



HAL
open science

NMDAR Encephalitis, a model of synaptopathy

Aude Chefdeville

► **To cite this version:**

Aude Chefdeville. NMDAR Encephalitis, a model of synaptopathy. Neuroscience. Université de Lyon, 2017. English. NNT : 2017LYSE1301 . tel-01885503

HAL Id: tel-01885503

<https://theses.hal.science/tel-01885503>

Submitted on 2 Oct 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



N°d'ordre NNT : 2017LYSE1301

THESE de DOCTORAT DE L'UNIVERSITE DE LYON

opérée au sein de
l'Université Claude Bernard Lyon 1

Ecole Doctorale N°476
Neurosciences et Cognition (NsCo)

Spécialité de doctorat : Neurosciences
Discipline : Neurobiologie

Soutenue publiquement le 11/12/2017, par :
Aude Chefdeville

L'encéphalite à auto-anticorps anti-NMDAR, un modèle de synaptopathie

Devant le jury composé de :

Nom, Prénom	Grade/Qualité Etablissement	Président.e
Castel, Hélène	Directrice de recherche INSERM	Rapporteuse
Jouvion, Grégory	Chargé de recherche Institut Pasteur	Rapporteur
Ricard, Damien	PU-PH HIA du Val de Grâce	Examineur
Wiar, Marlène	Directrice de recherche CNRS	Examinatrice
Honorat, Jérôme	PU-PH Hospices Civils de Lyon	Directeur de thèse
Desestret, Virginie	MCU-PH Hospices Civils de Lyon	Co-directrice de thèse
Malleret, Gaël	Chargé de recherche CNRS	Invité
Meyronet, David	MCU-PH Hospices Civils de Lyon	Invité

UNIVERSITE CLAUDE BERNARD - LYON 1

Président de l'Université

Président du Conseil Académique

Vice-président du Conseil d'Administration

Vice-président du Conseil Formation et Vie Universitaire

Vice-président de la Commission Recherche

Directrice Générale des Services

M. le Professeur Frédéric FLEURY

M. le Professeur Hamda BEN HADID

M. le Professeur Didier REVEL

M. le Professeur Philippe CHEVALIER

M. Fabrice VALLÉE

Mme Dominique MARCHAND

COMPOSANTES SANTE

Faculté de Médecine Lyon Est – Claude Bernard

Directeur : M. le Professeur G.RODE

Faculté de Médecine et de Maïeutique Lyon Sud – Charles Mérieux

Directeur : Mme la Professeure C. BURILLON

Faculté d'Odontologie

Directeur : M. le Professeur D. BOURGEOIS

Institut des Sciences Pharmaceutiques et Biologiques

Directeur : Mme la Professeure C. VINCIGUERRA

Institut des Sciences et Techniques de la Réadaptation

Directeur : M. X. PERROT

Département de formation et Centre de Recherche en Biologie Humaine

Directeur : Mme la Professeure A-M. SCHOTT

COMPOSANTES ET DEPARTEMENTS DE SCIENCES ET TECHNOLOGIE

Faculté des Sciences et Technologies

Directeur : M. F. DE MARCHI

Département Biologie

Directeur : M. le Professeur F. THEVENARD

Département Chimie Biochimie

Directeur : Mme C. FELIX

Département GEP

Directeur : M. Hassan HAMMOURI

Département Informatique

Directeur : M. le Professeur S. AKKOUCHE

Département Mathématiques

Directeur : M. le Professeur G. TOMANOV

Département Mécanique

Directeur : M. le Professeur H. BEN HADID

Département Physique

Directeur : M. le Professeur J-C PLENET

UFR Sciences et Techniques des Activités Physiques et Sportives

Directeur : M. Y.VANPOULLE

Observatoire des Sciences de l'Univers de Lyon

Directeur : M. B. GUIDERDONI

Polytech Lyon

Directeur : M. le Professeur E.PERRIN

Ecole Supérieure de Chimie Physique Electronique

Directeur : M. G. PIGNAULT

Institut Universitaire de Technologie de Lyon 1

Directeur : M. le Professeur C. VITON

Ecole Supérieure du Professorat et de l'Education

Directeur : M. le Professeur A. MOUGNIOTTE

Institut de Science Financière et d'Assurances

Directeur : M. N. LEBOISNE

Remerciements

Je tiens à remercier en premier lieu les membres de mon jury qui ont accepté de lire et d'évaluer ce travail et m'honorent de leur présence : Mesdames et Messieurs les Professeur.e.s et Docteur.e.s Hélène Castel, Grégory Jouvion, Damien Ricard et Marlène Wiart.

Je tiens également à remercier le Professeur Jérôme Honorat de m'avoir confié ce travail et le Docteur Virginie Desestret de l'avoir encadré. Je vous remercie tous les deux de la confiance que vous m'avez accordée.

Je veux aussi remercier le Docteur Gaël Malleret, pour m'avoir initié à l'étude du comportement, pour ses sages conseils et surtout pour son enthousiasme et son optimisme sans faille ; et le Docteur David Meyronet pour son expertise histologique et ses remarques pertinentes. Merci d'avoir accepté de lire ce travail et d'être présents en cette occasion.

Résumé

L'encéphalite à auto-anticorps anti-récepteurs NMDA (E-NMDAR) est une maladie neurologique auto-immune caractérisée par une présentation clinique d'encéphalite et la présence systématique d'IgG dirigés contre la sous-unité GluN1 des NMDAR. Un tératome ovarien est associé chez environ 40% des patients, suggérant un rôle de la tumeur dans l'immunisation. Nous avons mené une étude des caractéristiques histologiques de 27 tératomes ovariens associés à l'E-NMDAR qui a révélé la présence quasi-systématique d'un contingent de tissu neuroglial dans ces tératomes, avec une expression de GluN1 prédominant dans les cellules gliales. Des infiltrations massives du contingent neuroglial par des cellules immunitaires sont constamment observées dans les tératomes associés à l'E-NMDAR, mais pas dans les tératomes non associés à une encéphalite. L'association entre les contingents neurogliaux, dont certains (3/27) présentent un aspect histologique de gliomes, et l'infiltration par les cellules immunitaires suggèrent un rôle de cette association dans la rupture de la tolérance immunitaire. En second lieu, nous avons développé un modèle murin d'E-NMDAR par transfert passif d'anticorps anti-NMDAR (NMDAR-Ac) provenant de sérum ou de liquide céphalo-rachidien (LCR) de patients afin de tester leur pouvoir pathogène et leur impact sur le fonctionnement cérébral. Les NMDAR-Ac provenant de sérum de patients causent une simplification de la morphologie des épines dendritiques suggérant un effet sur la plasticité synaptique mais n'ont qu'un effet mineur sur mémoire. Enfin, nos résultats suggèrent un effet spécifique des NMDAR-Ac sur la microglie et son implication possible dans l'E-NMDAR.

Mots-clés : encéphalite à auto-anticorps anti-récepteurs NMDA, auto-immunité, tératome ovarien, mémoire, microglie

Abstract

Encephalitis with anti-NMDAR antibodies (NMDAR-AE) is a severe autoimmune neurological disorder, defined by a clinical presentation of encephalitis and the presence of CSF IgG targeting the GluN1 subunit of NMDA receptors. An underlying ovarian teratoma is commonly associated with this autoimmune disease suggesting a role for the tumor in immunopathogenesis. We investigated the histological specificities of NMDAR-AE associated teratoma to clarify their role in immunization against the NMDAR. We characterized the histopathological features of 27 ovarian teratomas associated with NMDAR-AE. All but one contained a nervous tissue component and GluN1 expression by teratomatous nervous tissue was more often glial in NMDAR-AE teratomas than in control teratomas. NMDAR-AE associated teratomas had consistent and massive infiltration of their neuroglial tissue component by immune cells. Association between infiltration of NMDAR-AE-associated teratomas by immune effector cells and particular glial features of its consistent neuroglial component suggests that this particular association may trigger the autoimmune pathology development. Secondly, we design a rodent model of NMDAR-AE by passive transfer of NMDAR-Abs from serum or CSF of patients to test NMDAR-Abs pathogenicity and investigate their impact on brain function. We showed that NMDAR-Abs from patients' serum induce a simplification of dendritic spine morphology but cause only minor behavioral impairment. Our results outlined differences between NMDAR-Abs from serum or from CSF. NMDAR-Abs induced modifications of microglial morphology also suggested involvement of new cellular player in NMDAR-AE pathogenesis.

Keywords: NMDAR encephalitis, autoimmunity, ovarian teratoma, memory, microglia

Plan

Abbreviations

Introduction ***p. 1***

I. FROM PARANEOPLASTIC NEUROLOGICAL SYNDROMES TO AUTOIMMUNE ENCEPHALITIDES **p. 2**

II. ANTI-NMDAR ENCEPHALITIS: CLINICAL ASPECTS **p. 4**

- II.1. Definition p. 4
- II.2. Frequency p. 4
- II.3. Epidemiology & Clinical Presentation p. 4
- II.4. Associated Neoplasms p. 5
- II.5. Diagnosis p. 5
- II.6. Ancillary Tests p. 6
- II.7. Treatment p. 6
- II.8. Recovery & Relapses p. 7
- II.9. Mortality & Causes of Death p. 7
- II.10. Antibody Subtypes & Clinical Relevance p. 7

III. THE N-METHYL-D-ASPARTATE RECEPTOR **p. 8**

- III.1. NMDAR Structure And Expression p. 8
 - III.1.1. NMDAR subunits and genes
 - III.1.2. NMDAR conformation
 - III.1.3. NMDAR expression patterns and properties
- III.2. NMDAR In Synaptic Plasticity And Memory p. 11
 - III.2.1. NMDAR-dependent long-term potentiation
 - III.2.2. NMDAR, from long-term potentiation to memory
- III.3. NMDAR Trafficking & Interactions With Synaptic Proteins p. 13
 - III.3.1. NMDAR localization and surface diffusion
 - III.3.2. Proteins interacting with NMDAR at the synapse
- III.4. Non-Neuronal NMDAR p. 15

IV. ANTI-NMDAR ENCEPHALITIS: INVESTIGATING PATHOGENIC MECHANISMS **p. 17**

- IV.1. Epitope Targeted By NMDAR-Abs p. 17

IV.2. Researching the Autoimmune Trigger	p. 18
IV.2.1. Association with ovarian teratoma in young female patients	
IV.2.2. Viral hypothesis	
IV.2.3. Genetic	
IV.3. Autoantibody Pathogenesis	p. 20
IV.3.1. Clinical arguments	
IV.3.2. <i>In vitro</i> & <i>Ex vivo</i> Experiments	
IV.3.3. <i>In vivo</i> Experiments	

Objectives ***p. 24***

I. STUDY 1	p. 25
-------------------	--------------

II. STUDY 2	p. 25
--------------------	--------------

Material & Methods ***p. 27***

I. STUDY 1	p. 28
-------------------	--------------

I.1. Patient's Samples	p. 28
I.2. Tumor Pathological Study	p. 28
I.3. Immunohistochemical Studies	p. 28
I.4. DNA Sequencing	p. 29
I.5. Statistical Analysis	p. 29

II. STUDY 2	p. 30
--------------------	--------------

II.1. Serum & CSF Samples Obtention	p. 30
II.2. Serum & CSF Samples Processing	p. 30
II.3. Animals	p. 30
II.4. Surgical Procedure	p. 31
II.5. Minocycline Treatment	p. 31
II.6. Behavioral Experiments	p. 31
II.6.1. Fear conditioning	
II.6.2. Spatial and object recognition.	
II.7. Rodent Brain Tissue Immunostaining	p. 34
II.8. Glial Cells Morphology Analysis	p. 35
II.9. Electron Microscopy	p. 36
II.10. Golgi Staining & Analysis	p. 37
II.11. Statistical Analysis	p. 37

I. STUDY 1**p. 39****I.1. Histological Characteristics of NMDAR-AE Associated Teratomas****p. 39****I.1.1. Grading****I.1.2. Consistent neural differentiation and neuroglial NMDAR expression in NMDAR-AE associated teratomas****I.1.3. Glioma-like features in NMDAR-AE teratomas****I.1.4. Characteristics of inflammatory infiltrates in contact with neuroglial tissue in NMDAR-AE associated teratomas****II. STUDY 2****p. 49****II.1. Preliminary Experiments: Validation of the Model****p. 49****II.1.1. Control of Human IgG diffusion in the hippocampus****II.1.2. Impact of the infusion site on fear memory****II.2. Effects of Purified IgG Infusion on Behavior and Dendritic Spines Morphology****p. 51****II.2.1. Behavioral effects of Ctrl IgG and NMDAR-AE IgG***II.2.1.1. Fear conditioning on mice treated during 7 days with IgG purified from serum of Ctrl or NMDAR-AE patients**II.2.1.2. Pool analysis of all fear conditioning experiments on mice treated during 7 days with IgG purified from serum of Ctrl or NMDAR-AE patients**II.2.1.3. Fear conditioning on mice treated during 14 days with IgG purified from serum of Ctrl or NMDAR-AE patients**II.2.1.4. Spatial and object recognition test on mice treated during 14 days with IgGs purified from serum of Ctrl or NMDAR-AE patients***II.2.2. Effect of NMDAR-Abs infusion on hippocampal and dendritic spine morphology***II.2.2.1. Analysis of hippocampal morphology by EM after treatment with Ctrl or NMDAR-AE IgGs during 7 days**II.2.2.2. Analysis of dendritic spines morphology by EM after treatment with Ctrl or NMDAR-AE IgGs during 7 days**II.2.2.3. Analysis of dendritic spines morphology by Golgi staining after treatment with Ctrl or NMDAR-AE IgGs during 14 days***II.3. Possible Inflammation after 7 Days of NMDAR-AE IgG Infusion****p. 61****II.3.1. Inflammation after treatment with Ctrl or NMDAR-AE IgGs during 7 days****II.3.2. Impact of minocycline on inflammation and behavior after treatment with Ctrl or NMDAR-AE IgGs during 7 days****II.4. Effects of Purified CSF Infusion on behavior****p. 66**

Discussion **p. 69**

I. STUDY 1 **p. 71**

II. STUDY 2 **p. 73**

- II.1. Infusion of Human IgG Purified From Serum Did Not Reproduce NMDAR-AE Symptoms p. 73
- II.2. Differences of NMDAR-Abs Effects from Serum or CSF? p. 75
- II.3. Human IgG Effect on Dendritic Spines Morphology p. 78
- II.4. An Effect of Human IgG From Healthy Subjects? p. 79
- II.5. A Role for Microglia In The Physiopathogenesis Of NMDAR-AE? p. 79

Conclusion **p. 80**

References **p. 81**

Appendices **p. 88**

Research Article - V. Desestret, **A. Chefdeville**, A. Viaccoz, C. Bost, F. Ducray, G. Picard, V. Rogemond, M. O. Chaffois, C. Blanc, C. Bardel, I. Treilleux, O. Pascual, J. C. Antoine, J. Y. Delattre, J. Honnorat (2015) CSF IgA antibodies are potential biomarkers for teratomas in anti-NMDAR encephalitis. *Neurology Neuroimmunology Neuroinflammation*, 2(6): e166

Review Article - **A. Chefdeville**, J. Honnorat, C. Hampe, V. Desestret (2016) Neuronal central nervous system syndromes probably mediated by autoantibodies. *European Journal of Neuroscience*, 43(12): 1535-52

Figures

Figure 1: Autoantibodies against « onconeural » antigens or synaptic antigens.	3
Figure 2: Clinical evolution of NMDAR-AE.	5
Figure 3: Clinical outcome of NMDAR-AE	6
Figure 4: Schematic representation of the N-methyl-D-aspartate receptor and subunit structure.	9
Figure 5: NMDAR-subunits expression pattern throughout development.	10
Figure 6: The identity of the GluN2 subunit controls the biophysical properties of NMDAR.	10
Figure 7: Glutamatergic receptors and synaptic plasticity pathways.	12
Figure 8: NMDAR are part of macromolecular complexes at excitatory synapses.	14
Figure 9: Proposed MDAR-Abs binding site.	17
Figure 10: Proposed model of NMDAR-Abs-induced alterations of NMDAR trafficking.	22
Figure 11: Schematic representation of the fear conditioning test apparatus and protocol.	32
Figure 12: Schematic representation of the spatial change and novel object recognition set-up.	34
Figure 13: Schematic representation of parameters used for dendritic spine morphology analysis based on EM images or Golgi stainings.	36
Figure 14: Gross structure of a control ovarian teratoma or teratomas associated with NMDAR-AE.	42
Figure 15: GluN1 expression by neuronal and glial cells in central nervous tissue from NMDAR-AE associated and control ovarian teratoma.	43
Figure 16: Neuroglial tissue from NMDAR-AE associated teratoma presenting histological features of gliomas.	44
Figure 17: Quantification of inflammatory infiltrates in NMDAR-AE-associated and control ovarian teratomas.	46
Figure 18: Prominent inflammatory infiltrates and germinal centers in NMDAR-AE-associated ovarian teratomas.	47
Figure 19: IgG and IgA deposits and producing cells in NMDAR-AE associated teratomas.	48
Figure 20: Diffusion of purified human IgGs into mice brain parenchyma after 7 days.	50
Figure 21: The infusion site does not impact mice behavioral performances in a fear conditioning test.	51
Figure 22: NMDAR-AE purified IgG do not impair mice behavioral performances in a fear conditioning test.	53
Figure 23: NMDAR-AE purified IgGs do not strongly impair mice behavioral performances in a fear conditioning test but Ctrl IgGs enhances auditory fear conditioning.	54
Figure 24: NMDAR-AE IgG infusion during 14 days does not impair mice behavioral performances in a fear conditioning test.	55
Figure 25: NMDAR-AE purified IgGs do not impair mice memory performances in a novel-object recognition test.	57
Figure 26: Ctrl IgGs and NMDAR-AE IgGs modify dendritic spines morphology.	60
Figure 27: Microglial cells present minor signs of activation after a 7 days infusion of IgGs from NMDAR-AE patients.	62
Figure 28: Microglial cells present minor signs of activation after 7 days of infusion of IgGs from	63

NMDAR-AE patients.

Figure 29: Glial cells do not present signs of activation after 14 days infusion of control IgGs or IgGs from NMDAR-AE patients. 64

Figure 30: Minocycline treatment did not modify microglial morphology nor fear memory of Ctrl IgGs or NMDAR-AE IgGs infused mice in a fear conditioning test. 65

Figure 31: CSF from NMDAR-AE patients does not impair mice behavioral performances in a fear conditioning test. 67

Figure 32: NMDAR-AE CSF did not impair mice memory performances in a novel-object recognition test. 68

Figure 33: NMDAR-AE IgGs infusion does not alter synaptic plasticity but CSF NMDAR-AE does (results from Dr. Bost). 74

Suppl. Figure 1: Nervous tissue in control ovarian teratomas. 91

Tables

Table 1: Primary and secondary antibodies used for immunostaining of mice brain tissue 35

Table 2: Histological features of NMDAR-AE associated ovarian teratomas 40

Table 3: Compared histological features of control and NMDAR-AE associated ovarian teratomas 41

Suppl. Table 1: References of antibodies used for immunohistochemistry and immunofluorescent stainings 90

Abbreviations

Ach = Acetylcholine

AchR = Acetylcholine receptor

AE = Autoimmune encephalitis

AMPA = α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

BSA = Bovine serum albumin

CaMKII = Calmoduline kinase II

CNS = Central nervous system

CSF = Cerebrospinal fluid

CXCL = Chemokine (C-X-C motif) ligand

DAPI = 4', 6-diamidino-2-phenylindole

EEG = Electroencephalography

EM = Electron Microscopy

E-NMDAR = Encéphalite à autoanticorps anti-NMDAR

EPSP = Excitatory post-synaptic potential

EphB2R= Ephrin B2 Receptor

EphB2 = Ephrin B2

HEK = Human embryonic kidney cells

HSV =Herpes Simplex Virus

HSVE = Herpes Simplex Virus Encephalitis

hIgG = Human immunoglobulin G

Ig = Immunoglobulin

IgG, IgA = Immunoglobulin G, immunoglobulin A

IL = Interleukine

LCR = Liquide céphalo-rachidien

LTD = Long-term depression

LTP = Long-term potentiation

MAGUK = Membrane associated guanylate kinase

mGluR = Metabotropic glutamate receptors

MRI = Magnetic resonance imaging

NGS = Normal goat serum

NMDA = N-methyl-D-aspartate

NMDAR = N-methyl-D-aspartate receptor

NMDAR-Abs = Autoantibodies directed against the GluN1 subunit of NMDAR from NMDAR-AE patients

NOR = Novel object recognition

PBS = Phosphate buffered saline

PSD = Post-synaptic density

PNS = Paraneoplastic neurological syndrome

Introduction

I. FROM PARANEOPLASTIC NEUROLOGICAL SYNDROMES TO AUTOIMMUNE ENCEPHALITIDES

Encephalitis is an inflammation of the brain caused by infection, autoimmunity or toxins. Although the main etiology of encephalitis remains viral infection, an increasing number of autoimmune encephalitides (AE), associated with autoantibodies targeting the central nervous system (CNS) have been described for the last three decades.

Autoantibodies targeting antigens of the CNS were initially discovered about 25 years ago in paraneoplastic neurological syndromes (PNS) such as neuropathy, limbic and/or brainstem encephalitis and cerebellar degeneration. In these disorders, target epitopes are intracellular (e.g. Hu or CRMP2) (Szabo *et al.* 1991), leaving limited access for antibodies to their target. Although these antibodies are present in the cerebrospinal fluid of patients, they do not appear to exercise a direct pathogenic action but are instead believed to be markers of a T-cell-mediated immune response against the corresponding antigens (reviewed in Lancaster & Dalmau, 2013). Cytotoxic neuronal damage causes symptoms that are not reversible with immune-suppressive treatments and human disease is not reproduced by passive transfer of patients' autoantibodies in rodent, further arguing for a non-pathogenic effect of these antibodies. These antibodies are collectively termed "onconeural antibodies" and each one is highly correlated with presence of a specific tumor (e. g. Hu antibodies with small-cells lung cancer) (Honnorat, 2006).

Identification of autoantibodies targeting cell-surface neuronal antigens started in 2000 with mGluR1 (Sillevis Smitt *et al.* 2000). The description in 2007 of a stereotyped neuropsychiatric syndrome associated with autoantibodies targeting the NMDAR and recognition that this disorder was largely misdiagnosed were the starting points of a highly dynamic field of research on (i) the identification of new autoantibodies targeting cell-surface neuronal antigens and (ii) description of associated syndrome and dissection of the pathophysiological mechanisms underlying the action of these autoantibodies.

Several key features differentiate this group of antigens from onconeural targets (*cf.* Fig. 1): 1) location of these antigens renders them vulnerable to direct antibody-mediated disruption; 2) associated neurological syndromes usually are immunotherapy-responsive and reversible, consistent with a direct pathogenic effect of antibodies without cytotoxic-induced neuronal loss; 3) tumor association is far less consistent.

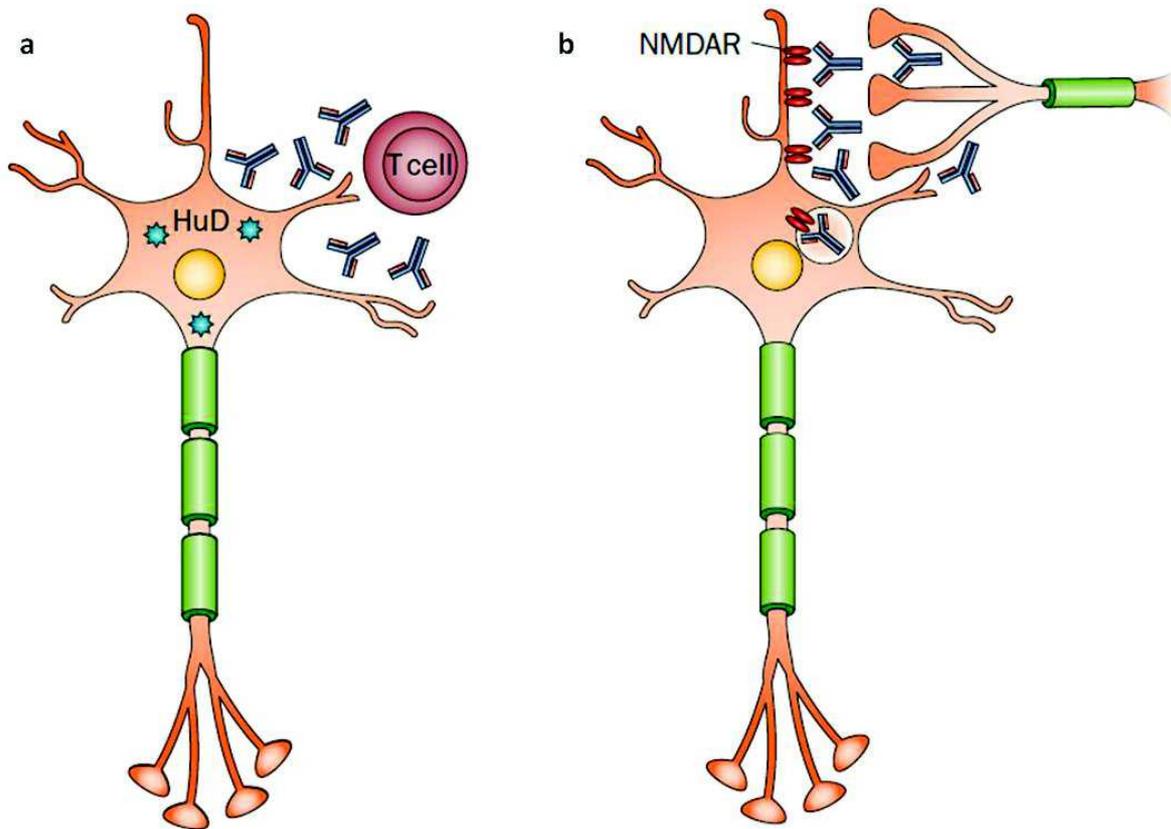


Figure 1: Autoantibodies against « onconeuronal » antigens or synaptic antigens. (a) « Onconeuronal » targets are intracellular and thus not directly accessible to autoantibodies. Autoantibodies are biomarkers of a T-cell mediated immune response against neurons. (b) Synaptic receptor function can be directly disrupted by autoantibodies. Adapted from Lancaster & Dalmau, 2012.

Encephalitides mediated by antibodies directed against cell-surface antigens are more common than paraneoplastic encephalitis (Granerod *et al.* 2010). To this day, more than 10 antibodies targeting neuronal cell-surface antigens have been identified (Chefdeville *et al.* 2016). Targets now include ionotropic receptors like the N-methyl-D-aspartate receptors, the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), the γ -aminobutyric acid receptor-B receptors (GABA_BR) and the glycine receptors; metabotropic receptors such as the metabotropic glutamate receptors mGluR1 et mGluR5 and γ -aminobutyric acid receptor-A receptors (GABA_AR); and proteins belonging to the voltage-gated potassium channels (VGKC) complex, namely leucine-rich glioma inactivated protein 1 (LGI1), contactin-associated protein-like 2 (Caspr2) and dipeptidyl-peptidase-like protein 6 (DPP6). Autoimmune encephalitides were identified as the third cause of encephalitis in 2010 (Granerod *et al.* 2010) and the frequency of NMDAR-AE surpasses that of individual viral etiologies in patient under 30 years old (Gable *et al.* 2012).

II. ANTI-NMDAR ENCEPHALITIS: CLINICAL ASPECTS

II.1. DEFINITION

Encephalitis associated with anti-NMDAR autoantibodies is a clinico-biological entity defined by the presence of two elements: a highly stereotyped clinical picture of limbic encephalitis, and presence of antibodies of the IgG subtype targeting the GluN1 subunit of the NMDAR in patient' CSF (Dalmau *et al.* 2008; Graus *et al.* 2016).

II.2. FREQUENCY

Limbic encephalitis associated with anti-NMDAR antibodies was firstly described in 2007 in 12 women with an associated ovarian teratoma by J. Dalmau *et al.* (Dalmau *et al.* 2007). The clinical syndrome associated with anti-NMDAR antibodies was then more comprehensively described in 2008 in a case series of a 100 patients (Dalmau *et al.* 2008). To date, almost a thousand human cases and one polar bear case have been described (Dalmau *et al.* 2008, Titulaer *et al.*, 2013, Prüss *et al.* 2015), indicating a relatively frequent disorder. Accordingly, NMDAR-AE was identified as the most common cause of encephalitis in young individuals (Gable *et al.* 2012). Among all autoimmune encephalitides, NMDAR encephalitis is the most frequent (Thomas *et al.* 2014).

II.3. EPIDEMIOLOGY & CLINICAL PRESENTATION

This disorder predominantly affects young woman (81%; Titulaer *et al.* 2013), although it can also affect men and children (Titulaer *et al.* 2013). A prodromal syndrome resembling a non-specific viral-like illness with headache, low-grade fever and gastrointestinal disorders is reported in 85% of patients (Dalmau *et al.* 2008), followed, in the next weeks, by the development of psychiatric symptoms (e.g. anxiety, agitation, delusional thoughts, hallucinations, personality or behavioral changes) and/or neurological symptoms (e. g. seizures, movement disorders, catatonia, speech deficit and short-term memory loss). Though the overall clinical presentation of NMDAR-AE is highly stereotyped (*cf.* Fig. 2), the modality of entry into the disease slightly differs according to patients' gender and age. As observed in the initial cohort of 12 women (Dalmau *et al.* 2007), about 70% of adult female patients present with psychiatric symptoms (Dalmau *et al.* 2007, 2008; Maat *et al.* 2013; Titulaer *et al.* 2013) whereas adult male patients more frequently present with seizures (Viaccoz *et al.* 2014). The majority of children under 12 years present with neurological symptoms, usually seizures or movement disorders (Armangué *et al.* 2013; Zekeridou *et al.* 2015). In adolescents aged between 12 and 18 years, the modality of clinical presentation varies between those of adults and children (Titulaer *et al.* 2013). Over the course of the disease, symptomatology evolves towards uniformization. 87% of patients develop symptoms belonging to four or more of the following categories within 4 weeks of disease onset: abnormal behavior & cognition, memory alterations, speech impairment, seizures, dyskinesias and movement disorder (the

Introduction

most characteristic being oro-facial dyskinesias), alteration of consciousness, autonomic instabilities and central hypoventilation (Titulaer *et al.* 2013).

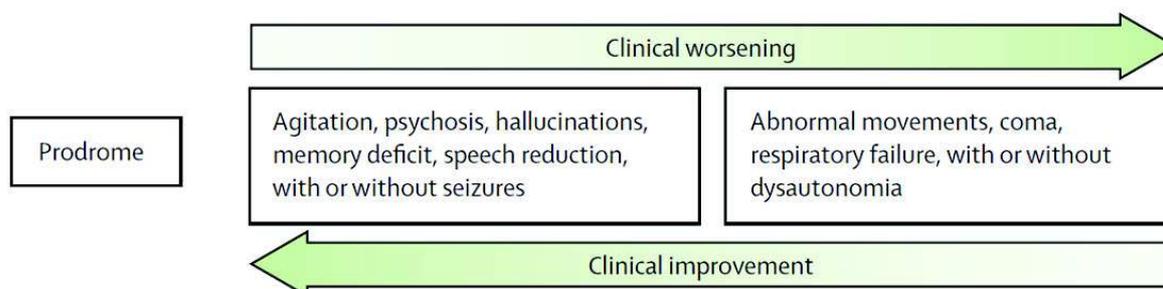


Figure 2: Clinical evolution of NMDAR-AE. Dalmau *et al.* 2011.

II.4. ASSOCIATED NEOPLASMS

Though NMDAR-AE was initially described as a paraneoplastic disorder (Dalmau *et al.* 2007), further studies revealed that and an underlying neoplasm is found only in about 40% of patients (Dalmau *et al.* 2008, Dalmau *et al.* 2011). Female patient aged between 12 years and 45 years constitute 93% of patients with underlying neoplasm (Titulaer *et al.* 2013) More than 90% of all tumors associated with anti-NMDAR encephalitis are ovarian teratoma (Titulaer *et al.* 2013). Other tumors described in association with NMDAR-AE include lung, breast and testicular tumors, extraovarian teratomas and ovarian carcinomas, thymic carcinoma and pancreatic cancer (Titulaer *et al.* 2013, Bost *et al.* In prep.).

II.5. DIAGNOSIS

Screening of the CSF for presence of IgG directed against the NMDAR is the central element in the diagnosis of NMDAR-AE. Detection of anti-NMDAR antibodies in the CSF and/or in the serum relies on two tests : 1) a specific staining pattern obtained upon immunohistochemical detection of NMDAR antibodies bound to rat brain section and 2) and antibodies binding in a cell-based assay with NMDAR-expressing HEK fixed cells (Dalmau *et al.* 2008; Gresa-Arribas *et al.* 2014). Sensitivity of NMDAR-Abs detection is higher using NMDAR-expressing fixed cells than live cells. A study of paired CSF/sera samples from 250 NMDAR-AE patients revealed that all CSF samples were positive for NMDAR-Abs whereas only 214 serum tested positive (Gresa-Arribas *et al.* 2014) indicating that sensitivity of NMDAR-Abs testing is higher in the CSF (Gresa-Arribas *et al.* 2014; Wang *et al.* 2015). Though high NMDAR-Abs titres in CSF and serum are associated with poorer outcome and presence of an associated ovaria teratoma (Desestret *et al.* 2015; Gresa-Arribas *et al.* 2014), NMDAR-Abs titres retain limited interest in clinical use other than as a diagnosis tool. Indeed CSF titres only roughly correlate with disease clinical severity and relapses are not always associated with titres changes (Gresa-Arribas *et al.* 2014). Correlation between serum titers and clinical severity is even poorer, with reported cases of recovery from NMDAR-AE with long-term follow-up still retaining high serum titres and absent or barely detectable CSF titres (Seki *et al.* 2008; Wang *et al.* 2015).

II.6. ANCILLARY TESTS

Ancillary tests such as MRI and EEG are abnormal in respectively 33% and 90% of patients with NMDAR-AE (Titulaer *et al.* 2013). Abnormal MRI findings are predominantly mild or transient signal hyperintensity on FLAIR/T2 sequence in the hippocampi, cerebral cortex, frontobasal and insular regions, basal ganglia, cerebellum and brainstem (Dalmau *et al.* 2008, Dalmau *et al.* 2011). EEG recordings usually show mild to severe polymorphic diffuse slowing of electrographic activity and electrographic seizures, with or without clinical correlates (Dalmau *et al.* 2008; Dalmau *et al.* 2011; Schmitt *et al.* 2012). A specific pattern named “extreme-delta brush pattern” (EDB) is observed in about 30% of NMDAR-LE patients, and may be a marker of a more severe disease course and a worse outcome (Schmitt *et al.* 2012). CSF examination is abnormal in 79% of patients (Dalmau *et al.* 2011, Titulaer *et al.* 2013), with CSF-specific oligoclonal bands in 60% of patients, moderate lymphocytic pleiocytosis and mildly increased protein concentration (Dalmau *et al.* 2011, Wang *et al.* 2015).

II.7. TREATMENT

Overall, 75% of patients with NMDAR-AE achieve full recovery or retain only mild deficits (mRS 0-2, corresponding to total absence of symptoms to slight disability preventing the patient to carry out some activities, *cf.* Fig. 3; Dalmau *et al.* 2008, Titulaer *et al.* 2013). First line immunotherapy, i. e. steroids, intravenous immunoglobulins (IVIg) and plasma exchange alone or combined, and tumor removal if applicable, result in substantial clinical improvement within 4 weeks in about 50% of patients (Titulaer *et al.* 2013). Early search for an underlying tumor is important, as tumor removal speeds up clinical improvement and decreases the risks of relapses (Titulaer *et al.* 2013). In patients not responding to first-line immunotherapy, using more aggressive second-line immunotherapy, i. e. rituximab and/or cyclophosphamides, is associated with a better outcome than continuation of first-line immunotherapy (Titulaer *et al.* 2013).

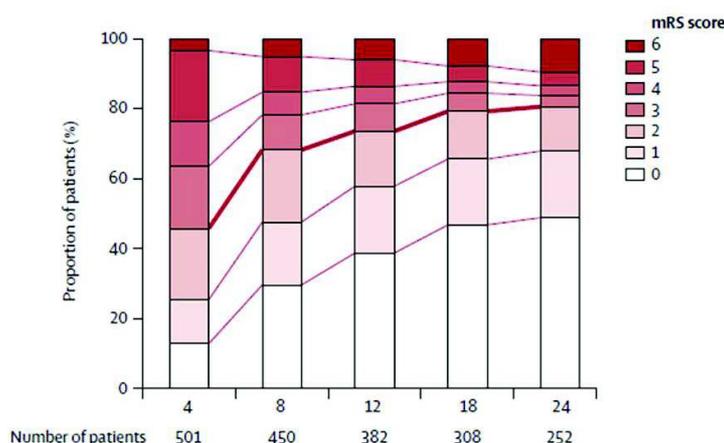


Figure 3: Clinical outcome of NMDAR-AE from the largest case serie of NMDAR-AE patients published in 2013 by Titulaer & coll. A mRS score of 0 indicates absence of any symptoms; 1: no significant disability,

ability to carry out all usual activities; 2: slight disability; 3: moderate disability but ability to walk unassisted; 4: moderate to severe disability, unable to walk unassisted; 5: severe disability, requiring constant nursing care; 6: death. Adapted from Titulaer *et al.* 2013.

II.8. RECOVERY & RELAPSES

Recovery from anti-NMDAR encephalitis is typically slow (Dalmau *et al.* 2011), occurring in stages following the reverse order from symptoms appearance (Dalmau *et al.* 2011). Relapses occur in 12 to 25% of patients (Dalmau *et al.* 2011, Titulaer *et al.* 2013). Relapses are often separated by month or year and are usually less severe than the initial episode (Dalmau *et al.* 2011, Titulaer *et al.* 2013). The use of immunotherapy decreases the risk of relapses and patient with a tumor treated early have a lower frequency of relapse (Dalmau *et al.* 2011, Titulaer *et al.* 2013).

II.9. MORTALITY & CAUSES OF DEATH

Estimated mortality for NMDAR-AE is around 5% (Dalmau *et al.* 2011, Titulaer *et al.* 2013), with reported causes of death including refractory status epilepticus, sudden cardiac arrest, acute respiratory distress, tumor progression and sepsis (Dalmau *et al.* 2011).

II.10. ANTIBODY SUBTYPES & CLINICAL RELEVANCE

Autoantibodies against the NMDAR specific of NMDAR-AE are immunoglobulins G (IgG-NMDAR-Abs). IgG1 is the subtype predominantly found in NMDAR-AE patients' CSF, either alone or combined with other IgG subtypes (e. g. IgG1 and IgG3; Tüzün *et al.* 2009). IgG3 alone have also been reported, but no IgG4 (Tüzün *et al.* 2009). However, other types of Ig directed against NMDAR have been described in the serum and/or CSF of patient with NMDAR-AE and other neurologic disorders (Prüss *et al.* 2012, Desestret, Chefdeville *et al.* 2015). Notably, IgA-NMDAR-Abs can be found in the CSF of NMDAR-AE patient in addition of IgG-NMDAR-Abs (Desestret, Chefdeville *et al.* 2015). IgM and IgA against the NMDAR have also been described in the serum of patients with mild cognitive impairment, dementia and schizophrenia and autoantibodies directed against the NMDAR have been detected in the serum of up to 3% of healthy individuals (Doss *et al.* 2014). Thus, the clinical relevance of NMDAR-Abs, especially if identified in the serum, in neurologic diseases other than NMDAR-AE remains unclear and the presence of IgG-NMDAR-Abs in the CSF appears to be only consistent hallmark of NMDAR-AE. Presence of these autoantibodies reflects an immunization process against the NMDAR, a glutamate receptor widely expressed throughout the CNS and a key player in normal brain function.

III. THE N-METHYL-D-ASPARTATE RECEPTOR

NMDAR are synaptic receptors belonging to a family of cation-permeable ion-channel receptors named ionotropic glutamate receptors (iGluRs), which can itself be subdivided into three families: NMDA receptors, AMPA receptors and kainate receptors (Traynelis *et al.* 2010).

NMDARs exhibit specific properties that distinguish them from other iGluRs and ligand-gated ion channel. First, their ion-channel is subjected to a voltage-dependent block by Mg²⁺ ion; second, their activation not only requires the presence of glutamate but also of a co-agonist (glycine or D-serine); third, they are highly Ca²⁺-permeable; fourth, they display an unusually slow kinetic of deactivation due to slow glutamate unbinding (Paoletti *et al.* 2013). Consequently, NMDAR activation induces a massive influx of Ca²⁺ in synapses but requires binding of the co-agonist and simultaneous glutamate release and membrane depolarization. This confers them the property of being co-incidence detector, able to activate only when the pre-synaptic element of an excitatory synapse release glutamate and the post-synaptic membranes is depolarized. NMDARs are therefore capable of detecting and “*converting specific patterns of neuronal activation into long-term changes in synapse structure and function that are thought to underlie higher cognitive function*” (Paoletti *et al.* 2013), such as learning and memory.

III.1. NMDAR STRUCTURE AND EXPRESSION

III.1.1. NMDAR subunits and genes

NMDARs assemble as heterotetramers that differ in subunits composition (*cf.* Fig. 4). To date, seven different subunits have been identified and classified into three categories based on their sequence homology: the GluN1 subunit, four distinct GluN2 subunits (GluN2A, GluN2B, GluN2C and GluN2D) encoded by four different genes, and two GluN3 subunit (GluN3A and GluN3B) arising from two separate genes. The GluN1 subunit is encoded by a single gene but has eight isoforms (GluN1-1a to GluN1-4a and GluN1-1b to GluN1-4b) formed by alternative splicing. Typical NMDAR heterotetramers function as an assembly associating two obligatory GluN1 subunits with two GluN2 subunits or a combination of GluN2 and GluN3 subunits (Paoletti *et al.* 2013). Though di-heteromeric GluN1/GluN2A and GluN1/GluN2B receptors represent an important fraction of juvenile and adult NMDARs, tri-heteromeric GluN1/GluN2A/GluN2B receptors also populate many regions of the adult brain, especially the hippocampus and cortex, with an estimated abundance ranging from 15% to >50% of the total NMDAR population (Al-Hallaq *et al.* 2007).

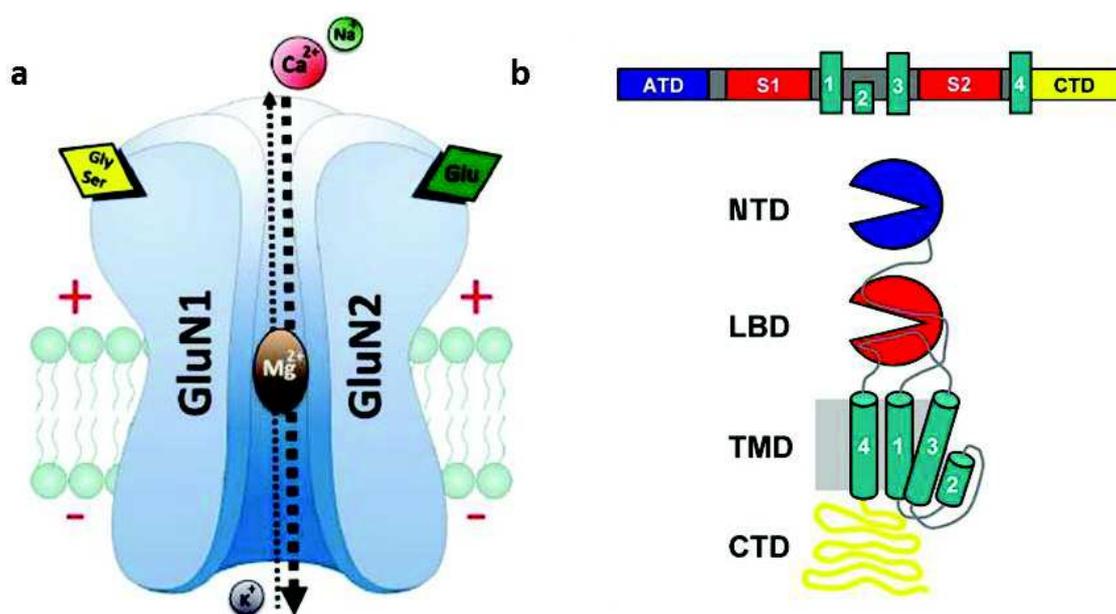


Figure 4: Schematic representation of the N-methyl-D-aspartate receptor and subunit structure. (a) At resting membrane potential, the NMDAR canal is blocked by an ion Mg^{2+} . NMDAR activation requires presence of a co-agonist, liberation of glutamate by the pre-synaptic element and generation of an excitatory post-synaptic potential. (b) Structure of an NMDAR subunit. NTD: N-terminal domain, LBD: ligand-binding domain, TMD: transmembrane domain, CTD: C-terminal domain. Wyllie *et al.* 2013.

III.1.2. NMDAR conformation

NMDAR subunits exhibit a modular structure similar to other iGluRs subunit. Each NMDAR subunit is comprised of two extracellular domains, the N-terminal domain (NTD) which exhibit a bilobar “clamshell-like” conformation and the ligand-binding domain (LBD, containing the binding sites for glycine or glutamate); one transmembrane domain (TMD) comprised of three membrane-spanning helices (M1, M3 and M4) and a re-entrant pore loop (M2) and a large intracellular C-terminal domain (CTD) (Traynelis *et al.* 2010). Contrary to other iGluRs, the NTD of NMDARs contains numerous binding sites for ions and small molecules, and is required to interact with other extracellular proteins (Traynelis *et al.* 2010). The CTD is the least conserved domain between NMDAR subunits and thus contains several subunit-specific regulation sites involved in receptor localization, trafficking and coupling to signaling molecules.

III.1.3. NMDAR expression patterns and properties

NMDAR subunit composition varies across CNS region during development (*cf.* Fig. 5). The obligatory GluN1 subunit is ubiquitously expressed from embryonic stage E14 to adulthood. The four GluN2 subunits show drastically different spatiotemporal expression patterns. Only GluN2B and GluN2D subunits are expressed in the embryonic brain. A developmental switch from GluN2B to GluN2A expression occurs after birth: GluN2A expression starts shortly after birth and increases steadily to become abundantly expressed in every CNS area in the adult (Monyer *et al.* 1994); concomitant to GluN2A progression, GluN2B expression peaks shortly after birth then becomes less prominent and

Introduction

restricted to the forebrain. GluN2D expression drops markedly after birth and is confined to the diencephalon and mesencephalon in the adult. Finally, GluN2C expression appears late in development (P10) and its expression is restricted to the cerebellum and the olfactory bulb (Monyer *et al.* 1994). GluN3A and GluN3B also display differential spatial expression profiles. GluN3A expression reaches a peak during early postnatal life then slowly declines whereas GluN3 expression progressively rises throughout development. At adult stages GluN3A expression declines while GluN3B is mainly expressed in the cerebellum, making GluN2A and GluN2B the predominant subunits in the higher structures of the adult brain, such as the hippocampus and cortex.

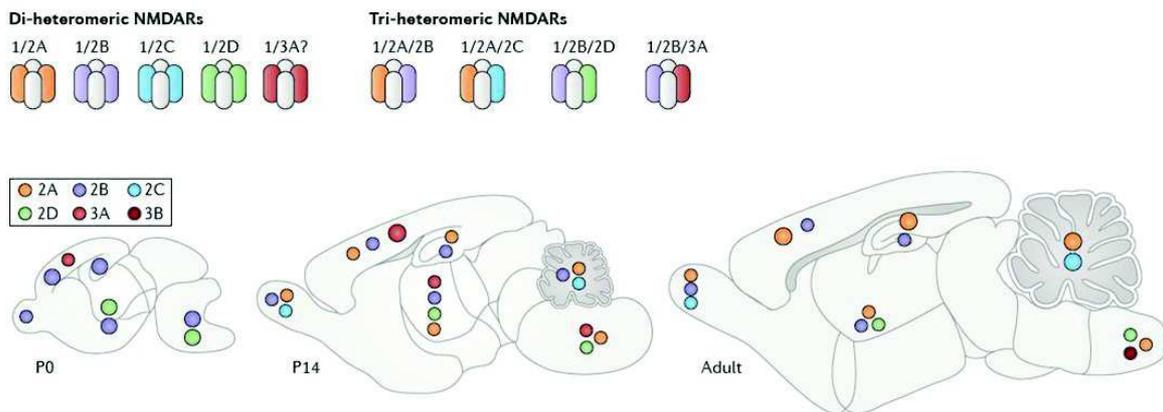


Figure 5: NMDAR-subunits expression pattern throughout development. Paoletti *et al.* 2013.

Subunit composition controls biophysical and pharmacological properties of NMDARs. Briefly di-heteromeric NMDAR containing GluN2A or GluN2B exhibit high conductance (40/50pS) and deactivation rate, high Ca^{2+} permeability and high sensitivity to Mg^{2+} blockade. On the contrary, GluN2A- and GluN2B-containing NMDARs have the lowest sensitivity to glutamate and glycine (Wyllie *et al.* 2013; *cf.* Fig. 6).

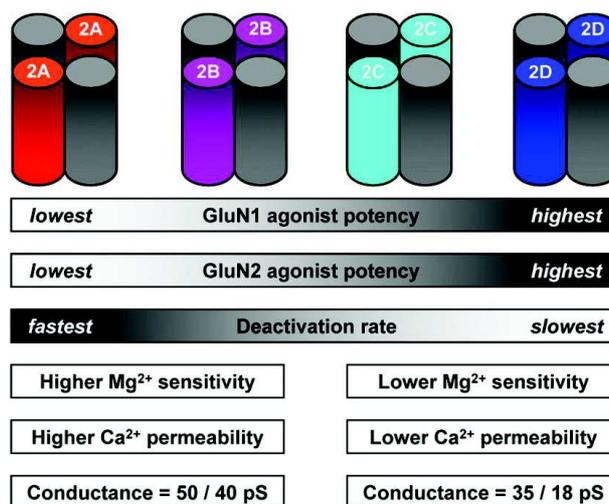


Figure 6: The identity of the GluN2 subunit controls the biophysical properties of NMDAR. Wyllie *et al.* 2013.

III.2. NMDAR IN SYNAPTIC PLASTICITY AND MEMORY

III.2.1. NMDAR-dependent long-term potentiation

Historically, the notion that synaptic strength changes during learning and memory was proposed by Cajal in 1911 (Cajal 1911), who believed that memory involved changes in the strength of neuronal connection, and refined by Hebb in 1949 (Hebb 1949), who proposed that synaptic strengthening occurred as a consequence of coincidental activity of pre- and post-synaptic elements. Bliss and Lomo launched the research field of long-term potentiation in 1973 in an article describing the rapid and persistent increase in the strength of hippocampal excitatory synapses following a brief, high-frequency stimulation (referred to as tetanus; Bliss & Lomo 1973; for a detailed historical review of LTP see Nicoll, 2017). Overall, long-term potentiation is a strengthening of synaptic connection producing a long-lasting increase in signal transmission between two neurons. Nowadays, multiple forms of LTP have been described (e. g. cerebellar LTP, Grasselli & Hansel, 2014). We will focus on NMDAR-dependent LTP in the hippocampus as this form of LTP has been thoroughly investigated (Bliss & Collingridge, 1993) and is highly relevant to NMDAR-AE as an encephalitis affecting the limbic system function. Due to their unique activation mechanisms, requiring both pre-synaptic release of glutamate and post-synaptic depolarization, NMDAR are the perfect “coincidence detectors”. Involvement of NMDAR LTP was demonstrated by Collingridge and coll. in 1983, even though basal synaptic transmission was found to rely mainly on AMPAR (Collingridge *et al.* 1983). As NMDAR are highly permeable to Ca²⁺, synaptic NMDAR activation allows a massive influx of Ca²⁺ into the post-synaptic element, usually located on a dendritic spines (Gray 1959). This event is referred to as LTP induction. Ca²⁺ entry through NMDAR activates CaMKII which is supposed to be both necessary and sufficient for LTP (Lisman *et al.* 2012). CaMKII interacts with AMPAR and PSD scaffolding proteins to promote AMPAR insertion at the synapse their insertion in the PSD (Hayashi *et al.* 2000; *cf.* Fig. 7). Notably, LTP is accompanied by an enlargement of dendritic spines (Matsuzaki *et al.* 2004) at least partially caused by CaMKII’s interaction with cytoskeleton (Okamoto *et al.* 2007). As a final step, long-term potentiation induces insertion of AMPAR into silent synapses (i. e. inactive synapses containing only NMDAR; Isaac *et al.* 1995, Liao *et al.* 1995) or promotes AMPAR insertion into already functional synapses (Oliet *et al.* 1996).

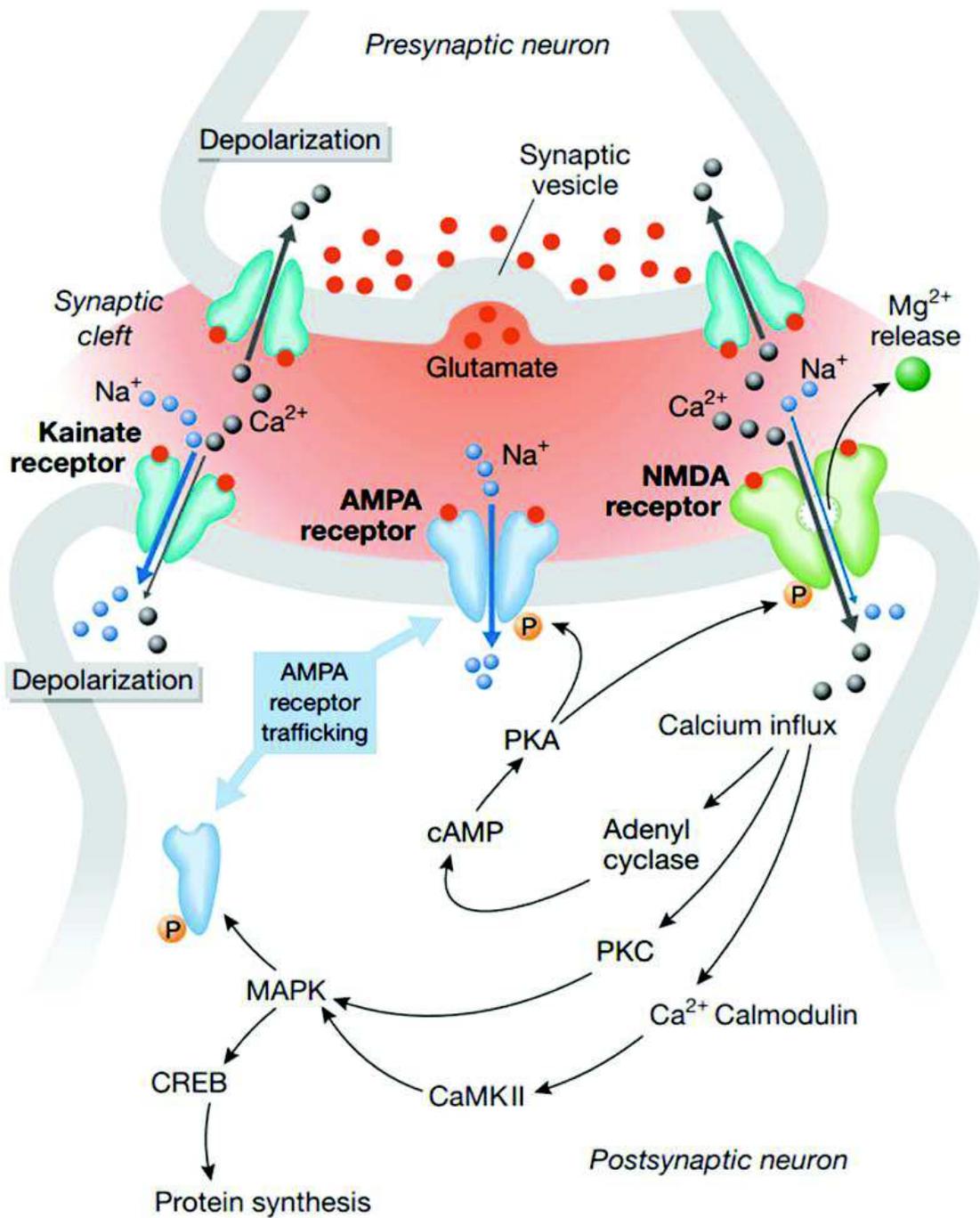


Figure 7: Glutamatergic receptors and synaptic plasticity pathways. From Voglis & Tavernarakis *et al.* 2006.

III.2.2. NMDAR, from long-term potentiation to memory

Long-term potentiation possesses all the characteristics expected of a cellular and molecular mechanism underlying learning and memory. Several studies were performed to establish a clear link between these phenomena. Interestingly, long-term potentiation induction and conditioned fear learning induced identical changes in field-potentials responses recorded in the lateral amygdala (Rogan *et al.* 1997). Ventricular administration of the NMDAR antagonist D-AP5 impaired spatial memory in rats (Morris *et al.* 1986). Finally, though knocking-out GluN1 expression is lethal in neonatal stages (Forrest *et al.* 1994) inducible knock-down of GluN1 suggested involvement of NMDAR in different steps of declarative and non-declarative memory (Cui *et al.* 2004, Cui *et al.* 2005). Evidence supporting a role for LTP as a basis for memory also comes from models of pathology, such as Alzheimer's disease (AD). Amyloid- β ($\alpha\beta$) alters NMDAR proper function (notably NMDARs' synaptic expression and trafficking; Snyder *et al.* 2005), impairs LTP *ex vivo* and interferes with the memory of a learned behavior when administered to rats (Shankar *et al.* 2008) for example.

III.3. NMDAR TRAFFICKING & INTERACTIONS WITH SYNAPTIC PROTEINS

III.3.1. NMDAR localization and surface diffusion

NMDAR can be present both at synaptic and extrasynaptic sites but tend to differ in their subunit composition. Neurons express GluN2B early in development (Monyer *et al.* 1994) both at synaptic and extra-synaptic sites (Tovar & Westbrook, 2016) while GluN2A-containing NMDARs appear later in development and appear to be more restricted to synapses, though they can also be present in the extrasynaptic membrane (Groc *et al.* 2006, Thomas *et al.* 2006). Studies on dissociated neuronal cultures found rapid exchanges between synaptic and extrasynaptic NMDARs subpopulations indicating existence of a dynamic organization of synaptic NMDAR in post-synaptic complexes (Bard *et al.* 2010, Tovar & Westbrook, 2002). However, little evidence of exchanges between synaptic and extrasynaptic pools were found in an *ex vivo* study using acute hippocampal slices (Harris & Pettit, 2007). Surface mobility of NMDARs differs according to the identity of the GluN2 subunit, with GluN2A-containing NMDARs being less mobile than GluN2B-containing ones both at synaptic and extrasynaptic sites (Groc *et al.* 2006). Moreover, GluN2B-NMDARs residency time at mature synapses is lower than GluN2A-NMDARs (Groc *et al.* 2006) due to differences in the interaction with scaffolding proteins between the two subunits.

