Evaluation of the Pathophysiological Mechanisms Underlying Anti-NMDA Receptor Encephalitis

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Introduction

Anti-NMDA receptor encephalitis is a rare autoimmune disease involving acute inflammation of the brain. It was first described in 2007 by Dalmau and colleagues\(^1\) and since then, a growing array of literature has reported the condition in a variety of clinical settings. Given its relatively recent discovery, accurate values of prevalence are currently unknown but estimates calculated in 2010 suggest that it accounts for at least 4% of all encephalitides\(^2\). Although the disease was previously thought to be solely a paraneoplastic complication (secondary to cancer) in women with ovarian teratomas (tumours), it is now known to occur in both men and women, with and without tumours\(^3\). Nonetheless it most commonly affects young women (below 30) with no past neuropsychiatric history.

The condition seems to follow a predictable progression of clinical manifestations, starting with non-specific viral like symptoms such as fever and headache and then progressing to a state of psychosis (impaired contact with reality)\(^4\), often involving hallucinations and severe confusion. Subsequently, involuntary body movements (dyskinesias) may develop, along with non-epileptiform seizures. The final phase is categorized by respiratory depression (inability to breath) and autonomic dysfunction. First line treatments include immunotherapy, corticosteroids and seizure control, as and when necessary.

Thorough investigation into the manner in which the disease functions (pathophysiology) has been carried out with varying degrees of success in recent years\(^5\) \(^6\). However the cellular mechanisms that underpin anti-NMDA receptor encephalitis are heavily debated, with many of the fine details of the processes concerned still unknown. The basis involves the abnormal production of antibodies - chemical weapons that usually facilitate immune defence against external pathogens\(^4\). These pugnacious agents target NMDA receptors on synapses (gaps between neurones) in the brain and thus render cerebral damage. This study aims to evaluate the evidence supporting the current models of the pathogeneses (causes) of the disease and it will therefore focus on antibody presence in the cerebrospinal fluid (CSF) – the fluid that baths the brain and spinal cord\(^4\) – and will then investigate the selective binding of the antibodies to NMDA receptors. Finally it will review the mechanisms of neuronal damage and how these relate to the symptomatic manifestations.

The Presence of Antibodies in the CSF

Empirical evaluation of the origin of anti-NMDA antibodies in the CSF (ultimately where they actuate their damage) leads to the consideration of two possible mechanisms. Primarily, passive access involves the diffusion of antibodies from the blood across a ‘pathologically disrupted blood-brain barrier (BBB)\(^5\). This cellular filter, separating the central nervous system from the circulatory system, normally prevents larger molecules from entering the brain\(^4\). A variety of reasons for such a collapse in integrity have been suggested, with the most likely answer being the effects of acute inflammation of the nervous system. Rabchevsksky et al report the use of Freund’s adjuvant (a solution containing attenuated mycobacteria) in mice to elicit an acute phase response - a subsequent increase in BBB permeability to tracer molecules was noted\(^7\). Likewise, the involvement of corticotropin releasing hormone on mast cells in acute stress has been shown to facilitate BBB penetration\(^8\). Given the inflammatory nature of encephalitis, it is likely that this is the principal manner by which antibodies gain access to the CSF. However, it is also possible that the autonomic dysfunction manifested in many patients during the later phases of the condition aids antibody
entry. For example, an increase in blood pressure would force larger proteins, such as antibodies, to extravasate (pass out of the blood\textsuperscript{3}) into the CSF.

Secondly, there is strong evidence to suggest that the presence of antibodies in the CSF is also due to intrathecal production (synthesis in the space under the arachnoid membranes in the CNS\textsuperscript{1}). Dalmau et al demonstrated that 53 out of 58 patients with the condition had at least partially preserved BBBs, whilst having a high concentration of antibodies in the CSF\textsuperscript{3}. Figure 1 demonstrates this – if there was totally passive diffusion between the CSF and blood, the antibody concentration in both would equilibrate. One can therefore surmise that plasma clones (immune cells that produce antibodies) present in the central nervous system are responsible for this phenomenon. Furthermore cyclophosphamide and rituximab\textsuperscript{9}, drugs used to eliminate dysfunctional immune cells, have been shown to be successful second line treatments in patients where immunotherapy has failed\textsuperscript{10}. They endeavour to destroy excess immunoglobulin (antibody) producing cells in the intrathecal space, alleviating the symptoms. Finally, electrophoresis (a process used to separate and identify proteins) has shown that certain antibodies were found in the CSF and not the blood, again advocating that passive diffusion is not the only explanation\textsuperscript{1}. However, more sophisticated analysis of the processes involved in antibody presence in the CSF hint at a combination of these two mechanisms in tandem. In the early phases of the disease, the BBB is temporarily disrupted, allowing specific B-cell (immune cells that divide to form plasma clones) to enter the brain. This assumption is supported by Fluid Attenuated Inversion Recovery Magnetic Resonance Imaging (FLAIR MRI)\textsuperscript{11}, an imaging technique used to differentiate between tissue types; it often shows cerebral cortical hyperintensities (bright spots) where both antibodies and B-cells can enter\textsuperscript{1}. Consequentially, these cells differentiate into plasma clones and produce antibodies from within the thecal space (a process driven by restimulation after antibodies bind to NMDA receptors).

