CHAPTER ELEVEN

Anti-NMDA Receptor Encephalitis: Clinical Features and Basic Mechanisms

David R. Lynch*,†,1, Amy Rattelle*, Yi Na Dong*, Kylie Roslin*, Amy J. Gleichman‡,2, Jessica A. Panzer*,†

*Children’s Hospital of Philadelphia, Philadelphia, PA, United States
†Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States
1Corresponding author: e-mail address: lynchd@mail.med.upenn.edu

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Abstract

In slightly more than 10 years, anti-NMDA receptor (NMDAR) encephalitis has changed from a rare paraneoplastic syndrome to the most common cause of nonviral encephalitis. It presents fulminantly with progressive psychosis, seizures, and autonomic dysfunction, leading to death if untreated. However, rapid recognition and treatment can lead to survival and a return to baseline levels of functioning in many patients. While initially associated with ovarian teratomas, it is now associated with other tumors and can reflect a postviral event. The antibodies to the NMDAR made in this syndrome are

2 Current address: Department of Neurology, David Geffen School of Medicine, University of California—Los Angeles, Los Angeles, CA, United States.
pathogenic and are directed at the extracellular domain of the GluN1 subunit. Such antibodies lead to internalization of NMDARs in model systems, leading to a physiological state characterized by NMDAR hypofunction. Analogous disorders, characterized by antibodies to other synaptic receptors, present with neurological and psychiatric dysfunction and also appear to reflect antibody-induced internalization of receptors. However, this simple pathophysiology may be too simplistic to reflect the complexity of events in anti-NMDAR encephalitis. Future scientific investigations may allow a more complete understanding of this disorder and improve treatment of anti-NMDAR encephalitis.

**ABBREVIATIONS**

AMPA  α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid  
anti-NMDARE  anti-NMDA receptor encephalitis  
NMDA  N-methyl D-aspartate  
PtAbs  anti-NMDARE patients’ antibodies

## 1. INTRODUCTION

In the context of this commemorative volume, it seems appropriate to remember what Sol Snyder, Chair of Neuroscience at Johns Hopkins for decades, taught one of us (D.R.L.) 35 years ago in graduate school. First, the core of many discoveries, particularly Sol’s, begins with a clinical observation that demands scientific explanation (e.g., the biological basis of opiate action). In addition, Sol’s work always stressed that crucial neuroscientific findings have implications outside the nervous system, and vice versa. Thus, during the time there of Dr. Lynch, work in the lab included not only collaborations with neurologists and psychiatrists but also endocrinologists, hypertension specialists, pathologists, and physiologists. Understanding all these fields prepares one for the breadth of modern investigation. Finally, through working with Sol, one comes to realize a collaborative spirit that must unite investigators and science advances. Crucial in this is that one can learn from one’s close collaborators and friends, but also from essentially all others in the field. These principles have exemplified the work of Sol over the past 50+ years.

The ongoing elucidation of the disorder now known as anti-NMDA receptor (NMDAR) encephalitis demonstrates all of these principles. First described as a clinical syndrome of acute onset psychosis followed by progressive, but treatable, encephalopathy in 2005, then linked to the NMDAR in 2007, anti-NMDAR encephalitis (anti-NMDARE) is now one of the most common nonviral encephalitides worldwide (Dalmau et al., 2007). With the
discovery of an increased incidence of anti-NMDARE also has come the realization of the seriousness of the disorder. Thus, there is an urgent need for increased clinical and scientific understanding that could lead to improved treatment. In this chapter, we will review the basic pharmacology and neurobiology of the NMDAR, the present understanding of the clinical phenomenology and pathophysiology of anti-NMDARE, and possible concepts and future directions for ameliorating the effect of this disorder.

2. NMDA RECEPTORS

Glutamate, the most important excitatory transmitter in the mammalian brain, acts synaptically at a series of receptors named for their prototypical agonist (Lynch & Guttmann, 2002). The major fast excitatory receptor in the mammalian brain is the AMPA receptor, a glutamate-gated sodium channel. In contrast, the other main postsynaptic ionotropic glutamate receptor, the NMDAR, is a ligand-gated cation channel that is highly permeable to calcium. Consequently, calcium entering through the NMDAR readily modifies the long-term biochemical proprieties of the cell and mediates the effects of intracellular calcium changes on both normal physiological events and pathophysiological processes. NMDARs are formed from four subunits: each receptor contains two GluN1 subunits in combination with two GluN2 or GluN3 subunits. GluN1, the so-called obligatory subunit, is found as eight different splice variants (GluN1–1a to GluN1–4a and GluN1–1b to GluN1–4b), while GluN2 and GluN3 are made from four (2A–2D) and two (A–B) genes, respectively (Monyer et al., 1992; Regan, Romero-Hernandez, & Furukawa, 2015; Traynelis et al., 2010; Zhu & Paoletti, 2015). GluN3 subunits are less well understood. GluN2 subunits bind glutamate or its competitive analogs (NMDA for example), while GluN1 and GluN3 bind the obligatory coagonist glycine. D-Serine substitutes for glycine in certain physiological situations or anatomic locations and may be the endogenous agonist at synaptic NMDARs (Papouin et al., 2012). Different subunit combinations create receptors with distinct physiological and pharmacological properties and are differentially expressed regionally and developmentally in vivo (Lynch & Guttmann, 2001).

