

Anti-DPPX encephalitis

Pathogenic effects of antibodies on gut and brain neurons

J. Piepgras*, **M. Höltje***, K. Michel, Q. Li, C. Otto, C. Drenckhahn, C. Probst, M. Schemann, S. Jarius, W. Stöcker, B. Balint, H.M. Meinck, R. Buchert, **J. Dalmau**, G. Ahnert-Hilger, **K. Ruprecht**⁺. *equal contribution; ⁺correspondent autor.

From the Department of Neurology (J.P., K.R.), Institute for Integrative Neuroanatomy (J.P., M.H., C.D., G.A.-H.), and Department of Nuclear Medicine (R.B.), **Charité–Universitätsmedizin Berlin**; and the Catalan Institution of Research and Advanced Studies (ICREA) and Biomedical Research Institute August Pi i Sunyer (IDIBAPS) (J.D.), Hospital Clinic, **University of Barcelona, Spain**.

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Introduction - Autoimmune encephalitis

- Symptoms can resemble infectious encephalitis
- Neurological and psychiatric symptoms
- Association to auto-antibodies against neuronal and synaptic proteins (NMDA Rec., AMPA Rec., GABA Rec.)
- Association means antibody-mediated or antibody as a biomarker (epiphenomenon)
- Responsive to immunotherapy
- Diagnosis by: clinical presentation, antibody testing, response to immunotherapy

Lancet Neurol 2016; 15: 391-404.

Ann N Y Acad Sci. 2015 March ; 1338(1): 94-114

DPPX encephalitis

- 2013 first published (Josep Dalmau's group)
- 28 patients reported (incl. this publication)
- Clinical:
 - Agitation, hallucination, confusion, myoclonus, tremor, seizures
 - Gastrointestinal symptoms (diarrhea,...)
- Associated with antibodies against DPPX
- immunotherapy-responsive syndrome

Neurology 2014;83:1797–1803.

DPPX protein

- dipeptidyl-peptidase-like protein 6 (DPPX or DPP6)
- Membrane glycoprotein, subunit of Kv4.2 potassium channel
- Function:
 - Increases surface expression (membrane localization) and channel conduction of Kv4.2 channels
 - Kv4.2 channel: A-type K⁺ current regulates electrical excitability -> dampens action potential firing -> influences synaptic integration and plasticity.

Neuron 2003;37:449-461

Ann Neurol 2013; 73:120-128



tissue expression of DPPX protein

- Expression in brain: hippocampus, cerebellum, striatum
- Expression in gastrointestinal tract: myenteric plexus

Ann Neurol 2013; 73:120-128

Aim of the study

- What are the pathogenic effects of anti-DPPX antibodies on gut and brain neurons (in vitro)?

