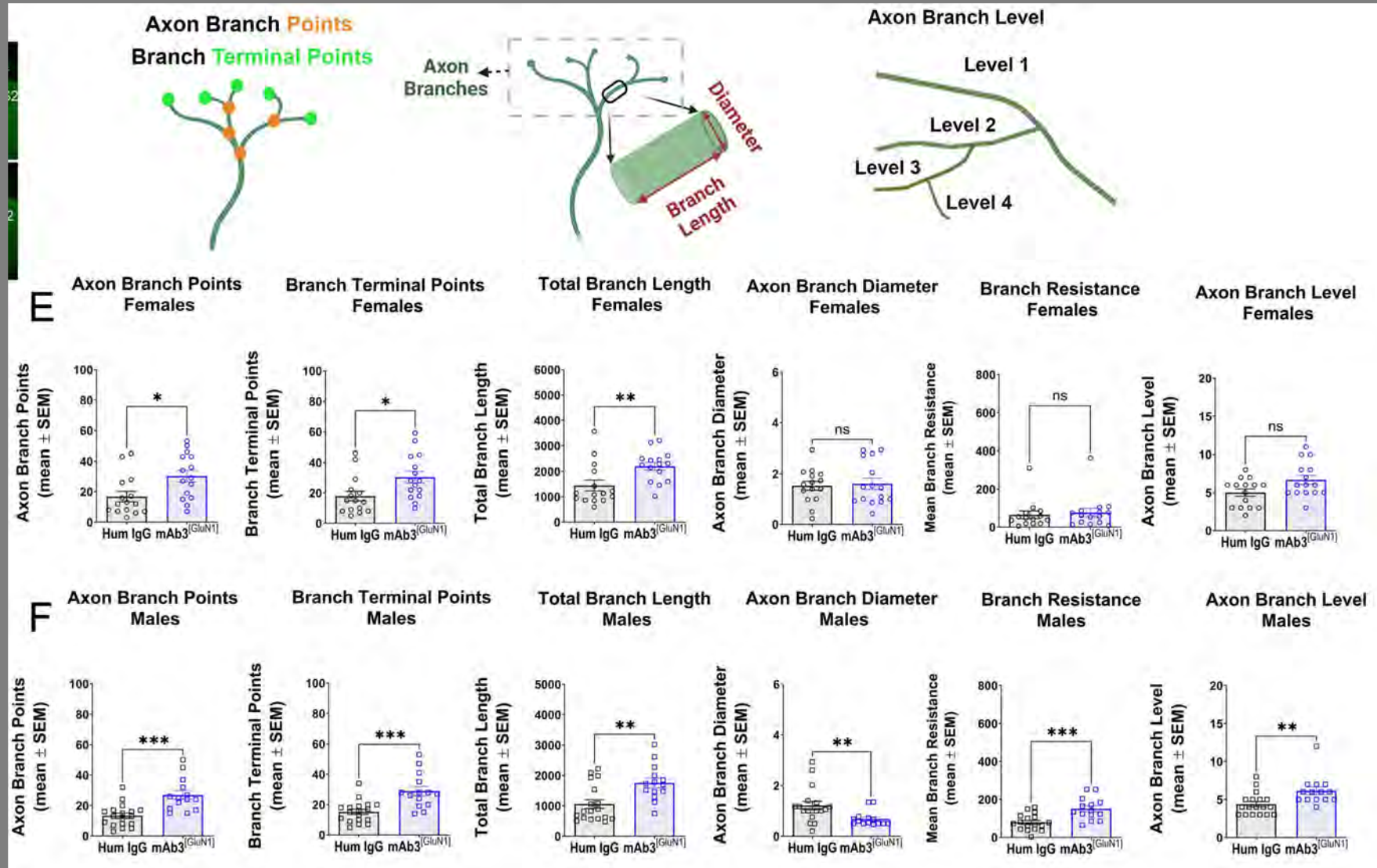
Branching away from Cortical Surface:

