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Mechanisms underlying autoimmune synaptic encephalitis leading to disorders of memory, behavior and cognition: insights from molecular, cellular and synaptic studies

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Abstract

Recently, several novel, potentially lethal, and treatment-responsive syndromes that affect hippocampal and cortical function have been shown to be associated with auto-antibodies against synaptic antigens, notably glutamate or GABA-B receptors. Patients with these auto-antibodies, sometimes associated with teratomas and other neoplasms, present with psychiatric symptoms, seizures, memory deficits, and decreased level of consciousness. These symptoms often improve dramatically after immunotherapy or tumor resection. Here we discuss studies of the cellular and synaptic effects of these antibodies in hippocampal neurons *in vitro* and preliminary work in rodent models. Our work suggests that patient antibodies lead to rapid and reversible removal of neurotransmitter receptors from synaptic sites, leading to changes in synaptic and circuit function that in turn are likely to lead to behavioral deficits. We also discuss several of the many questions raised by these and related disorders. Determining the mechanisms underlying these novel anti-neurotransmitter receptor encephalopathies will provide insights into the cellular and synaptic bases of the memory and cognitive deficits that are hallmarks of these disorders, and potentially suggest avenues for therapeutic intervention.

Keywords

synaptic plasticity; glutamate receptors; autoimmune; encephalitis; hippocampus

Many encephalitides once considered idiopathic are now thought to be immune mediated. One of these disorders predominantly affects structures of the limbic system, including medial temporal lobes, amygdala, hippocampus, and orbitofrontal cortex (Gultekin *et al.*, 2000; Posner & Dalmau, 2000; Graus & Dalmau, 2007). As a result, patients develop short-term memory deficits, emotional and behavioral disturbances such as confusion, irritability, depression, and sleep disturbances, as well as seizures and sometimes dementia (Gultekin *et al.*, 2000; Tüzün & Dalmau, 2007). For many years limbic encephalitis was invariably attributed to the paraneoplastic manifestation of cancers that express the target neuronal antigen, and the neurological deficits were considered refractory to treatments. These views have changed with the discovery of an expanding group of encephalitides that occur with or without cancer association, respond to immunotherapy, and range from focal limbic

dysfunction to a multifocal or diffuse encephalopathy. In contrast to classical paraneoplastic encephalitides in which the target antigens are intracellular and appear to be mediated by cytotoxic T-cell mechanisms, the novel group of disorders associates with autoantigens that are on the cell or synaptic surface and appear to be directly mediated by antibodies.

The pathogenic role of antibodies can be established using several criteria in vitro and in vivo. First, if antigens are membrane proteins, antibodies should have access to and bind extracellular antigenic epitopes in living cells and/or tissues. Second, that antibodies recognize a particular antigen should be assessed by expressing the antigen in heterologous cells, assayed by immunostaining or immunoprecipitation followed by Western blot. Third, antibodies cause structural and/or functional alterations of the target antigen that can be established in vitro in dissociated neuron cultures as well as in vivo after antibody infusion. Adjuncts to these approaches would include using affinity purified antibody to recapitulate the effects of human CSF or serum, as well as demonstrating that CSF or serum depleted of the particular antibody has no structural or functional effects on cells. In the particular case of antibodies against neurotransmitter receptors or ion channels, receptor/channel function may well be acutely or chronically altered by antibody treatment, leading secondarily to changes in cellular and/or synaptic function. Fourth, the clinical syndrome should mirror some or all of the phenotypes of pharmacological or genetic manipulation of the antigen. Fifth, passive transfer of disease-specific antibodies to animals should recapitulate the effects of the antibodies on the antigen as well as the clinical features of the disorder. Sixth, cellular and synaptic alterations, and clinical symptoms, should improve as antibody titer is reduced. For some autoimmune diseases such as myasthenia gravis or Lambert-Eaton syndrome, all of these criteria have been met; however, for many, only a subset has been confirmed (Table 1).

Anti-neurotransmitter receptor limbic encephalitides resemble the autoimmune syndromes of the neuromuscular synapse (e.g., myasthenia gravis) in that they can also occur with or without tumor association and are likely antibody-mediated (Table 1; Rudnicki & Dalmau, 2000; Phillips, 2003; Tormoehlen & Pascuzzi, 2008; Meriggioli & Sanders, 2009b). However, in autoimmune synaptic encephalitis the autoantigen is located behind the blood-brain-barrier (BBB), requiring that the antibodies or cells producing antibodies cross this barrier in order to cause neurological dysfunction (Figure 1). In some disorders the patients' cerebrospinal fluid (CSF) show lymphocytic pleocytosis and intrathecal synthesis of antibodies suggesting that after initial systemic immune activation by a tumor or unknown causes, there is an expansion of the immune response within the nervous system (Dalmau & Rosenfeld, 2008). The role of the immune response in the neurological symptoms is further supported by the correlation between antibody titers and symptoms, and the frequent response of the disorders to immunotherapies, including plasmapheresis, IVIg, corticosteroids, cyclophosphamide, or rituximab, a monoclonal antibody that depletes B cells.

The importance of these disorders is that they offer human models of brain-immune interactions in which the target antigens have critical roles in neuronal synaptic transmission and plasticity. Therefore, their study will improve our understanding of the effects of the antibodies at the cellular, synaptic and circuit levels, eventually impacting the clinical management of the patients. Here we describe the three most recently identified autoimmune synaptic encephalitides and the state of our understanding of the cellular and synaptic mechanisms, and discuss some of the many questions raised by these diseases.