The Binding of Antibodies to NMDA Receptors

Once the specific antibodies reach their target, a variety of mechanisms have been suggested regarding the manner in which they render neuronal damage (damage to nerve cells). It is first important to review the functional structure of the receptor being attacked and how the binding process occurs. The NMDA receptor is a heteromeric (non uniform) complex consisting of two extracellular (outside the cell) subunits; the NR1 subunit, where glycine binds and the NR2 subunit, where glutamate binds\textsuperscript{12}. Figure 2 illustratively differentiates between these extracellular domains. Extensive research into the binding of antibodies onto this receptor has been carried out using ELISA (Enzyme Linked Immunosorbent Assay), particularly by Dalmau and collegues\textsuperscript{3}. ELISA is an immunohistochemical technique (to do with the chemistry of the tissues of the immune system) that involves taking cells artificially made to express the NR1...
component, NR2 component or both. Then a purified form of the patient’s CSF with the specific antibodies is passed over these cells. Finally another antibody (with a fluorescent protein), binds onto the first antibody, only if it has bound onto the receptor. In short, if fluorescence can be seen under the microscope, the antibody has attached to the receptor. Notably, this technique can also be used to quantitatively assess the concentration of antibodies in the CSF. Results show that binding occurs principally to the NR1 subunit, as demonstrated by figure 3. As a control, separated NR1 subunits and CSF do not fluoresce, thus establishing that the two elements bind together when mixed. Hughes et al have demonstrated that once the antibodies bind to the NMDA receptors, no cell death or dendritic destruction occurs; instead alteration of the synapses takes place\(^6\). This claim is supported by in vitro (in the test tube) studies, which suggest that the number of neurones remains unchanged when the antibodies are passed over rat brain cells.

The first and most likely mechanism of neuronal dysfunction is a reduction in cluster density (number per unit area) of NMDA receptors on the post synaptic neurone. This has been determined both in vitro and in vivo (in a live organism). The latter can be seen in figure 4, with a significant reduction in the number of NMDA receptor clusters per 75 µm\(^2\) when patient CSF with antibodies (red) was exposed to live rat neurones, compared to the control study (black). However, the cluster density of other receptor types, such as gamma aminobutyric acid (GABA) receptors was unaltered, proving that these antibodies only act upon NMDA receptors. The fact that many patients with the condition make almost a full recovery, despite the grave severity of their symptoms, correlates with a theory involving synaptic modifications rather than a hypothesis involving cellular death\(^13\). The subcellular workings of such a mechanism were previously enigmatic, until a repertoire of ingenious experiments elucidated the situation. When in vitro rat hippocampal neurones are treated with the active binding domain of the NR1 specific antibodies (called Fab fragments) we would expect them to bind to NR1 subunits and cause them to cease to exist. Paradoxically this does not happen. Instead a reduction in cluster density of NR1 subunits can only be achieved when two or more binding domains are linked (via an anti-Fab fragment), in a molecular arrangement similar to a normal antibody (IgG). Therefore, two separate subunits must be cross-linked by an antibody and subsequently internalised from the cell surface membrane\(^6\). Figure 5 demonstrates this.
Another, albeit less likely mechanism, is the direct agonism (stimulation) or antagonism (blocking) of the NMDA receptor by the antibody, as if it were an exogenous pharmacological agent. Myasthenia gravis is a similar autoimmune condition, categorized by periodic voluntary muscular weakness\textsuperscript{14}. Its pathophysiology involves specific autoantibodies antagonising acetylcholine receptors on post synaptic neurones, thus inhibiting nerve transmission. Jahn et al have concluded that this interaction is similar to that of conventional acetylcholine receptor antagonists such as alpha bungarotoxin - the active component in the venom of the elapid snake\textsuperscript{15,16}. Similarly NMDA receptor antagonists, such as phencyclidine, have been shown to incite similar clinical manifestations as anti-NMDA receptor encephalitis. In fact, phencyclidine overdoses in rat models cause ‘gross motor ataxia’ (inability to coordinate voluntary muscles) and eventually lead to a ‘cataleptic freeze’ (a state of unresponsiveness), as demonstrated by Haggerty et al\textsuperscript{17}. These symptoms are not only also apparent in human models with phencyclidine overdoses but they are almost identical to those displayed in the present condition. This conclusive evidence renders direct antagonism a non-excludable mechanism.