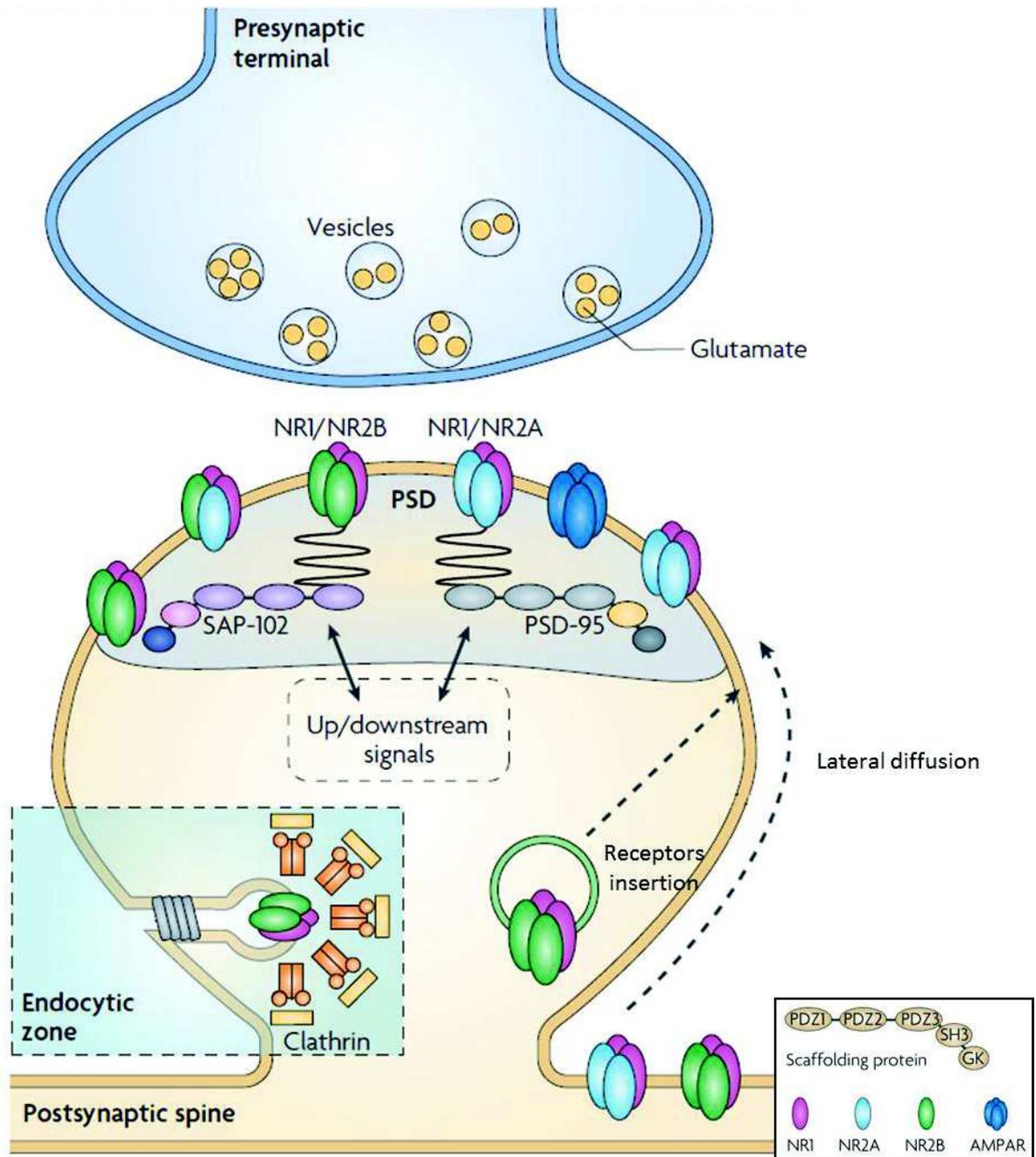


Figure 8: NMDAR are part of macromolecular complexes at excitatory synapses. NMDAR are associated with scaffolding proteins (e. g. PSD-95 or SAP-102) that regulate their lateral diffusion and link them directly or indirectly to a variety of signaling protein. Adapted from Lau & Zukin *et al.* 2007.

III.3.2. Proteins interacting with NMDAR at the synapse

At excitatory synapses, NMDARs associate with the scaffolding proteins of the post-synaptic density (*cf.* **Fig. 8**). In particular, GluN2A and GluN2B subunit of NMDAR contain a PDZ-binding domain in their C-terminus region allowing them to interact with MAGUK's PDZ domains but are also able to interact with MAGUKs independently of PDZ domains ([Chen *et al.* 2012](#)). MAGUKs PSD-95 and SAP-102 are present in most mature synapses. Under resting conditions PSD-95 forms almost immobile and organized structures in the postsynaptic density. SAP102 is more mobile and its expression extends beyond the postsynaptic density into the cytoplasm ([Zheng *et al.* 2010](#)). Their involvement in binding and stabilization of iGluRs at synapses has been extensively studied. Notably, knock-down of PSD-95 and/or SAP102 reduces both AMPAR and MMDAR-mediated baseline synaptic currents ([Levy *et al.* 2015](#)), diminishes the size of post-synaptic densities and increases the number of silent synapses ([Chen *et al.* 2015](#)). SAP102 is also involved in NMDAR trafficking prior to synaptic insertion ([Sans *et al.* 2005](#)) and in clearance of GluN2B-containing NMDAR from synapses ([Chen *et al.* 2012](#)). CaMKII is another critical interactor of NMDARs involved into synaptic plasticity ([Giese *et al.* 1998](#)) through its interaction with de C-terminal region of GluN2B ([Bayer *et al.* 2001](#), reviewed in [Lisman *et al.* 2012](#)). Mutated form of GluN2B preventing this binding impair LTP ([Barria & Malinow, 2005](#)). NMDARs also interact with other synaptic proteins through their extracellular domain. In particular, EphB2 receptors (EphB2R) interact with the extracellular domain of GluN1 ([Dalva *et al.* 2000](#)). EphB2R are transmembrane proteins with a tyrosine kinase activity dependent on liaison with their ligand named EphrinB2 ([Brückner *et al.* 1997](#)) that localize at the post-synaptic density in hippocampal neurons ([Sheffler-Collins & Dalva, 2012](#)). Notably, EphrinB2 treatment in cultivated neurons induces clustering of NMDAR at the synapse and recruitment of CaMKII ([Dalva *et al.* 2000](#)). Finally, a study revealed that EphB2R expression could be impaired by a β oligomers (a β o) and that this reduction of EphB2R expression reduced NMDAR currents and impaired hippocampal LTP ([Cissé *et al.* 2011](#)). Rescuing EphB2R expression restored NMDAR-dependent LTP and reversed memory impairments in an AD mouse model ([Cissé *et al.* 2011](#)). This elegant study by Cissé and coll. emphasizes the importance of NMDAR and its interactions with partner proteins on brain function, behavior and pathology.

III.4. NON-NEURONAL NMDAR

Albeit NMDAR are classically described as synaptic receptors, they are also expressed in glial cells, e. g. astrocytes and microglial cells, but also in non-neural cell types (reviewed in [Hogan-Cann & Anderson, 2016](#)). NMDAR expressed in glial cells display distinct structures and properties than neuronal NMDAR. Astrocytes express tri-heteromeric NMDAR comprised of GluN1, GluN2C or GluN2D and a GluN3 subunit ([Palygin *et al.* 2011](#)). This structure confers them a low sensibility to channel Mg²⁺ blockade allowing channel permeability even at resting membrane potentials ([Lalo *et al.* 2006](#), [Palygin *et al.* 2011](#)). Involvement of astrocytic NMDAR in gliotransmitter-mediated modulation of synaptic activity has been suggested. Experiments performed on cultured astrocytes also suggest that astrocytic NMDAR may also be involved in neuroinflammation processes by contributing to morphological changes observed in reactive astrogliosis and release of pro-inflammatory cytokines ([Gérard & Hansson, 2012](#)). NMDAR are also thought to be expressed by microglial cells. Several studies report expression of functional NMDAR

Introduction

by cultured microglial cells obtained from rodents at post-natal day 0 or 1 (Kaindl *et al.* 2012, Murugan *et al.* 2011). Recently, Kaindl and coll. suggested that NMDAR were also expressed by microglial cells in the mature rodent and human (Kaindl *et al.* 2012). However the lack of electrical response from microglia to localized puffs of either glutamate or NMDA in *ex vivo* hippocampal slices from adult mice lead to question this assertion (Eyo *et al.* 2014). To date, expression of NMDAR by mature microglial cells remains controversial. Finally, expression of GluN2B in normal human oocytes has been suggested (Tachibana *et al.* 2013). A more recent study did not report NMDAR expression in the healthy ovary but revealed NMDAR expression in ovarian carcinoma (North *et al.* 2015). Possible NMDAR expression in ovary is of particular interest in the context of NMDAR-AE as an ovarian teratoma is commonly found in young female patients.

IV. ANTI-NMDAR ENCEPHALITIS: INVESTIGATING PATHOGENIC MECHANISMS

IV.1. EPIOTOPE TARGETED BY NMDAR-Abs

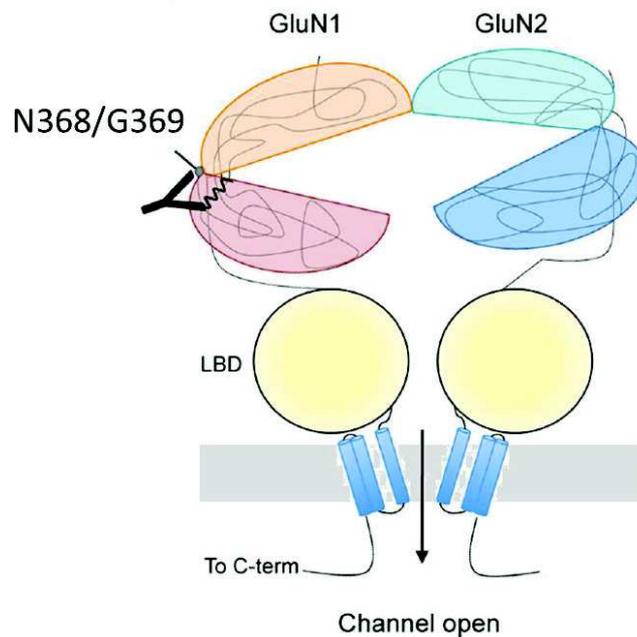


Figure 9: Proposed MDAR-Abs binding site. The epitope is located near the hinge of the clamshell forming the NTD. Identity of residus N368 & G369 are critical for antibody binding. A nearby α -helix spanning also seems to be involved. However, deletion of the top lobe of the NTD has variable effects on NMDAR-Abs binding, suggesting a degree of antibody heterogeneity. Adapted from Gleichman *et al.* 2012.

Autoantibodies directed against NMDAR target the obligatory subunit GluN1 and antibody binding is independent of the GluN1 splice variant and the NMDAR subunit composition (Dalmau *et al.* 2008, Gleichman *et al.* 2012). Epitope was located in the ATD of the GluN1 subunit (*cf.* Fig. 9), in a small region located near the hinge of the clamshell-like structures (Gleichman *et al.* 2012). Within this region, identity of the amino acid N368 is crucial for antibody binding. Residue N368 is involved in N-glycosylation and deamidation reactions that modify epitope three-dimensional structure altering antibody recognition. Nonetheless, glycosylation does not appear to be necessary of sufficient for antibody recognition (Gleichman *et al.* 2012) and the exact influence of post-transcriptional modifications on the epitope structure remain unclear. Deletion of the α -spanning located in the vicinity of the epitope but far removed in the primary sequence abolishes antibody staining, further strengthening the hypothesis of a conformational epitope (Gleichman *et al.* 2012). Deletion of the top lobe of the ATD however can lead to enhanced, decreased or abolished antibody binding, thus raising

the hypothesis of fairly small immunogenic region yet containing not one but several epitopes (Gleichman *et al.* 2012). Antibodies from different patient subgroups based on demographic or etiological factor showed the same staining patterns against GluN1-N368 mutants, suggesting that all anti-NMDAR encephalitis patients have common epitopic determinants (Gleichman *et al.* 2012).

IV.2. RESEARCHING THE AUTOIMMUNE TRIGGER

Although NMDAR-AE is now a well-characterized disorder, especially on the clinical aspects, mechanisms underlying the outbreak of the autoimmune reaction remain elusive. Observation of subsets of patients sharing common clinical features (e. g. presence of an ovarian teratoma, prior infection by a neurotropic virus) and analogy with other autoimmune disorders provide several hypotheses but the origin of break of the immune tolerance is unknown in a vast majority of cases.

IV.2.1. Association with ovarian teratoma in young female patients

Patients with an underlying neoplasm represent 40% of all NMDAR-AE patients (Titulaer *et al.* 2013). In this subset of patients, 93% are female, primarily aged between 12 and 45 years, and the associated neoplasm is an ovarian teratoma in more than 90% of cases (Titulaer *et al.* 2013), thus defining an homogeneous subgroup of patients sharing a significant clinical feature. This strong association suggests a role of the tumor in the immunopathogenesis of the disease. To the best of our knowledge, all but one histopathological studies of NMDAR-AE-associated ovarian teratoma demonstrated presence of nervous tissue and NMDAR expression in the associated ovarian teratomas (Dalmau *et al.* 2008, Tüzün *et al.* 2009), which might trigger the autoimmune reaction. However, 30% to 50% of sporadic ovarian teratoma contain nervous tissue (Marcial-Rojas *et al.* 1958) and studies report NMDAR expression in healthy ovary (Tachibana *et al.* 2013) and ovarian teratoma without NMDAR-AE association (Tabata *et al.* 2013), and ovarian teratoma are relatively frequent tumor whereas NMDAR-AE remains a rare disease. The mere presence of nervous tissue expressing NMDAR is not sufficient to induce the production of NMDAR-Abs (Mangler *et al.* 2013, Tabata *et al.* 2014), suggesting specificities of ovarian teratoma in NMDAR-AE patients. To date, histopathological investigations of associated teratomas have remained limited, due to the relative rarity of the disease. In the largest serie of 11 tumors by Tüzün *et al.* (Tüzün *et al.* 2009) as in the smaller ones (3 to 5 studied tumors by serie) (Dabner *et al.* 2012, Day *et al.* 2014, Tabata *et al.* 2014), NMDAR-AE-associated teratomas distinguished from control teratomas by marked intratumoral lymphoid infiltrate that colocalizing with nervous tissue. Interestingly, dysplastic neurons closely associated with prominent inflammatory infiltrates have been observed in 4 cases of NMDAR-AE-associated teratomas (Day *et al.* 2014). Interaction between abnormal neural elements and the immune system through lymphoid infiltrates might explain immunization against the NMDAR in a subset of patients but more studies are needed to fully characterize the specific features of NMDAR-AE associated ovarian teratoma.

IV.2.2. Viral hypothesis

Autoimmune disease can develop as post-infectious disorders (e. g. Guillain-Barré syndrome; [Willison et al. 2016](#)). Association of NMDAR-AE with infectious disease susceptible to trigger the breaking of immune tolerance has therefore been investigated. Herpes simplex virus encephalitis is one of the most commonly encountered viral encephalitis in developed countries. HSVE disease course is usually monophasic and includes altered mental status, fever and seizures. HSVE constitute a potential differential diagnosis of NMDAR-AE. CSF examination is required in both cases, with a positive HSV-PCR being indicative of HSVE and detection of NMDAR-Abs being indicative of NMDAR-AE. HSVE became of interest as a potential trigger for NMDAR-AE based on the observation that despite receiving appropriate treatment 10 to 25% of HSVE patients suffer from recurrences of neurological symptoms closely resembling those observed in NMDAR-AE. Interestingly, in most of cases of presumed relapses no replicating virus or viral DNA is detected in brain tissue or CSF ([Sköldenberg et al. 2006](#)). An analysis performed on archived CSF-samples from 44 patients with confirmed HSVE that revealed 11% also had IgG-NMDAR-Abs ([Prüss et al. 2012](#)) in serum or CSF. In 6% of cases, intrathecal production of IgG-NMDAR-Abs, a characteristic feature of NMDAR-AE, was demonstrated ([Prüss et al. 2012](#)). Given the relative rarity of autoimmune encephalitis and viral encephalitis, independent co-occurrence of these conditions appears unlikely ([Prüss et al. 2012](#)). Later study on 5 prospectively identified patients revealed absence of NMDAR-Abs at HSE onset but development of NMDAR-Abs during relapse with negative HSV-PCR and absence clinical evidence of HSVE recurrence ([Armangue et al. 2014](#)), suggesting a link between these entities. Two main hypothesis account for the development of autoantibodies in the context of viral infection: molecular mimicry, or antigen exposure due to nervous tissue damage in an environment primed by inflammation from the viral infection (reviewed in [Galli et al. 2017](#)). Molecular mimicry due to shared epitopes has been described in other autoimmune disorder (e. g. Guillain-Barré syndrome after *Campylobacter jejuni* infection; [Willison et al. 2016](#)) but appears unlikely in this case. Indeed, to this day, no common epitope has been identified. Moreover, patients' wide immune response, not only against NMDAR but also against other antigens ([Armangue et al. 2014](#)) is in line with the release of multiple neuronal antigen secondary to viral-induced nervous tissue damage. Finally the concomitant presence of NMDAR-Abs has been described in cases of Epstein-Barr virus, Human Herpes-virus 6 and Varicella Zoster virus and a case of NMDAR-AE presenting as a relapse of VZV infection has also been reported ([Linnoila et al. 2016](#)). Interestingly, among people contracting these viral infection, only a very small proportion eventually develop NMDAR-AE, thus suggesting specificities in these subjects, possibly genetic predisposition to autoimmunity.

IV.2.3. Genetic

Studies concerning underlying predisposing genetic factor for NMDAR-AE are scarce. A study investigating GRIN1 single-nucleotide polymorphisms, which might affect NMDAR-Abs' epitope and therefore impact disease susceptibility, found no difference in the frequency of SNP variants between NMDAR-AE patients and the general population ([Day et al. 2015](#)). However, as acknowledge by the authors, the study design was only powered to detect or exclude strong magnitude effect of GRIN1 polymorphisms on disease susceptibility and a moderate effect may have been overlooked ([Day et al.](#)

2015). A case of NMDAR-AE in a 3-years-old boy with a microdeletion at the short arm of chromosome 6 including the HLA-DPB1 and HLA-DPB2 genes was reported in 2011 (Verhelst *et al.* 2011). Link between autoimmunity and HLA polymorphisms have been reported several diseases but microdeletions of HLA genes has not been linked to autoimmune disorder and this finding has not been confirmed by larger case series. To date, available studies remain inconclusive and no genetic susceptibility to NMDAR-AE have been demonstrated but systematic studies on larges case series are needed to conclude.

IV.3. AUTANTIBODY PATHOGENESIS

Contrary to paraneoplastic neurological syndromes (PNS) associated with onconeural antibodies, in which autoantibodies are biomarkers of the disease without a pathogenic effect, autoantibodies directed against the NMDAR (NMDAR-Abs) are believed to play a causal role in NMDAR-AE. This hypothesis is initially based on clinical observations and has generated intense research over the past decade.

IV.3.1. Clinical arguments

The initial clinical evidence arguing for a pathogenic effect of NMDAR-Abs was the correlation between autoantibody titers and patients' symptoms (Dalmau *et al.* 2007, Dalmau *et al.* 2008). An extensive study on antibody NMDAR-Abs titers in serum and CSF confirmed an overall correlation between disease course and CSF antibody titers (Gresa-Arribas *et al.* 2014), yet also reported that a significant decrease in CSF titres occurs in 30% of cases despite a poor clinical outcome, and that in 26% of patients with clinical relapses, recovery or deterioration were not associated with a significant CSF titres change (Gresa-Arribas *et al.* 2014). Histopathological studies performed on brain biopsy or at autopsy report deposits of IgG and reactive microgliosis, predominantly in the hippocampus (Dalmau *et al.* 2008, Tüzün *et al.* 2009). Lymphocytic infiltrates were scarce but perivascular cuffing mainly comprised of B cells and plasma cells were identified (Tüzün *et al.* 2009). No deposits of complements were found (Tüzün *et al.* 2009). Little evidence of neuronal loss were reported (Tüzün *et al.* 2009). Interestingly, NMDAR-AE bears resemblance to symptoms caused by abuse of NMDAR-antagonist recreational drugs (e. g. ketamine, phencyclidine; Weiner *et al.* 2000) supporting the hypothesis of a clinical syndrome mediated by a disruption of NMDAR function by NMDAR-Abs. Efficacy of therapeutic strategies aiming at reducing the concentration of Ig in the plasma (i. e. plasma exchange) or targeting B lymphocytes (i. e. rituximab) is another argument for NMDAR-Abs pathogenicity. Taken together, there are strong clinical evidence supporting a pathogenic action of NMDAR-Abs, but some discrepancies indicate that there may be some other(s) mechanism(s) involved. For example, studies on chemokines and cytokines in the CSF of NMDAR-AE patients report elevated concentration of CXCL13 (Byun *et al.* 2016, Kothur *et al.* 2016, Leypoldt *et al.* 2015, Liba *et al.* 2016), a B-cell attracting chemokine, but also elevated levels of T-cells related interleukines IL17A, IL6, IFN γ et TNF α and chemokine CXCL10 (Byun *et al.* 2016, Kothur *et al.* 2016, Liba *et al.* 2016) arguing for the involvement of other components of the immune system. Furthermore, patients' long recovery time (Dalmau *et al.* 2011) is not consistent with a "pharmacological action" of NMDAR-Abs alone and suggest a sustained alteration of the neuronal network function either secondary to the exposure to NMDAR-Abs or due to a pathogenic mechanism persisting even after autoantibody removal.

IV.3.2. *In vitro* & *Ex vivo* Experiments

In order to investigate NMDAR-Abs pathogenicity, the first step was to demonstrate their ability to disrupt NMDAR function *in vitro*. Early studies showed that application of patients' CSF or purified IgG from patient's serum on neuronal cultures specifically decreased surface and total GluN1 clusters density on a reversible and titer-dependent fashion (Dalmau *et al.* 2008), without affecting other synaptic components, neuronal morphology or causing neuronal loss (Hughes *et al.* 2010, Moscato *et al.* 2014). Anti-NMDAR antibody binding do not appear to target specifically excitatory or inhibitory neurons (Moscato *et al.* 2014). Antibody-mediated reduction of NMDAR clusters density is independent of the presence of complement (Hughes *et al.* 2010) but appears to rely on the cross-linking ability of autoantibodies. A series of experiments performed by Hughes and coll. revealed that NMDAR-antibodies mediated decrease of NMDAR clusters relies upon the presence of the Fc domain of anti-NMDAR IgG that allows cross-linking of NMDAR (Hughes *et al.* 2010, Moscato *et al.* 2014), suggesting an alteration of NMDAR surface diffusion by anti-NMDAR antibodies. In accordance with those results, subsequent collaborative work between our team and Groc and coll. confirmed the effect of anti-NMDAR antibodies onto NMDAR surface diffusion and revealed a differential effect on GluN2A and GluN2B containing NMDAR (Mikasova *et al.* 2012; cf. Fig. 10). Indeed, patients' antibodies decreased extrasynaptic GluN2B-NMDAR surface mobility but increased surface diffusion of GluN2A-NMDAR which are usually confined to synaptic area, allowing them to laterally escape from synapses and accumulate in the extrasynaptic compartment (Mikasova *et al.* 2012) leading to their internalization and degradation. Moreover, anti-NMDAR antibodies block interaction between NMDAR and EphrinB2 receptor (EPHB2R). Activation of EPHB2R by its ligand EphrinB2 prevents the increased surface diffusion of GluN2A-NMDAR and decreased GluN1 clusters density (Mikasova *et al.* 2012). Binding of anti-NMDAR antibodies to NMDAR do not appear to affect their post-endocytic trafficking in cultured hippocampal neurons (Moscato *et al.* 2014). These studies provide a working model explaining the acute molecular mechanisms underlying NMDA-Abs ability to alter NMDAR function. This ability was confirmed by several studies performing functional investigations *in vitro* using electrophysiological recordings. Whole-cell patch clamp recordings showed that treatment with patients' CSF decreases NMDAR-mediated component of mEPSC with no effect on frequency or amplitude, indicating that presynaptic release probability is unaffected (Hughes *et al.* 2010). Furthermore, decrease of NMDAR mediated current seems to be caused only by internalization of NMDAR receptor and not by an antagonistic effect of patients' antibody binding since (non cross-linking) F(ab) fragment did not affect mEPSC in neuronal hippocampal cultures (Moscato *et al.* 2014). Patients' CSF with titer NMDAR antibodies application for 15 minutes also decreases neuronal network activity *in vitro* (Jantzen *et al.* 2013). Finally, two studies reported an alteration of NMDAR mediated plasticity by NMDAR antibodies as they prevented induction chemical LTP (Mikasova *et al.* 2012) and electrophysiologically induced LTP in neuronal cell cultures (Zhang *et al.* 2012).

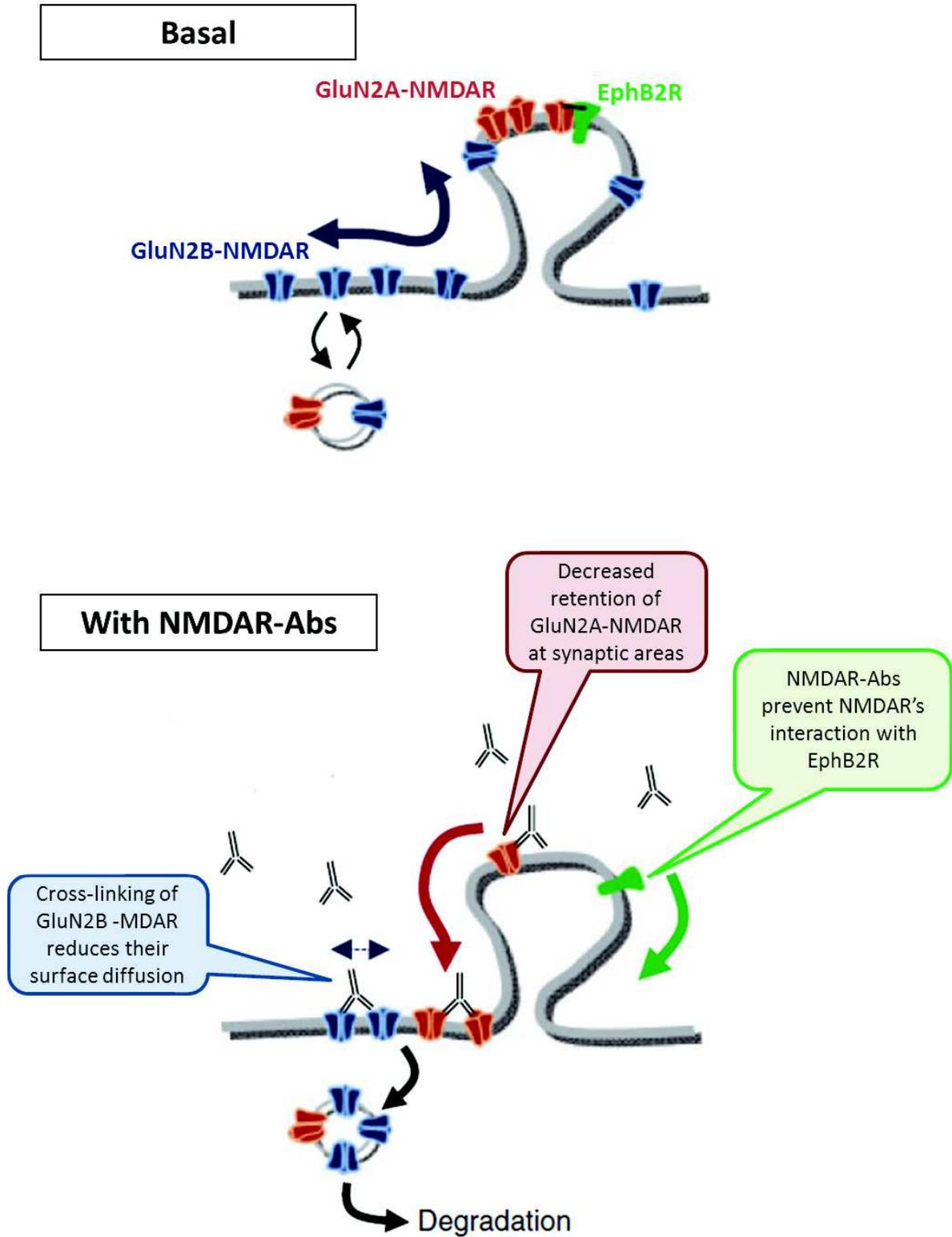


Figure 10: Proposed model of NMDAR-Abs-induced alterations of NMDAR trafficking. Adapted from Mikasova *et al.* 2012.

IV.3.3. *In vivo* Experiments

Effect of NMDAR-Abs *in vivo* was assessed performing intrathecal punctual injection or sustained infusion of patients' CSF, or less often purified IgG, in several studies since 2010. Separate authors reported a reduction of NMDAR clusters after intrathecal administration of NMDAR-Abs from patients' CSF or serum (Hughes *et al.* 2010, Mikasova *et al.* 2012, Planaguma *et al.* 2014). Notably, a study performing continuous intrathecal infusion of patients' CSF during 14 days revealed a behavioral effect of NMDAR-Abs presenting resemblance to the clinical syndrome and correlated to the kinetic of reduction of NMDAR clusters. Briefly, standardized tests to assess memory, anhedonic behavior, depressive-like behavior, anxiety, aggressiveness and locomotor activity were performed. Results showed gradually increasing memory deficits that were maximal on day 18 (4 days after patients' CSF infusion was stopped), depressive like behavior and anhedonic behavior. No effect NMDAR antibodies were found on anxiety, aggressiveness and locomotor activity (Planaguma *et al.*, 2014). Hippocampal LTP was altered by patients' CSF infusion (Planagumà *et al.* 2016). These effects were reversible upon cessation of patients' CSF infusion. Impaired memory (assessed by a Morris water maze) and hippocampal LTP alteration was also reported after a single injection of NMDAR-AE patient's CSF into rat dentate gyrus (Würdemann *et al.* 2016). As observed *in vitro*, EphrinB2 reverses NMDAR-Abs induced reduction of NMDAR clusters (Mikasova *et al.* 2012, Planaguma *et al.* 2016), rescues NMDAR-dependent LTP and prevents NMDA-Abs induced memory deficits in mice (Planaguma *et al.* 2016). Seizures and abnormal movements, which are two prominent features of NMDAR-AE were not observed in those models. The epileptogenic effect of NMDAR-Abs was specifically investigated in a model of single injection of NMDAR-Abs purified from patients' serum into mice hippocampus (Wright *et al.* 2015). No spontaneous seizures were recorded upon administration of NMDAR-Abs, however injection of both NMDAR-Abs and control antibodies increased sensibility to drug-induced seizures, tough seizures elicited in NMDAR-Abs treated mice were more frequent and more severe (Wright *et al.* 2015). Microdialysis after hippocampal injection of NMDAR antibodies in rats revealed an increase in extracellular glutamate concentration (Manto *et al.* 2010). Furthermore, NMDAR antibodies injection in the motor prefrontal cortex increases motor stimulation after high-frequency stimulation (Manto *et al.* 2011). Both observations support the hypothesis of overactivity of glutamatergic pathways which might play a role in the epileptic activity observed in patients. Taken together, this body of studies suggests that administration of NMDAR-Abs from NMDAR-AE patients' CSF or from patients' serum in some studies can reproduce parts of the clinical syndrome observed in NMDAR-AE, thus arguing for a pathogenic role of NMDAR-Abs.

Objectives

Objectives

This thesis work focused on improving the understanding of the immunopathological mechanisms underlying the recently identified NMDAR-AE. NMDAR-AE is an auto-immune disease affecting the central nervous system. We chose to tackle both the “autoimmune” aspect of the disease by investigating one of the possible mechanisms of immunization and the “neurological” aspect by studying the impact of NMDAR-Abs exposure on brain function in a rodent model.

I. STUDY 1

Though presence of NMDAR-Abs of the IgG subtype in the CSF is the hallmark of NMDAR-AE, another NMDAR-Abs of another subtype, IgA, are also found in the CSF of about 40% percent of patients. I joined an ongoing **study investigating the clinical significance of IgA-NMDAR-Abs in the CSF** that demonstrated that CSF IgA-NMDAR-Abs could be used as a biological marker for the presence of an ovarian teratoma (see “CSF IgZ NMDAR antibodies are potential biomarkers for teratomas in anti-NMDAR encephalitis” by [Desestret, Chefdeville et al. 2015](#) cf. Appendices). In young females, NMDAR-AE is consistently associated with an ovarian teratoma ([Titularer et al. 2013](#)) and this underlying neoplasm was suggested to play a causative role in the breaking of the immune self-tolerance. However, ovarian teratoma are common tumors and are rarely associated with autoimmune disorder, suggesting specificities in NMDAR-AE patients’ teratomas. We thus **investigated the histological specificities of NMDAR-AE associated teratoma in order to clarify their possible involvement in the immunization against the NMDAR.**

II. STUDY 2

In 2014, based on previously published results from our team ([Mikasova et al. 2012](#)) and others ([Dalmau et al. 2008](#), [Hughes et al. 2010](#)), our work hypothesis was that NMDAR-Abs either purified from patients’ serum or in patient’s CSF disrupt NMDAR function and are the major pathogenic element in NMDAR-AE. Thus, we defined three objectives:

1) **Design an experimental animal model of NMDAR-AE and bring further evidence of NMDAR-Abs pathogenicity.** In 2014, a substantial corpus of evidence suggesting a pathogenic role of NMDAR-Abs in NMDAR-AE had been published based on *in vitro* experiments but results obtained *in vivo* were scarce. Thus we aimed at designing an experimental mouse model of NMDAR-AE by continuous passive infusion of NMDAR-Abs to reproduce at least part of NMDAR-AE symptoms in order to prove NMDAR-Abs pathogenicity and to have a model to investigate the impact of NMDAR-Abs on brain function. To evaluate possible pathogenic effect of NMDAR-Abs on mice, we performed behavioral experiments focused on assessment of memory performances as there are evidence of NMDAR involvement in memory processes and clinical observations report memory impairment in NMDAR-AE patients.

2) **Investigate the impact of NMDAR-Abs on NMDAR function, especially on synaptic plasticity.** The decision to focus on synaptic plasticity was based on the general knowledge of NMDAR involvement in

Objectives

these processes (*cf.* Introduction §III.2.1.) and on clinical observations. We used a morphological approach studying the shape of dendritic spines while an electrophysiological study investigating the effect of NMDAR-Abs on LTP was performed in parallel in the laboratory (Bost, 2017).

3) Investigate other possible mechanisms of NMDAR pathogenicity with a focus on microglial cells activation. Microglial cells are the resident immune cells of the central nervous system and several immunopathological studies on NMDAR-AE patients' brain samples report prominent microglial activation (Dalmau *et al.* 2008, Tüzün *et al.* 2009). NMDAR-Abs might affect microglia directly by binding onto microglial NMDAR (as authors have reported that microglial cells express NMDAR, though this is still debated, *cf.* Introduction §III.4.; Kaindl *et al.* 2012, Eyo *et al.* 2014) or interact with microglial cells through their Fc receptor (Vedeler *et al.* 1994). Conversely, microglial cells could be activated indirectly via a cross-talk with neurons, as observed in other pathological conditions (Eyo *et al.* 2014). If confirmed, a further question was to determine if glial cells activation was protective or harmful to neurons in NMDAR-AE.

Material & Methods

I. STUDY 1

I.1. PATIENT'S SAMPLES

To be included, patients had to meet the recognized criteria for limbic encephalitis associated with the presence in CSF of IgG directed against NMDAR ([Dalmau et al. 2008](#), [Titulaer et al., 2013](#)). Between September 2007 and June 2014, the French Reference Center for Autoimmune Encephalitis identified 163 cases of NMDAR-LE. In 37 cases (23%) an associated ovarian teratoma was identified. With standard protocol approval and patient consent, we collected 27 formalin-fixed paraffin-embedded (FFPE) samples of the resected ovarian teratomas. Forty control cases were derived through retrospective review of ovarian teratomas resected in 2013 at the Gynaecology Hospital of Lyon and analysed by the Department of Pathology (Hospices Civils de Lyon, Groupement Hospitalier Est).

I.2. TUMOR PATHOLOGICAL STUDY

Four μm -thick FFPE tissue sections were stained with hematoxylin-phloxine-saffron (HPS). A referent pathologist (IT) assessed the histological features of ovarian teratomas, identified their various mixed germ cell components and analysed the maturity of neuroglial elements when present. When an immature tissue with neural differentiation was present, grading of immature teratomas was realized according to the World Health Organization (WHO) classification. All available slides of each tumor were examined at low magnification and the inflammatory infiltrates in contact or inside the nervous component were assessed semi-quantitatively as absent (0), low (+), moderate (++), or high (+++). Cases containing nervous tissue elements presenting the pathological features of neuroglial tumors were further examined by neuropathologists (K. M., A.V., D.M.) and were described using World Health Organization terminology for neuroglial tumors ([Louis et al. 2016](#)).

I.3. IMMUNOHISTOCHEMICAL STUDIES

Immunohistochemistry was performed on serial sections using automated staining system (BenchMark XT; Ventana and DISCOVERY XT automate; Roche Diagnostic), developed with diaminobenzine, and counterstained with hematoxylin, except for the GluN1 subunit of NMDAR that was immunostained with a manual protocol (detailed in Supplemental method). Inflammatory cells were characterized by performing immunostainings specific for B cells (CD20), T cells (CD3), and mature dendritic cells (DC-Lamp). Neural elements within teratomas were analysed using neuronal markers (neurofilament (NF), chromogranin A (ChromoA) and glial markers (GFAP, Olig2), completed by routine immunomarkers dedicated to glial tumor phenotyping (PS100, EMA, ATRX, IDH1, INA, CD34). Manual multiparametric immunofluorescence staining was performed to detect IgG and IgA producing cells in the GFAP+ glial component. Antibodies used, concentrations, and antigen retrieval procedures are detailed in Supplemental Table 1.

I.4. DNA SEQUENCING

Next-generation sequencing (NGS) was performed for 1 patient on nervous tissue component of a paraffin-embedded teratoma sample as described in the Supplemental method. The following 10 genes were sequenced: *ATRX*, *BRAF*, *CDKN2A*, *HIST1H3B/C*, *H3F3A*, *IDH1*, *IDH2*, *TERT*, *TP53*. Chromosomal deletion on 7q, 7p, 9p, 10p, 10q and 1p/19q co-deletion were also investigated.

I.5. STATISTICAL ANALYSIS

Statistical analysis were performed using the R software version (<https://cran.r-project.org/>). Numerical variable (i. e. age at symptoms presentation) were compared using the Mann-Whitney test. p-values for teratoma maturity, nervous tissue, GluN1 expression and presence of inflammatory comparisons were obtained using the Fisher exact test for contingency tables with Bonferroni correction for multiple testing. p-values inferior to 0.05 before Bonferroni adjustments were considered significant.

II. STUDY 2

II.1. SERUM & CSF SAMPLES OBTENTION

Purified IgG from serum and CSF from either NMDAR-AE patients or control subjects were used. Samples were provided by the French Reference Center of Autoimmune Encephalitis and the NeuroBioTec Hospices Civils de Lyon BRC (France, AC-2013-1867, NFS96-900). Samples selection criteria were: 1) a clear clinical picture of NMDAR-AE excluding cases with atypical clinical presentation; 2) samples obtained at NMDAR-AE presentation before any immunomodulating treatment; 3) strong NMDAR-Abs reactivity assessed in cell-based assay and immunostaining on rodent brain section. All patient samples were obtained from adult female. Control serum samples were obtained from healthy subject by the Etablissement Français du Sang. Control CSF samples (Ctrl CSF) were obtained from the Service de Biochimie (GHE Hôpital Neurologique Pierre Wertheimer) from patients without neurological or inflammatory systemic disease. CSF samples from nine NMDAR-AE patients and 5 control subjects were pooled for experiments.

II.2. SERUM & CSF SAMPLES PROCESSING

For the experiments, two batches of pooled serum samples from 3 NMDAR-AE patients were used and one batch of serum samples from 3 healthy subjects was used. IgG were purified from patients and control serum samples. Serum samples (1mL) were incubated with 1mL Protein-A coated beads (Protein A-Sepharose 4B Fast Flow from *Staphylococcus aureus*, P9424, Sigma) in chromatography column (064046, Dutscher) for two hour at room temperature. After washes, IgG were eluted with glycine (0.1M, pH 2.8) and neutralized in Tris-HCL (1,5M, pH 8.8). IgG purified from serum were then dialyzed (Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 3mL, 87730, Pierce) overnight at 4°C against PBS and sterilized by filtration with 0.22µm filters. CSF samples from patients and control subjects were simple dialyzed (Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 3mL, 87730, Pierce) against PBS overnight at +4°C and sterilized by filtration. For the experiments, pooled CSF samples from 9 patients and 5 control subjects were used. GluN1 antibody titer in the NMDAR-AE CSF samples was assessed in a cell-based assay on HEK cells transfected to express GluN1/GluN2 and was >1/640.

II.3. ANIMALS

All experiments were performed on C57BL/6J male mice, obtained from Janvier Labs and aged 5 weeks at reception. Mice were housed under à 12h/12h light/dark cycle (lights on at 7:00AM), in a temperature-controlled environment (21±2°C) with access *ad libitum* to food and tap water. Animals were granted an acclimation period of 7 to 14 days before the beginning of any experiment. Mice were housed in groups of 5 to 8 individuals in standard plastic cages before surgery. After surgery, mice were housed in individual cages and received daily visits to assess their health, behavior and well-being. All

animal experiments were approved by the Université Claude Bernard Lyon 1 ethic and animal care committee (protocols filed under the numbers DR2013-12 and DR2015-50).

II.4. SURGICAL PROCEDURE

Intra-cerebral infusion of IgG or CSF were performed using osmotic pumps (Alzet) with the following characteristics: model 1007D, volume 100 μ L, flow rate 0.5 μ L/h for 7 infusions and model 2002, volume 200 μ L, flow rate 0.5 μ L/h for 14 infusions. The day before surgery, samples concentration was adjusted to 2mg/mL for purified IgG and to 0.04mg/mL for CSF. The osmotic pump was loaded with the appropriate volume of sample and connected to a brain infusion catheter (Brain Infusion Kit II, Alzet) under sterile conditions and left overnight in NaCl 0.7% at 37°C. The next day, mice were deeply anesthetized with isoflurane (Iso-Vet, Piramal HealthCare) and placed in a stereotaxic frame. Brain infusion catheter was inserted in the medial septum (antero-posterior: Bregma+0.25mm; lateral: Bregma+0.5mm; depth: skull surface-3mm) and secured to the skull with dental cement. For one set of experiments, cannulas were implanted in the lateral ventricle (antero-posterior: Bregma+0.22mm; lateral: Bregma+1mm; depth: skull surface-3mm). The osmotic pump was subcutaneously implanted on the back of the animal. Appropriate infusion site was assessed visually at brain cryo-sectioning and human antibodies diffusion in the brain parenchyma was controlled by immunostaining.

II.5. MINOCYCLINE TREATMENT

Animals received daily intra-peritoneal injection of either minocycline (50mg/kg/day) or vehicle (NaCl 0,9%). Intra-peritoneal injections were performed daily starting the day after surgery and until euthanasia. Injections were performed 90 minutes after light onset and at least 1 hour before behavioral experiments. Minocycline Hydrochloride (M9511, Sigma) solution (5g/L in NaCl 0,9%) was prepared daily and pH was adjusted to 7.4. Minocycline solution and vehicle (NaCl 0,9%) were sterilized by filtration prior to injection.

II.6. BEHAVIORAL EXPERIMENTS

Mice were handled daily for 7 or 14 days before the beginning of behavioral experiments. Fear conditioning was performed at days 5 and 6 after surgery for mice implanted with a 7-days osmotic pump and at days 11 and 12 after surgery for mice implanted with a 14-days osmotic pump. Spatial and object recognition was only performed on mice implanted with a 14-days osmotic pump at day 10 after the surgery. All behavioral experiments were performed between 7:00AM and 11:00AM.

II.6.1. Fear conditioning

The fear conditioning protocol was comprised of 3 sessions for each animal: training, contextual fear conditioning test and auditory fear conditioning (**Fig 11**). Each session lasted 3 minutes. Two different apparatus were used for fear conditioning experiment. We were kindly granted access to the first apparatus by the team "Forgetting" from the Centre de Recherche en Neurosciences de Lyon from 2014

Material & Methods

to the end of 2016 when we acquired our own apparatus. The first apparatus consisted of a square cage with transparent plastic walls and a grid floor wired to a shock generator (Context A). A dim light was provided in the experimental room. For auditory fear conditioning, 3 cage-walls were changed for a wall with circle drawings, a striped wall and a squared one and the grid-floor was replaced by a transparent plastic floor (Context B). Each trial was video-recorded using a camera and freezing, defined as a complete lack of movement besides respiratory movement, was automatically scored using the FearFactor software. The second fear conditioning apparatus was used from the beginning of 2016 and consist of a square-cage (20x20x40cm) with transparent plastic walls and a grid floor wired to a shock generator (Context A), placed inside a soundproof chamber (Ugo Basile). Light inside the conditioning chamber is provided by a white light placed in an upper corner and light intensity was set à 30 Lux. For auditory fear conditioning, 3 cage-walls were changed for a grey wall, a striped one and a squared one and the grid-floor was replaced by a white plastic floor (Context B). Each trial was recorded using a camera (Basler ACE asA1300-60gm) and freezing, defined as a complete lack of movement besides respiratory movement, was automatically scored using the EthoVision XT 11.0 software (Noldus).

In the training session, the mice was placed in a previously unknown environment (Context A) and allowed to explore the cage for 2 minutes. An auditory cue (2800Hz, 60db) was then presented for 30 seconds and co-terminated with a 2 seconds footshock (0.7mA). Mice were returned to their home cage 30s later. Contextual fear conditioning was assessed 24 hours after training. Mice were returned to the training cage (Context A) and monitored during 3 minutes. Auditory fear conditioning was tested 1 hour after the contextual fear conditioning test. Mice were placed inside the altered fear conditioning cage (Context B) and allowed to explore it freely for 1 minute. The auditory cue was then presented for two minutes and freezing was assessed during cue presentation. The chamber was cleaned with 70% ethanol (v/v) before each trial and the olfactory context was modified by cleaning the cage with 1% ammonia (v/v) before each auditory fear conditioning session.

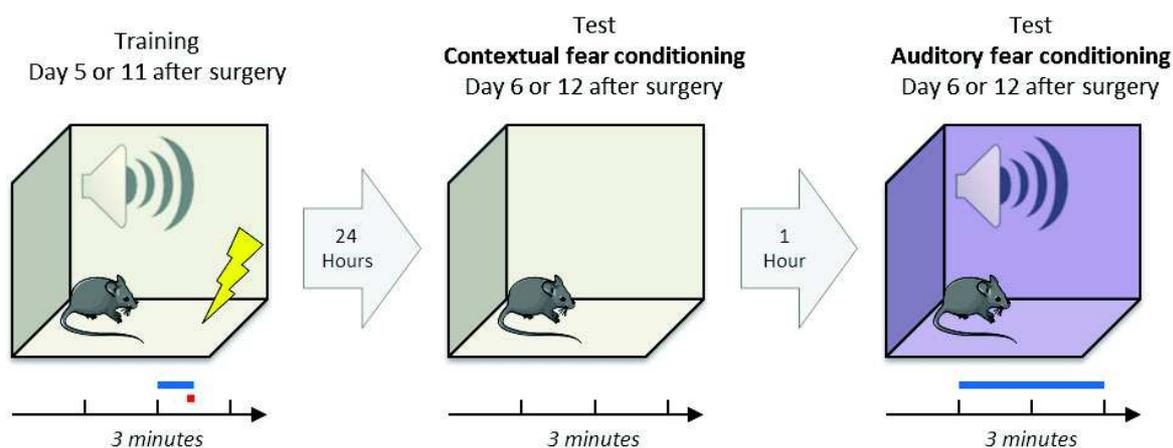


Figure 11: Schematic representation of the fear conditioning test apparatus and protocol. The blue bar represents the sound and the red bar represents the electric shock.

II.6.2. Spatial and object recognition.

The spatial and object recognition protocol was adapted from Buhot & Naili (Buhot & Naili, 1995). Each animal was submitted to seven successive sessions in the arena, each lasting 6 minutes, separated by 3 minutes inter-sessions intervals during which the animal was replaced in its home cage. The arena consisted of a circular open-field (60cm in diameter) with a white floor and transparent 30cm-high plastic walls. Four objects were used: a 50mL glass-bottle half-filled with blue liquid (Object A), a red conical plastic lamp (Object B), a rectangular plastic-brick construction (Object C), and a 40mL plastic culture flask filled with water (Object D). Each session was recorded by a camera (Basler ACE asA1300-60gm) placed above the arena and connected to a video-tracking system (EthoVision XT 11.0, Noldus). The experimenter and the video-tracking system were separated from the experimental room by a white curtain during the experiment. White-noise was diffused to drown out outside noises.

During the first session, the arena remained empty. During sessions 2 to 4, three different objects (A, B and C) were placed at three corners of a virtual square shape inside the arena, the fourth corner remaining empty. In the fifth session, the object C was moved to the diagonally opposed and previously empty corner. No change was made during the sixth session. In the seventh session, the object B was replaced by a novel object (D). During the first session, locomotor activity in an open-field and reaction to a novel environment were analyzed. Object exploration and habituation were assessed during sessions 2 to 4 and session 6. Reaction to the moved-object and the new-objects were measured in session 5 and 7.

Object exploration was automatically scored by the EthoVision XT 11.0 video-tracking software (Noldus). C57BL/6 mice were detected by the system as a black form on a white background. The video-tracking software calculated the barycenter of the form for each image and scored object-exploration when the barycenter of the mice was in a 4 cm radius of the object (Fig 12). However, this type of analysis does not discriminate between actual exploration of the object and any other activity in the defined area (e.g. grooming near an object). Thus, activities others than object exploration in the objects area were scored by a blind-experimenter and subtracted to the amount of time in object area calculated by EthoVision XT 11.0 (Noldus).

To assess the reaction to a spatial change or to a novel object, an exploratory-index of the moved-object or the new-objects was calculated during session 5 and 7 respectively, as follow: the exploration time of the moved or the new object was divided by the mean of the sum of the exploration of the two familiar objects, e.g. $\text{Exploration time}_{\text{ObjectD}} / [(\text{Exploration Time}_{\text{ObjectA}} + \text{Exploration Time}_{\text{ObjectC}}) / 2]$ for the exploratory-index of the moved objects during the seventh session. Notable, as in the protocol published by Léger and coll. mice spending less than 30s exploring the objects during the familiarization session were excluded from further analysis (Leger *et al.* 2013).

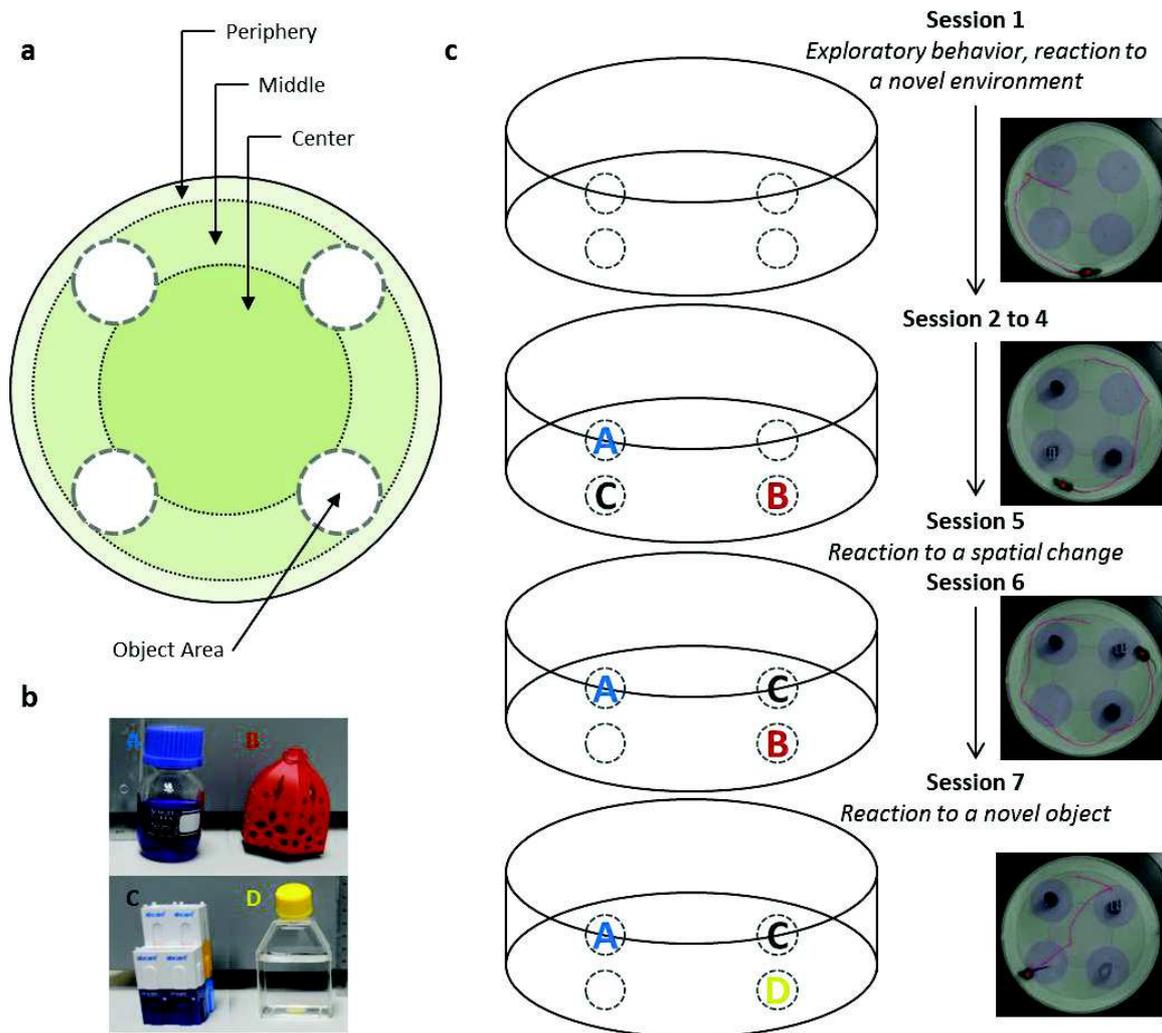


Figure 12: Schematic representation of the spatial change and novel object recognition set-up. The arena is subdivided in several virtual area (a) and mice movements and exploration is video-tracked and scored throughout the different areas. Four objects were selected for the spatial change and novel object recognition experiment (b). The protocol is comprised of 7 sessions in which several behaviors are recorded (c).

II.7. RODENT BRAIN TISSUE IMMUNOSTAINING

After 7 or 14 days of purified IgG or CSF infusion, mice were killed by intra-peritoneal injection of a lethal dose of pentobarbital. For immunostainings, intra-cardiac perfusion of 7mL of iced (+4°C) NaCl 0.9% laced with Heparin followed by intra-cardiac perfusion of cold (+4°C) paraformaldehyde (4% v/v in phosphate buffer 0.1M) was performed. Brains were harvested and post-fixed in 4% paraformaldehyde at +4°C for two hours, cryoprotected in sucrose (30% m/v in PBS) for forty-eight hours at +4°C and cryo-sectioned into 20µm-thick sections. 20µm-thick free-floating sections were immunolabeled for astrocytes and microglial cells markers and human IgG (hIgG) to assess the diffusion of the purified human IgG into the brain parenchyma. For immunostaining astrocytes and microglia, sections were

blocked in 4% NGS, 1% BSA, 0.3% Triton X-100 in PBS at room temperature during 1 hour and incubated with primary antibodies against GFAP and Iba1 (*cf.* **Table 1**) diluted in blocking solution at +4°C *overnight*. After 3 washes in 0.3% Triton X-100 in PBS, slices were incubated in secondary antibodies (*cf.* **Table 1**) diluted in blocking solution at room temperature during 2 hours, washed and counterstained with DAPI. For immunostaining of hIlgG, sections were blocked in 4% NGS, 1% BSA, 0.3% Triton X-100 in PBS at room temperature during 1 hour and incubated with antibody against hIlgG (*cf.* **Table 1**) diluted in 0.3% Triton X-100 in PBS at room temperature during 1 hour, washed, and counterstained with DAPI.

Table 1: Primary and secondary antibodies used for immunostaining of mice brain tissue

Antibody	Manufacturer	Catalogue N°	Host	Dilution	Specific Molecules or Cell Types Stained
GFAP	BD Biosciences	556330	Mouse	1:1000	Astrocytes
Iba1	Wako	019-19741	Rabbit	1:1000	Myeloid cells lineage
Goat-anti-Mouse 555	LifeTechnologies	A21424	Goat	1:1000	Mouse Ig
Goat-anti-Rabbit 488	LifeTechnologies	A11029	Goat	1:1000	Rabbit Ig
Goat-anti-Human 555	LifeTechnologies	A21435	Goat	1:1000	Human Ig

II.8. GLIAL CELLS MORPHOLOGY ANALYSIS

To analyze the morphology of glial cells, five stacks of 20 serial optical sections (Δz 0.24 μ m) were acquired in the ipsilateral (left) hippocampus under a 63x objective lens (Zeiss Plan Apochromat NA1.4) mounted on a Zeiss Imager Z1 microscope equipped with an ApoTome. Analysis of microglial morphology was performed on using the freely available software ImageJ for the preliminary experiment. Briefly, images were filtered for background noise and a maximal intensity projection was performed. Images were then manually thresholded. Images names were coded so that the experimenter was unaware of experimental conditions. The analysis protocol was then modified in order to use an automatic thresholding procedure. Analysis of the area occupied by glial cells and fluorescence intensity was therefore performed using the free software Icy (<http://icy.bioimageanalysis.org/>). A maximal-intensity projection of each stack was made and a thresholding procedure based on the K-mean method was performed to isolate glial cells from background. Area occupied by glial cells and fluorescence intensity were measured as indicators of glial cells activation (Beynon & Walker, 2012).

II.9. ELECTRON MICROSCOPY

Electron microscopy was performed on mice infused during 7 days with purified IgG from either control subjects or NMDAR-AE patients. Mice were deeply anesthetized by intra-peritoneal injection of pentobarbital. Intra-cardiac perfusion of 50mL of an iced (+4°C) solution of paraformaldehyde (2%v/v) and glutaraldehyde (2%v/v) in PBS. Brains were harvested one hour after intra-cardiac perfusion and post-fixed in perfusion solution for four hours at +4°C then transferred in PBS. Sectioning, electron microscopy preparation and imaging was performed by Serge Marty at l'Institut du Cerveau et de la Moëlle Epinière. Morphological measurements of the CA1 region of the hippocampus (height of the *stratum radiatum*, number of neuronal nuclei in the *stratum radiatum*, synaptic density, length of the PSD) were obtained on semi-thick section. Serial thin sections were used to create reconstructions of dendritic spines and allowed analysis of the volume and morphology (**Fig 13a**). Dendritic spines were classified into pedunculated spines (i. e. with a clearly defined 'neck') or sessile spines (i. e. without a clearly defined neck) based on measurement of the width of the head of the spines and of the width of the neck.