Anti-NMDA receptor encephalitis

A new, severe, potentially lethal, and treatment-responsive disorder, anti-NMDA receptor encephalitis was reported within the last several years by Dalmau and colleagues (Dalmau *et al.*, 2007; Sansing *et al.*, 2007). Patients are usually young women, but also include men, without a past medical history of interest, who, often after prodromic symptoms of mild hyperthermia, headache, or a viral-like process, develop sudden behavioral and personality changes for which they are often seen by psychiatrists (Dalmau *et al.*, 2007; Sansing *et al.*, 2007). This clinical presentation is usually followed by seizures, decreased level of consciousness, abnormal movements (orofacial and limb dyskinesias, dystonia, choreoathetosis), autonomic instability (fluctuating blood pressure, cardiac rhythms, and temperature), and sometimes hypoventilation. MRI is frequently normal, but in about 40% of patients inflammation is transiently identified in hippocampus, cerebral or cerebellar cortex, and subcortical regions (Dalmau *et al.*, 2008; Davies *et al.*, 2010; Kataoka *et al.*, 2008; Gable *et al.*, 2009b; Kataoka *et al.*, 2009; Niehusmann *et al.*, 2009). Patients have serum and CSF antibodies that react with brain antigens predominantly expressed in the hippocampus (Dalmau *et al.*, 2008). In two large cohorts comprising 181 patients, including young adults and children, neurologic improvement was correlated with a decrease in antibody titer (Dalmau *et al.*, 2008; Florance *et al.*, 2009). Overall, about 75% of the patients had dramatic or substantial recoveries despite the severity or long duration of symptoms; 19% had partial or limited improvement, and 6% died. Analyses of brain biopsies in 14 cases and autopsy of three patients showed microgliosis, occasional inflammatory B-cell and plasma cell infiltrates, and very rare T-cell infiltrates, in contrast to other paraneoplastic syndromes in which cytotoxic T-cell infiltrates are prominent (Stein-Wexler *et al.*, 2005). In the autopsy studies the most prominent microglial activation localized in the hippocampus. Most patients with this disorder were previously categorized as “encephalitis of unknown etiology” (Iizuka *et al.*, 2008; Gable *et al.*, 2009a). It is likely that many patients died without a diagnosis or recovered with empiric treatment with immunotherapy. In fact, the little autopsy material that is available for analysis comes from cases diagnosed retrospectively using archived tissue, serum or CSF. The current mortality rate of anti-NMDA receptor encephalitis is ~3%, usually as a result of complications during the stage that patients require intensive care and ventilatory support (Dalmau *et al.*, 2008; Florance *et al.*, 2009; Gable *et al.*, 2009).

When patient antibodies are used to stain rodent brain sections, immunoreactivity is observed in the neuropil of the hippocampus, with less staining in cortex, striatum and cerebellum (Dalmau *et al.*, 2008). When used to stain live cultured hippocampal neurons, patient antibodies react with surface antigens localized to synapses. Additional studies, including immunoprecipitation followed by mass spectroscopy, led to the identification of the NR1 subunit of NMDA receptor as the target autoantigen. NMDA receptors are usually formed from heteromers of two NR1 and two NR2 subunits (Kendrick *et al.*, 1996; Laube & Kiderlen, 1997). There are four NR2 subunits (NR2A-D), which have 50-70% sequence identity in the extracellular domain; NR1 is ubiquitously distributed in the brain (Monyer *et al.*, 1994; Standaert *et al.*, 1994; Waxman & Lynch, 2005). Domain swapping and other experiments showed that the epitope was located at the N-terminal extracellular domain of NR1 (Dalmau *et al.*, 2008; Gleichman *et al.*, 2009). Since NR1 is ubiquitously expressed in brain as an obligate subunit of functional NMDA receptors (Monyer *et al.*, 1994; Standaert *et al.*, 1994; Waxman & Lynch, 2005), it remains unclear why patient NR1 antibodies preferentially label hippocampus rather than all brain regions. This binding pattern may reflect the relative high density of NMDA receptors in the hippocampus or a differential posttranslational modification of NR1 in different brain regions (Gleichman *et al.*, 2009).

We have recently shown that patients' antibodies cause a selective, titer-dependent decrease of NMDA receptor surface density, synaptic localization, and currents *in vitro*, via antibody mediated capping and internalization (Figure 2; Hughes *et al.*, 2010), while overall cellular structure and synaptic density were largely unchanged. Moreover, these effects were reversible: after 3 days of treatment with patients' CSF, followed by 4 days of treatment with control CSF, NMDA receptor cluster density and synaptic localization recovered to levels seen in control cultures. In addition, NMDA receptor density was dramatically reduced in the hippocampus of rats infused with patients' antibodies and in the hippocampus of autopsied patients (Hughes *et al.*, 2010). These studies demonstrated that patients' NR1 antibodies reversibly alter the number and distribution of glutamate receptors in neurons, resulting in a decrease in glutamatergic synaptic function. The lack of neuronal death and the reversibility of the effects of antibodies on cultured neurons may explain, in part, the frequent recovery of patients with this disorder. It is unclear, however, whether the prolonged time of recovery (usually several months) represents persistence of the immune response in the brain, slow recovery of circuit dysfunction caused by the decrease of synaptic NMDA receptors, or both.

Other autoimmune synaptic encephalitides

A goal of our ongoing work is to determine whether other syndromes may involve antibodies to other surface or synaptic antigens. Currently, ~90% of patients with limbic encephalitis of non-viral etiology that we have studied have well-defined immune responses against neuronal antigens (Bataller & Dalmau, 2004; Bataller *et al.*, 2007; Graus *et al.*, 2008). The importance of antibodies to cell surface or synaptic proteins was shown in a recent study in which these antibodies were found to be more prevalent than antibodies to intracellular antigens described in paraneoplastic disorders (54% versus 24%; Graus *et al.*, 2008)). A study of 1570 patients with diffuse encephalitis by the California Encephalitis Project showed that in only 30% could a final diagnosis be established (viral, bacterial, prion, parasitic, fungal; Glaser *et al.*, 2006). A pilot study examining a group of cases selected by subphenotype ("encephalitis, psychosis, and dyskinesias") showed that 50% had NMDA receptor antibodies (Gable *et al.*, 2009a). This suggests that other antibodies to currently unknown antigens may occur in the remaining cases.