Specific properties make NMDARs conceptually distinct from other ligand-gated ion channels. First, NMDARs require two agonists (glutamate and glycine) to open. NMDARs are also blocked by magnesium in a voltage-dependent manner; membrane depolarization from adjacent AMPA receptors is needed to open NMDARs (Kupper, Ascher, & Neyton, 1996;
Traynelis et al., 2010). A variety of regulated posttranslational modifications also influence receptor opening and localization. These unusual features of the NMDAR allow it to be a coincidence detector in the nervous system in that it opens only when a series of events occur; this makes it a crucial modulator of long-term change. Thus interference with NMDAR function represents a likely site for pathophysiological events to occur in human disease.

In addition, NMDARs are heterogeneous in the manner in which they signal downstream of the receptor. Calcium entering through the NMDAR can activate many pathways and be either neuroprotective or neurotoxic. At present, most paradigms suggest that synaptic NMDARs are protective against neuronal toxicity, while extrasynaptic NMDAR activation leads to cell death. This paradigm is based on results from excitotoxicity, in which NMDAR overactivation leads to neurotoxicity. Although there are combinations of different GluN receptor subunits that are more likely to be synaptic vs extrasynaptic, this varies between brain regions so that the properties of synaptic and extrasynaptic receptors do not always correlate with subunit combinations. Consequently, the differentiation between synaptic and extrasynaptic receptors is defined anatomically rather than pharmacologically.

Until the discovery of anti-NMDARE, the toxicology of NMDARs involved two types of diseases. The first are those of excitotoxicity, in which excess glutamatergic stimulation through the NMDAR leads to acute or chronic neuronal death. Excitotoxicity has been implicated in numerous disorders, including acute insults such as stroke and epilepsy and chronic diseases like Huntington’s, Parkinson’s, and Alzheimer’s diseases. In addition, although less explored, NMDAR hypofunction can lead to human diseases or clinical syndromes. Schizophrenia has features of NMDAR hypofunction, as do forms of developmental disability such as autism (Balu & Coyle, 2015; Coyle, 2012; Schade & Paulus, 2016). The well-established roles of NMDARs in synaptic plasticity and learning lend credence to the idea that NMDAR hypofunction may play a role in these developmental disorders. Given the many varied and crucial roles of every component of the receptor, it is therefore to be expected that interfering with the NMDAR via antibody binding would have dramatic neurological consequences.

3. ANTI-NMDAR ENCEPHALITIS

3.1 Essential Clinical Features

Anti-NMDARE was initially described as a paraneoplastic process, in which the immune reaction to a tumor leads to cross-reacting sequelae in the brain
(Dalmau et al., 2007). In the initial description, a cohort of young women presented with mental status changes of acute psychosis including severe short-term memory loss. This cohort overall contained 12 young women with teratomas (benign tumors containing all three germ cell layers): 11 teratomas were ovarian and 1 mediastinal. Over the course of weeks, neurological function worsened until individuals developed ongoing psychosis, dyskinesias, autonomic dysfunction, decreased consciousness, hypventilation, and death (if untreated). There was also a high frequency of response to plasmapheresis or other forms of immunotherapy, leading to patients recovering with no memory of the events or the illness. This is suggestive of antibodies to a specific protein mediating the events, as T cell-mediated immunity seen in classical paraneoplastic processes is far less responsive. In individuals with antibody-mediated paraneoplastic processes, when patient sera or cerebrospinal fluid (CSF) is applied to tissue sections, antibodies from the sera react with the particular antigen involved in the process in the brain. The immunological properties of the antibodies in patients’ serum and CSF in this new syndrome were distinct from those found in individuals with other forms of limbic encephalitis, as when sera from the individuals with teratomas were applied to brain sections, they recognized an antigen found in high levels in the cortex and particularly hippocampus, with lower levels in brainstem and cerebellum.