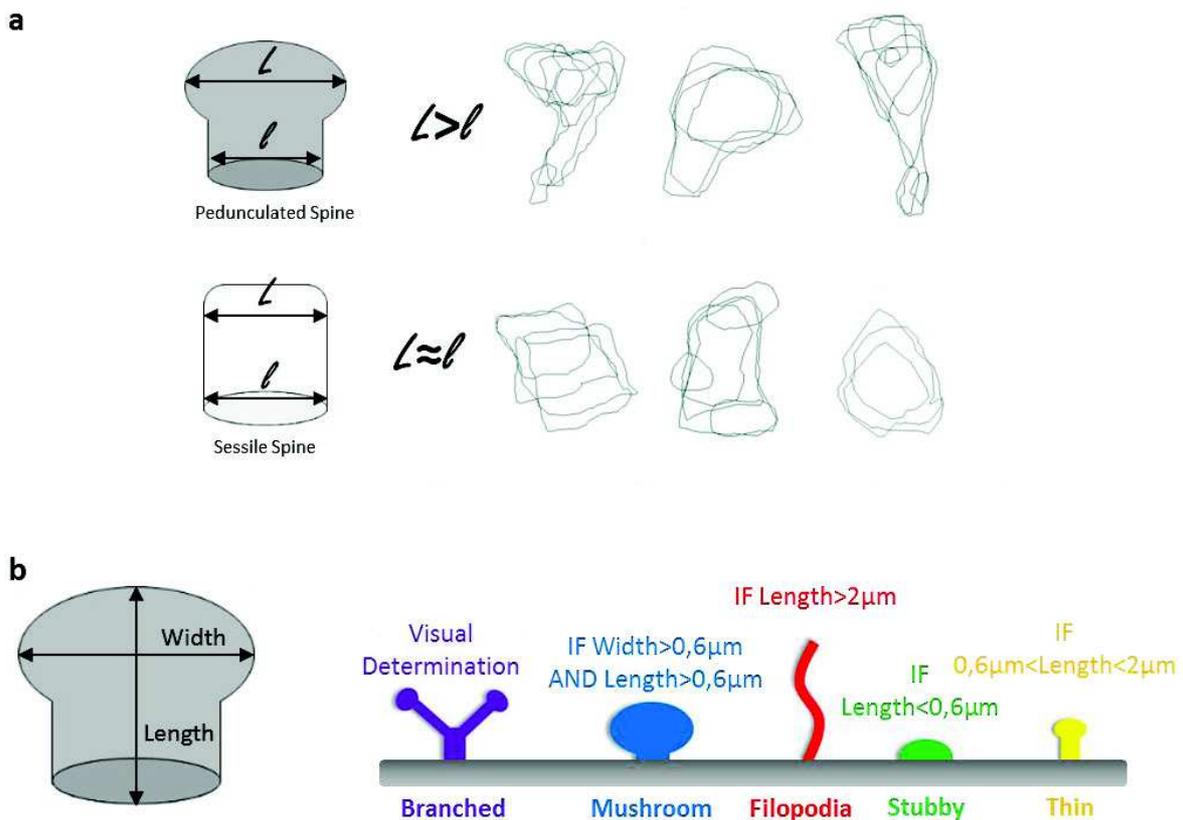


Figure 13: Schematic representation of parameters used for dendritic spine morphology analysis based on MET images or Golgi stainings. Dendritic spine morphology analysis based on MET images (a) allowed classification of spines into sessile or pedunculated spines based on the width of the head of the spines (L) and the width of the neck of the spines (l). Spines stained using the Golgi techniques (b) were classified into 5 categories based on the width of the head and the length of the spine.

II.10. GOLGI STAINING & ANALYSIS

For Golgi staining, mice infused with control or patients' purified IgG during 14 days were deeply anesthetized with isoflurane (Iso-Vet, Piramal HealthCare) and decapitated. Brains were harvested, the two hemispheres were separated and the left hemisphere was stained using the FD Rapid GolgiStain kit (FDNeuroTechnologies) according to the manufacturer instructions. The right hemisphere was snap-frozen by immersion in 2-methylbutane at -35°C during 2 minutes and kept at -80°C for later studies. Golgi staining was performed using the FD Rapid GolgiStain kit (FDNeuroTechnologies) according to the manufacturer instructions. Image stacks were acquired from the CA1 region of the dorsal hippocampus (Bregma+1.28 to Bregma+2.12) using a Leica SP5 confocal microscope equipped with a x63 objective. Based on power-analysis results obtained by Pillai and coll. (Pillai *et al.* 2012), 7 to 9 neurons per mice from 3 mice per experimental groups were analyzed. Spine density and dendritic spine morphology was measured on three to four 15- μ m sections of secondary or tertiary ramification of the main apical dendritic shaft located at least 100 μ m away from the soma (Pillai *et al.* 2012). Analysis of the morphology of dendritic spines was adapted from the method published by Risher and coll. using the freely available Reconstruct software (<http://synapses.clm.utexas.edu>; Risher *et al.* 2014). The head-size and the length of each spine were measured through the image stacks using the Reconstruct software and classified according to their morphology (Fig 13b) in Microsoft Excel using the following equation:

$$=SI(NB.SI(SPINE;"*branch*");"branch";SI(NB.SI(SPINE;"*NA*");"NA";SI((WIDTH>0,6)*(LENGTH>0,6);"mush";SI(LENGTH>2;"filo";SI(LENGTH<0,6);"stubby";SI(LENGTH<2;"thin"))))))$$

Several dendritic spines counts and measurements were performed by were performed to ensure reproducibility of this method and to confirm that the semi-automated classification closely reproduced a visual classification. For analysis, mice ID were encoded and all acquisition and analysis were performed by an experimenter blinded to experimental groups.

II.11. STATISTICAL ANALYSIS

Statistical analysis were performed using the GraphPad Prism software version 7. Numerical variable (i. e. % of freezing) were compared using the Mann-Whitney test when comparing between two groups and with Kruskal-Wallis test with Dunn's post-hoc test for multiples comparison. p-values inferior to 0.05 were considered significant.

Results

I. STUDY 1

Encephalitis with anti-NMDA receptors (NMDAR) antibodies (NMDAR-AE) is a recently described, severe autoimmune neurological disorder, defined by a clinical presentation of encephalitis and presence of CSF IgG targeting the GluN1 subunit of the NMDAR (Dalmau *et al.* 2008). An underlying neoplasm is found in about 40% of patients, primarily in young females, and this associated tumor is an ovarian teratoma in 90% of the cases (Dalmau *et al.* 2011; Titulaer *et al.* 2013). This strong association suggests a role of the tumor in the immunopathogenesis of the autoimmune disease. Histopathological studies reporting presence of neuroglial tissue expressing NMDAR in ovarian teratoma associated with NMDAR-AE (Dalmau *et al.* 2008, Tüzun *et al.* 2009) raised the hypothesis that immunization against the NMDAR might be triggered by NMDAR expression by teratoma neuroglial elements. However, later studies including sporadic teratomas without associated NMDAR-AE shown that presence of an ovarian teratoma with neuroglial tissue expressing NMDAR is not sufficient to induce anti-NMDAR auto-immune response (Tabata *et al.* 2014, Mangler *et al.* 2013), suggesting teratoma specificities in NMDAR-AE patients. To date, histopathological investigations of ovarian teratomas associated with NMDAR-AE have remained limited, due to the relative rarity of the disease. Ovarian teratomas of NMDAR-AE patients seem to distinguish from sporadic ovarian teratomas by marked intratumoral lymphoid infiltrate colocalizing with mature neuroglial elements (Tüzun *et al.* 2009, Dabner *et al.* 2012, Day *et al.* 2014, Tabata *et al.* 2014). To further characterize the pathological phenotype of ovarian teratomas associated with NMDAR-AE, the French Reference Center of Paraneoplastic Neurological Syndromes and Autoimmune Encephalitis has collected twenty-seven NMDAR-AE-associated ovarian teratomas samples and conducted the analysis of their histological and immune cell infiltrate characteristics.

I.1. HISTOLOGICAL CHARACTERISTICS OF NMDAR-AE ASSOCIATED TERATOMAS

Description of the histological features of the clinico-pathological cohort of 27 NMDAR-AE ovarian teratomas are detailed in the **Table 2** and compared with the 40 control sporadic ovarian teratomas in the **Table 3**.

Results

Table 2: Histological features of NMDAR-AE associated ovarian teratomas

Case #	Age at AE Diagnosis	Teratoma	Cystic central nervous tissue/Solid central nervous tissue	Peripheral nerve and ganglia/enteric ganglia	Inflammatory infiltrates in contact with central nervous tissue	Presence of germinal centers in contact with central nervous tissue
1	35	Immature (Grade 1)	Yes/Yes	No/Yes	++	0
2	20	Immature (Grade 2)	Yes/Yes	Yes/No	+++	0
3	19	Immature (Grade 2)	No/Yes	Yes/Yes	+	0
4*	38	Mature	Yes/Yes	No/No	+	0
5*	22	Mature	No/Yes	Yes/No	+	1
6*	15	Mature	Yes/Yes	No/No	+	1
7	32	Mature	Yes/No	No/No	+	0
8	31	Mature	Yes/Yes	No/No	++	0
9	28	Mature	Yes/No	No/No	++	0
10	26	Mature	No/Yes	No/No	++	1
11	44	Mature	Yes/Yes	Yes/Yes	++	0
12	22	Mature	No/No	No/No	Irrelevant	Irrelevant
13	26	Mature	Yes/Yes	No/No	++	0
14	28	Mature	Yes/Yes	No/No	+++	1
15	24	Mature	Yes/No	Yes/No	++	0
16	32	Mature	Yes/No	Yes/Yes	++	0
17	22	Mature	No/Yes	No/No	0	0
18	25	Mature	Yes/Yes	No/Yes	++	1
19	24	Mature	Yes/Yes	Yes/No	+++	1
20	18	Mature	Yes/Yes	Yes/No	+	1
21	15	Mature	No/Yes	Yes/No	+++	1
22	19	Mature	No/Yes	No/No	++	1
23	16	Mature	No/Yes	No/No	++	1
24	25	Mature	Yes/No	No/No	++	1
25	29	Mature	Yes/No	No/No	+++	0
26	23	Mature	No/Yes	No/Yes	+	1
27	28	Mature	No/Yes	No/Yes	+++	1

* Cases presenting histologic features resembling glioma

Results

Table 3: Compared histological features of control and NMDAR-AE associated ovarian teratomas

	NMDAR-AE Teratomas (n=27)	Control Teratomas (n=40)	p-value
Age, y, median (range)	25(15-44)	31(18-53)	0.00104
Side R/L/Bilateral	12/13/1	19/13/7	
<i>1 missing data in each group</i>			
Mature teratoma, n (%)	24/27 (88.9%)	40/40 (100%)	NS
Immature teratoma, n (%)	3/27 (11.1%)	0/40 (0%)	
Nervous tissue, n (%)	26/27 (96%)	15/40 (38%)	4,85.10 ⁻⁷
Central	26/27 (96%)	15/15 (100%)	NS
<i>Solid</i>	19/27 (70%)	9/15 (60%)	NS
<i>Cystic</i>	17/27 (63%)	12/15 (80%)	NS
Choroid plexus	12/27 (44%)	5/15 (33%)	NS
Peripheral	9/27 (33%)	7/15 (46%)	NS
Enteric	6/27 (22%)	1/15 (6%)	NS
Neuronal markers expression by neural element when present, n(%)*			
ChromoA+	10/17 (59%)	6/12 (50%)	NS
NF+	7/17 (41%)	7/13 (54%)	NS
GluN1 expression by neural element, n(%)*			
Neuronal	12/22 (55%)	9/14 (64%)	NS
<i>Nuclear</i>	7/12 (58%)	3/14 (21%)	NS
<i>Cytoplasmic</i>	11/12 (92%)	9/14 (64%)	NS
Glial	16/22 (73%)	4/14 (29%)	0.015
<i>Nuclear</i>	16/16 (100%)	4/4 (100%)	NS
<i>Cytoplasmic</i>	13/16 (81%)	3/4 (75%)	NS
Inflammatory infiltrates in contact to neural element, n(%)*			
<i>B cells (CD20+)</i>	19/23 (83%)	-	
<i>T cells (CD3+)</i>	22/23 (96%)	-	
<i>Mature dendritic cells (DClamp+)</i>	11/24 (46%)	-	
<i>Germinative centers</i>	13/26 (50%)	1/15 (7%)	0.006
Teratomas displaying histological feature of CNS tumours, n(%)	3/26	0/15 (0%)	NS

*Percentages are calculated on the number of assessable cases. NS: not significant.

I.1.1. Grading

Twenty-four out of the 27 analyzed NMDAR-AE ovarian teratomas were mature mixed germ cell tumor (also referred as dermoid cysts), composed of tissue derived from ectoderm, endoderm, and mesoderm (**Fig 14a**). The 3 remaining teratomas associated with NMDAR-AE contained foci of immature neural tissues (neuroepithelial tubules and neural blastema) and were thus diagnosed as immature ovarian teratoma (two grade 2 and one grade 1) (**Fig 14b**). All (40/40) control ovarian teratomas were mature dermoid cysts (**Fig 14c**).

Results

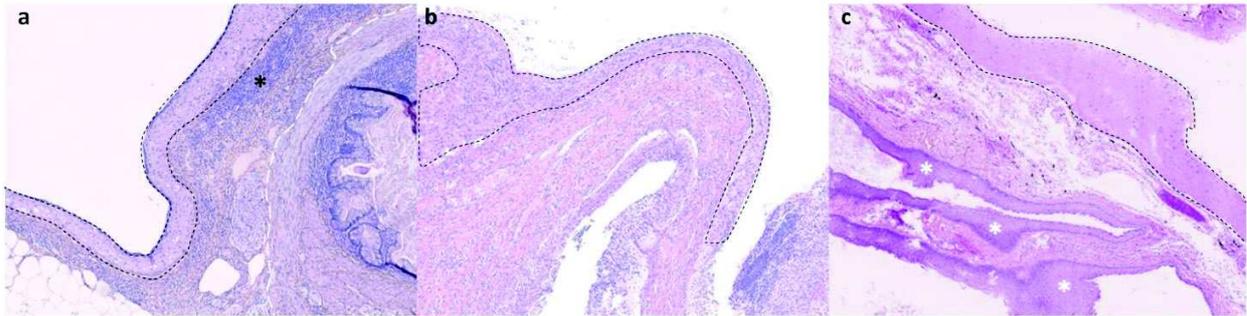


Figure 14: Gross structure of control or NMDAR-AE-associated ovarian teratomas. Ovarian teratomas are multi-tissular tumors. (a & b) Representative samples of mature (a) and immature (b) ovarian teratomas associated with NMDAR-AE. Among others, these samples contain nervous tissue (outlined, black-dotted lines) in close proximity with inflammatory infiltrates (black asterisk), mucosa tissue organized in a digestive track-like manner (outlined, white-dotted line), connective tissue and dermoid epithelia (white asterisks). (c) Example of a control teratoma. Low magnification.

1.1.2. Consistent neural differentiation and neuroglial NMDAR expression in NMDAR-AE associated teratomas

Nervous tissue, was present in all but one NMDAR-AE associated teratoma (26/27, 96%) while only 15/40 (38%) of control teratomas contained nervous tissue ($p < 0.001^{***}$). When present, the nervous component always contained a central nervous tissue differentiation. Central nervous tissue appeared either as a solid component forming a mass or lining the inner wall of a cystic cavity, and could contain choroid plexuses. Presence of solid mass of central nervous tissue was more common in NMDAR-AE-associated teratoma (respectively 19/27 (70%) vs. 9/15 (60%)). Peripheral and enteric nervous tissue could be also present (nerves and ganglia in 9/27 NMDAR-AE-associated teratoma vs. 7/15 control teratomas and myenteric plexuses in 6/27 NMDAR-AE-associated teratoma vs. 1/15 control teratomas). No different frequencies of central, peripheral or enteric neural differentiation were observed between NMDAR-AE-associated and sporadic ovarian teratomas. When present, neural elements were characterized by neuronal (ChromoA and NF) immunostaining revealing ChromoA⁺ neurons and NF⁺ neurites, respectively in 10/17 (59%) and 7/17 (41%) analyzed NMDAR-AE associated teratomas and 6/12 (50%) and 7/13 (54%) control teratomas. All analyzed teratomatous neural elements contained GFAP⁺ astrocytes. Thus, no difference in cell composition, based on these immunostainings for glial and neuronal cells was observed in the nervous tissue, when present.

The expression of the GluN1 subunit of the NMDAR was detected by immunohistochemistry in the neural elements of 18/22 (82%) analyzable NMDAR-AE associated teratoma and 11/14 (79%) analyzable control teratomas. GluN1 was expressed by neuron and glial cells and anti-GluN1 immunostaining was cytoplasmic or nuclear (**Table 3** and **Fig 15a-b**). GluN1 was similarly expressed by neurons in the nervous contingent of teratomas associated with NMDAR-AE and control teratomas (respectively in 12/22 (55%) and 9/14 (64%) of analyzable cases)). GluN1⁺ neurons in neural elements of NMDAR-AE associated teratomas exhibited cytoplasmic GluN1 expression in 11/12 (92%) of the cases, while 9/14 (64%) GluN1⁺ neurons in control teratomas did. Nuclear GluN1 neuronal expression was respectively detected in 7/12

Results

(58%) and 3/14 (21%) of NMDAR-AE-associated and control teratomas containing neural elements. Notably, GluN1 was also expressed by glial cells of astrocytic morphology both in NMDAR-AE associated and control teratoma (**Fig 15c-d**). This glial expression of GluN1 was slightly more common in the nervous tissue of NMDAR-AE associated ovarian teratomas (16/22 (73%) vs. 4/14 (29%), $p=0.015$). Co-immunolabelling of GluN1 and GFAP confirmed nuclear (in all cases) and cytoplasmic expression of GluN1 by astrocytes in respectively 81% and 75% of NMDAR-AE teratomas and control cases) (**Fig15e-j**).

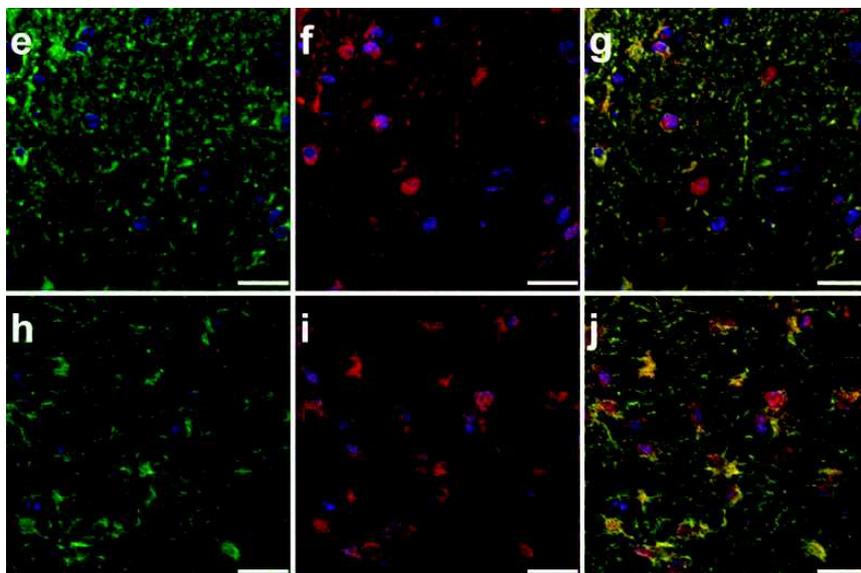
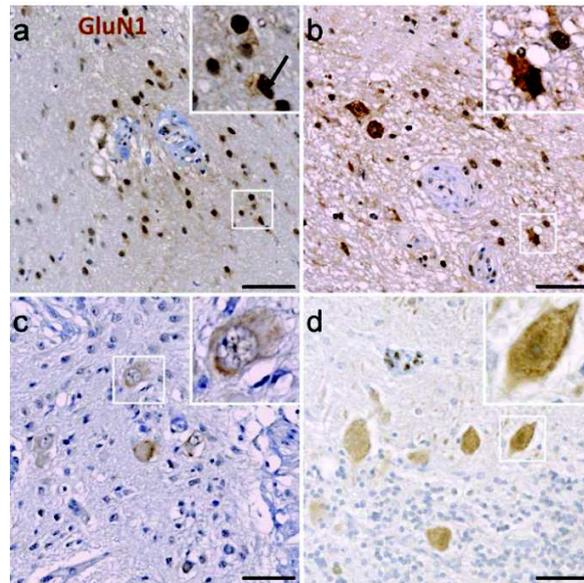


Figure 15: GluN1 expression by neuronal and glial cells in central nervous tissue from NMDAR-AE associated and control ovarian teratoma. Representative GluN1 immunostaining of ovarian teratoma-associated with NMDAR-AE (a,c) or sporadic ovarian teratoma (b, d). GluN1 expression by glial cells and neuropil in a NMDAR-AE associated ovarian teratoma (a). GluN1 expression by astrocytic cells is cytoplasmic but also sometimes nuclear (a, see lenticular nuclear inclusions on magnification, designated

Results

by an arrow). GluN1 expression by glial cells, neuropil and cell bodies of neurons in a sporadic ovarian teratoma (b). Neuronal expression of GluN1 is detected both in NMDAR-AE-associated ovarian teratoma (c) and in control teratoma (d). Note the cerebellar-like organisation of the nervous tissue in (d). Co-immunofluorescence stainings for GFAP and GluN1 (e-j) confirms GluN1 expression by astrocytes in the nervous tissue from ovarian teratoma. Scale bar: 50µm

1.1.3. Glioma-like features in NMDAR-AE teratomas

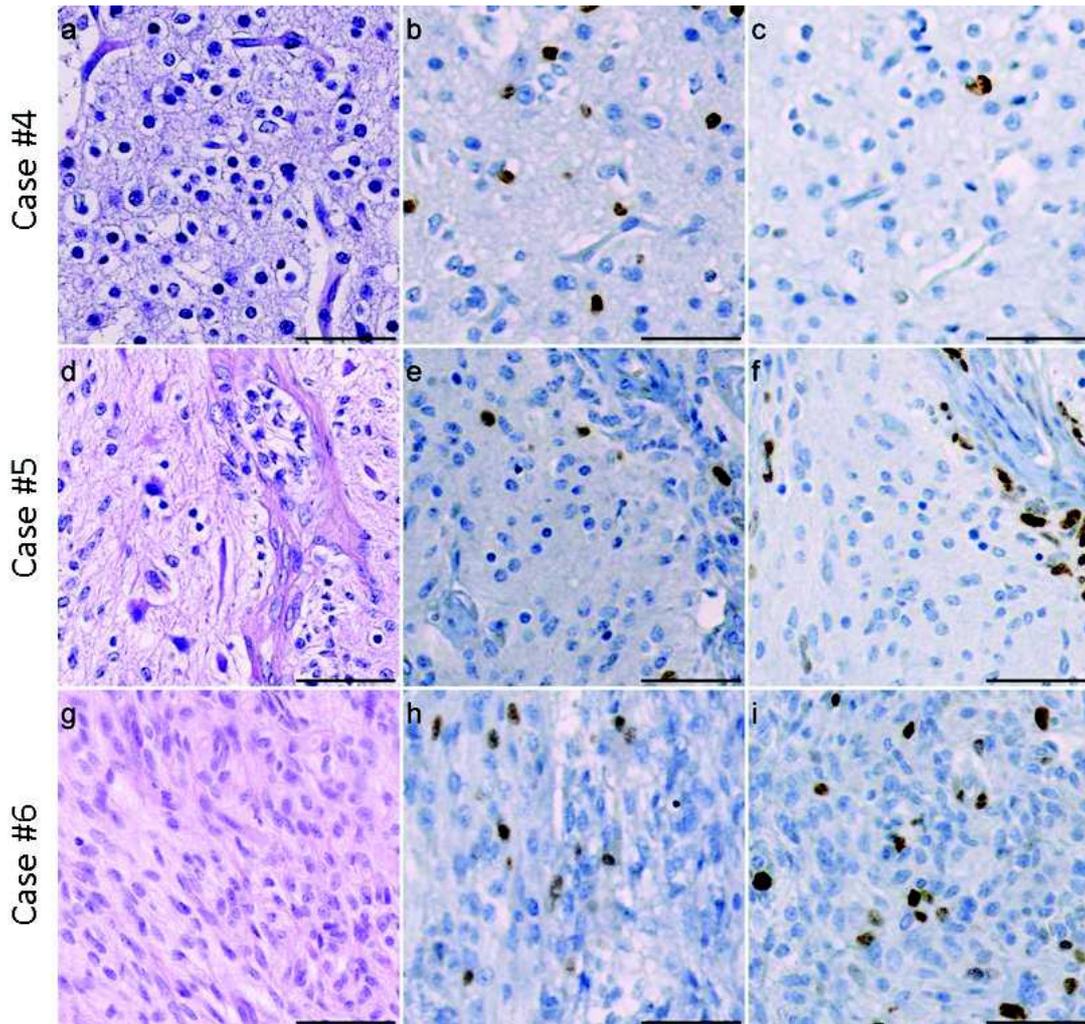


Figure 16: Neuroglial tissue from NMDAR-AE associated teratoma presenting histological features of gliomas. Case #4 (a-d): mature ovarian teratoma containing neuroglial tissue, with monotonous cells surrounded by a perinuclear cytoplasmic halo mixed with a vascular network consisting of short geometrically-arranged capillary segments (a, see magnification on the lower corner), (b) high magnification on “fried-egg” oligodendrocytes-like cells. Tumor cells show positivity for Olig2 expression (c) but low Ki-67 proliferation index (d), consistent with the histopathological observations of an oligodendroglioma. Case #5 (e-h): elevated cellular density, with clusters of ganglion cells (e, see magnification on the lower corner) and proliferation of aligned oligodendrocytes-like cells (f, higher magnification). These features appear similar to the histological features of a ganglioglioma. Case #6 (i-l): nervous tissue inside an ovarian teratoma with highly elevated cellular density (a, see magnification on

Results

the lower corner) and pleiomorphic or poorly differentiated cells (j, higher magnification) with elevated Ki67 proliferation index (I), consistent the histopathological aspect of a malignant glioma. Scale bar: 100µm and 10µm for inserts.

Neuroglial tissue exhibited histopathological features of central nervous system neuroglial tumor in 3 cases of mature ovarian teratoma with NMDAR-AE (patient #4, #5 and #6). The histopathological phenotypes of the solid mature nervous tissues of these 3 cases were respectively consistent with an oligodendroglioma, a ganglioglioma and a malignant glioma. Detailed examination of the nervous tissue from the teratoma resected from case #4 revealed a proliferation of monotonous cells with round and uniform nuclei surrounded by a perinuclear cytoplasmic halo mixed with a vascular network consisting of short, geometrically-arranged, capillary segments (**Fig 16a-d**), consistent with an oligodendroglioma. Tumor cells showed positivity for Olig2, PS100 and ATRX expressions. Ki-67 proliferation index was <5%. Histological study of the nervous tissue component from case #5 demonstrated elevated cellular density with presence of clusters of ChromoA+ ganglion cells and alignments of oligo-like cells (**Fig 16e-h**), evoking the histological features of a ganglioglioma. Finally, in case #6, the nervous tissue component presented strong transformations, with highly elevated cellular density and abundant pleomorphic cells, which were sometimes positive for Olig2, PS100 or EMA (**Fig 162i-l**). Ki-67 proliferation index was focally elevated. This histological phenotype was reminiscent of malignant glioma. In all those three cases, no immature neural tissue was found. Genetic alterations commonly associated with gliomas were searched by NGS analysis focused on the nervous component of the teratoma for one case (#5). No mutation commonly associated with gliomagenesis was found. Medical NMDAR-AE history and outcome of these 3 cases of NMDAR-AE were classical and further described in the Supplemental data.

1.1.4. Characteristics of inflammatory infiltrates in contact with neuroglial tissue in NMDAR-AE associated teratomas

NMDAR-AE associated teratomas strikingly differ from control teratoma by a systematic immune cell infiltration closely adjacent to the neuroglial tissue (26/26 vs 2/15 control teratomas, $p < 0.001^{***}$). Inflammatory infiltrates were moderate to high in 17/26 (65%), while only one control case's inflammatory infiltrate was rated moderate and the others (14/15, 93%) were rated low or absent (**Fig 17** and Suppl. Fig 1). These inflammatory infiltrates were composed of CD3+ T-cells and CD20+ B-cells in similar proportions (**Fig 18**). In 50% of NMDAR-AE associated ovarian-cases, immune cell infiltrates in the nervous tissue component were organized in germinal centers (13/26 vs. 1/15 control teratomas, NS) (**Fig 18**). Consistently, mature dendritic cells expressing DC-LAMP were detected in 46% of cases with NMDAR-AE associated ovarian teratoma. Multiple immunofluorescence staining revealed the presence of IgG and IgA deposits and IgG or IgA-producing cells in contact with the neuroglial tissue of 17/19 (89%) of NMDAR-AE associate teratomas (**Fig 19**).

Results

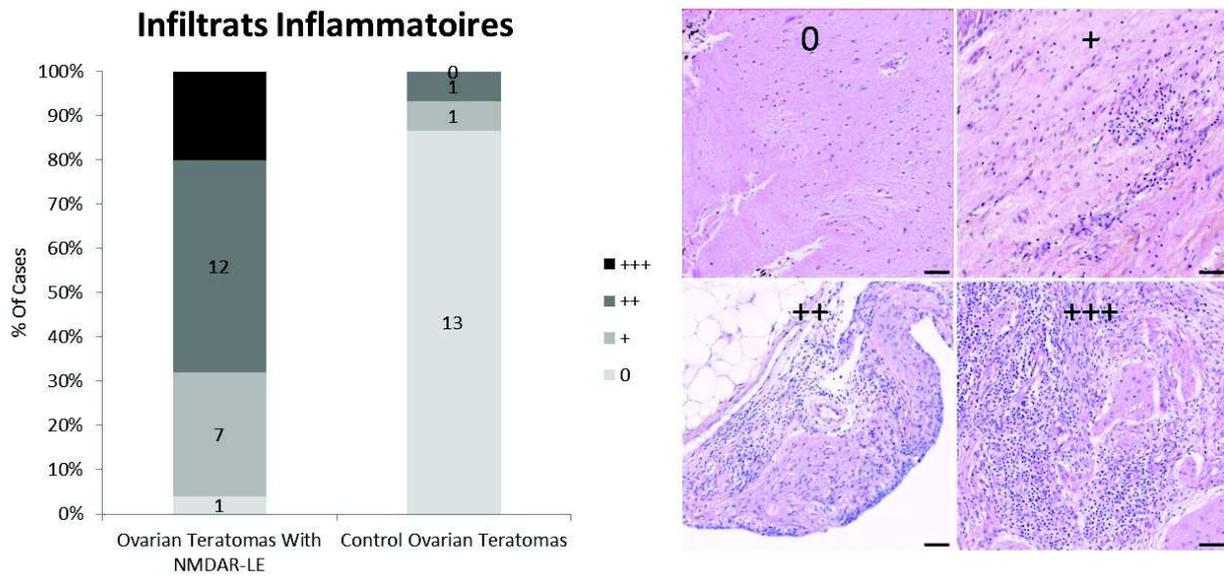


Figure 17 Quantification of inflammatory infiltrates in NMDAR-AE-associated and control ovarian teratomas. Paraffin-embedded sections of ovarian teratoma stained with HPS. Microphotograph show the nervous tissue component of several ovarian teratoma with different degree of inflammatory infiltrates. Scale bars: 50 μ m.

Results

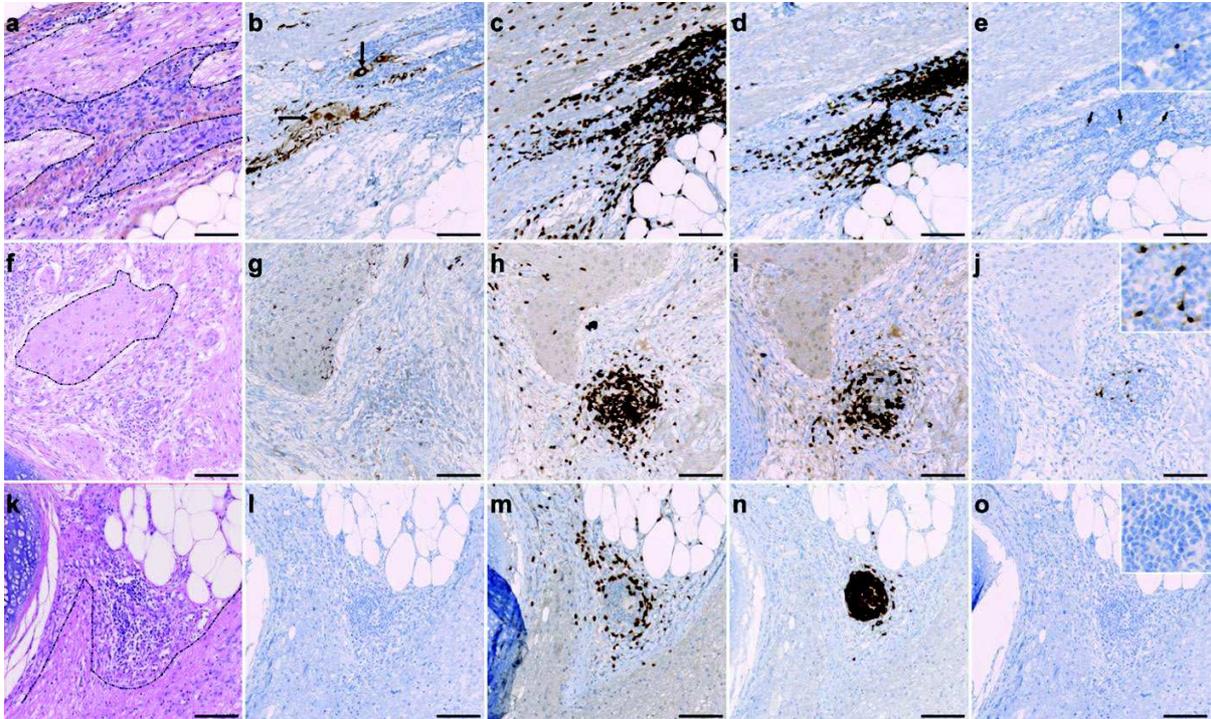


Figure 18 Prominent inflammatory infiltrates and germinal centers in NMDAR-AE-associated ovarian teratomas. Paraffin-embedded sections of ovarian teratoma stained with HPS (a, f, k) and immunolabeled with the neuronal marker NF (b, g, l), CD3 T-cells marker (c, h, m), CD20 B-cells marker (d, i, n) and the mature dendritic cells marker DC-LAMP (e, j, o). (a-e): diffuse inflammatory infiltrate composed of T-cells and B-cells, but without DC (e), closely apposed to neural tissue (a, dotted area) containing NF-stained neurons (b, white arrow). (f-j): a germinal center next to a small focus of neuroglial tissue (f, dotted area) with segregated B- et T-cell component and mature DC-LAMP+ dendritic cells (j, see higher magnification in the upper right corner). (k-o) lymphoid infiltrates composed of clearly organized and segregated T-cells (m) and B-cells (n) close to a foci of neuroglial tissue (k, dotted area). No mature DC-LAMP+ cells were detected (o, see higher magnification in the upper right corner). Scale bars: 50 μ m.

Results

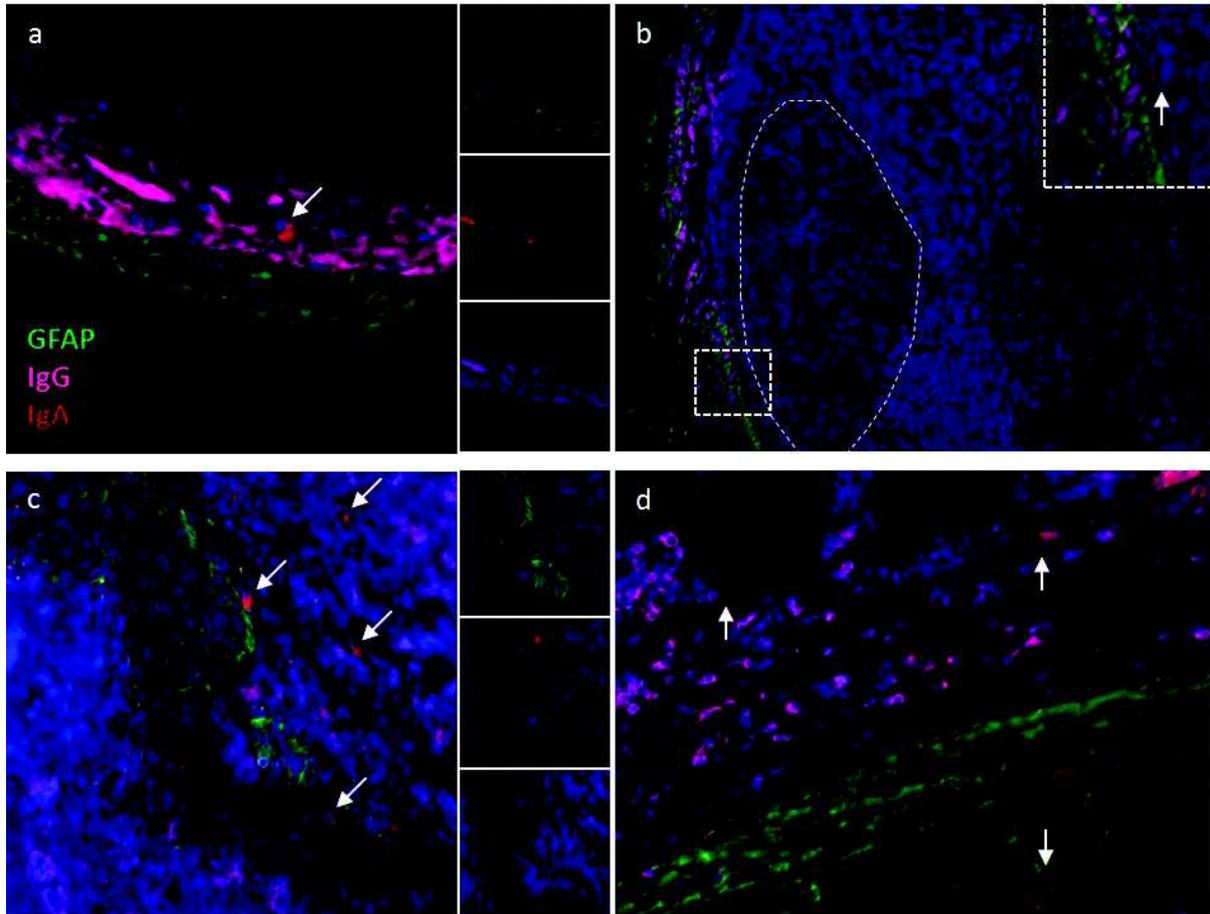


Figure 19: IgG and IgA deposits and producing cells in NMDAR-AE associated teratomas. (a) Representative GFAP/IgG/IgA immunofluorescence (IF) stainings of NMDAR-AE associated teratomas showing IgG deposits and IgG-producing cells (in purple) along a GFAP+ band of neuroglial tissue (in green). See isolated IgA-producing cells in red (arrowed). Hoechst was used to visualize nuclei (blue) and pictures in the left correspond to individual channels. (b) Representative IF staining for IgG (purple) and IgA (red) of inflammatory infiltrates harboring a germinal center (circled) of NMDAR-AE teratoma neighboring neuroglial tissue (GFAP+ in green). IgG deposits are more intense along the GFAP+ neural and diffuse in the inflammatory infiltrates. The bottom right picture at high magnification shows IgG deposits (purple) in neural tissue (green) and an isolated IgA+ cells (red, arrowed). (c) GFAP/IgG and IgA IF staining focused on GFAP+ neuroglial islands surrounded by immune cells and diffuse IgG deposits (purple) and some isolated IgA+ cells (red, arrowed). Hoechst was used to visualize nuclei (blue) and pictures in the left correspond to individual channels. (d) Representative GFAP/IgG and IgA IF staining showing amount of individualized IgG+ cells (purple) in contact with GFAP+ neural elements (green) in a NMDAR-AE associated teratoma. Note some diffuse IgA+ deposits (red) in the immune infiltrate and the adjacent neural tissue.

II. STUDY 2

II.1. PRELIMINARY EXPERIMENTS: VALIDATION OF THE MODEL

In order to investigate a possible pathogenic effect of NMDAR-Abs, we designed a model of passive transfer of NMDAR-Abs from NMDAR-AE patients directly infused into mice brain parenchyma to mimic NMDAR-Abs production by plasma cells infiltrating the CNS. We then assessed the diffusion of human antibodies into mice brain parenchyma and evaluated the effect of the surgery on behavior. Over the year 2016, I performed 66 surgeries to implant an infusion cannula using a stereotaxic frame and a subcutaneous placement of the osmotic mini-pump. 9 mice (13%) were not included in all planned experiments: 3 died in surgery or immediately after (in one case, it was due to a malfunction of the anesthesia apparatus), 1 died within days of surgery and 5 were excluded because the cannula disconnected from the mini-pump, often in the day following the training session of the fear conditioning protocol, maybe because of agitation following the electric shock. Notably, success rate of this protocol improves with increased surgery training as the following year, 32 out 33 surgeries performed were a success. Thus, with adequate training, infusion cannula implantation and subcutaneous placement of the osmotic mini-pump is a reliable procedure.

II.1.1. Control of Human IgG diffusion in the hippocampus

NMDAR-AE profoundly alters behavior and causes a wide range of symptoms in patients. Though several of these symptoms (e. g. anxiety, depression) could be translated into mice and be investigated using behavioral tests, we chose to focus our investigations on memory impairments. Indeed, memory impairments are consistent in NMDAR-AE patients and NMDAR involvement in memory processes has been widely investigated. We hypothesized that IgGs purified from the serum of patients with NMDAR-AE would cause memory impairments resembling the memory deficit observed in patients and investigated possible memory impairment using two behavioral tests, fear conditioning on mice treated during 7 or 14 days and spatial and novel object recognition on mice treated during 14 days.

The diffusion of the infused control or NMDAR-AE IgGs into the brain parenchyma was assessed by immunostaining human IgGs on mice brain slices. Human IgGs diffusion into the brain parenchyma was clear both in Ctrl-IgGs and NMDAR-AE IgGs infused mice whereas there was no human IgG staining in mice infused with the vehicle (PBS; **Fig 20**), as expected. The staining pattern was similar between mice infused with Ctrl-IgGs and NMDAR-AE IgGs with a visible staining of the cortex and subcortical structures (thalamus, striatum and amygdala) and a stronger staining in the hippocampus. Diffusion was often slightly asymmetrical, with the ipsilateral side (left) more intensely stained than the contralateral side of the infusion point. Though there was some inter-individual variability, IgGs diffusion was clearly visible until Bregma -3mm in the antero-posterior axis. In some cases, IgGs staining was very low and this correlated with a directly observed infusion point in the ventricle. Due to this variability, and to compare our model with a model of intraventricular infusion developed by another group ([Planagumà et al. 2014](#)),

Results

we investigated the impact of the infusion site (i. e. medial septum vs. lateral ventricle) on the behavior of mice.

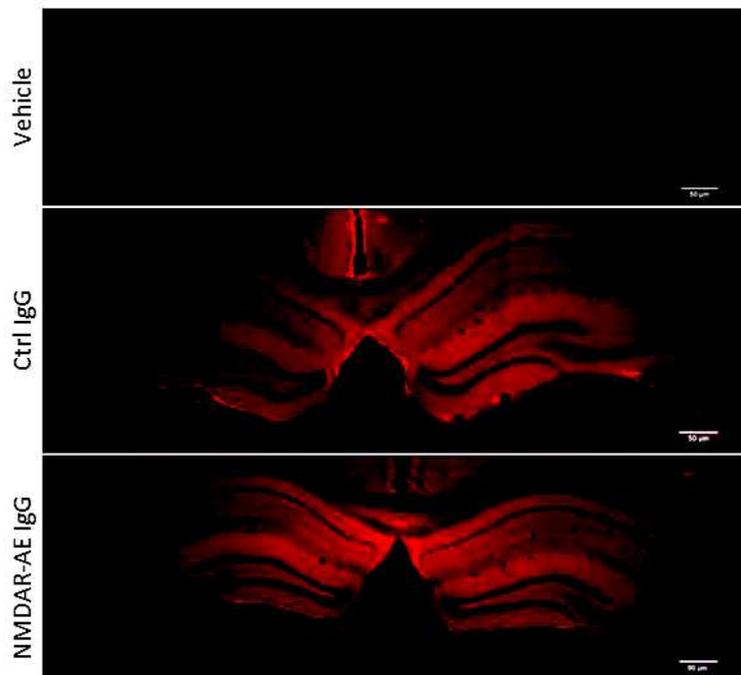


Figure 20: Diffusion of purified human IgGs into mice brain parenchyma after 7 days. Mice were infused with vehicle (upper panel) or purified IgGs from control subjects or NMDAR-AE patients (two lower panels). The catheter was placed in the medial septum and the osmotic pump delivered the solution during 7 days. IgGs were immunostained with anti-human IgGs coupled to Alexa 555 to assess the diffusion. A clear human IgGs staining was until Bregma -3mm both after infusion of Ctrl- or NMDAR-AE IgGs. Scale bar: 50µm.

II.1.2. Impact of the infusion site on fear memory

To investigate a possible effect of the infusion site on behavior, mice were infused with purified IgGs from healthy subjects (Ctrl IgGs) either in the medial septum or in the lateral ventricle (**Fig 21a**) during 7 days. The behavioral impact of surgery and location of the infusion catheter was assessed in a fear conditioning test. Time spent freezing was considered as an index of fear memory. Performances were also compared with those of non operated mice (not implanted (NI) experimental group). Non implanted mice's contextual and auditory fear memory was slightly better than implanted mice but there were no statistical differences between groups (**Fig 21b&c**). Having established that the location of the catheter does not affect behavioral performances for mice receiving Ctrl IgGs infusion, we also assessed the impact of catheter implantation site for mice receiving NMDAR-AE IgGs infusion. Mice receiving intra-septal or intra-ventricular infusion or NMDAR-AE IgGs presented similar amount of freezing behavior both in contextual and auditory fear conditioning tests. Therefore, fear memory might be impaired when mice undergo surgery but the location of the infusion catheter (medial septum vs. lateral ventricle) does not have a major effect on fear memory. All the following experiments were performed on mice infused

Results

in the medial septum. Except in rare cases where the infusion cannula was not properly secured to the skull and therefore disconnected before the end of the infusion time, all operated mice were included in the behavioral analysis, even if the observed *post-mortem* infusion site was in the lateral ventricle.

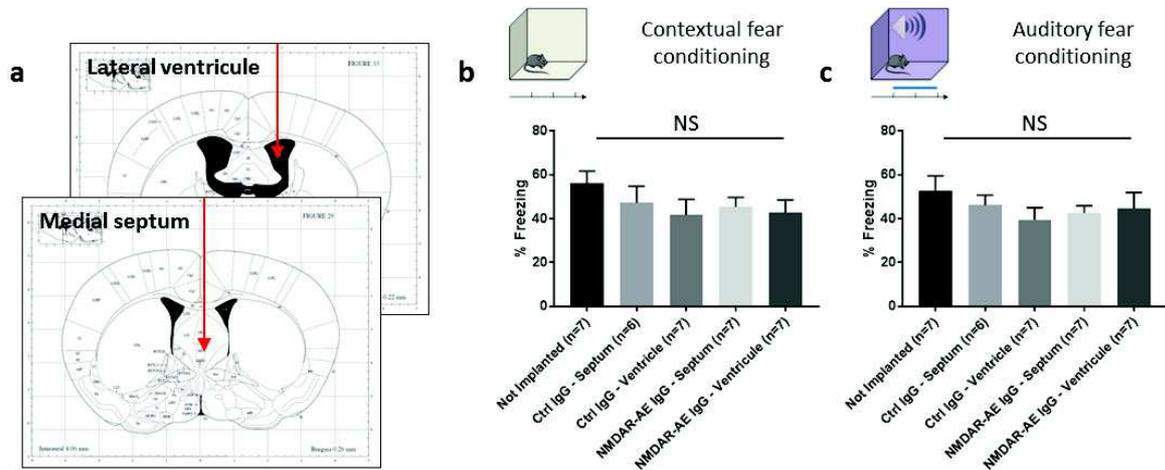


Figure 21: The infusion site does not impact mice behavioral performances in a fear conditioning test. Mice were infused with purified IgGs from control subjects during 7 days in the medial septum or the lateral ventricle (a) or did not receive any surgery. Surgery and the infusion site did not significantly impact behavior in contextual (b) or auditory (c) conditioning. N represents the number of mice per group. Horizontal bars represent mean \pm SEM.

II.2. EFFECTS OF PURIFIED IgG INFUSION ON BEHAVIOR AND DENDRITIC SPINES MORPHOLOGY

As stated previously, our work hypothesis was that NMDAR-Abs had an active pathogenic role in NMDAR-AE based on results mostly obtained from *in vitro* experiments by our group (Mikasova *et al.* 2012) or others (Dalmau *et al.* 2008, Hughes *et al.* 2010). These results were obtained either using NMDAR-Abs from patients' serum or CSF. Specifically, treatment of cultured neurons with purified IgGs from NMDAR-AE patients' serum or NMDAR-AE patients' CSF decreased NMDAR synaptic content to the same extent (Hughes *et al.* 2010). Therefore, as it is easier to obtain large amount of IgGs from serum than from CSF, we used IgG purified from serum in first intention to develop an NMDAR-AE mouse model. Using this model, we researched behavioral impairments and investigated changes in dendritic spines morphology that could possibly be induced by NMDAR-Abs.

II.2.1. Behavioral effects of Ctrl IgG and NMDAR-AE IgG

II.2.1.1. Fear conditioning on mice treated during 7 days with IgG purified from serum of Ctrl or NMDAR-AE patients

Two separate experiments of fear conditioning were performed on mice treated during 7 days with vehicle, Ctrl IgGs or NMDAR-AE IgGs, using two different apparatus (Fig 22). The initial experiment was performed on 9 to 15 mice per group and included a group who did undergo any surgery (Not Implanted;

Results

Fig 22a&Xb). When compared to mice which did not undergo surgery, mice receiving vehicle infusion seemed to exhibit a slightly reduced amount of freezing both in contextual (Not Implanted: $28.13 \pm 3.6\%$ vs. Vehicle: $22.84 \pm 2.7\%$, Kruskal-Wallis test $p=0.211$ **Fig 22a**) and auditory (Not Implanted: $48.81 \pm 6.9\%$ vs. Vehicle: $43.85 \pm 4.6\%$, Kruskal-Wallis test: $p=0.0419^*$, Dunn's multiple comparison test: Not Implanted vs. Vehicle $p>0.99$; **Fig 22b**) fear conditioning test. This observation is suggestive of an effect of the surgical procedure and in line with results previously described. Ctrl IgGs treatment seemed to increase freezing behavior compared to Vehicle-treated mice both in contextual (Vehicle: $22.84 \pm 2.7\%$ vs. Ctrl IgG: $30.48 \pm 2.6\%$, Kruskal-Wallis test $p=0.211$; **Fig 22a**) and auditory fear conditioning (Vehicle: $43.85 \pm 4.6\%$ vs. Ctrl IgG: $63.81 \pm 4.5\%$, Kruskal-Wallis test: $p=0.0419^*$, Dunn's multiple comparison test: vehicle vs. Ctrl IgG $p=0.0503$; **Fig 22b**) and this effect was partly with infusion of NMDAR-AE IgGs ($25.39 \pm 2.4\%$ of freezing in contextual fear conditioning, Kruskal-Wallis test $p=0.211$; and $53.06 \pm 5.1\%$ of freezing in auditory fear conditioning, Kruskal-Wallis test: $p=0.0419^*$, Dunn's multiple comparison test: Ctrl IgG vs. NMDAR-AE IgG $p=0.9622$; **Fig 22a&b**). These results were suggestive both of an unexpected positive effect of Ctrl IgGs on memory performance in a fear conditioning test and of a slight memory impairment in animals treated with NMDAR-AE IgGs. A second experiment was later performed to confirm these observations and though none of these effects reached statistical significance levels they closely reproduced in the second experiment (**Fig 22c&d**).

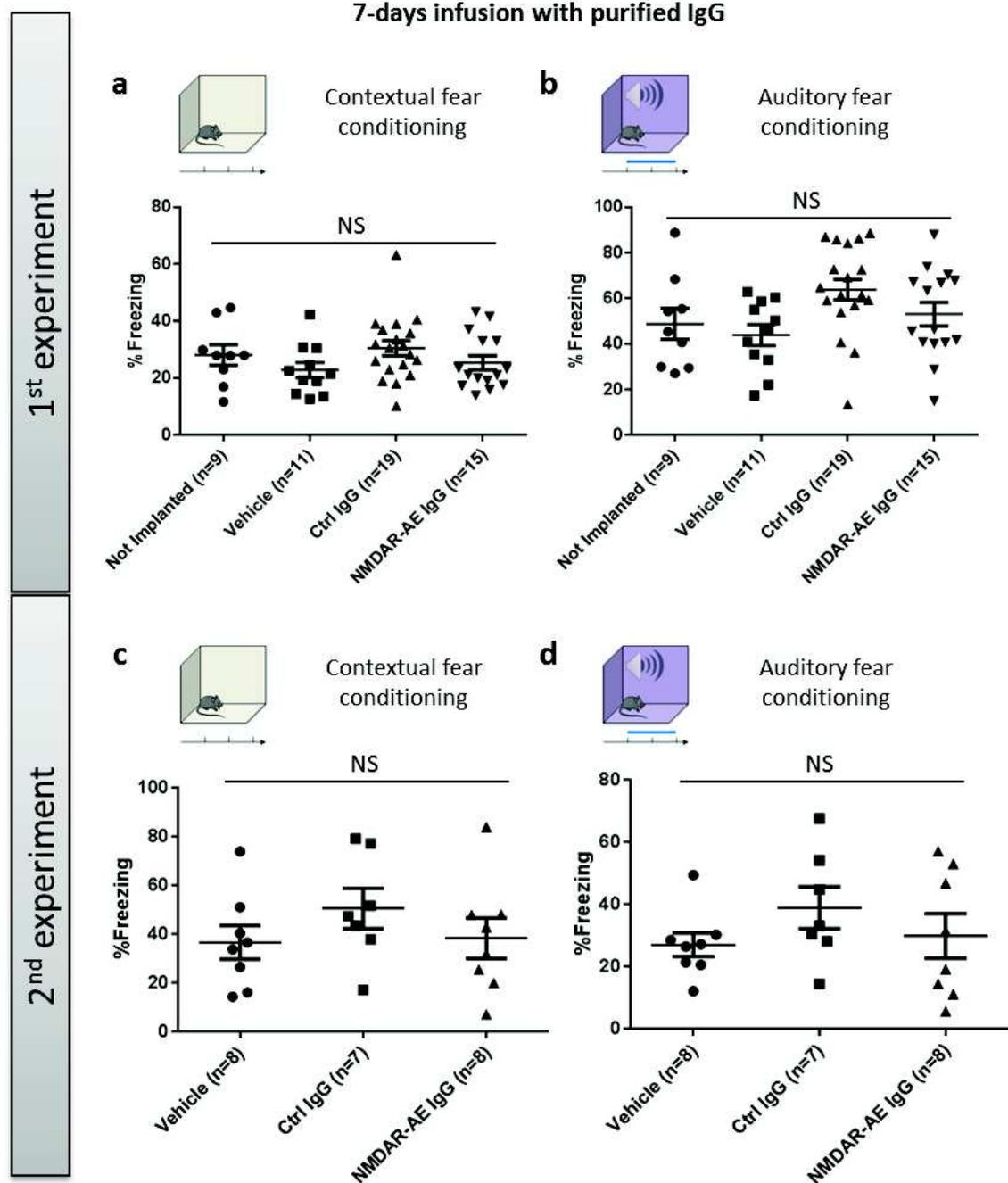


Figure 22: NMDAR-AE purified IgG do not impair mice behavioral performances in a fear conditioning test. Mice were infused with vehicle or purified IgGs from control subjects or from NMDAR-AE patients during 7 days and subjected to a fear conditioning protocol in two separate experiments. Notable, two different apparatus were used for the experiments but in both cases infusion with control IgGs (2mg/mL) or with IgGs from NMDAR-AE patients (2mg/mL) did not modify mice freezing behavior in contextual (a, c) or auditory fear conditioning (b, d). N represents the number of mice per group. Horizontal bars represent mean \pm SEM.

Results

II.2.1.2. Pool analysis of all fear conditioning experiments on mice treated during 7 days with IgG purified from serum of Ctrl or NMDAR-AE patients

Finally, to ensure that lack of statistical significance was not due to the limited number of animals in each study, results from animal receiving intra-septal infusion from Fig 21 and from the two experiments described above (*cf.* Fig 18) were gathered and analyzed together in Fig 23. No statistically significant impairment in fear memory between was observed in mice treated with NMDAR-AE IgG in the contextual (**Fig 23a**) or auditory test (**Fig 23b**), though the small decrease in the freezing time in the NMDAR-AE treated group compared to the Ctrl IgGs treated group ($33.05\pm 3\%$ vs. $38\pm 3.1\%$ respectively in contextual fear conditioning, Kruskal-Wallis & Dunn's multiple comparisons $p=0.72$, **Fig 23a**; and $44.5\pm 3.6\%$ vs. $55.08\pm 3.6\%$ respectively in auditory fear conditioning, Kruskal-Wallis & Dunn's multiple comparisons $p=0.34$, **Fig 23b**) was suggestive of a minor contextual fear memory impairment. Intriguingly, a positive effect of Ctrl IgG compared to vehicle infusion on auditory fear conditioning was also revealed by this grouped analysis ($55.08\pm 3.6\%$ vs. $36.74\pm 3.63\%$ respectively in auditory fear conditioning, Kruskal-Wallis & Dunn's multiples comparisons $p=0.0112^*$; **Fig 23b**).

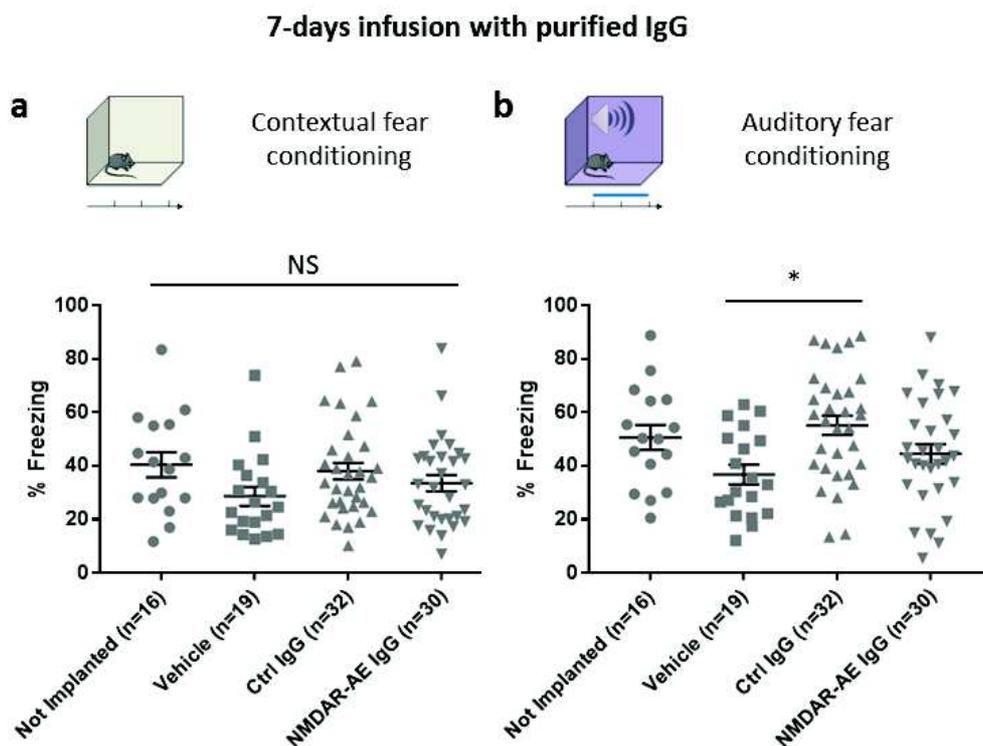


Figure 23: NMDAR-AE purified IgGs do not strongly impair mice behavioral performances in a fear conditioning test but Ctrl IgGs enhances auditory fear conditioning. All mice infused with either vehicle or purified IgGs from control subjects or from NMDAR-AE patients during 7 days and subjected to a fear conditioning test were pooled for statistical analysis (a, b). Infusion with control IgGs (2mg/mL) enhances auditory fear conditioning (b) but infusion with IgGs from NMDAR-AE patients (2mg/mL) did not cause major modification in mice freezing behavior in contextual (a) or auditory fear conditioning (b). N represents the number of mice per group. Horizontal bars represent mean \pm SEM.

