

# Opsoclonus myoclonus syndrome: a novel approach to antigen identification and disease modeling

**10<sup>th</sup> International workshop on OMS**  
**Abington 7<sup>th</sup> October 2022**

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# Autoimmune encephalitis

“LIMBIC ENCEPHALITIS” AND ITS ASSOCIATION WITH  
CARCINOMA

BY

J. A. N. CORSELLIS, G. J. GOLDBERG<sup>1</sup> AND A. R. NORTON<sup>2</sup>

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**1968 - 2000**

**Intracellular antibodies (Hu, Ma2, Ri..)**

Limbic encephalitis

Adults with cancer

Bad response to immunotherapy, cytotoxic T c

**2001 “seronegative” limbic encephalitis**

Response to immunotherapy, cancer

**2005-2007**

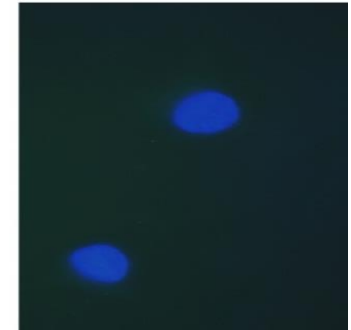
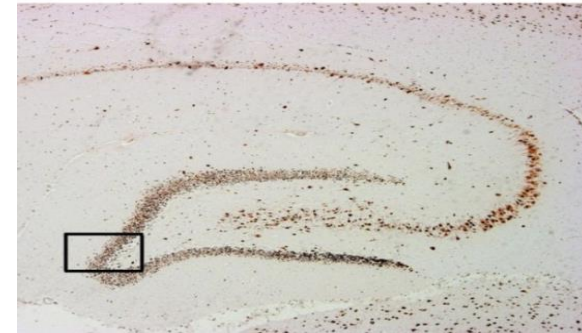
**Antibodies against neuronal surface antigens**

Limbic encephalitis or diffuse involvement

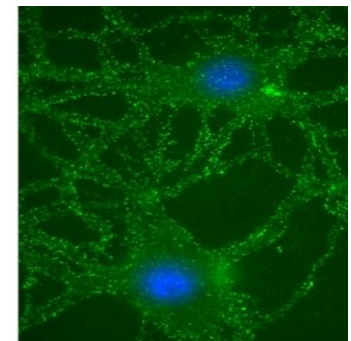
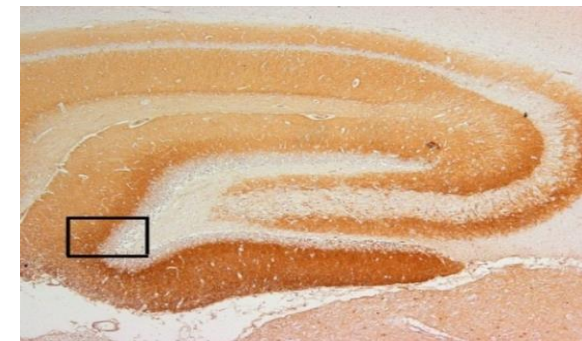
All ages, with or without cancer

Good response to immunotherapy

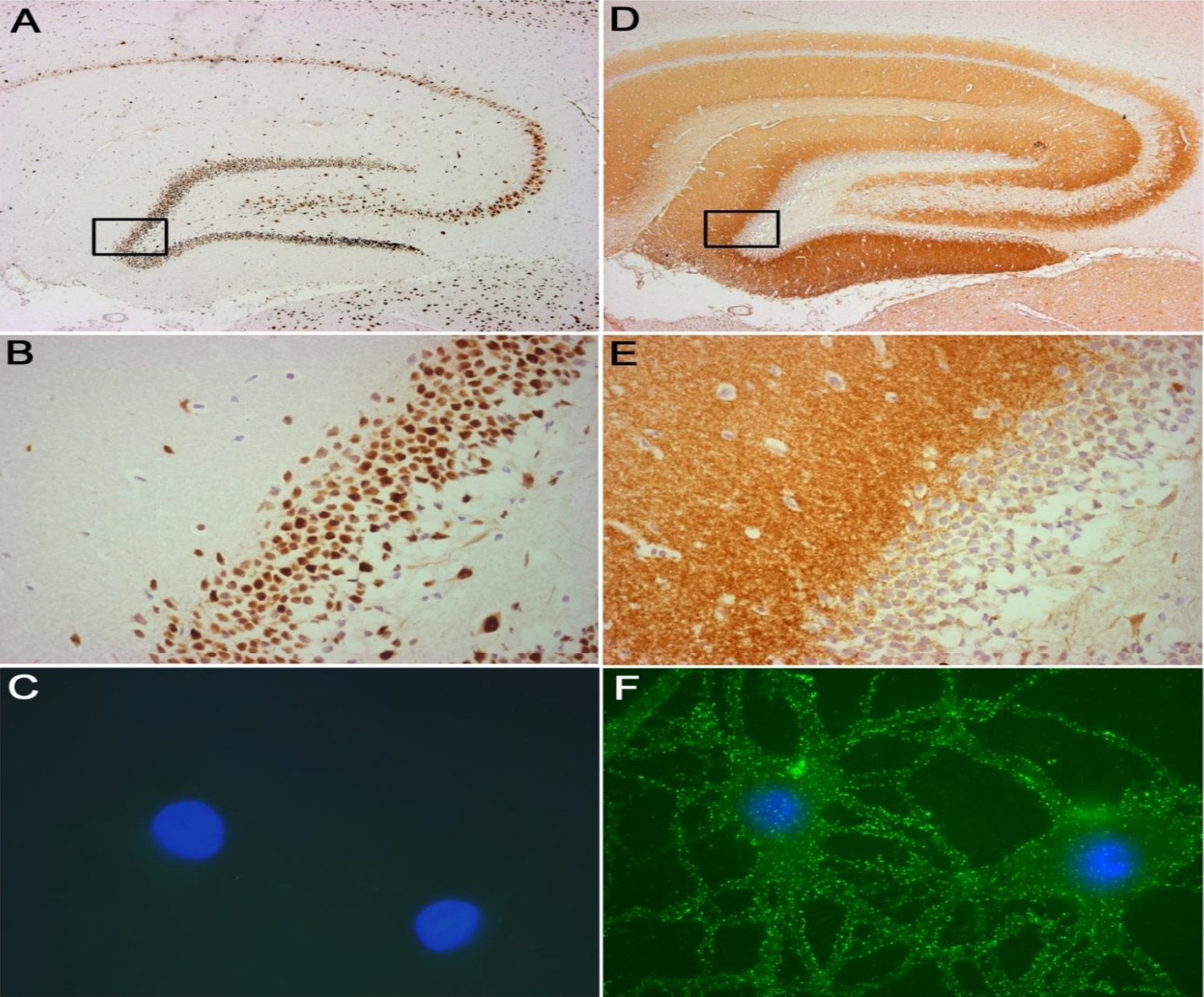
Brain 1968; 91:481-498



Lancaster et al.  
Neurology, 2011; 77:179-89.



# Antibodies to intracellular and cell-surface antigens



Intracellular,  
e.g., Hu

Cell-surface, e.g.,  
NMDA receptor

Lancaster et al.  
Neurology, 2011; 77:179-  
89.

# Disorders of synaptic/cell autoimmunity

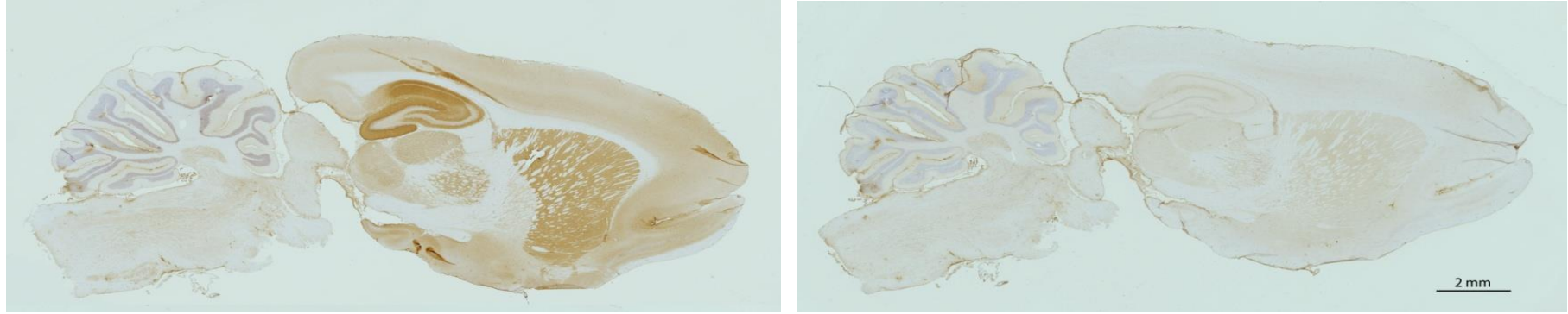
Antigen	Syndrome and main features
NMDA	Anti-NMDAR encephalitis
AMPA	Limbic encephalitis, psychosis
GABA-B	Limbic encephalitis with prominent seizures, status
LGI1	Limbic encephalitis, myoclonus, hyponatremia
Caspr2	Encephalitis and/or neuromyotonia
mGluR5	Ophelia syndrome
DPPX (Kv4.2)	Hallucinations, agitation, myoclonus, tremor, seizures, diarrhea
mGluR1	Cerebellitis (+/- Hodgkin's Disease)
GlyR	Stiff-person syndrome, hyperekplexia, PERM
<u>Dopamine 2 (D2)</u> (not reported by all groups)	<i>Basal ganglia encephalitis, Sydenham's chorea</i>
<u>GABA-A</u>	Encephalitis with status epilepticus, refractory seizures
Igln-5	Taupathy, sleep disorder
<u>Neurexin-alfa</u>	<b>Encephalitis +/- seizures +/- movement disorder</b>

\*in children

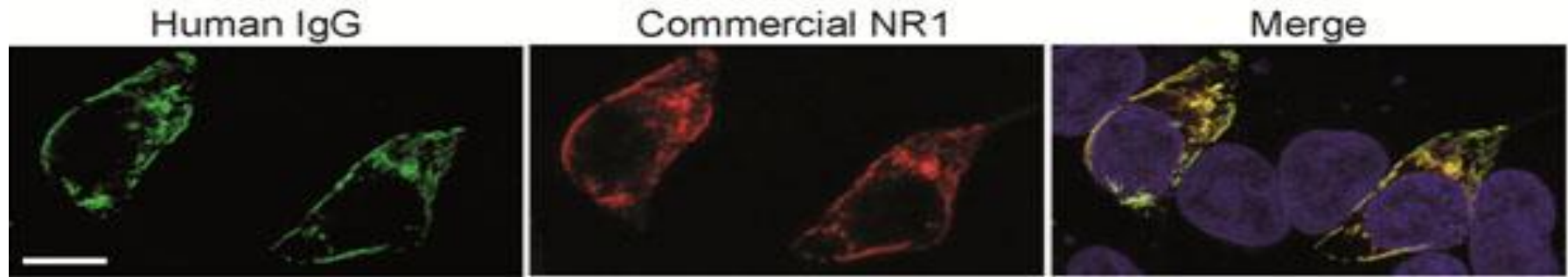
Adapted from Lancaster and Dalmau, Nat Review Neurol 2012;8:380-90