In a search for the novel antigen, the distribution closely resembled that of the NMDAR. The role of the NMDAR as the autoantigen was shown by the introduction of GluN1/GluN2 heteromeric receptors into heterologous cells; the NMDAR-transfected cells were recognized by patients’ serum and CSF, while untransfected cells or cells transfected with other antigens were not. As all subjects in this initial cohort contained antibodies to GluN1/GluN2 heteromers, this led to a definition of anti-NMDARE as an appropriate clinical syndrome in the context of serum or CSF antibodies staining specific items:

1. Reactivity to brain involving the hippocampus.
2. Cell-surface staining of cultured cortical or hippocampal neurons.
3. Staining of heterologous cells transfected with GluN1 and GluN2 (forming GluN1–GluN2 heteromers of the NMDAR) in a pattern matching that of other antibodies to GluN1 (Fig. 1).

These three features characterize the antibody response of anti-NMDARE.

Using the criteria defined in the initial report, the number of identified patients with anti-NMDARE has expanded rapidly and has revised much of the concept of anti-NMDARE (Dalmau et al., 2008). Although the
recognition of anti-NMDARE occurred in young adults with paraneoplastic processes, not all individuals have identified tumors and anti-NMDARE is found in all age groups (Armangue et al., 2013; Florance et al., 2009; Rainey, Gholkar, & Cheesman, 2014; Titulaer et al., 2013). Anti-NMDAR encephalitis also occurs in children and in older adults. If anti-NMDARE is viewed as a nonviral encephalitis, then its frequency surpasses that of any identified virus, and it becomes the most common single form of nonviral encephalitis identified (Gable, Sheriff, Dalmau, Tilley, & Glaser, 2012; Kamei et al., 2009; Prüss et al., 2010). Thus anti-NMDARE is not a rare disease limited to neurooncology, but instead a far more common disorder affecting thousands of people every year.

With time, the identification of large cohorts demonstrates a continuum of findings across patients with anti-NMDARE (Dalmau et al., 2008; Zekeridou et al., 2015). In general, patients with anti-NMDARE are young (mean age 23 in an early large series) and predominantly women, matching the sex difference in many immune disorders. The presentation is dominantly psychiatric (particularly in adults), in association with memory difficulties, autonomic instability, seizures, and hypoventilation in greater than half of adult patients. More than 30%–60% of patients have neoplasms, mainly ovarian teratomas. However, in contrast to the earliest series, only
75% of subjects recover to their baseline level of functioning; 25% have residual functional deficits (Thomas et al., 2013; Titulaer et al., 2013). In addition, neuropsychological testing reveals persistent cognitive dysfunction in many patients, and people still die on occasion from anti-NMDARE (Chi et al., 2017; McKeon et al., 2017). In broadening the features of the disease, a variety of new presentations have appeared. These include unilateral dyskinesia movements in children, status epilepticus, isolated epilepsy, fever of unknown origin, irritable insomnia, exercise-induced foot weakness, late-onset autism, anorexia nervosa, and, rarely, opsoclonus myoclonus (Baizabal-Carvallo, Stocco, Muscal, & Jankovic, 2013; Creten et al., 2011; Hur, 2015; Johnson, Henry, Fessler, & Dalmau, 2010; Kurian, Lalive, Dalmau, & Horvath, 2010; Labate, Quattrone, Dalmau, & Gambardella, 2013; Mechelhoff et al., 2015; Player et al., 2015). Interestingly, isolated seizures as a presentation are more frequent in men than women for reasons that are unclear. Similarly, a large number of novel tumors are associated with anti-NMDAR, including hepatic neuroendocrine tumors, uterine neuroendocrine tumors, small cell lung cancer, and carcinoma of unknown origin (Afanasiev et al., 2016; Hara et al., 2011; Jeraiby, Depince-Berger, Bossy, Antoine, & Paul, 2016; Wu, He, Zhang, Shi, & Zhang, 2016).

The pathology of anti-NMDARE is consistent with a paraneoplastic limbic encephalitis. Inflammatory infiltrates are found in the hippocampus, and there is a specific decrease in the number of NMDARs in the hippocampus. Complement-mediated mechanisms are not prominent in anti-NMDARE, showing that it is likely a direct effect of antibodies on NMDARs that mediate the disease process (Martinez-Hernandez et al., 2011). Plasma cell infiltration of the CNS though does occur and may be crucial to the disease process (Dale, Pillai, & Brilot, 2013).