$$-180 < Y2 < 0$$



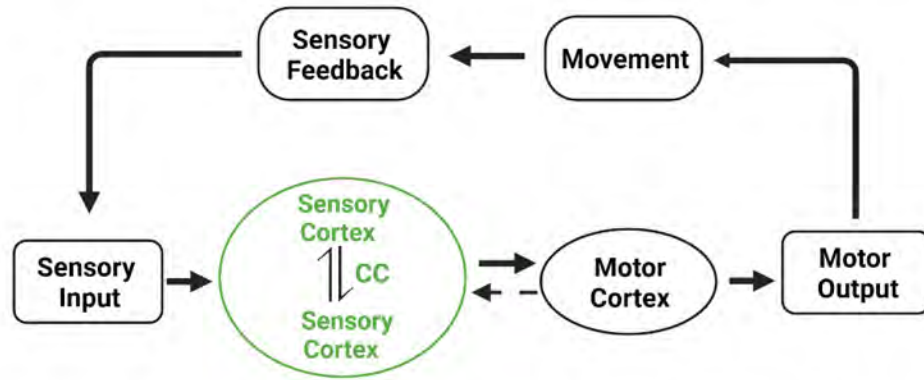
There are a range of significant anatomic defects in cells in the somatosensory cortex that were exposed to NMDAR mAb for only 8 days even months later

Now, what we did is prepared these mice and waited until they were 4 months old and studied the anatomy of the circuit

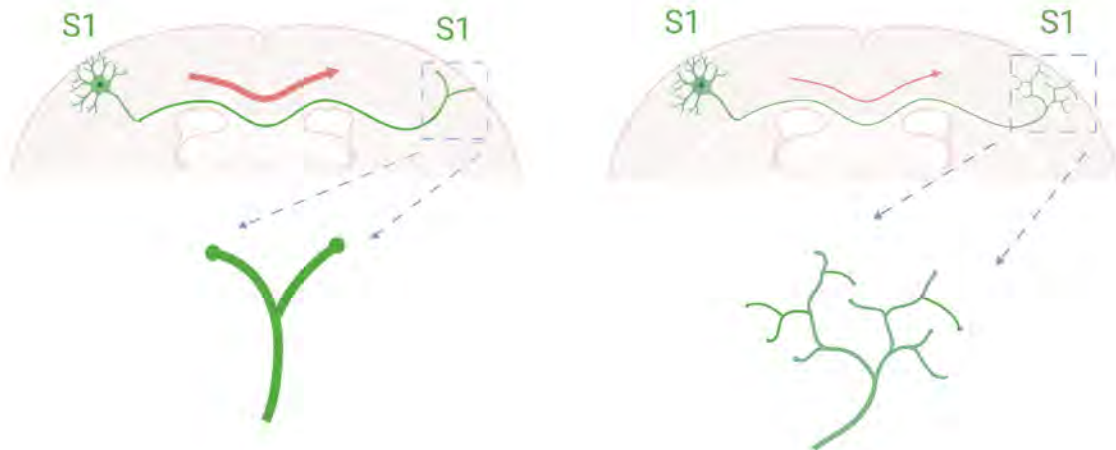


What is significance of all of these anatomic changes however?