# Characterization of neuronal surface antibodies

Screening

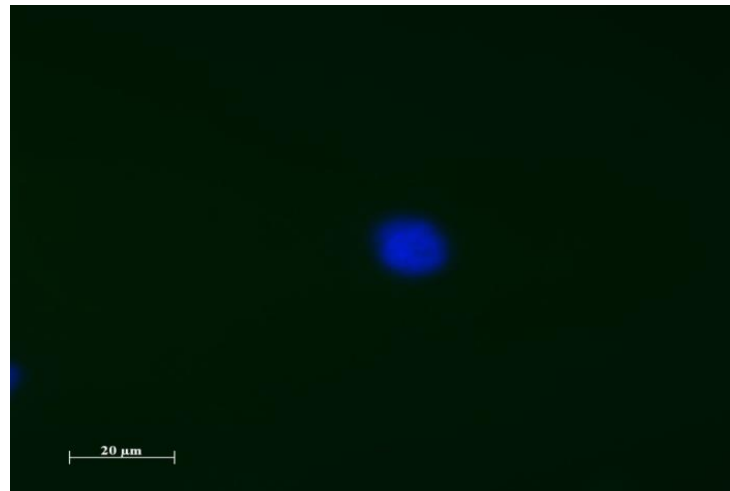
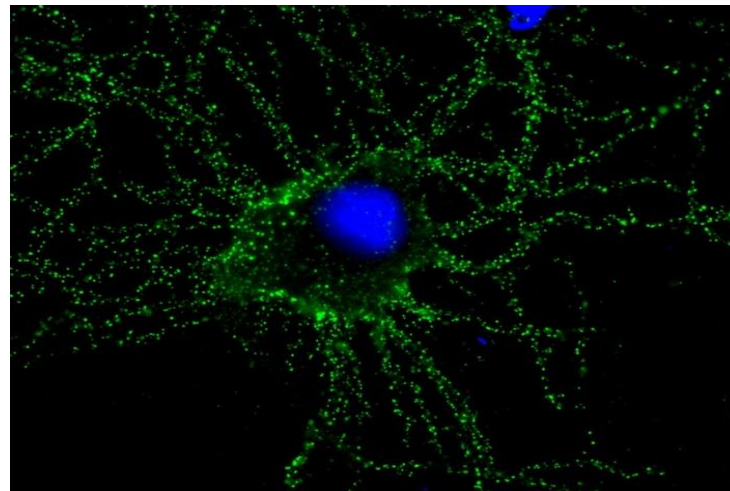


Confirmation

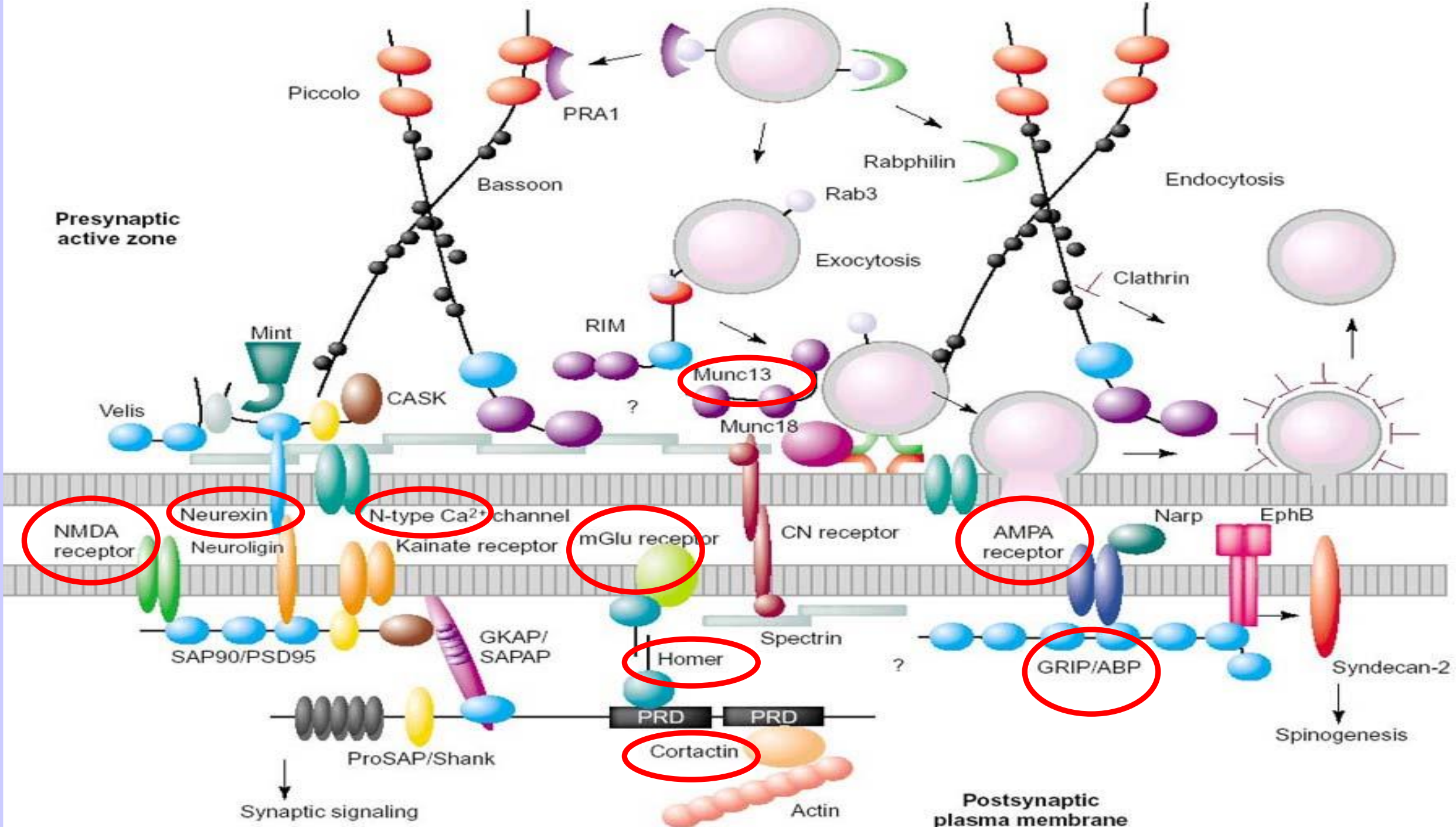


Known antigen  
e.g. NMDAR

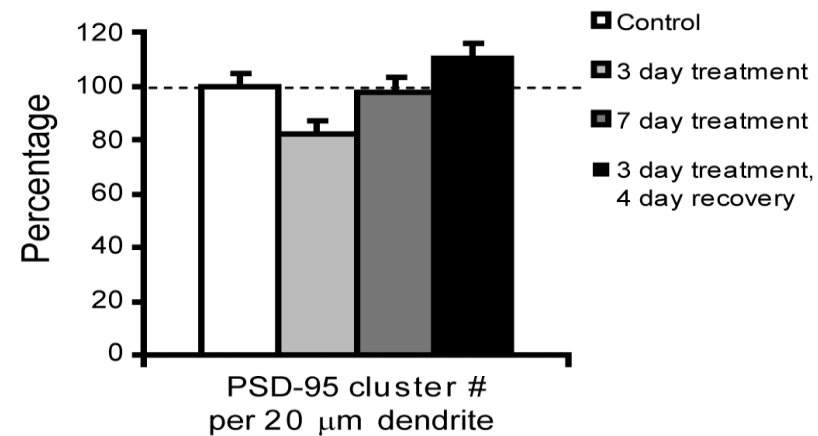
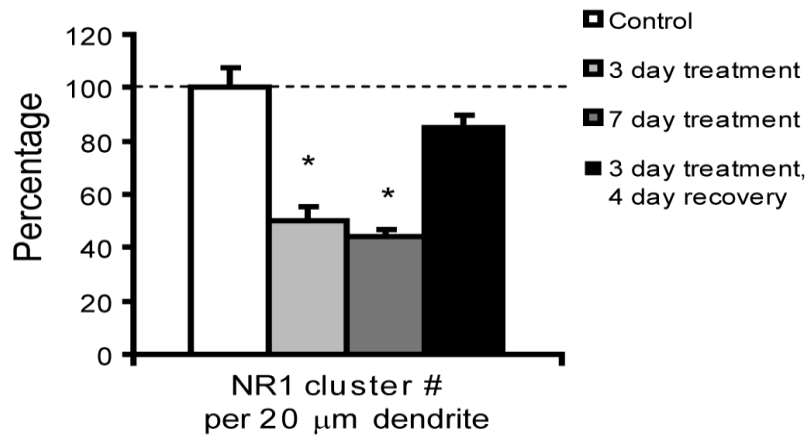
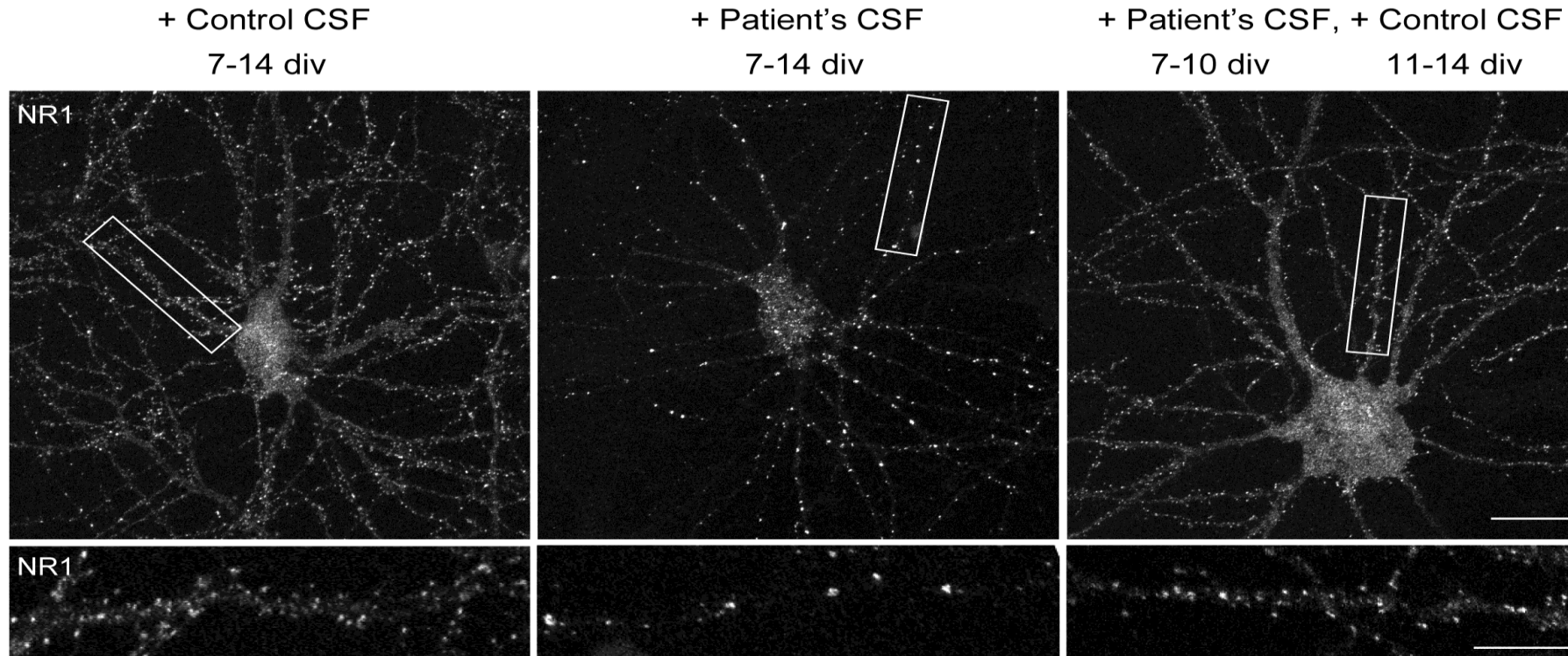
Unknown  
antigen