Results

II.2.1.3. Fear conditioning on mice treated during 14 days with IgG purified from serum of Ctrl or NMDAR-AE patients

We then decided to test if a prolonged exposure to Ctrl IgGs or NMDAR-AE IgGs would result in stronger effect on memory. Mice were infused with vehicle, Ctrl IgGs or NMDAR-AE IgGs during 14 days and their memory performances were assessed in a spatial-change and object-recognition protocol 11 after surgery and in a fear conditioning test 13 days after surgery. No statistically significant differences were observed between group in the contextual (Vehicle: $21.58 \pm 4.2\%$, Ctrl IgG: $23.12 \pm 2.7\%$, NMDAR-AE IgG $18.27 \pm 2.32\%$, Kruskal-Wallis test $p=0.3971$) or auditory (Vehicle: $25.63 \pm 3.8\%$, Ctrl IgG: $20.45 \pm 2.042\%$, NMDAR-AE IgG $20.12 \pm 2.7\%$, Kruskal-Wallis test $p=0.3191$) fear conditioning test after 14 days of vehicle or Ctrl IgG or NMDAR-AE IgG infusion (**Fig 24a&b**).

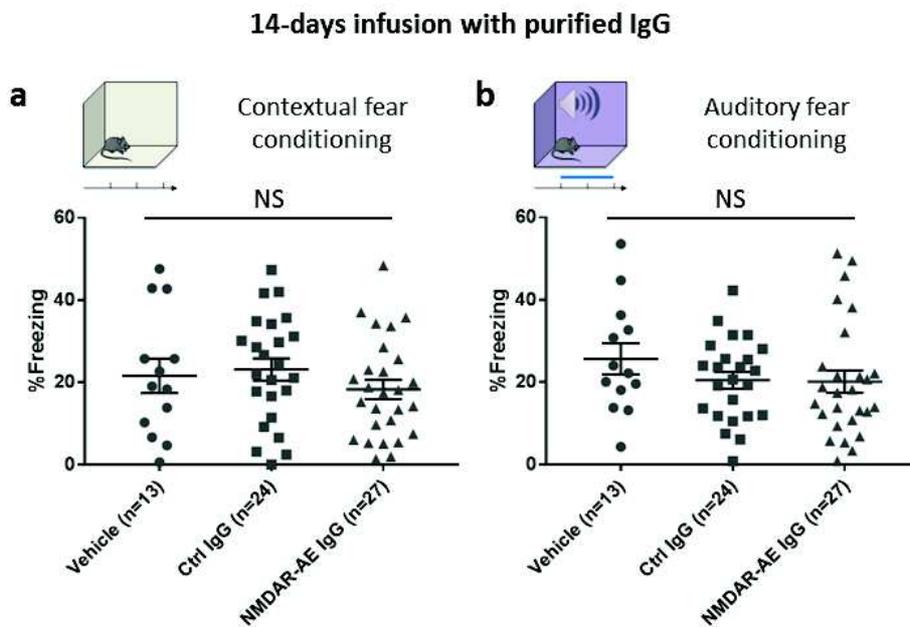


Figure 24: NMDAR-AE IgG infusion during 14 days does not impair mice behavioral performances in a fear conditioning test. Mice were infused with purified IgGs from control subjects or from NMDAR-AE patients during 14 days (a, b). Infusion with control IgGs (2mg/mL) or with IgGs from NMDAR-AE patients (2mg/mL) did not modify mice freezing behavior in contextual (a) or auditory fear conditioning (b). N represents the number of mice per group. Horizontal bars represent mean \pm SEM.

II.2.1.4. Spatial and object recognition test on mice treated during 14 days with IgGs purified from serum of Ctrl or NMDAR-AE patients

Another memory test was performed on mice infused with vehicle, Ctrl IgGs or NMDAR-AE IgGs during 14 days to i) explore other memory processes: spatial memory, which depends on hippocampus proper function and object recognition; and ii) to confirm results obtained in the fear conditioning. In order to ensure that potential differences observed in object exploration would be not caused by differences in

Results

animal mobility, the distance moved and the velocity of the mice were recorded during the first session of the object-recognition protocol. No differences were observed between the Ctrl IgGs-treated group or the NMDAR-AE IgGs-treated group regarding the mean distance moved (**Fig 25a**) or the mean velocity (**Fig 25b**) per group. During the first session, the arena was subdivided into 3 virtual zones, namely the periphery, the middle zone and the center zone (*cf.* Fig 12a in the Material & Methods Section). As anxious animals tend to avoid wide-open spaces and stay near arena walls, the average time exploring each zone was computed for mice group and used as an index for anxiety. No differences in the time spent exploring the periphery, middle or center zone were observed (**Fig 25c**). As indicated in the Material & Method section (§II.6.2.), mice spending less than 30 seconds exploring the objects during all three habituation sessions were excluded from analysis. Hence, one mouse from the vehicle-treated group and two mice from the NMDAR-AE IgGs treated group were excluded from analysis. Exploration index in the spatial-change recognition test was higher than 1 for all tested group indicating a higher exploration of the moved-object but not statistically different between any groups, though slightly lower in the NMDAR-AE IgGs group (vehicle: 1.6 ± 0.3 ; Ctrl IgG: 1.6 ± 0.2 ; NMDAR-AE IgG: 1.3 ± 0.2 , Kruskal-Wallis test $p=0.5448$; **Fig 25d**). As expected with a more salient change, mean exploration index in the novel-object recognition test was higher than in the spatial-change recognition test for all groups (vehicle: 1.8 ± 0.3 ; Ctrl IgG: 2.3 ± 0.3 ; NMDAR-AE IgG: 1.8 ± 0.2 Kruskal-Wallis test $p=0.4185$; **Fig 25e**) but there were no statistically relevant differences between groups, leading to the conclusion that NMDAR-AE IgGs infusion do not cause spatial and novelty recognition memory impairment.

Results

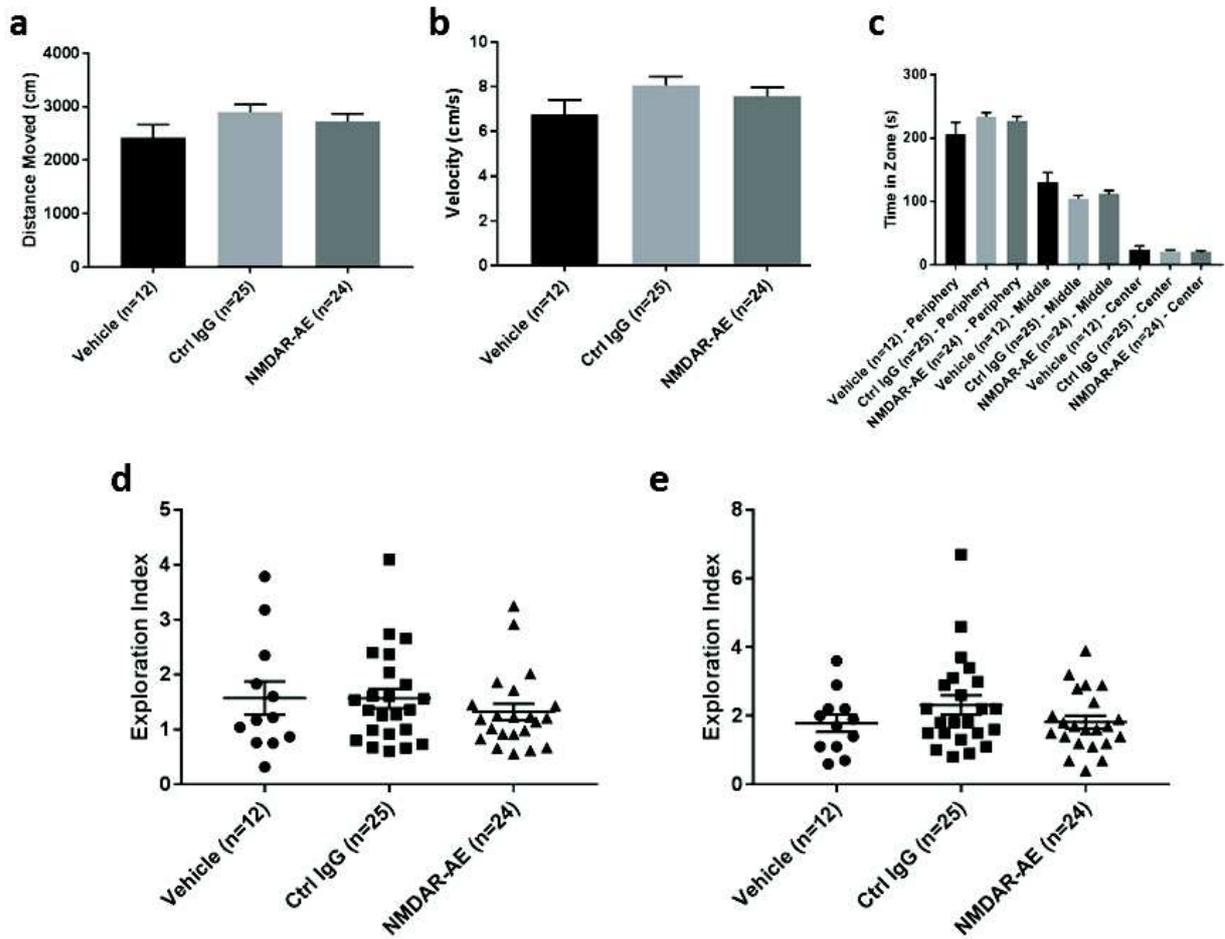


Figure 25: NMDAR-AE purified IgGs do not impair mice memory performances in a novel-object recognition test. Mice were infused with vehicle or purified IgGs from control subjects or NMDAR-AE patients during 14 days. Distance moved (a), velocity (b) and arena exploration (c) were assessed during the first session of the protocol (*cf.* Fig 8 in the Material & Methods). NMDAR-AE IgGs infusion did not alter spontaneous activity, mobility and exploration of a new environment (a-c). NMDAR-AE IgGs infusion did not significantly impair mice memory performances in a spatial-change (d) of novel-object recognition test (e). N represents the number of mice per group. Data are represented as mean±SEM.

Taken together, behavioral experiments performed on mice infused with vehicle, or Ctrl IgGs or NMDAR-AE IgGs during 7 or 14 days revealed a positive effect of Ctrl IgGs on memory performances in auditory fear conditioning but did not show an effect of NMDAR-AE IgGs on memory, even though a small but not statistically significant decrease in memory performances was consistently observed in this group compared to mice treated with Ctrl IgGs.

Results

II.2.2 Effect of NMDAR-Abs infusion on hippocampal and dendritic spine morphology

II.2.2.1. Analysis of hippocampal morphology by EM after treatment with Ctrl or NMDAR-AE IgGs during 7 days

Some pathologies involving dysfunction of NMDAR, such as AD, also results in major change in cerebral morphology, with neuronal death and brain shrinkage. Neuronal loss was rarely reported in early studies of NMDAR-AE (Tuzün *et al.* 2009) but recent neuroimaging studies suggest discrete atrophy and alteration of hippocampal microstructural integrity in NMDAR-AE patients (Finke *et al.* 2015). We thus studied hippocampal morphology in our animal model of passive transfer of NMDAR-Abs. EM imaging of the hippocampus was performed in our model in collaboration with Dr. S. Marty from the Institut du Cerveau et de la Moëlle Epinière. NMDAR-AE IgGs treatment during 7 days (Fig 26a&b) did not modify the height of the *stratum radiatum* in the CA1 region of the hippocampus, or the numbers of neuronal nuclei compared to Ctrl-IgGs infusion. NMDAR-Abs treatment does not alter the gross morphology of the hippocampus. These results are in line with the full recovery achieved by most NMDAR-AE patients (Titulaer *et al.* 2013) which suggest that if a neuronal loss occurs in this disease, it remains limited; as well as with previous *in vitro* results which did not found NMDAR-Abs-induced cytotoxicity (Hughes *et al.* 2010).

II.2.2.2. Analysis of dendritic spines morphology by EM after treatment with Ctrl or NMDAR-AE IgGs during 7 days

The shape of dendritic spines is believed to play a role in the efficacy of neuronal transmission and to be an important component underlying neural plasticity. Therefore, we investigated the morphology of dendritic spine in mice treated with purified IgG using two processing and imaging methods: electron microscopy followed by reconstruction of spine morphology and Golgi staining and measurement of spine length and head width. EM imaging of the hippocampus and 3D-reconstruction of dendritic spines morphology was performed on mice treated during 7 days with Ctrl-IgGs or NMDAR-AE IgGs (Fig 26a & 26b) as a preliminary experiment in collaboration with Serge Marty. Reconstruction of spine morphology based on EM imaging allowed classification of dendritic spines into sessile or pedunculated spines. Treatment with NMDAR-AE IgGs during 7 days increased the proportion of sessile spines compared with treatment with Ctrl IgGs suggesting that NMDAR-AE IgGs caused a simplification of dendritic spine morphology with loss of clearly defined spine-head (Fig 26b). We completed this analysis by performing Golgi staining on brain samples from mice infused with Ctrl IgGs or NMDAR-AE IgGs during 14 days to investigate a possible time-dependent effect of NMDAR-AE IgGs on dendritic spine morphology.

II.2.2.3. Analysis of dendritic spines morphology by Golgi staining after treatment with Ctrl or NMDAR-AE IgGs during 14 days

Modification of dendritic spine morphology was further investigated after 14 days of infusion with either vehicle, or Ctrl IgGs or NMDAR-AE IgGs by performing Golgi staining on the hippocampus region and classifying spines into 5 different shapes (branched, mushroom, filopodia and thin; Fig 26c &d). Compared to vehicle infusion, treatment with Ctrl IgGs decreased spine density (vehicle: 1.867 ± 0.054

Results

spines/ μm vs. Ctrl IgG: 1.584 ± 0.081 spines/ μm , Kruskal-Wallis & Dunn's multiples comparisons test $p=0.0153^*$; **Fig 26e**) and increased the number of mushroom spines (vehicle: 0.412 ± 0.033 mushroom/ μm vs. Ctrl IgG: 0.708 ± 0.051 mushroom/ μm , Kruskal-Wallis & Dunn's multiples comparisons test $p < 0.0001^{***}$; **Fig 26f**). Interestingly, this indicates an effect of Ctrl IgGs on spine morphology. When compared to Ctrl IgGs treatment, NMDAR-AE IgGs exposition decreases the number of mushroom spines (Ctrl IgG: 0.708 ± 0.051 mushroom/ μm vs NMDAR-AE IgG: 0.473 ± 0.034 mushroom/ μm , Kruskal-Wallis & Dunn's multiples comparisons $p=0.0069^*$; **Fig 26f**) and increases the number of stubby (Ctrl IgG: 0.2 ± 0.029 vs NMDAR-AE IgG: 0.383 ± 0.035 stubby/ μm , Kruskal-Wallis & Dunn's multiples comparisons $p=0.0001^{***}$; **Fig 26g**) and thin spines (Ctrl IgG: 0.616 ± 0.071 vs NMDAR-AE IgG: 1.01 ± 0.05 stubby/ μm , Kruskal-Wallis & Dunn's multiples comparisons $p=0.0002^{***}$; **Fig 26h**). There were no differences in spine density and morphology between vehicle-infused or NMDAR-AE IgGs infused mice except for an increased density of stubby spines in the NMDAR-AE IgGs-treated group (vehicle: 0.25 ± 0.019 vs NMDAR-AE IgG: 0.383 ± 0.035 stubby/ μm , Kruskal-Wallis & Dunn's multiples comparisons $p=0.0432^*$).

To conclude, Ctrl IgGs diminish spine density but increase the number of mushroom spines, while NMDAR IgGs induce a simplification of dendritic spine morphology reflected by an increase of sessile spines after 7 days of treatment and a decreased of mushroom spine density accompanied by an increase of thin and stubby spines after 14 days of treatment. Taken together, these results show an effect of Ctrl IgGs on dendritic spines morphology and could either indicate a lack of effects of NMDAR-AE IgGs or an effect opposed to the effects induced by Ctrl IgGs.

Results

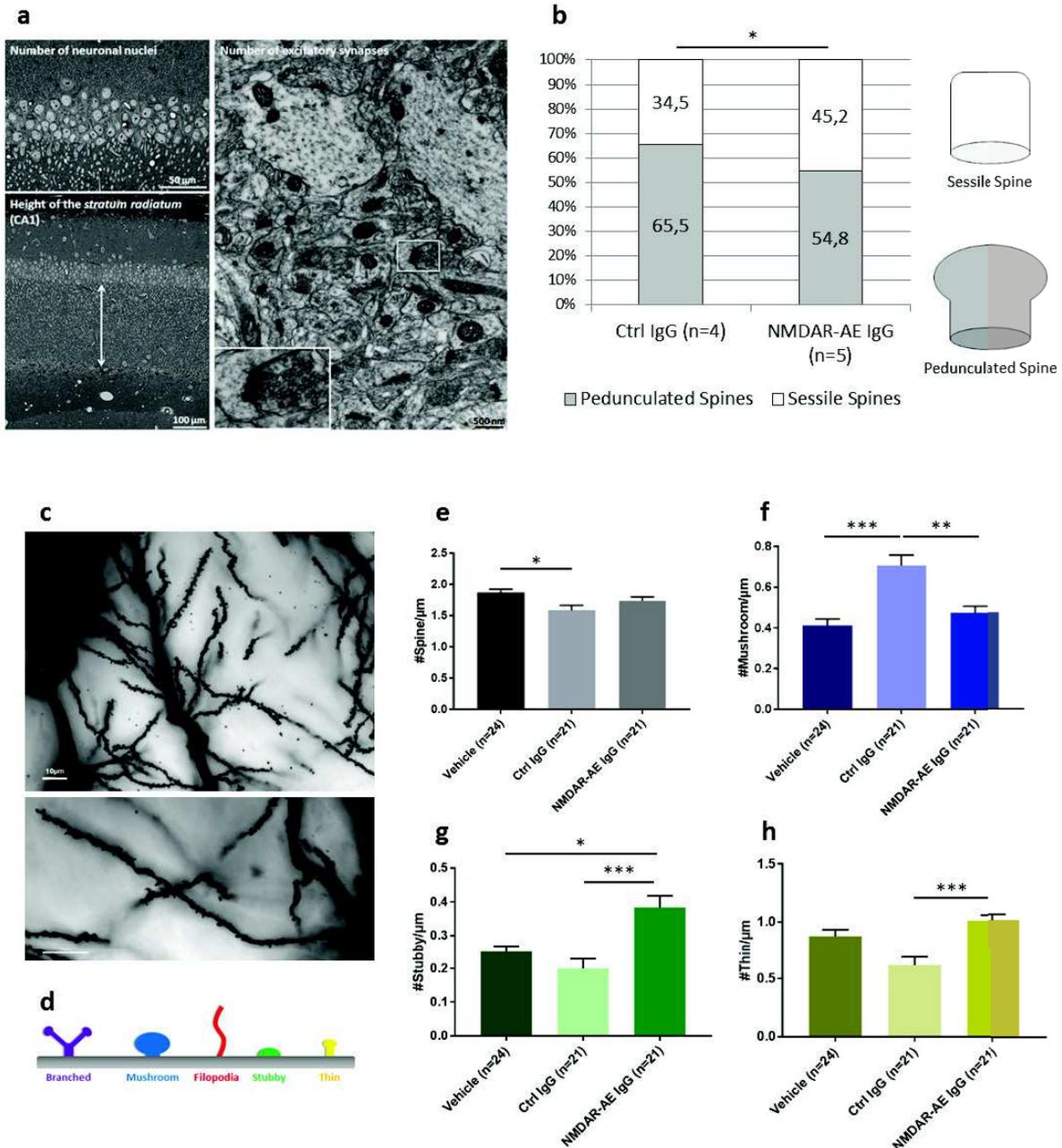


Figure 26: Ctrl IgGs and NMDAR-AE IgGs modify dendritic spines morphology. MET imaging of the hippocampus and 3D-reconstruction of dendritic spines morphology was performed on mice treated during 7 days with Ctrl-IgGs or NMDAR-Ae IgGs (a & b). NMDAR-AE IgGs treatment did not modify the height of the *stratum radiatum* in the CA1 region of the hippocampus, or the numbers of neuronal nuclei or excitatory synapses (a). 3D-reconstruction based on MET imaging allowed classification of dendritic spines into sessile or pedunculated spines. Treatment with NMDAR-AE IgGs during 7 days increased the proportion of sessile spines (b). Golgi stainings were performed after a 14-days infusion of vehicle or Ctrl-IgGs or NMDAR-IgGs (c). Spines were classified into 5 categories according to their morphology (d; see also Fig 13 of the M&M section). Branched and filopodia represented a low number of spines and are

Results

therefore not represented here. Spine density was reduced in the Ctrl-IgGs treated mice (e). Ctrl-IgGs treatment appeared to increase the number of mushroom spines (f) but decreases the number of thin spines (h). NMDAR-AE IgGs treatment increased the number of stubby spines (f) but did not change the number of mushroom (f) or thin (h) spines compared to vehicle infusion. N represents the number of mice per group in (b) and the number of dendrites analyzed in (e) to (h). Dendrites from 7 to 10 neurons from 3 mice per group were analyzed. Horizontal bars represent mean±SEM.

II.3. POSSIBLE INFLAMMATION AFTER 7 DAYS OF NMDAR-AE IGG INFUSION

II.3.1. Inflammation after treatment with Ctrl or NMDAR-AE IgGs during 7 days

Microgliosis, i. e. morphological changes and proliferation of microglial cells have been reported in brain biopsies (Dalmau *et al.* 2008) and *post-mortem* analysis of brain tissue of NMDAR-AE patients (Tüzün *et al.* 2009). Inflammatory reaction after infusion of human IgGs could also explain the effect observed on dendritic spine morphology and behavior after infusion of Ctrl IgGs. To study this hypothesis, we investigated the morphology of glial cells (microglia and astrocytes) after infusion of human IgGs in mice brain parenchyma. Indeed, it is now well established that glial cells activation translates into morphological changes (Beynon & Walker 2012). A preliminary experiment investigating the morphology of microglial cell based on immunostaining for Iba1 in vehicle- or Ctrl IgGs- or NMDAR-AE IgGs-treated mice revealed an increase in the area occupied by microglial cells (Fig 27) in the hippocampus contralateral to the infusion site. These results were reproduced and confirmed in a second independent experiment. We performed immunostaining for a microglial cells marker (Iba1) and an astrocytes marker (GFAP) and quantified the area and intensity of immunostaining in the ipsilateral hippocampus after 7 (Fig 28) or 14 days (Fig 29) of infusion. Interestingly, a small increase in the intensity (vehicle: 127.5±16.8; Ctrl IgG: 133.5±10.83; NMDAR-AE IgG: 163.5±14.58, Kruskal-Wallis test: p=0.1664; Fig 28b) and area (vehicle: 659.6±45.63; Ctrl IgG: 624.4±52.83; NMDAR-AE IgG: 820±59.49; Kruskal-Wallis test: p=0.0411*, Dunn's multiple comparison test: Ctrl IgG vs. NMDAR-AE IgG p=0.0694 Fig 28c) occupied by microglial cells after a 7-days infusion of NMDAR-AE IgGs, reflecting a modification of microglial morphology suggestive of microglial activation. However, this effect did not reach statistical significance using Dunn's multiple comparison test. Furthermore, this augmentation was reduced or absent after a 14-days infusion of Ctrl or NMDAR-AE-IgGs (Fig 29c& 29d). Concerning astrocytes, we observed no statistically significant increase in the area stained by GFAP or the intensity of the staining after 7 (Fig 28d&e) or 14 days (Fig 29d&e) of NMDAR-AE IgGs infusion. Noteworthy, the effect of Ctrl IgGs infusion compared to vehicle infusion was only tested after 7 days (Fig 28), but infusion of purified human IgGs in mice brain did not appear to affect glial cells morphology.

Results

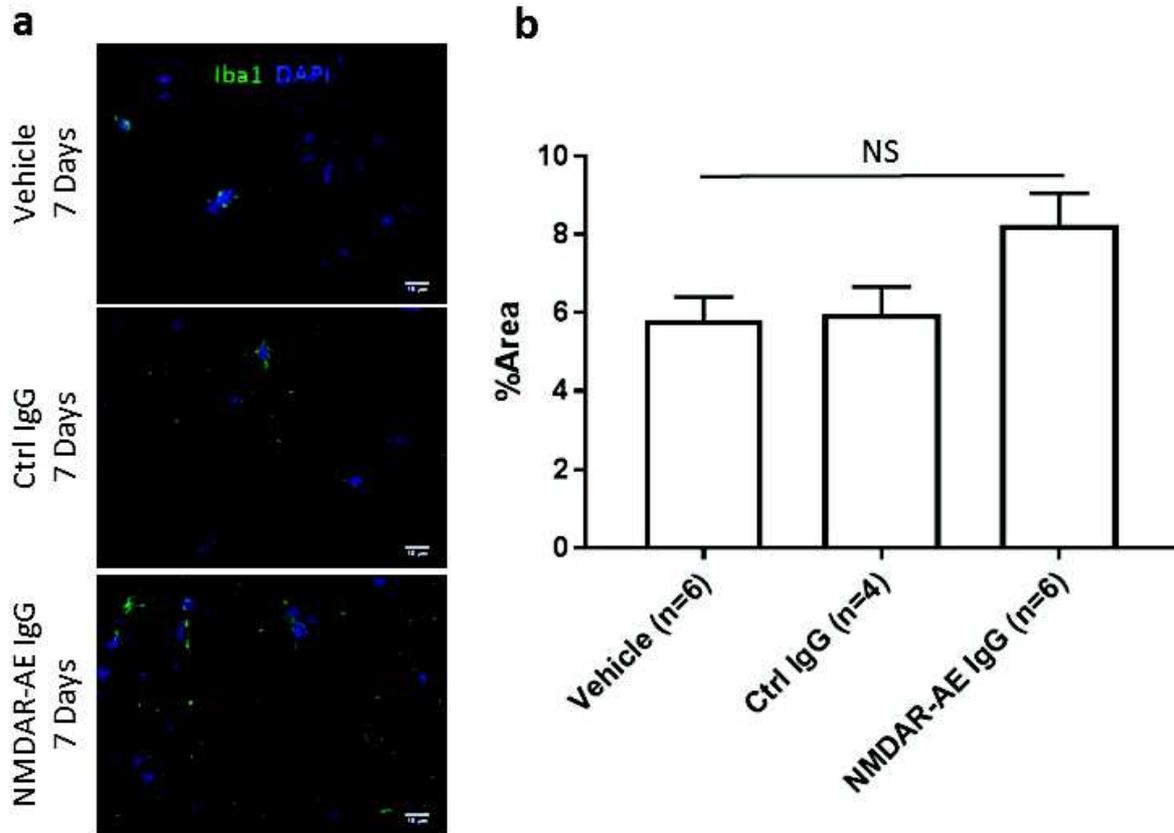


Figure 27: Microglial cells present minor signs of activation after a 7 days infusion of IgGs from NMDAR-AE patients. Mice were infused with vehicle or IgGs from control subjects or from NMDAR-AE patients during 7 days and immunostainings for a microglial-specific marker (Iba1) was performed (a). The area (b) of Iba1 staining was quantified for each experimental group. Scale bar: 10µm. Horizontal bars represent mean±SEM.

Results

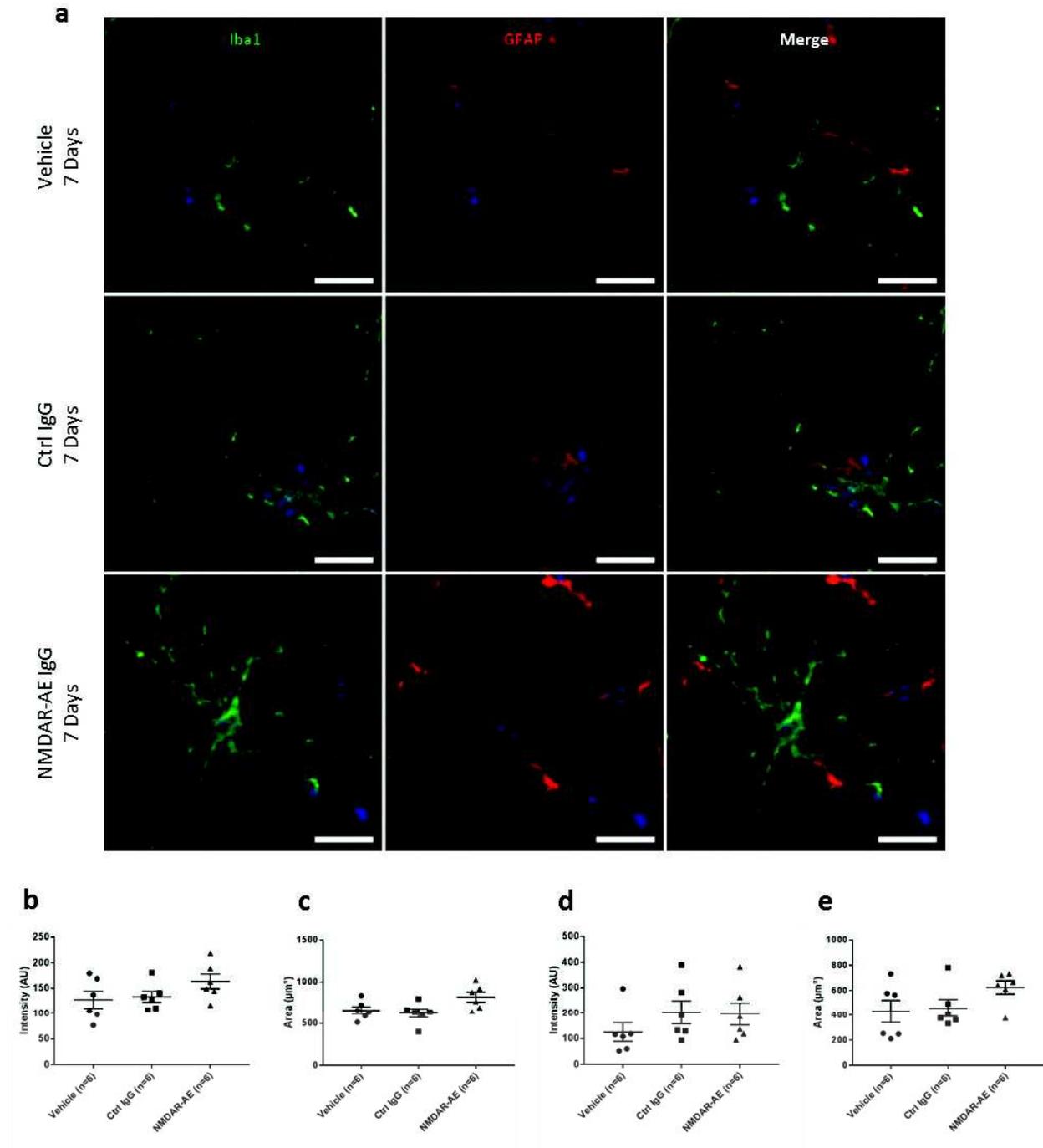


Figure 28: Microglial cells present minor signs of activation after 7 days of infusion of IgGs from NMDAR-AE patients. Mice were infused with vehicle or IgGs from control subjects or from NMDAR-AE patients during 7 days and cell-type specific immunostainings were performed (a). Note in the hyper ramified morphology of the microglial cell (in green) in the lower panel. The intensity (b) and area (c) of Iba1 staining and the intensity (d) and area (e) of GFAP staining were quantified for each experimental group. Scale bar: 10µm. Horizontal bars represent mean±SEM.

Results

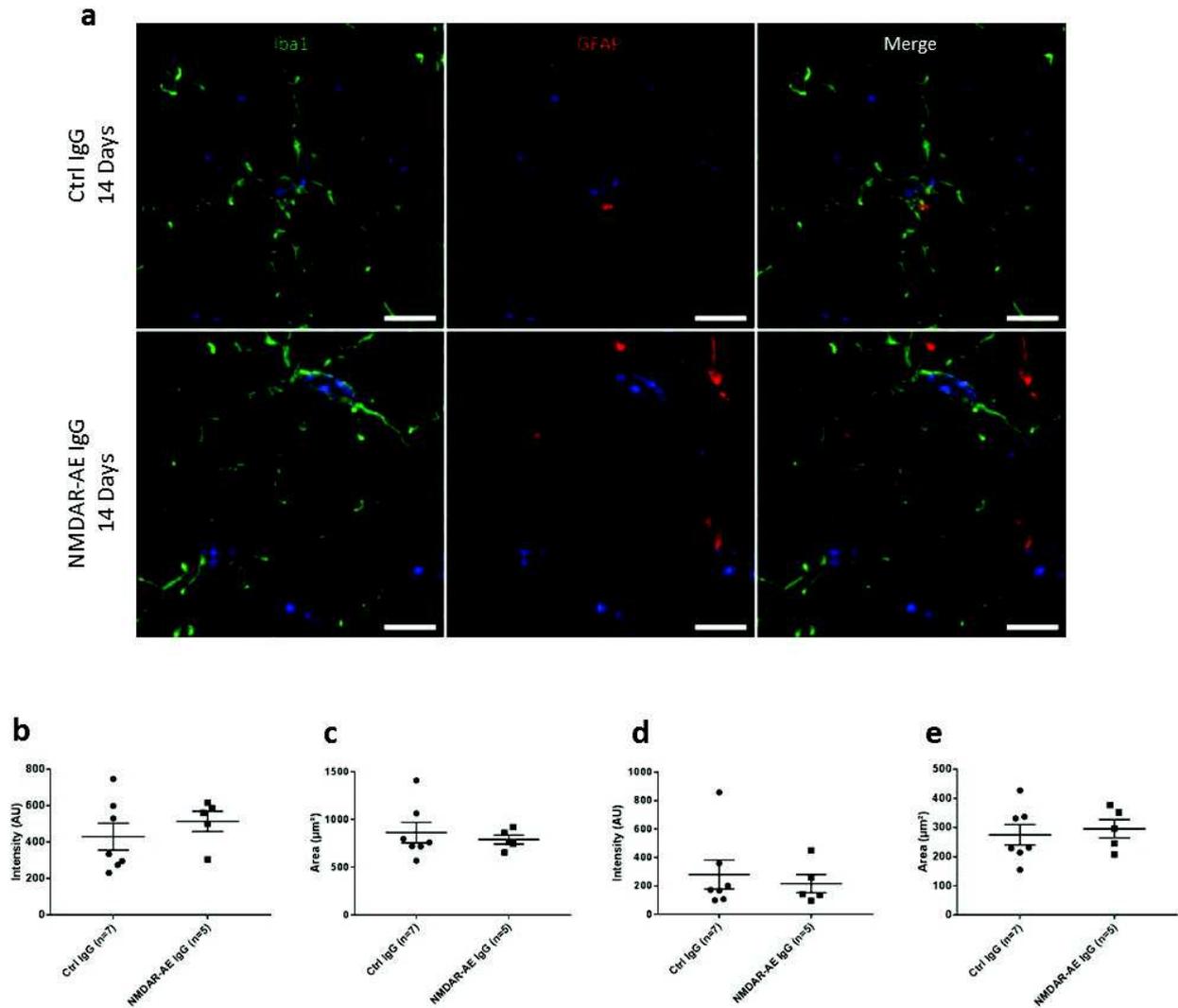


Figure 29: Glial cells do not present signs of activation after 14 days infusion of control IgGs or IgGs from NMDAR-AE patients. Mice were infused with IgGs from control subjects or from NMDAR-AE patients during 14 days and cell-type specific immunostainings were performed (a). The intensity (b) and area (c) of Iba1 staining and the intensity (d) and area (e) of GFAP staining were quantified for each experimental group. Scale bar: 10µm. Horizontal bars represent mean±SEM.

II.3.2. Impact of minocycline on inflammation and behavior after treatment with Ctrl or NMDAR-AE IgGs during 7 days

Finally, we tested if a modulation of microglial activity using minocycline could impact behavior. Mice were treated with either vehicle, or Ctrl or NMDAR-AE purified IgGs during 7 days and received daily intra-peritoneal injection of either minocycline (50mg/kg/day) or vehicle (NaCl 0,9%) (**Fig 30**). Effect of minocycline on microglial activation following IgGs treatment was assessed by performing immunostainings for Iba1 and analysis of microglial morphology. No clear effect on microglial

Results

morphology was observed after minocycline treatment (**Fig 30a&b**). As inflammation can affect memory performances mice treated with minocycline were also subjected to a fear conditioning test (**Fig 30c&d**). Consistent with the lack of clear effect of minocycline on microglial morphology, no effect of minocycline on mice memory performances were observed. However, effects of vehicle or Ctrl IgGs or NMDAR-AE IgGs infusion on fear memory (**Fig 30c&d**) were in line with previous results (see §II.2.1.2. Fig 19 in the Results section).

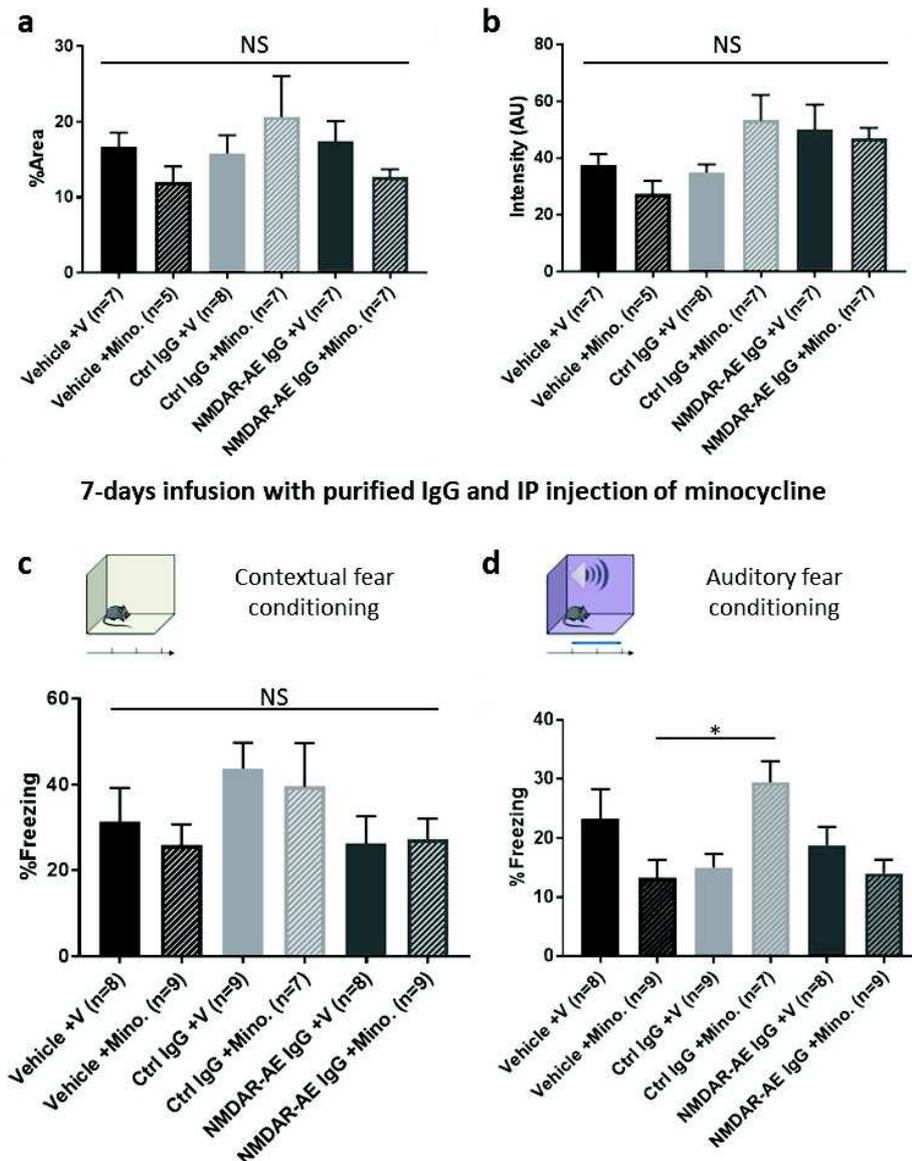


Figure 30: Minocycline treatment did not modify microglial morphology nor fear memory of Ctrl IgGs or NMDAR-AE IgGs infused mice in a fear conditioning test. Mice were infused with vehicle or purified IgGs from control subjects (2mg/mL) or from NMDAR-AE patients (2mg/mL) during 7 days and received daily injection of minocycline (-M, 50mg/kg/day) or vehicle (+V, NaCl 0,9%) (a-d). Immunostainings for a microglial-specific marker (Iba1) was performed and the area (a) and intensity (b) of Iba1 staining was

Results

quantified for each experimental group. Memory was assessed in a contextual (c) and an auditory (d) fear conditioning test. Data are presented as mean±SEM.

II.4. EFFECTS OF PURIFIED CSF INFUSION ON BEHAVIOR

All our results using infusion of NMDAR-Abs purified from patient's serum clearly did not reproduced NMDAR-AE behavioral impairment, despite a clear effect of NMDAR-AE IgGs *in vitro* on dendritic spines morphology. However, studies demonstrating that NMDAR-Abs impaired memory in mouse model obtained this result by infusing dialyzed CSF from NMDAR-AE patients (Planagumà *et al.* 2014) instead of IgG purified from serum. We therefore investigated the effect of CSF infusion from control subjects (Ctrl CSF) or from NMDAR-AE patients (NMDAR-AE CSF during 7 or 14 days) on fear memory and in the spatial-change and novel-object recognition paradigm.

Infusion of CSF from NMDAR-AE patients did not alter contextual or auditory fear memory after 7 (CSF Ctrl IgG: 40.5±5.13%; CSF NMDAR-AE: 41.15±4.35% for contextual fear conditioning, Mann-Whitney test: p=0.9185; **Fig 31a**; and Ctrl IgG: 36.35±4.73%; CSF NMDAR-AE: 28.38±4.03% for auditory fear conditioning, Mann-Whitney test: p=0.2572; **Fig 31b**) or 14 days (CSF Ctrl IgG: 51.31±9.3%; CSF NMDAR-AE: 48.98±10.61% for contextual fear conditioning, Mann-Whitney test: p=0.9551; **Fig 31b**; and Ctrl IgG: 28.96±2.23%; CSF NMDAR-AE: 41.82±7.62% for auditory fear conditioning, Mann-Whitney test: p=0.2319; **Fig 31c**) of infusion. Mice mobility in the spatial and object recognition test after control or NMDAR-AE CSF infusion during 14 days was verified based on the distance moved and the velocity during the first session of the object recognition protocol. Exploration of the different part of the arena during the first session was used as an index for anxiety. In both cases, no differences were observed between the two experimental groups (**Fig32a-c**). Two mice from the NMDAR-AE CSF treated group, but not from the control group, were excluded from analysis due to a lack of exploration (less than 30 seconds per trial) during the object-familiarization sessions. Exploration index in the spatial-change recognition test appeared higher in the NMDAR-AE CSF treated group compared to the Ctrl CSF group but not in the novel-object recognition test (**Fig 32 d&e**) but no statistical analysis were performed due to the very limited number of animals (n= 3 to 6 per group) and these observations must be considered with caution.

Results

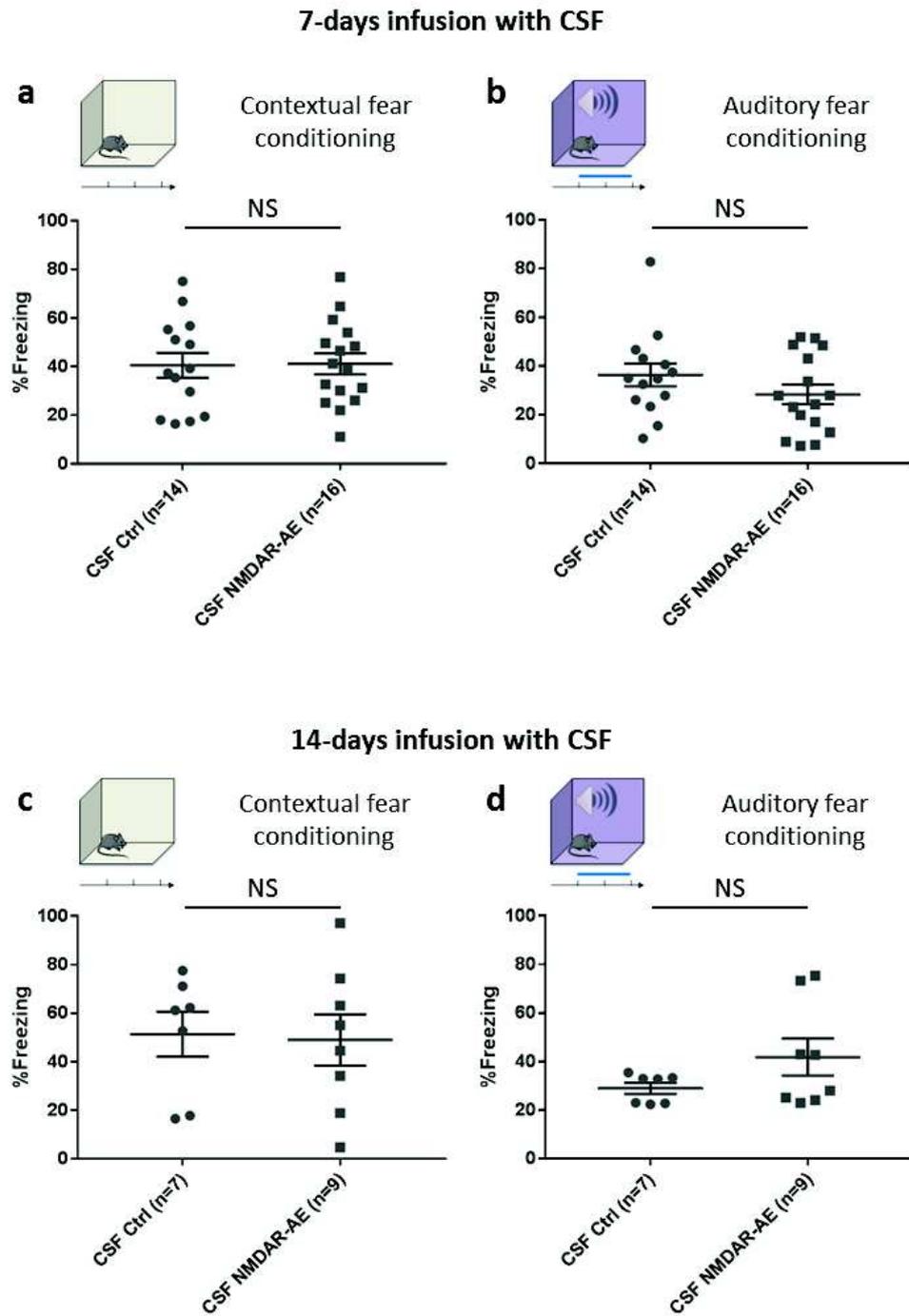


Figure 31: CSF from NMDAR-AE patients does not impair mice behavioral performances in a fear conditioning test. Mice were infused with CSF from control subjects or from NMDAR-AE patients during 7 (a, b) or 14 days (c, d). Infusion with control CSF or from NMDAR-AE patients did not modify mice freezing behavior in contextual (a, c) or auditory fear conditioning (b, d). Noteworthy, the number of mice tested after a 14-days infusion with CSF was limited (n=7 to 9). Horizontal bars represent mean \pm SEM.

Results

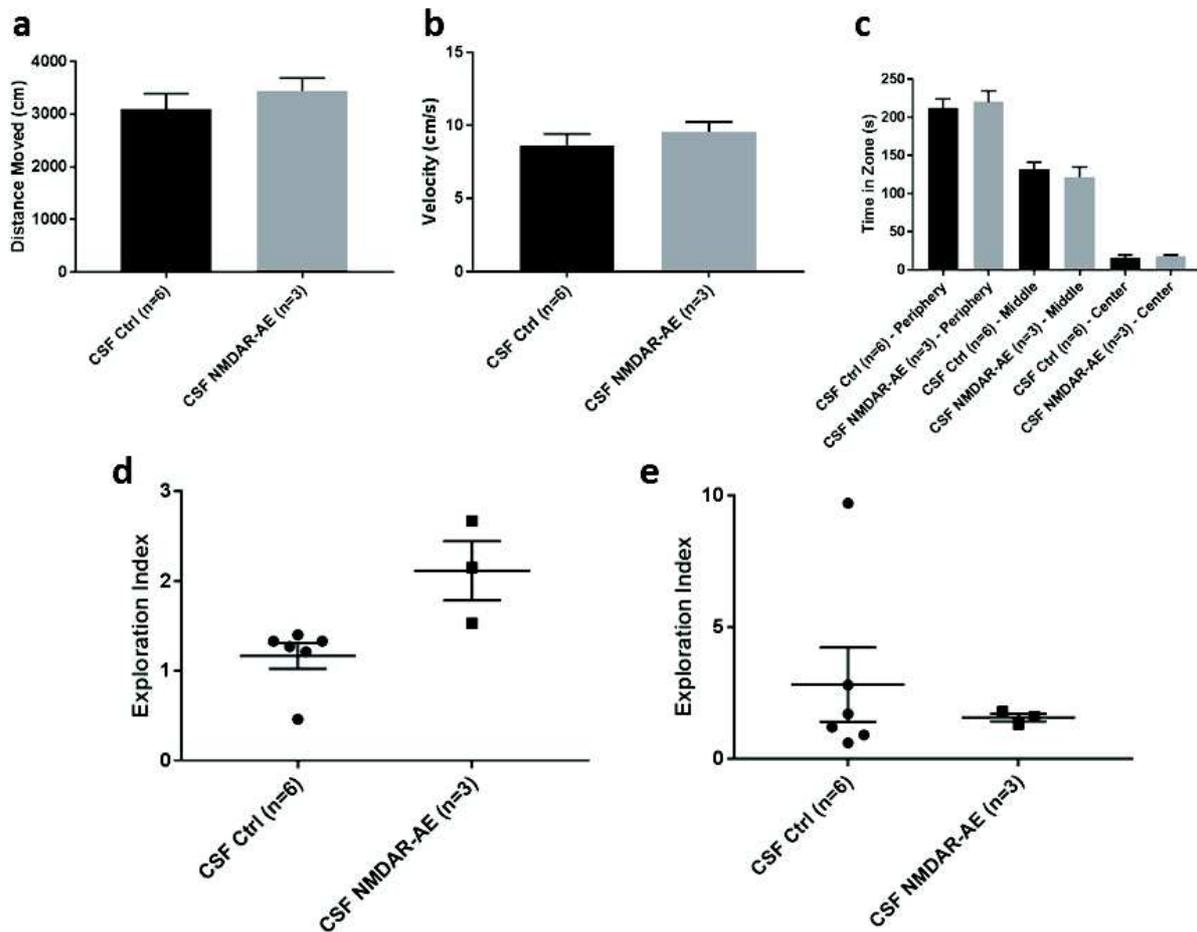


Figure 32: NMDAR-AE CSF did not impair mice memory performances in a novel-object recognition test. Mice were infused with CSF from control subjects or NMDAR-AE patients during 14 days. Distance moved (a), velocity (b) and arena exploration (c) were assessed during the first session of the protocol. NMDAR-AE CSF infusion did not alter spontaneous activity, mobility and exploration of a new environment but appeared to enhanced mice memory performances in the spatial-change test (d) but not in the novel-object recognition test (e). Noteworthy, the number of mice tested after a 14-days infusion with CSF was limited (n=6 to 3) and therefore not statistical analysis were performed. N represents the number of mice per group. Data are represented as mean±SEM.

Discussion

Discussion

Autoantibodies targeting synaptic antigens can exert a pathogenic effect, as it has been demonstrated, for instance in *myasthenia gravis*, an autoimmune neuromuscular disease in which autoantibodies target the nicotinic acetylcholine receptors (AChRs). The direct pathogenic action of AChR-Abs is supported by the reproduction of the disease in a rodent model both by passive transfer of patients' autoantibodies and active immunization with muscle AChR (Keijzers *et al.* 2014). On the contrary, in most of paraneoplastic neurological syndrome, evidence of autoantibodies pathogenicity (e. g. Hu, Yo) are lacking and other mechanisms of pathogenicity, relying on T-cell immunity are believed to be involved. Though NMDAR-AE was initially described as a paraneoplastic neurological syndrome (Dalmau *et al.* 2007), NMDAR-Abs were identified as the probable pathogenic factor very early (Dalmau *et al.* 2008). Since then, a growing body of evidence has suggested a direct pathogenic role of NMDA-Abs on the NMDAR but most of these evidence came from *in vitro* experiments (Dalmau *et al.* 2008, Hughes *et al.* 2010, Manto *et al.* 2010, Manto *et al.* 2011, Mikasova *et al.* 2012) and an animal model of the disease was still lacking to confirm the pathogenicity of NMDAR-Abs and study their impact on brain function. In 2014, Planaguma *et al.* published an animal model with infusion of NMDAR-Abs from human patients in mice brain using osmotic pumps, but this model did not present all the aspects of the disease (Planaguma *et al.*, 2014). Moreover, most of researches on NMDAR-AE physiopathology focused on NMDAR-Abs, but investigation on the involvement of other potential cellular and molecular players are scarce. About 25% of NMDAR-AE patients retain severe deficits or die, and for patients who recover, recovery is a month-long process, with relapses in 12 to 25% of patients (Dalmau *et al.* 2011, Titulaer *et al.* 2013). Thus a better understanding of NMDAR-AE physiopathology is critical. Another remaining question is the origin of the breaking of immune tolerance triggering NMDAR-AE, which is believed to be the associated ovarian teratoma in a subpopulation of patients (38% of patients, young women), but this immune trigger is still unknown in most patients. In this context, my thesis work was organized in two studies. The aim of the first study was to corroborate the role of teratoma in immunization and to identify possible tumor specificities causing the abnormal immunization. The second study aimed at creating an animal model of NMDAR-AE by passive transfer of NMDAR-Abs to test their pathogenicity and impact on brain function.

I. STUDY 1

In this study, we characterized the histopathological features of 27 ovarian teratomas associated with NMDAR-AE (3 immature and 24 mature teratomas). This was the first study performed on a sizable number of cases. Previous studies were limited to a small number (n=3 to 11) of teratomas (Tabata *et al.* 2013, Tüzün *et al.* 2009). Our cohort, lead us to better describe the specificities characterizing these teratoma. Notably that a nervous tissue component was present in all but one NMDAR-AE associated ovarian teratoma, while only 40% of control teratoma contained nervous tissue. GluN1 expression by teratomatous nervous tissue was more often glial in NMDAR-AE teratomas than in control teratomas (73% vs 29%) and a striking particularity was that 3 out of 27 NMDAR-AE-associated mature teratomas contained neuroglial tissue exhibiting histopathological features of central nervous system neuroglial tumor, while it was exceptionally reported in the literature (Day *et al.* 2014, Kleinman *et al.* 1993, Yadav *et al.* 1999). We confirmed another characteristic feature of NMDAR-AE associated teratomas that was the prominent infiltration of their nervous tissue component by immune cells.

In our case series, immature ovarian teratomas accounted for 11% of NMDAR-AE associated ovarian teratomas while in previously reported case series, immature ovarian teratoma accounted for 18 to 20% of all NMDAR-AE associated ovarian teratomas (Tuzun *et al.* 2009, Dabner *et al.* 2012, Day *et al.* 2014). Bearing in mind that these values were obtained on a limited number of cases, this still suggests a higher representation of immature teratoma in NMDAR-AE associated teratoma compared to sporadic cases in which immature teratoma only represent about 3% of all ovarian teratomas (Caruso *et al.* 1971; Nogales *et al.* 2014). Nogales and coll. suggested that immature teratomas were proportionally overrepresented in association with NMDAR-AE because they more frequently contained neural tissue that has the potential to be immature (Nogales *et al.* 2014). However, based on our results and previously published observations we suggest that other specific features of the teratomatous nervous tissue may better distinguish between NMDAR-AE associated teratoma and sporadic teratomas (Days *et al.* 2014).

In our cohort, neural tissue was found in all but one of ovarian teratomas from patients with NMDAR-AE and in 38% of control teratomas. Accordingly, presence of nervous tissue reported in about 30% of mature ovarian teratoma (Caruso *et al.* 1971, Khan *et al.* 2014) indicating that presence of nervous tissue may be a necessary but not sufficient condition to trigger the breaking of immune tolerance causing NMDAR-AE. Systematic NMDAR expression by nervous tissue in NMDAR-AE associated ovarian teratoma has been suggested in 2008 by Dalmau and coll. and in 11 NMDAR-AE associated teratomas in 2009 by Tüzün and coll. (Dalmau *et al.* 2008, Tüzün *et al.* 2009). In this study, we report a positive immunostaining for GluN1 in 82% of NMDAR-AE associated cases, but this lower proportion may be imputed to different sensibility between staining techniques and GluN1 antibody. Notably, contrary to Tüzün and coll., we only used a commercial GluN1 antibody and not anti-NMDAR IgGs purified from patients' serum to detect GluN1 expression in teratoma samples (Tüzün *et al.* 2009). Interestingly, we also report GluN1 expression in 79% of control teratoma, a proportion that is similar to the one observed in NMDAR-AE associated ovarian teratomas. GluN1 expression in sporadic teratoma was also reported in the 3 control cases of Tuzun and coll. (Tüzün *et al.* 2009) indicating that the mere ectopic expression of

Discussion

GluN1 is not sufficient to trigger the break in immune-tolerance leading to NMDAR-AE. Though NMDAR expression in healthy ovary remains controversial ([Tachibana et al. 2013](#), [North et al. 2015](#)), GluN1 and GluN2B expression was already reported in epithelial ovarian carcinoma samples ([North et al. 2015](#)) without association with NMDAR-AE. We observed that GluN1 was expressed by glial cells in the nervous component of ovarian teratoma associated or not with NMDAR-AE. GluN1 expression by human astrocytes was already described ([Conti et al. 1996](#), [Lee et al. 2010](#)) but to the best of our knowledge, this is the first description of glial GluN1 expression in ovarian teratoma. This glial GluN1 expression was slightly more common in NMDAR-AE-associated ovarian teratoma than in sporadic ones, which might suggest the cell type expressing NMDAR could be involved in the outbreak of NMDAR-AE.