A The Workflow of Bilateral Sensory-Motor Integration

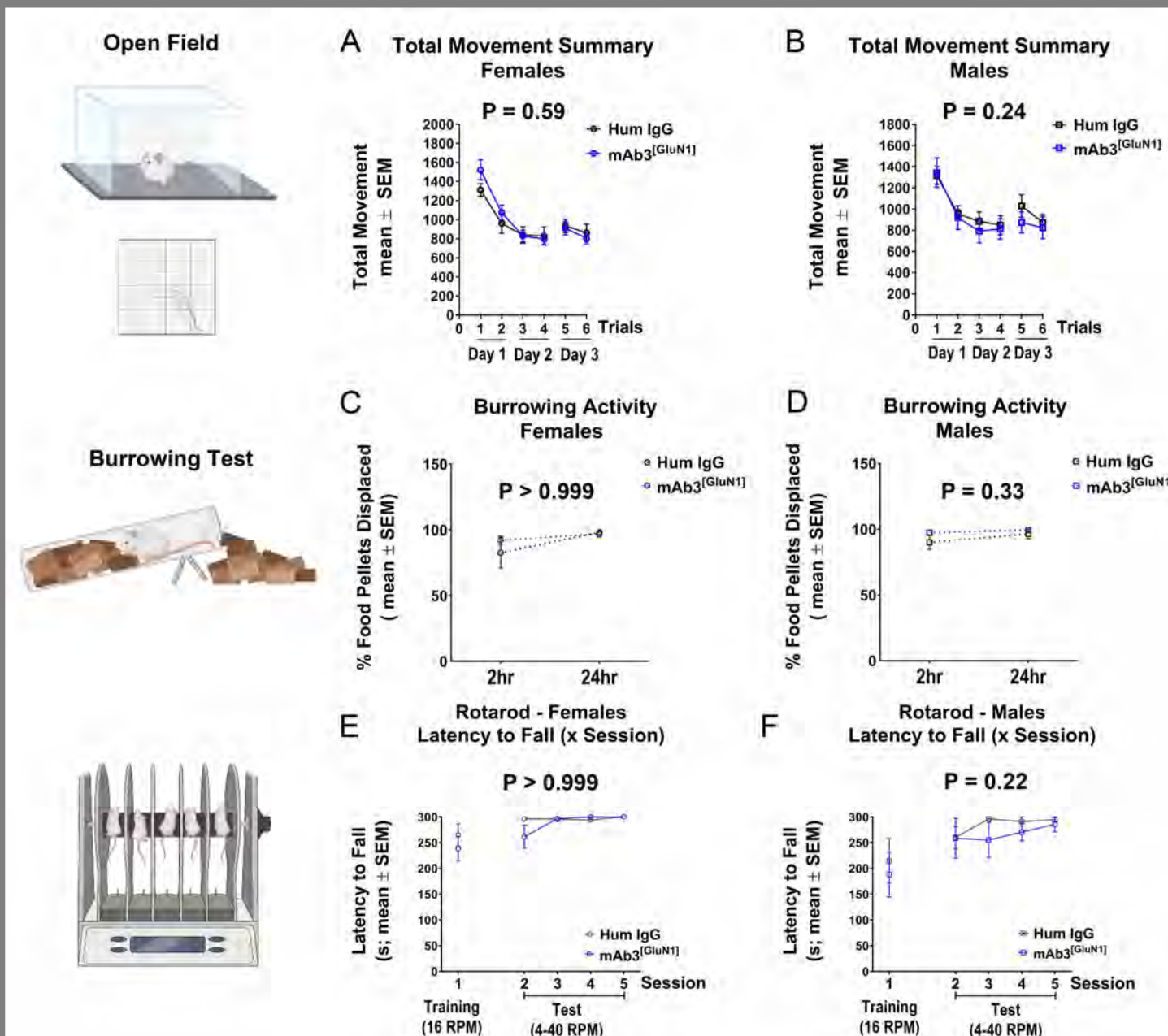


B Reduced diameter, increased branch complexity in S1 callosal axon terminals increase the signal propagation failures



The role of callosal circuits is to coordinate sensory and motor feedback to allow coordinated movement, the anatomic changes we see predict deficits in complex movements.