Relapses can occur in anti-NMDARE (Gabilondo et al., 2011; Kayser, Titulaer, Gresa-Arribas, & Dalmau, 2013; Ramanathan, Wong, & Fung, 2013). However, while antibody titer correlates with severity, improvement may not be fully correlated with falling titer as intrathecal synthesis can remain for years after functional recovery (Alexopoulos, Kosmidis, Dalmau, & Dalakas, 2011; Dalmau, Lancaster, Martinez-Hernandez, Rosenfeld, & Balice-Gordon, 2011; Gresa-Arribas et al., 2014; Hansen et al., 2013; Wali, Cai, Rossor, & Clough, 2011). This suggests that mechanisms unrelated to NMDAR biology may play some role in recovery, particularly in individuals with spontaneous recovery and those with persistent intrathecal synthesis.
3.2 Relationship With Viral Infection

While the trigger for anti-NMDARE is neoplastic in greater than half of individuals, viral illness appears to precede this disorder in another large set of individuals. Paraneoplastic anti-NMDARE is less common in children, a group in which viral exposure is more common. Although direct links to viral illness are most commonly found with herpes virus infection, it has also been noted with varicella zoster, measles virus, and other viruses (Grillo & da Silva, 2013; Ioannidis, Papadopoulos, Koufou, Parissis, & Karacostas, 2015, Phan et al., 2016; Solis, Salazar, & Hasbun, 2016). In many cases of anti-NMDARE, a link to viral illness is missed, but might account for the seasonal variability in anti-NMDARE in children (Adang, Lynch, & Panzer, 2014). In addition, in some situations viral infection is remote, and anti-NMDARE represents a late sequelae. However, the most well-defined postviral association of anti-NMDARE occurs in herpes encephalitis, in which an initial viral encephalitis leads to a secondary anti-NMDARE (Nosadini et al., 2017). Such individuals are recognized as persons in which the initial presentation is consistent with herpes encephalitis (with PCR confirmation), but is followed by persistent and advancing neurological sequelae (Geoghegan et al., 2016; Hacohen et al., 2014; Höftberger, Armande, Leyboldt, Graus, & Dalmau, 2013; Leypoldt et al., 2013). These events include both cognitive events and movement disorder (chorea). Treatment thus must be aimed not only at the original viral encephalitis but also at the secondary immune phenomenon of anti-NMDARE.

With the growing frequency of anti-NMDARE, it is possible that other disorders could be linked to anti-NMDARE. The first is schizophrenia, based on the hypothesis that a subset of this disorder could represent chronic anti-NMDARE. While some studies have detected anti-NMDAR antibodies in schizophrenic patients, the type of antibodies seems to differ and the number of patients is low (Pollak, McCormack, Peakman, Nicholson, & David, 2014; Tsutsui et al., 2012). Thus, it seems unlikely that chronic anti-NMDARE represents a major subgroup of schizophrenic patients (de Witte et al., 2015). Similarly, studies show no large association between anti-NMDAR antibodies and epilepsy of unknown origin. In addition, there have been some links between anti-NMDARE and other immune disorders such as progressive systemic sclerosis (Suzuki et al., 2011). Isolated patients have been identified with multiple sclerosis and neuromyelitis optica following anti-NMDARE, and a true association appears to be present based on some series (Baheerathan et al., 2017; Ran
et al., 2017; Titulaer et al., 2014; Uzawa et al., 2012; Zoccarato et al., 2013). As immune disorders frequently coassociate, an association of anti-NMDARE with other immune-mediated disorders would not be surprising.

### 3.3 Treatment

One of the most exciting aspects of anti-NMDARE is its response to therapy. Even in the early studies the majority of patients return to relatively normal function with basic immunotherapies such as plasmapheresis or IV IgG (DeSena et al., 2015). In addition, a number of reports also demonstrate spontaneous recovery, usually after extended periods of time (Evoli et al., 2012; Iizuka et al., 2008). Still the quality of recovery on average reflects the speed with which definitive treatment occurs (Breese, Dalmau, Lennon, Apiwattanakul, & Sokol, 2010; Byrne, McCoy, Lynch, Webb, & King, 2014; Kurian, Fluss, & Korff, 2012).

In those individuals with anti-NMDARE as a paraneoplastic process, the first consideration in treatment is tumor removal (Iizuka et al., 2008). Ovarian teratomas do not typically metastasize and are usually benign, so tumor removal can be accomplished expeditiously in most situations. As these tumors contain neural tissue leading to production of NMDAR antibodies, then this step should lead to falling antibody levels and ideal clinical response.