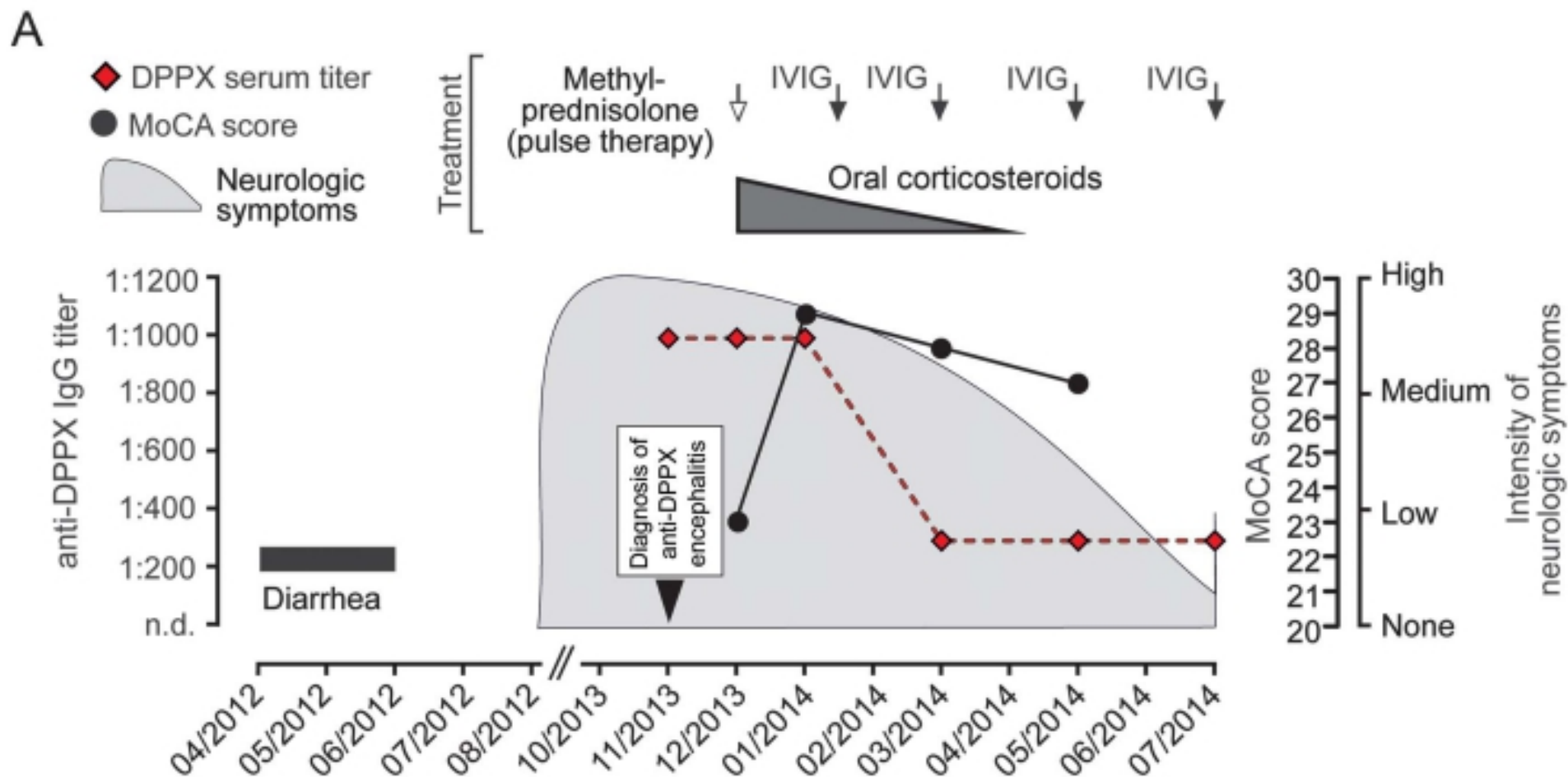
Methods

- case report of newly identified patient, 3 more patient sera
- Serology: Cell-based assay positive for anti-DPPX antibodies
- Serum IgG purification: Protein G HP columns
- Immunofluorescence: tissue: biomosaic chip (rat cerebellum, monkey colon), mouse hippocampus or ileum, guinea pig ileum, mouse primary hippocampal neurons (cell culture)
- electrophysiology / neuroimaging: neuronal activity of guinea pig myenteric or human submucous plexus prep.
- cell culture: mouse primary hippocampal neuronal cell culture
- Immunoblot of neuronal membrane fraction preparations

Methods

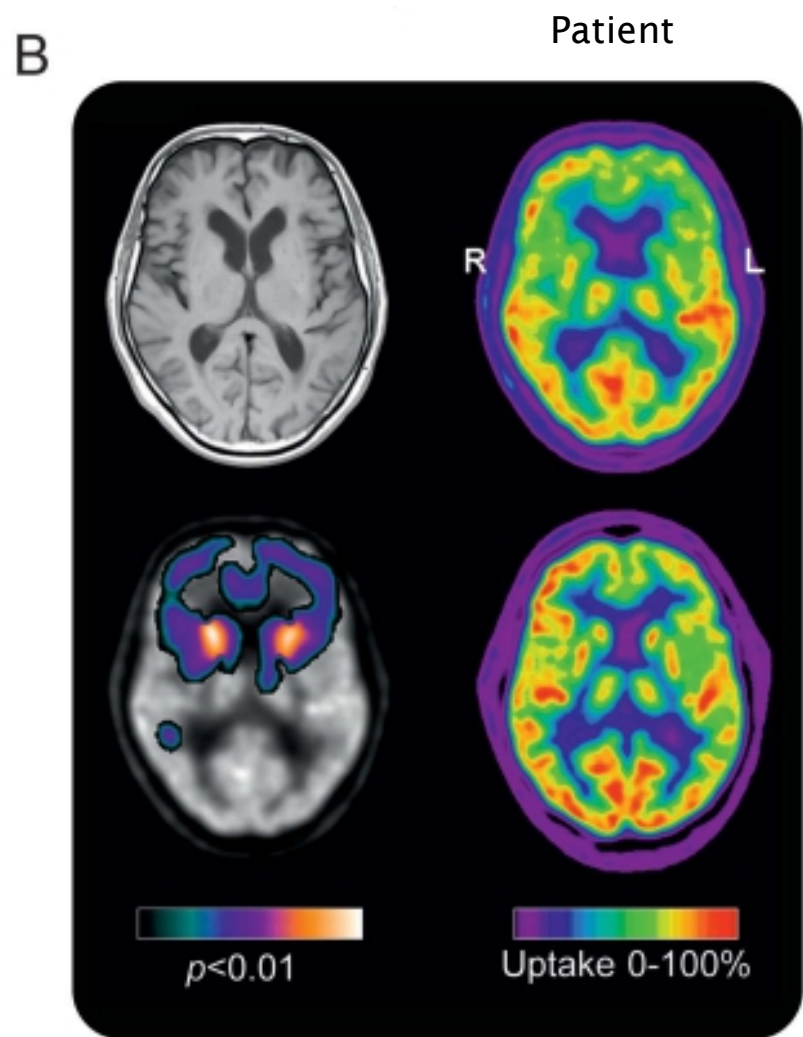
Case report of a new DPPX encephalitis patient:

Figure 1 Clinical and paraclinical findings of a novel patient with anti-dipeptidyl-peptidase-like protein 6 encephalitis