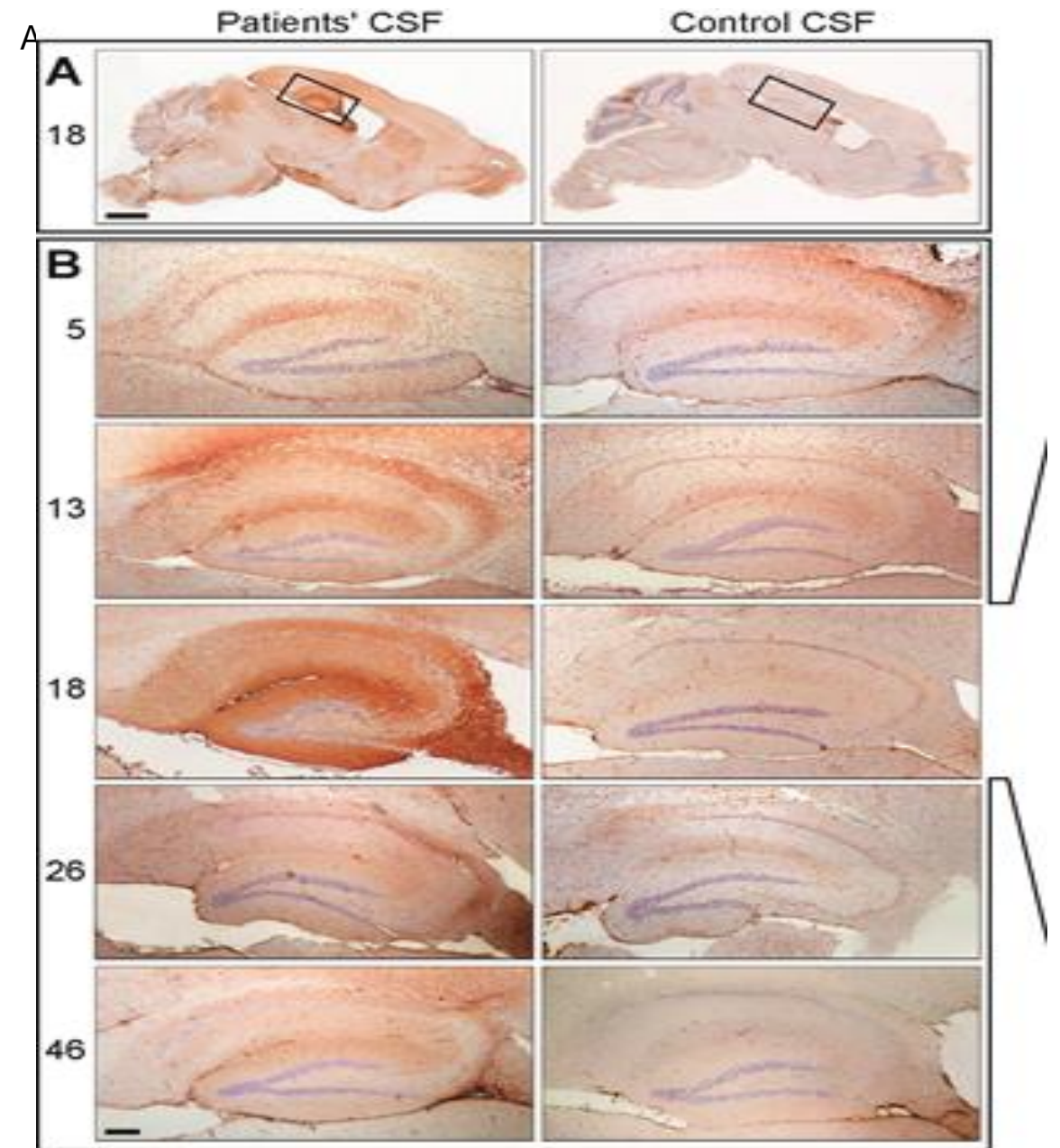
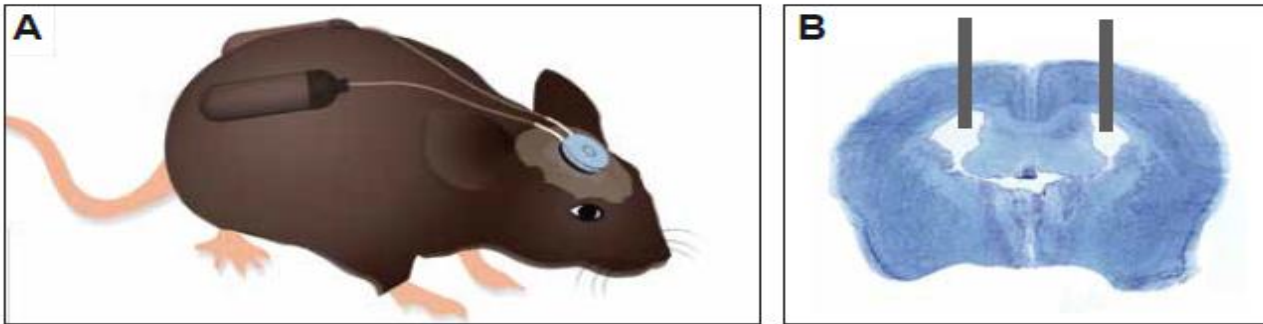
Immunoprecipitation



# Patient's antibodies decrease the clusters of NMDAR



# Anti-NMDAR murine model: antibodies are pathogenic



But...

Not all treatment-responsive immune mediated disorders associate with antibodies (at least nowadays)

Paraneoplastic Anti-*N*-methyl-D-aspartate  
Receptor Encephalitis Associated with  
Ovarian Teratoma

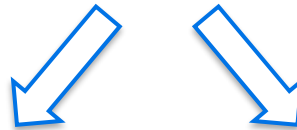
Dalmau et al., Ann Neurol 2007;61:25-36



3600 Patients with suspected autoimmune  
encephalitis



249 with teratoma

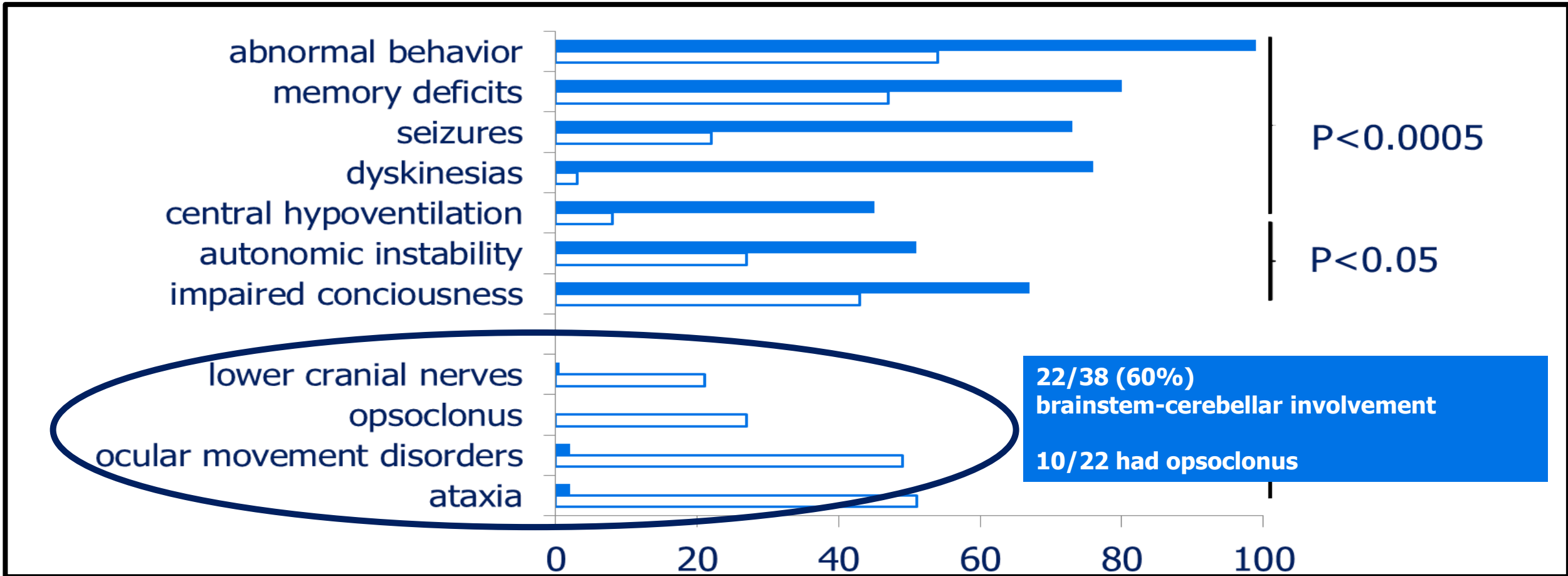


211 NMDAR antibody positive (anti-NMDAR  
encephalitis)

38 NMDAR  
antibody negative

?

# Symptoms of patients with teratoma with and without NMDAR antibodies



■ 211 cases with NMDAR antibodies

□ 38 cases without NMDAR antibodies

# Brainstem-cerebellar syndrome and teratoma

- 20/22 (91%) women
- Median age: 28 years (12-41)
- 18 developed neurological symptoms before tumor diagnosis and 2 post tumor removal
- All but one had mature teratomas
- CSF: 14 pleocytosis, 9 elevated level of proteins, 5/7 BOC+
- MRI: abnormal in 6/19 patients
- 10 (45%) developed opsoclonus

## Subgroup of opsoclonus

- All 10 were women (median age, 27 years; 15-32)
- 4/10 prodromal symptoms
- Associated symptoms: 9 ataxia, 7 myoclonus, 4 decreased level of consciousness, 3 altered behavior, 2 seizures
- CSF: 7 pleocytosis, 3 high protein concentration; 3/3 OCB
- MRI abnormal in 2/9 patients

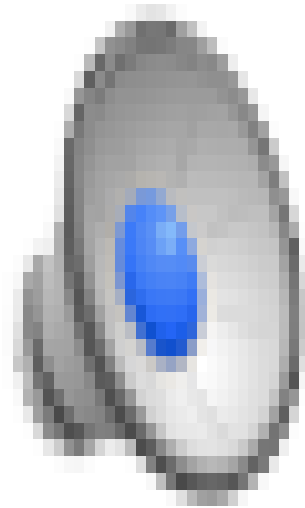
## Treatment

- All 10 patients: steroids, IVIG or/and plasma exchange
- 1 Rituximab, 1 rituximab and azathioprine

## Outcome

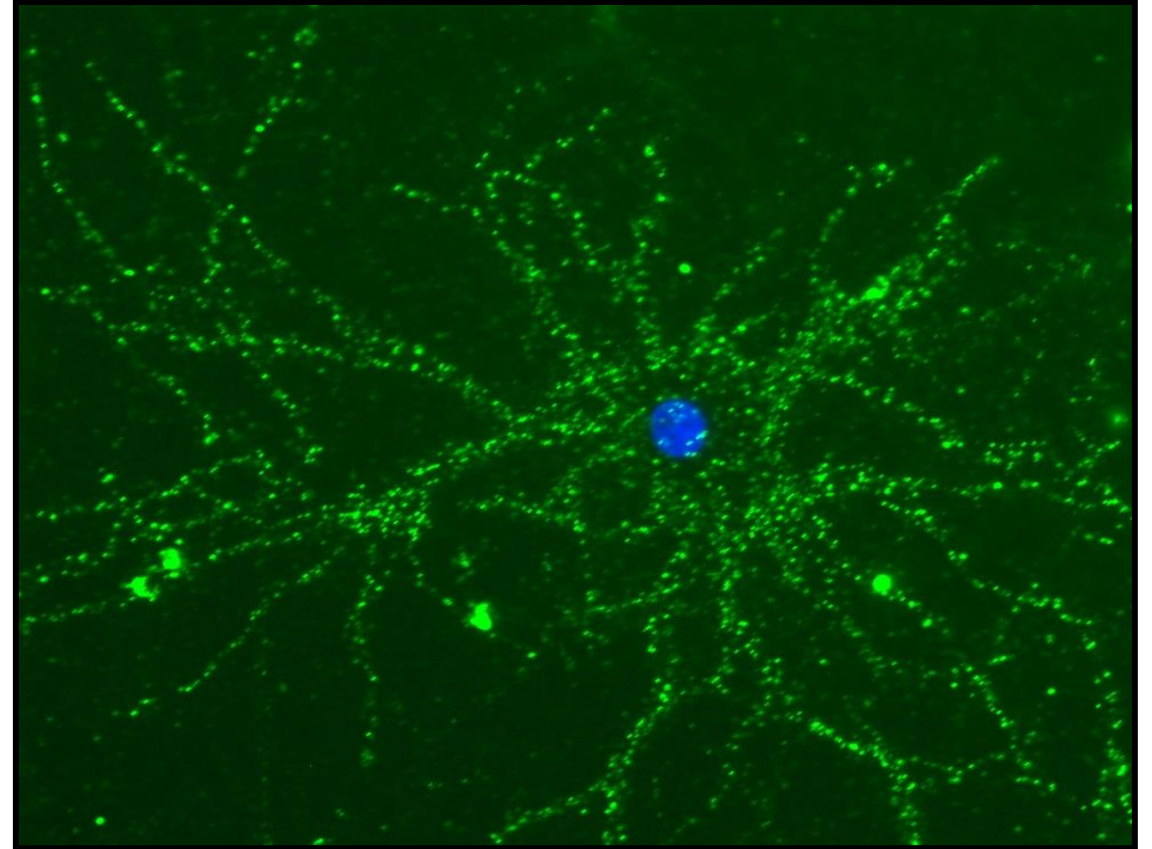
- 8/10 complete recovery
- 2 partial improvement (dysarthria and ataxia at 13 and 15 months of follow up)