Finally the complement cascade is an unlikely, but not impossible, mechanism for the symptoms in anti-NMDA receptor encephalitis. It involves components of the innate (unchanging) immune system, being summoned by immunoglobular activity\textsuperscript{18}. Certain proteins activate an array of other intermediates in a cascade, with the final products including such molecules as the membrane attack complex (MAC), which inserts itself in plasma membranes and cause cell lysis (bursting). Whitney et al describe a related autoimmune condition known as Rasmussen’s encephalitis - a rare but degenerative epileptiform encephalopathy that operates via malfunctioning antibodies\textsuperscript{19}. They have found certain complement precursors, such as C4 and C8, in the brains of patients suffering from the condition. Despite this, no evidence of an abnormal complement system has yet been found in anti-NMDA receptor encephalitis patients\textsuperscript{1}. This, coupled with the aforementioned data suggesting no neuronal death (a necessary indication of the complement cascade), makes it an improbable mechanism. At this present stage however, it is worth noting that the lack of more detailed biochemical information renders us incapable of decisively concluding which mechanism is the correct one. To that end, these three explanations are not necessarily mutually exclusive – they could, at least in part, synergize to render the neuronal dysfunction categorized by the condition.

**Explanation of the Symptoms**

This final section of the pathogenesis will concentrate on relating the cellular mechanisms of the condition to the clinical manifestations seen in the majority of patients. Amnesia is almost totally ubiquitous across all reported cases of the condition - this paradigm hints at the nature and primary location of the NMDA receptor modification\textsuperscript{10}. With the highest density of NR1 subunits in the limbic system, in particular the hippocampus\textsuperscript{20}, it is no surprise that anterograde amnesia (the inability to form new memories during the course of the disease) is a common side effect, given the role played by this area of the brain in the strengthening of synaptic pathways. For instance, Meredith et al report a complete lack of recall in a 16 year old male over the three month period in which he suffered from the condition\textsuperscript{21}.

A central concept in the formation of new memories is long-term potentiation; the strengthening of synaptic pathways when they are repeatedly activated, as described by Cooke et al\textsuperscript{22}. If a synapse expresses both NMDA receptors and AMPA (2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic
acid) receptors, glutamate (a neurotransmitter) released from the presynaptic neurone can bind onto both, but only the AMPA receptor will open if the signal is weak - allowing a small amount of sodium ions into the postsynaptic neurone. However, if the signal is of sufficient strength, enough sodium ions will be allowed into the cell to expel a magnesium ion blocking the NMDA receptor. The NMDA receptor will now also allow ions, including calcium, to flow through it. These calcium ions activate an intracellular cascade, resulting in two major consequences. Firstly more AMPA receptors are inserted into the postsynaptic membrane and secondly these receptors are phosphorylated. This facilitates cell depolarization and therefore a signal of a weaker strength is now sufficient to activate the neurone; one of the fundamental concepts in memory formation. If there is a lack of functional NMDA receptors, as in the present condition, ionic currents due to these receptors are reduced. This however cannot be investigated directly. Instead the total current delivered to the postsynaptic neurone can be measured and then the NMDA receptor can be blocked with APV (2-amino-5-phosphonopentanoic acid); the two values can then be subtracted from each other. Figure 6 shows the reduction in NMDA receptor mediated current when CSF from patients suffering from the condition is suspended on hippocampal cells for 24 hours. This is clear evidence for the inability of patients to strengthen synaptic pathways and thus form new memories throughout the duration of the disease.

A pathological evaluation of the neuropsychiatric symptoms exhibited by patients can also be achieved with careful consideration of the synaptic mechanisms of the NMDA receptor. In recent years, the general consensus behind schizophrenia has shifted from one primarily involving the dopaminergic system to that of glutamatergic dysregulation. Anti-NMDA receptor encephalitis causes a similar state of psychosis to that of schizophrenia, namely one involving paranoia, hallucinations and delusional episodes. It is therefore no coincidence that NMDA hypofunction (low function) is associated with both conditions. Further, Javitt and colleagues describe how NMDA receptor antagonists (such as phencyclidine) can induce schizophrenic manifestations in normal humans. This adds extra credence to the theory that fewer competent NMDA receptors are associated with the condition.