In the last 2 years, a second form of immune mediated encephalitis in which patients' serum and CSF antibodies are directed against AMPA receptors was identified (Lai *et al.*, 2009). Most patients develop a clinical picture of limbic encephalitis including confusion, agitation, seizures, and severe short-term memory deficits. Sometimes patients present with a rapidly progressive abnormal behavior that resembles acute psychosis. Patients are usually women older than 50 years, and 70% had an underlying tumor, usually lung or breast cancer or tumors of the thymus that express AMPA receptors. Immunotherapy and treatment of the tumor, if detected, usually results in neurological recovery. The neurological disorder has a tendency to relapse, and for these patients the outcome depends of how well each relapse is controlled.

Staining of live cultured hippocampal neurons with patients' serum or CSF showed that patient antibodies recognized cell surface antigens that were localized to synapses. Immunoprecipitation followed by mass spectrometry demonstrated that the target antigens were the GluR1 and/or GluR2 subunits of the AMPA receptors. AMPA receptors mediate most of the fast excitatory synaptic transmission in the brain (Shepherd & Huganir, 2007) and the majority are heterotetramers composed of GluR1, 2, 3 or 4 subunits that are expressed in a region-specific manner (Palmer *et al.*, 2005). GluR1/2 and GluR2/3 levels are high in hippocampus and other limbic regions (Sprengel, 2006), similar to the distribution of immunostaining with patients' antibodies. Preliminary analyses suggest that the location of

the epitope is the N-terminal extracellular domain of these AMPA receptor subunits (Gleichman *et al.*, 2009). None of these patients' antibodies reacted with GluR3, a subunit identified as an autoantigen in some patients with Rasmussen's encephalitis (Rogers *et al.*, 1994).

Treatment of rat hippocampal neurons with patients' antibodies resulted in a significant decrease in the synaptic localization of AMPA receptor clusters, without a decrease in overall synaptic density or NMDA receptor clusters (Lai *et al.*, 2009). Moreover, these effects were reversible: after 3 days of treatment with patients' CSF containing GluR1/GluR2 antibodies, followed by 4 days of treatment with control CSF, AMPA receptor cluster density and synaptic localization recovered to levels seen in control cultures (Lai *et al.*, 2009).

A third subtype of autoimmune encephalitis associated with antibodies against the γ -aminobutyric acid-B (GABA_B) receptor was also recently identified (Lancaster *et al.*, 2009). The median age of a cohort of 15 patients was 62 years (24-75); 8 were men. All presented with early and prominent seizures; other symptoms, as well as MRI and EEG findings, were consistent with predominant limbic dysfunction. Forty-seven percent of patients had small cell lung cancer (SCLC), and 40% showed propensity to autoimmunity. Cancer screening and demographic data indicate the disorder also occurs in patients without cancer. Neurological improvement occurred in 60% of the patients and was correlated with prompt immunotherapy and tumor control. Staining of live neurons showed that all patients serum and CSF had antibodies against a cell surface antigen. Immunoprecipitation and mass spectroscopy demonstrated that the autoantigen was the B1 subunit of the GABA_B receptor, a metabotropic receptor that when disrupted causes seizures and memory dysfunction (Prosser *et al.*, 2001; Schuler *et al.*, 2001).

Three syndromes have been shown to be associated with antibodies against voltage-gated potassium channels (Kleopa *et al.*, 2006). Neuromyotonia is a peripheral nervous system disorder characterized by muscle hyperactivity, while Morvan's syndrome, in addition to peripheral muscle hyperactivity, has autonomic and central nervous system manifestations, including insomnia, hallucinations, anxiety, delirium, and memory loss (Kleopa *et al.*, 2006). The third, limbic encephalitis, consists only of central nervous system symptoms with no peripheral dysfunction (Kleopa *et al.*, 2006). Similar to the encephalitides presented above, this type of limbic encephalitis can be paraneoplastic but also more frequently occurs in the absence of a tumor (Buckley *et al.*, 2001; Vincent *et al.*, 2004; Geschwind *et al.*, 2008). Patients experience psychiatric and neurological symptoms including short-term memory loss, disorientation, memory loss, agitation, hallucinations, and seizures, as well as excessive secretions (Buckley *et al.*, 2001; Thieben *et al.*, 2004; Vincent *et al.*, 2004; Geschwind *et al.*, 2008). In addition, voltage gated potassium channel antibodies have also been reported in patients with isolated seizures and rapidly progressive dementia, sometimes suggesting a prion disease (Geschwind *et al.*, 2008).

A number of other synaptic autoimmune disorders have been identified. These disorders affect neurological functions other than memory, behavior and cognition, and therefore are only briefly described here. Two patients with cerebellar ataxia were found to have antibodies against mGluR1 (Smitt *et al.*, 2007). Autonomic neuropathy, a disorder affecting various autonomic nervous system functions, can associate with antibodies against ganglionic acetylcholine receptors (Vernino *et al.*, 1998; Vernino *et al.*, 2000). Patients with Miller-Fisher syndrome, a variant of Guillain-Barre causing extraocular paralysis, produce antibodies against ganglioside type GQ1b that cause a complement-mediated block of neuromuscular transmission (Plomp *et al.*, 1995). Antibodies against glycine receptors have been reported in one patient with Progressive Encephalomyelitis, Rigidity, and Myoclonus

(PERM), resulting in muscle spasms and rigidity, facial muscle weakness, and gaze palsies (Hutchinson *et al.*, 2008). While these syndromes are of clinical interest, the underlying cellular mechanisms remain to be determined.