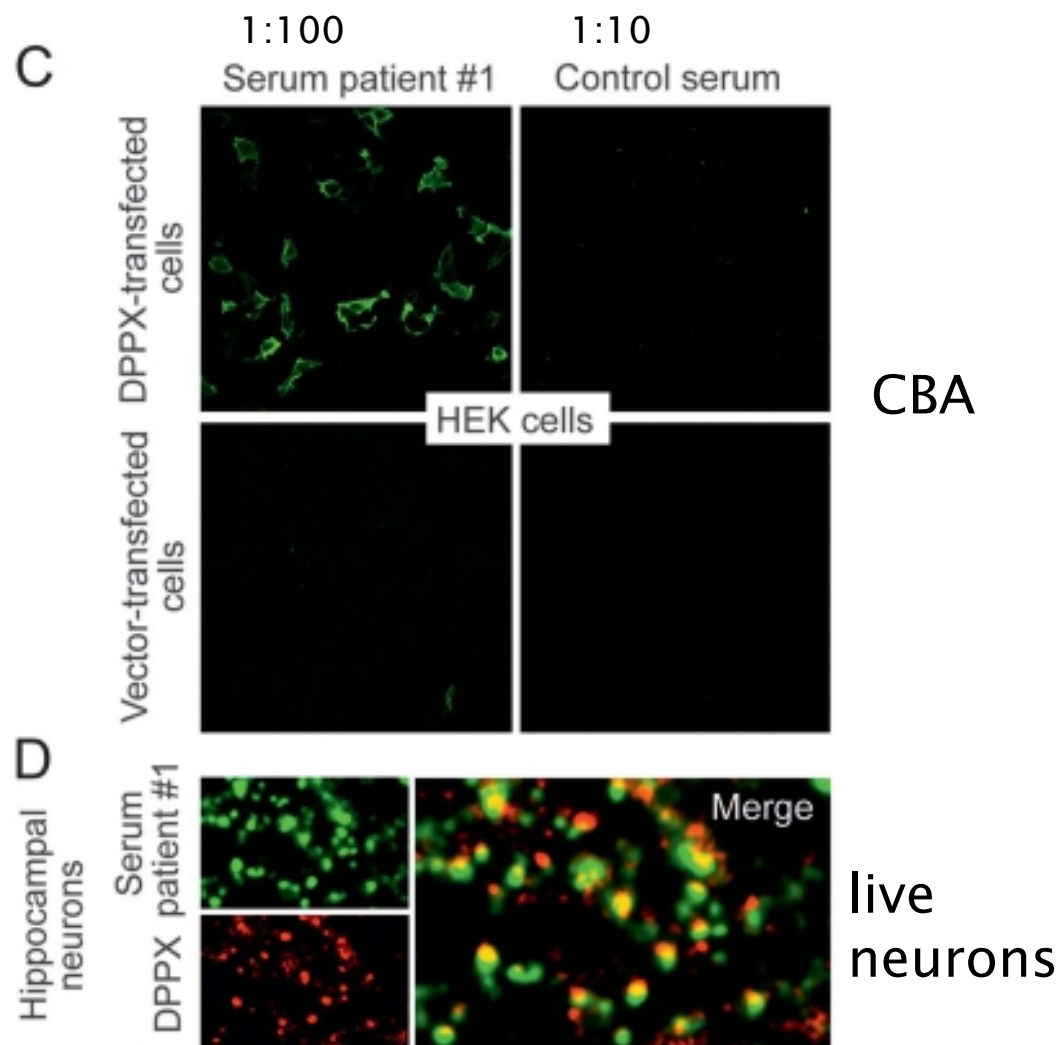
MoCA – Montreal Cognitive Assessment

MRI, FDG-PET (synaptic activity, reduced caudate nuclei+frontal)



Voxel-based testing vs healthy control group
Brighter = lower activity

Control subject



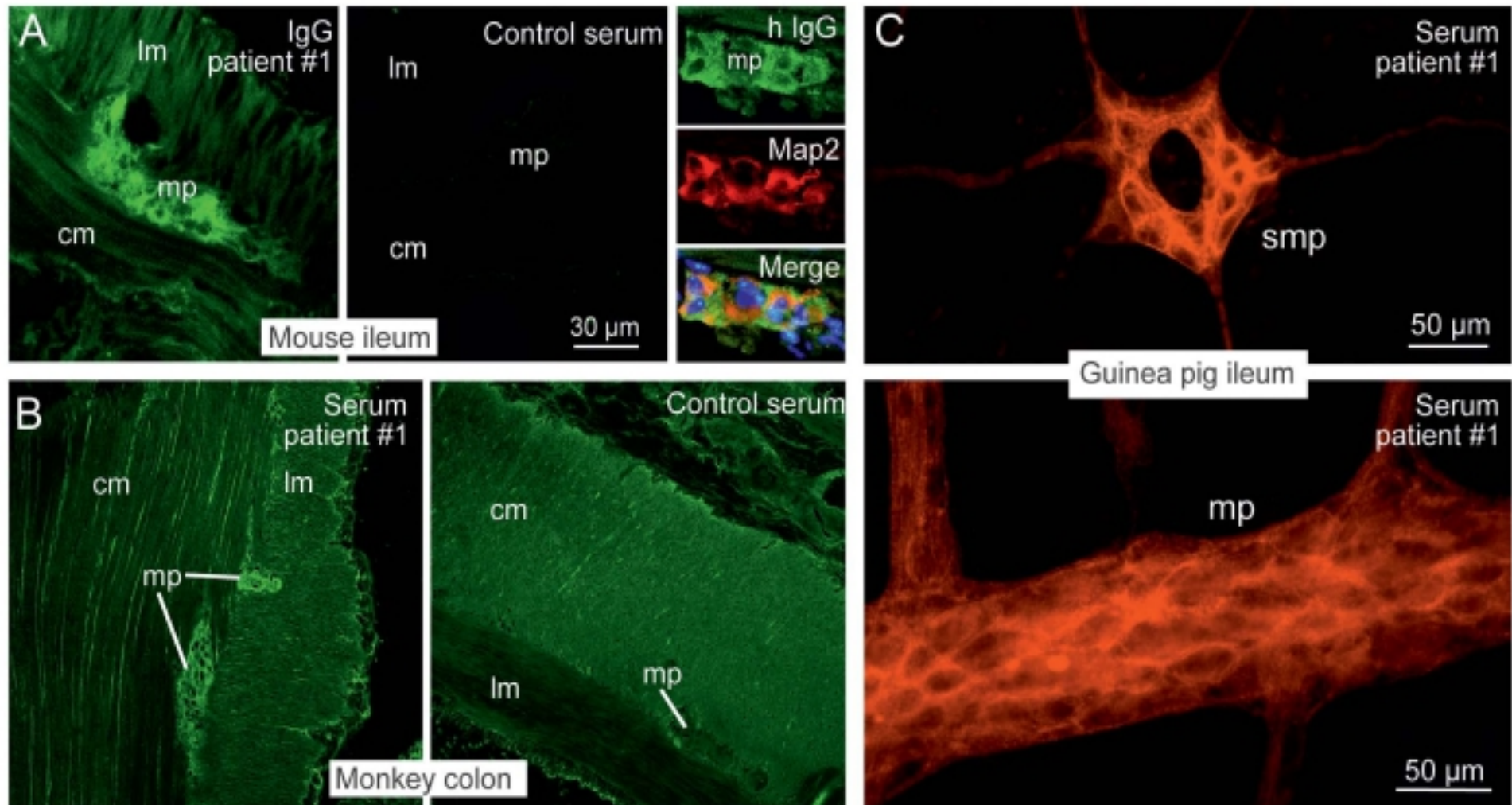
Results

Anti-DPPX antibody containing sera cause hyperexcitability of enteric nervous system neurons