# Opsoclonus and teratoma without NMDAR antibodies



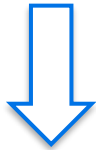
# Antibody findings

- None of the patients had known neuronal surface or intracellular antibodies.
- 3/22 patients (2 with opsoclonus) had light reactivity against unknown neuronal surface antigens



# Paraneoplastic opsoclonus

Children  
< 3 years



50%  
neuroblastoma

Adolescents and young adults



Idiopathic or post-infectious

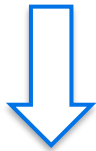
Adults  
≥ 45 years



Cancers lung, breast, ovary

# Paraneoplastic opsoclonus

Children  
< 3 years



50%  
neuroblastoma

Adolescents and young adults



**Teratoma**  
(highly responsive to treatment)

Adults  
≥ 45 years



Cancers lung, breast, ovary

Original Investigation

# Clinical and Immunological Features of Opsoclonus-Myoclonus Syndrome in the Era of Neuronal Cell Surface Antibodies

Thaïs Armangué, MD, PhD; Lidia Sabater, PhD; Estefanía Torres-Vega, BSc; Eugenia Martínez-Hernández, MD, PhD; Helena Ariño, MD; Mar Petit-Pedrol, BSc; Jesús Planagumà, PhD; Luis Bataller, MD, PhD; Josep Dalmau, MD, PhD; Francesc Graus, MD, PhD

- 114 adult patients with OMS
  - Analysis of clinical risk factors for paraneoplastic OMS
  - Analysis of serum/CSF for neuronal surface / intracellular Ab
  - Additional laboratory techniques (cerebellar cell cultures in addition to hippocampal cell cultures, IgM/IgA)

# Clinical risk factors for paraneoplastic OMS in adults

Table. Clinical Features of Patients With P-OMS and I-OMS

Characteristic	No. (%)		P Value
	P-OMS (n = 45)	I-OMS (n = 69)	
Age, median (IQR), y	54 (45-65) <sup>a</sup>	38 (31-50)	<.001
Female	24 (53)	38 (55)	.86
Prodromal and/or infection	6 (13)	23 (33)	.02
Autoimmune background	3 (7)	11 (16)	.14
Presenting symptoms			
Acute vertigo	22 (49)	47 (68)	.04
Subacute ataxia or myoclonus	11 (24)	8 (12)	.07
Other symptoms <sup>b</sup>	12 (27)	14 (20)	.43
Additional symptoms at any time			
Encephalopathy	13 (29)	7 (10)	.01
Cranial nerve palsy	7 (16)	4 (5)	.02
Severe behavioral changes	6 (13)	4 (6)	.16
CSF studies (n = 90)	(n = 34)	(n = 56)	
Abnormal	24 (71)	42 (75)	.65
WBC count >5/ $\mu$ L	16 (47)	34 (61)	.21
Protein >45 mg/dL	14 (41)	18 (32)	.38
Follow-up, median (IQR), mo (n = 81)	(n = 38)	(n = 43)	
Duration, median (IQR), mo	14 (6-27)	20 (8-45)	.36
Poor outcome, mRS score >2 <sup>c</sup>	23 (61)	7 (16)	<.001
OMS relapse	9 (24)	3 (7)	.04

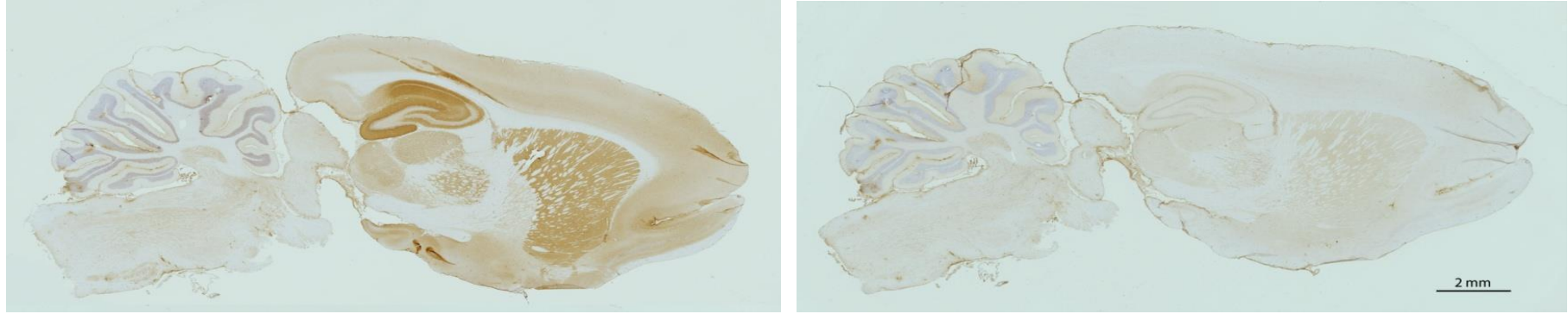
# Characterization of neuronal surface antibodies in a large Cohort of OMS

Screening

Immunochemistry

Rat brain tissue:

- Perfused tissue (intracellular)
- Non perfused (surface)
- IgG, IgA, IgM



Confirmation by CBA

and westernblot

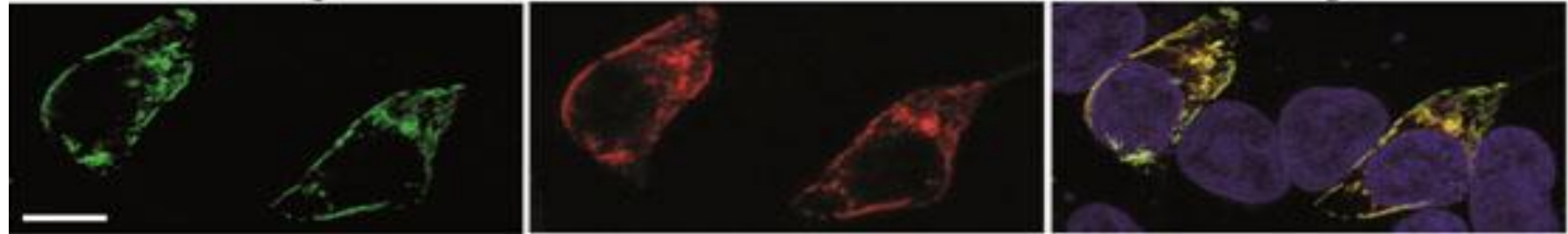
additional Ab in all:

GlyR, MOG

Human IgG

Commercial NR1

Merge

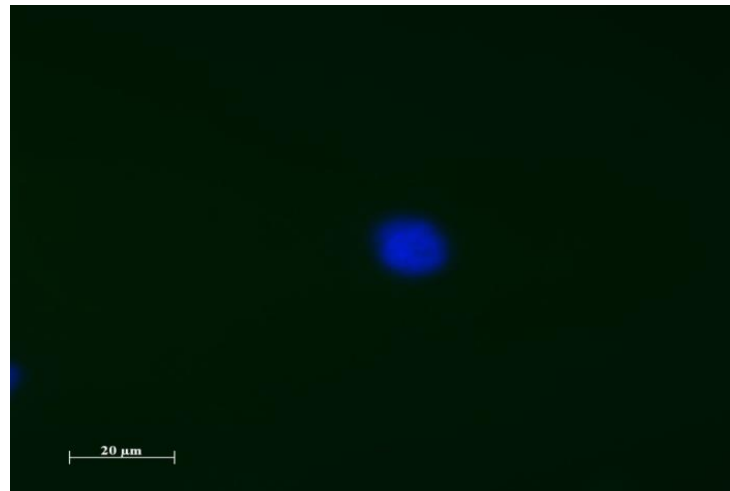
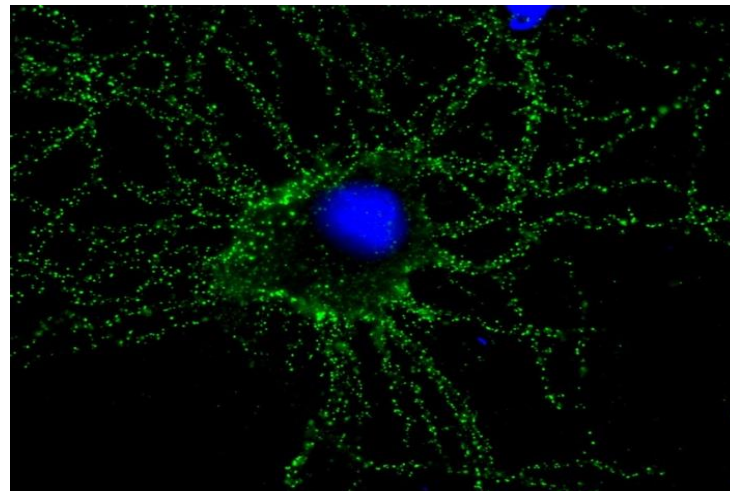


Hippocampal

neurons

Granular cerebellar

neurons

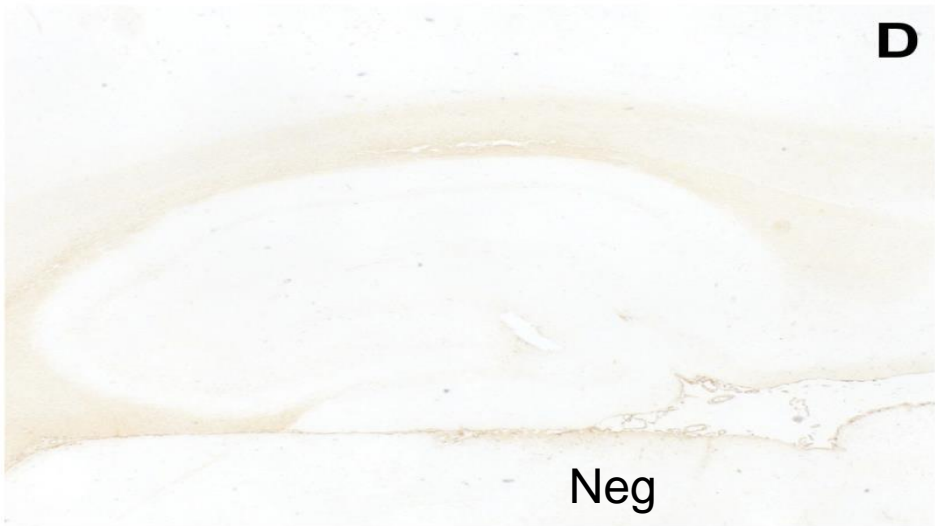
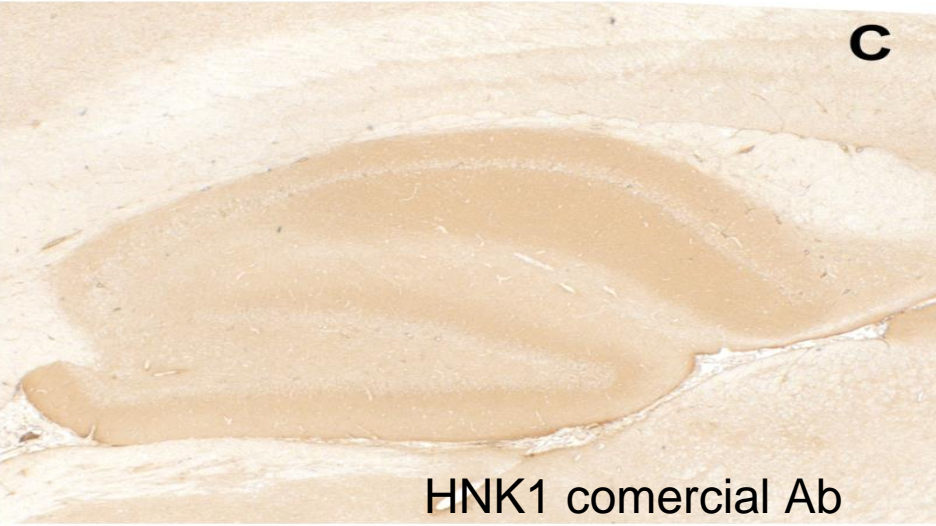
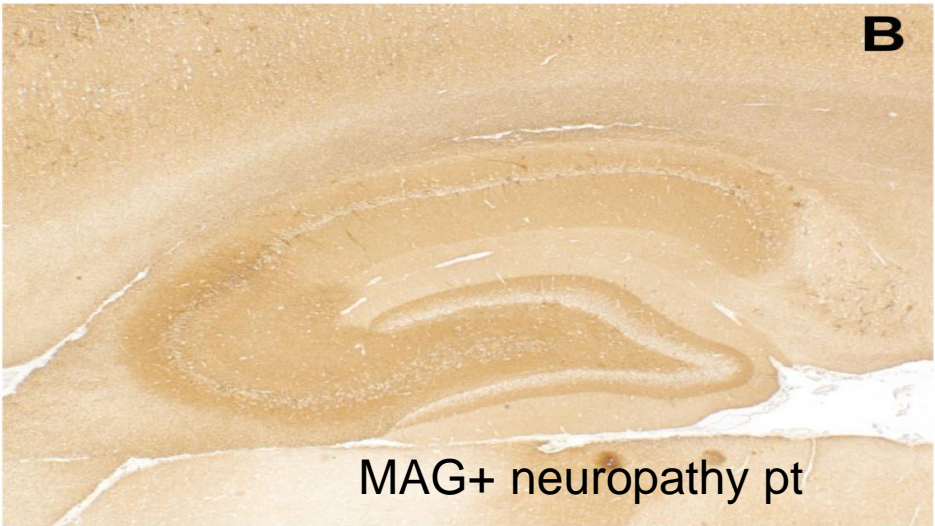