In three NMDAR-AE-associated mature ovarian teratoma, the nervous component presented the histological features of CNS tumor and were thus classified using the WHO classification for neuroglial tumor, acknowledging that this classification was developed for CNS tumor as oligodendroglioma (case #4), ganglioglioma (case #5) and malignant glioma (case #6). Foci of nervous tissue forming histologic pattern reminiscent of glioblastoma multiforme in ovarian teratoma were described as soon as 1960 ([Thurlbeck & Scully 1960](#)), however, less than 25 cases of mature ovarian teratoma containing nervous tissue presenting histological features of glioma (mostly glioblastoma and oligodendroglioma) have been described since, making these extremely rare events ([Kleinman et al. 1993](#), [Yadav et al. 1999](#), [Day et al. 2014](#)). In 2014, Day and coll. reported 4 cases of NMDAR-AE-associated ovarian teratoma presenting histological phenotypes of neuroglial tumor and we added 3 more in the present study. In one sample, we further investigated the molecular phenotype of this glioma-like contingent. No gliomatous mutations were detected, suggesting that adult CNS gliomagenesis pathways are not involved in the glioma-like tissue. These abnormal glial aspects were associated with massive intratumoral immune response. Indeed, as other authors have done ([Tuzun et al. 2009](#), [Dabner et al. 2012](#), [Tabata et al. 2013](#), [Day et al. 2014](#)), we report a massive and systematic infiltration of NMDAR-AE associated ovarian teratoma by immune cells directly in contact or in the near vicinity of nervous tissue components. As Dabner and coll. we often observed lymphoid aggregates composed of B- and T-cells segregated and clearly organized as reactive germinal centers in teratoma associated with NMDAR-AE ([Dabner et al. 2012](#)). We also further revealed that immune infiltrates in NMDAR-AE associated teratoma frequently contained mature DC in close proximity with nervous tissue. These features are characteristics of tertiary lymphoid organs that have already been described in other tumor and are believed to be generated as a result of local antigen presentation in a context of chronic inflammation and to perpetuate adaptive immune responses providing local source for antibody production (reviewed in [Hughes et al. 2016](#)). We observed IgGs and IgA deposits and producing cells in the nervous tissue of NMDAR-teratomas. This presence of the effectors of the B cell response in the tumor associated with this B-mediated auto-immune strengthened the idea that in young females with NMDAR-AE and ovarian teratoma, the ovarian teratoma is the origin of the anti-NMDAR immune reaction. Further studies will aim at refining the characterization of the immune infiltrates, and research other specificities, possibly genetic, underlying the development of autoimmunity in these patients. Moreover, an active model of immunization will be required to examine every aspect of the immunization process.

II. STUDY 2

II.1. INFUSION OF HUMAN IgGs PURIFIED FROM SERUM DID NOT REPRODUCE NMDAR-AE SYMPTOMS

Several clinical and experimental evidences support the hypothesis of NMDAR-Abs direct pathogenic effect on NMDAR-Abs (Dalmau *et al.* 2008, Hughes *et al.* 2010, Manto *et al.* 2010, Manto *et al.* 2011, Mikasova *et al.* 2012). Exposure to NMDAR-Abs reduces NMDAR expression on cultured neurons (Hughes *et al.* 2010) and decreases their retention time at the synapse (Mikasova *et al.* 2012), thus promoting their internalization. In *ex vivo* experiments, short exposure of acute brain slice to NMDAR-Abs causes a hyper-glutamatergic state (Manto *et al.* 2010, Manto *et al.* 2011). IgGs-NMDAR-Abs are found in the CSF and serum of all NMDAR-AE patients and the clinical syndrome resemble symptoms caused by abuse of NMDAR-antagonist recreational drugs (Weiner *et al.* 2000, Dalmau *et al.* 2011) supporting the hypothesis of a clinical syndrome mediated by a disruption of NMDAR function by NMDAR-Abs. Experimentally, NMDAR-Abs reduces the synaptic expression of NMDAR in cultured neurons and decreases the number of NMDAR cluster in rat brain parenchyma (Hughes *et al.* 2010, Planagumà *et al.* 2014). Therefore, our work hypothesis was that NMDAR-Abs from NMDAR-AE patients exert a pharmacologic and pathogenic effect on NMDAR function and that their administration to mice would reproduce symptoms observed in NMDAR-AE patient. However, contrary to our hypothesis, treatment of mice with NMDAR-AE IgGs purified from NMDAR-AE patients' serum did not induce memory impairment in a fear conditioning test after a 7-days treatment or both in a fear conditioning and a spatial and object recognition test after a 14-days treatment, though a trend toward an NMDAR-AE IgGs induced memory impairment was observed in each experiment. Results from several experiments were pooled and analyzed together to check the existence of an effect that might have been missed due to low statistical power. The trend toward NMDAR-AE IgGs memory impairment remained, but the strongest effect that emerged was the positive impact of Ctrl IgGs on memory performances in auditory fear conditioning.

These results concur with those obtained by Dr. Bost (**Fig 29**) in an electrophysiological study of synaptic plasticity performed in parallel on the same rodent model (Bost, 2017). In this study, the NMDAR-mediated component of synaptic transmission was studied as well as synaptic plasticity in a protocol of theta-burst-induced long-term potentiation at the CA1-CA3 synapses on acute brain slices from mice treated with Ctrl IgGs or NMDAR-AE IgGs during 7 or 14 days. Results revealed no statistically significant effect of NMDAR-AE IgGs compared to control IgGs both after 7 or 14 days of infusion on either the NMDAR-component of synaptic transmission or synaptic plasticity (**Fig 29**). However, an effect of Ctrl IgGs was also observed in the electrophysiological study: Ctrl IgGs infusion decreases LTP amplitude compared to vehicle infusion level.

Discussion

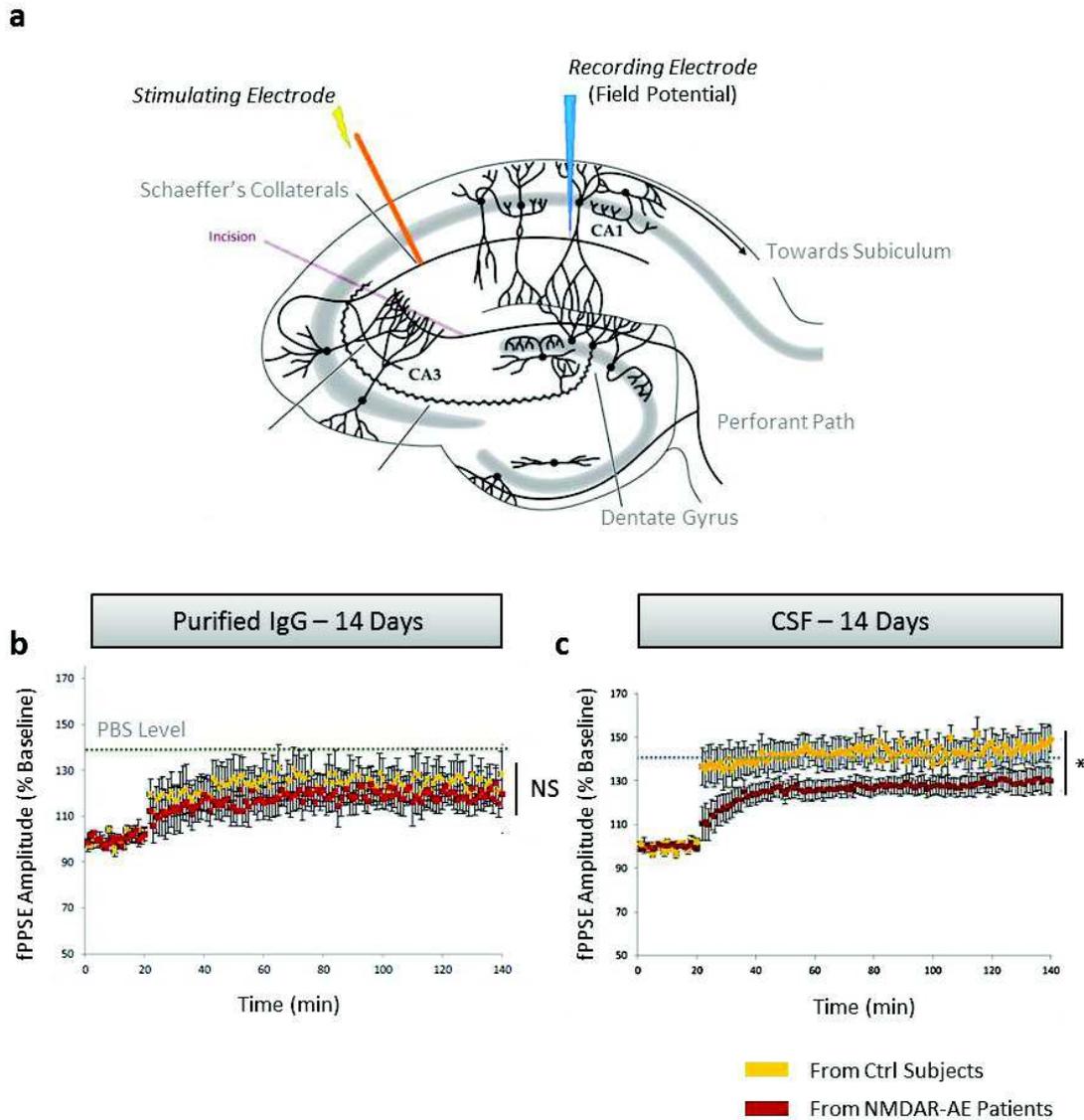


Figure 33: NMDAE-AE IgGs infusion does not alter synaptic plasticity but CSF NMDAR-AE does (results from Dr. Bost). Experiments were performed using the same animal model, infused with either purified IgGs from serum or CSF samples. Treatment with samples from Ctrl subjects is represented in yellow. Treatment with samples from NMDAR-AE patients is represented in red. Long-term potentiation was induced on acute hippocampal slices using a theta-burst stimulation and field potentials were recorded in the CA1 region of the hippocampus. NMDAE-AE IgGs infusion does not alter synaptic plasticity compared to Ctrl IgGs infusion after 7 (Bost, 2017) or 14 days (a). Note that Ctrl IgGs infusion decreases LTP amplitude compared to vehicle infusion level (dotted line, a). CSF NMDAR-AE impairs synaptic plasticity after 14 days of infusion. Adapted from Bost, 2017.

Treatment with IgGs purified from serum of healthy subject (Ctrl IgG) was used as a control condition for treatment with IgGs purified from the serum of NMDAR-AE patients (NMDARAE IgG) and thus containing NMDAR-Abs. Absence of NMDAR-Abs in Ctrl IgGs samples was checked in a cell-based assay. However, both in NMDAR-AE IgGs samples and in Ctrl samples, the identity of antigens targeted by IgGs that were

Discussion

not directed against the NMDAR is unknown and depends on which antigens each subject was exposed to during its lifetime. In order to limit this variability, Ctrl IgGs samples came from three healthy subjects and NMDAR-AE IgGs samples were pooled from three NMDAR-AE patients. An ideal control to investigate specifically the effect of NMDAR-Abs would have been to use NMDAR-AE IgGs samples depleted from NMDAR-Abs, for example by absorption of NMDAR-Abs by successive incubation with HEK transfected cells expressing NMDAR, as has been for *in vitro* experiments by Moscato and coll. (Moscato *et al.* 2014). Limited supplies of NMDAR-AE IgGs samples and large amounts of samples required for continuous brain infusions are limits to the application of this approach to an *in vivo* model. A shortcoming of this study is the lack of positive control. Possible controls include NMDAR antagonists (e. g. ketamine) but also commercial NMDAR antibodies, though their action mechanism might differ from NMDAR-Abs from NMDAR-AE patients as they target different epitopes of the NMDAR (Wüderman *et al.* 2016).

II.2. DIFFERENCES OF NMDAR-Abs EFFECTS FROM SERUM OR CSF?

Interestingly, at the end of year 2014, Planagumà *et al.* reported that NMDAR-Abs impaired synaptic long-term plasticity and caused anhedonic and depressive-like behavior as well as memory deficit using a model of bilateral intraventricular infusion of NMDAR-AE patients' CSF infusion in C57BL/6 mice (Planagumà *et al.* 2014). Subsequent studies mostly confirmed the effect of NMDAR-AE patients' CSF on synaptic plasticity (Planagumà *et al.* 2016, Wüdermann *et al.* 2016) and on memory (Li *et al.* 2015, Wüdermann *et al.* 2016). Notably, several-key features differ in our animal model of passive immunization with NMDAR-Abs from NMDAR-AE patients compared to other published models where an effect was observed : infusion site, biological samples used for treatment (i. e. IgGs purified from serum in our model, vs. CSF samples in other models), IgGs concentration, behavioral tests performed, experimental time frame.

Other published model of NMDAR-AE used either a single CSF injection in hippocampus parenchyma (Wüdermann *et al.* 2016) or continuous infusion in the lateral ventricle (Planagumà *et al.* 2015, Li *et al.* 2015) while our approach is based on continuous infusion of purified IgGs from serum in the medial septum. A wide diffusion of NMDAR-Abs in the brain likely increases their potential pathological role. Hence, human IgGs diffusion in mice brain parenchyma was verified in our model, with a focus on hippocampus due to its involvement in episodic memory, which is altered in NMDAR-AE patients (Dalmau *et al.* 2008). Even though a single infusion cannula was used in our model, human IgGs diffusion in mice brain parenchyma was wide in both hemisphere, concerned both cortical and sub-cortical structures and was clearly seen in the hippocampus, especially in the dorsal regions, but also in ventral hippocampal region. A single infusion cannula in the medial septum is therefore enough to obtain a wide diffusion of purified human IgGs in mice brain parenchyma. An experiment was performed to assess the impact of the infusion site (medial septum vs. lateral ventricles) both on synaptic plasticity by Dr. Bost and on behavior on mice treated during 7 days with Ctrl IgGs or NMDAR-AE IgGs at 2mg/mL through a single infusion cannula placed either in the medial septum or in the left lateral ventricle. Though the number of animal enrolled in the study was limited, the location of the infusion site had no effect on synaptic plasticity or learning in a fear conditioning test, thus ruling out any major impact of the infusion

Discussion

site. Another possible issue linked to the choice of the infusion site is the damaged cause to the target structure. The implantation procedure is designed minimize surrounding lesions to the surrounding area, but as the medial septum send cholinergic, glutamatergic, and GABAergic projections to the hippocampus and cortex (Colom *et al.* 2005, Unal *et al.* 2015) and is involved in learning and spatial memory, and especially in contextual cues processing in conditioned fear learning (Calandreau *et al.* 2007), placement of an infusion cannula in this structure raised concern of a possible lesional effect on synaptic plasticity and behavior. However, placement of an infusion cannula in the medial septum did not alter synaptic plasticity in the electrophysiological study (Bost, 2017) and did not cause significant behavioral impairment in conditioned fear learning, indicating that this was not an issue in this model. Several tests were used to investigate the effect of NMDAR-Abs on memory in rodent models, assessing different memory processes underlied by different brain structures and networks. These differences might explain discrepancies between published results but also with our results. Planagumà *et al.* assessed mice memory performances in a novel object recognition test, either in an open-field or in a V-maze, 3 hours after sample exposure and reported significant impairments after 18 of treatment with NMDAR-AE patients' CSF (Planagumà *et al.* 2014). Other studies on the effects of NMDAR-AR patients' CSF reported spatial memory impairments in the water-maze test after single intra-hippocampal injection (Wüdermann *et al.* 2016) or continuous intra-ventricular infusion (Li *et al.* 2015), but no alteration of object recognition memory (Li *et al.* 2015; though the experimental design of this particular study does not allow to conclude if the absence of effect on memory recognition is due to the type of memory tested or to the time point at which tests were conducted). Notably, all experiments investigating behavioral impact of NMDAR-Abs were performed using high-titer CSF samples from NMDAR-AE patients. Our experiments included contextual and auditory fear conditioning with a retention time of 24 hours and additionally, mice implanted with a 14 days osmotic mini-pump were subjected to a spatial and object recognition protocol at day 11. Brain structures and networks involved in these tasks differ. Fear conditioning notably implicates the amygdale and hippocampus (reviewed in Sanders *et al.* 2003). Spatial and object recognition involve a network of brain regions that includes the peri-rhinal cortex, the medial prefrontal cortex, the hippocampus and the medial dorsal thalamus, but spatial recognition rely more heavily on hippocampus involvement while object recognition implicates predominantly the peri-rhinal cortex (Aggleton *et al.* 1997, reviewed in Warburton & Brown, 2015). Contrary to what was previously reported, we did not observe significant memory performances alterations in conditioned fear responses. To our knowledge, the impact of NMDAR-Abs on fear responses had not been investigated previously. However, we did not observe memory performances alterations in object recognition or spatial recognition either. Other than the use of IgGs purified from serum instead of CSF sample, a possible explanation for this difference is the short retention time (minutes vs. hours in Planagumà *et al.* 2014) used in our spatial and object recognition tests. A shorter retention time might require less involvement of NMDAR-mediated processes. However, previously published NMDAR-Abs mediated memory impairments were accompanied by long-term potentiation impairments (Planagumà *et al.* 2016, Wüdermann *et al.* 2016) absent in our model, indicating that the absence of memory alteration in our model are probably due to a lack or too small effect of NMDAR-Abs from serum.

Discussion

Memory deficits reported by Planagumà and coll. gradually worsened until 18 days after initiation of treatment with NMDAR-AE patients' CSF (Planagumà *et al.* 2014), suggesting that the impact of NMDAR-Abs on behavior is related to the time of exposure. Accordingly, Li and coll. reported spatial memory deficits after 14 days of NMDAR-AE patients' CSF (Li *et al.* 2015). In these experiments, it is always unclear if the absence of memory deficits prior to 14 days of infusion is due to the limited time of infusion or to the differences in memory processes tested (Li *et al.* 2015). In our case, behavioral experiments were performed either 6 days or 11 to 13 days after the onset of the infusion, and only a slight non-significant but reproducible decrease in memory performances was observed with NMDAR-AE IgGs treatment. It is therefore possible that this decrease corresponds to the beginning of NMDAR-Abs induced memory alteration. However, as the amplitude of memory impairments remained stable between tests performed after 6 days or those performed after 13 days in mice treated with NMDAR-AE IgGs, the progression of alteration might be slower in mice treated with IgGs purified from serum, or possibly reach a plateau.

The major difference between our rodent model of passive immunization using NMDA-Abs and others (Planagumà *et al.* 2014, Li *et al.* 2015, Wüdermann *et al.* 2016) is the biological samples used to obtain NMDAR-Abs. IgGs-NMDAR-Abs are always present in NMDAR-AE patients' CSF but also in patient's serum although at lower titers (Dalmau *et al.* 2008). Serum obtention from NMDAR-AE patients' is a simple and non-invasive procedure while CSF requires a lumbar puncture and only allows for small samples withdrawal. Moreover, several studies reported that *in vitro* IgGs-NMDAR-Abs from patients' serum reduced NMDAR expression to the same extent as CSF (Hughes *et al.* 2010) and altered their trafficking (Mikasova *et al.* 2012) suggesting a biological effect of purified IgGs from anti-NMDAR AE patients. Therefore, in first intention, we used IgGs purified from NMDAR-AE patients' serum for passive immunization. However, infusion of NMDAR-AE IgGs did not alter synaptic plasticity or impaired memory in our model, while patients' CSF infusion seems to cause major impairments of both synaptic plasticity and memory (Planagumà *et al.* 2014, 2016). Differences concerning both the quantity and the quality of NMDAR-Abs from serum or CSF might explain these discrepancies. As there are no method available to measure the concentration of NMDAR-Abs in samples containing other types of IgGs, titers are used to estimate their amount. NMDAR-Abs titers are lower in patients' serum than in CSF (Dalmau *et al.* 2008) and NMDAR-Abs effect on NMDAR expression is titer-dependent both *in vitro* and *in vivo* (Hughes *et al.* 2010). Therefore, NMDAR-Abs may not represent a proportion of total serum IgGs important enough to induce a strong effect on synaptic plasticity (Bost, 2017) and memory, and part of their effect might be masked by the effect of Ctrl IgGs. Moreover, the amount of NMDAR-Abs might not be the only difference between NMDAR-Abs from serum and CSF. The epitope targeted by NMDAR-Abs was initially investigated using samples of NMDAR-AE patients' CSF and identified in a small region of the ATD of the GluN1 subunit (Gleichman *et al.* 2012). Interestingly, a later study investigating the epitope of NMDAR-Abs from serum samples of NMDAR-AE patients revealed that 2 of them targeted a different epitope (Castillo-Gómez *et al.* 2016). Although all NMDAR-Abs were able to decrease NMDAR surface expression, irrespective of their epitope (Castillo-Gomez *et al.* 2016, Würdemann *et al.* 2016), this argues toward differences in the "quality" as well as quantity of NMDAR-Abs between serum and CSF, which might also explain part of the differences in our results and those obtained by Planagumà and coll. Alterations of brain function following treatment with CSF from NMDAR-AE patients appear to develop more quickly

Discussion

than using IgGs purified from serum. Indeed, NMDAR-AE CSF treatment significantly impaired synaptic plasticity after 14 days of treatment (Bost, 2017). Alteration of synaptic plasticity after NMDAR-AE CSF infusion is an important step toward validation of our model and indicates that discrepancies with published data are due to the nature of samples used and possibly from time of exposure. Indeed, impairment of synaptic plasticity might require a longer time before affecting memory performances, as no impairment were recorded after 13 days of NMDAR-AE CSF treatment. Altogether, the discrepancies observed between our results and previously published ones appear to result mainly from the difference in samples used to obtain NMDAR-Abs and outline possible differences between NMDAR-Abs from patients' serum or CSF.

II.3. HUMAN IgGs EFFECT ON DENDRITIC SPINES MORPHOLOGY

Dendritic spine morphology is believed to be the morphological correlate of synaptic plasticity (Bourne & Harris, 2007). NMDAR-AE IgGs treatment caused a simplification of dendritic spines morphology, with loss of the clearly defined spine-head and neck. Dendritic spines morphology, especially spine-neck morphology act on neuronal communication by compartmentalizing Ca^{2+} diffusion and signaling (Noguchi *et al.* 2005) and modulating the propagation of membrane potential (Araya *et al.* 2006). Mushroom spines density was decreased in NMDAR-AE IgGs treated mice and stubby and thin spines density was increased. Mushroom spines contains larger postsynaptic densities able to anchor more AMPAR than thin spines which makes them functionally stronger (Matsuzaki *et al.* 2004) and are also more stable over time (Holtmaat *et al.* 2005). Synaptic plasticity and long-term potentiation is associated with spines enlargement and growth (Engert & Bonhoeffer, 1999) while long-term depression is associated with dendritic spines shrinkage (Zhou *et al.* 2004). Altogether, this decrease of mushroom spine density following NMDAR-AE IgGs treatment is consistent with NMDAR-Abs-induced long-term potentiation impairment reported in several studies (Mikasova *et al.* 2012, Planagumà *et al.* 2016, Würdermann *et al.* 2016) though in our case, long-term potentiation was not significantly impaired (Bost, 2017). Alternatively, these changes in dendritic spines morphology could be compensatory mechanisms to regulate NMDAR surface diffusion altered by NMDAR-Abs (Mikasova *et al.* 2012). Indeed, lateral diffusion of AMPAR depends on spine morphology and is restricted at the spine-neck (Ashby *et al.* 2006). Loss of spine neck might facilitate NMDAR entry at the synapse. Alteration of dendritic spines morphology by NMDAR-Abs is of special interest in the context of NMDAR-AE. Indeed, recovery from this disease is long even after removal of NMDAR-Abs (Titularer *et al.* 2013) and a progressive process of reversal of morphologic alteration might underlie reversal of symptoms. To conclude, we have revealed both an effect of Ctrl IgG and NMDAR-AE IgG on dendritic spines morphology. The simplification of dendritic spines morphology caused by NMDAR-Abs might explain the slow recuperation experienced by patients.

II.4. AN EFFECT OF HUMAN IgGs FROM HEALTHY SUBJECTS?

Interestingly, this study revealed several somewhat paradoxical effects of infusion of human IgGs purified from the sera of healthy subject in mice brain parenchyma. Ctrl IgGs infusion reduced dendritic spines density but increased the density of mushroom spines and a concentration-dependent alteration of hippocampal LTP by Ctrl IgGs infusion was observed by Dr. Bost (Bost, 2017) though *in vivo*, Ctrl IgGs infusion enhanced memory performances in auditory fear conditioning. IgGs improve novelty recognition and odor discrimination in piglets (Goncharova *et al.* 2017), indicating an involvement of IgGs on brain development. On the contrary, increased brain IgGs concentration in the context of neurodegenerative disease impairs learning and memory through activation of FcγR (Fernandez-Vizarra *et al.* 2012). As our, these studies outline the importance of IgGs for normal brain function and possible consequences of their deregulation. This is of importance in NMDAR-AE but also for paraneoplastic neurological syndromes, as these patients have increased IgGs concentration in their CSF which could impact their brain function even without direct access to their antigens.

II.5. A ROLE FOR MICROGLIA IN THE PHYSIOPATHOGENESIS OF NMDAR-AE?

Study of IgGs-mediated changes in glial cells morphology allowed tackling of both the question of a FcγR-mediated glial cells activation not specific to NMDAR-Abs and the possible existence of an NMDAR-Abs specific glial cells activation. Gross analysis of glial cells morphology in mice revealed discrete and transient changes of microglial morphology in mice treated with NMDAR-AE IgGs but not with Ctrl IgGs. Microglial cell express FcγR (Vedeler *et al.* 1994) and could interact with Ctrl IgGs through their Fc domain, but the observed effect appeared specific to NMDAR-AE IgGs treatment. Though more refined analysis are necessary to confirm this result and clarify the functional significances of the morphological changes of microglial cells in the context, this is of special interest as microglial are the resident immune cell of the CNS but are also involved in synaptic elimination, plasticity and learning (reviewed in Salter & Beggs, 2014). A study suggests that microglial cells could express functional NMDAR (Kaindl *et al.* 2012) and while this is debated (Eyo *et al.* 2014), it raises the hypothesis of a direct effect of NMDAR-Abs on microglial cells. Alternatively, microglial cells could be indirectly “activated” by NMDAR-Abs *via* their effect on neuronal activity and synaptic transmission. Indeed, microglial cells are sensitive to neuronal and synaptic activity and NMDAR are involved in microglia-neuron communication (Eyo *et al.* 2014). Conversely, microglial cells are able to modulate synaptic NMDAR signaling by releasing IL-1β (Wang *et al.* 2008), a pro-inflammatory cytokine. Chemokine and cytokine signaling in NMDAR-AE might also be detected by microglia. Notably, microglial cell express low-level of CXCL13 receptor (Flynn *et al.* 2003) a chemokine which concentration is increased in the CSF of NMDA-AE patients (Leypoldt *et al.* 2014). Systemic inflammation can also increase FcγR expression by microglial cells, thereby lowering the signaling threshold for IgGs-mediated cell activation (Lunnon *et al.* 2011). Microglial activation was initially observed on brain biopsies (Dalmau *et al.* 2008) and autopsies (Tüzun *et al.* 2009) of NMDAR-AE patients, but this study suggests new mechanisms by which they could be involved in the physiopathology of the disease and outline of their role in NMDAR-AE.

Conclusion

This study questions the almost-established view of NMDAR-Abs pathogenicity. By using samples from NMDAR-AE patients instead of commonly used CSF samples, we show that the amount and the origin of NMDAR-Abs impact their pathological effects. Moreover, we described alteration of fine neuronal morphology which was not predicted by *in vitro* experiments and could be at the root of lengthy recovery experienced by NMDAR-AE patients. Ongoing work focuses on the comparison of NMDAR-Abs impact on neuronal morphology, synaptic plasticity and behavior depending on their origin and after an extended period of exposition and after a recovery period. An improved characterization of serum and CSF samples appears also needed notably to determine if our serum NMDAR-Abs target the same epitopes as NMDAR-Abs from CSF samples. Preliminary results on microglial activation need to be confirmed by refined analysis of microglial morphology and use of other microglial activation markers before thorough investigations of signaling processes involved. Nonetheless, these results are really interesting raise the possibility of the involvement of new actors in the physiopathology of NMDAR-AE *via* unexpected mechanisms, e. g. NMDAR-Abs activation of microglial cells. Finally, creation of an active model of immunization is the next step necessary to further study the immune processes causing NMDAR-AE. We have brought new evidence of the involvement of the ovarian teratoma in the genesis of NMDAR-AE. Though associated teratomas only concern 38% of patients, it provides basis to create an animal model of active immunization, for example by implanting cultured tumor cells expressing the NMDAR. This would allow studying of the different steps of the immune system reaction to this NMDAR ectopic expression and to tests critical parameter (e. g. B or T lymphocytes infiltration, cytokines and chemokines expression, leakage of BHE) in order to identify those causing the break of immune tolerance leading to NMDAR-AE.

References

References

- Aggleton, J. P., Keen, S., Warburton, E. C., & Bussey, T. J. (1997). Extensive cytotoxic lesions involving both the rhinal cortices and area TE impair recognition but spare spatial alternation in the rat. *Brain Research Bulletin*, *43*(3), 279–87.
- Al-Hallaq, R. A., Conrads, T. P., Veenstra, T. D., & Wenthold, R. J. (2007). NMDA Di-Heteromeric Receptor Populations and Associated Proteins in Rat Hippocampus. *Journal of Neuroscience*, *27*(31), 8334–8343.
- Araya, R., Jiang, J., Eisenthal, K. B., & Yuste, R. (2006). The spine neck filters membrane potentials. *Proceedings of the National Academy of Sciences*, *103*(47), 17961–17966.
- Armangue, T., Leypoldt, F., Málaga, I., Raspall-Chaure, M., Marti, I., Nichter, C., ... Dalmau, J. (2014). Herpes simplex virus encephalitis is a trigger of brain autoimmunity. *Annals of Neurology*, *75*(2), 317–23.
- Armangué, T., Titulaer, M. J., Málaga, I., Bataller, L., Gabilondo, I., Graus, F., & Dalmau, J. (2013). Pediatric anti-N-methyl-D-aspartate receptor encephalitis - Clinical analysis and novel findings in a series of 20 patients. *Journal of Pediatrics*, *162*(4).
- Ashby, M. C., Maier, S. R., Nishimune, A., & Henley, J. M. (2006). Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *26*(26), 7046–55.
- Bard, L., Sainlos, M., Bouchet, D., Cousins, S., Mikasova, L., Breillat, C., ... Groc, L. (2010). Dynamic and specific interaction between synaptic NR2-NMDA receptor and PDZ proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(45), 19561–6.
- Barria, A., & Malinow, R. (2005). NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron*, *48*(2), 289–301.
- Bayer, K. U., De Koninck, P., Leonard, A. S., Hell, J. W., & Schulman, H. (2001). Interaction with the NMDA receptor locks CaMKII in an active conformation. *Nature*, *411*(6839), 801–5.
- Beynon, S. B., & Walker, F. R. (2012). Microglial activation in the injured and healthy brain: What are we really talking about? Practical and theoretical issues associated with the measurement of changes in microglial morphology. *Neuroscience*, *225*, 162–171.
- Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, *361*(6407), 31–9.
- Bliss, T.V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* *232*, 331–356.
- Bost, C. (2017) Encéphalites à anticorps anti-NMDAR : étude clinique et mécanistique.
- Bourne, J., & Harris, K. M. (2007). Do thin spines learn to be mushroom spines that remember? *Current Opinion in Neurobiology*, *17*(3), 381–386.
- Brückner, K., Pasquale, E. B., & Klein, R. (1997). Tyrosine phosphorylation of transmembrane ligands for Eph receptors. *Science (New York, N.Y.)*, *275*(5306), 1640–3.
- Buhot, M. C., & Naïli, S. (1995). Changes in exploratory activity following stimulation of hippocampal 5-HT1A and 5-HT1B receptors in the rat. *Hippocampus*, *5*(3), 198–208.
- Byun, J.-I., Lee, S.-T., Moon, J., Jung, K.-H., Sunwoo, J.-S., Lim, J.-A., ... Lee, S. K. (2016). Distinct intrathecal interleukin-17/interleukin-6 activation in anti-N-methyl-d-aspartate receptor encephalitis. *Journal of Neuroimmunology*, *297*, 141–7.
- Cajal, S.R. (1911). *Histologie du Systeme Nerveux de l'Homme et des Vertebres*, Swanson, N., and Swanson, L.W. (trans.) (Oxford University Press).
- Calandreau, L., Jaffard, R., & Desmedt, A. (2007). Dissociated roles for the lateral and medial septum in elemental and contextual fear conditioning. *Learning & Memory*, *14*(6), 422–429.
- Caruso, P. A., Marsh, M. R., Minkowitz, S., & Karten, G. (1971). An intense clinicopathologic study of 305 teratomas of the ovary. *Cancer*, *27*(2), 343–8.
- Castillo-Gómez, E., Oliveira, B., Tapken, D., Bertrand, S., Klein-Schmidt, C., Pan, H., ... Hollmann, M. (2016). All naturally occurring autoantibodies against the NMDA receptor subunit NR1 have pathogenic potential irrespective of epitope and immunoglobulin class. *Molecular Psychiatry*.
- Chefdeville, A., Honnorat, J., Hampe, C. S., Desestret, V., & Maccaferri, G. (2016). Neuronal central nervous system syndromes probably mediated by autoantibodies. *European Journal of Neuroscience*, *43*(12), 1535–1552.
- Chen, B. S., Gray, J. a., Sanz-Clemente, A., Wei, Z., Thomas, E. V., Nicoll, R. a., & Roche, K. W. (2012). SAP102 Mediates Synaptic Clearance of NMDA Receptors. *Cell Reports*, *2*(5), 1120–1128.
- Chen, X., Levy, J. M., Hou, A., Winters, C., Azzam, R., Sousa, A. A., ... Reese, T. S. (2015). PSD-95 family MAGUKs are essential for anchoring AMPA and NMDA receptor complexes at the postsynaptic density. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(50), E6983-92.
- Cissé, M., Halabisky, B., Harris, J., Devidze, N., Dubal, D. B., Sun, B., ... Mucke, L. (2011). Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature*, *469*(7328), 47–52.
- Collingridge, G. L., Kehl, S. J., & McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *The Journal of Physiology*, *334*,

References

- 33–46.
- Colom, L. V., Castaneda, M. T., Reyna, T., Hernandez, S., & Garrido-sanabria, E. (2005). Characterization of medial septal glutamatergic neurons and their projection to the hippocampus. *Synapse*, *58*(3), 151–164.
- Cui, Z., Lindl, K. A., Mei, B., Zhang, S., & Tsien, J. Z. (2005). Requirement of NMDA receptor reactivation for consolidation and storage of nondeclarative taste memory revealed by inducible NR1 knockout. *The European Journal of Neuroscience*, *22*(3), 755–63.
- Cui, Z., Wang, H., Tan, Y., Zaia, K. A., Zhang, S., & Tsien, J. Z. (2004). Inducible and reversible NR1 knockout reveals crucial role of the NMDA receptor in preserving remote memories in the brain. *Neuron*, *41*(5), 781–93.
- Dabner, M., McCluggage, W. G., Bundell, C., Carr, A., Leung, Y., Sharma, R., & Stewart, C. J. R. (2012). Ovarian teratoma associated with anti-N-methyl D-aspartate receptor encephalitis: a report of 5 cases documenting prominent intratumoral lymphoid infiltrates. *International Journal of Gynecological Pathology: Official Journal of the International Society of Gynecological Pathologists*, *31*(5), 429–37.
- Dalmau, J., Gleichman, A. J., Hughes, E. G., Rossi, J. E., Peng, X., Lai, M., ... Lynch, D. R. (2008). Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *The Lancet Neurology*, *7*(12), 1091–1098.
- Dalmau, J., Lancaster, E., Martinez-Hernandez, E., Rosenfeld, M. R., & Balice-Gordon, R. (2011). Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *The Lancet Neurology*, *10*(1), 63–74.
- Dalmau, J., Tüzün, E., Wu, H. Y., Masjuan, J., Rossi, J. E., Voloschin, A., ... Lynch, D. R. (2007). Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Annals of Neurology*, *61*(1), 25–36.
- Dalva, M. B., Takasu, M. A., Lin, M. Z., Shamah, S. M., Hu, L., Gale, N. W., & Greenberg, M. E. (2000). EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell*, *103*(6), 945–56.
- Day, G. S., Laiq, S., Tang-Wai, D. F., & Munoz, D. G. (2014). Abnormal Neurons in Teratomas in NMDAR Encephalitis. *JAMA Neurology*, *71*(6), 1–8.
- Day, G. S., Prüss, H., Benseler, S. M., Paton, T. A., Paterson, A. D., & Andrade, D. M. (2015). GRIN1 polymorphisms do not affect susceptibility or phenotype in NMDA receptor encephalitis. *Neurology® Neuroimmunology & Neuroinflammation*, *2*(5), e153.
- Desestret, V., Chefdeville, A., Viacoz, A., Bost, C., Ducray, F., Picard, G., ... Honnorat, J. (2015). CSF IgA NMDAR antibodies are potential biomarkers for teratomas in anti-NMDAR encephalitis. *Neurology*, *2*(6), 1–8.
- Doss, S., Wandinger, K.-P., Hyman, B. T., Panzer, J. A., Synofzik, M., Dickerson, B., ... Prüss, H. (2014). High prevalence of NMDA receptor IgA/IgM antibodies in different dementia types. *Annals of Clinical and Translational Neurology*, *1*(10), 822–32.
- Engert, F., & Bonhoeffer, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature*, *399*(6731), 66–70.
- Eyo, U. B., Peng, J., Swiatkowski, P., Mukherjee, A., Bispo, A., & Wu, L.-J. (2014). Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *34*(32), 10528–40.
- Fernandez-Vizarra, P., Lopez-Franco, O., Mallavia, B., Higuera-Matas, A., Lopez-Parra, V., Ortiz-Munoz, G., ... Gomez-Guerrero, C. (2012). Immunoglobulin G Fc receptor deficiency prevents Alzheimer-like pathology and cognitive impairment in mice. *Brain*, *135*(9), 2826–2837.
- Flynn, G., Maru, S., Loughlin, J., Romero, I. A., & Male, D. (2003). Regulation of chemokine receptor expression in human microglia and astrocytes. *Journal of Neuroimmunology*, *136*(1–2), 84–93.
- Forrest, D., Yuzaki, M., Soares, H. D., Ng, L., Luk, D. C., Sheng, M., ... Curran, T. (1994). Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. *Neuron*, *13*(2), 325–38.
- Gable, M. S., Sheriff, H., Dalmau, J., Tilley, D. H., & Glaser, C. a. (2012). The frequency of autoimmune N-methyl-D-aspartate receptor encephalitis surpasses that of individual viral etiologies in young individuals enrolled in the california encephalitis project. *Clinical Infectious Diseases*, *54*(7), 899–904.
- Galli, J., Clardy, S. L., & Piquet, A. L. (2017). NMDAR Encephalitis Following Herpes Simplex Virus Encephalitis. *Current Infectious Disease Reports*, *19*(1), 1. <http://doi.org/10.1007/s11908-017-0556-y>
- Gérard, F., & Hansson, E. (2012). Inflammatory activation enhances NMDA-triggered Ca²⁺ signalling and IL-1 β secretion in primary cultures of rat astrocytes. *Brain Research*, *1473*, 1–8.
- Giese, K. P., Fedorov, N. B., Filipkowski, R. K., & Silva, A. J. (1998). Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science (New York, N.Y.)*, *279*(5352), 870–3.
- Gleichman, A. J., Spruce, L. A., Dalmau, J., Seeholzer, S. H., & Lynch, D. R. (2012). Anti-NMDA receptor encephalitis antibody binding is dependent on amino acid identity of a small region within the GluN1 amino terminal domain. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *32*(32), 11082–94.
- Goncharova, K., Lozinska, L., Arevalo Sureda, E., Woliński, J., Weström, B., & Pierzynowski, S. (2017). Importance of neonatal immunoglobulin transfer for hippocampal development and behaviour in the newborn pig. *PLOS ONE*, *12*(6), e0180002.
- Granerod, J., Ambrose, H. E., Davies, N. W. S., Clewley, J. P.,

References

- Walsh, A. L., Morgan, D., ... Crowcroft, N. S. (2010). Causes of encephalitis and differences in their clinical presentations in England: A multicentre, population-based prospective study. *The Lancet Infectious Diseases*, *10*(12), 835–844.
- Grasselli, G., & Hansel, C. (2014). Cerebellar long-term potentiation: cellular mechanisms and role in learning. *International Review of Neurobiology*, *117*, 39–51.
- Graus, F., Titulaer, M. J., Balu, R., Benseler, S., Bien, C. G., Cellucci, T., ... Dalmau, J. (2016). A clinical approach to diagnosis of autoimmune encephalitis. *The Lancet Neurology*, *15*(4), 391–404.
- Gresa-Arribas, N., Titulaer, M. J., Torrents, A., Aguilar, E., McCracken, L., Leypoldt, F., ... Dalmau, J. (2014). Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: A retrospective study. *The Lancet Neurology*, *13*(2), 167–177.
- Groc, L., Heine, M., Cousins, S. L., Stephenson, F. A., Lounis, B., Cognet, L., & Choquet, D. (2006). NMDA receptor surface mobility depends on NR2A-2B subunits. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(49), 18769–74.
- Harris, A. Z., & Pettit, D. L. (2007). Extrasynaptic and synaptic NMDA receptors form stable and uniform pools in rat hippocampal slices. *The Journal of Physiology*, *584*(Pt 2), 509–19.
- Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Poncer, J. C., & Malinow, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science (New York, N.Y.)*, *287*(5461), 2262–7.
- Hogan-Cann, A. D., & Anderson, C. M. (2016). Physiological Roles of Non-Neuronal NMDA Receptors. *Trends in Pharmacological Sciences*, *37*(9), 750–67.
- Holtmaat, A. J. G. D., Trachtenberg, J. T., Wilbrecht, L., Shepherd, G. M., Zhang, X., Knott, G. W., & Svoboda, K. (2005). Transient and persistent dendritic spines in the neocortex in vivo. *Neuron*, *45*(2), 279–91.
- Honorat, J. (2006). Onconeural antibodies are essential to diagnose paraneoplastic neurological syndromes. *Acta Neurologica Scandinavica. Supplementum*, *183*, 64–8.
- Hughes, E. G., Peng, X., Gleichman, A. J., Lai, M., Zhou, L., Parsons, T. D., ... Balice, R. J. (2010). Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *The Journal of Neuroscience* *30*(17), 5866–5875.
- Isaac, J. T., Nicoll, R. A., & Malenka, R. C. (1995). Evidence for silent synapses: implications for the expression of LTP. *Neuron*, *15*(2), 427–34.
- Jantzen, S. U., Ferrea, S., Wach, C., Quasthoff, K., Illes, S., Scherfeld, D., ... Dihné, M. (2013). In vitro neuronal network activity in NMDA receptor encephalitis. *BMC Neuroscience*, *14*, 17.
- Khan MM, Sharif N, Ahmad S. Morphological spectrum of mature ovarian teratoma. *Gomal J Med Sci* 2014; 12:76-80.
- Kaindl, A. M., Degos, V., Peineau, S., Gouadon, E., Chhor, V., Loron, G., ... Gressens, P. (2012). Activation of microglial N-methyl-D-aspartate receptors triggers inflammation and neuronal cell death in the developing and mature brain. *Annals of Neurology*, *72*(4), 536–49.
- Keijzers, M., Nogales-Gadea, G., & de Baets, M. (2014). Clinical and scientific aspects of acetylcholine receptor myasthenia gravis. *Current Opinion in Neurology*, *27*(5), 552–7
- Kleinman, G. M., Young, R. H., & Scully, R. E. (1993). Primary neuroectodermal tumors of the ovary. A report of 25 cases. *The American Journal of Surgical Pathology*, *17*(8), 764–78.
- Kothur, K., Wienholt, L., Mohammad, S. S., Tantsis, E. M., Pillai, S., Britton, P. N., ... Dale, R. C. (2016). Utility of CSF Cytokine/Chemokines as Markers of Active Intrathecal Inflammation: Comparison of Demyelinating, Anti-NMDAR and Enteroviral Encephalitis. *PLoS One*, *11*(8), e0161656.
- Lalo, U., Palygin, O., North, R. A., Verkhatsky, A., & Pankratov, Y. (2011). Age-dependent remodelling of ionotropic signalling in cortical astroglia. *Aging Cell*, *10*(3), 392–402.
- Lancaster, E., & Dalmau, J. (2013). NIH Public Access, *8*(7), 380–390.
- Leger, M., Quiedeville, A., Bouet, V., Haelewyn, B., Boulouard, M., Schumann-Bard, P., & Freret, T. (2013). Object recognition test in mice. *Nature Protocols*, *8*(12), 2531–7.
- Levy, J. M., Chen, X., Reese, T. S., & Nicoll, R. A. (2015). Synaptic Consolidation Normalizes AMPAR Quantal Size following MAGUK Loss. *Neuron*, *87*(3), 534–48.
- Leypoldt, F., Höftberger, R., Titulaer, M. J., Armangué, T., Gresa-Arribas, N., Jahn, H., ... Dalmau, J. (2015). Investigations on CXCL13 in Anti-N-Methyl-D-Aspartate Receptor Encephalitis. *JAMA Neurology*, *72*(2), 180.
- Li, Y., Tanaka, K., Wang, L., Ishigaki, Y., Kato, N. Induction of Memory Deficit in Mice with Chronic Exposure to Cerebrospinal Fluid from Patients with Anti-N-Methyl-DAspartate Receptor Encephalitis. *Tohoku J. Exp. Med.*, 2015, 237, 329-338
- Liao, D., Hessler, N. A., & Malinow, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature*, *375*(6530), 400–4.
- Liba, Z., Kayserova, J., Elisak, M., Marusic, P., Nohejlova, H., Hanzalova, J., ... Sediva, A. (2016). Anti-N-methyl-D-aspartate receptor encephalitis: the clinical course in light of the chemokine and cytokine levels in cerebrospinal fluid. *Journal of Neuroinflammation*, *13*(1), 55.
- Linnoila, J. J., Binnicker, M. J., Majed, M., Klein, C. J., & McKeon, A. (2016). CSF herpes virus and autoantibody profiles in the evaluation of encephalitis. *Neurology(R) Neuroimmunology & Neuroinflammation*, *3*(4), e245.
- Lisman, J.E., and Harris, K.M. (1993). Quantal analysis and synaptic anatomy—integrating two views of

References

- hippocampal plasticity. *Trends Neurosci.* 16, 141–147.
- Lisman, J., Yasuda, R., & Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. *Nature Reviews. Neuroscience*, 13(3), 169–82.
- Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., ... Ellison, D. W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathologica*, 131(6), 803–20.
- Lunnon, K., Teeling, J. L., Tutt, A. L., Cragg, M. S., Glennie, M. J., & Perry, V. H. (2011). Systemic inflammation modulates Fc receptor expression on microglia during chronic neurodegeneration. *Journal of Immunology (Baltimore, Md. : 1950)*, 186(12), 7215–24.
- Maat, P., de Graaff, E., van Beveren, N. M., Hulsenboom, E., Verdijk, R. M., Koorengel, K., ... Sillevis Smitt, P. (2013). Psychiatric phenomena as initial manifestation of encephalitis by anti-NMDAR antibodies. *Acta Neuropsychiatrica*, 1–9. <http://doi.org/10.1111/acn.12013>
- Mangler, M., Trebesch de Perez, I., Teegen, B., Stöcker, W., Prüss, H., Meisel, A., ... Speiser, D. (2013). Seroprevalence of anti-N-methyl-D-aspartate receptor antibodies in women with ovarian teratoma. *Journal of Neurology*, 260(11), 2831–5. <http://doi.org/10.1007/s00415-013-7074-0>
- Manto, M., Dalmau, J., Didelot, A., Rogemond, V., & Honnorat, J. (2010). In vivo effects of antibodies from patients with anti-NMDA receptor encephalitis: further evidence of synaptic glutamatergic dysfunction. *Orphanet Journal of Rare Diseases*, 5(1), 31. <http://doi.org/10.1186/1750-1172-5-31>
- Manto, M., Dalmau, J., Didelot, A., Rogemond, V., & Honnorat, J. (2011). Afferent facilitation of corticomotor responses is increased by IgGs of patients with NMDA-receptor antibodies. *Journal of Neurology*, 258(1), 27–33. <http://doi.org/10.1007/s00415-010-5674-5>
- Marcial-Rojas RA, Medina R. Cystic teratomas of the ovary: a clinical and pathological analysis of two hundred sixty-eight tumors. *AMA Arch Pathol.* 1958;66(4):577-589.
- Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. R., & Kasai, H. (2004a). Structural basis of long-term potentiation in single dendritic spines. *Nature*, 429(6993), 761–766. <http://doi.org/10.1038/nature02617>
- Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. R., & Kasai, H. (2004b). Structural basis of long-term potentiation in single dendritic spines. *Nature*, 429(6993), 761–6. <http://doi.org/10.1038/nature02617>
- Mikasova, L., De Rossi, P., Bouchet, D., Georges, F., Rogemond, V., Didelot, A., ... Groc, L. (2012). Disrupted surface cross-talk between NMDA and Ephrin-B2 receptors in anti-NMDA encephalitis. *Brain*, 135(5), 1606–1621. <http://doi.org/10.1093/brain/aws092>
- Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., & Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron*, 12(3), 529–540. [http://doi.org/10.1016/0896-6273\(94\)90210-0](http://doi.org/10.1016/0896-6273(94)90210-0)
- Monyer, H. N., Burnashev, D. J., Laurie, B., Sakmann, B., & Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four {NMDA} receptors. *Neuron*, 12, 529–540.
- Morris, R. G., Anderson, E., Lynch, G. S., & Baudry, M. (n.d.). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature*, 319(6056), 774–6. <http://doi.org/10.1038/319774a0>
- Moscato, E. H., Peng, X., Jain, A., Parsons, T. D., Dalmau, J., & Balice-Gordon, R. J. (2014). Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. *Annals of Neurology*, 76(1), 108–119. <http://doi.org/10.1002/ana.24195>
- Murugan, M., Sivakumar, V., Lu, J., Ling, E.-A., & Kaur, C. (2011). Expression of N-methyl D-aspartate receptor subunits in amoeboid microglia mediates production of nitric oxide via NF-κB signaling pathway and oligodendrocyte cell death in hypoxic postnatal rats. *Glia*, 59(4), 521–39. <http://doi.org/10.1002/glia.21121>
- Nicoll, R. A. (2017). A Brief History of Long-Term Potentiation. *Neuron*, 93(2), 281–290. <http://doi.org/10.1016/j.neuron.2016.12.015>
- Nogales, F. F., Dulcey, I., & Preda, O. (2014). Germ cell tumors of the ovary: an update. *Archives of Pathology & Laboratory Medicine*, 138(3), 351–62. <http://doi.org/10.5858/arpa.2012-0547-RA>
- Noguchi, J., Matsuzaki, M., Ellis-Davies, G. C. R., & Kasai, H. (2005). Spine-Neck Geometry Determines NMDA Receptor-Dependent Ca²⁺ Signaling in Dendrites. *Neuron*, 46(4), 609–622. <http://doi.org/10.1016/j.neuron.2005.03.015>
- North, W. G., Liu, F., Tian, R., Abbasi, H., & Akerman, B. (2015). NMDA receptors are expressed in human ovarian cancer tissues and human ovarian cancer cell lines. *Clinical Pharmacology: Advances and Applications*, 7, 111–7. <http://doi.org/10.2147/CPAA.S90367>
- Okamoto, K.-I., Narayanan, R., Lee, S. H., Murata, K., & Hayashi, Y. (2007). The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure. *Proceedings of the National Academy of Sciences of the United States of America*, 104(15), 6418–23. <http://doi.org/10.1073/pnas.0701656104>
- Oliet, S. H., Malenka, R. C., & Nicoll, R. A. (1996). Bidirectional control of quantal size by synaptic activity in the hippocampus. *Science (New York, N.Y.)*, 271(5253), 1294–7. Retrieved from

References

- <http://www.ncbi.nlm.nih.gov/pubmed/8638114>
- Palygin, O., Lalo, U., & Pankratov, Y. (2011). Distinct pharmacological and functional properties of NMDA receptors in mouse cortical astrocytes. *British Journal of Pharmacology*, *163*(8), 1755–66. <http://doi.org/10.1111/j.1476-5381.2011.01374.x>
- Paoletti, P., Bellone, C., & Zhou, Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nature Reviews. Neuroscience*, *14*(6), 383–400. <http://doi.org/10.1038/nrn3504>
- Pillai, A. G., de Jong, D., Kanatsou, S., Krugers, H., Knapman, A., Heinzmann, J.-M., ... Touma, C. (2012). Dendritic morphology of hippocampal and amygdalar neurons in adolescent mice is resilient to genetic differences in stress reactivity. *PLoS One*, *7*(6), e38971. <http://doi.org/10.1371/journal.pone.0038971>
- Planagumà, J., Haselmann, H., Mannara, F., Petit-Pedrol, M., Grünwald, B., Aguilar, E., ... Dalmau, J. (2016). Ephrin-B2 prevents N-methyl-D-aspartate receptor antibody effects on memory and neuroplasticity. *Annals of Neurology*, *80*(3), 388–400. <http://doi.org/10.1002/ana.24721>
- Planaguma, J., Leyboldt, F., Mannara, F., Gutierrez-Cuesta, J., Martin-Garcia, E., Aguilar, E., ... Dalmau, J. (2014). Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain*, *138*(1), 94–109.
- Prüss, H., Finke, C., Höltje, M., Hofmann, J., Klingbeil, C., Probst, C., ... Wandinger, K. P. (2012). N-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. *Annals of Neurology*, *72*(6), 902–911.
- Risher, W. C., Ustunkaya, T., Singh Alvarado, J., & Eroglu, C. (2014). Rapid Golgi Analysis Method for Efficient and Unbiased Classification of Dendritic Spines. *PLoS ONE*, *9*(9), e107591.
- Rogan, M. T., Stäubli, U. V., & LeDoux, J. E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. *Nature*, *390*(6660), 604–7.
- Salter, M. W., & Beggs, S. (2014). Sublime microglia: Expanding roles for the guardians of the CNS. *Cell*, *158*(1), 15–24.
- Sanders, M. J., Wiltgen, B. J., & Fanselow, M. S. (2003). The place of the hippocampus in fear conditioning. *European Journal of Pharmacology*, *463*(1–3), 217–223.
- Sans, N., Wang, P. Y., Du, Q., Petralia, R. S., Wang, Y.-X., Nakka, S., ... Wenthold, R. J. (2005). mPins modulates PSD-95 and SAP102 trafficking and influences NMDA receptor surface expression. *Nature Cell Biology*, *7*(12), 1179–90.
- Schmitt, S. E., Pargeon, K., Frechette, E. S., Hirsch, L. J., Dalmau, J., & Friedman, D. (2012). Extreme delta brush: a unique EEG pattern in adults with anti-NMDA receptor encephalitis. *Neurology*, *79*(11), 1094–100.
- Seki, M., Suzuki, S., Iizuka, T., Shimizu, T., Nihei, Y., Suzuki, N., & Dalmau, J. (2008). Neurological response to early removal of ovarian teratoma in anti-NMDAR encephalitis. *Journal of Neurology, Neurosurgery, and Psychiatry*, *79*(3), 324–6.
- Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., ... Selkoe, D. J. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature Medicine*, *14*(8), 837–42.
- Sheffler-Collins, S. I., & Dalva, M. B. (2012). EphBs: an integral link between synaptic function and synaptopathies. *Trends in Neurosciences*, *35*(5), 293–304.
- Sheng, M., Cummings, J., Roldan, L. a, Jan, Y. N., & Jan, L. Y. (1994). Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. *Nature*, *368*(6467), 144–147.
- Sillevis Smitt, P., Kinoshita, A., De Leeuw, B., Moll, W., Coesmans, M., Jaarsma, D., ... Shigemoto, R. (2000). Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. *The New England Journal of Medicine*, *342*(1), 21–7.
- Sköldenberg, B., Aurelius, E., Hjalmarsson, A., Sabri, F., Forsgren, M., Andersson, B., ... Rosengren, L. (2006). Incidence and pathogenesis of clinical relapse after herpes simplex encephalitis in adults. *Journal of Neurology*, *253*(2), 163–70.
- Snyder, E. M., Nong, Y., Almeida, C. G., Paul, S., Moran, T., Choi, E. Y., ... Greengard, P. (2005). Regulation of NMDA receptor trafficking by amyloid-beta. *Nature Neuroscience*, *8*(8), 1051–8.
- Szabo, a, Dalmau, J., Manley, G., Rosenfeld, M., Wong, E., Henson, J., ... Furneaux, H. M. (1991). HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. *Cell*, *67*(2), 325–333.
- Tabata, E., Masuda, M., Eriguchi, M., Yokoyama, M., Takahashi, Y., Tanaka, K., ... Hara, H. (2014). Immunopathological significance of ovarian teratoma in patients with anti-N-methyl-d-aspartate receptor encephalitis. *European Neurology*, *71*(1–2), 42–8.
- Tachibana, N., Kinoshita, M., Saito, Y., & Ikeda, S. (2013). Identification of the N-Methyl-D-aspartate receptor (NMDAR)-related epitope, NR2B, in the normal human ovary: implication for the pathogenesis of anti-NMDAR encephalitis. *The Tohoku Journal of Experimental Medicine*, *230*(1), 13–6.
- Thomas, C. G., Miller, A. J., & Westbrook, G. L. (2006). Synaptic and extrasynaptic NMDA receptor NR2 subunits in cultured hippocampal neurons. *Journal of Neurophysiology*, *95*(3), 1727–34.
- Thomas, L., Mailles, A., Desestret, V., Ducray, F., Mathias, E., Rogemond, V., ... Vuillemet, F. (2014). Autoimmune N-methyl-D-aspartate receptor encephalitis is a differential diagnosis of infectious encephalitis. *Journal of Infection*, *68*(5), 419–425.
- Thurlbeck, W., M., Scully, R., E., (1960) Solid Teratome Of The Ovary A Clinicopathological Analysis of 9 Cases.