However, anti-NMDARE is an immune disease at the time it presents in all patients. Thus, immunomodulation must begin in parallel with tumor removal, including plasmapheresis, IV IgG, and potentially corticosteroids as first-line therapies. In some individuals, improvement does not occur and bolder therapies including rituximab and cyclophosphamide may be used; these of course carry greater risk (Hachiya et al., 2013; Ikeguchi et al., 2012; Ishiura et al., 2008; Kashyape et al., 2012). A novel approach is the use of a proteosomal inhibitor for therapy, though the mechanisms for such protection is not fully known (Scheibe et al., 2017). Perhaps the most difficult aspect of anti-NMDAR treatment is ascertaining who requires more aggressive therapy. Early series emphasize the surprisingly large number of individuals who return to baseline in anti-NMDAR, while more recent perspectives have addressed the substantial portion who remain disabled. Treating such refractory individuals earlier might improve outcome. However, identifying such individuals early in their course (when they might be most responsive) is challenging and to this point no specific identifiers of whom are most likely to be refractory to therapy have been defined.
4. BASIC MECHANISMS OF ANTI-NMDARE

In the initial characterizations of anti-NMDAR encephalitis, the crucial step was identification of the antigen. While this step happened mainly by chance, the physiological properties of the NMDAR suggest it as a potential antibody target based on the clinical phenomena. NMDAR blockade using agents like phencyclidine leads to acute psychosis, a phenotype matching that of typical anti-NMDARE (Javitt & Zukin, 1991). In addition, NMDA knockdown in mice leads to hypoventilation, the cause of death in many patients with anti-NMDARE (Forrest et al., 1994). Finally, NMDAR modulation is linked to control of dyskinesias, an aspect that could be associated with the prominent dyskinesia in anti-NMDARE (Blanchet, Papa, Metman, Mouradian, & Chase, 1997). Thus, studies identifying NMDARs as the autoantigen in this disorder are consistent with the expected features of dysfunctional NMDARs.

Biochemically, the site of epitope recognition for the antibodies of anti-NMDARE is on GluN1, the glycine-binding subunit. Expression of GluN1 in heterologous systems, such as HEK cells, is necessary and sufficient for binding of patients’ antibodies (PtAbs). There is no difference among any naturally occurring GluN1 splice variants in their ability to be recognized by PtAbs. Expression of GluN2 subunits (as performed in the initial studies) may alter the recognition of the epitope on GluN1, perhaps by altering the conformation of the GluN1 subunit, but is not required for epitope formation. A variety of manipulations to the GluN1 subunit alter epitope recognition (Gleichman, Spruce, Dalmau, Seeholzer, & Lynch, 2012). Development of immunoreactivity to PtAbs is blocked by tunicamycin, an agent altering glycosylation of proteins, and removal of a single glycosylation site (N368) removes the epitope of GluN1. However, alternative mutations that remove glycosylation but not N368 still retain the ability to be recognized by PtAbs. Thus, multiple mutations in the region around N368 remove epitope recognition, but they do not correlate perfectly with glycosylation, suggesting that this region creates a conformation of GluN1 that is necessary for generating epitope recognition. It also does not prove that this region is sufficient for immunoreactivity; the smallest portion of GluN1 that still generates immunoreactivity includes the full N-terminal domain linked to a transmembrane region, a much larger region that that which is necessary. These data are most easily interpreted as showing that GluN1 contains a conformational epitope in the first 381 amino acids of
GluN1 that requires the region near amino acid 368 to develop proper conformation.

Anti-NMDARE antibodies also alter the physiology of the receptor and of glutamatergic transmission. In patch-clamp experiments, receptors with mutant GluN1s having shorter open times bind lower levels of PtAbs, and PtAbs appear to bind to an open form of the receptor. This could lead receptors to remain in an open conformation when the receptor is bound. This would explain the observation that intracellular calcium levels in neurons increase with low-level exposure to PtAbs, suggesting an initial effect of PtAbs in activating NMDARs before decreasing by 24 h in culture (Fig. 2). Such an observation also might explain some of the discrepancies in the distribution of immunoreactivity to PtAbs in the brain. While the initial studies suggested lower levels of binding to cerebellar NMDARs, this result has not always been replicated. However, cerebellar receptors open less frequently than forebrain NMDAR under similar activation paradigms. As different studies have not closely matched protocols for incubation with antibody, the level of activation across different studies might explain this variability. The effects of PtAbs are also apparent in larger physiological contexts, as they

![Fig. 2](image_url)

**Fig. 2** Effect of PtAbs on neuronal calcium levels. Cultured cortical neurons were exposed to PtAbs (IgG) for 24 h. Levels of intracellular calcium were lower in PtAbs treated cells, consistent with NMDAR hypofunction. ***The basal Ca\(^{2+}\) levels are \(P < 0.0001\) (T-test).
facilitate afferent cortical motor response when injected into rodent brain, consistent with glutamatergic dysfunction in cortical neurons (Manto, Dalmau, Didelot, Rogemond, & Honnorat, 2010). They also produce cognitive loss in rodents, similar to the features of anti-NMDARE (Planagumà et al., 2016). Finally, such antibodies suppress synaptic plasticity in vitro, consistent with blocking the role of the NMDAR in many paradigms of synaptic modification.