- Via indirect immunofluorescence and neuroimaging

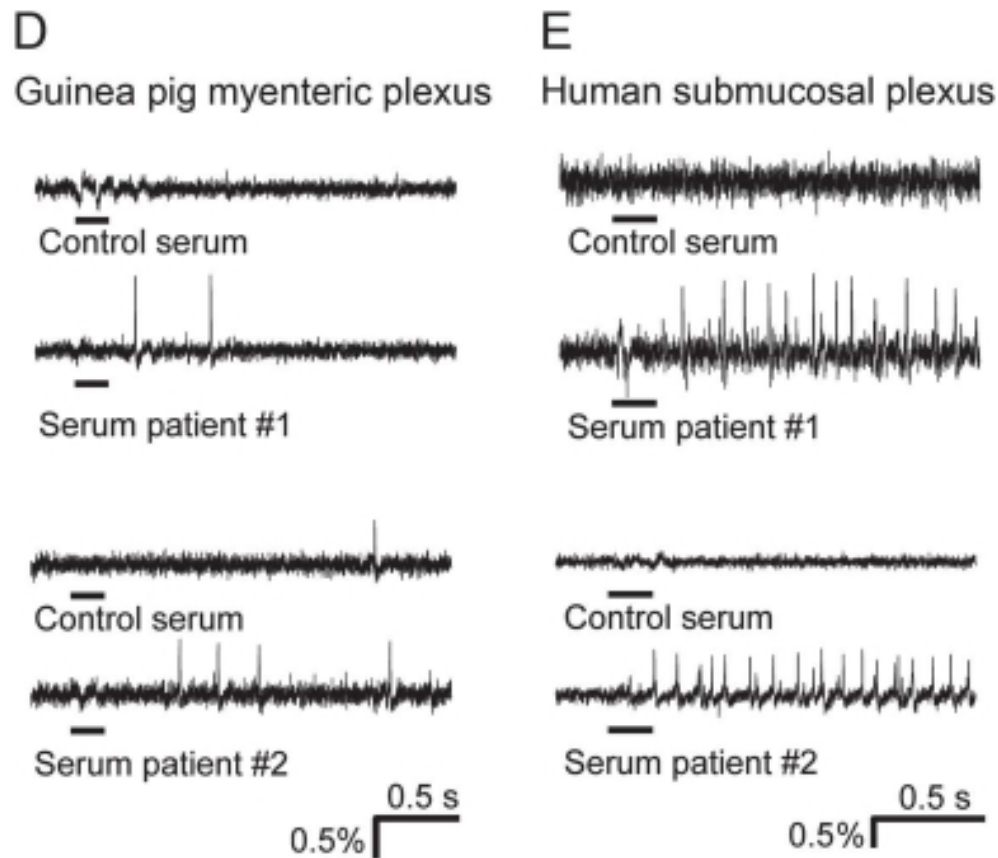
Figure 2

Confirmed binding of serum antibodies

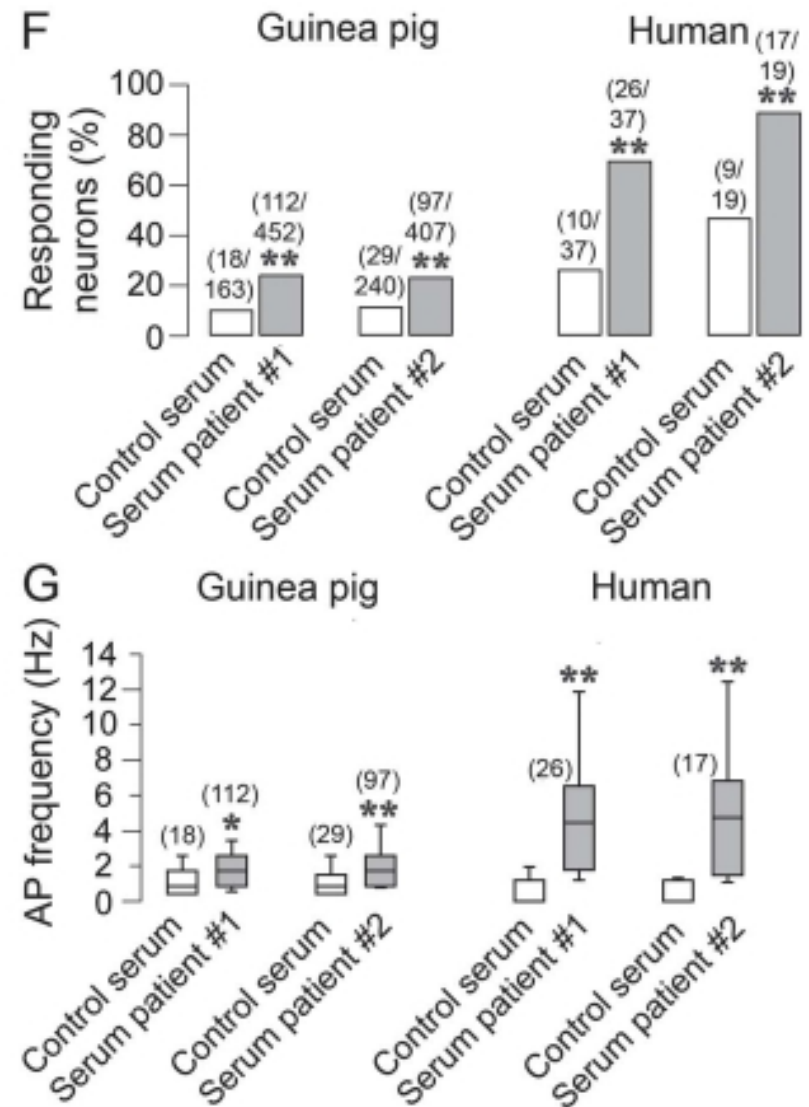


mp - myenteric plexus
smp - submucous plexus

Electrical activity of neurons

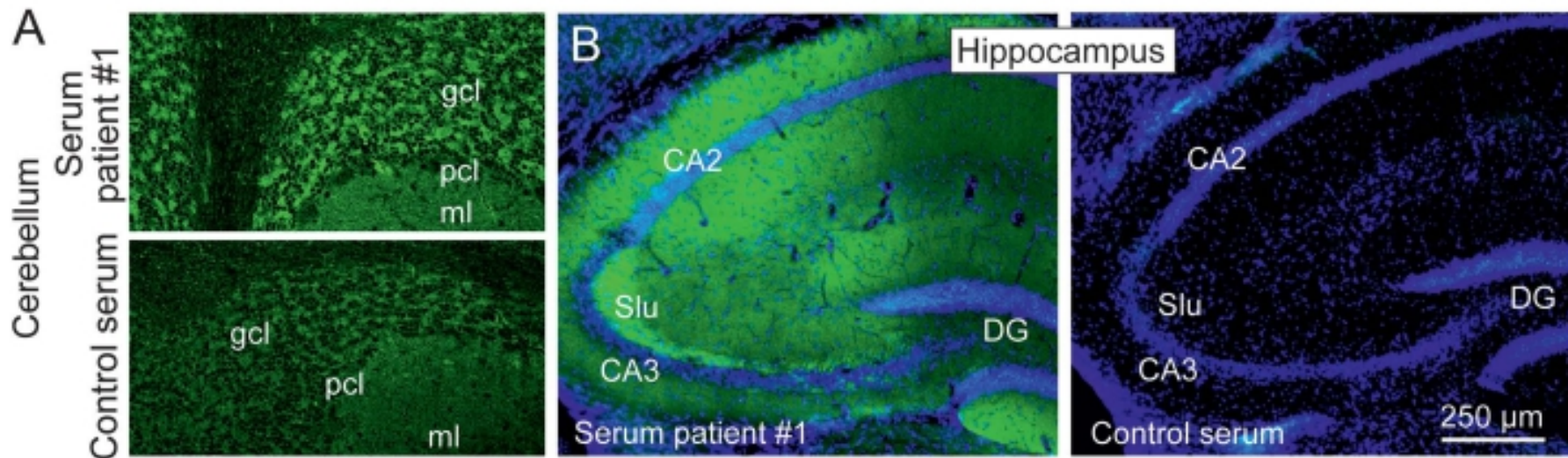


% (dF/F) change in fluorescence intensity
linearly related to membrane potential changes



Binding of anti-DPPX serum to excitatory and inhibitory synapses of CNS neurons and association with Kv4.2

Figure 3 Staining of CNS neurons by anti-dipeptidyl-peptidase-like protein 6 serum



Rat cerebellum

Mouse hippocampus

Live cultured neurons (mouse hippocampus),
stained with serum/purified IgG + Map2 (somatodendritic marker)