Immunoprecipitation

# New Neuronal cell surface and intracellular antibodies in 114 adult OMS

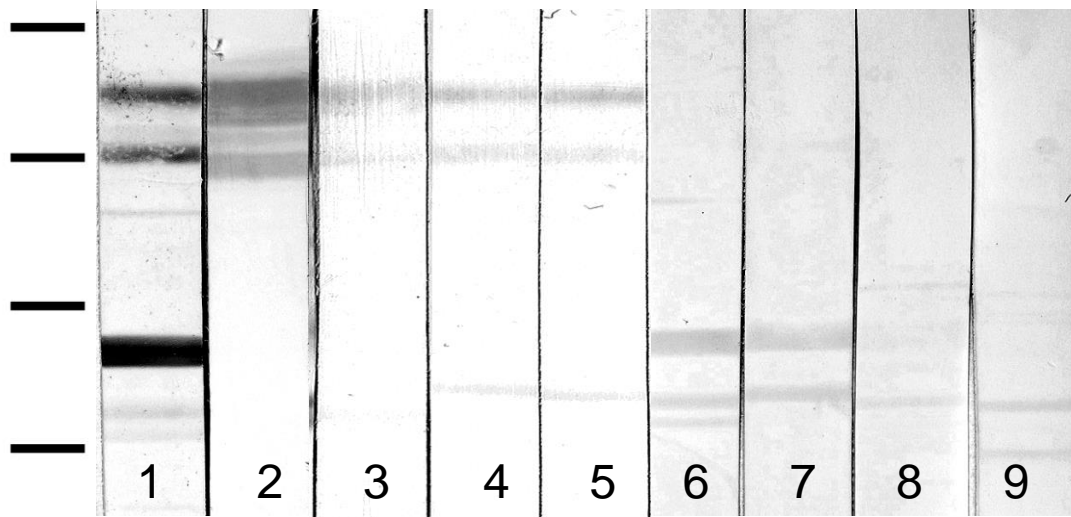
- 13 (11%) had onconeurological/intracellular antibodies
  - 7/10 patients with breast cancer had Ri/ANNA2
  - Other: Hu (lung cancer)
- 12 (11%) had neuronal surface antibodies
  - 3 unknown antigens
  - 9 GlyR Ab → predominated in lung cancer (21% vs 5%)
  - We found similar frequency GlyR Ab (20%) in lung cancer without OMS
- IgM studies → identified a new epitope → HNK1, in 3 patients with lung cancer (2 with concomitant GAD65 antibodies)

OPSOCLONUS AND IgM ISOTYPE



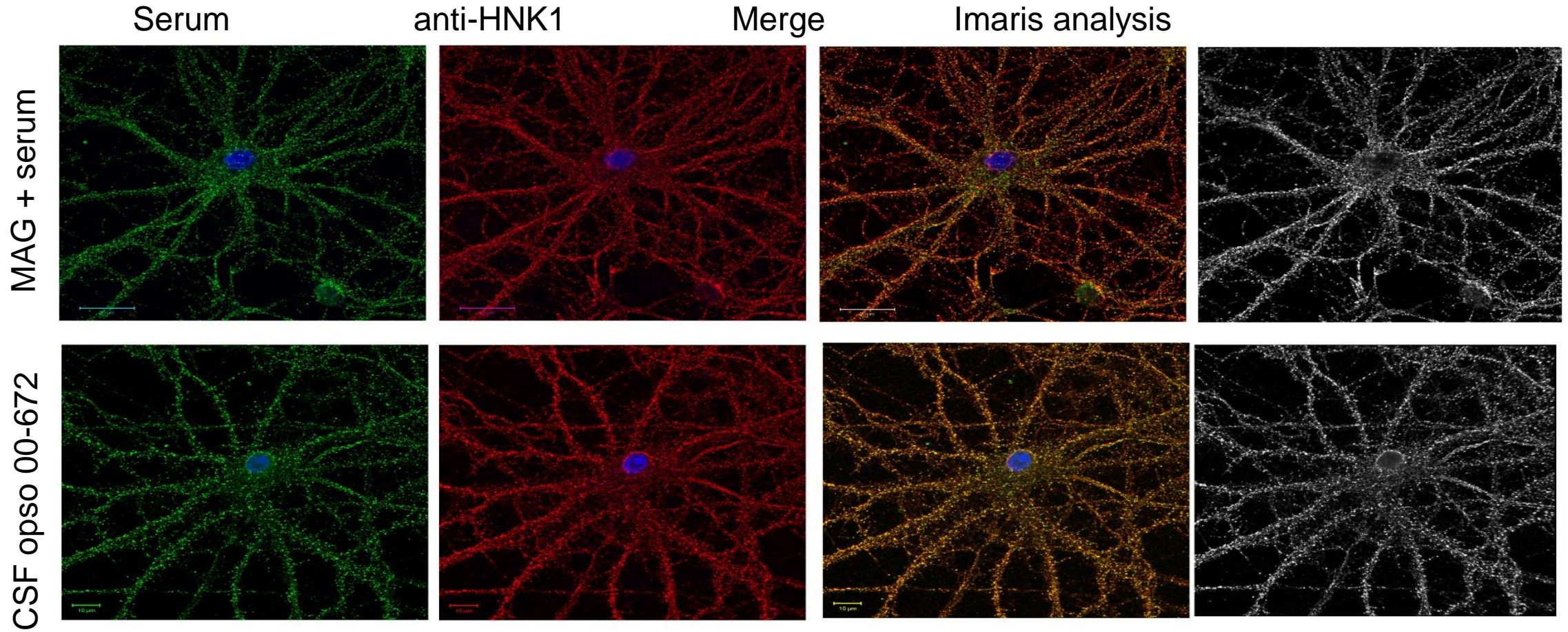
## OPSOCLONUS AND IgM ISOTYPE

WB of human myelin extract



1. MAG IgM C+
2. HNK1 ab
3. Opso csf 11-2184
4. Opso csf 02-835
5. Opso csf 00-672
6. Opso serum 11-2183
7. Opso serum 02-834
8. Opso serum 00-671
9. NHS control

## COLOCALIZATION OF THE REACTIVITY WITH HNK1 IN HC NEURONS



IgM isotype

Armangue et al. JAMA Neurol 2016

# Antibodies in 45 Children with OMS with or without neuroblastoma

- 3 had Hu antibodies (all neuroblastoma)
- 3 reactivity in live neurons suggestive of antibodies against unknown antigens
- None had GlyR or IgM HNK1 antibodies

ARTICLE

OPEN ACCESS

# Glutamate receptor $\delta 2$ serum antibodies in pediatric opsoclonus myoclonus ataxia syndrome

Georgina Berridge, BSc (Hons), David A. Menassa, DPhil, Teresa Moloney, PhD, Patrick J. Waters, PhD, Imogen Welding, BMBCh, Selina Thomsen, Sameer Zuberi, MD, Roman Fischer, PhD, A. Radu Aricescu, PhD, Michael Pike, FRCPCH, Russell C. Dale, PhD, Benedikt Kessler, PhD, Angela Vincent, FRCPATH, Ming Lim, PhD,\* Sarosh R. Irani, MRCP, DPhil,\* and Bethan Lang, PhD\*

## Correspondence

Prof. Lang  
bethan.lang@ndcn.ox.ac.uk

*Neurology*<sup>®</sup> 2018;0:e1-e10. doi:10.1212/WNL.0000000000006035

# Absence of GluD2 Antibodies in Patients With Opsoclonus-Myoclonus Syndrome

Mar Petit-Pedrol, PhD, Mar Guasp, MD, Thais Armangue, MD, PhD, Cinzia Lavarino, PhD, Andres Morales La Madrid, MD, PhD, Albert Saiz, MD, PhD, Francesc Graus, MD, PhD, and Josep Dalmau, MD, PhD

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*Neurology*® 2021;96:e1082-e1087. doi:10.1212/WNL.00000000000011410

**Table 1** General Features of Adult and Pediatric Patients With Opsoclonus-Myoclonus Syndrome

	Pediatric patients	Adult patients
<b>Number of patients</b>	45	158
<b>Median age, y (IQR)</b>	2 (1–6)	43 (31–59)
<b>Female (%)</b>	21/45 (47)	86/158 (54)
<b>Tumor associated (%)</b>	10/45 (22) <sup>a</sup>	53/158 (34) <sup>b</sup>
<b>No tumor (%)</b>	35/45 (78)	105/158 (66)
<b>Cell surface antibodies</b>	3 unknown	15 (8 GlyR, 2 NMDAR, 1 GABA <sub>B</sub> R, 1 AMPAR, 3 neuronal surface unknown) <sup>d</sup>
<b>Intracellular antibodies</b>	3 Hu	16 (8 Ri, 3 Ma2, 2Hu, 1CRMP5, 1Zic, 1GAD65)
<b>GluD2 pattern of brain immunostaining<sup>c</sup></b>	0/45	0/158
<b>GluD2 CBA-specific reactivity using 2 plasmids and 2 immunofluorescence methods</b>	0/45	0/158