While *in vitro* approaches have been useful to establish the effects of antibodies to NMDA, AMPA, and GABA_{B1} receptors on neurons and in particular on synapses, *in vivo* models will be needed to establish the relationship between the effects of each antibody on synapse and circuit function, and the changes in behavior, memory and cognition that are hallmarks of these disorders. Below we discuss several of many outstanding questions that, when addressed, will provide new insights into the basic neuroscience of synaptic plasticity as well as the clinical understanding of autoimmune encephalitides.

Paraneoplastic and non-paraneoplastic mechanisms of autoimmune synaptic encephalitides

Anti-NMDA, -AMPA, and -GABA_{B1} receptor encephalitides are often paraneoplastic syndromes. In this setting the presence of a tumor that expresses these receptors likely contributes to breaking immune tolerance. However, other unknown immunological triggers may be involved, particularly in patients without tumor. A propensity toward autoimmunity is suggested by the frequent occurrence of other immune responses, and in the case of anti-NMDA receptor encephalitis, an apparent predominance in ethnic groups (African-American, Asian, Latinos; Gable *et al.*, 2009a).

As in other autoimmune diseases, a number of mechanisms could potentially account for the immune response in the absence of a tumor. Cross reactivity of antibodies against different antigens can occur if the epitopes are sufficiently similar. Guillain-Barré syndrome is an autoimmune peripheral neuropathy affecting axons and myelin sheaths (Hahn, 1998; Hughes & Cornblath, 2005). The disorder is frequently preceded by an infection, often by *Campylobacter jejuni* (Rees *et al.*, 1995; Alios, 1997; McCarthy & Giesecke, 2001). Patients' serum antibodies react with peripheral nerve gangliosides as well as lipooligosaccharide from *Campylobacter jejuni* (Oomes *et al.*, 1995; Ang *et al.*, 2004; Yuki *et al.*, 2004). Other examples include Sydenham's chorea and systemic lupus erythematosus (SLE). Sydenham's chorea is characterized by abnormal movements, hypotonia, and neuropsychiatric symptoms, and characteristically occurs after an infection by group A streptococci (Marques-Dias *et al.*, 1997; Kirvan *et al.*, 2006a). Antibodies from these patients react with gangliosides expressed in the basal ganglia and cross-react with group A streptococcal N-acetyl-glucosamine (Bronze & Dale, 1993; Kirvan *et al.*, 2003; Kirvan *et al.*, 2006b). In SLE, patients with neuropsychiatric symptoms and sometimes asymptomatic family members harbor antibodies to double stranded DNA that also cross react with a single epitope present in the extracellular region of NR2A and NR2B of the NMDA receptor (DeGiorgio *et al.*, 2001; Kowal *et al.*, 2006a).

Given that most paraneoplastic disorders are triggered by small tumors at initial stages of the disease, it is possible that an immune response resulting in antibody synthesis decreases the size or eliminates the tumor by antibody binding and complement mediated cytotoxicity. Thus at the time of diagnosis, antibodies are present, but no tumor is detected. A better understanding of the events that trigger the immune response in anti-NMDA receptor encephalitis and other autoimmune encephalitides will be important in both treatment and prevention of these and other related disorders.

The source and brain access of autoantibodies to synaptic antigens

Two possible, not mutually exclusive, mechanisms that explain the presence of synaptic autoantibodies in the CNS include passive IgG crossing of the BBB, and intrathecal or cerebral synthesis of antibodies by plasma cells (see Figure 1).

The first possibility is that antibodies synthesized in the periphery cross a pathologically disrupted BBB, or through regions where the BBB is normally leaky (area postrema) or more susceptible to systemic changes (e.g., stress, high blood pressure). Several methods for experimentally increasing BBB permeability have been described in the literature, including focal ultrasound (Kinoshita *et al.*, 2006), hypertonic solute (Neuwelt *et al.*, 1988; Doolittle *et al.*, 2000), and lipopolysaccharide (Xiao *et al.*, 2001). More physiologically relevant models of BBB disruption include peripheral inflammation (Rabchevsky *et al.*, 1999; Huber *et al.*, 2001), acute stress (Esposito *et al.*, 2002), and epinephrine (Huerta *et al.*, 2006). Several studies have shown that peripherally administered antibodies can access the brain following these breaches in BBB integrity. Iodinated antibodies injected into rats were detected in the brain following osmotic opening of the BBB (Neuwelt *et al.*, 1988). Kinoshita *et al.* showed that focal sonication caused BBB disruption allowing intravenously injected dopamine receptor antibodies to enter the brain and bind to antigen at sites of barrier breakdown (Kinoshita *et al.*, 2006).

The second possibility is that antibodies are synthesized intrathecally. Most patients with anti-NMDA, -AMPA or -GABA_B receptor encephalitis have an increased ratio of CSF to serum IgG concentration, indicating intrathecal synthesis of antibodies. Moreover, protein electrophoretic analyses of the CSF of these patients often demonstrates multiple distinct bands of IgG that are absent in serum (oligoclonal bands), suggesting the presence of plasma cell clones within the thecal space that secrete distinct immunoglobulins (Dalmau *et al.*, 2008). Extensive clinical and immunological data from patients with anti-NMDA receptor encephalitis suggest a model in which both passive crossing and intrathecal synthesis of antibodies occur. Given that most patients present with prodromal symptoms (hyperthermia, undetermined viral-like infection) it is likely that the BBB is transiently disrupted. This is supported by studies showing transient FLAIR MRI changes involving cortical or subcortical regions. Additionally, after systemic immune activation by a NMDA receptor-expressing tumor or other unknown factors, memory B-cells that are able to cross a normal BBB will undergo re-stimulation, antigen-driven affinity maturation, clonal expansion, and differentiation into NMDA receptor antibody-secreting plasma cells. This mechanism, that has been involved in other autoimmune diseases such as multiple sclerosis (Hauser *et al.*, 2008), would explain the detection of intrathecal synthesis of antibodies in most patients with anti-NMDA receptor encephalitis. Moreover, the occurrence of both passive BBB transfer and intrathecal synthesis of antibodies explains the increasing symptom refractoriness to predominant serum IgG depleting strategies (IVIg, plasma exchange) during the course of the disease. Patients that do not respond to these treatments often improve with cyclophosphamide and rituximab that are more effective in reducing intrathecal synthesis of antibodies. A better understanding of the site and dynamics of antibody synthesis during the course of these disorders is crucial for improving treatment methods and delivery.