While PtAbs alter the physiology of the receptor, their direct pathogenicity more likely reflects antibody-induced changes in synaptic NMDAR levels. PtAbs induce internalization of NMDARs from synapses, without loss of associated synaptic proteins. This internalization correlates with antibody titer and requires receptor cross-linking, as Fab fragments of antibody do not lead to receptor internalization, while exogenously cross-linked Fab’ fragments do (Hughes et al., 2010). After internalization, receptors are destroyed in a proteasome–dependent manner, suggesting that proteosomal inhibition may be one avenue for treatment (Fig. 3). Synaptic NMDAR currents decrease, but other synaptic receptors are spared. In addition, a key aspect of the pathophysiology is the movement of NMDAR to extrasynaptic sites by PtAbs; this along with the internalization is blocked by the activation/presence of EphB receptors (Mikasova et al., 2012). Thus, the selective internalization of NMDAR receptors can be controlled by synaptic messenger systems in addition to PtAbs. Cellular mechanisms also block the full effect of internalization by mobilizing a compensatory increase in inhibitory mechanisms (Moscato et al., 2014). Thus, the events involved in creation of the NMDAR hypofunction state of anti-NMDARE require both PtAbs-based events and neuronal reactions.

Are these PtAbs truly the cause of the disease or merely an epiphenomenon? Ongoing experiments demonstrate the pathogenicity of the antibodies, as they meet the basic principles of Koch’s postulates for demonstrating causal relationships as applied to this form of limbic encephalitis. First, antibodies with specific anti-NMDAR reactivity have been found in all individuals with anti-NMDARE, though this is somewhat circular as such antibodies define the specific type of limbic encephalitis. The antibodies have been partially purified from patients to antibody types (IgG vs IgA) and retain their basic biochemical properties; they continue to react with NMDAR in transfected cells and on the surface of cultured neurons (Desestret et al., 2015). In addition, such antibodies lead to internalization of receptors and to behavioral changes in mice, consistent with PtAbs reproducing the phenotype of the disease. Finally, cloning of B cells from patients’ CSF produces antibodies that recapitulate the features of
disease when injected into animals (Kreye et al., 2016). Further preliminary reports suggest that antibodies derived from peripheral B cells from patients also recognize NMDAR and have some of the features of pathogenic antibodies. Consequently, substantial data show that the PtAbs are pathogenic as they essentially satisfy all of Koch’s postulates for disease reproduction.

4.1 NMDAR Antibodies From Other Human Disorders

While the PtAbs associated with anti-NMDAR E have a defined target specificity, other antibodies to NMDAR have been characterized from other
disorders and occasionally from normal people. Recent reports suggest that some individuals produce IgA antibodies to the NMDAR in association with a clinical syndrome of dementia (Doss et al., 2014). Though their exact epitope specificity is unknown, they lead to cell death in culture and do not have the selectivity of anti-NMDARE antibodies for synaptic structures. They are also found in individuals with a broad variety of dementias, thus lacking the clinical specificity associated with typical anti-NMDARE. Thus, in contrast to PtAbs of NMDARE that are selective for a specific clinical phenotype, synaptic pattern, and epitope specificity, the meaning of the IgA antibodies in these dementias is unclear.

A second type of anti-NMDAR antibody is associated with systemic lupus erythematosus (SLE). Such antibodies recognize GluN2A/2B subunits and parallel the presence of clinical neuropsychiatric syndromes in SLE (Fragoso-Loyo et al., 2008; Harrison, Ravdin, & Lockshin, 2006; Lapteva et al., 2006). However, there is little evidence of a causal nature of the antibodies, and the basic science data on their epitopes, and how they alter synaptic function and animal models is limited. Thus these most likely represent markers of CNS SLE rather than direct causes of CNS dysfunction.

Still, while clinical phenotyping and substantial data dissociate the effects of PtAbs from anti-NMDARE from other forms of NMDAR antibodies found in humans, it remains possible that other NMDAR antibodies could be pathogenic in specific situations. In model systems such as heterologous systems, all human NMDAR antibodies to extracellular epitopes appear to be capable of internalizing NMDAR (Castillo-Gómez et al., 2016). While this seems to conflict with the clinical data and the remainder of the basic science data on PtAbs in anti-NMDARE, there are several possible ways to reconcile these results. First, in anti-NMDAR and other immune disorders, the blood–brain barrier allows increased penetration of antibodies into the CNS allowing plasma cells to reside there. While blood–brain barrier opening is clearly a feature of anti-NMDARE, what leads to this is unclear. Creation of anti-NMDAR antibodies outside of anti-NMDARE may not be associated with blood–brain barrier dysfunction. In addition, there may be concentration differences in antibody accumulation in vivo between PtAbs of anti-NMDARE and other antibodies. Finally, while internalization is part of the pathogenesis of anti-NMDARE, it is possible that other events are also required. The immune response of anti-NMDARE is not monoclonal. Other anti-NMDARE antibodies besides those that lead to internalization may facilitate or even be necessary for complete disease
pathogenesis. The diversity of the immune response in anti-NMDARE remains incompletely explored, but might provide a perspective on this concept.