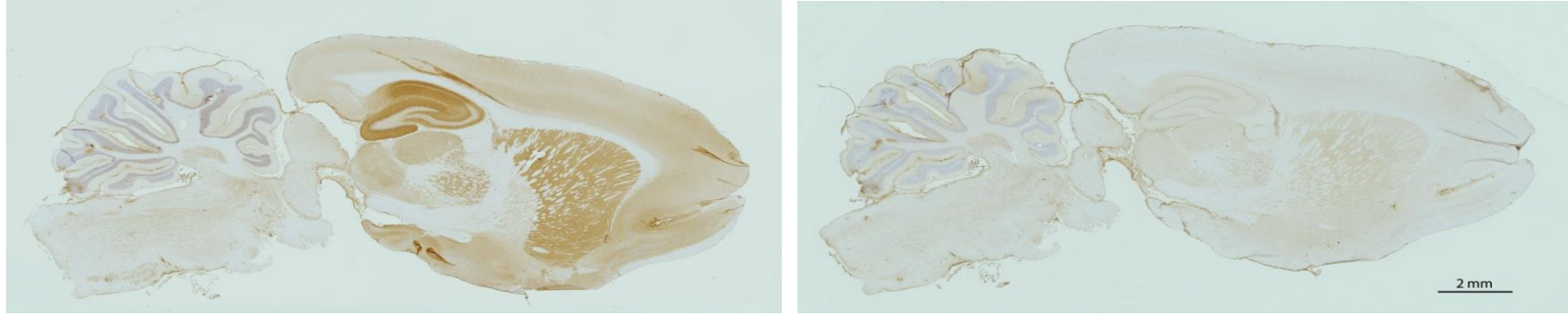
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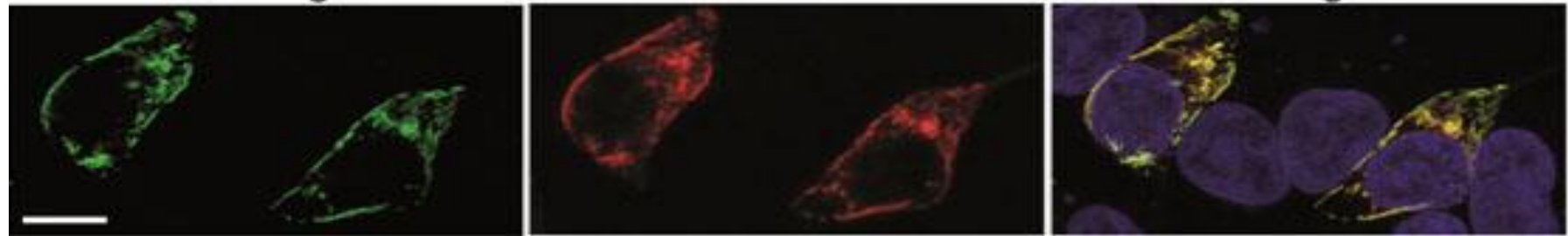
and westernblot

additional Ab in all:  
GlyR, MOG

Human IgG

Commercial NR1

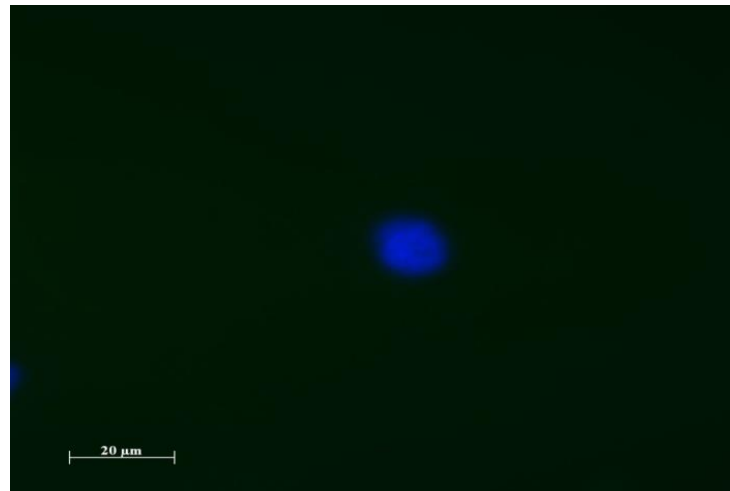
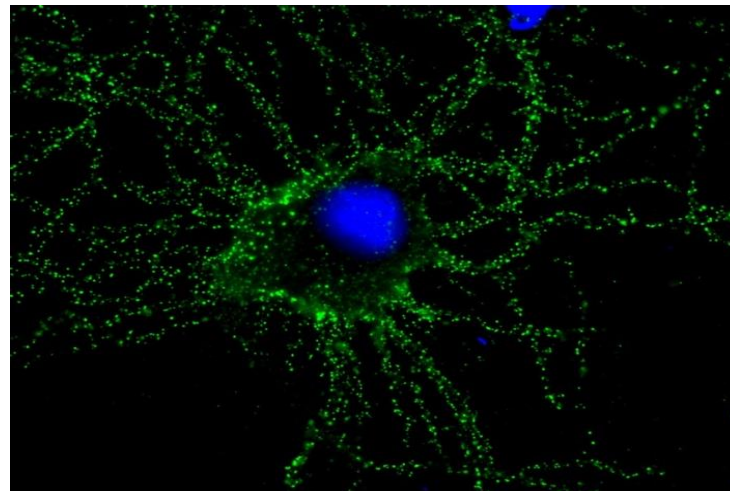
Merge



Unknown antigens:

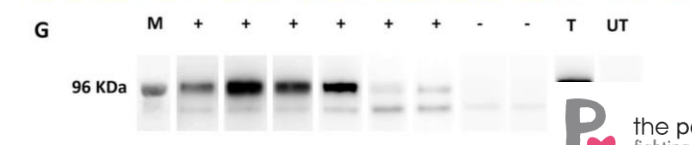
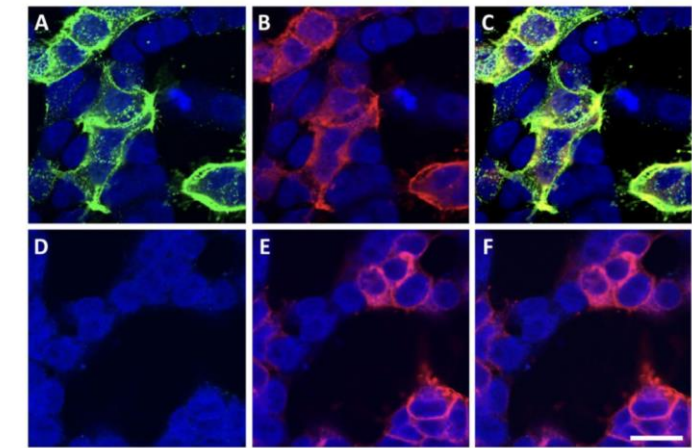
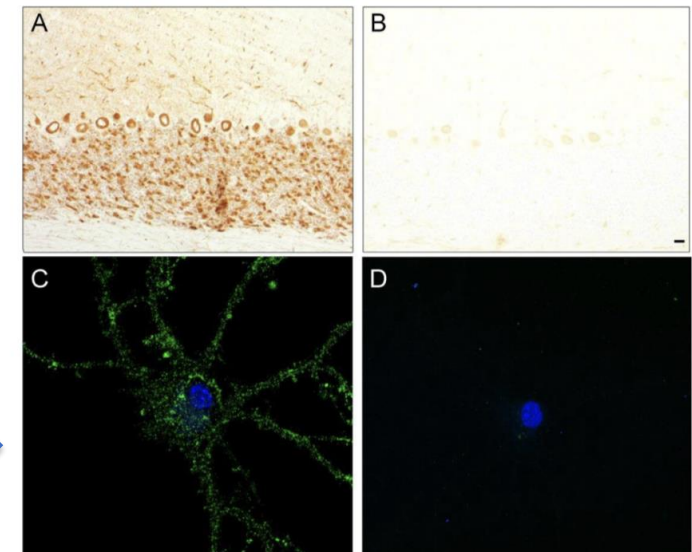
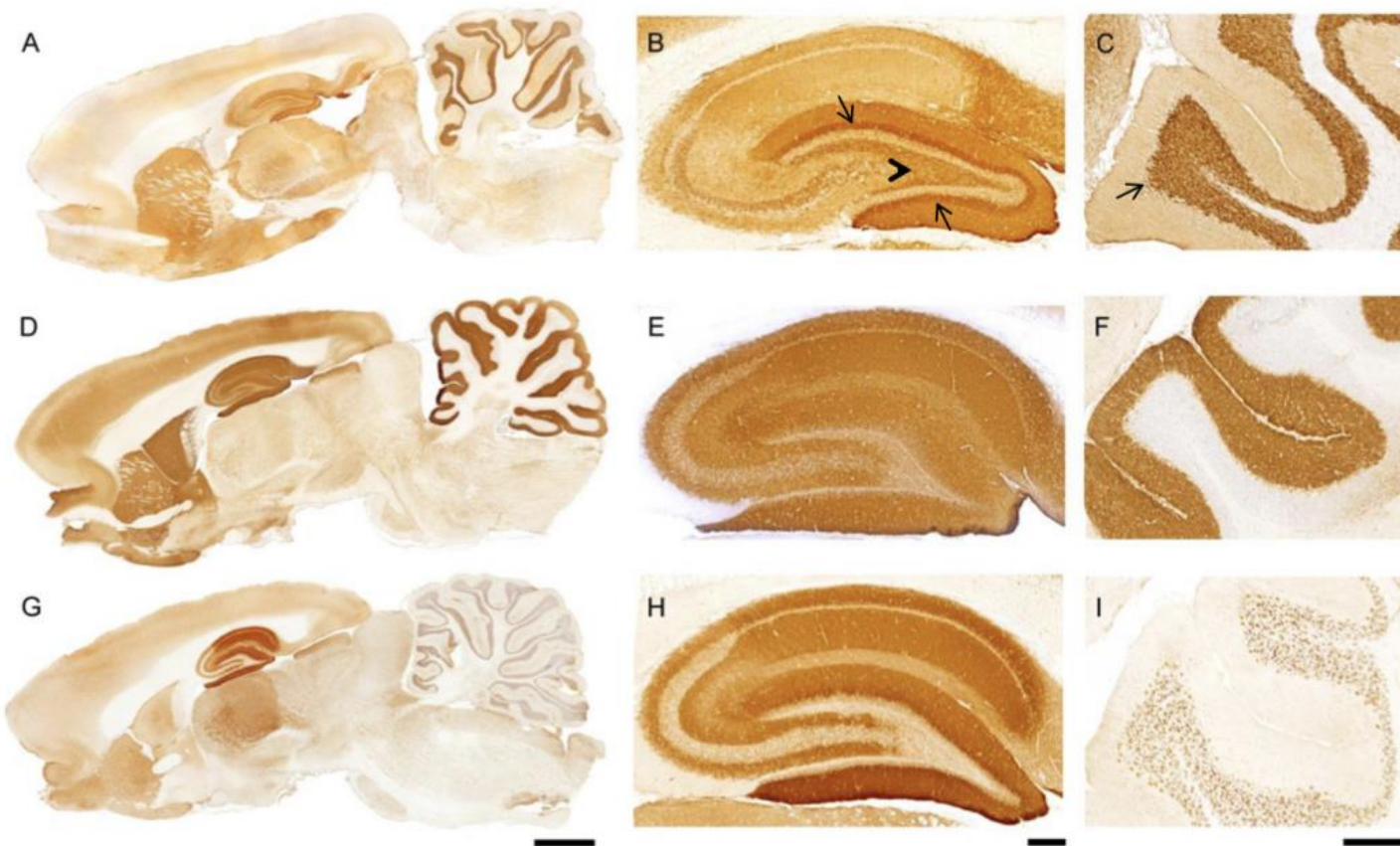
Hippocampal neurons