References

- Cancer July-August 1960 Vol. 13 804-811
- Titulaer, M. J., McCracken, L., Gabilondo, I., Armangué, T., Glaser, C., Iizuka, T., ... Dalmau, J. (2013). Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: An observational cohort study. *The Lancet Neurology*, *12*(2), 157–165.
- Tovar, K. R., & Westbrook, G. L. (2002). Mobile NMDA receptors at hippocampal synapses. *Neuron*, *34*(2), 255–64.
- Tovar, K. R., & Westbrook, G. L. (2016). Modulating synaptic NMDA receptors. *Neuropharmacology*.
- Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., ... Dingledine, R. (2010). Glutamate Receptor Ion Channels: Structure, Regulation, and Function. *Pharmacological Reviews*, *62*(3), 405–496.
- Tüzün, E., Zhou, L., Baehring, J. M., Bannykh, S., Rosenfeld, M. R., & Dalmau, J. (2009). Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. *Acta Neuropathologica*, *118*(6), 737–743.
- Unal, G., Joshi, A., Viney, T. J., Kis, V., & Somogyi, P. (2015). Synaptic Targets of Medial Septal Projections in the Hippocampus and Extrahippocampal Cortices of the Mouse. *Journal of Neuroscience*, *35*(48), 15812–15826.
- Vedeler, C., Ulvestad, E., Grundt, I., Conti, G., Nyland, H., Matre, R., & Pleasure, D. (1994). Fc receptor for IgG (FcR) on rat microglia. *Journal of Neuroimmunology*, *49*(1–2), 19–24.
- Verhelst, H., Verloo, P., Dhondt, K., De Paepe, B., Menten, B., Dalmau, J., & Van Coster, R. (2011). Anti-NMDA-receptor encephalitis in a 3 year old patient with chromosome 6p21.32 microdeletion including the HLA cluster. *European Journal of Paediatric Neurology: EJPN: Official Journal of the European Paediatric Neurology Society*, *15*(2), 163–6.
- Viaccoz, A., Desestret, V., Ducray, F., Picard, G., Cavillon, G., Rogemond, V., ... Honnorat, J. (2014). Clinical specificities of adult male patients with NMDA receptor antibodies encephalitis. *Neurology*, *82*(7), 556–563.
- Wang, P., Rothwell, N. J., Pinteaux, E., & Brough, D. (2008). Neuronal injury induces the release of pro-interleukin-1beta from activated microglia in vitro. *Brain Research*, *1236*, 1–7.
- Wang, R., Guan, H. Z., Ren, H. T., Wang, W., Hong, Z., & Zhou, D. (2015). CSF findings in patients with anti-N-methyl-d-aspartate receptor-encephalitis. *Seizure*, *29*, 137–142.
- Warburton, E. C., & Brown, M. W. (2015). Neural circuitry for rat recognition memory. *Behavioural Brain Research*, *285*, 131–9.
- Weiner, A. L., Vieira, L., McKay, C. A., & Bayer, M. J. (2000). Ketamine abusers presenting to the emergency department: a case series. *The Journal of Emergency Medicine*, *18*(4), 447–51.
- Willison, H. J., Jacobs, B. C., & van Doorn, P. A. (2016). Guillain-Barré syndrome. *Lancet (London, England)*, *388*(10045), 717–27.
- Wright, S., Hashemi, K., Stasiak, L., Bartram, J., Lang, B., Vincent, A., & Upton, A. L. (2015). Epileptogenic effects of NMDAR antibodies in a passive transfer mouse model. *Brain: A Journal of Neurology*, *138*(Pt 11), 3159–67.
- Würdemann, T., Kersten, M., Tokay, T., Guli, X., Kober, M., Rohde, M., ... Kirschstein, T. (2016). Stereotactic injection of cerebrospinal fluid from anti-NMDA receptor encephalitis into rat dentate gyrus impairs NMDA receptor function. *Brain Research*, *1633*, 10–18.
- Yadav, A., Lellouch-Tubiana, A., Fournet, J. C., Quazza, J. E., Kalifa, C., Sainte-Rose, C., & Jaubert, F. (1999). Glioblastoma multiforme in a mature ovarian teratoma with recurring brain tumours. *Histopathology*, *35*(2), 170–3.
- Zekeridou, A., Karantoni, E., Viaccoz, A., Ducray, F., Gitiaux, C., Villega, F., ... Honnorat, J. (2015). Treatment and outcome of children and adolescents with N-methyl-d-aspartate receptor encephalitis. *Journal of Neurology*, 1859–1866.
- Zhang, Q., Tanaka, K., Sun, P., Nakata, M., Yamamoto, R., Sakimura, K., ... Kato, N. (2012). Suppression of synaptic plasticity by cerebrospinal fluid from anti-NMDA receptor encephalitis patients. *Neurobiology of Disease*, *45*(1), 610–5.
- Zheng, C.-Y., Petralia, R. S., Wang, Y.-X., Kachar, B., & Wenthold, R. J. (2010). SAP102 is a highly mobile MAGUK in spines. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *30*(13), 4757–66.
- Zhou, Q., Homma, K. J., & Poo, M. (2004). Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron*, *44*(5), 749–57.

Appendices

SUPPLEMENTAL MATERIAL & METHODS

Immunohistochemistry

Four μm FFPE tumor sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol, followed by antigen retrieval in 0.01M citrate buffer (ph=6). After blocking in 1% NGS, 1% BSA, 0.3% Triton X-100 in PBS, sections were incubated with the primary antibody (Table X) overnight at +4°C, thoroughly washed, then incubated with a biotinylated secondary antibodies (REF). Staining was developed using DAB chromogen (Vectastain ABC Kits, Vector Laboratories). Slides were mildly counterstained in hematoxylin, dehydrated and mounted in DEPEX. A section of human cerebellum was used as a positive control, and primary antibodies were omitted in negative control.

Immunofluorescence (IF) staining

Teratoma sections were deparaffinized and rehydrated and antigen retrieval in 0.01M citrate buffer (ph=6) was performed. After blocking in 1% NGS, 1% BSA, 0.3% Triton X-100 in PBS, sections were incubated with primary antibodies (Suppl. Table 1) overnight at +4°C, extensively washed and then incubated with a secondary antibodies for 1h at room temperature. Fluorescence was developed using streptavidin coupled to a fluorochrome. Autofluorescence was eliminated by incubation with 0.1% Sudan Black B (Merck) in 70% ethanol. Nuclei were stained with DAPI and slides were mounted with FluorPreserve. A section of human cerebellum was used as a positive control, and primary antibodies were omitted in negative control.

Next-generation sequencing

Next-generation sequencing was performed at l'Institut du Cerveau et de la Moëlle Epinière à Paris. Genomic DNA was extracted from the nervous tissue component of a paraffin-embedded teratoma sample. DNA library was prepared using the Kapa DNA Library Preparation Kits (Roche). Hybridization was performed at 47°C during 72 hours with primers designed using the SeqCapEZ (Roche) and hybridized sequences were isolated following the manufacturer protocol. After amplification, next generation sequencing was performed on the NextSeq500 from Illumina according to manufacturer specification (Illumina, San Diego, California).

SUPPLEMENTAL RESULTS

Clinical description of NMDAR-AE cases presenting glioma-like features teratomas

Case #4

A thirty-eight years old women presented with gait instability and diplopia. A prodromal episode of flu-like symptoms and headaches a month before initial symptoms was reported, otherwise medical history was unremarkable. Brain MRI was normal. The patient was confused, agitated and presented signs of cognitive impairment. Fluctuating levels of consciousness and dysautonomia motivated her transfer to the ICU, and later in the course of the disease she also developed seizures and dyskinesia. The patient improved after resection of a teratoma on the left ovary and treatment with IVIg and corticoids. Neurologic recovery was complete after 4 months and patient remained symptom-free at the 2-years

Appendices

follow-up. Gross examination of the resected mass revealed a multi-tissular mature ovarian teratoma containing bones, fat, neuroglial tissue and choroid plexuses. Histological study identified a proliferation of monotonous glial cells with round and uniform nuclei surrounded by a perinuclear cytoplasmic halo mixed with a delicate branching network of capillaries consistent with an oligodendroglioma.

Case #5

A twenty-two years-old women presented in the hospital with abnormal movements, generalized seizures, and dysautonomia and central hypoventilation necessitating transfer to the ICU and ventilation support. She had close antecedents of behavioral and psychiatric disorder treated with neuroleptics. First MRI was normal but EEG showed abnormalities suggestive of encephalopathy. Pelvic echography revealed the presence of a mass on the left ovary and a dermoid cyst was resected. The patient was treated with 6 rounds of IVIg and corticoids. Neurological examination was normal after 6 months. Upon histologic examination, the cyst contained bone, fat, skin, and neural tissue. In the neural component, elevation of the cellular density, with clusters of ganglion cells and proliferation of aligned oligodendrocytes consistent with the histologic features of a ganglioglioma was observed.

Case #6

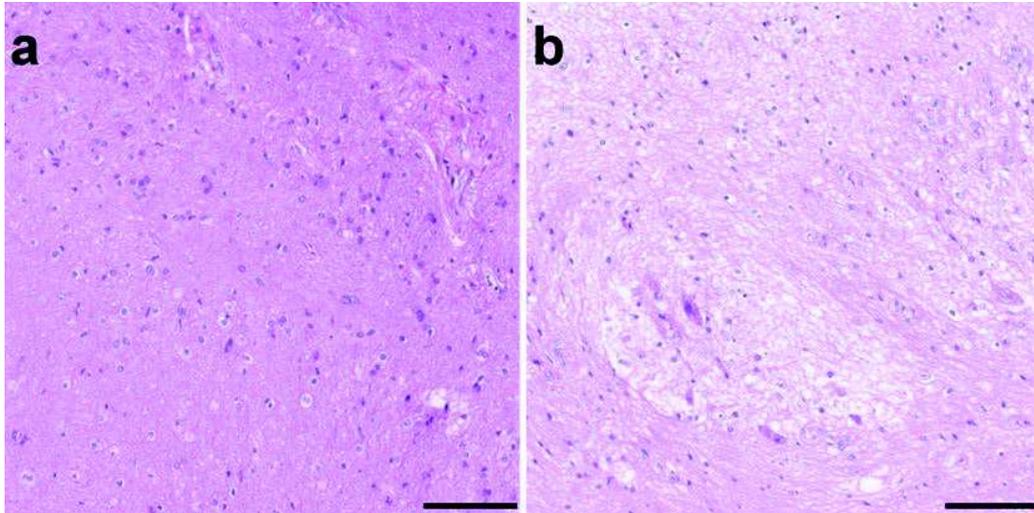
A sixteen-year old girl initially presented with behavioral disorder, agitation and disinhibition. Her condition worsened and she developed movement disorder, confusion, catatonia, mutism, fluctuating levels of consciousness and dysautonomia. Patient was initially treated by corticoids followed by IVIg and plasma exchange and underwent surgical resection of a mass on the right ovary. Upon diagnosis of NMDAR-AE, treatment with second-line immunotherapy (rituximab and cyclophosphamide) was started. The patient responded to treatment and was completely free from symptoms at the 12-months follow-up. Macroscopically, the excised mass measured 4 cm in diameter. Histopathological study revealed a multi-tissular mature teratoma, containing skin, bone, fat, a tooth, choroid plexuses and neural tissue with strong transformations, with highly elevated cellular density and abundant pleomorphic cells and focally elevated proliferation index consistent with histological finding expected in a malignant glioma.

SUPPLEMENTAL TABLE

Antibody	Producer	Catalog #	Dilution	Cell Type Stained
CD3	Roche	2GV6	Pre-diluted	T lymphocytes
CD20	Dako	L26	1:200	B lymphocytes
Chromogranin A				Neuron (perikaryon), neuroendocrine cell
DC-Lamp	Dendritics	1010E1.01	1:100	Mature dendritic cell
GFAP	Dako for IHC	Z0334	1:500	Astrocytes
	BD Biosciences for IF	556330	1:500	
GluN1	Millipore	R1JHL	1:250 for IHC 1:100 for IF	NMDAR-GluN1 subunit
IgA	Dako		1/200	IgA deposits and IgA-producing cells
IgG	Southern Biotech		1/500	IgG deposits and IgG-producing cells
Ki-67				Proliferating cells
Neurofilament				Neurons (axon)
Olig2				Oligodendrocytic lineage

Suppl. Table 1: References of antibodies used for immunohistochemistry and immunofluorescent stainings

SUPPLEMENTAL FIGURE



Suppl. Figure 1: Nervous tissue in control ovarian teratomas. Paraffin-embedded sections of ovarian teratoma stained with HPS. Nervous tissue in control teratoma do not present high cellular density and are seldom infiltrated by immune cells. Scale bars: 50 μ m.

CSF IgA NMDAR antibodies are potential biomarkers for teratomas in anti-NMDAR encephalitis

OPEN

Virginie Desestret, MD, PhD
Aude Chefdeville, MSc
Aurélien Viaccoz, MD
Chloe Bost, MSc
François Ducray, MD, PhD
Géraldine Picard
Veronique Rogemond, PhD
Marie-Océane Chaffois
Charlotte Blanc, MSc
Claire Bardel, PhD
Isabelle Treilleux, MD, PhD
Olivier Pascual, PhD
Jean-Christophe Antoine, MD
Jean-Yves Delattre, MD
Jerome Honnorat, MD, PhD

Correspondence to
Dr. Honnorat:
jerome.honnorat@chu-lyon.fr

Supplemental data
at Neurology.org/nn

ABSTRACT

Objective: To evaluate the presence of immunoglobulin A (IgA) subtype of anti-NMDA receptor (NMDAR) antibodies (IgA-NMDAR-Abs) in the CSF of patients with immunoglobulin G (IgG)-NMDAR-Ab encephalitis and to describe the potential association with a specific clinical pattern.

Methods: The retrospective analysis for the presence of IgA-NMDAR-Abs in 94 CSF samples from patients with anti-NMDAR encephalitis diagnosed between October 2007 and February 2014 was conducted at the French Reference Centre on Paraneoplastic Neurological Syndrome. This observational study compared 39 patients with both IgA- and IgG-NMDAR-Abs to 55 patients with only IgG-NMDAR-Abs.

Results: In the retrospective cohort, 41% of the patients with NMDAR-Ab encephalitis had both CSF IgG- and IgA-NMDAR-Abs. Approximately half of the IgA-NMDAR-Ab-positive patients (18/38, 49%) definitively possessed associated tumors, primarily ovarian teratomas (17/18, 94%), compared with only 5% (3/55) of the patients in the IgA-NMDAR-Ab-negative group ($p < 0.001$). In the adult female population at risk for ovarian teratoma, the detection of CSF IgA-NMDAR-Ab positivity showed 85% sensitivity, 70% specificity, a 57% positive predictive value, and a 90% negative predictive value for the diagnosis of ovarian teratoma. No other specific clinical features or clinical outcome were associated with CSF IgA-NMDAR-Ab positivity.

Conclusion: These results suggest that in patients with IgG-NMDAR-Ab encephalitis, CSF IgA-NMDAR-Abs could be used as a biological marker for the presence of an ovarian teratoma.

Neurol Neuroimmunol Neuroinflamm 2015;2:e166; doi: 10.1212/NXI.000000000000166

GLOSSARY

Ab = antibody; **CBA** = cell-based assay; **ICU** = intensive care unit; **IgA** = immunoglobulin A; **IgG** = immunoglobulin G; **IgM** = immunoglobulin M; **IQR** = interquartile range; **mRS** = modified Rankin Scale; **NMDAR** = NMDA receptor; **PBS** = phosphate-buffered saline; **QAlb** = albumin quotient; **QIg** = immunoglobulins quotient.

Anti-NMDA receptor (NMDAR) encephalitis is a severe autoimmune neuropsychiatric disorder characterized by the circulating immunoglobulin G (IgG) subtype of anti-NMDAR autoantibodies (IgG-NMDAR-Abs). IgG-NMDAR-Abs are directly involved in the pathophysiology of this encephalitis, leading to the selective and reversible internalization of cell-surface NMDARs.^{1–3} Diagnosis is based on the presence of highly specific IgG-NMDAR-Abs in the CSF.^{4,5} Immunoglobulin A (IgA) (IgA-NMDAR-Abs) or immunoglobulin M (IgM) (IgM-NMDAR-Abs) directed against NMDAR have also been reported in the sera of some patients with dementia, schizophrenia, affective disorders, Parkinson disease, stroke, or amyotrophic lateral sclerosis, or in healthy controls,^{6–8} but these findings have an unclear diagnostic value. Some authors suggested

From the Lyon Neuroscience Research Center (V.D., A.C., C. Bost, F.D., V.R., C. Blanc, O.P., J.-C.A., J.H.), INSERM U1028/CNRS UMR 5292; Université de Lyon—Université Claude Bernard Lyon 1 (V.D., A.C., C. Bost, F.D., V.R., C. Bardel, O.P., J.H.); the Department of Neurology D (V.D.) and French Reference Center on Paraneoplastic Neurological Syndrome (A.V., C. Bost, F.D., G.P., V.R., M.-O.C., C. Blanc, J.-C.A., J.-Y.D., J.H.), Hospices Civils de Lyon, Hôpital Neurologique, Bron; Service de Biostatistique (C. Bardel), Hospices Civils de Lyon, CNRS UMR 5558; Laboratoire de Biométrie et Biologie Evolutive (C. Bardel), Equipe Biostatistique-Santé, Villeurbanne; the Department of Biopathology (I.T.), Centre de Recherche en Cancérologie de Lyon; Service de Neurologie (J.-C.A.), CHU de Saint-Etienne et Université de Lyon, Saint-Etienne; and Service de Neurologie Mazarin (J.-Y.D.), Groupe Hospitalier Pitié-Salpêtrière, APHP, Université Pierre et Marie Curie-Paris 6, Centre de Recherche de l'Institut du Cerveau et de la Moelle Épinrière, UMR S975, CNRS, UMR 7225, Paris, France.

Funding information and disclosures are provided at the end of the article. Go to Neurology.org/nn for full disclosure forms. The Article Processing Charge was paid by INSERM–DRLYS–FACTURATION.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially.

that under some circumstances, IgA-NMDAR-Abs could be pathogenic and that IgA-NMDAR-Abs would be present in the serum of 31% of patients with IgG-NMDAR-Ab encephalitis.⁶

The aim of the present study was to evaluate the presence of IgA-NMDAR-Abs in the CSF of patients with IgG-NMDAR-Ab encephalitis and determine the potential association with a specific clinical pattern. The results suggested that although CSF IgA-NMDAR-Abs could be used as biological markers for the presence of ovarian teratoma, these markers do not have much influence on the clinical course of the disease.

METHODS Patients and NMDAR-Ab detection. We systematically tested 94 CSF samples of patients with NMDAR-Ab encephalitis (25 children [age <12 years] and 69 adult patients) diagnosed between October 2007 and February 2014 at the French Reference Centre on Paraneoplastic Neurological Syndrome for presence of IgA-NMDAR-Abs. This observational cohort study was based on patients diagnosed with NMDAR-Ab encephalitis using the CSF rather than the serum because of the better quality of the results and the observation of a 3% false-positive rate of serum IgG-NMDAR-Abs in healthy patients. All analyzed CSF were the first available CSF obtained by a lumbar puncture realized for diagnostic purpose (median delay between the onset of the disease and the lumbar puncture: 30 days; interquartile range [IQR] 18–56) before any immunosuppressive treatment.

CSF IgG-NMDAR-Abs detection. For a diagnosis of NMDAR-Ab encephalitis, the CSF analysis had to fulfill the following previously established criteria for the presence of IgG-NMDAR-Abs⁹: (1) CSF samples must produce a specific pattern of neuropil immunostaining in the rat brain hippocampus; and (2) CSF samples must yield a positive cell-based assay (CBA) using HEK293 cells expressing both the GluN1 and GluN2B subunits of the NMDAR (figure 1).

For immunohistochemistry, rat brains were fixed in 4% paraformaldehyde for 1 hour at 4°C, washed in 0.1 M phosphate buffer, placed in sucrose 30% at 4°C until brain sink, and frozen. Twelve-micrometer-thick frozen rat brain sections were realized and were serially incubated with phosphate-buffered saline (PBS) for 10 minutes, in PBS containing 3% normal goat serum and 3% bovine serum albumin for 1 hour and the patient's CSF (1:10) overnight at room temperature. The bound patient antibodies were visualized using Alexa488-conjugated anti-human IgG (Life Technologies, St. Aubin, France) diluted at 1/1,500 at room temperature for 1 hour and fluorescence microscopy (AxioPhot; Zeiss, Oberkochen, Germany).

CBA was performed on HEK cells overexpressing the GluN1 (fused with the green fluorescent protein) and the GluN2b subunits of the NMDAR. Cells, fixed with 4% paraformaldehyde, were permeabilized in PBS containing 0.2% gelatin and 0.1% triton and then incubated with the patient's CSF (1:10) for 90 minutes. The bound antibodies were visualized using Cy3-conjugated anti-human IgG (CyTM3 AffiniPure Goat Anti-Human IgG; Jackson ImmunoResearch, West Grove, PA).

CSF IgA-NMDAR-Ab detection. Ninety-four CSF of patients with NMDAR-Ab encephalitis were available to study the presence of IgA-NMDAR-Ab. In order to detect such autoantibodies, we used the same CBA described above using a

Cy3-conjugated anti-human IgA (Jackson ImmunoResearch). For all positive patients by CBA, we controlled IgA-NMDAR-Abs immunostained rat brain hippocampus with a specific pattern of neuropil (figure 1). Absence of cross-reactivity between the secondary anti-human IgA and IgG was checked on purified patient IgG as previously described.²

Titration of CSF Ig-NMDAR-Abs. IgG-NMDAR-Ab and IgA-NMDAR-Ab titers were determined using a CBA and serial dilutions of CSF until the reactivity was no longer detectable. The assigned titer value is indicative of the last dilution in which the positivity was detected. Two investigators independently evaluated the endpoint dilution (C.B. and V.R.).

Detection of intrathecal antibody synthesis. The IgG, IgA, and albumin concentrations in the CSF and serum were evaluated using nephelometry (IMMAGE Immunochemistry Systems; Beckman-Coulter, Hialeah, FL). The CSF/serum albumin quotient (QAlb) was used to evaluate the integrity of the CSF-blood barrier. The QAlb is age-dependent: the upper reference limit is calculated for each patient.¹⁰

To characterize intrathecal immunoglobulin synthesis, the immunoglobulins quotient (QIg = [Ig_{CSF}/Ig_{serum}]) was calculated and compared to the corresponding QLim. QLim represents the maximum QIg that can be expected at a given QAlb in the absence of intrathecal immunoglobulin synthesis.¹⁰

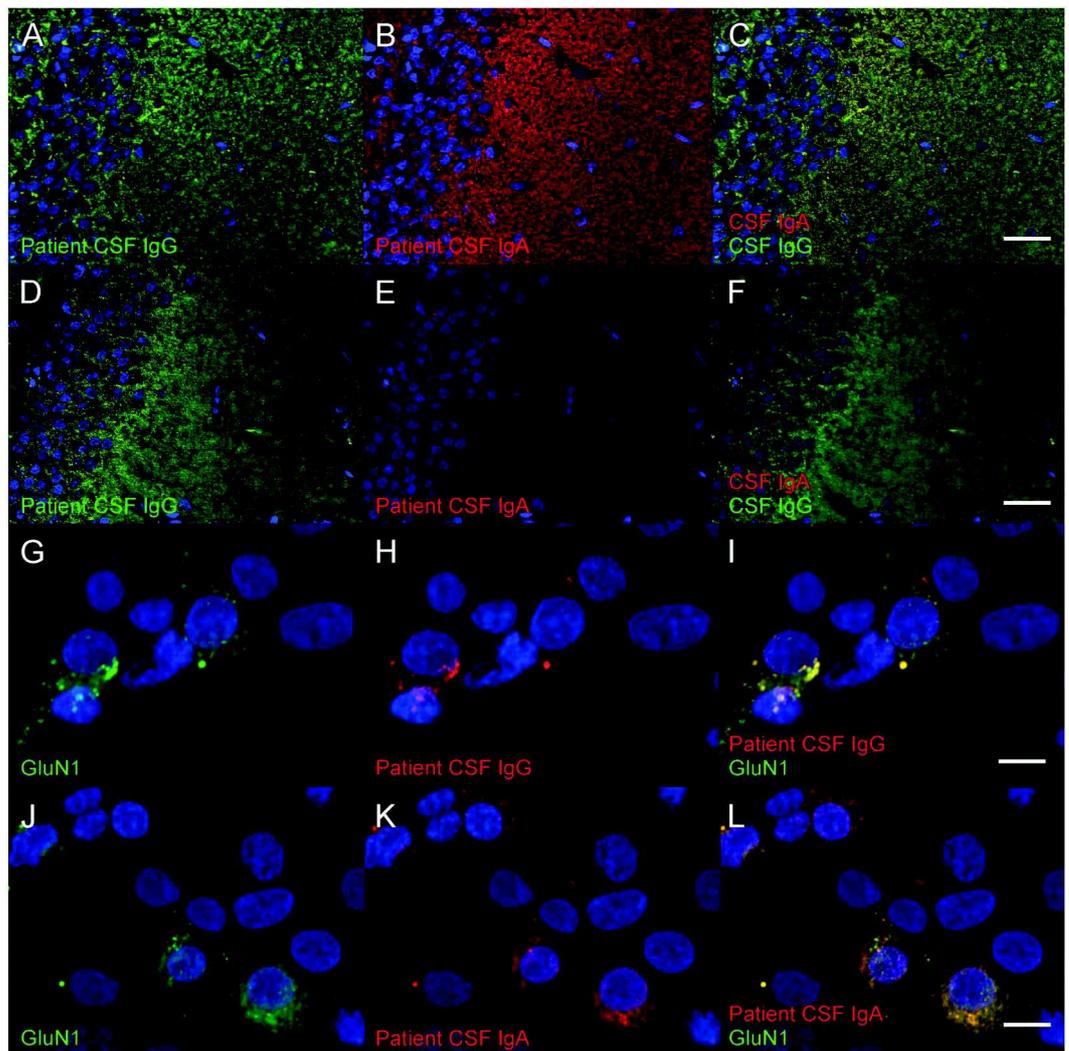
Standard protocol approval, registration, and patient consent. Written consent was obtained from all patients, and this study was approved by the institutional review board of the University Claude Bernard Lyon 1 and Hospices Civils de Lyon. The biological samples were obtained from NeuroBioTec Hospices Civils de Lyon BRC (France, AC-208-73 NFS96-900).

Definition of clinical events, ancillary tests, treatment, and evolution. Detailed clinical data on acute disease stages were obtained at the time of biological diagnosis, and data regarding disease clinical course were collected during follow-up examinations. Clinical events classification was detailed previously.¹¹

Prodromal symptoms were considered to be signs or symptoms arising prior to the first neurologic symptoms. These symptoms were subdivided into the following subgroups: (1) headache, (2) gastrointestinal symptoms, (3) infection signs, and (4) other. The first neurologic symptoms were considered to be the first clinical signs after the prodromal state, when present, or the first neurologic symptoms. The subsequent symptoms were clinical signs that arose after the first symptoms, with a delay interval of at least 24 hours. All symptoms were categorized into the following subgroups: (1) behavioral and psychiatric features, (2) seizure, (3) cognitive dysfunction (which included anterograde amnesia, speech disorder, and alteration of mental status), (4) movement disorders, (5) fluctuating level of consciousness, (6) dysautonomia, and (7) a subgroup called other for symptoms that did not correspond to any of the previously described features. The results from the initial ancillary examinations (MRI, CSF analysis, EEG, and tumor screening) were also compiled. Follow-up information was collected at regular intervals (3, 6, 9, 12, 18, and 24 months) after diagnosis. Neurologic disability was assessed using the modified Rankin Scale (mRS). Recovery was defined as occurring when the patient's mRS score was determined to be 0, 1, or 2 (mRS 0–2).

Teratoma samples analysis. When an associated teratoma was diagnosed, paraffin-embedded samples were collected. Four-micrometer-thick sections were cut and stained with hematoxylin-phloxine-saffron. A reference pathologist (I.T.) assessed the composition of each teratoma in different tissues, particularly the

Figure 1 Reactivity of the patients' antibodies with rat brain and HEK cell-based assays



Rat hippocampal dentate gyrus neuropils were stained with patient CSF; the reactivity was revealed using anti-human immunoglobulin G (IgG) (in green) or immunoglobulin A (IgA) (in red) (A–F, scale bar = 50 μ m). The CSF of the first patient (A–C) contained both IgG–NMDA receptor (NMDAR)–antibodies (Abs) (A) and IgA–NMDAR–Abs (B), both showing the same pattern of neuropil staining (C). The CSF of the second patient (D–F) only contained IgG–NMDAR–Abs (D). No IgA–NMDAR–Abs was detected (E, F). Human epithelial kidney (HEK) cells were transfected to express green fluorescent protein (GFP)–tagged GluN1 (green; G, J) and were then incubated with CSF from patients; the reactivity was revealed using anti-human IgG (red; H) or anti-human IgA (red; K) (G–L, scale bars = 10 μ m). (I, L) The merged image of GFP–GluN1 and IgG or IgA reactivity, respectively. The nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI).

nervous system parenchyma and respiratory and digestive mucosae (sites of possible IgA production).

Statistical analysis. Statistical analysis was performed using the R software version 3.1.1 (<http://www.r-project.org/>). The *p* values for demographic information, symptoms, ancillary tests results, and the clinical severity assessment (mRS, intensive care unit [ICU] admission, death) were obtained using the Fisher exact test for contingency tables with Bonferroni correction for multiple testing. A multivariate analysis was performed using the logistic regression technique including variables with a *p* value <0.05 before Bonferroni adjustment. The logistic mixed model was fitted using the package lme4 version 1.1–7 (<http://CRAN.R-project.org/package=lme4>). Comparisons of the medians and NMDAR–Ab titers were performed using the Wilcoxon rank-sum test (Mann–Whitney test). Because of a skewed distribution, we

used a log transformation for the titers. A multivariate linear regression model, including the presence of a teratoma as cofactor, was used to assess the relationship between CSF IgA–NMDAR–Ab titers and CSF IgG–NMDAR–Ab titers. Analysis of factors affecting outcome (recovery defined as mRS 0–2) was performed using a logistic binary mixed model including a random effect per patients (on the intercept only) and 4 fixed effects (sex, CSF IgA–NMDAR–Ab status, ICU admission, and time [modeled as a quadratic function]).

RESULTS Detection of CSF IgA–NMDA–Abs and associated clinical presentation. The cohort of 94 IgG–NMDAR–Ab encephalitis patients (median age 19 years, range 1–76) with available CSF comprised 25 children (median age 6 years, range 1–12; 17 girls

Table Comparison of the clinical presentation of the 39 IgA-NMDAR-Ab-positive patients with NMDAR-Ab encephalitis to the 55 IgA-NMDAR-Ab-negative patients

	IgA-NMDAR-Ab-positive (n = 39)	IgA-NMDAR-Ab-negative (n = 55)	p Value
Age, y, median (range)	22 (2-76)	17 (1-66)	NS
Women	34 (87)	44 (80)	NS
Children (age <12 y)	6 (15)	19 (34)	NS
Caucasian	29 (74)	33 (60)	NS
Prodromal symptoms^a			
Assessable cases	37	51	
Podroms	20 (54)	26 (51)	NS
Headache	13 (35)	9 (17)	NS
Infectious diseases	8 (21)	11 (21)	NS
Gastrointestinal signs	6 (16)	4 (8)	NS
Others ^b	5 (13)	3 (6)	NS
Oncologic status			
Assessable cases	38	55	
Tumors	18 (47)	3 (5)	<0.001
Ovarian teratoma	17 (44)	3 (5)	<0.001
Other ^c	1 (2)	0 (0)	NS
First symptoms^a			
Assessable cases	39	54	
Behavior and psychiatric features	24 (61)	23 (43)	NS
Seizures	10 (26)	24 (44)	NS
Cognitive dysfunction	12 (30)	10 (18)	NS
Anterograde amnesia	3 (7)	1 (2)	NS
Speech disorders	3 (7)	4 (7)	NS
Alteration of mental status	8 (20)	6 (14)	NS
Movement disorders	0 (0)	2 (3)	NS
Others ^d	3 (8)	6 (11)	NS
Subsequent symptoms^a			
Assessable cases	39	53	
Behavior and psychiatric features	30 (77)	45 (84)	NS
Seizures	26 (67)	46 (86)	NS
Cognitive dysfunction	38 (97)	50 (94)	NS
Anterograde amnesia	27 (69)	28 (52)	NS
Speech disorders	21 (53)	34 (64)	NS
Alteration of mental status	28 (71)	35 (66)	NS
Movement disorders	30 (77)	34 (64)	NS
Fluctuating level of consciousness	26 (67)	33 (62)	NS
Dysautonomia	22 (56)	17 (32)	NS
Other symptoms ^e	7 (18)	10 (19)	NS
Paraclinic examinations (acute phase)^a			
MRI			
Assessable cases	36	49	
Abnormal	14 (39)	17 (35)	NS

Continued

and 8 boys), 61 female participants (median age 21 years, range 14–48), and 8 men (median age 33 years, range 22–76). The CSF samples obtained from these 94 individuals showed that 39 individuals (41%) were positive for IgA-NMDAR-Abs (figure 1). Among the 39 IgA-NMDAR-Ab-positive patients, 30 patients were adult women, 3 patients were adult men, and 6 patients were children (age <12 years; 4 girls and 2 boys). We compared the 39 IgA-NMDAR-Ab-positive patients with the 55 IgA-NMDAR-Ab-negative patients identified in the cohort (table). The median age, sex ratio, and percentage with Caucasian origin were similar in both groups. The children tended to be underrepresented in the IgA-NMDAR-Ab-positive group (15% vs 34% in the IgA-NMDAR-Ab-negative group, without reaching significance after Bonferroni adjustment). The incidences of prodromal signs and the first symptoms were similar in both groups. The complete clinical pattern was also similar in both groups. The incidence of abnormalities in ancillary tests (MRI, EEG, and CSF) and the clinical severity (mRS at onset, ICU admission, death) were similar in both populations. Ninety-one patients (39 IgA-NMDAR-Ab-positive and 52 IgA-NMDAR-Ab-negative individuals) had a follow-up (median 24 months, range 3–24). The global progression and recovery were similar in the groups (figure 2). In multivariable analysis, no effect of the CSF IgA-NMDAR-Ab status on the time to recovery was identified.

Associated tumors. The positivity of CSF IgA-NMDAR-Abs is independently associated with the presence of an ovarian teratoma (odds ratio 10.59, confidence interval 2.81–52.88; $p = 0.0012$). Approximately half of the IgA-NMDAR-Ab-positive patients with a known oncologic status (18/38, 47%) had a definite associated tumor, primarily ovarian teratomas (17/18, 94%), compared with 5% (3/55) in the IgA-NMDAR-Ab-negative group ($p < 0.001$). In the adult women, ovarian teratomas were present in 17/29 (58%) of the IgA-NMDAR-Ab-positive women vs 3/31 (9.6%) of the IgA-NMDAR-Ab-negative women ($p = 0.001$). The histologic nature of the ovarian cystic lesion observed through CT scan was unknown for one IgA-NMDAR-Ab-positive woman who died before the end of the diagnostic assessment for an ovarian tumor and surgical excision. Among the men, only one individual had a tumor (a perineal schwannoma)¹¹ and was positive for IgA-NMDAR-Abs. Among the children, no underlying tumor was detected in the groups (19 IgA-NMDAR-Ab-negative and 6 IgA-NMDAR-Ab-positive children). In the adult female population at risk for ovarian teratoma, the CSF positivity of IgA-NMDAR-Ab detection showed 85% sensitivity,

Table Continued		IgA-NMDAR-Ab-positive (n = 39)	IgA-NMDAR-Ab-negative (n = 55)	p Value
CSF				
Assessable cases		37	48	
Abnormal		32 (86)	43 (88)	NS
EEG				
Assessable cases		33	48	
Abnormal		31 (94)	42 (87)	NS
Epileptiform abnormalities^f		7 (23)	16 (38)	NS
Clinical severity^a				
Assessable cases		39	53	
ICU		27 (69)	32 (60)	NS
Death		1 (3)	1 (2)	NS
Initial mRS				
mRS 0		0 (0)	0 (0)	NS
mRS 1–2		0 (0)	0 (0)	NS
mRS 3–4		9 (23)	18 (35)	NS
mRS 5		30 (77)	34 (65)	NS

Abbreviations: Ab = antibody; ICU = intensive care unit; IgA = immunoglobulin A; mRS = modified Rankin Scale; NMDAR = NMDA receptor; NS = not significant.

Values are n (%) unless noted otherwise.

^aNo. on assessable cases.

^bAsthenia, anorexia, myalgia, cervicgia, photophobia.

^cPerineal schwannoma.

^dDiplopia, walking problem, upper limb and face pain, sleep disorder, gait ataxia.

^eWalking problem, cerebellar syndrome, pyramidal syndrome, rigidity, neuroleptic malignant syndrome, nystagmus, acouphobia, sensory and motor disorder, myelitis.

^fClustered seizures, status epilepticus, critical activities.

70% specificity, a 57% positive predictive value, and a 90% negative predictive value for the diagnosis of ovarian teratoma.

All 20 teratomas were detected after the diagnosis of encephalitis, except for the teratoma in one patient who was diagnosed after pelvic ultrasound examination with ovarian cysts of unknown nature at 3 years prior to the onset of encephalitis. The median time between the first neurologic symptom and the surgical removal of the teratoma was 32 days (range 6–386). This median delay was similar between the IgA-NMDAR-Ab-positive and IgA-NMDAR-Ab-negative patients (34 days, range 6–386; and 30 days, range 10–83, respectively). In 2 patients, an early body CT scan did not detect a cystic ovarian tumor, which was subsequently diagnosed (a few days later) through pelvic MRI and endovaginal ultrasound, indicating estimated tumor sizes of 7 and 18 mm. In 4 patients, teratomas were detected at several months (from 6 to 10 months) after the onset of neurologic symptoms and the diagnosis of the NMDAR-Ab encephalitis. However, 3 of them had a diagnostic assessment for an ovarian tumor during the first weeks of the disease; the assessment comprised at least one CT scan that was considered

normal. The last patient did not have a paraclinical checkup before 10 months after the onset of the encephalitis. These 4 patients were IgA-NMDAR-Ab positive.

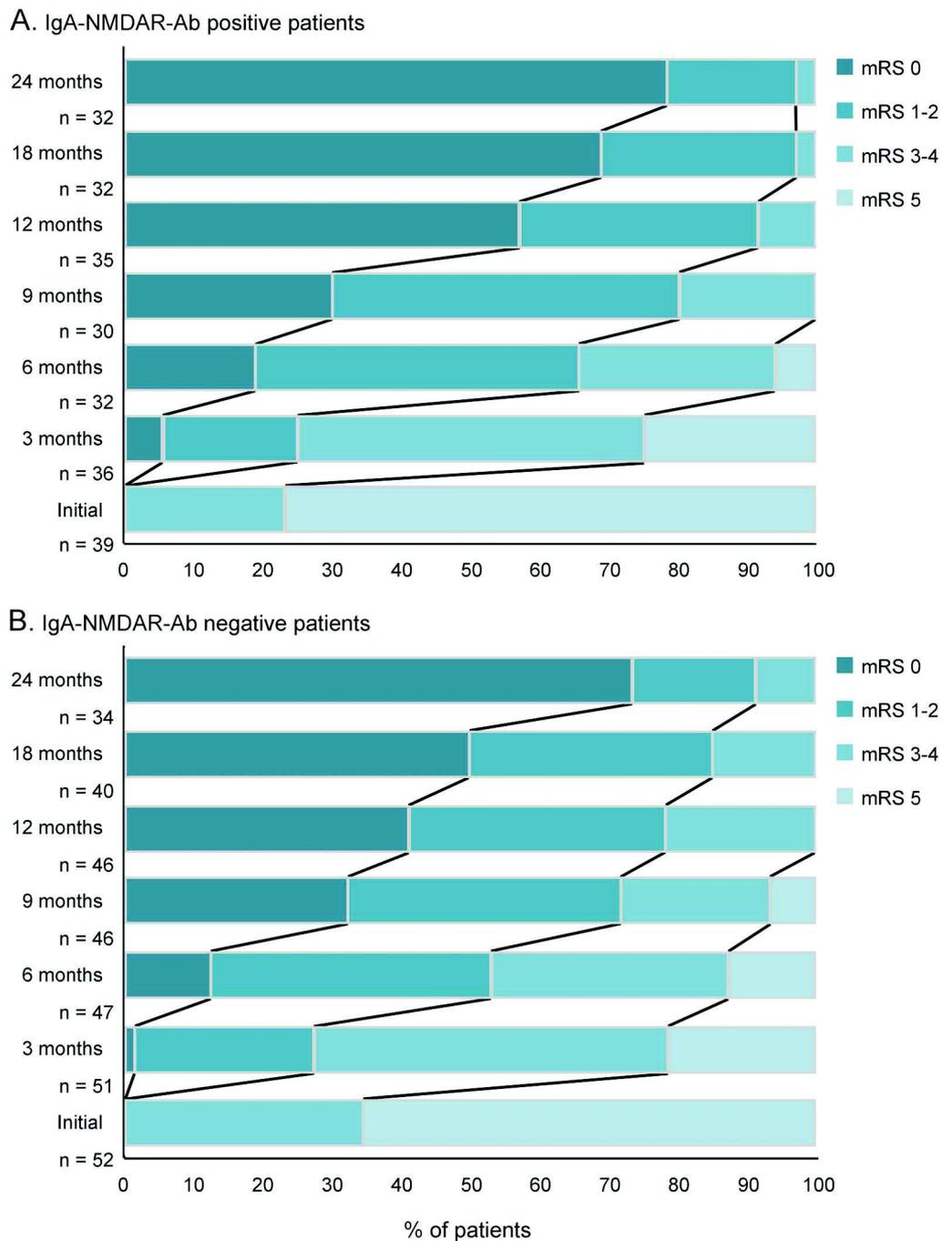
Histologic analysis of teratoma samples. Nineteen of the 20 diagnosed ovarian teratomas in the cohort were collected at the French Reference Centre on Paraneoplastic Neurological Syndrome. Sixteen tumors were from IgA-NMDAR-Ab-positive patients and 3 from IgA-NMDAR-Ab-negative patients. The mean tumor size was similar in the IgA-NMDAR-Ab-positive and IgA-NMDAR-Ab-negative patients (2.71 ± 1.20 cm and 2.16 ± 1.26 cm, respectively). Histologic examination of all cases showed teratomatous lesions composed of derivatives of all 3 germ cell layers. Two cases showed neuroepithelial foci corresponding to an immature glial component. A mature glial component was present in all cases. Among the 16 teratomas associated with IgA-NMDAR-Ab, 2 tumors were immature, and 13/16 tumors (81%) contained mucosal epithelium (gastrointestinal [9/16] or respiratory [9/16] epithelium). The 3 teratomas not associated with IgA-NMDA-Ab were mature and contained mucosal epithelium (gastrointestinal [2/3] or respiratory [1/3]) (figure e-1 at Neurology.org/nn).

CSF NMDAR-Ab titers and intrathecal immunoglobulin synthesis. We examined the CSF IgG-NMDAR-Ab titers at diagnosis in 12 patients with IgA-NMDAR-Ab (6/12 with a teratoma) and 12 patients without IgA-NMDAR-Ab (2/12 with a teratoma). The CSF IgG-NMDAR-Ab titers were significantly higher in the patients positive for IgA-NMDAR-Ab (median 320; IQR [50–560] vs 40 [20–140], $p = 0.0136$, Mann-Whitney test) and in the patients with a teratoma (320; IQR [100–640] vs 40 [20–160], $p = 0.0081$). The CSF IgA-NMDAR-Ab titers were significantly lower than the CSF IgG-NMDAR-Ab titers (median 30; IQR [12.5–80] vs 320 [50–560], $p = 0.0037$). In IgA-NMDAR-Ab-positive patients, the CSF IgA-NMDAR-Ab titers were linearly related to the CSF IgG-NMDAR-Ab titers ($r^2 = 0.54$, $p = 0.012$).

The IgG and IgA quotients were assessed in 4 patients with IgA-NMDAR-Ab. Two patients had an associated teratoma, while the other 2 patients did not. The QAlb was consistently inferior to the upper age-related reference, reflecting the integrity of the CSF–blood–brain barrier in all cases. Intrathecal IgG synthesis ($QIgG > QLim$) was detected in 3/4 patients. The only patient without IgG intrathecal synthesis had no associated teratoma. Intrathecal IgA synthesis ($QIgA > QLim$) was only detected in the 2 patients without teratomas.

DISCUSSION The present study evaluates the presence of IgA-NMDAR-Ab in the CSF of patients

Figure 2 Clinical outcome after extended follow-up



The clinical outcome was measured using the modified Rankin Scale (mRS) at 0, 3, 6, 9, 12, 18, and 24 months after the onset of NMDA receptor (NMDAR)-antibody (Ab) encephalitis in immunoglobulin A (IgA)-NMDAR-Ab-positive (A) and IgA-NMDAR-Ab-negative (B) patients.

with NMDAR-Ab encephalitis and determines their relative contribution to this syndrome. In the retrospective cohort, 41% of the patients with NMDAR-Ab encephalitis had both CSF IgG- and IgA-NMDAR-Abs. This CSF IgA-NMDAR-Ab-positive rate was consistent with the 30% serologic IgA-NMDAR-Ab-positive rate previously reported in NMDAR-Ab encephalitis⁶ and markedly higher than the false-positive rate observed in large

seroprevalence studies.⁷ No significant differences in clinical and paraclinical presentation, severity, and prognosis according to the IgA status were identified. No specific clinical features were associated with CSF IgA-NMDAR-Ab positivity, except that ovarian teratomas were much more frequent in patients with CSF IgA-NMDAR-Abs. These results suggest that CSF IgA-NMDAR-Abs could be biomarkers for the presence of an ovarian

teratoma, without influencing the clinical course of this disease.

The absence of a clinical influence of IgA-NMDAR-Ab status is not in favor of a particular pathogenic role for IgA-NMDAR-Abs in the neurologic symptoms, although it was suggested by other authors.⁶ The specific role of IgA and IgG autoantibodies has previously been discussed in other autoantibody-mediated diseases. In the mucous membrane pemphigoid, the dual circulating antibody response with IgA in addition to IgG has been associated with a more severe disease,¹² although studies have not confirmed the importance of IgA antibodies as a prognostic indicator.¹³ Thus, the clinical relevance of these IgA-Abs remains controversial, and if present, might be weak, with an uncertain pathogenesis.

The site of IgA production is another nonelucidated issue. The absence of intrathecal secretion of IgA-NMDAR-Abs in IgA-positive patients with a teratoma should be confirmed but could suggest a peripheral antibody response when a teratoma is present. One hypothesis could be that teratomas directly trigger the production of the IgA serotype of immunoglobulins in the periphery. We also observed that CSF IgA-NMDAR-Ab positivity was associated with higher CSF IgG-NMDAR-Ab titers. When available, CSF IgA-NMDAR-Ab titers seem to be proportional to the CSF IgG NMDAR-Ab titers. This result suggests that IgA status might primarily reflect the intensity of humoral autoimmunity against the NMDAR, and this response could be particularly activated in the periphery through the presence of teratomas. Indeed, previous studies have shown that patients with an ovarian teratoma have significantly higher titers of IgG-NMDAR-Abs than those of patients without teratoma.⁴ Nevertheless, we observed 3 patients with NMDAR-Ab encephalitis and a teratoma but without CSF IgA-NMDAR-Abs, suggesting that the humoral reaction might differ according to the patient. We did not observe any difference in the histopathologic examination of the teratoma according to the patient IgA status among the 19 analyzed teratomas. Specifically, the presence of gastrointestinal or respiratory mucosa in the teratoma was not sufficient to explain the observed IgA-NMDAR-Ab production. Thus, the teratoma-associated mechanism leading to IgA production remains unclear, and another external agent, such as a viral infection, could be involved.

A practical issue of the present study is the value of the CSF IgA-NMDAR-Ab status as an indicator of an associated teratoma in the context of NMDAR-Ab encephalitis. At the individual level, there is no available predictive surrogate marker for the association of an ovarian tumor or a primary tumor elsewhere. In

the present study, initial radiologic examinations did not reveal an ovarian teratoma in 5 cases. In clinical practice, the detection of a small teratoma (mean tumor size of 2.3 cm in the present study) in a patient with a severe neurologic state requiring respiratory assistance and sedation is typically difficult but must be considered a high priority. Because of the observed 85% sensitivity and 90% negative predictive value in the adult female population at risk for ovarian teratomas, we suggest that CSF-IgA-NMDAR-Ab status could be a predictive indicator for managing the patient screening for an associated teratoma. For an IgA-NMDAR-Ab-positive woman, when early radiologic pelvic investigations are negative, a second screening with pelvic CT or MRI or endovaginal ultrasound should be conducted promptly and repeated if negative. Future prospective studies are needed to further demonstrate the usefulness of CSF IgA-NMDAR-Ab status as a biomarker for a teratoma in anti-NMDAR-Abs encephalitis.

AUTHOR CONTRIBUTIONS

Virginie Desestret: analysis or interpretation of the data, drafting or revising the manuscript for intellectual content. Aude Chedeville: acquisition and analysis of data. Aurélien Viaccoz: analysis and interpretation of the data. Chloé Bost: acquisition and analysis of data. François Ducray: drafting or revising the manuscript for intellectual content. Géraldine Picard: acquisition and analysis of the data. Véronique Rogemond: acquisition, analysis, or interpretation of the data. Marie-Océane Chaffois: acquisition and analysis of the data. Charlotte Blanc: acquisition and analysis of the data. Claire Bardel: statistical analysis of data. Isabelle Treilleux: analysis and interpretation of data. Olivier Pascual: drafting or revising the manuscript for intellectual content. Jean-Christophe Antoine: drafting or revising the manuscript for intellectual content. Jean-Yves Delattre: drafting or revising the manuscript for intellectual content. Jérôme Honnorat: analysis or interpretation of the data, design or conceptualization of the study, drafting or revising the manuscript for intellectual content.

ACKNOWLEDGMENT

The authors thank Julien Bancel (Immunology Laboratory, Hôpital E. Herriot, Lyon, France) for providing guidance and advice.

STUDY FUNDING

Supported by a grant from the Fédération pour la Recherche sur le Cerveau (FRC).

DISCLOSURE

V. Desestret, A. Chedeville, A. Viaccoz, C. Bost, F. Ducray, G. Picard, V. Rogemond, M.-O. Chaffois, and C. Blanc report no disclosures. C. Bardel received research support from La Ligue Contre le Cancer. I. Treilleux and O. Pascual report no disclosures. J.-C. Antoine received travel funding from le Laboratoire Français des Biotechnologies et du Fractionnement, le CSL Behring, Genzyme, and is an associate editor for *Revue Neurologique*. J.-Y. Delattre is on the editorial board for *The Oncologist* and received research support from Institut National du Cancer, Ligue Nationale Contre le Cancer. J. Honnorat reports no disclosures. Go to Neurology.org/nn for full disclosures.

Received July 14, 2015. Accepted in final form September 9, 2015.

REFERENCES

1. Hughes EG, Peng X, Gleichman AJ, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci* 2010;30:5866–5875.

2. Mikasova L, De Rossi P, Bouchet D. Disrupted surface cross-talk between NMDA and Ephrin-B2 receptors in anti-NMDA encephalitis. *Brain* 2012;135:1606–1621.
3. Planaguma J, Leyboldt F, Mannara F, et al. Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain* 2014;138:94–109.
4. Gresa-Arribas N, Titulaer MJ, Torrents A, et al. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. *Lancet Neurol* 2014;13:167–177.
5. Lancaster E, Leyboldt F, Titulaer MJ, et al. IgG antibodies to the NMDA receptor are distinct from IgA and IgM responses. *Ann Neurol* 2015;77:183.
6. Prüss H, Klingbeil C, Probst C, et al. IgA NMDA receptor antibodies are markers of synaptic immunity in slow cognitive impairment. *Neurology* 2012;78:1743–1753.
7. Dahm L, Ott C, Steiner J, et al. Seroprevalence of auto-antibodies against brain antigens in health and disease. *Ann Neurol* 2014;76:82–94.
8. Doss S, Wandinger KP, Hyman BT, et al. High prevalence of NMDA receptor IgA/IgM antibodies in different dementia types. *Ann Clin Transl Neurol* 2014;1:822–832.
9. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* 2008;7:1091–1098.
10. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. *J Neurol Sci* 2001;184:101–122.
11. Viacoz A, Desestret V, Ducray F, et al. Clinical specificities of adult male patients with NMDA receptor antibodies encephalitis. *Neurology* 2014;82:556–563.
12. Setterfield J, Shirlaw PJ, Kerr-Muir M, et al. Mucous membrane pemphigoid: a dual circulating antibody response with IgG and IgA signifies a more severe and persistent disease. *Br J Dermatol* 1998;138:602–610.
13. Cozzani E, Drosera M, Parodi A, Carrozzo M, Gandolfo S, Reborja A. Frequency of IgA antibodies in pemphigus, bullous pemphigoid and mucous membrane pemphigoid. *Acta Derm Venereol* 2004;84:381–384.

REVIEW

Neuronal central nervous system syndromes probably mediated by autoantibodies

Aude Chefdeville,^{1,2} Jérôme Honnorat,^{1,2,3,5} Christiane S. Hampe⁴ and Virginie Desestret^{1,2,3,5}

¹Institut NeuroMyoGène, INSERM U1217/UMR CNRS 5310, Lyon, France

²Université de Lyon, Lyon, France

³French Reference Center on Paraneoplastic Neurological Syndrome, F-69677 Bron, France

⁴Department of Medicine, University of Washington, Seattle, WA, USA

⁵Department of Neurology, Hospices Civils de Lyon, Hôpital Neurologique, F-69677 Bron, France

Keywords: antibodies, cerebellitis, encephalitis, NMDAR, synaptopathies

Edited by Gianmaria Maccaferri

Received 13 December 2015, revised 9 February 2016, accepted 15 February 2016

Abstract

In the last few years, a rapidly growing number of autoantibodies targeting neuronal cell-surface antigens have been identified in patients presenting with neurological symptoms. Targeted antigens include ionotropic receptors such as *N*-methyl-D-aspartate receptor or the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, metabotropic receptors such as mGluR1 and mGluR5, and other synaptic proteins, some of them belonging to the voltage-gated potassium channel complex. Importantly, the cell-surface location of these antigens makes them vulnerable to direct antibody-mediated modulation. Some of these autoantibodies, generally targeting ionotropic channels or their partner proteins, define clinical syndromes resembling models of pharmacological or genetic disruption of the corresponding antigen, suggesting a direct pathogenic role of the associated autoantibodies. Moreover, the associated neurological symptoms are usually immunotherapy-responsive, further arguing for a pathogenic effect of the antibodies. Some studies have shown that some patients' antibodies may have structural and functional *in vitro* effects on the targeted antigens. Definite proof of the pathogenicity of these autoantibodies has been obtained for just a few through passive transfer experiments in animal models. In this review we present existing and converging evidence suggesting a pathogenic role of some autoantibodies directed against neuronal cell-surface antigens observed in patients with central nervous system disorders. We describe the main clinical symptoms characterizing the patients and discuss conflicting arguments regarding the pathogenicity of these antibodies.

Introduction

In the last few years, a rapidly growing number of patients with non-infectious, autoimmune encephalitis associated with autoantibodies targeting the central nervous system (CNS) have been described. The first anti-neuronal autoantibodies – anti-Hu (Szabo *et al.*, 1991), anti-Yo, anti-CV2/CRMP5 (Honnorat *et al.*, 1996) and anti-Ri (Pittock *et al.*, 2003) – were identified about 25 years ago in paraneoplastic neurological syndromes (PNSs). These anti-neuronal autoantibodies recognize intracellular antigens and therefore do not appear to be directly pathogenic, but may be used as diagnostic biomarkers of cancer (Honnorat, 2006; Rasputnig *et al.*, 2011). Cytotoxic neuronal damage and neuronal death are responsible for symptoms found in these PNSs and immunization with the paraneoplastic antigen does not induce disease in rodents, further supporting a non-pathogenic role of the respective autoantibodies (Sillevis Smitt *et al.*, 1995). Rather, the antibodies may reflect a T-cell-mediated immune response against neurons (Bien *et al.*, 2012). On the other

hand, an increasing number of autoantibodies targeting neuronal cell-surface antigens have been identified recently in patients presenting with neurological symptoms resembling PNSs. Targets include ionotropic receptors including *N*-methyl-D-aspartate receptor (NMDAR), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), γ -aminobutyric acid (GABA)_AR and glycine receptor (GlyR); metabotropic receptors such as mGluR1, mGluR5 and GABA_BR; and proteins belonging to the voltage-gated potassium channel (VGKC) complex, namely Lgi1 and Caspr2. Several key features differentiate patients with cell-surface autoantibodies from patients with autoantibodies recognizing intracellular targets: (i) associated neurological symptoms are usually immunotherapy-responsive and reversible, suggesting a direct pathogenic effect of antibodies without cytotoxically induced neuronal loss; and (ii) tumour association is far less consistent. Encephalitis associated with antibodies directed against cell-surface antigens such as NMDAR or VGKC complex are much more common than PNSs with intracellular targets and were identified to account for ~8% of encephalitis cases in a population-based prospective study in England (Granerod *et al.*, 2010). The location of cell-surface antigens allows their recognition by autoantibodies on the intact cell and evidence

Correspondence: Professor Jérôme Honnorat, ³French Reference Center on Paraneoplastic Neurological Syndrome, as above.
E-mail: jerome.honnorat@chu-lyon.fr

suggests that these antibodies may play a direct pathogenic role in the neurological and psychiatric symptoms of patients with encephalitis. The purpose of this review is to present existing and converging evidence suggesting a pathogenic role of some autoantibodies directed against neuronal cell-surface antigens observed in patients with encephalitis. We describe the main clinical symptoms characterizing the patients and discuss conflicting arguments regarding the pathogenicity of these antibodies.

Encephalitis associated with antibodies directed against NMDAR

Definition and clinical presentation

Encephalitis associated with anti-NMDAR autoantibodies is a clinico-biological entity defined by a clinical presentation of encephalitis, and the presence of IgG antibodies in the cerebrospinal fluid (CSF) that are directed against the GluN1 subunit of the NMDAR (Dalmau *et al.*, 2008). Detection of anti-NMDAR antibodies relies on two tests: a specific immunohistochemical staining pattern on rat brain section with patients' IgG; and patients' IgG binding to NMDAR-expressing HEK cells in a cell-based assay (Dalmau *et al.*, 2008; Gresa-Arribas *et al.*, 2014; Viacoz *et al.*, 2014). Encephalitis with anti-NMDAR antibodies was initially described in 2007 in 12 women with associated ovarian teratoma (Dalmau *et al.*, 2007). The clinical presentation was more comprehensively described 1 year later in 100 autoantibody-positive encephalitis patients (Dalmau *et al.*, 2008). To date, several hundred cases have been published (Titulaer *et al.*, 2013), indicating a relatively frequent disorder. Accordingly, NMDAR encephalitis was identified as the most common cause of non-infectious encephalitis in patients under 30 years old (Gable *et al.*, 2012). Among all autoimmune encephalitides, NMDAR encephalitis is the most frequent (Thomas *et al.*, 2014). The clinical disorder, predominantly affecting young women, shows a progression of similar symptoms (Titulaer *et al.*, 2013), usually starting with a prodromal syndrome including headache, low-grade fever or non-specific viral-like illness followed by the development of psychiatric symptoms (anxiety, agitation, delusional thoughts, hallucinations, and personality or behavioural changes) and/or neurological symptoms (seizures, movement disorders, catatonia, alteration of mental state, speech impairment and rapid development of short-term memory loss) within the next days or weeks. During the course of the disease, patients often develop decreased responsiveness, abnormal movements (the most characteristic being orofacial dyskinesia) and autonomic instabilities (Dalmau *et al.*, 2008, 2011). Only a few adult male patients have been described and tend to present with seizures as first symptoms whereas female patients usually present with psychiatric symptoms (Viacoz *et al.*, 2014). Psychiatric symptoms and abnormal movements are more prominent in children (Florance *et al.*, 2009). A good recovery is generally observed in about 80% of patients but is typically slow, occurring in stages following the reverse order from symptom appearance (Dalmau *et al.*, 2011; Titulaer *et al.*, 2013; Gresa-Arribas *et al.*, 2014). In the largest published series, more than 75% of patients recovered fully or retained only mild deficits (Dalmau *et al.*, 2008). An underlying neoplasm is found in about 40% of patients, primarily in females aged between 12 and 45 years (Dalmau *et al.*, 2011; Titulaer *et al.*, 2013). More than 90% of all tumours associated with anti-NMDAR encephalitis are ovarian teratoma, while the frequency of an underlying tumour is less than 5% in men and children (Titulaer *et al.*, 2013). All immunopathological studies of teratomas associated with NMDAR encephalitis revealed the

presence of GluN1-expressing nervous tissue (Dalmau *et al.*, 2008; Tüzün *et al.*, 2009). In more than 60% of cases, no tumour is found and mechanisms leading to the breaking of immunological tolerance remain unclear. In a few cases, anti-NMDAR antibodies have been observed a few weeks after a typical herpes simplex encephalitis, suggesting that viral encephalitis may sometimes trigger anti-NMDAR auto-immunity (Prüss *et al.*, 2012; Armangué *et al.*, 2013; Hacoheh *et al.*, 2014) (Fig. 1).