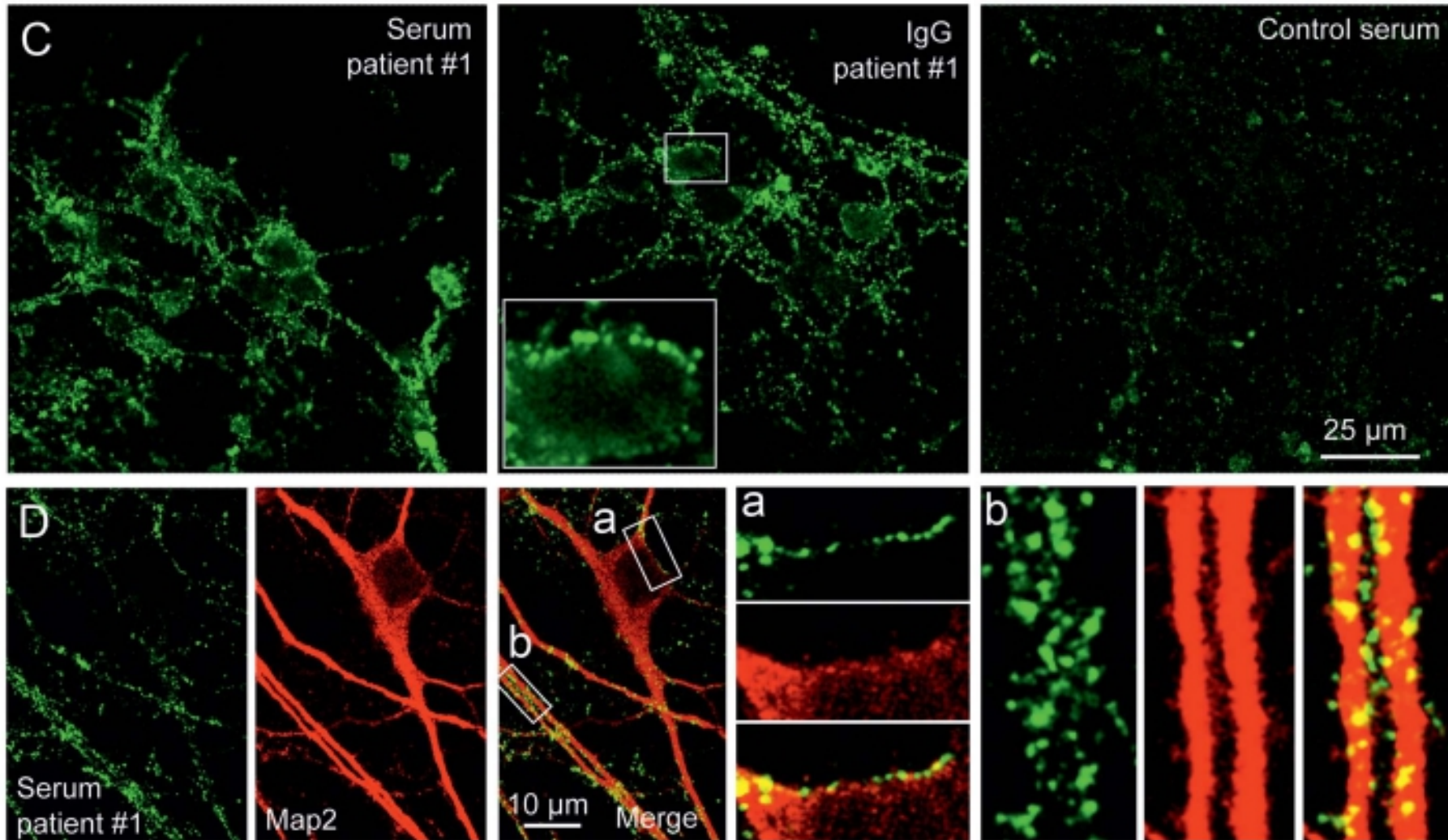


Fig.4 Live cultured neurons,

- Serum + Synapsin (general synaptic marker)
- + VGLUT (excitatory synapses, vesicular Glutamat transporter)
- + VGAT (inhibitory synapses, vesicular GABA transporter)