Granular cerebellar neurons



Immunoprecipitation

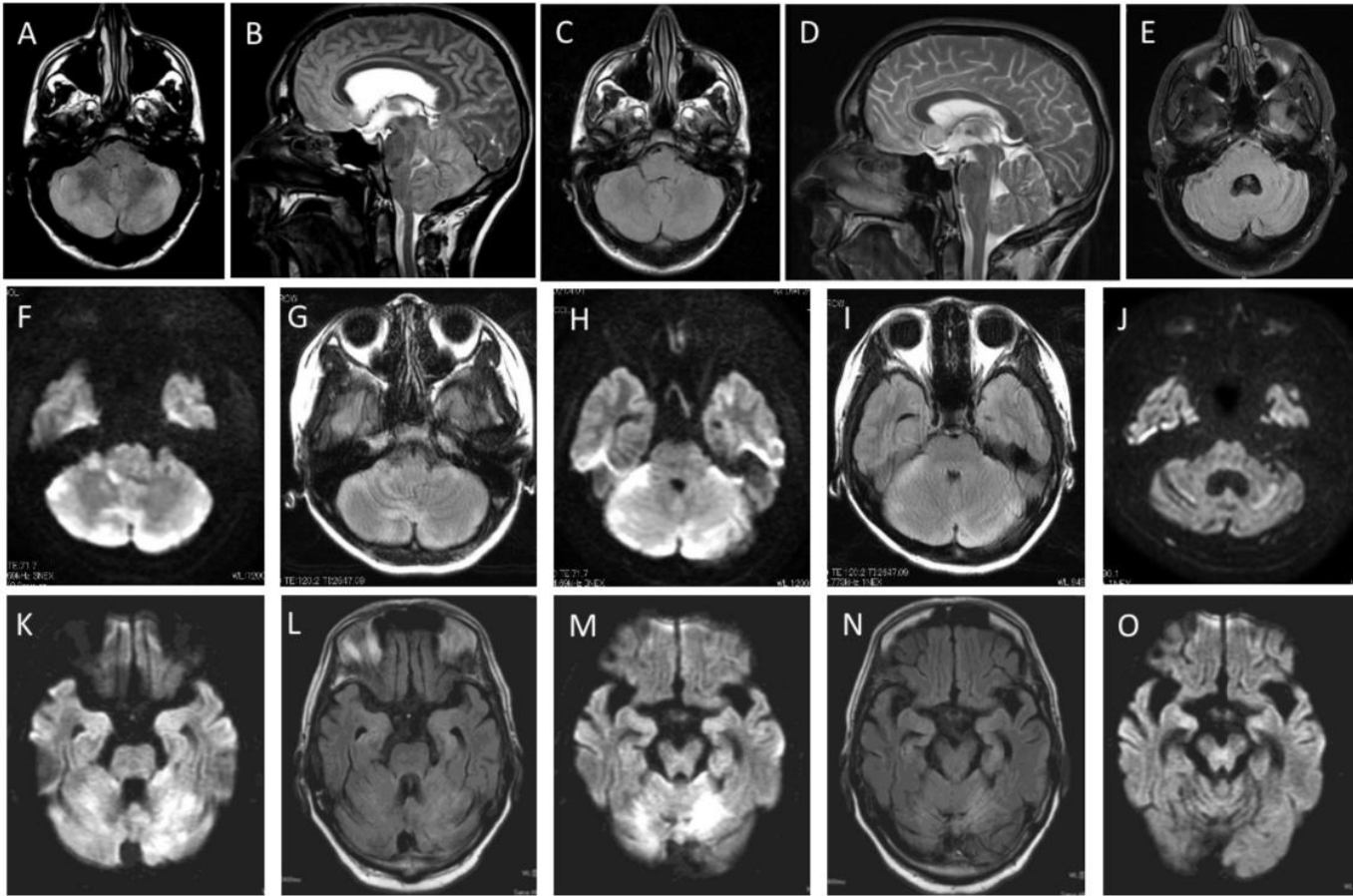
# GluK2 (Glutamate kainate receptor subunit 2): a new neuronal surface antibody in OMS and cerebellar involvement



Sera and CSF from 2 patients with similar brain immunostaining were used to precipitate the antigen from cultures of rat cerebellar neurons

Landa et al. Ann Neurol 2021;90:101-117

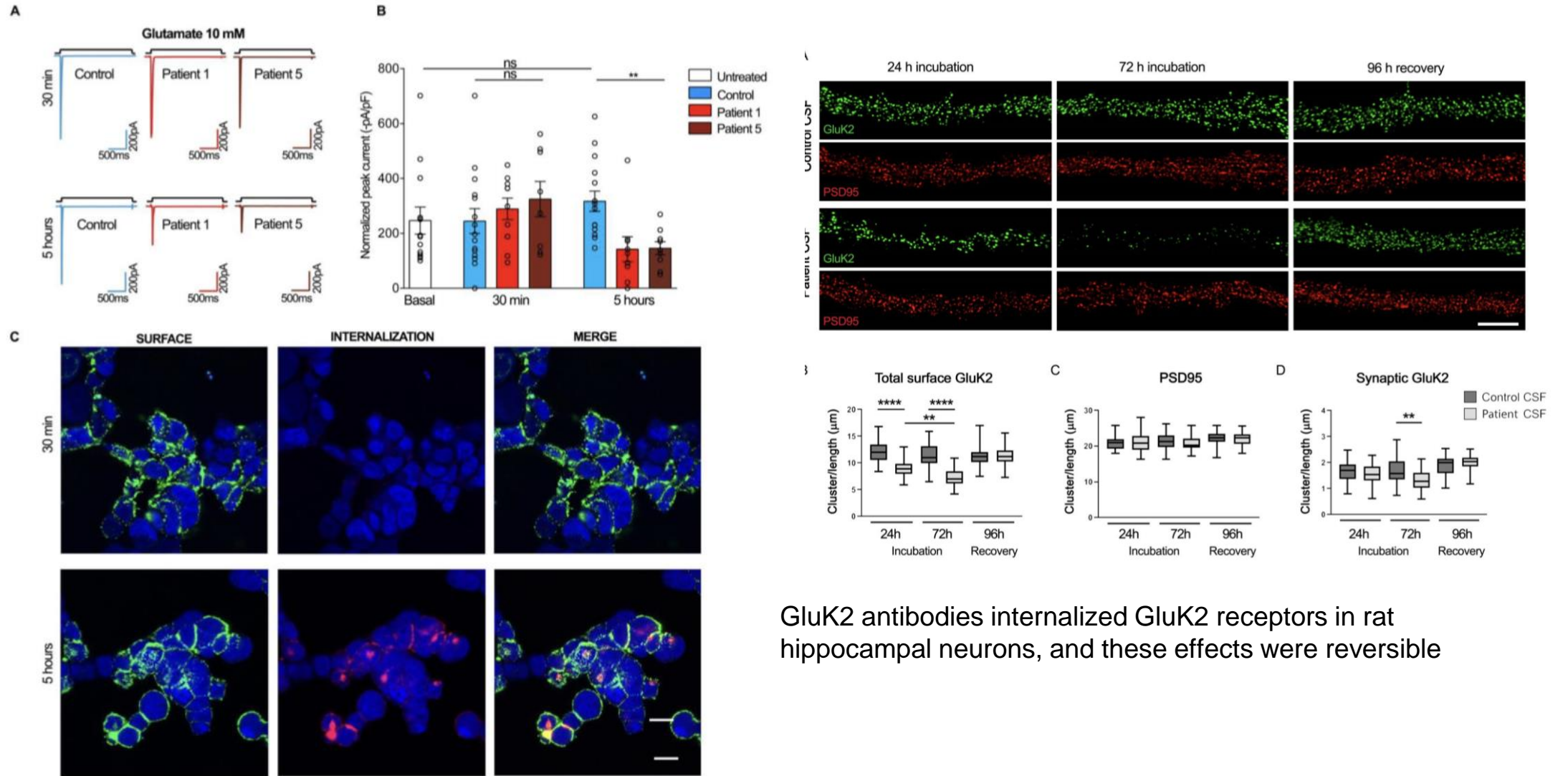
## GluK2-Ab can be found in OMS but also in cerebellitis



CBA testing >600 patients:

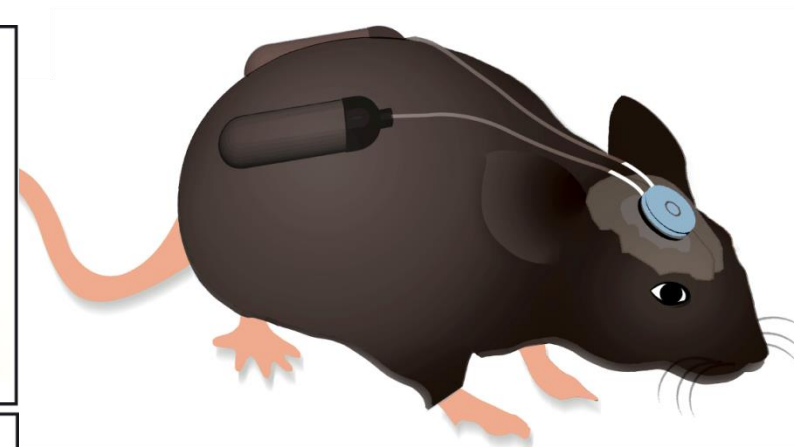
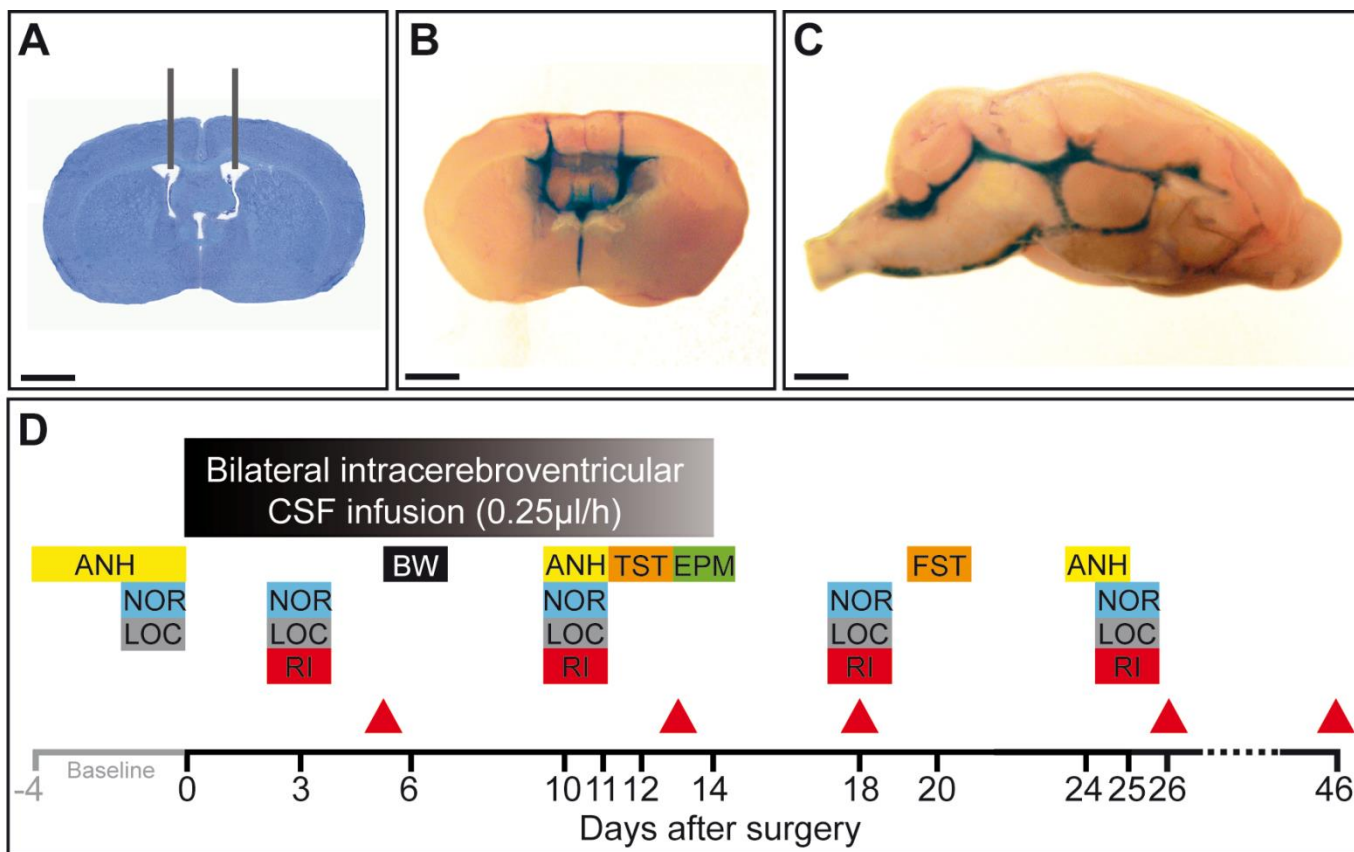
- 8 patients with GluK2-Ab only
  - 7 predominant cerebellar  
(1 OMS, 6 cerebellitis -> 2 edema compres IV v)
- 6 patients with GluK2-and concurrent Ab
  - 5 AMPAR, 1 NMDAR