As the disease progresses, dyskinesias (especially orofacial) can occur, along with other dystonic body posturing. This can also be explained by the aforementioned hypothesis of NMDA hypofunction and Ilkiw et al have demonstrated that ketamine (another NMDA receptor antagonist) in cats can elicit similar involuntary responses. Many patients also experience seizures, which seems somewhat paradoxical on first inspection. Surely, preventing glutamate (the major excitatory neurotransmitter in the brain) from acting by removing NMDA receptors will reduce cortical excitation and thus decrease the likelihood of epileptiform activity? This enigma can be resolved by analysing the downstream effects on neuronal circuitry. By inhibiting excitatory neurones connected to inhibitory neurones (associated with GABA) from firing, we overall decrease inhibitory effects, or in other words, increase excitation. This therefore leads to episodic seizures throughout the latter stages of the condition. A comparable analogy would be taking one’s foot off the brakes (that normally inhibit movement) in a car when travelling downhill – the vehicle gains velocity (excitation). Finally this also explains why benzodiazepines such as midazolam, which enhance GABAergic (inhibitory) activity, are successful in treating the seizures.
Although a full recovery occurs in the majority of patients, up to 25% of cases result in severe complications or death\(^5\). The most common cause of fatality amongst patients with anti-NMDA receptor encephalitis is central hypoventilation and overwhelming autonomic dysfunction in the critical and final phase. Thus, a thorough consideration of the neurological roots of such manifestations is vital if treatments and preventions are to be realised. Forrest et al have demonstrated that genetically modified mice neonates (newly born), lacking the NR1 subunit of the NMDA receptor died shortly after birth due to respiratory depression\(^27\). The importance of the NMDA receptor in ventilation is accredited to its ubiquitous expression across the brain, including regions in the brainstem such as the nucleus tractus solitaire (NTS) that are heavily associated with breathing regulation\(^28\). Likewise reduced hypoxic (low oxygen) sensitivity with NMDA receptor antagonists such as dizocilpine has been shown in rat models\(^29\). The lack of functional NMDA receptors and the subsequent hypoventilation mandate respiratory support throughout this phase of the condition. Likewise, NMDA receptors coexist with AMPA receptors in second order neurones in the brainstem involved in autonomic control\(^30\). They receive signals from baroreceptors (blood pressure receptors) along with the heart and the lungs; therefore their disruption clearly affects the regulation of autonomic processes.

**Conclusion**

Extensive research into the processes involved in anti-NMDA receptor encephalitis will no doubt continue in the near future. Hopefully these efforts will come to fruition with a greater understanding of the pathophysiology of the condition and consequently, more accurate guidelines regarding treatment schedules. Primarily, the presence of antibodies in the CSF can be explained by a combination of passive diffusion from the blood and intrathecal production. It is therefore not surprising that treatment options, such as immunotherapy, involve the artificial removal of antibodies from the serum and the destruction of immunoglobulin producing cells in the CSF. A novel approach however may include the restoration of BBB competency, yet this approach remains a hypothetical ideal.

Once the specific antibodies bind to the NR1 subunits, the most likely explanation is the internalisation of the receptors into the neurone. Subsequently, a reduction in receptor cluster density on the post synaptic neurone occurs. However, direct antagonism of the receptors also remains a possibility, given that it would also lead to glutamatergic hypofunction of neurones in the limbic system of the brain. Finally the range of clinical symptoms - from amnesia and psychosis to seizures and hypoventilation - can be explained by such a theory, given the pivotal role of NMDA receptors in each instance.

Future attempts should not only focus on pinpointing the exact mechanisms involved in anti-NMDA receptor encephalitis but also in expanding awareness and knowledge of the disease, so clinicians worldwide can identify and diagnose patients rapidly. Once such aims have been accomplished, it will only be a matter of time before contemporary treatments will evolve and fatalities will be minimized.
References

Figure References

- Figure 1 – Analysis of the concentration of NR1 specific antibodies in the CSF and the serum. See reference 3
- Figure 2 – An activated NMDA Receptor. Wikipedia 2007 http://en.wikipedia.org/wiki/File:Activated_NMDAR.PNG
- Figure 3 – Immunolabeling of neuronal NR1 Clusters. See reference 3
- Figure 4 - Effect of infusion of patient CSF with varying antibody titer on NMDAR cluster density in CA1. See reference 6
- Figure 5 - Diagram that outlines the effect of each treatment on surface receptor clusters. See reference 6
- Figure 6 - Effect of patient antibodies on NMDA receptor-mediated synaptic currents. See reference 6