Mechanisms underlying antibody pathogenic effects on the target receptors

Several mechanisms may account for the pathogenicity of autoantibodies in these disorders. These include agonizing or antagonizing the receptor, causing receptor internalization and degradation resulting in diminished receptor function, or stimulating complement-mediated

neuronal damage. Each of these effects has been demonstrated in autoimmune diseases of the nervous system, and in some, more than one of these effects contributes to the disease process.

The first possibility is that patient anti-receptor antibodies agonize or antagonize the receptor. NR2 antibodies from patients with SLE cause neuronal death when injected into mouse brain; this effect is attenuated by treatment with the NMDA receptor blocker, MK-801, suggesting the antibodies mediate cell death by enhancing channel activation (DeGiorgio *et al.*, 2001). Conversely, application of nicotinic acetylcholine receptor (nAChRs) antibodies from myasthenia gravis patients to outside-out patches of mouse myotubes caused an acute block of AChR currents that became irreversible with time (Jahn *et al.*, 2000a). With respect to patient NMDA and AMPA receptor antibodies, we have not yet found clear evidence supporting direct agonism or antagonism of receptor function. However, the fact that the epitope for both NMDA receptor and AMPA receptor antibodies is in the N-terminus raises the possibility that autoantibodies could have direct functional effects. The ligand binding domain for both channels is also in the N-terminus, and conformational changes are thought to couple ligand binding to channel opening (Armstrong & Gouaux, 2000; Sobolevsky *et al.*, 2009). Therefore, patient antibodies could sterically hinder ligand binding or enhance its effects. In addition, N-terminal binding sites for channel modulators such as zinc and polyamines may be obscured by patients' antibodies (Rassendren *et al.*, 1990; Herin & Aizenman, 2004; Paoletti & Neyton, 2007). Whole cell recording experiments during acute application of antibodies will allow this issue to be resolved.

The second possibility is that patient anti-receptor antibodies cause receptor internalization and degradation, resulting in diminished receptor function. AChR antibodies from patients with myasthenia gravis cause a loss of surface AChRs by cross-linking and internalization (Drachman *et al.*, 1978). Cross-linking and internalization of voltage gated calcium channels by autoantibodies has also been shown to occur in patients with Lambert-Eaton syndrome (Nagel *et al.*, 1988a; Peers *et al.*, 1993a). In anti-NMDA receptor encephalitis, we have shown that the loss of surface NMDA receptors is the result of antibody mediated cross-linking and internalization (see Figure 2). Treatment of cultured neurons with monovalent Fab fragments generated from patients' antibodies did not induce receptor internalization, but subsequent crosslinking with anti-Fab antibodies recapitulated the decrease caused by intact patient NMDA receptor antibodies (Hughes *et al.*, 2010). These data demonstrate that patients' antibodies produce both structural and functional changes at the synapse. Determining the range of effects of patients' antibodies (short-term, long-term, and dynamics of recovery) will provide insight into the synaptic basis of the memory and behavioral changes seen in these patients, and help to establish the roles of NMDA receptor signaling in human behavior and cognition.

The third possibility is that patient anti-receptor antibodies cause complement-mediated neuronal damage or death. Muscle biopsies from patients with myasthenia gravis have revealed extensive deposits of components of the complement cascade (Engel *et al.*, 1977; Sahashi *et al.*, 1980). Autopsy and *in vitro* studies have also linked complement activation with Rasmussen's encephalitis and neuromyelitis optica, the later characterized by antibodies to aquaporin-4 (Whitney *et al.*, 1999; Lucchinetti *et al.*, 2002; Waters *et al.*, 2008). IgG1 and IgG3, subclasses of IgG capable of activating complement, are the main IgG types of NMDA and AMPA receptor antibodies. In anti-NMDA receptor encephalitis, we have not found evidence of deposits of complement in autopsies of patients. In light of the substantial recoveries made by many of these patients, extensive neuronal damage due to complement activation seems unlikely. Furthermore, it is unclear whether the elements of the complement cascade that are present in the central nervous system are sufficient to induce

complement-mediated lysis. Further studies are needed to determine the degree of involvement of complement mediated mechanisms in the brain and tumor of patients with synaptic autoimmune encephalitis.

Homeostatic compensatory changes in response to antibody-mediated decrease of receptor levels

Compensatory mechanisms at the cellular and synaptic level have been shown to occur in autoimmune disorders of the nervous system in humans and in experimental model systems. Studies from mouse models of myasthenia gravis and patients' tissue have shown an enhanced rate of synthesis of AChRs and increased expression levels of the α , β , δ , and ϵ subunits of the AChR, as well as increased acetylcholine release upon stimulation (Wilson *et al.*, 1983; Guyon *et al.*, 1994; Plomp *et al.*, 1995; Guyon *et al.*, 1998). Purkinje cells treated with IgG from patients with Lambert-Eaton syndrome show a loss of P/Q-type voltage gated calcium channel currents and a concomitant increase in R-type currents (Pinto *et al.*, 1998). Deletion of the α -1a subunit of the P/Q-type channel in mice causes age related ataxia and muscle weakness and results in enhanced L- and N-type calcium channel currents in Purkinje cells (Jun *et al.*, 1999).