4.2 Other Synaptic Encephalitides

Anti-NMDARE serves as a model for other antibody-generated disorders of the synapse, collectively called “synaptic encephalitides,” in which the pathophysiology reflects antibody binding to synaptic neurotransmitter receptors rather than more destructive traditional immune responses (Panzer, Gleichman, & Lynch, 2014; Rosenfeld & Dalmau, 2011). The most well-known of these (besides anti-NMDARE) is anti-AMPA receptor encephalitis. Like NMDAR, AMPA receptors are tetrameric complexes made from four receptor subunits (GluA1–4) that are assembled into homomeric (identical subunits) or heteromeric (differing subunits) receptors. Most AMPA receptors in vivo are GluA1/GluA2 or GluA2/GluA3 heterotetramers (Geiger et al., 1995; Sans et al., 2003; Wenthold, Petralia, Blahos, & Niedzielski, 1996). Distinct genes encode GluA1–4, all of which have multiple splice variants (Traynelis et al., 2010). AMPA receptors activate and deactivate faster than NMDAR (ms timescale) and thus control fast glutamatergic neurotransmission in most synapses. AMPA receptor activation removes the magnesium block on NMDARs, thus allowing AMPA receptors to modulate NMDAR-mediated events.

Anti-AMPA receptor encephalitis presents distinctly from anti-NMDARE, being primarily a disorder of memory (De Bruijn & Titulaer, 2016). Subjects present with memory issues and seizures, but lack much of the psychotic behavior (Höftberger et al., 2015). As in anti-NMDARE, about 50% of AMPAR encephalitis is paraneoplastic, and the antibodies give rise to internalization of AMPA receptors with sparing of other glutamatergic receptors, analogous to the events of anti-NMDARE (Lai et al., 2009; Peng et al., 2015). However, anti-AMPAR encephalitis is much less common, with less than 30 identified patients. In addition, the receptor regions controlling epitope recognition are less specific as antibodies can be to GluA2 subunits, GluA1 subunits, or sometimes both. This contrasts with anti-NMDARE, in which receptor recognition of PtAbs can be modulated selectively by the small region around N368. In antibodies from a large group of AMPAR encephalitis patients, the epitope resides in the N-terminus of the subunit based on epitope mapping using both deletion mutants and fusion proteins, but cannot be further localized (Gleichman, Panzer, Baumann, Dalmau, &
Lynch, 2014). In addition, the exact receptor conformation appears less critical for epitope recognition in AMPA receptor encephalitis, as PtAbs recognize not only heterologous receptors but also denatured portions of receptor found in fusion proteins. Unfortunately, while AMPA receptor encephalitis contributes to the concept of the importance of synaptic encephalitides, the small number of patients prohibits further mechanistic characterization at this time.

Other synaptic encephalitides have been identified to nonglutamatergic receptors. These include anti-GABAB receptor encephalitis, anti-mGluR1 receptor encephalitis, and anti-mGluR5 encephalitis (Boronat, Sabater, Saiz, Dalmau, & Graus, 2011; Höftberger, Titulaer, et al., 2013; Jarius & Wildemann, 2015; Lancaster et al., 2011; Petit-Pedrol et al., 2014; Spatola et al., 2017). In general, these are less well characterized than anti-NMDAR encephalitis but appear to have similar mechanisms, and internalization of receptors is a crucial component of the pathophysiology. They can have very specific clinical syndromes attached to them such as the “Ophelia syndrome” associated with mGluR5 antibodies. In addition, a variety of clinical syndromes such as Rasmussen’s encephalitis (GluA3) and opsoclonus myoclonus (unknown antigens) exist in which subgroups of patients exist with antibodies to synaptic components (Gurcharran & Karkare, 2017). Such disorders likely represent overlapping syndromes rather than a specific disease, and the mechanisms of these disorders may or may not match the antibody-dependent internalization of receptors found in anti-NMDARE.