Antigen

NMDAR belong to the family of ionotropic glutamate receptors (iGluRs). They are involved in excitatory neurotransmission and are the pivots of synaptic plasticity (e.g. long-term potentiation and long-term depression) underlying cognitive functions such as learning and memory. Functional NMDAR are heterotetramers consisting of two obligatory GluN1 subunits and GluN2 or GluN3 subunits. Antibodies of patients with anti-NMDAR encephalitis target the obligatory subunit GluN1 (Dalmau *et al.*, 2008; Gleichman *et al.*, 2012). Each NMDAR subunit comprises two extracellular domains, the amino-terminal domain (ATD) and the ligand-binding domain, one transmembrane domain and a large intracellular domain (Paoletti *et al.*, 2013). Remarkably, this subunit structure is conserved for all members of the iGluR family (Traynelis *et al.*, 2010). Antibodies of patients with anti-NMDAR encephalitis target a conformational epitope located in the ATD of the GluN1 subunit, in a small region located near the hinge of a clamshell-like structure (Gleichman *et al.*, 2012). Within this region, the amino acid N368 was identified as crucial for patients' anti-NMDAR antibody binding (Gleichman *et al.*, 2012).

Antibody pathogenicity

Strong clinical arguments support the hypothesis of an antibody-mediated pathogenicity in anti-NMDAR encephalitis. First, pharmacological antagonists of NMDAR (e.g. ketamine) may induce specific clinical symptoms similar to those presented by patients with anti-NMDAR encephalitis. Moreover, the majority of patients respond well to plasma exchanges and intravenous immunoglobulins, often with full recovery (Titulaer *et al.*, 2013). Furthermore immunotherapy targeting an antibody producing CD20+ B-cells (e.g. Rituximab) improves patients' recovery (Titulaer *et al.*, 2013). Substantial evidence points toward an intrathecal synthesis of autoantibodies in anti-NMDAR encephalitis. Indeed, in patients with preserved blood-brain barrier integrity, antibody titres are higher in the CSF than in the serum (Dalmau *et al.*, 2007, 2008). Accordingly, several immunopathological studies reported deposits of IgG, B-cells and plasma cells cuffing in the brain perivascular spaces (Dalmau *et al.*, 2007, 2008; Tüzün *et al.*, 2009; Hughes *et al.*, 2010; Camdessanché *et al.*, 2011). Although anti-NMDAR antibodies are of the subclasses IgG1 and IgG3 that are capable of complement activation, no deposits of complement were found (Tüzün *et al.*, 2009; Hughes *et al.*, 2010). Taken together, these data support the hypothesis of an antibody-mediated pathogenicity, without major involvement of T-cells or complement-mediated cytotoxicity.

Incubation of neuronal cultures with patients' NMDAR autoantibodies caused a selective and reversible decrease in NMDAR surface density and synaptic localization without affecting other synaptic components, neuronal morphology or causing neuronal loss (Hughes *et al.*, 2010; Moscato *et al.*, 2014). The effect size correlated with patients' autoantibody titres. The mechanism of this decrease is selective antibody-mediated capping and internalization

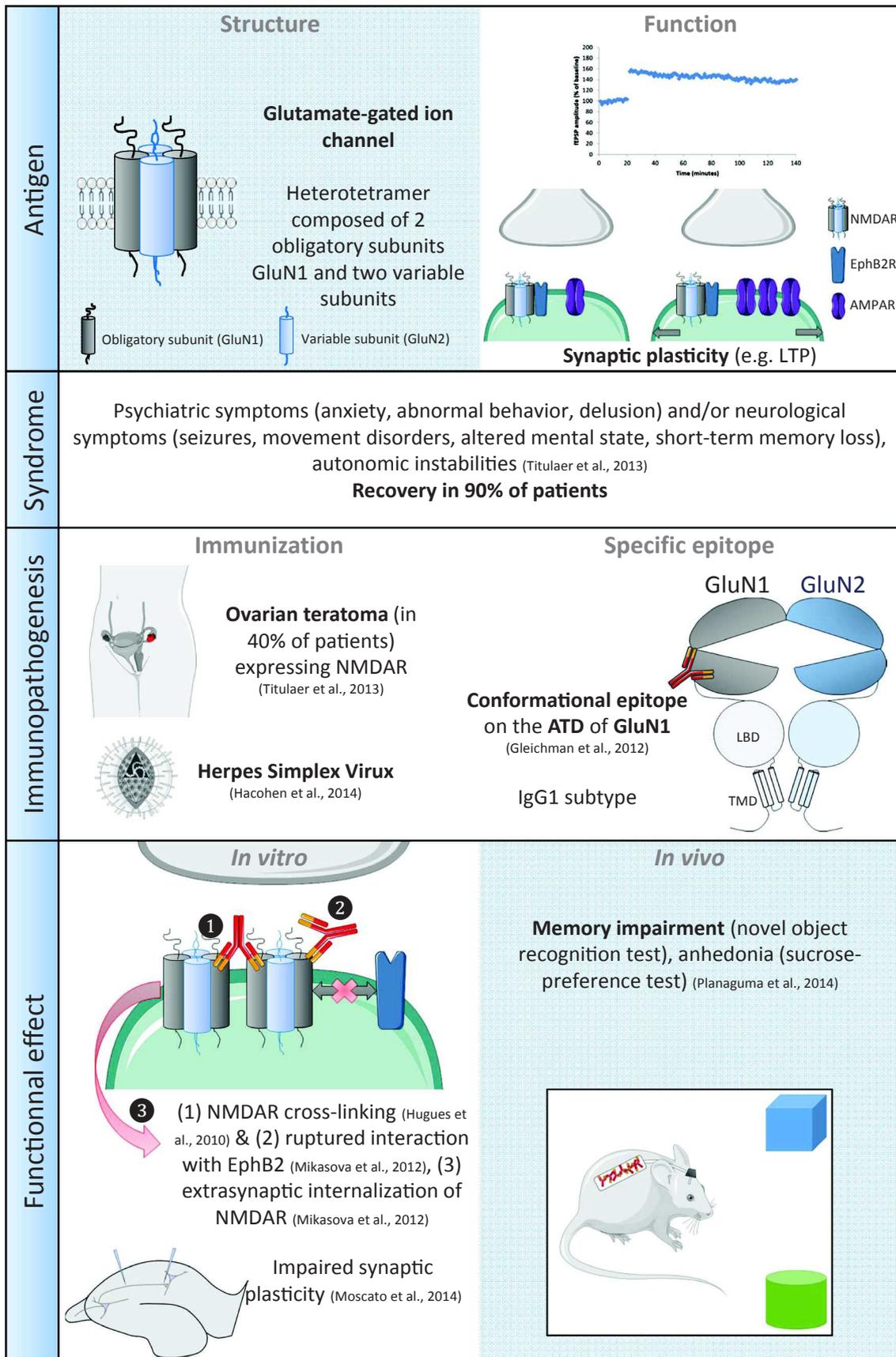


FIG. 1. Example of encephalitis associated with anti-NMDAR autoantibodies.

of surface NMDAR (Hughes *et al.*, 2010). NMDAR antibody binding does not appear to distinguish between excitatory and inhibitory neurons (Moscato *et al.*, 2014). Interestingly, patients' antibody-mediated decrease of NMDAR clusters relies upon the presence of the Fc domain of anti-NMDAR IgG that allows cross-linking of NMDAR (Hughes *et al.*, 2010; Moscato *et al.*, 2014) and suggests an antibody-mediated alteration of NMDAR surface diffusion. In accordance with those results, a subsequent study confirmed the effect of patients' NMDAR antibodies on NMDAR surface diffusion by disruption of NMDAR and EphrinB2 receptor (EPHB2R) interactions (Mikasova *et al.*, 2012) leading to NMDAR internalization. Activation of EPHB2R by its ligand EphrinB2 prevents patients' NMDAR antibodies' effects on their target (Mikasova *et al.*, 2012). Data from several electrophysiological recordings performed in *in vitro* and *ex vivo* studies point toward a functional effect of patients' NMDAR antibodies. Whole cell-patch clamp recordings showed that application of patients' CSF decreased the NMDAR-mediated component of excitatory currents (Hughes *et al.*, 2010). A decrease of the NMDAR-mediated current seems to be caused only by internalization of NMDAR and not by an antagonistic effect (Moscato *et al.*, 2014). Acute application of patients' CSF also decreased neuronal network activity in neuronal cultures (Jantzen *et al.*, 2013). Finally, two studies reported an alteration of NMDAR-mediated plasticity by patients' NMDAR antibodies as they prevented induction of chemical long-term potentiation (LTP) (Mikasova *et al.*, 2012) and electrophysiologically induced LTP in neuronal cell cultures (Zhang *et al.*, 2012).

Independent studies reported a reduction of NMDAR clusters after intrathecal administration of NMDAR antibodies (Hughes *et al.*, 2010; Mikasova *et al.*, 2012; Planaguma *et al.*, 2014). Interestingly, this effect was partly reversed by co-injection of EphrinB2 (Mikasova *et al.*, 2012) confirming the above described *in vitro* studies. Microdialysis after hippocampal injection of patients' NMDAR antibodies in rodents revealed an increase in extracellular glutamate concentration (Manto *et al.*, 2010). Furthermore, injection of the motor prefrontal cortex with patients' NMDAR antibodies increased motor stimulation after high-frequency stimulation (Manto *et al.*, 2011a). Both studies support the hypothesis of induction of hyperactivity of glutamatergic pathways by patients' NMDAR antibodies. Recently, an *in vivo* study (Planaguma *et al.*, 2014) demonstrated that continuous intrathecal infusion of patients' CSF induced behavioural changes in mice (memory impairment, depressive-like and anhedonic behaviour; all symptoms also observed in patients). These effects were reversible upon cessation of patients' CSF infusion. No effect of patients' CSF infusion was observed on anxiety, aggressiveness and locomotor activity (Planaguma *et al.*, 2014).

To summarize, anti-NMDAR encephalitis is the best-described autoimmune encephalitis and appears to be a disorder much more common than initially expected. A direct pathogenic effect of NMDAR antibodies is suggested by altered behaviour in mice partly mimicking anti-NMDAR encephalitis symptoms after infusion with patients' CSF (Planaguma *et al.*, 2014), clinical data, patients' outcome, and other *in vitro* and *in vivo* studies. The possible mechanisms of NMDAR antibody action on NMDAR have started to be elucidated *in vitro* (Dalmau *et al.*, 2008; Hughes *et al.*, 2010; Gleichman *et al.*, 2012; Mikasova *et al.*, 2012), arguing for an antigenic modulation. Yet studies on the long-term effect of NMDAR antibodies on neuronal networks and pathways underlying synaptic plasticity are still lacking. Although clinical data such as treatment response and histopathological studies are not in favour of the involvement of cellular-mediated cytotoxicity, further experimental research is still needed to confirm this impression.

Encephalitis associated with antibodies directed against AMPAR

Definition and clinical presentation

Limbic encephalitis associated with antibodies directed against AMPAR was identified in 2009 in 10 of 109 patients with limbic encephalitis (Lai *et al.*, 2009). Subsequently, three studies describing a few cases were reported (Graus *et al.*, 2010; Hofberger *et al.*, 2015; Joubert & Honnorat, 2015). The majority of patients were women (60%) with a median age around 60 years (Lai *et al.*, 2009; Graus *et al.*, 2010; Hofberger *et al.*, 2015; Joubert & Honnorat, 2015). Clinical features are more variable than in anti-NMDAR encephalitis. The most common presentation reported is classical limbic encephalitis dominated by short-term memory loss, confusion and abnormal behaviour (Lai *et al.*, 2009; Hofberger *et al.*, 2015). Diffuse encephalitis with limbic dysfunction associated with various other symptoms (e.g. seizures, psychiatric manifestations, ataxia, abnormal movements, aphasia and neuropathy) has also been described (Spatola *et al.*, 2014; Hofberger *et al.*, 2015). A few patients presented with fulminant encephalitis (Wei *et al.*, 2013; Joubert & Honnorat, 2015), and a few cases of atypical presentation with prominent psychiatric symptoms resembling acute psychosis have also been reported (Graus *et al.*, 2010; Hofberger *et al.*, 2015). Additional associated antibodies recognizing cell-surface (GABA_BR, NMDAR, LGI1, VGCC), intracellular (SOX1, Hu) or synaptic (amphiphysin, GAD) targets have been identified in about 30% of these patients (Lai *et al.*, 2009; Hofberger *et al.*, 2015; Joubert *et al.*, 2015). Overlapping immune responses may complicate clinical presentations and explain some aspects of the clinical heterogeneity observed in patients with anti-AMPA encephalitis (Lai *et al.*, 2009; Hofberger *et al.*, 2015). Associated tumours (thymus, lung, breast) were observed in 65% of patients with anti-AMPA encephalitis (Lai *et al.*, 2009; Hofberger *et al.*, 2015) and these tumours expressed AMPAR (Lai *et al.*, 2009). This finding supports the hypothesis of a break in immune tolerance induced by ectopic expression of AMPAR by the underlying neoplasm. However, anti-AMPA encephalitis can occur without an identified tumour (Lai *et al.*, 2009). In these cases, the immune trigger remains unknown. Patients usually respond to immunotherapy although relapses may occur (Lai *et al.*, 2009). Aggressive immunotherapy significantly reduces risks of relapses (Hofberger *et al.*, 2015). Regarding clinical presentation and severity, the outcome is heterogeneous and the presence of an underlying tumour seems to be associated with a poor outcome (Hofberger *et al.*, 2015).

Antigen

Like NMDAR, AMPAR belong to the family of iGluRs. AMPAR mediate fast excitatory transmission in the brain. They are required for basal synaptic transmission and are involved in the mechanisms of synaptic plasticity (Santos *et al.*, 2009). AMPAR are heterotetramers composed of four subunits (GluA1 to GluA4) (Traynelis *et al.*, 2010). Patients' antibodies are IgG of uncharacterized subtype targeting GluA1 and/or GluA2 subunits of AMPAR, with a higher incidence of GluA2 antibodies (Lai *et al.*, 2009; Hofberger *et al.*, 2015). Like NMDAR subunits, each AMPAR subunit is composed of two extracellular domains, the ATD and the ligand-binding domain, a transmembrane domain and an intracellular domain. The bottom lobe of the ATD is the main receptor epitope recognized by AMPAR antibodies (Gleichman *et al.*, 2014). This region matches the location of the epitope on the NMDAR in patients with anti-NMDAR encephalitis, suggesting that the bottom lobe of the

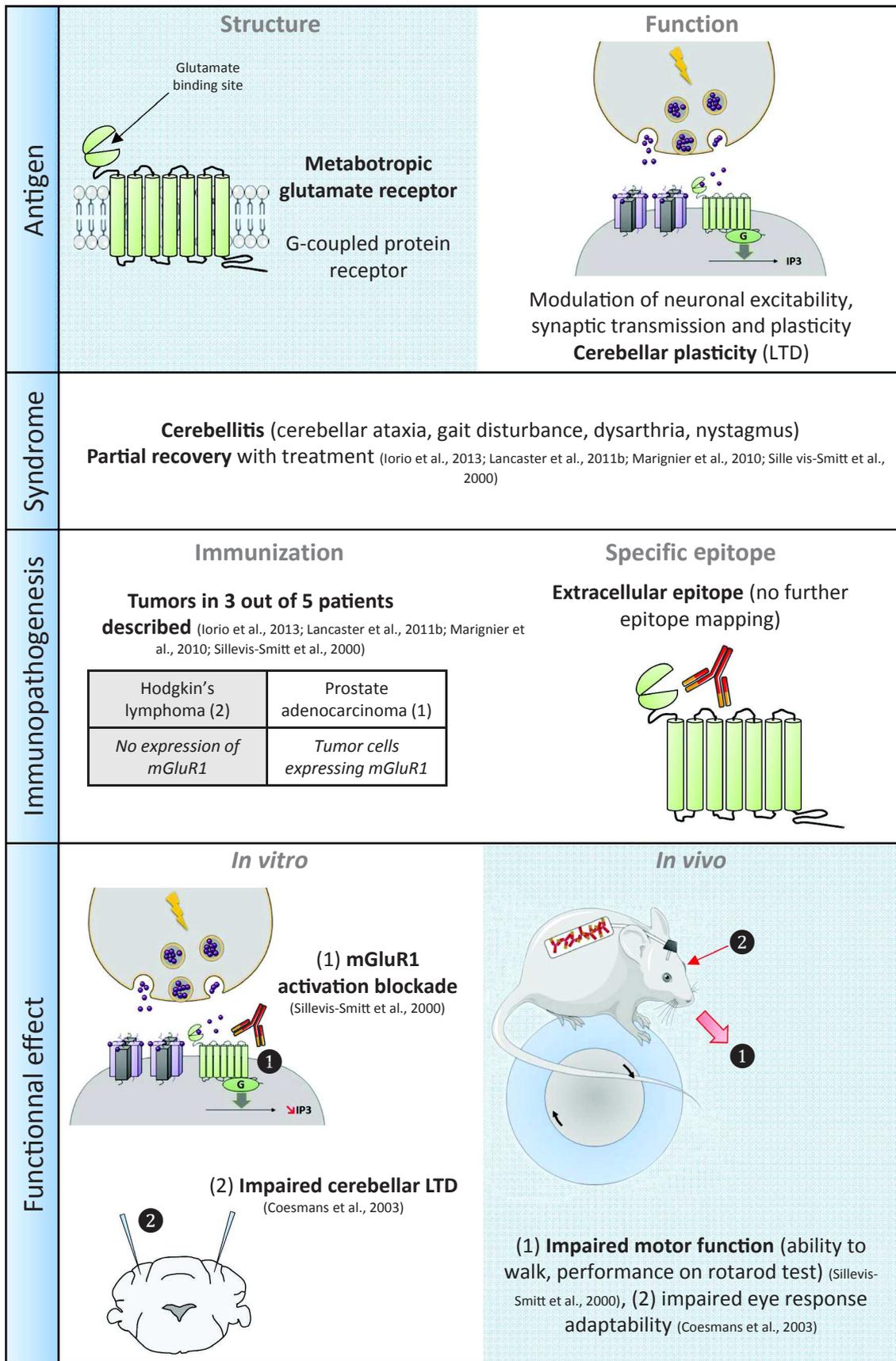


FIG. 2. Example of cerebellitis associated with anti-mGluR1 autoantibodies.

iGluRs might be particularly antigenic (Gleichman *et al.*, 2014). The possible detection of AMPAR antibodies by western blot suggests a non-conformational epitope (Gleichman *et al.*, 2014).

Antibody pathogenicity

As in patients with anti-NMDAR encephalitis, clinical data tend to support the hypothesis of AMPAR-antibody-mediated pathogenicity. Clinical improvement after immune therapy occurred concurrent with a decline in CSF antibody titre, supporting a pathogenic role of the antibodies (Wei *et al.*, 2013). Some evidence indicates that patients' AMPAR antibody synthesis is intrathecal; antibody titres were higher in the CSF than in the serum (Lai *et al.*, 2009) and some patients have antibodies only detected in CSF (Hoftberger *et al.*, 2015). Moreover, serum AMPAR antibodies differ in their epitope specificity from CSF antibodies (Gleichman *et al.*, 2014). Concerning the pathological effects of antibodies on affected brain structures, diffuse cortical atrophy was observed in a few patients with anti-AMPA encephalitis (Joubert & Honnorat, 2015), suggesting a possible neuronal loss in these patients. However, neuropathological data to support this hypothesis are lacking.

Patients' CSF specifically decreases the number of AMPAR and alter their synaptic localization in cultured neurons. This effect is reversible upon removal of antibodies (Lai *et al.*, 2009). *In vitro*, patients' AMPAR antibodies do not seem to alter other components of glutamatergic synapses, or excitatory synapse density or cell viability (Lai *et al.*, 2009; Peng *et al.*, 2015). Cross-linking assay using patients' F(ab) fragment antibodies or full patient antibodies have not been published and the mechanisms leading to this AMPAR internalization have not yet been demonstrated. Functionally, patients' AMPAR antibodies specifically alter AMPAR-mediated synaptic transmission, as demonstrated by the reduction of amplitude and frequency of excitatory current recordings in cultured neurons treated with patients' CSF (Gleichman *et al.*, 2014; Peng *et al.*, 2015). Interestingly, patients' AMPAR antibodies also reduced inhibitory currents and increased neuronal excitability in cultured neurons, suggesting a compensatory decrease of inhibitory synaptic strength leading to a homeostatic increase in intrinsic neuronal excitability to counteract the effects of patients' AMPAR antibodies (Peng *et al.*, 2015). This imbalance between excitatory and inhibitory transmission has been suggested to account for seizures observed in some patients suffering from AMPAR encephalitis (Peng *et al.*, 2015). No published *in vivo* experimental data are available regarding a possible role of patients' AMPAR antibodies.

To summarize, contrary to anti-NMDAR encephalitis patients, the clinical presentations of anti-AMPA encephalitis are more variable. This heterogeneity could reflect a wider diversity of pathological mechanisms in anti-AMPA encephalitis that remains to be elucidated. While *in vitro* data strongly suggest a possible direct pathological effect of patients' AMPAR antibodies, the proof of their pathogenicity remains lacking and other immune factors such as T-cells or some cytokines could be also involved in the pathogenesis.

Encephalitis associated with antibodies directed against the GABA_AR

Clinical presentation

GABA_AR antibodies have been recently described in patients with encephalitis and refractory seizures or status epilepticus (Ohkawa *et al.*, 2014; Petit-Pedrol *et al.*, 2014; Pettingill *et al.*, 2015). Two subgroups of patients can be differentiated based on immunological

and clinical criteria. The first group presents with encephalitis and refractory seizures and high titres of serum and CSF GABA_AR antibodies. The second group presents with diverse clinical features (encephalitis with seizures, stiff-person syndrome and opsoclonus–myoclonus) and low titres of GABA_AR antibodies present only in the serum (Pettingill *et al.*, 2015). No strong tumour association has been reported in this disorder. These initial reports suggest a treatment-responsive disorder but the novelty of this discovery needs certitude regarding treatment response and patient outcome.

Antigen

GABA_ARs belong to the family of heteropentameric ligand-gated ion channels, which also includes the glycine receptor. Various subunit combinations generate structurally and functionally distinct GABA_AR subtypes with different pharmacology and channel gating properties (Luscher *et al.*, 2011). Synaptic GABA_ARs are composed of three α subunits ($\alpha 1$ – $\alpha 3$), two β subunits ($\beta 2$ or $\beta 3$) and a single $\gamma 2$ subunit and mediate most fast inhibitory neurotransmissions in the adult brain (Jacob *et al.*, 2008). Mutations in GABA_AR subunits associate with genetic epilepsy syndrome in humans (Macdonald *et al.*, 2010). In patients with encephalitis and high GABA_AR antibody serum titres, the antibodies are mainly of the IgG1 or rarely IgG3 subtype with subunit specificity ($\alpha 1$ or $\beta 3$ or $\gamma 2$) (Ohkawa *et al.*, 2014; Petit-Pedrol *et al.*, 2014; Pettingill *et al.*, 2015). No further epitope mapping has been reported. In patients with low GABA_AR antibody serum titre, the antibodies were mainly of the IgM subtype without subunit specificity (Pettingill *et al.*, 2015).

Antibody pathogenicity

Patients with anti-GABA_AR encephalitis tend to improve when treated with immunosuppressors and at least in one case clinical improvement was correlated with reduction of GABA_AR antibody titre in the serum (Pettingill *et al.*, 2015). Studies linking clinical evolution to CSF antibody titres are still lacking. Patients' CSF and sera reduce the synaptic density of GABA_AR when applied to neuronal culture with no effect on other synaptic components (Ohkawa *et al.*, 2014; Petit-Pedrol *et al.*, 2014; Pettingill *et al.*, 2015). This effect is not mediated by complement activation (Ohkawa *et al.*, 2014; Pettingill *et al.*, 2015). A functional effect of GABA_AR antibodies on inhibitory synaptic currents *in vitro* has been demonstrated by whole-cell patch clamp (Ohkawa *et al.*, 2014). However, effects of patients' antibodies on extrasynaptic GABA_AR are still unclear, with some contradictory data (Ohkawa *et al.*, 2014; Petit-Pedrol *et al.*, 2014) and there are as yet no *in vivo* studies published on the effect of GABA_AR antibodies.

To summarize, the clinical manifestation of encephalitis with anti-GABA_ARs seem to be dependent on the CSF autoantibody titre. *In vitro* data suggest a potential structural and functional effect of the patients' CSF antibodies on their target antigen by a mechanism of antigenic modulation, similar to that demonstrated for other antibodies directed against the ionotropic channel receptor, but this effect must be confirmed by other studies.

Encephalitis associated with antibodies directed against Lgi1

Clinical presentation

Anti-Lgi1 antibodies were initially described in a cohort of patients with 'VGKC-Ab'-associated encephalitis (Shillito *et al.*,

1995; Lai *et al.*, 2010). Indeed, autoantibodies called 'anti-VGKC antibodies' were first described in patients with neuromyotonia, and later in patients with autoimmune limbic encephalitis and Morvan's syndrome (Lee *et al.*, 1998; Buckley *et al.*, 2001). Further studies revealed that the target of these autoantibodies was not the potassium channel itself but other proteins interacting with the channel, namely contactin-associated protein-like 2 (Caspr2) and leucine-rich, glioma inactivated 1 (Lgi1) (Irani *et al.*, 2010; Lai *et al.*, 2010; Lancaster *et al.*, 2011a).

Lgi1 antibodies are predominantly encountered in patients with a clinical picture of autoimmune encephalitis (Lai *et al.*, 2010; Klein *et al.*, 2013; Ohkawa *et al.*, 2013). Age of onset is usually in the sixth decade but can vary from 20 to 80 years (Lai *et al.*, 2010; Shin *et al.*, 2013; Malter *et al.*, 2014). Both limbic (anterograde amnesia, behavioural/psychiatric disturbances, seizures) and extra-limbic symptoms (motor, cerebellar, extrapyramidal involvement) can be observed. Epilepsy is found in 80% of the patients and may represent the initial symptom (Lai *et al.*, 2010; Klein *et al.*, 2013; Shin *et al.*, 2013; Malter *et al.*, 2014). Insomnia, paradoxical sleep disorders, severe bradyarrhythmias and hyponatraemia are other typical features (Lai *et al.*, 2010; Klein *et al.*, 2013; Kim *et al.*, 2014; Malter *et al.*, 2014). Importantly, abnormal movements called facio-brachial dystonic seizures are closely associated with anti-Lgi1 encephalitis (Irani *et al.*, 2011, 2013). Interestingly, facio-brachial seizures respond only poorly to anti-epileptic drugs, but are highly responsive to immunotherapy (Irani *et al.*, 2011, 2013). Anti-Lgi1 encephalitis seems to be associated with a poor cognitive outcome and frequent evolution to hippocampal atrophy (Malter *et al.*, 2014), but no prospective studies are available. Relapses occur in 10% of patients (Lai *et al.*, 2010; Kim *et al.*, 2014; Malter *et al.*, 2014). Aggressive and prolonged immunotherapy seems important to relieve symptoms and prevent relapses (Irani *et al.*, 2013; Kim *et al.*, 2014). The prevalence of cancers varies among different studies but does not appear to exceed 20% of patients (Irani *et al.*, 2010; Klein *et al.*, 2013; Shin *et al.*, 2013; Malter *et al.*, 2014).

Antigen

Lgi1 is a secreted synaptic protein expressed in neural tissues. It interacts with the synaptic receptors ADAM22 and ADAM23 to create a trans-synaptic complex bridging post-synaptic glutamatergic AMPAR and pre-synaptic Kv1.1 (Fukata *et al.*, 2010). Lgi1 was proposed to act as a regulating factor for neuronal excitability at synapses. Indeed, Lgi1 mutations in humans are related to a hereditary epileptic syndrome (Ottman *et al.*, 1995) and Lgi1-knockout mice develop a phenotype of lethal epilepsy (Fukata *et al.*, 2010). Anti-Lgi1 antibodies are of the IgG4 type, as demonstrated by subclass-specific staining (Irani *et al.*, 2012). The results of epitope mapping performed using patients' serum antibodies suggest that different parts of the protein are recognized by these antibodies (Ohkawa *et al.*, 2013).

Antibody pathogenicity

The clinical symptoms of anti-Lgi1 encephalitis suggest CNS hyperexcitability, similar to what is reported in Lgi1 genetic deletion models (human hereditary epileptic syndrome and mutant mice), suggesting that patients' antibodies may alter Lgi1 functions leading to neuronal hyperexcitability. *In vitro*, Lgi1 antibodies were shown to impair Lgi1 binding to ADAM22 and to decrease surface expression of post-synaptic AMPAR in a reversible and dose-dependent

manner (Ohkawa *et al.*, 2013). Although anti-Lgi1 antibodies are mainly of the IgG4 subtype (Irani *et al.*, 2012) and thus unlikely to fix complement, deposition of complement on neuronal membranes has been demonstrated in the brain of patients with anti-Lgi1 encephalitis, suggesting the involvement of complement-dependent cytotoxicity (Bien *et al.*, 2012). Moreover, Lgi1 antibodies fixed complement on the surfaces of Lgi1-transfected cells (Irani *et al.*, 2012). These observations might explain the more frequent occurrence of hippocampal atrophy associated with Lgi1 antibodies compared to other anti-cell surface antigen antibodies associated with autoimmune encephalitis, such as anti-NMDAR or anti-Caspr2 antibodies. No *in vivo* experimental data arguing for Lgi1 antibody pathogenicity are available.

Encephalitis associated with antibodies directed against Caspr2

Clinical-resentation

Anti-Caspr2 antibodies (Caspr2 Abs) were initially described in eight 'VGKC Ab' patients with encephalitis and/or peripheral nervous system symptoms (Shillito *et al.*, 1995; Irani *et al.*, 2010). Caspr2 Abs correlate with the presence of peripheral symptoms, mostly neuromyotonia and Morvan's syndrome (Shillito *et al.*, 1995; Irani *et al.*, 2010; Lancaster *et al.*, 2011a; Klein *et al.*, 2013). By definition, CNS involvement is excluded in patients with pure neuromyotonia. Morvan's syndrome, by contrast, is a rare autoimmune disorder presenting neuromyotonia features together with marked dysautonomic symptoms (profuse sweating, tachycardia, genito-urinary dysfunction), complete disruption of sleep organization and encephalopathy (visual hallucinations, delusion and impaired vigilance) (Irani *et al.*, 2012). Caspr2 Abs are found in most of Morvan's syndromes, either alone or with moderately elevated anti-Lgi1 Abs (Irani *et al.*, 2012; Ohkawa *et al.*, 2013). Nevertheless, Caspr2 Abs can also be found in patients with pure limbic encephalitis, whose specific clinical pattern and prognosis remain to be precisely determined (Lancaster *et al.*, 2011a; Malter *et al.*, 2014). Differences in epitope specificities and location of the production of autoantibodies have been hypothesized to account for such a variety of clinical presentations. An associated malignant thymoma is observed in more than 50% of Caspr2 Ab-positive neuromyotonia/Morvan's syndrome patients (Irani *et al.*, 2012), while the prevalence of tumours is much lower in patients with pure limbic encephalitis (Lai *et al.*, 2010; Klein *et al.*, 2013; zShin *et al.*, 2013; Malter *et al.*, 2014). Patients with Caspr2 Ab-associated syndrome seem to have low titre of multiple autoantibodies such as Lgi1 Abs, anti-DCC or anti-DPP10 antibodies (Ohkawa *et al.*, 2013). The pathogenic role of these antibodies has not yet been evaluated.

Antigen

Caspr2 is a membrane cell adhesion molecule that clusters voltage-gated potassium channels (Kv1.1/1.2) at the juxtaparanodes of myelinated axons and may regulate axonal excitability (Poliak, 2003; Labasque & Faivre-Sarrailh, 2010). Experimental findings suggest that Caspr2 may also be present in the CNS at pre-synaptic sites where it may interact with the pre-synaptic Kv1 channels (Zweier *et al.*, 2009). Caspr2 was also suggested to play a role in synapse formation and dendritic arborization (Labasque & Faivre-Sarrailh, 2010). Polymorphisms of the CASPR2 gene (CNTNAP2) have notably been described in the autistic spectrum disorders (Anderson *et al.*, 2012).

Antibody pathogenicity

A recent *in vitro* study, using Caspr2 Abs from patients affected by pure limbic encephalitis, determined that Caspr2 Abs from patients' CSF mainly recognize the N-terminal Discoïdin and LamininG1 modules of Caspr2 and strongly target inhibitory interneurons (Pinatel *et al.*, 2015). Functional assays indicated that limbic encephalitis with Caspr2 Abs may induce alteration of Gephyrin clusters at inhibitory synaptic contacts (Pinatel *et al.*, 2015). This work provides new insight into the potential pathogenic effect of Caspr2 Abs in central hyperexcitability that may be related to perturbation of inhibitory interneuron activity. However, *in vivo* studies are still lacking to assess the potential pathogenic effects of Caspr2 Abs according to the different associated clinical presentations.

To conclude, despite the overlap of clinical syndromes, neurological symptoms specific to Lgi1 or Caspr2 Abs begin to emerge, but further work is needed to understand the exact role of the respective autoantibodies (Table 1).

Encephalitis associated with antibodies directed against GABA_BR

Clinical presentation

Antibodies against GABA_BR were described in an initial series of 15 patients (Lancaster *et al.*, 2010) and subsequently followed by several case series (Boronat *et al.*, 2011; Höftberger *et al.*, 2013b; Jeffery *et al.*, 2013; Kim *et al.*, 2014). Patients with GABA_BR antibodies usually present a pure limbic encephalitis with prominent severe seizures or status epilepticus (Lancaster *et al.*, 2010; Höftberger *et al.*, 2013b). Other rare clinical presentations such as opsoclonus–myoclonus or cerebellar ataxia have also been described (Lancaster *et al.*, 2010; Höftberger *et al.*, 2013b; Jarius *et al.*, 2013; Defelipe-Mimbrera *et al.*, 2014). About 50% of patients with GABA_BR antibodies present an underlying small-cell lung carcinoma (Höftberger *et al.*, 2013b). In this paraneoplastic subgroup, the median age of patients is higher (around 60 years) with a shorter overall survival (Höftberger *et al.*, 2013b). Almost all patients achieve partial to complete neurological recovery with immunotherapy and appropriate oncological treatment (Lancaster *et al.*, 2010; Höftberger *et al.*, 2013b; Jeffery *et al.*, 2013; Kim *et al.*, 2014). As observed with anti-NMDAR encephalitis, recovery might be improved using more aggressive immunotherapy targeting B-cells and antibody production (e.g. rituximab) (Kim *et al.*, 2014). Interestingly, as observed in patients with GABA_AR antibodies, a subset of patients with low titres of GABA_BR antibodies in the serum or the CSF and with heterogeneous clinical syndrome has also been described (Lancaster *et al.*, 2010; Jeffery *et al.*, 2013). These patients present with additional antibodies (CV2/CRMP5, ANNA-1 or ANNA-3) revealing a larger anti-neuronal immunization and their prognosis is less favourable in spite of treatment (Jeffery *et al.*, 2013).

Antigen

The GABA_BRs are transmembrane metabotropic G-protein coupled receptors that trigger cAMP cascades and thereby regulate specific ion channel properties. GABA_BRs have been found to play a key role in regulating membrane excitability and synaptic transmission in the brain. Indeed, they can stimulate the opening of K⁺ and Ca²⁺ channels and elicit both presynaptic and slow postsynaptic inhibition (Benarroch, 2012; Gassmann & Bettler, 2012). GABA_BR is an obligatory heterodimer formed by 2 subunits (GABA_B1 and

GABA_B2) (Bettler, 2004; Gassmann & Bettler, 2012). Patients' antibodies target the GABA_B1 subunit and are mainly of the IgG1 subtype, but IgG2 and IgG3 also occur (Lancaster *et al.*, 2010). Failure of GABA_BR detection by patient antibody in immunoblots suggests that the antibodies are directed against a conformational epitope (Höftberger *et al.*, 2013b). Recent deletion mapping experiments suggest that patients' anti-GABA_BR antibodies bind to alternatively spliced Sushi domains present in the presynaptically localized GABA_B1A isoform (Jain *et al.*, 2015).

Antibody pathogenicity

Aside from the good neurological outcome under immunosuppressive treatment, no *in vivo* experimental data are available on the direct neuronal pathogenicity of GABA_BR antibodies. However, the above *in vitro* experiments by Jain *et al.* (2015) showed that patients' anti-GABA_BR antibodies block baclofen-mediated activation of the GABA_BR without decreasing GABA_BR surface expression. These data suggest that anti-GABA_BR antibodies interfere with GABA-mediated inhibition, thereby contributing to seizures.

Encephalomyelitis associated with antibodies directed against the glycine receptor

Clinical presentation

Glycine receptor (GlyR) antibodies were first described in association with progressive encephalomyelitis rigidity and myoclonus (Hutchinson *et al.*, 2008). Stiff-person syndrome (SPS), defined as axial rigidity with or without hyperreflexia and/or autonomic disturbances (Ciccotto *et al.*, 2013), progressive encephalomyelitis with rigidity and myoclonus (PERM or SPS with brainstem involvement) are the most common clinical presentations of patients with GlyR antibodies (McKeon *et al.*, 2013; Carvajal-González *et al.*, 2014). However, a strong phenotypic association is lacking, because GlyR antibodies could also be associated with demyelinating phenotypes including optic neuritis (Woodhall *et al.*, 2013; Martinez-Hernandez *et al.*, 2015). SPS and PERM symptoms are acute or subacute in 70% of cases (Carvajal-González *et al.*, 2014). In the largest described cohort of patients, median age at symptom onset was 50 years (range: 1–75 years) with equal male/female presence (Carvajal-González *et al.*, 2014). Contrary to patients with GlyR antibody-negative SPS/PERM, antibody-positive patients improved markedly with symptomatic treatment and immunotherapy (McKeon *et al.*, 2013; Carvajal-González *et al.*, 2014) although relapses and relapsing/remitting courses are frequent (Carvajal-González *et al.*, 2014). Associated tumours (mainly thymoma and lymphoma, but also malignant melanoma and metastatic breast cancer) have been reported in a few patients (Clerinx *et al.*, 2011; McKeon *et al.*, 2013; Bourke *et al.*, 2014; Carvajal-González *et al.*, 2014). A clear causality link is lacking and the majority of cases are not paraneoplastic.

Antigen

GlyRs are chloride pentameric channels composed of a variable arrangement of α and β subunits (Legendre, 2001; Betz & Laube, 2006). GlyRs are distributed mainly in the spine and brainstem and have a prominent role in the inhibitory modulation of motor, visual, auditory and autonomic networks (Lynch, 2004; Dutertre *et al.*, 2012). GlyR antibodies are predominantly of the IgG1 subclass, intrathecally synthesized and directed against an extracellular epitope

TABLE 1. Main autoantibodies suspected to play a direct role in neurological symptoms

Antigen	Nature of the antigen	Number of described cases	Clinical syndrome	Tumour association	Described mechanisms
NMDAR	Ionotropic Glutamate Receptor	> 500 (Titulaer <i>et al.</i> , 2013)	Psychiatric symptoms (anxiety, abnormal behaviour, delusion) and/or neurological symptoms (seizures, movement disorders, altered mental state, short-term memory loss), autonomic instabilities (Titulaer <i>et al.</i> , 2013).	Age-dependent, up to 40% (ovarian teratoma) in young women (Titulaer <i>et al.</i> , 2013)	Antibodies disrupt NMDAR function by cross-linking and internalization of receptors (Hughes <i>et al.</i> , 2010; Mikasova <i>et al.</i> , 2012). <i>In vitro</i> : reduced surface expression (Hughes <i>et al.</i> , 2010), disrupted interaction with EphB2R (Mikasova <i>et al.</i> , 2012). <i>In vivo</i> : hyperactivity of glutamatergic pathway (Manto <i>et al.</i> , 2010), reduced number of NMDAR clusters (Hughes <i>et al.</i> , 2010), impaired memory and depression-like behaviour (Planaguma <i>et al.</i> , 2014).
AMPA	Ionotropic Glutamate Receptor	≈58 (Lai <i>et al.</i> , 2009; Bataller <i>et al.</i> , 2010; Graus <i>et al.</i> , 2010; Wei <i>et al.</i> , 2013; Gleichman <i>et al.</i> , 2014; Spatola <i>et al.</i> , 2014; Hofberger <i>et al.</i> , 2015; Joubert <i>et al.</i> , 2015)	Four distinct clinical presentations: classical limbic encephalitis, diffuse encephalitis, fulminant encephalitis, psychiatric presentation (Hofberger <i>et al.</i> , 2015; Joubert <i>et al.</i> , 2015)	34% (thymus, breast, lung)	<i>In vitro</i> : antibody-induced internalization of AMPAR (Lai <i>et al.</i> , 2009; Peng <i>et al.</i> , 2015), reduced excitatory transmission (Gleichman <i>et al.</i> , 2014).
mGluR1	Metabotropic Glutamate Receptor	5 (Sillevis Smitt <i>et al.</i> , 2000; Marignier <i>et al.</i> , 2010; Lancaster <i>et al.</i> , 2011b; Iorio <i>et al.</i> , 2013)	Cerebellitis with cerebellar ataxia, balance and gait disturbances, dysarthria and nystagmus	2 cases with Hodgkin's lymphoma (Sillevis Smitt <i>et al.</i> , 2000), 1 case with prostate adenocarcinoma (Iorio <i>et al.</i> , 2013)	<i>In vitro</i> : blockage of mGluR1 activation (Sillevis Smitt <i>et al.</i> , 2000), reduced cerebellar LTD (Coesmans <i>et al.</i> , 2003). <i>In vivo</i> : severe but transient ataxia induced by patients' IgG infusion in mice (Sillevis Smitt <i>et al.</i> , 2000), impaired compensatory eye movement (Coesmans <i>et al.</i> , 2003).
mGluR5	Metabotropic Glutamate Receptor	4 (Lancaster <i>et al.</i> , 2011b; Mat <i>et al.</i> , 2013; Pruss <i>et al.</i> , 2014)	Limbic encephalitis	3 cases with Hodgkin's lymphoma (Lancaster <i>et al.</i> , 2011b; Mat <i>et al.</i> , 2013)	No experimental study available.
GABA _A R	Ligand-gated ion Channel	≈35 (Ohkawa <i>et al.</i> , 2014; Petit-Pedrol <i>et al.</i> , 2014; Pettingill <i>et al.</i> , 2015)	Encephalitis with refractory seizures	2 cases with thymoma (Ohkawa <i>et al.</i> , 2014), 1 case with a neuroepithelial tumour (Pettingill <i>et al.</i> , 2015)	<i>In vitro</i> : reduction of GABA _A R synaptic density (Ohkawa <i>et al.</i> , 2014; Petit-Pedrol <i>et al.</i> , 2014; Pettingill <i>et al.</i> , 2015), reduced inhibitory currents (Ohkawa <i>et al.</i> , 2014).
GABA _B R	Metabotropic Receptor	≈67 (Lancaster <i>et al.</i> , 2010; Boronat <i>et al.</i> , 2011; Höftberger <i>et al.</i> , 2013a,b; Jeffery <i>et al.</i> , 2013; Kim <i>et al.</i> , 2014)	Limbic encephalitis with prominent seizures	50% (small-cell lung cancer) (Höftberger <i>et al.</i> , 2013a,b)	<i>In vitro</i> : blockage of GABA _B R activation by baclofen without decrease of surface GABA _B Rs (Jain <i>et al.</i> , 2015)
GlyR	Chloride pentameric channel	≈77 (Hutchinson <i>et al.</i> , 2008; Clerinx <i>et al.</i> , 2011; Mas <i>et al.</i> , 2011; Turner <i>et al.</i> , 2011; Iizuka <i>et al.</i> , 2012; Damásio <i>et al.</i> , 2013; Kyskan <i>et al.</i> , 2013; McKeon <i>et al.</i> , 2013; Carvajal-González <i>et al.</i> , 2014; Kenda <i>et al.</i> , 2015; Bourke <i>et al.</i> , 2014; Carvajal-González <i>et al.</i> , 2014)	Stiff-person syndrome & progressive encephalomyelitis with rigidity and myoclonus	≈20% (thymoma, lymphoma, malignant melanoma, breast cancer)	<i>In vitro</i> : internalization of GlyR by GlyR in recombinant cellular model (Wuerfel <i>et al.</i> , 2014).

(continued)

TABLE 1 (continued)

Antigen	Nature of the antigen	Number of described cases	Clinical syndrome	Tumour association	Described mechanisms
LGII	Secreted Protein	> 150 (Irani <i>et al.</i> , 2010, 2011; Lai <i>et al.</i> , 2010; Ohkawa <i>et al.</i> , 2013; Shin <i>et al.</i> , 2013)	Encephalitis with limbic (amnesia, behavioural alterations, seizures) and extra-limbic symptoms	No consistent tumour association	<i>In vitro</i> : patients' LGII antibodies impair LGII binding to partner proteins and decrease synaptic AMPAR expression (Ohkawa <i>et al.</i> , 2013)
Caspr2	Adhesion Protein	95 (Irani <i>et al.</i> , 2010, 2012; Lancaster <i>et al.</i> , 2011a; Klein <i>et al.</i> , 2013)	Limbic encephalitis, Morvan's syndrome	50% (thymoma) (Irani <i>et al.</i> , 2012)	<i>In vitro</i> : Caspr2 antibodies from patients with limbic encephalitis alter gephyrin clusters (Pinatel <i>et al.</i> , 2015).
DPPX	Membrane glycoprotein	28	Pronounced gastrointestinal symptoms, agitation, hallucinations, confusion, myoclonus, tremor and seizures, sleep-disturbance, PERM-like syndrome	No tumour associations reported	<i>In vitro</i> : patient's IgG reduce expression of DPPX and Kv4.2 in cultured neurons (Piepgras <i>et al.</i> , 2015).
Amphiphysin	Synaptic vesicle protein	> 35 (Antoine <i>et al.</i> , 1999; Murinson & Guarnaccia, 2008; Moon <i>et al.</i> , 2014)	Stiff-person syndrome	≈90% (breast cancer)	<i>In vivo</i> : transfer of IgG from patients to rat causes muscles stiffness and spasms (Sommer <i>et al.</i> , 2005).
GAD	GABA synthesis enzyme	> 100 (Saiz <i>et al.</i> , 2008; Ariño <i>et al.</i> , 2015; Fouka <i>et al.</i> , 2015)	Limbic encephalitis, cerebellitis	Usually none. Associated with neuroendocrine tumours and lung cancer (Ariño <i>et al.</i> , 2014) in 2 case series (Saiz <i>et al.</i> , 2008; Ariño <i>et al.</i> , 2014).	<i>In vitro</i> : autoantibodies uptake in heterologous cell systems expressing GAD-65 (Hampe <i>et al.</i> , 2013). <i>In vivo</i> : altered behaviour induced by GAD antibodies transfer to rodents (Manto <i>et al.</i> , 2007, 2015; Hampe <i>et al.</i> , 2013).
VGCC	Calcium channel	> 40 (Mason <i>et al.</i> , 1997; Lorenzoni <i>et al.</i> , 2008; Bürk <i>et al.</i> , 2010; Ogawa <i>et al.</i> , 2011)	Cerebellar ataxia	Small cell lung carcinoma	<i>In vivo</i> : passive transfer of patients' antibodies to mice induces acute ataxia (Martín-García <i>et al.</i> , 2013).
Tr/DNER	Neuron-specific signalling protein	> 40 (Graus <i>et al.</i> , 1997; de Graaff <i>et al.</i> , 2012; Probst <i>et al.</i> , 2015)	Cerebellar ataxia	≈90% (Hodgkin's lymphoma)	No experimental study available.

on the GlyR α subunit, probably common to the three isoforms of the subunit (Carvajal-González *et al.*, 2014).

Antibody pathogenicity

GlyR antibody titres in serum and CSF may correlate with the clinical course (Hutchinson *et al.*, 2008; Iizuka *et al.*, 2012). In one case, rituximab treatment (anti-CD20 antibody) led to a marked clinical improvement, suggesting a B-cell-mediated pathology. Interestingly, an increase in CSF GABA levels and a decrease in glutamate level suggestive of a compensatory mechanism have been reported in one patient (Carvajal-González *et al.*, 2014). Recently, the ability of patients' GlyR antibodies to induce GlyR internalization in HEK-transfected cells was reported (Carvajal-González *et al.*, 2014), but no *in vivo* experimental data arguing for GlyR antibody pathogenicity are available.

Other autoimmune encephalitis associated with pathogenic antibodies

Encephalitis associated with antibodies directed against mGluR5

Four patients with limbic encephalitis and antibodies against mGluR5 were recently described (Lancaster *et al.*, 2011b; Mat

et al., 2013; Pruss *et al.*, 2014). All presented with pure limbic encephalitis. This syndrome was previously described and named Ophelia syndrome by Carr (1982). The first described patients with mGluR5 antibodies were three patients with Hodgkin's lymphoma (Lancaster *et al.*, 2011b; Mat *et al.*, 2013), but no tumour was observed in a recently published case (Pruss *et al.*, 2014). Patients appear to respond well to immunosuppressive treatment (Lancaster *et al.*, 2011b; Mat *et al.*, 2013; Pruss *et al.*, 2014) and this recovery correlates with serum antibody titre reduction (Pruss *et al.*, 2014), indirectly arguing for a link between mGluR5 antibodies and pathogenicity. However, no *in vitro* or *in vivo* experimental data on the effect of mGluR5 antibodies have been reported.

Encephalitis associated with antibodies directed against DPPX

Antibodies against dipeptidyl-peptidase-like protein X (DPPX) (DPP6), an accessory subunit of Kv4.2 potassium channels, were detected in four patients with hallucinations and confusion associated with symptoms reflecting CNS hyperexcitability (e.g. myoclonus, tremor, hyperreflexia and seizures) (Boronat *et al.*, 2013). Subsequently, three additional patients with anti-DPPX antibodies were reported with a syndrome resembling PERM (Balint *et al.*, 2014). Until today, 21 patients with this disorder have been described (Tobin *et al.*, 2014; Piepgras *et al.*, 2015). Interestingly, half of the reported encephalitic patients with

anti-DPPX had prominent gastrointestinal symptoms, mainly severe diarrhoea, but also constipation (Boronat *et al.*, 2013; Balint *et al.*, 2014; Tobin *et al.*, 2014). DPPX is a membrane glycoprotein that critically determines the conductance of neuronal Kv4.2 channels and thus regulates membrane excitability (Kim *et al.*, 2008; Kaulin *et al.*, 2009). DPPX expression is spread in the hippocampus, striatum and cerebellum, and remarkably in the myenteric plexus, which may explain the characteristic gastrointestinal symptoms of anti-DPPX encephalitis. DPPX-knockout mice have hyperexcitability of CNS neurons (Boronat *et al.*, 2013; Balint *et al.*, 2014). Recently, it was observed that patients' purified IgG reduced expression of DPPX and Kv4.2 channels on hippocampal neurons and demonstrated a significantly increased activity of enteric neurons after application of anti-DPPX sera to myenteric plexus preparations (Piepgras *et al.*, 2015). These *in vitro* data suggest that downregulation of DPPX and Kv4.2 by a patient's anti-DPPX antibodies results in CNS and enteric neuron hyperexcitability, which may underlie the neuropsychiatric and gastrointestinal manifestations of anti-DPPX encephalitis. Therefore, these first *in vitro* data support a pathogenic role of anti-DPPX antibodies in this newly identified autoimmune encephalitis.

CNS syndromes associated with antibodies directed against amphiphysin

Amphiphysin antibodies were first described in patients with paraneoplastic SPS (Camilli *et al.*, 1993), although they can also be found in other neurological manifestations such as limbic encephalitis, dysautonomia and cerebellar dysfunction (Pittock *et al.*, 2005; Moon *et al.*, 2014). Classically, patients with SPS and anti-amphiphysin antibodies are female with a median age at symptom onset around 60 years and associated breast cancer (Murinson & Guarnaccia, 2008). These patients improve with immunotherapy and tumour removal (Murinson & Guarnaccia, 2008). An underlying tumour (lung cancer, ovarian cancer, gastric cancer) can also be found in some cases of non-SPS associated with amphiphysin antibodies and some clinical improvement can be achieved through immunotherapy (Moon *et al.*, 2014). Amphiphysin is an intracellular synaptic vesicle protein (Lichte *et al.*, 1992) involved in clathrin-mediated endocytosis. Alteration of GABAergic neurotransmission induced by patients' anti-amphiphysin antibodies has been reported (Geis *et al.*, 2010). Moreover, transfer of amphiphysin antibodies from patients to rodents causes stiffness and muscle spasms (Sommer *et al.*, 2005). Taken together, these data suggest that amphiphysin antibodies might be pathogenic although the mechanisms of antibody action on their intracellular target remain unknown.

Encephalitis and cerebellitis associated with antibodies directed against glutamate decarboxylase (GAD)

Clinical presentation

GAD autoantibodies associated with neurological syndromes were initially described by Solimena *et al.* (1988). Several neurological syndromes have been described in association with high titres of GAD autoantibodies, including SPS, cerebellar ataxia, epilepsy and limbic encephalitis (Saiz *et al.*, 2008; Ali *et al.*, 2011; Alexopoulos & Dalakas, 2013; Fouka *et al.*, 2015; Gresa-Arribas *et al.*, 2015). While GAD autoantibodies are generally not associated with cancer, they can occur as a paraneoplastic event (Saiz *et al.*, 2008; Ariño

et al., 2015). Patients with limbic encephalitis associated with GAD autoantibodies usually do not respond well to treatment (Malter *et al.*, 2010). Patients with cerebellar ataxia are mainly women with a median age around 55 years (Ariño *et al.*, 2014). Clinical presentation is insidious or subacute (Honnorat *et al.*, 2001; Ariño *et al.*, 2014) with the most common presenting symptom being gait ataxia, followed by limb ataxia, dysarthria and nystagmus (Ariño *et al.*, 2014). In contrast to limbic encephalitis patients, good outcome (modified Rankin scale < 3) with immunotherapy has been reported in 50% of patients with cerebellar ataxia (Ariño *et al.*, 2014). Finally, patients with SPS associated with GAD antibodies appear to have the best probability of clinical improvement compared to patients who developed another GAD-associated neurological syndrome (Ariño *et al.*, 2015).

Antigen

GAD is the rate-limiting enzyme implicated in the synthesis of GABA, which is the major inhibitory neurotransmitter in the CNS (Buddhala *et al.*, 2009). Two isoforms of GAD (65 and 67 kDa) are expressed predominantly in the brain. GAD-65 can be found concentrated in presynaptic terminals and appears to be the dominant target of patients' autoantibodies that seem to recognize mainly the decarboxylase catalytic domain of GAD-65 (Gresa-Arribas *et al.*, 2015). Although GAD-65 is an intracellular enzyme, it has been suggested that autoantibodies may interact with their target during the synaptic release event (Gresa-Arribas *et al.*, 2015). GAD-65 antibody titres are significantly higher in patients with cerebellar ataxia than in patients with SPS associated with GAD antibodies (Gresa-Arribas *et al.*, 2015). Notably, GAD autoantibodies present in patients with SPS and cerebellar ataxia recognize an epitope distinct from that recognized by GAD autoantibodies present in patients with type 1 diabetes mellitus or limbic encephalitis (Manto *et al.*, 2015), arguing for GAD-65 antibodies recognizing disease-specific epitopes.

Antibody pathogenicity

In vitro and *in vivo* experiments with purified IgGs of patients with GAD-65 autoantibodies and SPS or cerebellar ataxia further supported the hypothesis that GAD-65 autoantibodies target and inhibit GAD-65 function in these diseases (Manto *et al.*, 2007, 2011b; Holmøy & Geis, 2011; Hampe *et al.*, 2013; Hansen *et al.*, 2013). Moreover, the administration of a monoclonal GAD-65 antibody directed against the epitope recognized by SPS patients disrupted *in vitro* the association of GAD with GABAergic synaptic vesicles and depressed the inhibitory synaptic transmission in cerebellar slices (Manto *et al.*, 2015). *In vivo*, this monoclonal antibody induced decreased exploratory behaviour and impaired locomotor function in rats (Manto *et al.*, 2007, 2015). These findings support a specific pathological role of GAD-65 autoantibodies in the pathogenesis of SPS and cerebellar ataxia. In contrast, no effect of GAD-65 antibodies from a patient with limbic encephalitis was observed *in vitro* on GABAergic neurotransmission (Stemmler *et al.*, 2015).

To summarize, numerous evidence suggests a direct role of GAD-65 antibodies in some associated neurological syndromes, but the antibodies may not always play a direct role. Differences in epitope specificities according to the neurological syndromes may explain at least in part the spectrum of anti-GAD-associated neurological disorders (Mitoma *et al.*, 2016). Strong experimental evidence exists in favour of a pathogenic effect of GAD-65 antibodies in SPS and cerebellar ataxia.

Cerebellitis associated with antibodies directed against mGluR1

Clinical presentation

Since their first description in 2000, mGluR1 autoantibodies have been reported in five patients with cerebellitis. All patients experienced severe cerebellar ataxia with balance and gait disturbances, dysarthria and nystagmus. In two cases, mGluR1 cerebellitis was associated with a Hodgkin's lymphoma (Sillevis Smitt *et al.*, 2000) and in one case with a prostate adenocarcinoma (Iorio *et al.*, 2013). In the two remaining published cases, no underlying tumours were found in spite of an extensive examination (Marignier *et al.*, 2010; Lancaster *et al.*, 2011b), raising the hypothesis of a 'primary' autoimmune-mediated disorder for which mechanisms leading to cross-reactivity remain unknown. Treatment allows only limited recovery (Sillevis Smitt *et al.*, 2000; Marignier *et al.*, 2010; Lancaster *et al.*, 2011b; Iorio *et al.*, 2013) (Fig. 2).

Antigen

mGluR1 is a metabotropic glutamate receptor localized postsynaptically in somatodendritic domains and strongly expressed in hippocampus and cerebellum (Martin *et al.*, 1992). In Purkinje cells, mGluR1 is critically involved in long-term depression (LTD) and motor coordination (Ichise *et al.*, 2000). Interestingly, mGluR1-knockout mice exhibit cerebellar symptoms and impaired cerebellar LTD (Aiba *et al.*, 1994). A role for mGluR1 in eye response movement adaption has also been reported (Shutoh *et al.*, 2002). The mGluR1 epitope is located extracellularly as indicated by the detection of patients' antibodies in cell-based assay experiments, but no further epitope mapping has yet been published. Although there is a strong sequence homology between mGluR1 and other members of the mGluR family (for instance mGluR5), antibodies do not cross-react (Lancaster *et al.*, 2011b). The IgG subtypes of patient antibodies have not been reported.