These observations raise the possibility that homeostatic mechanisms occur in anti-NMDA receptor and anti-AMPA receptor encephalitis, though this remains to be demonstrated. Support for this idea comes from the changes in synaptic strength observed after pharmacological blockade of glutamate receptors. Several studies have shown that, after 48 hours of NMDA or AMPA receptor blockade, mEPSC amplitude is enhanced (Turrigiano *et al.*, 1998; Sutton *et al.*, 2006).

Effects of maternal antibodies on fetal development and subsequent behavior

While the symptoms of anti-NMDA receptor encephalitis are largely similar between children and adults, children tend to have less severe autonomic problems and increased speech dysfunction (Florance *et al.*, 2009; Lebas *et al.*, 2009). These differences, along with a subset of patients who were diagnosed during pregnancy, raise questions about the effects of anti-receptor antibodies on the developing brain, and the long-term consequences of fetal or pediatric exposure to the antibodies.

Several other autoimmune diseases are associated with abnormalities in offspring. Children born to mothers with SLE, especially male children, have higher rates of developmental and learning disabilities than children born to unaffected mothers (McAllister *et al.*, 1997; Ross *et al.*, 2003). In mice, in utero exposure to antibodies from SLE patient serum results in morphological and behavioral abnormalities in offspring (Lee *et al.*, 2009). Maternal myasthenia gravis is often associated with arthrogryposis multiplex congenita, a condition caused by lack of fetal movements that is associated with joint contractures and muscle weakness in offspring (Polizzi *et al.*, 2000). Interestingly, asymptomatic women who have children with this condition may harbor anti-AChR antibodies. Serum from these women contains antibodies only to a fetal subunit of the AChR, and is therefore specifically harmful to the fetus rather than the mother (Vincent *et al.*, 1995; Riemersma *et al.*, 1996). These studies highlight the devastating effects maternal autoantibodies can have on fetal development.

Studies of mothers of autistic children also raise the possibility that maternal antibodies may impact fetal development. Several studies of these women suggested that they harbor anti-neuronal antibodies in their serum, although the antigen(s) is unknown. For example, serum

from a woman with an autistic child and a child with a severe language disorder contained antibodies that bound rodent neurons and caused behavioral and motor abnormalities in mice exposed to the serum as embryos (Dalton *et al.*, 2003). In addition, serum from mothers with autistic children caused hyperactivity and stereotypic behavior in rhesus monkeys exposed prenatally to the serum (Martin *et al.*, 2008). These results show that asymptomatic women may have circulating neuronal antibodies that have access to the fetal brain and may affect brain development.

Two women that developed anti-NMDA receptor encephalitis at 14 and 17 week of pregnancy, when the specific transplacental transfer of IgG1 and IgG3 is not yet fully developed, delivered apparently normal newborns. Studies performed in one of the babies showed a lack of antibodies in serum and CSF (Kumar *et al.*, 2010). However, given the importance of synaptic activity, in particular glutamatergic and GABAergic transmission during neural circuit development, *in utero* exposure to antibodies from patients with anti-NMDA receptor encephalitis, or other autoimmune encephalitides, may potentially affect normal fetal brain development resulting in neurological and behavioral disturbances in offspring. Thus establishing a mechanistic link between anti-receptor antibodies, access to the developing brain, effects on synapses and circuits and ultimately behavior, assayed across the lifespan, will be important for resolving these issues in a broad spectrum of disorders.

Relating the effects of synaptic receptor antibodies to neurological symptoms

Glutamate binding to NMDA receptor and AMPA receptor is crucial for synaptic transmission and plasticity. Pharmacological blockade or genetic reduction of NMDA receptor or AMPA receptors has been shown to alter measures of learning and memory and other behaviors in animal models (Nishikawa *et al.*, 1991; Mohn *et al.*, 1999; Kapur & Seeman, 2002; Krystal *et al.*, 2002b; Nabeshima *et al.*, 2006; Large, 2007; Schmitt *et al.*, 2007; Labrie *et al.*, 2008). The balance between excitatory and inhibitory synaptic inputs is also altered, and this has been shown to affect circuit function and behavior (Prange *et al.*, 2004; Hensch & Fagiolini, 2005; Levinson & El-Husseini, 2005; Murphy *et al.*, 2005; Epsztein *et al.*, 2006; Fritschy, 2008; Kehrer *et al.*, 2008). In addition, NMDA (Olney *et al.*, 1999; Coyle *et al.*, 2003; Coyle & Tsai, 2004; Kapoor *et al.*, 2006; Lindsley *et al.*, 2006; Stahl, 2007b; a; Shim *et al.*, 2008) and/or AMPA (Noga & Wang, 2002; Makino *et al.*, 2003; Magri *et al.*, 2006; Wiedholz *et al.*, 2008; Zavitsanou *et al.*, 2008) receptor hypofunction has been proposed to be part of the pathophysiological mechanisms underlying schizophrenia.

It's interesting to consider why patients with anti-NMDA receptor antibodies develop a complex syndrome that includes psychosis, learning and memory dysfunction, abnormal movements, autonomic instability and frequent hypoventilation, while those with AMPA receptor antibodies preferentially develop psychiatric and amnesic symptoms. Studies using genetic deletion of NMDA receptor or AMPA receptor subunits in mouse models provide some insight into this issue. While NR1 knockout mice die shortly after birth due to hypoventilation (Li *et al.*, 1994), mice with spatially restricted NR1 deletion can survive into adulthood (Nakazawa *et al.*, 2004). CA1-specific NR1 knockouts have impaired spatial and temporal memory and a loss of CA1 LTP (Tsien *et al.*, 1996). Mice with an inducible, reversible knockout of NR1 in forebrain show impairment in the maintenance of long-term memory if NR1 expression is turned off during the memory storage phase. In addition to memory deficits, targeted manipulation of NR1 expression can result in schizophrenia-like symptoms. Hypomorphic expression of NR1 leads to increased stereotypic behavior and decreased sociability, while early postnatal loss of NR1 in a subset of cortical and