5. NMDARE: A NEW CONCEPTUALIZATION

Given that many patients with anti-NMDARE have poor recovery despite aggressive treatment with potentially risky immunotherapies, innovative treatments are needed. Such treatments must be based on a better understanding of the neurobiology of the disorder. The published dogma is that anti-NMDAR encephalitis is a disease of global NMDAR hypofunction, in many ways analogous to schizophrenia. However, published data conflict with this hypothesis including the findings that PtAbs can prolong NMDAR channel open time and appear to activate the receptor initially before leading to a later NMDAR hypofunction state. In addition, antibodies have decreased affinity for GluN1 mutants with shorter channel open time. These data support the possibility that PtAbs act as NMDAR
agonists in some situations and that antibody impact may vary depending on NMDAR open conformation and subtype. At active excitatory synapses, PtAbs may preferentially bind to open synaptic (often GluN2A-containing) NMDARs. In contrast, on tonically active inhibitory neurons, extrasynaptic NMDARs are more likely to be open from glutamate spillover and may be preferentially bound by PtAbs. This concept suggests that PtAbs cause a shift from synaptic to extrasynaptic NMDAR signaling specifically at the sites of greatest NMDAR activity (Fig. 4). This hypothesis can account for unexplained clinical features of anti-NMDAR encephalitis: (1) it is known that treatment with NMDAR agonists, excess extrasynaptic NMDAR signaling, and inhibitory neuron dysfunction all can trigger seizures, a surprising clinical feature of a purely NMDAR hypofunction state; and (2) both dyskinesias and catatonia are associated with excessive extrasynaptic GluN2B signaling. Thus, this hypothesis has the potential to explain the core symptoms of anti-NMDAR encephalitis in a way that simple NMDAR hypofunction does not (Table 1). Thus as Dr. Snyder has demonstrated throughout his career, scientific and clinical hypotheses are intimately inter-related in a way that drives both forward.

**Fig. 4** Proposed pathophysiology of anti-NMDARE. Application of PtAbs leads to increased opening of synaptic receptors (lightning bolt) (GluN1/2A receptors more commonly) followed by their movement to extrasynaptic sites and/or internalization. Extrasynaptic (more commonly GluN1/2B) receptors open less frequently and are unaffected by PtAbs. This leads to an imbalance between extrasynaptic and synaptic receptors that could account for the complex neurological features of anti-NMDARE.
6. CONCLUSION

For a disorder as common as anti-NMDARE, there is surprisingly little basic science information available. As the outcome is not always benign, such information is crucial to advancing therapy and preventing adverse outcomes. Early intervention based on the properties of antibody–NMDAR interactions could provide a logical methodology for improving long-term outcome.

There are many questions to be addressed including the following:

1. Is there any selectivity to the in vivo recognition process of NMDAR by PtAbs? In vitro systems suggest that all receptors containing a GluN1 subunit should be recognized by PtAbs, but the stereotypic presentation of the disease suggests that it is not that simple. If subtypes of NMDAR are the key targets, does it reflect differing subunits, differing synaptic locations, differing activation states, or other properties?

2. How diverse is the immune response in anti-NMDARE and which antibodies mediate disease? While single monoclonal antibodies appear capable of generating the crucial phenomena of anti-NMDARE, antibody production is almost certainly polyclonal. There could be multiple types of antibody involved in disease pathogenesis, or perhaps the diagnostic antibodies do not necessarily match the true disease generating antibodies. This might explain instances of recovery without disappearance of immunoreactivity to NMDAR.

3. Is internalization the only event mediating pathogenesis? While the role of internalization in vitro is well defined, the observation that NMDAR antibodies other than those found in anti-NMDARE can also cause internalization suggests that other factors may be involved. Such factors could include opening the blood–brain barrier, or other actions at the

<table>
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<th>Table 1</th>
<th>Proposed relationship between synaptic effects and symptoms in anti-NMDARE</th>
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<td>NMDAR Dysregulation</td>
<td>Proposed Symptoms</td>
</tr>
<tr>
<td>Agonist effect on NMDARs</td>
<td>Seizures</td>
</tr>
<tr>
<td>Synaptic NMDAR hypofunction</td>
<td>Amnesia, psychosis, hypoventilation</td>
</tr>
<tr>
<td>Extrasynaptic NMDAR hyperfunction</td>
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<tr>
<td>Neuronal network imbalance with impaired intraneuronal activity</td>
<td>Seizures</td>
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NMDAR such as direct receptor antagonism. Answering this question almost certainly requires better knowledge of the diversity of the immune response in anti-NMDARE.

4. Are there downstream mechanisms for interaction? One of the crucial events of excitotoxicity is a differential effect on downstream pathways, which provides a novel methodology for facilitating neuronal repair in excitotoxic disorders. Does a similar diversity in messenger systems exist after internalization that might be modulated? The modest success of proteosomal inhibition could represent a way to modulate such pathways.

Through further scientific investigations, many of these questions may be answered. This should lead to new understanding of anti-NMDARE and related disorders and facilitate development of definitive therapies.

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CONFLICT OF INTEREST

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REFERENCES


FURTHER READING