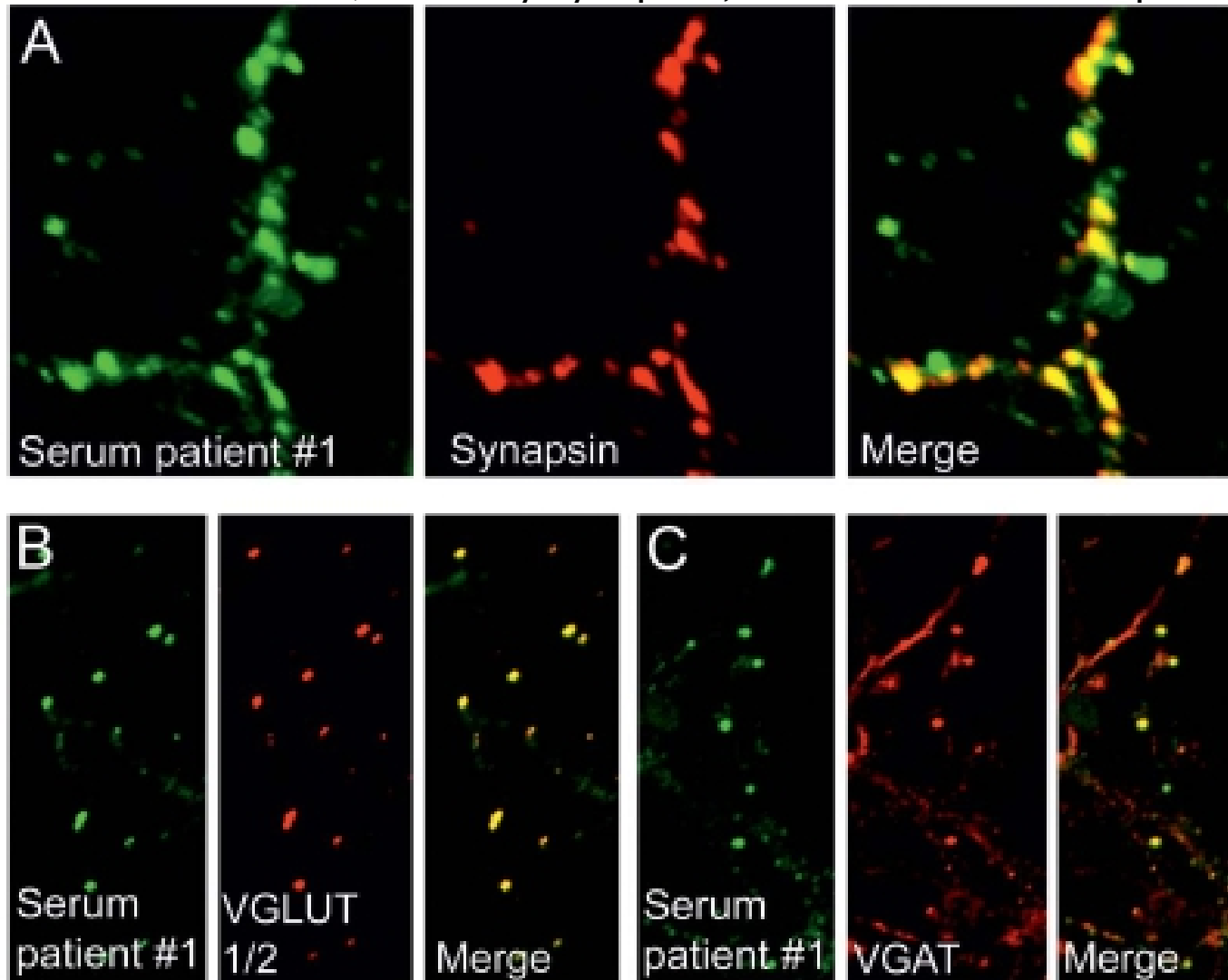
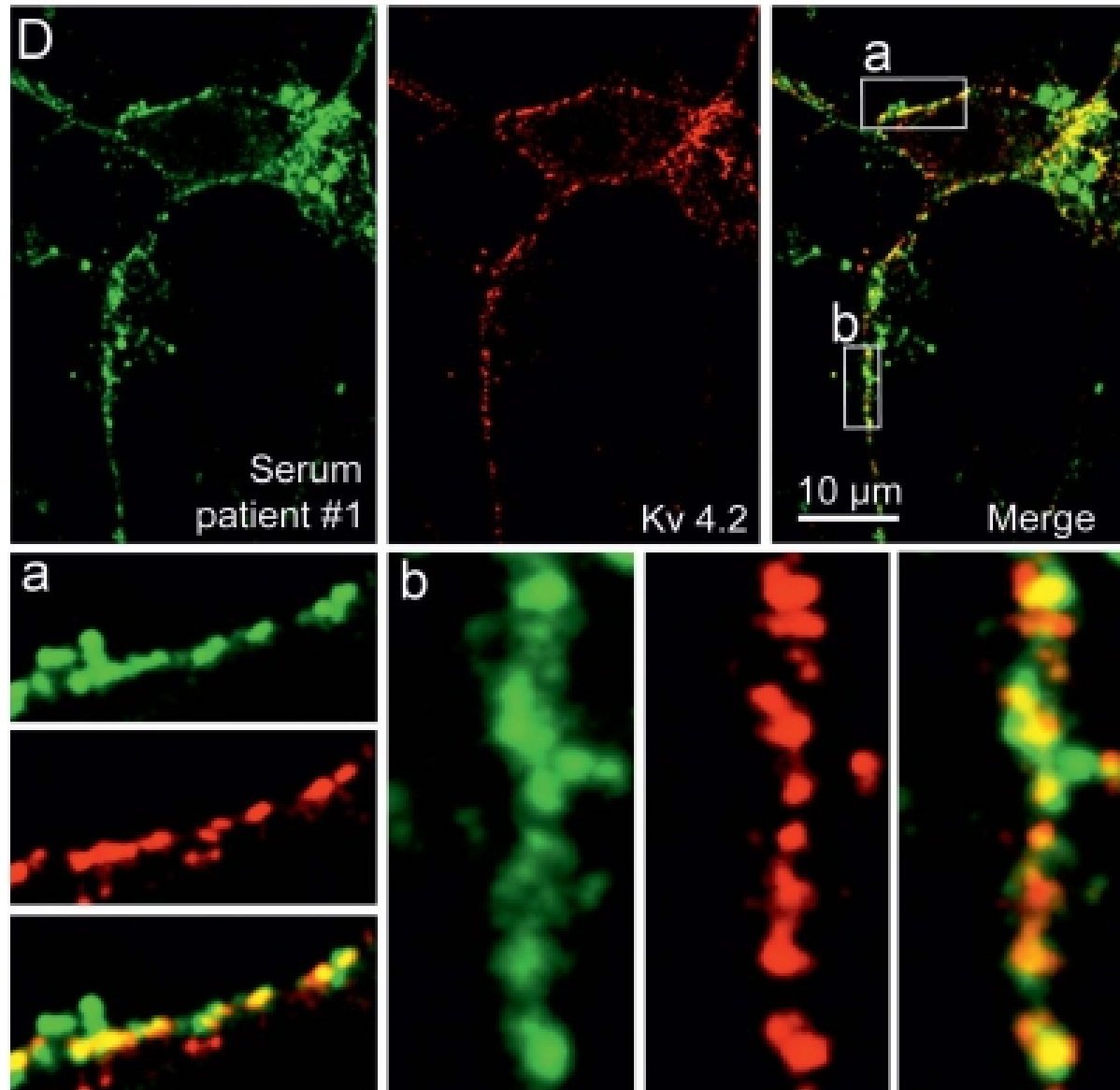