hippocampal interneurons results in decreased pre-pulse inhibition and increased social isolation-induced anxiety (Mohn *et al.*, 1999; Belforte *et al.*, 2010). Moreover, subanesthetic doses of NMDA receptor blockers such as phencyclidine and ketamine are psychotomimetic, and they recapitulate many of the positive and negative signs of schizophrenia in humans and rodents as well as repetitive orofacial movements, autonomic instability and seizures. (Luby *et al.*, 1962; Krystal *et al.*, 1994; Lahti, 2001; Krystal *et al.*, 2002a) The remarkable similarity between these phenotypes, the effect of patients' antibodies resulting in a dramatic decrease of synaptic NMDA receptor clusters and function, and the reduced levels of NMDA receptors in autopsied patients, support an antibody-mediated pathogenesis of anti-NMDA receptor encephalitis, and strengthen the NMDA receptor hypofunction hypothesis of schizophrenia (Belforte *et al.*, 2010).

The consequences of loss of AMPA receptor expression have also been studied in mouse models. Spatial learning and memory are largely unaffected in GluR1 knockout mice despite the fact that LTP is reduced in CA1 and CA3 (Zamanillo *et al.*, 1999) and working memory is diminished (Reisel *et al.*, 2002; Sanderson *et al.*, 2007). GluR2 knockout mice show reduced exploration and impaired motor coordination. In these animals, AMPA receptor mediated synaptic transmission is reduced, but LTP is enhanced (Jia *et al.*, 1996a; Gerlai *et al.*, 1998). GluR2 knockout mice also have increased cell death (Feldmeyer *et al.*, 1999; Oguro *et al.*, 1999), possibly due to excitotoxicity related to increased, compensatory insertion of GluR1 homomeric AMPA receptors. While AMPA receptor subunit knockout mice have not provided a satisfying explanation for the role of AMPA receptors in synaptic plasticity related to learning and memory, the fact that patients with AMPA receptor antibodies have short-term learning and memory deficits argues that further studies at the circuit and behavioral levels are warranted.

GABA_{B1} receptor knockout mice display a variety of neurological and behavioral abnormalities, including spontaneous seizures, enhanced anxiety, hyperactivity, hyperalgesia, and impaired memory (Schuler *et al.*, 2001; Prosser *et al.*, 2001), suggesting dysfunction of the limbic system. Consistent with these experimental data, patients with anti-GABA_{B1} receptor antibodies present with an encephalitis that associates with early and prominent seizures, confusion, agitation, behavioral problems and severe short-term memory deficit along with MRI abnormalities predominantly involving the hippocampi. Interestingly, both GABA_{B1} receptor knock out mice and mice treated with a GABA_{B1} receptor antagonist, CGP56433A, exhibit antidepressant-like behavior in a forced swim test and a learned helplessness paradigm (Mombereau *et al.*, 2004; Nakagawa *et al.*, 1999), suggesting that GABA signaling may have disparate effects on different aspects of mood such as depression and anxiety. Combined with animal studies, these patients can provide rich insight into the role of GABA_{B1} receptor signaling in memory, behavior, and cognition.

Conclusions

We have begun to obtain a better cellular- and synaptic-level understanding of a new and remarkable group of immune-mediated behavioral and memory disorders. On the clinical side, we would like to know the frequency of these antibodies in patients with milder or *form frustes* of the syndromes (e.g., predominant psychosis, isolated refractory seizures), and whether the effects of antibodies on glutamate and GABA receptors, and synapses, vary according to different subgroups of patients, improving the diagnostic and treatment strategies. It is likely that the effects of antibodies on children (or antibody effects on immature hippocampal synapses) are different from those on adults (or on mature hippocampal synapses), and this may account for some of the behavioral differences between adults and children. Another critical question is the optimal type of immunotherapy at different stages of the disease, and the duration of treatment. In current clinical practice, most patients receive intravenous immunoglobulins, plasma exchange, and corticosteroids as

the first line of therapy. When these fail, Rituximab (a B-cell depleting monoclonal antibody) and cyclophosphamide are increasingly being used in an attempt to modify the levels of antibodies behind the BBB. However, it is unclear whether or how these treatments modify the effects of antibodies on synapses.

On the basic neuroscience side, a major goal will be to develop and test rodent models in a battery of behavioral tests designed to assay hippocampal, amygdala, cortical and cerebellar function in each disorder. In this way, we can begin to relate the cellular, synaptic, and circuit effects of patients' antibodies to behavioral deficits in learning, memory, and other cognitive and motor manifestations.

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Figure 1. Mechanisms involved in the presence of antibodies in the central nervous system

A – B: In a subset of anti-NMDA receptor encephalitis patients, an immune response is initiated against neuronal antigens expressed by an ovarian teratoma or another tumor (A). In another subset of patients, the immunological trigger is unknown (B).

The ectopic expression of NMDA receptors by a tumor or other mechanisms (e.g., molecular mimicry) break down immune tolerance by a sequence of mechanisms including antigen presentation by T or dendritic cells generating memory B-cells (1) and antibody-producing plasma cells (2). Systemically synthesized antibodies can bind to NMDA receptors present in the tumor and a disrupted or leaky blood brain barrier (BBB) (4). Disruption of the BBB is likely to occur early in the disease process. In addition, memory B-cells (likely also activated T-cells) that are able to cross a normal BBB (5), will undergo re-stimulation (resident antigen presenting cells or T-cells, 6), antigen-driven affinity maturation, clonal expansion, and differentiation into NMDA receptor antibody-secreting plasma cells (7). Antibodies produced within the CNS by plasma cells (8) or reaching the CNS by BBB disruption (4) bind to extracellular epitopes of NMDA receptor (9) causing subsequent dysfunction and internalization (see Figure 2).