Antibody pathogenicity

The rarity of the disease does not allow a conclusion to be made regarding a pathogenic role of the associated autoantibody. Interestingly, antibodies to Homer-3, which co-localizes with and organizes mGluR1 at synapses in the dendritic spines of the Purkinje cells, have been reported in two exceptional patients who presented with clinical symptoms similar to patients with mGluR1-ab antibodies (Zuliani *et al.*, 2007; Höftberger *et al.*, 2013a). In one case of anti-mGluR1 cerebellitis, early immunosuppressive treatment led in one patient to a reduction of mGluR1 antibody titres in serum and CSF and recovery (Sillevis Smitt *et al.*, 2000) but the other described patients developed cerebellar atrophy and responded only partially to treatment (Coemans *et al.*, 2003; Marignier *et al.*, 2010; Lancaster *et al.*, 2011b; Iorio *et al.*, 2013). In the only reported post-mortem study of brain tissue, Purkinje cell loss was observed, suggesting involvement of cytotoxic mechanisms although no cytotoxic CD8⁺ T-lymphocytes were observed (Coemans *et al.*, 2003). Patients' antibodies are able to block mGluR1 activation in mGluR1-expressing CHO cells (Sillevis Smitt *et al.*, 2000). Accordingly, slice recordings and whole-cells patch clamp recordings indicate that patients' mGluR1 antibodies reduce Purkinje neuron excitability and spontaneous firing rate (Coemans *et al.*, 2003). Extracellular bath application onto cultured embryonic Purkinje cells but not intracellular microinjection reduces LTD, further confirming that patients' mGluR1 antibodies are directed against the extracellu-

lar part of the receptor and that they can block mGluR1 activation and impair synaptic plasticity (Coemans *et al.*, 2003). Passive transfer of patients' mGluR1 antibodies into mouse brain provoked transient ataxia and this behavioural effect was abolished by patients' CSF pre-absorption on CHO cells expressing mGluR1 α , confirming that this effect was directly caused by mGluR1 IgG (Sillevis Smitt *et al.*, 2000). Moreover, antibody infusion into the flocculus of mice acutely and reversibly decreases compensatory eye movements in a way similar to the impairment of saccadic eye movements observed in patients (Coemans *et al.*, 2003). These experimental models provided the ultimate proof of the pathogenicity of patients' mGluR1 antibodies.

To summarize, cerebellitis associated with mGluR1 is an exceptional disorder for which the pathogenicity of the patients' antibodies has been clearly demonstrated. The metabotropic nature of the target of this antibody-mediated neurological disease is unique in this group of autoimmune encephalitides that generally share ion channel dysfunction as a common pathogenesis. Elucidation of the cellular consequences of this singular antibody-mediated metabotropic receptor dysfunction needs further investigation.

Cerebellitis associated with antibodies directed against the VGCC

Antibodies targeting the P/Q-type of the voltage-gated calcium channels (VGCC-Abs) are associated with Lambert-Eaton myasthenic syndrome, cerebellar ataxia or both (Lennon *et al.*, 1995). Cerebellar ataxia associated with VGCC-Abs is essentially paraneoplastic, associated with small cell lung carcinoma expressing functional VGCC (Meriney *et al.*, 1996). The accepted hypothesis for paraneoplastic cases proposes that the immune adapted reaction against the tumour triggers the autoimmune response. VGCC-Ab-associated cerebellar ataxia is usually subacute with symmetrical gait and limb ataxia, dysarthria and nystagmus. However, anti-VGCC cerebellar ataxia may sometimes develop progressively, mimicking idiopathic sporadic late-onset ataxia (Bürk *et al.*, 2010). Patients respond poorly to immunotherapy. This poor response is imputed to the early and diffuse loss of Purkinje cells observed in these patients (Fukuda *et al.*, 2003). In contrast, some case reports suggest that patients with the rare non-paraneoplastic VGCC-Ab-associated cerebellar ataxia may respond well to immunotherapy (Rigamonti *et al.*, 2014). Numerous observations support the hypothesis of a direct pathogenic role of VGCC-Abs in the development of cerebellar ataxia: P/Q-type VGCCs, which are involved in neurotransmitter release in the synaptic cleft (Simms & Zamponi, 2014), are prominent in the cerebellum (Hillman *et al.*, 1991), and mutations of P/Q VGCCs cause ataxia in mice (Hillman *et al.*, 1991; Fletcher & Lennon, 2003). Post-mortem brain examination revealed a diffuse loss of Purkinje cells along with Bergmann's gliosis, with only minor or absent lymphocytic perivascular infiltration (Fukuda *et al.*, 2003), arguing against a major role of cellular immunity. Furthermore, P/Q type VGCC density is decreased by 70–80% in the cerebellum of VGCC-Ab cerebellar ataxia patients compared to control subjects (Fukuda *et al.*, 2003). Indirect experimental arguments were provided by a study using a polyclonal antibody generated against a major epitope in the P/Q-type VGCCs that inhibited VGCC function in neuronal and recombinant VGCCs, altered cerebellar synaptic transmission, and conferred the phenotype of cerebellar ataxia (Liao *et al.*, 2008). More importantly, passive immunization of mice with antibodies from VGCC-Ab cerebellar ataxia patients induced acute ataxia in mice, demonstrating the pathogenic potential of VGCC-Abs (Martín-García *et al.*, 2013). Questions remain concerning the

variability of the clinical manifestations associated with VGCC-Abs between patients. Differences in the production site of VGCC-Abs and in antibody epitope specificities may provide an explanation for this variability.

Cerebellitis associated with antibodies directed against Tr/DNER

Anti-Tr/Delta/notch-like epidermal growth factor-related receptor (DNER) antibodies are associated with paraneoplastic cerebellar degeneration and Hodgkin's lymphoma (Graus *et al.*, 1997; de Graaff *et al.*, 2012; Greene *et al.*, 2014). More than 40 patients have been reported with symptoms typically including progressive nystagmus, limb ataxia, dysarthria and gait ataxia (Trotter *et al.*, 1976; Graus *et al.*, 1997; Bernal *et al.*, 2003; de Graaff *et al.*, 2012; Greene *et al.*, 2014; Probst *et al.*, 2015). Even with successful Hodgkin disease treatment, ataxia correlated with cerebellar atrophy is irreversible in many patients (de Graaff *et al.*, 2012). DNER, the actual target antigen of anti-Tr antibodies, is a CNS-specific Notch ligand transmembrane protein expressed on Purkinje cells (de Graaff *et al.*, 2012). During development, DNER is involved in the crucial interaction between Purkinje cells and Bergmann's glia (Eiraku *et al.*, 2005). Anti-Tr/DNER antibodies are mainly of the IgG1 and IgG3 subclass (Bernal *et al.*, 2003) and intrathecally produced (Graus *et al.*, 1997). Their main epitopes have been mapped to an N-glycosylated extracellular region of DNER (de Graaff *et al.*, 2012). While it is plausible that DNER antibodies act by disrupting DNER-Notch signalling, this remains to be confirmed. Thus, proof of the pathogenicity of anti-DNER antibodies is still lacking and the exact pathogenic mechanisms of this disorder remain to be demonstrated.

Conclusions

The rapidly expanding types of autoimmune encephalitis have stimulated fruitful clinical and fundamental investigations, aiming to specify the role of the autoantibodies associated with these disorders (Table 1). Interestingly, some of these autoantibodies, in general targeting ionotropic channels or their partner proteins, define clinical syndromes that resemble the models of pharmacological or genetic disruption of the corresponding antigen, arguing for a pathogenic role of the associated autoantibodies. Different studies have shown that patients' antibodies may have structural and functional *in vitro* effects on the targeted antigens. However, definitive proof of the pathogenicity of these autoantibodies has been obtained for only a few antibodies by passive transfer experiments in murine models reproducing the human disease symptoms. Examples for autoantibodies with a direct effect are mGluR1R, NMDAR, amphiphysin, VGCC and GAD-65 antibodies. However, although strong evidence points toward a dysfunction of the targeted antigen's signalling and the synaptic apparatus that regulates it, the molecular cascades and cellular dynamics by which these autoantibodies induce pathological processes remain often poorly understood. In diseases for which no direct pathogenic role for associated autoantibodies has been demonstrated, patients often show great variability of the clinical presentations, which may possibly reflect a more complex pathogenicity. In these cases, the autoantibodies may serve as disease biomarkers and for characterization of the involvement of the immune system in the disease. However, they may present only one factor among other immune agents. Further clinico-biological correlation and experimental investigations will be necessary to understand the mechanisms of the underlying immune reaction.

Finally, these autoimmune encephalitides offer unique models of brain-immune interactions in which the targeted antigens have critical roles in synaptic transmission and plasticity. Therefore, studies of the role of each associated autoantibody should open new avenues to understand the molecular mechanisms of some neurological and psychiatric disorders. These rare diseases with specific antibodies may provide novel experimental tools to decipher the role of the targeted synaptic proteins and their involvement in neurological and psychiatric dysfunctions observed in patients.

Acknowledgements

The authors received granted support from the Fédération pour la Recherche sur le Cerveau (FRC), the Agence Nationale pour la Recherche (ANR-14-CE15-0001) and the LECMA-Vaincre Alzheimer (pilot grant 12751). Images from Servier Medical Art (<http://www.servier.fr/smart/banque-dimages-powerpoint>), which are covered by the Creative Commons Attribution 3.0 France, were used in the figures.

Abbreviations

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ATD, amino-terminal domain; Caspr2, contactin-associated protein-like 2; CNS, central nervous system; CRMP5, collapsin response mediator protein 5; CSF, cerebrospinal fluid; DNER, Delta/notch-like epidermal growth factor-related receptor; DPPX, dipeptidyl-peptidase-like protein X; EPHB2R, ephrin B2 receptor; GABA, γ -aminobutyric acid; GAD, glutamate decarboxylase; GlyR, glycine receptor; iGluR, ionotropic glutamate receptor; Lgi1, leucine-rich glioma inactivated 1; LTD, long-term depression; LTP, long-term potentiation; mEPSC, miniature excitatory post-synaptic current; mGluR, metabotropic glutamate receptor; mIPSC, miniature inhibitory post-synaptic current; NMDA, *N*-methyl-D-aspartate; PERM, progressive encephalomyelitis with rigidity and myoclonus; PNS, paraneoplastic neurological syndrome; SPS, stiff-person syndrome; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel.

References

- Aiba, A., Kano, M., Chen, C., Stanton, M.E., Fox, G.D., Herrup, K., Rosenmund, C., Stevens, C. & Tonegawa, S. (1994) Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell*, **79**, 377–388.
- Alexopoulos, H. & Dalakas, M.C. (2013) Immunology of stiff person syndrome and other GAD-associated neurological disorders. *Exp. Rev. Clin. Immunol.*, **9**, 1043–1053.
- Ali, F., Rowley, M., Jayakrishnan, B., Teuber, S., Gershwin, M.E. & Mackay, I.R. (2011) Stiff-person syndrome (SPS) and anti-GAD-related CNS degenerations: protean additions to the autoimmune central neuropathies. *J. Autoimmun.*, **37**, 79–87.
- Anderson, G.R., Galfin, T., Xu, W., Aoto, J., Malenka, R.C. & Sudhof, T.C. (2012) Candidate autism gene screen identifies critical role for cell-adhesion molecule CASPR2 in dendritic arborization and spine development. *Proc. Natl. Acad. Sci.*, **109**, 18120–18125.
- Antoine, J.C., Absi, L., Honnorat, J., Boulesteix, J.-M., De Broucker, T., Vial, C., Butler, M., De Camilli, P. & Michel, D. (1999) Anti-amphiphysin antibodies are associated with various paraneoplastic neurological syndromes and tumors. *Arch. Neurol.*, **56**, 172–177.
- Ariño, H., Gresa-Arribas, N., Blanco, Y., Martínez-Hernández, E., Sabater, L., Petit-Pedrol, M., Rouco, I., Bataller, L., Dalmau, J., Saiz, A. & Graus, F. (2014) Cerebellar ataxia and glutamic acid decarboxylase antibodies: immunologic profile and long-term effect of immunotherapy. *JAMA Neurol.*, **71**, 1009–1016.
- Ariño, H., Höftberger, R., Gresa-Arribas, N., Martínez-Hernández, E., Arman-gue, T., Krüger, M.C., Arpa, J., Domingo, J., Bojan, R., Bataller, L., Saiz, A., Dalmau, J. & Graus, F. (2015) Paraneoplastic neurological syndromes and glutamic acid decarboxylase antibodies. *JAMA Neurol.*, **72**, 1–8.
- Arman-gue, T., Titulaer, M.J., Málaga, I., Bataller, L., Gabilondo, I., Graus, F. & Dalmau, J. (2013) Pediatric anti-N-methyl-D-aspartate receptor encephalitis – clinical analysis and novel findings in a series of 20 patients. *J. Pediatr.*, **162**, 850–856.

- Balint, B., Jarius, S., Nagel, S., Haberkorn, U., Probst, C., Blocker, I.M., Bahtz, R., Komorowski, L., Stöcker, W., Kastrup, A., Kuthe, M. & Meinck, H.M. (2014) Progressive encephalomyelitis with rigidity and myoclonus: a new variant with DPPX antibodies. *Neurology*, **82**, 1521–1528.
- Bataller, L., Galiano, R., Garcia-Escrig, M., Martinez, B., Sevilla, T., Blasco, R., Vilchz, J.J. & Dalmau, J. (2010) Reversible paraneoplastic limbic encephalitis associated with antibodies to the ampa receptor. *Neurology*, **74**, 265–267.
- Benarroch, E.E. (2012) GABAB receptors: structure, functions, and clinical implications. *Neurology*, **78**, 578–584.
- Bernal, F., Shams'ili, S., Rojas, I., Sanchez-Valle, R., Saiz, A., Dalmau, J., Honnorat, J., Silveis-Smitt, P. & Graus, F. (2003) Anti-Tr antibodies as markers of paraneoplastic cerebellar degeneration and Hodgkin's disease. *Neurology*, **60**, 230–234.
- Bettler, B. (2004) Molecular structure and physiological functions of GABA_B receptors. *Physiol. Rev.*, **84**, 835–867.
- Betz, H. & Laube, B. (2006) Glycine receptors: recent insights into their structural organization and functional diversity. *J. Neurochem.*, **97**, 1600–1610.
- Bien, C.G., Vincent, A., Barnett, M.H., Becker, A.J., Blümcke, I., Graus, F., Jellinger, K.A., Reuss, D.E., Ribalta, T., Schlegel, J., Sutton, I., Lassman, H. & Bauer, J. (2012) Immunopathology of autoantibody-associated encephalitis: clues for pathogenesis. *Brain*, **135**, 1622–1638.
- Boronat, A., Sabater, L., Saiz, A., Dalmau, J. & Graus, F. (2011) GABA_B receptor antibodies in limbic encephalitis and anti-GAD-associated neurological disorders. *Neurology*, **76**, 795–800.
- Boronat, A., Gelfand, J.M., Gresa-Arribas, N., Jeong, H.-Y., Walsh, M., Roberts, K., Martinez-Hernandez, E., Rosenfeld, M.R., Balice-Gordon, R., Graus, F., Rudy, B. & Dalmau, J. (2013) Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels. *Ann. Neurol.*, **73**, 120–128.
- Bourke, D., Roxburgh, R., Vincent, A., Cleland, J., Jeffery, O., Dugan, N., Abernethy, D., King, A. & Anderson, N. (2014) Hypoventilation in glycine-receptor antibody related progressive encephalomyelitis, rigidity and myoclonus. *J. Clin. Neurosci.*, **21**, 876–878.
- Buckley, C.J., Oger, J., Clover, L., Tüzün, E., Carpenter, K., Jackson, M. & Vincent, A. (2001) Potassium channel antibodies in two patients with reversible limbic encephalitis. *Ann. Neurol.*, **50**, 73–78.
- Buddhala, C., Hsu, C.-C. & Wu, J.-Y. (2009) A novel mechanism for GABA synthesis and packaging into synaptic vesicles. *Neurochem. Int.*, **55**, 9–12.
- Bürk, K., Wick, M., Roth, G., Decker, P. & Voltz, R. (2010) Antineuronal antibodies in sporadic late-onset cerebellar ataxia. *J. Neurol.*, **257**, 59–62.
- Camdessanché, J.P., Streichenberger, N., Cavillon, G., Rogemond, V., Jousserand, G., Honnorat, J., Convers, P. & Antoine, J.C. (2011) Brain immunohistopathological study in a patient with anti-NMDAR encephalitis. *Eur. J. Neurol.*, **18**, 929–931.
- Camilli, P.De., Thomas, A., Cofelli, R., Folli, F., Lichte, B., Piccolo, G., Meinck, H.M., Autoni, M., Fasseta, G., Bottazon, G., Bates, D., Carlidge, N., Solimena, M. & Kilimann, M.W. (1993) The synaptic vesicle-associated protein amphiphysin is the 128-kD autoantigen of stiff man syndrome with breast cancer. *J. Exp. Med.*, **178**, 2219–2223.
- Carr, I. (1982) The Ophelia syndrome: memory loss in Hodgkin's disease. *Lancet*, **1**, 844–845.
- Carvajal-González, A., Leite, M.I., Waters, P., Woodhall, M., Coutinho, E., Balint, B., Lang, B., Pettingill, P., Carr, A., Sheerin, U.M., Press, R., Lunn, M.P., Lim, M., Maddison, P., Meinck, H.M., Vandenberghe, W. & Vincent, A. (2014) Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes. *Brain*, **137**, 2178–2192.
- Ciccotot, G., Blaya, M. & Kelley, R.E. (2013) Stiff person syndrome. *Neurol. Clin.*, **31**, 319–328.
- Clerinx, K., Breban, T., Schrooten, M., Leite, M.I., Vincent, A., Verschakelen, J., Tousseyn, T. & Vandenberghe, W. (2011) Progressive encephalomyelitis with rigidity and myoclonus: resolution after thymectomy. *Neurology*, **76**, 303–304.
- Coesmans, M., Silveis Smitt, P., Linden, D.J., Shigemoto, R., Hirano, T., Yamakawa, Y., Van Alphen, A.M., Luo, C., Van Der Geet, J.N., Kros, J.M., Gaillard, C.A., Frens, M.A. & De Zeeuw, C.I. (2003) Mechanisms underlying cerebellar motor deficits due to mGluR1-autoantibodies. *Ann. Neurol.*, **53**, 325–336.
- Dalmau, J., Tüzün, E., Wu, H.Y., Masjuan, J., Rossi, J.E., Voloschin, A., Baehring, J.M., Shimazaki, H., Koide, R., King, D., Mason, W., Sansing, L.H., Dichter, M.A., Rosenfeld, M.R. & Lynch, D.R. (2007) Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann. Neurol.*, **61**, 25–36.
- Dalmau, J., Gleichman, A.J., Hughes, E.G., Rossi, J.E., Peng, X., Lai, M., Dessain, S.K., Rosenfeld, M.R., Balice-Gordon, R. & Lynch, D.R. (2008) Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol.*, **7**, 1091–1098.
- Dalmau, J., Lancaster, E., Martínez-Hernandez, E., Rosenfeld, M.R. & Balice-Gordon, R.J. (2011) Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol.*, **10**, 63–74.
- Damásio, J., Leite, M.I., Coutinho, E., Waters, P., Woodhall, M., Santos, M.A., Carrilho, I. & Vincent, A. (2013) Progressive encephalomyelitis with rigidity and myoclonus. *JAMA Neurol.*, **70**, 498.
- Defelipe-Mimbrera, A., Masjuan, J., Corral, Í., Maria, L., Graus, F. & García-barragán, N. (2014) Opsoclonus – myoclonus syndrome and limbic encephalitis associated with GABA_B receptor antibodies in CSF. *J. Neuroimmunol.*, **272**, 91–93.
- Dutertre, S., Becker, C.-M. & Betz, H. (2012) Inhibitory glycine receptors: an update. *J. Biol. Chem.*, **287**, 40216–40223.
- Eiraku, M., Tohgo, A., Ono, K., Kaneko, M., Fujishima, K., Hirano, T. & Kengaku, M. (2005) DNER acts as a neuron-specific notch ligand during Bergmann glial development. *Nat. Neurosci.*, **8**, 873–880.
- Fletcher, C.F. & Lennon, V.A. (2003) Do calcium channel autoantibodies cause cerebellar ataxia with Lambert-Eaton syndrome? *Ann. Neurol.*, **53**, 5–7.
- Florance, N.R., Davis, R.L., Lam, C., Szperka, C., Zhou, L., Ahmad, S., Campen, C.J., Moss, H., Peter, N., Gleichman, A.J., Glaser, C.A., Lynch, D.R., Rosenfeld, M.R. & Dalmau, J. (2009) Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. *Ann. Neurol.*, **66**, 11–18.
- Fouka, P., Alexopoulos, H., Akrivou, S., Trohatou, O., Politis, P.K. & Dalakas, M.C. (2015) GAD65 epitope mapping and search for novel autoantibodies in GAD-associated neurological disorders. *J. Neuroimmunol.*, **281**, 73–77.
- Fukata, Y., Lovero, K.L., Iwanaga, T., Watanabe, A., Yokoi, N., Tabuchi, K., Shigemoto, R., Nicoll, R.A. & Fukata, M. (2010) Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proc. Natl. Acad. Sci.*, **107**, 3799–3804.
- Fukuda, T., Motomura, M., Nakao, Y., Shiraishi, H., Yoshimura, T., Iwanaga, K., Tsuchihata, M. & Eguchi, K. (2003) Reduction of P/Q-type calcium channels in the postmortem cerebellum of paraneoplastic cerebellar degeneration with Lambert-Eaton myasthenic syndrome. *Ann. Neurol.*, **53**, 21–28.
- Gable, M.S., Sheriff, H., Dalmau, J., Tilley, D.H. & Glaser, C.A. (2012) The frequency of autoimmune N-methyl-D-aspartate receptor encephalitis surpasses that of individual viral etiologies in young individuals enrolled in the California encephalitis project. *Clin. Infect. Dis.*, **54**, 899–904.
- Gassmann, M. & Bettler, B. (2012) Regulation of neuronal GABA_B receptor functions by subunit composition. *Nat. Rev. Neurosci.*, **13**, 380–394.
- Geis, C., Weishaupt, A., Hallermann, S., Grunewald, B., Wessig, C., Wulsch, T., Reif, A., Byts, N., Beck, M., Jablonka, S., Boettger, M.K., Üçeyler, N., Fouquet, W., Gerlach, M., Meinck, H.M., Sirén, A.L., Sigrist, S.J., Toyka, K.V., Heckmann, M. & Sommer, C. (2010) Stiff person syndrome-associated autoantibodies to amphiphysin mediate reduced GABAergic inhibition. *Brain*, **133**, 3166–3180.
- Gleichman, A.J., Spruce, L.A., Dalmau, J., Seeholzer, S.H. & Lynch, D.R. (2012) Anti-NMDA receptor encephalitis antibody binding is dependent on amino acid identity of a small region within the GluN1 amino terminal domain. *J. Neurosci.*, **32**, 11082–11094.
- Gleichman, A.J., Panzer, J.A., Baumann, B.H., Dalmau, J. & Lynch, D.R. (2014) Antigenic and mechanistic characterization of anti-AMPA receptor encephalitis. *Ann. Clin. Transl. Neurol.*, **1**, 180–189.
- de Graaff, E., Hulszenboom, E., Van Den Berg, R., Demmers, J., Lugtenburg, P.J., Hoogenraad, C.C. & Silveis-Smitt, P. (2012) Identification of delta/notch-like epidermal growth factor-related receptor as the Tr antigen in paraneoplastic cerebellar degeneration. *Ann. Neurol.*, **71**, 815–824.
- Granerod, J., Ambrose, H.E., Davies, N.W.S., Clewley, J.P., Walsh, A.L., Morgan, D., Cunningham, R., Zuckerman, M., Mutton, K.J., Solomon, T., Ward, K.N., Lunn, M.P., Irani, S.R., Vincent, A., Brown, D.W. & Crowcroft, N.S. (2010) Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect. Dis.*, **10**, 835–844.
- Graus, F., Dalmau, J., Valldeoriola, F., Ferrer, I., Rene, R., Marin, C., Vecht, C.J., Arbuzo, T., Targa, C. & Moll, J.W. (1997) Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. *J. Neuroimmunol.*, **74**, 55–61.

- Graus, F., Boronat, A., Xifro, X., Boix, M., Svingelj, V., Garcia, A., Palomino, A., Sabater, L., Alberch, J. & Saiz, A. (2010) The expanding clinical profile of anti-AMPA receptor encephalitis. *Neurology*, **74**, 857–859.
- Greene, M., Lai, Y., Baella, N., Dalmau, J. & Lancaster, E. (2014) Antibodies to Delta/notch-like epidermal growth factor-related receptor in patients with anti-Tr, paraneoplastic cerebellar degeneration, and Hodgkin lymphoma. *JAMA Neurol.*, **71**, 1003–1008.
- Gresa-Arribas, N., Titulaer, M.J., Torrents, A., Aguilar, E., McCracken, L., Leypoldt, F., Gleichman, A.J., Balice-Gordon, R., Rosenfeld, M.R., Lynch, D., Graus, F. & Dalmau, J. (2014) Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. *Lancet Neurol.*, **13**, 167–177.
- Gresa-Arribas, N., Ariño, H., Martínez-Hernández, E., Petit-Pedrol, M., Sabater, L., Saiz, A., Dalmau, J. & Graus, F. (2015) Antibodies to inhibitory synaptic proteins in neurological syndromes associated with glutamic acid decarboxylase autoimmunity. *PLoS One*, **10**, e0121364.
- Hacohen, Y., Deiva, K., Pettingill, P., Waters, P., Siddiqui, A., Chretien, P., Menson, E., Lin, J.P., Tardieu, M., Vincent, A. & Lim, M.J. (2014) N-methyl-D-aspartate receptor antibodies in post-herpes simplex virus encephalitis neurological relapse. *Mov. Disord.*, **29**, 90–96.
- Hampe, C.S., Petrosini, L., De Bartolo, P., Caporali, P., Cutuli, D., Laricchiuta, D., Foti, F., Radtke, J.R., Vidova, V., Honnorat, J. & Manto, M. (2013) Monoclonal antibodies to 65 kDa glutamate decarboxylase induce epitope specific effects on motor and cognitive functions in rats. *Orphanet J. Rare Dis.*, **8**, 82.
- Hansen, N., Grünwald, B., Weishaupt, A., Colaço, M.N., Toyka, K.V., Sommer, C. & Geis, C. (2013) Human stiff person syndrome IgG-containing high-titre anti-GAD65 autoantibodies induce motor dysfunction in rats. *Exp. Neurol.*, **239**, 202–209.
- Hillman, D., Chen, S., Aung, T.T., Cherksey, B., Sugimori, M. & Llinas, R.R. (1991) Localization of P-type calcium channels in the central-nervous-system. *Proc. Natl. Acad. Sci. USA*, **88**, 7076–7080.
- Hoftberger, R., van Sonderen, A., Leypoldt, F., Houghton, D., Geschwind, M., Gelfand, J., Paredes, M., Sabater, L., Saiz, A., Titulaer, M.J., Graus, F. & Dalmau, J. (2015) Encephalitis and AMPA receptor antibodies: novel findings in a case series of 22 patients. *Neurology*, **84**, 2403–2412.
- Höftberger, R., Sabater, L., Ortega, A., Dalmau, J. & Graus, F. (2013a) Patient with Homer-3 antibodies and cerebellitis. *JAMA Neurol.*, **70**, 506–509.
- Höftberger, R., Titulaer, M.J., Sabater, L., Dome, B., Rózsás, A., Hegedus, B., Hoda, M.A., Laszlo, V., Ankersmit, H.J., Harms, L., Boyero, S., de Felipe, A., Saiz, A., Dalmau, J. & Graus, F. (2013b) Encephalitis and GABA_B receptor antibodies: novel findings in a new case series of 20 patients. *Neurology*, **81**, 1500–1506.
- Holmøy, T. & Geis, C. (2011) The immunological basis for treatment of stiff person syndrome. *J. Neuroimmunol.*, **231**, 55–60.
- Honorat, J. (2006) Onconeural antibodies are essential to diagnose paraneoplastic neurological syndromes. *Acta Neurol. Scand.*, **113**, 64–68.
- Honorat, J., Antoine, J.C., Derrington, E., Aguera, M. & Belin, M.F. (1996) Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. *J. Neurol. Neurosurg. Ps.*, **61**, 270–278.
- Honorat, J., Saiz, A., Giometto, B., Vincent, A., Brieva, L., de Andres, C., Maestre, J., Fabien, N., Vighetto, A., Casamitjana, R., Thivolet, C., Tavolato, B., Antoine, J., Trouillas, P. & Graus, F. (2001) Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies. *Arch. Neurol.*, **58**, 225.
- Hughes, E.G., Peng, X., Gleichman, A.J., Lai, M., Zhou, L., Tsou, R., Parsons, T.D., Lynch, D.R., Dalmau, J. & Balice-Gordon, R.J. (2010) Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J. Neurosci.*, **30**, 5866–5875.
- Hutchinson, M., Waters, P., McHugh, J., Gorman, G., O’Riordan, S., Connolly, S., Hager, H., Yu, P., Becker, C.M. & Vincent, A. (2008) Progressive encephalomyelitis, rigidity and myoclonus: a novel glycine receptor antibody. *Neurology*, **71**, 1291–1292.
- Ichise, T., Kano, M., Hashimoto, K., Yanagihara, D., Nakao, K., Shigemoto, R., Katsuki, M. & Aiba, A. (2000) mGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. *Science*, **288**, 1832–1835.
- Iizuka, T., Leite, M.I., Lang, B., Waters, P., Urano, Y., Miyakawa, S., Hamada, J., Sakai, F., Mochizuki, H. & Vincent, A. (2012) Glycine receptor antibodies are detected in progressive encephalomyelitis with rigidity and myoclonus (PERM) but not in saccadic oscillations. *J. Neurol.*, **259**, 1566–1573.
- Iorio, R., Damato, V., Mirabella, M., Vita, M.G., Hulsenboom, E., Plantone, D., Bizzaro, A., Del Grande, A. & Silveis Smitt, P. (2013) Cerebellar degeneration associated with mGluR1 autoantibodies as a paraneoplastic manifestation of prostate adenocarcinoma. *J. Neuroimmunol.*, **263**, 155–158.
- Irani, S.R., Alexander, S., Waters, P., Kleopa, K.A., Pettingill, P., Zuliani, L., Peles, E., Buckley, C., Lang, B. & Vincent, A. (2010) Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan’s syndrome and acquired neuromyotonia. *Brain*, **133**, 2734–2748.
- Irani, S.R., Michell, A.W., Lang, B., Pettingill, P., Waters, P., Johnson, M.R., Schott, J.M., Armstrong, R.J., S Zagami, A., Bleasel, A., Somerville, E.R., Smith, S.M. & Vincent, A. (2011) Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann. Neurol.*, **69**, 892–900.
- Irani, S.R., Pettingill, P., Kleopa, K.A., Schiza, N., Waters, P., Mazia, C., Zuliani, L., Watanabe, O., Lang, B., Buckley, C. & Vincent, A. (2012) Morvan syndrome: clinical and serological observations in 29 cases. *Ann. Neurol.*, **72**, 241–255.
- Irani, S.R., Stagg, C.J., Schott, J.M., Rosenthal, C.R., Schneider, S.A., Pettingill, P., Pettingill, R., Waters, P., Thomas, A., Voets, N.L., Cardoso, M.J., Cash, D.M., Manning, E.N., Lang, B., Smith, S.J., Vincent, A. & Johnson, M.R. (2013) Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain*, **136**, 3151–3162.
- Jacob, T.C., Moss, S.J. & Jurd, R. (2008) GABA_A receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat. Rev. Neurosci.*, **9**, 331–343.
- Jain, A., Lancaster, E., Dalmau, J. & Balice-Gordon, R.J. (2015) Autoantibodies in the CSF of Anti-GABA_B receptor encephalitis patients block activation of GABA_B receptors in vitro. *Ann. Neurol. Special Issue: 2015 Annual Meetings*, **78**(S19), S77.
- Jantzen, S.U., Ferrea, S., Wach, C., Quasthoff, K., Illes, S., Scherfeld, D., Hartung, H.P., Seitz, R.J. & Dihné, M. (2013) In vitro neuronal network activity in NMDA receptor encephalitis. *BMC Neurosci.*, **14**, 17.
- Jarius, S., Steinmeyer, F., Knobel, A., Streitberger, K., Hotter, B., Horn, S., Heuer, H., Schreiber, S.J., Wilhelm, T., Trefzer, U., Wildemann, B. & Ruprecht, K. (2013) GABAB receptor antibodies in paraneoplastic cerebellar ataxia. *J. Neuroimmunol.*, **256**, 94–96.
- Jeffery, O.J., Lennon, V.A., Pittock, S.J., Gregory, J.K., Britton, J.W. & McKeon, A. (2013) GABAB receptor autoantibody frequency in service serologic evaluation. *Neurology*, **81**, 882–887.
- Joubert, B. & Honnorat, J. (2015) Autoimmune channelopathies in paraneoplastic neurological syndromes. *Biochim. Biophys. Acta*, **1848**, 2665–2676.
- Joubert, B., Kerschen, P., Zekeridou, A., Desestret, V., Rogemond, V., Chaffois, M.O., Ducray, F., Larrue, V., Daubail, B., Idbaih, A., Psimaras, D., Antoine, J.C., Delattre, J.Y. & Honnorat, J. (2015) Clinical spectrum of encephalitis associated with antibodies against the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. *JAMA Neurol.*, **72**, 1163–1169.
- Kaulin, Y.A., De Santiago-Castillo, J.A., Rocha, C.A., Nadal, M.S., Rudy, B. & Covarrubias, M. (2009) The dipeptidyl-peptidase-like protein DPP6 determines the unitary conductance of neuronal Kv4.2 channels. *J. Neurosci.*, **29**, 3242–3251.
- Kenda, J., Svingelj, V., Rodi, Z., Koritnik, B., Graus, F. & Kojić, M. (2015) Glycine receptor antibodies and progressive encephalomyelitis with rigidity and myoclonus with predominant motor neuron degeneration – expanding the clinical spectrum. *J. Neurol. Sci.*, **353**, 177–178.
- Kim, J., Nadal, M.S., Clemens, A.M., Baron, M., Jung, S.C., Misumi, Y., Rudy, B. & Hoffman, D.A. (2008) Kv4 accessory protein DPPX (DPP6) is a critical regulator of membrane excitability in hippocampal CA1 pyramidal neurons. *J. Neurophysiol.*, **100**, 1835–1847.
- Kim, T.J., Lee, S.T., Shin, J.-W., Moon, J., Lim, J.A., Byun, J.I., Shin, Y.W., Lee, K.J., Jung, K.H., Kim, Y.S., Park, K.I., Chu, K. & Lee, S.K. (2014) Clinical manifestations and outcomes of the treatment of patients with GABAB encephalitis. *J. Neuroimmunol.*, **270**, 45–50.
- Klein, C.J., Lennon, V.A., Aston, P.A., McKeon, A., O’Toole, O., Quek, A. & Pittock, S.J. (2013) Insights from LGI1 and CASPR2 potassium channel complex autoantibody subtyping. *JAMA Neurol.*, **70**, 229.
- Kyskan, R., Chapman, K., Mattman, A. & Sin, D. (2013) Antiglycine receptor antibody and encephalomyelitis with rigidity and myoclonus (PERM) related to small cell lung cancer. *BMJ Case Rep.*, **2013**, 1–3.
- Labasque, M. & Faivre-Sarrailh, C. (2010) GPI-anchored proteins at the node of Ranvier. *FEBS Lett.*, **584**, 1787–1792.
- Lai, M., Hughes, E.G., Peng, X., Zhou, L., Gleichman, A.J., Shu, H., Matà, S., Kremens, D., Vitaliani, R., Geschwind, M.D., Bataller, L., Kalb, R.G.,

- Davis, R., Graus, F., Lynch, D.R., Balice-Gordon, R.J. & Dalmau, J. (2009) AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann. Neurol.*, **65**, 424–434.
- Lai, M., Huijbers, M.G.M., Lancaster, E., Graus, F., Bataller, L., Balice-Gordon, R.J., Cowell, J.K. & Dalmau, J. (2010) Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol.*, **9**, 776–785.
- Lancaster, E., Lai, M., Peng, X., Hughes, E.G., Constantinescu, R., Raizer, J., Friedman, D., Skeen, M.B., Grisold, W., Kimura, A., Ohta, K., Iizuka, T., Guzman, M., Graus, F., Moss, S.J., Balice-Gordon, R. & Dalmau, J. (2010) Antibodies to the GABA_B receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol.*, **9**, 67–76.
- Lancaster, E., Huijbers, M.G.M., Bar, V., Boronat, A., Wong, A., Martinez-Hernandez, E., Wilson, C., Jacobs, D., Lai, M., Walker, R.W., Graus, F., Bataller, L., Illa, I., Markx, S., Strauss, K.A., Peles, E., Scherer, S.S. & Dalmau, J. (2011a) Investigations of caspr2, an autoantigen of encephalitis and neuromyotonia. *Ann. Neurol.*, **69**, 303–311.
- Lancaster, E., Martinez-Hernandez, E., Titulaer, M.J., Boulos, M., Weaver, S., Antoine, J.C., Liebers, E., Kornblum, C., Bien, C.G., Honnorat, J., Wong, S., Xu, J., Contractor, A., Balice-Gordon, R. & Dalmau, J. (2011b) Antibodies to metabotropic glutamate receptor 5 in the Ophelia syndrome. *Neurology*, **77**, 1698–1701.
- Lee, E.K., Maselli, R.A., Ellis, W.G. & Agius, M.A. (1998) Morvan's fibrillary chorea: a paraneoplastic manifestation of thymoma. *J. Neurol. Neurosurg. Ps.*, **65**, 857–862.
- Legendre, P. (2001) The glycinergic inhibitory synapse. *Cell. Mol. Life Sci.*, **58**, 760–793.
- Lennon, V.A., Kryzer, T.J., Griesmann, G.E., O'Suilleabhain, P.E., Winkler, A.J., Woppmann, A., Miljanich, G.P. & Lambert, E.H. (1995) Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes. *N. Engl. J. Med.*, **332**, 1467–1475.
- Liao, Y.J., Safa, P., Chen, Y.R., Sobel, R.A., Boyden, E.S. & Tsien, R.W. (2008) Anti-Ca²⁺ channel antibody attenuates Ca²⁺ current and mimics cerebellar ataxia *in vivo*. *Proc. Natl. Acad. Sci. USA*, **105**, 2705–2710.
- Lichte, B., Veh, R.W., Meyer, H.E. & Kilimann, M.W. (1992) Amphiphysin, a novel protein associated with synaptic vesicles. *EMBO J.*, **11**, 2521–2530.
- Lorenzoni, P.J., Scola, R.H., Lang, B., Kay, C.S.K., Teive, H.A.G., Kowacs, P.A. & Werneck, L.C. (2008) Cerebellar ataxia in non-paraneoplastic Lambert-Eaton myasthenic syndrome. *J. Neurol. Sci.*, **270**, 194–196.
- Luscher, B., Fuchs, T. & Kilpatrick, C.L. (2011) GABA_A receptor trafficking-mediated plasticity of inhibitory synapses. *Neuron*, **70**, 385–409.
- Lynch, J.W. (2004) Molecular structure and function of the glycine receptor chloride channel. *Physiol. Rev.*, **84**, 1051–1095.
- Macdonald, R.L., Kang, J.-Q. & Gallagher, M.J. (2010) Mutations in GABA_A receptor subunits associated with genetic epilepsies. *J. Physiol.*, **588**(Pt 11), 1861–1869.
- Malter, M.P., Helmstaedter, C., Urbach, H., Vincent, A. & Bien, C.G. (2010) Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. *Ann. Neurol.*, **67**, 470–478.
- Malter, M.P., Frisch, C., Schoene-Bake, J.C., Helmstaedter, C., Wandinger, K.-P.P., Stoeker, W., Urbach, H., Surges, R., Elger, C.E., Vincent, A.V. & Bien, C.G. (2014) Outcome of limbic encephalitis with VGKC-complex antibodies: relation to antigenic specificity. *J. Neurol.*, **261**, 1695–1705.
- Manto, M.U., Laute, M.-A., Aguera, M., Rogemond, V., Pandolfo, M. & Honnorat, J. (2007) Effects of anti-glutamic acid decarboxylase antibodies associated with neurological diseases. *Ann. Neurol.*, **61**, 544–551.
- Manto, M., Dalmau, J., Didelot, A., Rogemond, V. & Honnorat, J. (2010) In vivo effects of antibodies from patients with anti-NMDA receptor encephalitis: further evidence of synaptic glutamatergic dysfunction. *Orphanet J. Rare Dis.*, **5**, 31.
- Manto, M., Dalmau, J., Didelot, A., Rogemond, V. & Honnorat, J. (2011a) Afferent facilitation of corticomotor responses is increased by IgGs of patients with NMDA-receptor antibodies. *J. Neurol.*, **258**, 27–33.
- Manto, M.U., Hampe, C.S., Rogemond, V. & Honnorat, J. (2011b) Respective implications of glutamate decarboxylase antibodies in stiff person syndrome and cerebellar ataxia. *Orphanet J. Rare Dis.*, **6**, 3.
- Manto, M., Honnorat, J., Hampe, C.S., Guerra-Narbona, R., López-Ramos, J.C., Delgado-García, J.M., Saitow, F., Suzuki, H., Yanagawa, Y., Mizusawa, H. & Mitoma, H. (2015) Disease-specific monoclonal antibodies targeting glutamate decarboxylase impair GABAergic neurotransmission and affect motor learning and behavioral functions. *Front. Behav. Neurosci.*, **9**, 1–14.
- Marignier, R., Chenevier, F., Rogemond, V., Sillevs Smitt, P., Renoux, C., Cavillon, G., Androdias, G., Vukusic, S., Graus, F., Honnorat, J. & Confavreux, C. (2010) Metabotropic glutamate receptor type 1 autoantibody-associated cerebellitis. *Arch. Neurol.*, **67**, 627–630.
- Martin, L.J., Blackstone, C.D., Hagan, R.L. & Price, D.L. (1992) Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron*, **9**, 259–270.
- Martinez-Hernandez, E., Sepulveda, M., Rostásy, K., Höftberger, R., Graus, F., Harvey, R.J., Saiz, A. & Dalmau, J. (2015) Antibodies to aquaporin 4, myelin-oligodendrocyte glycoprotein, and the glycine receptor $\alpha 1$ subunit in patients with isolated optic neuritis. *JAMA Neurol.*, **72**, 187–193.
- Martín-García, E., Mannara, F., Gutiérrez-Cuesta, J., Sabater, L., Dalmau, J., Maldonado, R. & Graus, F. (2011) Anti glycine-receptor P/Q type voltage-gated calcium channel antibodies from paraneoplastic cerebellar degeneration cause ataxia in mice. *J. Neuroimmunol.*, **261**, 53–59.
- Mas, N., Saiz, A., Leite, M.I., Waters, P., Baron, M., Castano, D., Sabater, L., Vincent, A. & Graus, F. (2011) Anti glycine-receptor encephalomyelitis with rigidity. *J. Neurol. Neurosurg. Ps.*, **82**, 1399–1401.
- Mason, W.P., Graus, F., Lang, B., Honnorat, J., Delattre, J.Y., Valldeoriola, F., Antoine, J.C., Rosenblum, M.K., Rosenfeld, M.R., Newsom-Davis, J., Posner, J.B. & Dalmau, J. (1997) Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert-Eaton myasthenic syndrome. *Brain*, **120**, 1279–1300.
- Mat, A., Adler, H., Merwick, A., Chadwick, G., Gullo, G., Dalmau, J. & Tubridy, N. (2013) Ophelia syndrome with metabotropic glutamate receptor 5 antibodies in CSF. *Neurology*, **80**, 1349–1350.
- McKeon, A., Martinez-Hernandez, E., Lancaster, E., Matsumoto, J.Y., Harvey, R.J., McEvoy, K.M., Pittock, S.J., Lennon, V.A. & Dalmau, J. (2013) Glycine receptor autoimmune spectrum with stiff-man syndrome phenotype. *JAMA Neurol.*, **70**, 44.
- Meriney, S.D., Hulsizer, S.C., Lennon, V.A. & Grinell, A.D. (1996) Lambert-Eaton myasthenic syndrome immunoglobulins react with multiple types of calcium channels in small-cell lung carcinoma. *Ann. Neurol.*, **40**, 739–749.
- Mikasova, L., De Rossi, P., Bouchet, D., Georges, F., Rogemond, V., Dideot, A., Meissirel, C., Honnorat, J. & Groc, L. (2012) Disrupted surface cross-talk between NMDA and Ephrin-B2 receptors in anti-NMDA encephalitis. *Brain*, **135**, 1606–1621.
- Mitoma, H., Adhikari, K., Aeschlimann, D., Chattopadhyay, P., Hadjivassiliou, M., Hampe, C.S., Honnorat, J., Joubert, B., Kakei, S., Lee, J., Manto, M., Matsunaga, A., Mizusawa, H., Nanri, K., Shanmugarajah, P., Yoneda, M. & Yuki, N. (2016) Consensus paper: neuroimmune mechanisms of cerebellar ataxias. *Cerebellum*, **15**, 213–232.
- Moon, J., Lee, S.-T., Shin, J.-W., Byun, J.-I., Lim, J.-A., Shin, Y.W., Kim, T.J., Lee, K.J., Park, K.I., Jung, K.H., Jung, K.Y., Lee, S.K. & Chu, K. (2014) Non-stiff anti-amphiphysin syndrome: clinical manifestations and outcome after immunotherapy. *J. Neuroimmunol.*, **274**, 209–214.
- Moscato, E.H., Peng, X., Jain, A., Parsons, T.D., Dalmau, J. & Balice-Gordon, R.J. (2014) Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. *Ann. Neurol.*, **76**, 108–119.
- Murinson, B.B. & Guarnaccia, J.B. (2008) Stiff-person syndrome with amphiphysin antibodies: distinctive features of a rare disease. *Neurology*, **71**, 1955–1958.
- Ogawa, E., Sakakibara, R., Kawashima, K., Yoshida, T., Kishi, M., Tateno, F., Kataoka, M., Kawashima, T. & Yamamoto, M. (2011) VGCC antibody-positive paraneoplastic cerebellar degeneration presenting with positioning vertigo. *Neurol. Sci.*, **32**, 1209–1212.
- Ohkawa, T., Fukata, Y., Yamasaki, M., Miyazaki, T., Yokoi, N., Takashima, H., Watanabe, M., Watanabe, O. & Fukata, M. (2013) Autoantibodies to epilepsy-related LGI1 in limbic encephalitis neutralize LGI1-ADAM22 interaction and reduce synaptic AMPA receptors. *J. Neurosci.*, **33**, 18161–18174.
- Ohkawa, T., Satake, S., Yokoi, N., Miyazaki, Y., Ohshita, T., Sobue, G., Takashima, H., Watanabe, O., Fukata, Y. & Fukata, M. (2014) Identification and characterization of GABA_A receptor autoantibodies in autoimmune encephalitis. *J. Neurosci.*, **34**, 8151–8163.
- Ottman, R., Risch, N., Hauser, W.A., Pedley, T.A., Lee, J.H., Barker-Cummings, C., Lustenberger, A., Nagle, K.J., Lee, K.S., Scheuer, M.L. & Wilhelmson, K.C. (1995) Localization of a gene for partial epilepsy to chromosome 10q. *Nat. Genet.*, **10**, 56–60.
- Paoletti, P., Bellone, C. & Zhou, Q. (2013) NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat. Rev. Neurosci.*, **14**, 383–400.
- Peng, X., Hughes, E.G., Moscato, E.H., Parsons, T.D., Dalmau, J. & Balice-Gordon, R.J. (2015) Cellular plasticity induced by anti- α -amino-3-

- hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor encephalitis antibodies. *Ann. Neurol.*, **77**, 381–398.
- Petit-Pedrol, M., Armangué, T., Peng, X., Bataller, L., Cellucci, T., Davis, R.L., McCracken, L., Martínez-Hernández, E., Mason, W.P., Krüer, M.C., Ritacco, D.G., Grisold, W., Meaney, B.F., Alcalá, C., Sillevs-Smitt, P., Titulaer, M.J., Balice-Gordon, R., Graus, F. & Dalmau, J. (2014) Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABA_A receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. *Lancet Neurol.*, **13**, 276–286.
- Pettingill, P., Kramer, H.B., Coebergh, J.A., Pettingill, R., Maxwell, S., Nibber, A., Malaspina, A., Jacob, A., Irani, S.R., Buckley, C., Lang, B., Waters, P. & Vincent, A. (2015) Antibodies to GABA_A receptor $\alpha 1$ and $\gamma 2$ subunits: clinical and serologic characterization. *Neurology*, **84**, 1233–1241.
- Piepgas, J., Holtje, M., Michel, K., Li, Q., Otto, C., Drenckhahn, C., Probst, C., Schemann, M., Jarius, S., Stöcker, W., Balint, B., Meinck, H.M., Buchert, R., Dalmau, J., Ahnert-Hilger, G. & Ruprecht, K. (2015) Anti-DPPX encephalitis: pathogenic effects of antibodies on gut and brain neurons. *Neurology*, **85**, 890–897.
- Pinatel, D., Hivert, B., Boucraut, J., Saint-Martin, M., Rogemond, V., Zoupi, L., Karageorgos, D., Honnorat, J. & Faivre-Sarrailh, C. (2015) Inhibitory axons are targeted in hippocampal cell culture by anti-Caspr2 autoantibodies associated with limbic encephalitis. *Front. Cell. Neurosci.*, **9**, 265.
- Pittock, S.J., Lucchinetti, C.F. & Lennon, V.A. (2003) Anti-neuronal nuclear autoantibody type 2: paraneoplastic accompaniments. *Ann. Neurol.*, **53**, 580–587.
- Pittock, S.J., Lucchinetti, C.F., Parisi, J.E., Benarroch, E.E., Mokri, B., Stephan, C.L., Kim, K.K., Kilimann, M.W. & Lennon, V.A. (2005) Amphiphysin autoimmunity: paraneoplastic accompaniments. *Ann. Neurol.*, **58**, 96–107.
- Planaguma, J., Leypoldt, F., Mannara, F., Gutierrez-Cuesta, J., Martín-García, E., Aguilar, E., Titulaer, M.J., Petit-Pedrol, M., Jain, A., Balice-Gordon, R., Lakadamyali, M., Graus, F., Maldonado, R. & Dalmau, J. (2014) Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain*, **138**, 94–109.
- Poliak, S. (2003) Juxtaparanodal clustering of Shaker-like K⁺ channels in myelinated axons depends on Caspr2 and TAG-1. *J. Cell. Biol.*, **162**, 1149–1160.
- Probst, C., Komorowski, L., de Graaff, E., van Coevorden-Hameete, M., Rogemond, V., Honnorat, J., Sabeter, L., Graus, F., Jarius, S., Voltz, R., Wildemann, B., Franciotta, D., Blöcker, I.M., Schlumberger, W., Stöcker, W. & Sillevs Smitt, P.A. (2015) Standardized test for anti-Tr/DNER in patients with paraneoplastic cerebellar degeneration. *Neurol. Neuroimmunol. Neuroinflamm.*, **2**, e68.
- Pruss, H., Rothkirch, M., Kopp, U., Hamer, H.M., Hagge, M., Sterzer, P., Saschenbrecker, S., Stöcker, W., Harms, L. & Endres, M. (2014) Limbic encephalitis with mGluR5 antibodies and immunotherapy-responsive prosopagnosia. *Neurology*, **83**, 1384–1386.
- Prüss, H., Finke, C., Höljtje, M., Hofmann, J., Klingbeil, C., Probst, C., Borowski, K., Ahnert-Hilger, G., Harms, L., Schwab, J.M., Ploner, C.J., Komorowski, L., Stoecker, W., Dalmau, J. & Wandinger, K.P. (2012) N-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. *Ann. Neurol.*, **72**, 902–911.
- Rasputnig, M., Vedeler, C.A. & Storstein, A. (2011) Onconeural antibodies in patients with neurological symptoms: detection and clinical significance. *Acta Neurol. Scand. Suppl.*, **124**, 83–88.
- Rigamonti, A., Lauria, G., Stanzani, L., Mantero, V., Andreatta, F. & Salmaggi, A. (2014) Non-paraneoplastic voltage-gated calcium channels antibody-mediated cerebellar ataxia responsive to IVIG treatment. *J. Neurol. Sci.*, **336**, 169–170.
- Saiz, A., Blanco, Y., Sabater, L., González, F., Bataller, L.L., Casamitjana, R., Ramió-Torrentà, L. & Graus, F. (2008) Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain*, **131**, 2553–2563.
- Santos, S.D., Carvalho, A.L., Caldeira, M.V. & Duarte, C.B. (2009) Regulation of AMPA receptors and synaptic plasticity. *Neuroscience*, **158**, 105–125.
- Shillito, P., Molenaar, P.C., Vincent, A., Leys, K., Zheng, W., Van den Berg, R., Plomp, J.J., Van Kempen, G.T., Chauplannaz, G. & Wintzen, A.R. (1995) Acquired neuromyotonia: evidence for autoantibodies directed against K_v channels of peripheral nerves. *Ann. Neurol.*, **38**, 714–722.
- Shin, Y.-W., Lee, S.T.S.K.S.-T., Shin, J.-W., Moon, J., Lim, J.-A.A., Byun, J.I.I., Kim, T.J., Lee, K.J., Kim, Y.S., Park, K.I., Jung, K.H., Lee, S.K. & Chu, K. (2013) VGKC-complex/LGI1-antibody encephalitis: clinical manifestations and response to immunotherapy. *J. Neuroimmunol.*, **265**, 75–81.
- Shutoh, F., Katoh, A., Kitazawa, H., Aiba, A., Itoharu, S. & Nagao, S. (2002) Loss of adaptability of horizontal optokinetic response eye movements in mGluR1 knockout mice. *Neurosci. Res.*, **42**, 141–145.
- Sillevis Smitt, P.A., Manley, G.T. & Posner, J.B. (1995) Immunization with the paraneoplastic encephalomyelitis antigen HuD does not cause neurologic disease in mice. *Neurology*, **45**, 1873–1878.
- Sillevis Smitt, P., Kinoshita, A., De Leeuw, B., Moll, W., Coesmans, M., Jaarsma, D., Henzen-Logmans, S., Vecht, C., De Zeeuw, C., Sekiyama, N., Nakanishi, S. & Shigemoto, R. (2000) Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. *N. Engl. J. Med.*, **342**, 21–27.
- Simms, B.A. & Zamponi, G.W. (2014) Neuronal voltage-gated calcium channels: structure, function, and dysfunction. *Neuron*, **82**, 24–45.
- Solimena, M., Folli, F., Denis-Donini, S., Comi, G.C., Pozza, G., De Camilli, P. & Vicari, A.M. (1988) Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. *N. Engl. J. Med.*, **318**, 1012–1020.
- Sommer, C., Weishaupt, A., Brinkhoff, J., Biko, L., Wessig, C., Gold, R. & Toyka, K.V. (2005) Paraneoplastic stiff-person syndrome: passive transfer to rats by means of IgG antibodies to amphiphysin. *Lancet*, **365**, 1406–1411.
- Spatola, M., Stojanova, V., Prior, J.O., Dalmau, J. & Rossetti, A.O. (2014) Serial brain 18FDG-PET in anti-AMPA receptor limbic encephalitis. *J. Neuroimmunol.*, **271**, 53–55.
- Stemmler, N., Rohleder, K., Malter, M.P., Widman, G., Elger, C.E., Beck, H. & Surges, R. (2015) Serum from a patient with GAD65 antibody-associated limbic encephalitis did not alter GABAergic neurotransmission in cultured hippocampal networks. *Front. Neurol.*, **6**, 1–7.
- Szabo, A., Dalmau, J., Manley, G., Rosenfeld, M., Wong, E., Henson, J., Posner, J.B. & Furneaux, H.M. (1991) HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. *Cell*, **67**, 325–333.
- Thomas, L., Mailles, A., Desestret, V., Ducray, F., Mathias, E., Rogemond, V., Didelot, A., Marignier, S., Stahl, J.P., Honnorat, J. & Vuillemet, F. (2014) Autoimmune N-methyl-D-aspartate receptor encephalitis is a differential diagnosis of infectious encephalitis. *J. Infect.*, **68**, 419–425.
- Titulaer, M.J., McCracken, L., Gabilondo, I., Armangué, T., Glaser, C., Iizuka, T., Honig, L.S., Benseler, S.M., Kawachi, I., Martínez-Hernández, E., Aguilar, E., Gresa-Arribas, N., Ryan-Flourance, N., Torrents, A., Saiz, A., Rosenfeld, M.R., Balice-Gordon, R., Graus, F. & Dalmau, J. (2013) Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol.*, **12**, 157–165.
- Tobin, W.O., Lennon, V.A., Komorowski, L., Probst, C., Clardy, S.L. & Appendino, J.P. (2014) DPPX potassium channel antibody frequency, clinical accompaniments, and outcomes in 20 patients. *Neurology*, **83**, 1797–1803.
- Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J. & Dingledine, R. (2010) Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.*, **62**, 405–496.
- Trotter, J.L., Hendin, B.A. & Osterland, C.K. (1976) Cerebellar degeneration with Hodgkin disease. An immunological study. *Arch. Neurol.*, **33**, 660–661.
- Turner, M.R., Irani, S.R., Leite, M.I., Nithi, K., Vincent, A. & Ansorge, O. (2011) Progressive encephalomyelitis with rigidity and myoclonus: glycine and NMDA receptor antibodies. *Neurology*, **77**, 439–443.
- Tüzün, E., Zhou, L., Baehring, J.M., Bannykh, S., Rosenfeld, M.R. & Dalmau, J. (2009) Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. *Acta Neuropathol.*, **118**, 737–743.
- Viaccoz, A., Desestret, V., Ducray, F., Picard, G., Cavillon, G., Rogemond, V., Antoine, J.C., Delattre, J.Y. & Honnorat, J. (2014) Clinical specificities of adult male patients with NMDA receptor antibodies encephalitis. *Neurology*, **82**, 556–563.
- Wei, Y.C., Liu, C.H., Lin, J.J., Lin, K.J., Huang, K.L., Lee, T.H., Chang, Y.J., Peng, T.I., Lin, K.L., Chang, T.Y., Chang, C.H., Kuo, H.C., Chang, K.H., Cheng, M.Y. & Huang, C.C. (2013) Rapid progression and brain atrophy in anti-AMPA receptor encephalitis. *J. Neuroimmunol.*, **261**, 129–133.
- Woodhall, M., Coban, A., Waters, P., Ekizoğlu, E., Kürtüncü, M., Shugaiv, E., Türkoğlu, R., Akman-Demir, G., Eraksoy, M., Vincent, A. & Tüzün, E. (2013) Glycine receptor and myelin oligodendrocyte glycoprotein antibodies in Turkish patients with neuromyelitis optica. *J. Neurol. Sci.*, **335**, 221–223.
- Wuerfel, E., Bien, C.G., Vincent, A., Woodhall, M. & Brockmann, K. (2014) Glycine receptor antibodies in a boy with focal epilepsy and episodic behavioral disorder. *J. Neurol. Sci.*, **343**, 180–182.

- Zhang, Q., Tanaka, K., Sun, P., Nakata, M., Yamamoto, R., Sakimura, K., Matsui, M. & Kato, N. (2012) Suppression of synaptic plasticity by cerebrospinal fluid from anti-NMDA receptor encephalitis patients. *Neurobiol. Dis.*, **45**, 610–615.
- Zuliani, L., Sabater, L., Saiz, A., Baiges, J.J., Giometto, B. & Graus, F. (2007) Homer autoimmunity in subacute idiopathic cerebellar ataxia. *Neurology*, **68**, 239–240.
- Zweier, C., de Jong, E.K., Zweier, M., Orrico, A., Ousager, L.B., Collins, A.L., Bijlsma, E.K., Oortveld, M.A., Ekici, A.B., Reis, A., Schenck, A. & Rauch, A. (2009) CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in *Drosophila*. *Am. J. Hum. Genet.*, **85**, 655–666.