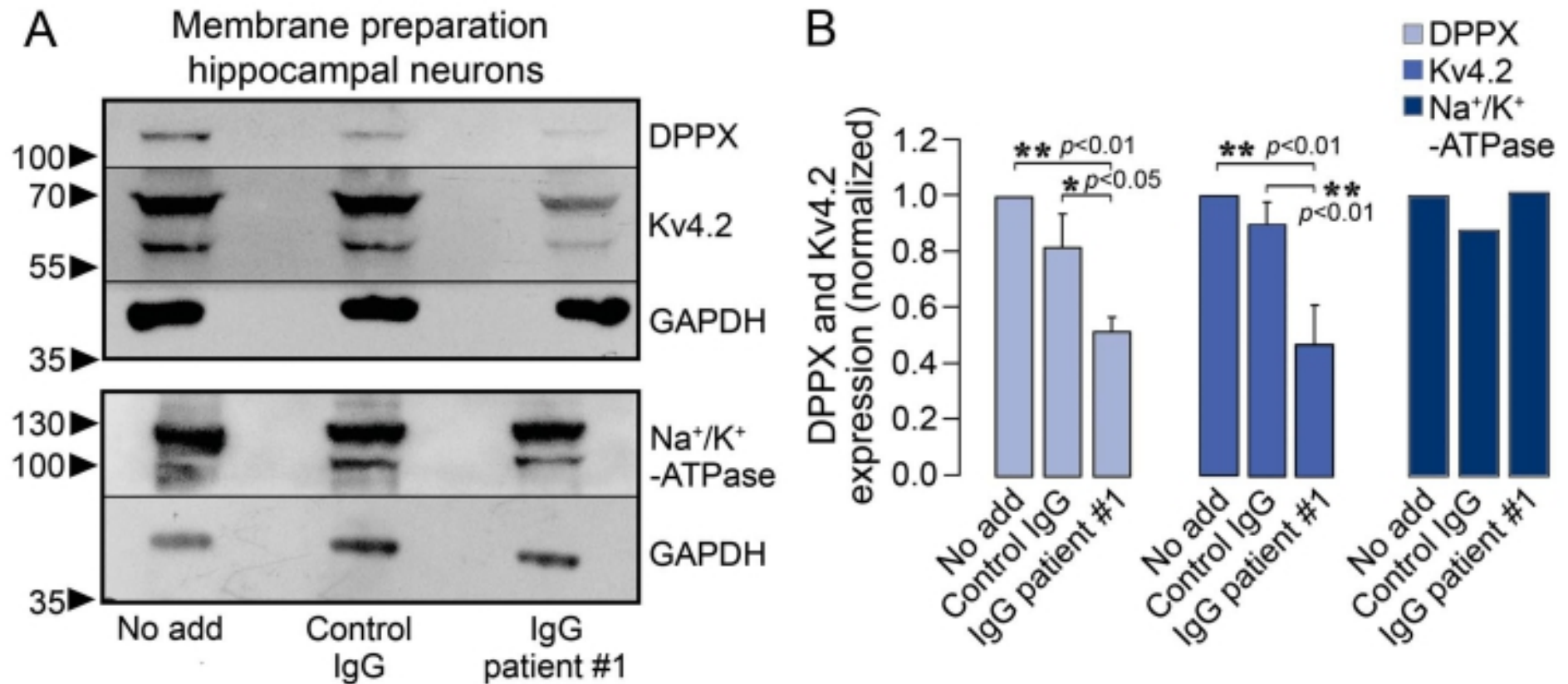