Figure 2. Mechanisms underlying cellular and synaptic effects of anti-NMDA receptor antibodies

A-C: AMPA and NMDA receptors are localized in the postsynaptic membrane and are clustered at the postsynaptic density (A). Patient antibodies in the CNS bind selectively to NMDA receptors at the synapse as well as extrasynaptic receptors. This binding leads to receptor cross-linking (B). NMDA receptors that have been bound and cross-linked by antibodies are internalized, resulting in a decrease of surface, synaptically localized NMDA receptors. Other synaptic components, such as postsynaptic AMPA receptor clusters, PSD-95, as well as presynaptic terminals, dendrite branches, dendritic spines and cell viability, are unaffected (C). Thus patient anti-NMDA receptor antibodies lead to a rapid, selective excision of NMDA receptors from neuronal membranes. This effect is titer-dependent and reverses after antibody titers are reduced (not shown).

D-E: Rodent cultured neurons treated with control CSF or patient's CSF for 3 days, and subsequently stained for postsynaptic NR1 to label NMDA receptor clusters, VGlut to label presynaptic sites, and PSD-95 as a postsynaptic marker. Note that patient's CSF cause a decrease in dendritic NR1 cluster density, while VGlut and PSD-95 cluster density remain unchanged.

F-G: Whole-cell patch recordings of miniature excitatory postsynaptic currents (mEPSCs), which consist of a fast AMPA receptor-mediated component and a later, slower NMDA receptor-mediated component that is APV sensitive. Compared to neurons incubated with CFS control (F), those treated with CSF from a patient with NMDA receptor antibodies show a selective loss of the APV-sensitive NMDA receptor component (E).

Table 1

Immune mediated disorders of the synapse

Central Nervous System Disorders					
Antigen	Syndrome / Symptoms	Patients' antibodies effects on receptors	Criteria *	Clinical References	Molecular References
NR1 subunit of NMDA receptor	Anti-NMDA receptor encephalitis: psychosis, behavioral and memory deficits, dyskinesias, language reduction (in children), seizures, and autonomic instability	Decreased synaptic NMDARs; decreased NMDAR mediated mEPSCs	1-4, 6	Dalmau <i>et al.</i> , 2007	Dalmau <i>et al.</i> , 2008; Hughes <i>et al.</i> , 2010
GluR1/2 subunit of AMPAR	Anti-AMPA receptor encephalitis: short-term memory deficits, seizures, psychiatric manifestations; tendency to relapse.	Decreased synaptic AMPARs; decreased AMPAR mediated mEPSCs	1-3, 6	Lai <i>et al.</i> , 2009	Lai <i>et al.</i> , 2009
GABA _{B1} receptor	Anti-GABA _{B1} receptor encephalitis: seizures, short-term memory deficits	Pending characterization	1-2, 4	Lancaster <i>et al.</i> , 2009	
NR2A/NR2B subunits of NMDA receptor	Neuropsychiatric systemic lupus erythematosus: cognitive deficits, psychosis, memory deficits, mood disorder, seizure, headache	NMDAR agonism	1, 3, , 5	Brey <i>et al.</i> , 2002	DeGiorgio <i>et al.</i> , 2001; Kowal <i>et al.</i> , 2006b
Voltage - gated K ⁺ channel	Neuromyotonia, limbic encephalitis, Morvan's syndrome Muscle spasticity, memory deficit, insomnia, hallucinations.		1, 2, 4, (5 only features of neuromyotonia), 6	Vincent <i>et al.</i> , 2004	Kleopa <i>et al.</i> , 2006; Shillito <i>et al.</i> , 1995; Rho <i>et al.</i> , 1999
Peripheral Nervous System Disorders					
Nicotinic acetylcholine receptors	Myasthenia gravis: muscle weakness, fatigue	Decreased synaptic AChRs at neuromuscular junctions; decreased AChR mediated currents	1-6	Dau <i>et al.</i> , 1977; Meriggioli & Sanders, 2009a	Toyka <i>et al.</i> , 1975; Lennon <i>et al.</i> , 1985; Missias <i>et al.</i> , 1997; Jahn <i>et al.</i> , 2000b; Leite <i>et al.</i> , 2008; Lang & Vincent, 2009
Voltage -gated Ca ²⁺ channel	Lambert-Eaton syndrome: muscle weakness	Decreased presynaptic channels at neuromuscular junctions; decreased neurotransmitter release	1-6	Leys <i>et al.</i> , 1989; Motomura <i>et al.</i> , 2000; Pourmand, 2009	Fukunaga <i>et al.</i> , 1983; Lang <i>et al.</i> , 1983; Takamori <i>et al.</i> , 2000; Kim, 1985; Molenaar, 2008; Nagel <i>et al.</i> , 1988; Peers <i>et al.</i> , 1993b; Pinto <i>et al.</i> , 2002
Ganglionic nicotinic acetylcholine receptor	Peripheral neuropathy; autonomic nervous system dysfunction	Reduction in AChR current due to antibody crosslinking	1-6	Vernino <i>et al.</i> , 1998; Vernino <i>et al.</i> , 2000	Xu <i>et al.</i> , 1999; Vernino <i>et al.</i> , 2004; Wang <i>et al.</i> , 2007; Vernino <i>et al.</i> , 2008

* In order to establish a pathogenic role for anti-receptor antibodies, at least six issues should be addressed: 1. Antibodies bind to an extracellular epitope in the target receptor. 2. Antibodies recognize the receptor when it is overexpressed in a heterologous cell system.

3. Antibodies block or alter the function of the receptor. 4. Clinical features of the autoimmune disease are similar to the phenotype resulting from genetic or pharmacological manipulation of the receptor. 5. Passive transfer of patients' receptor-specific immunoglobulin to animals results in a phenotype that recapitulates the clinical features of the disease. 6. Antibody mediated cellular and synaptic alterations, and clinical symptoms, should improve as antibody titer is reduced.