Mice do well in straightforward simple measures of motor function

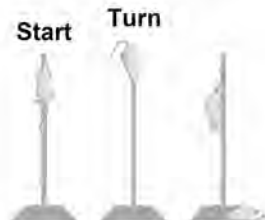


But fail in more complicated tasks requiring motor coordination

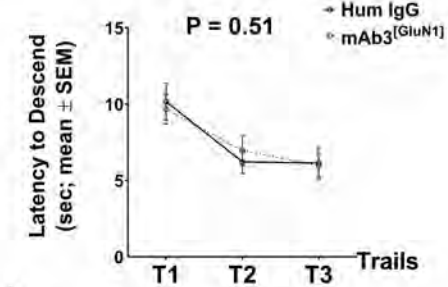
Facing Down Pole Test



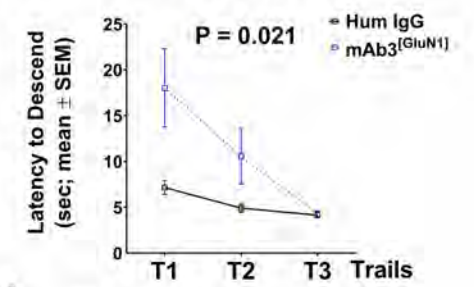
Facing Up Pole Test



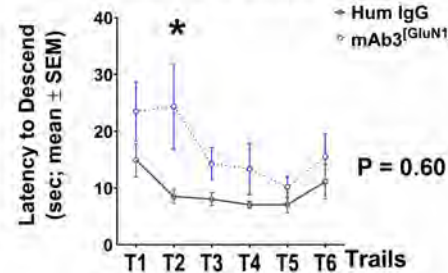
G Pole Test - Facing Down Females
Latency to Descend



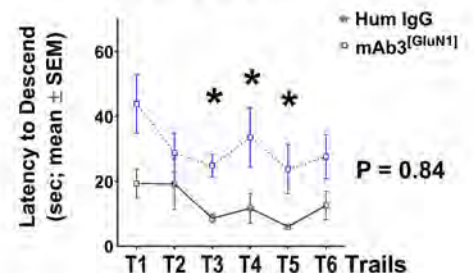
H Pole Test - Facing Down Males
Latency to Descend



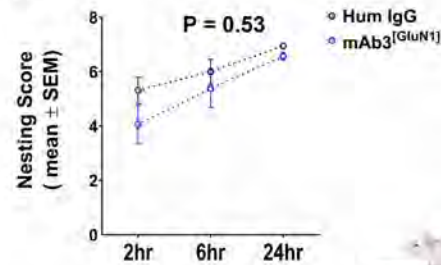
L Pole Test - Facing Up Females
Latency to Descend



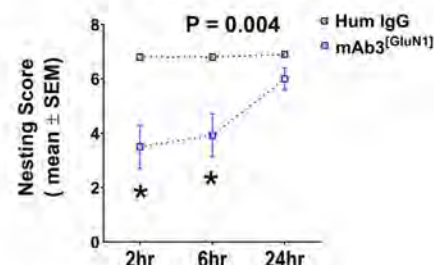
M Pole Test - Facing Up Males
Latency to Descend



A Nesting - Females



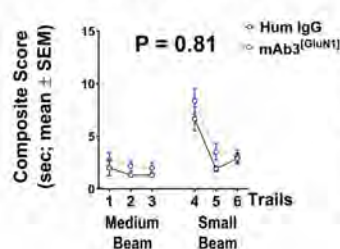
B Nesting - Males



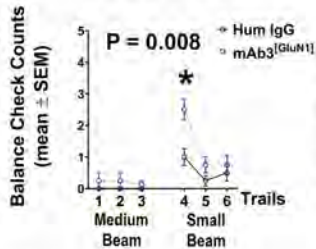
Nest building Test



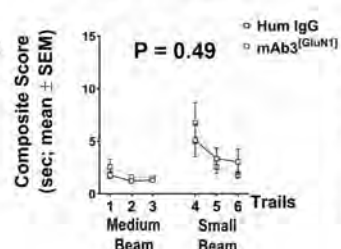
C Balance Beam - Females
Composite Score



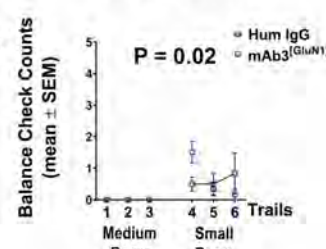
D Balance Beam - Females
Balance Checks



E Balance Beam - Males
Composite Score



F Balance Beam - Males
Balance Checks



Such as nest building, balance checking and turning on a pole to walk down

Conclusions

1. During cortical development anti-NMDAR antibodies cause pathfinding defects leading to formation of faulty circuits with disrupted neuronal morphology.
2. This occurs even with only brief exposure to pathogenic antibody. This may underlie some of the catastrophic changes seen in children with NMDAR AE
3. In a mouse model these defects lead to very long-term behavioral deficits reflecting defects in the circuits which are disrupted.

ACKNOWLEDGEMENTS

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