Fig.4 Live cultured neurons, stained with Serum + Kv4.2



Anti-DPPX serum decreases membrane expression of DPPX and Kv4.2 in hippocampal neurons

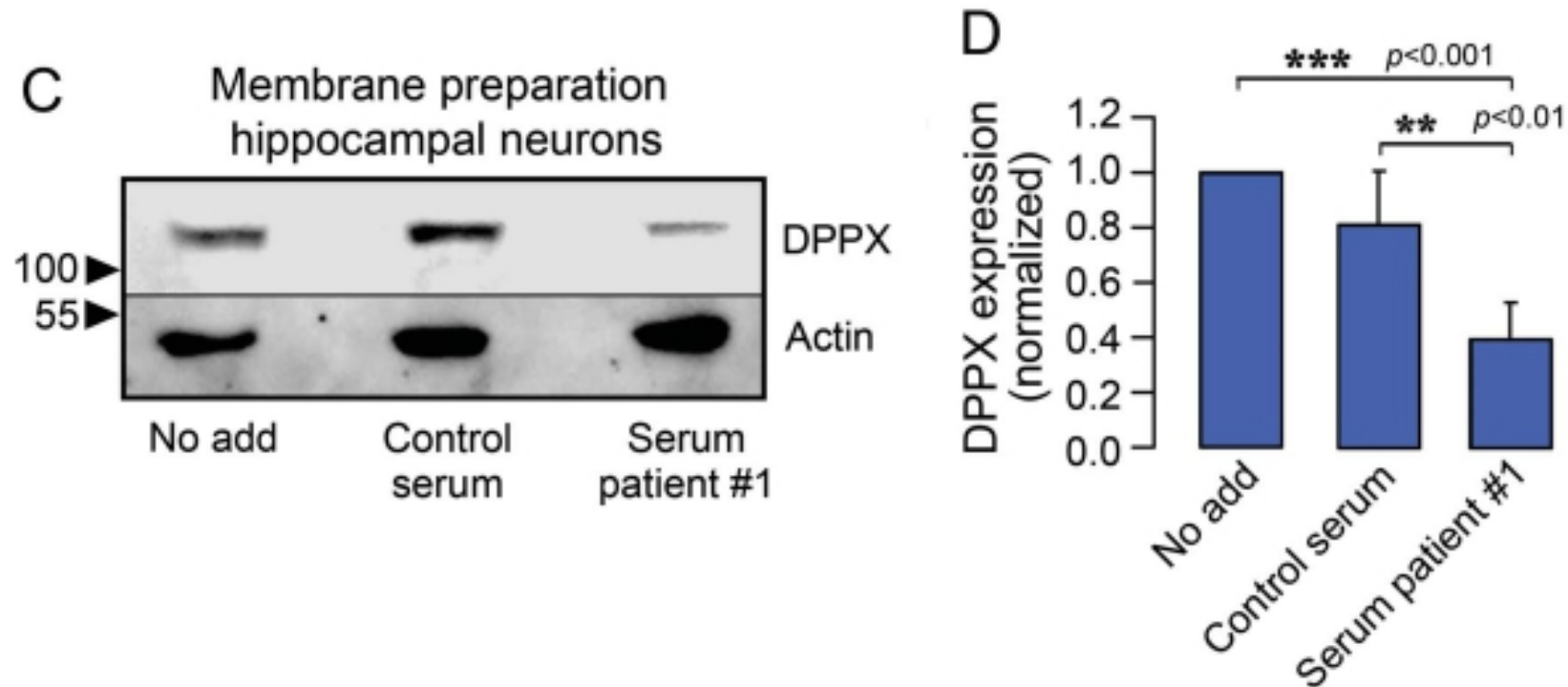
Fig.5 Neurons preincubated with **purf. IgG** (3d) before preparation, Membrane fractions of cultured neurons.



No nonspecific effect to cell surface proteins like Na⁺/K⁺-ATPase

Quantification in relation to GAPDH signal, normalized to untreated cells; data are means ± SEM (2-3 experiments)

Fig.5 Neurons preincubated with **Serum** (3 days) before preparation, Membrane fractions of cultured neurons.



Discussion

- Case report: clinical findings similar to patients of first publication (4 patients); sleep disturbance also noted in 9/20 patients
- DPPX antibody immediate effects on gut neurons: hyperexcitability of gut neurons
 - may lead to diarrhea symptoms
 - clinical hypomotility chronic effect (loss or exhaustion of enteric neurons)
- DPPX antibodies decrease DPPX protein and cause hyperexcitability; DPPX knockout mice show enhanced neuronal excitability,
- Reduced DPPX protein+reduced Kv4.2 in membranes may be due to interfering DPPX-mediated membrane targeting of Kv4.2

Conclusion

- Results are clues for potential pathogenic mechanisms of DPPX antibodies in DPPX encephalitis:
 - Gut: immediate hyperexcitability
 - Brain: reduced protein (DPPX+Kv4.2) at membrane
- Results support a pathogenic role of DPPX antibodies in DPPX encephalitis

My opinion

- Fullfilled their objective: Characterization of some antibody effects
- Direct causality still needed to be proofen
- New information supports pathogenic mechanisms
- In vivo data needed; electrophysiology data on brain neurons
- Synaptic localization (excitatory+inhibitory) of DPPX not known?
- Many experiments, many different methods / tissue used
- Methods description only online (supplements)