# GluK2-Ab are pathogenic



GluK2 antibodies internalized GluK2 receptors in rat hippocampal neurons, and these effects were reversible

# An animal model for OMS: learning from antibody mediated encephalitis



Planagumà,  
Leyboldt, Mannara  
et al., Brain 2014

**Group 0 = Control Group receiving 14 d intraventricular infusion with undiluted CSF of control individuals**

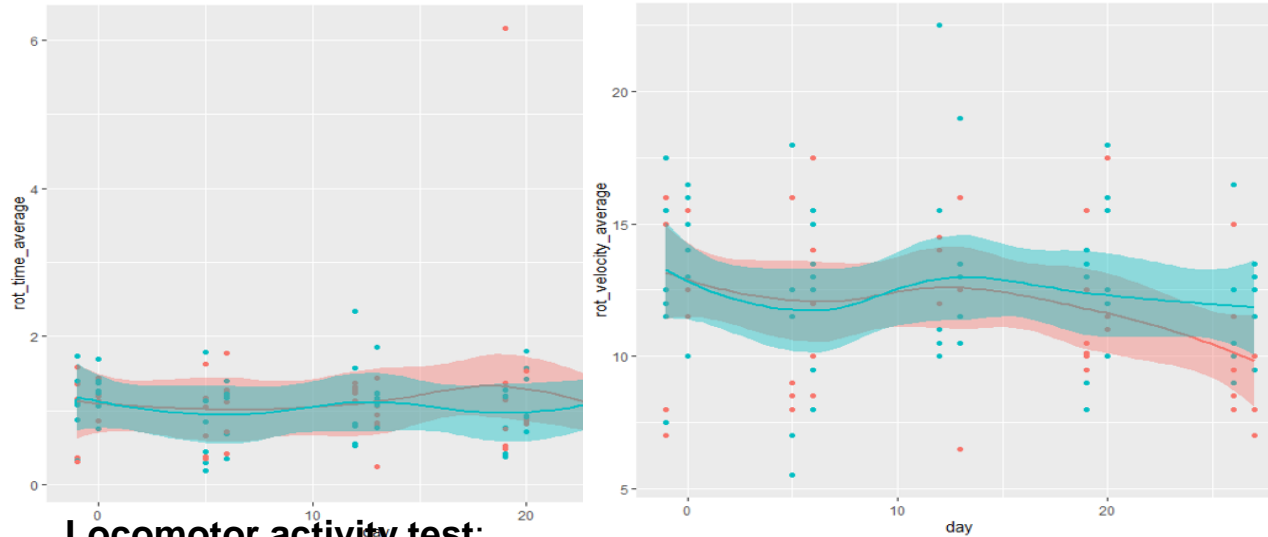
**Groups 1/2/3 = Treated groups receiving 14 d intraventricular infusion with purified IgG from serum of patients with OMS (1-children neuroblastoma-OMS, 2- idiopathic-OMS, 3-adult cancer OMS)**

Armangué et al.  
Work in progress

# Animal testing in OMS



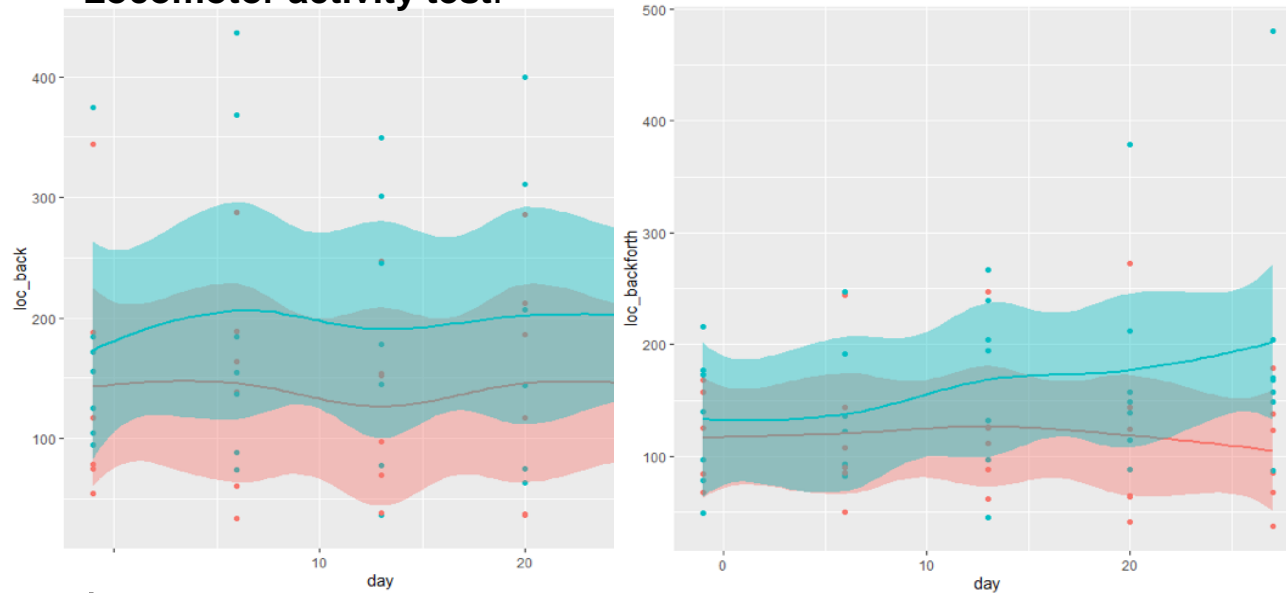
### Accelerating-Rotarod test:



**Group 0 (n=6) = Control Group receiving 14 d intraventricular infusion with undiluted CSF of control individuals**  
**Group 1 (n=7) = Treated group receiving 14 d intraventricular infusion with purified IgG from serum of children neuroblastoma-OMS**

(rot\_time ; **p=0,2414** rot\_velocity; **p=0,1247**)

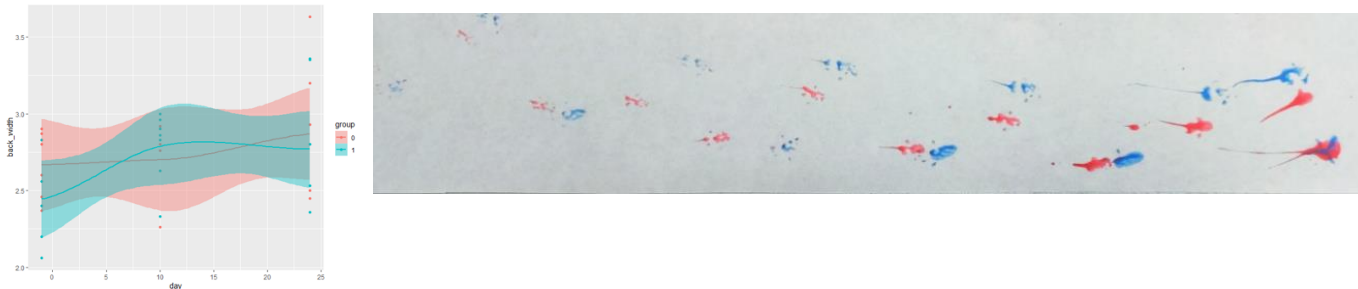
### Locomotor activity test:



(loc\_back **p=0,5818**; loc\_front **p= 0,5323**; loc\_backforth; **p=0,0585**  
 loc\_rearings; **p=0,6449**)

Armangué et al. Work in progress

### Gait Analysis (footprint) test:



(left\_front\_stride  $p=0,5704$  ; right\_front\_stride  $p=0,2644$ ; left\_back\_stride  $p=0,2317$  ; right\_back\_stride  $p=0,3516$  ; front\_width;  $p=0,0522$   
 back\_width;  $p=0,6599$ )

**Group 0 (n=6) = Control Group receiving 14 d intraventricular infusion with undiluted CSF of control individuals**

**Group 1 (n=7) = Treated group receiving 14 d intraventricular infusion with purified IgG from serum of children neuroblastoma-OMS**

### Beam balance test:



(beam\_time  $p=0,2513$  ; beam\_slips  $p=0,1351$ )

Armangué et al. Work in progress

# Conclusions and reflexions

- Young patients with teratoma can develop several forms of encephalitis without NMDAR antibodies among which a brainstem-cerebellar syndrome predominates (including OMS highly responsive to immunotherapy/tumor removal)
- Strongly current evidence supports OMS as an antibody-mediated disease (B cell expansion, good response to rituximab)
- Some OMS patients, mainly paraneoplastic, have autoantibodies.
- Comprehensive studies in a large OMS cohort of pediatric and adult OMS found new neuronal surface antibodies (GlyR, GAD65, IgM HNK1, and GluK2)
- Although we don't find a common neuronal surface antibody, previous experience in "seronegative limbic encephalitis" is the example that we have to continue looking for it! And we keep looking!

# Thanks!



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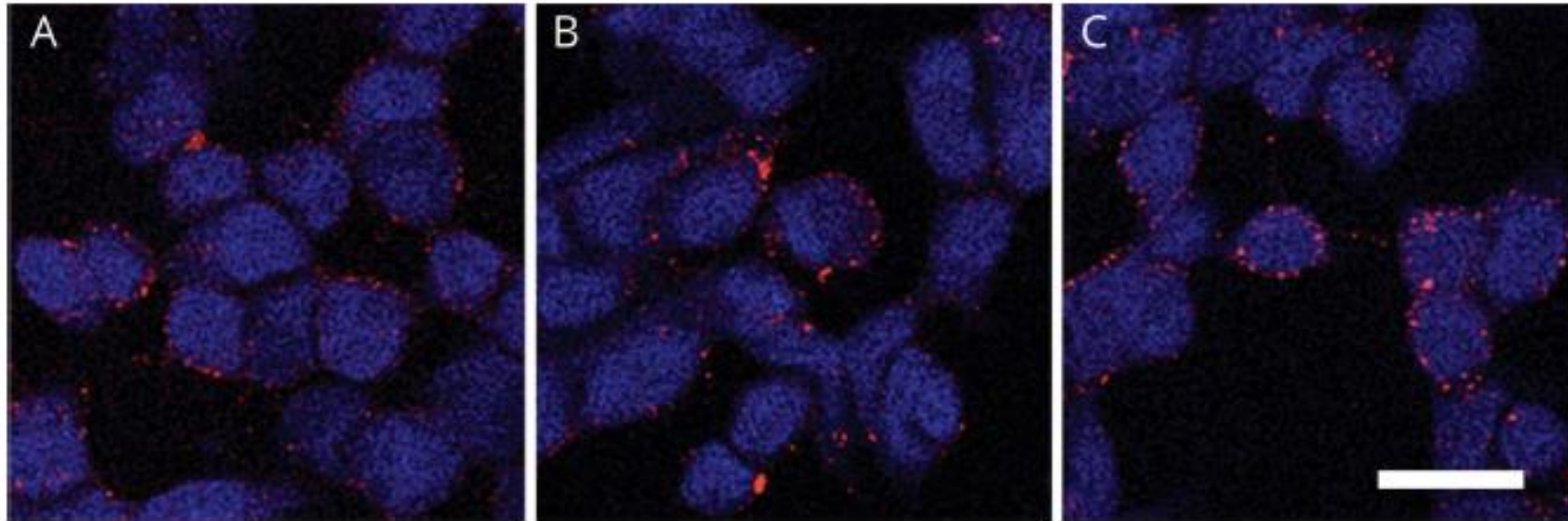
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## Figure 2 Immunoabsorption of Serum of a Patient With Equivocal Reactivity



(A) Equivocal glutamate receptor delta 2 (GluD2) reactivity (using the 3-step technique) of serum from a patient with opsoclonus-myoclonus syndrome. This reactivity is not altered by preabsorption of the serum with HEK293T cells expressing GluD2 (B) or nonexpressing GluD2 (C), indicating that the reactivity is not GluD2-specific. Scale bar = 20  $\mu\text{m}$ .

## Treatment

- 15/19 (79%) immunotherapy and tumor removal (13)
- 2 tumor removal without immunotherapy
- 2 were not treated

## Outcome (median follow-up 15 months; 3-84)

- 14/19 (74%) full recovery
- 3/19 (16%) partial improvement
- 2 no improvement