# ROLES OF ANTIBODIES TARGETING NEUROTRANSMITTER RECEPTORS AND COMPLEMENT IN THE MODULATION OF GLUTAMATERGIC HIPPOCAMPAL NEUROTRASMISSION: RELEVANCE TO AUTOIMMUNE SYNAPTOPATHIES

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**Introduction:** Autoantibodies recognizing NMDA receptors are involved in the development of autoimmune encephalitis, typified by progressive psychosis and seizures. These autoantibodies recognize outer sequences of the GluN subunits, causing NMDA receptors hypofunction and increasing their internalization from plasma membranes, without affecting neurons viability. Complement is a key driver of inflammation and it is produced by glial cells and neurons in central nervous system. A dysregulation in the complement cascade occurs in several central diseases including psychosis. In particular, immune complexes (i.e. antibody/receptor) can increase complement activation through the classic pathway, but the question on whether complement could trigger anti-GluN-mediated synaptic derangements is still open. The aim of our study was to evaluate i) whether selective anti-GluN antibodies can modulate the function of NMDA receptors in hippocampal nerve endings and their mediated control of glutamate release and ii) whether anti-GluN antibodies can affect complement mediated responses.

**Material and methods:** Mouse hippocampal synaptosomes were pre-incubated with selective anti-GluN antibodies recognizing the outer sequence of the NMDA receptor proteins and labeled with [$^3$H]D-aspartate ([$^3$H]D-Asp). We evaluated the effect of these antibodies on [$^3$H]D-Asp release evoked by NMDA and mouse complement, in comparison with control synaptosomes. Concomitantly, we investigated the expression of GluN subunit proteins with confocal microscopy and biotinylation and immunoblotting analysis.

**Results:** The selective anti-GluN antibodies don't modify glutamate exocytosis elicited by depolarizing stimuli but prevent the NMDA autoreceptors-evoked release of [$^3$H]D-Asp from mouse hippocampal nerve endings. Incubation of synaptosomes with anti-GluN antibodies increase the internalization of the NMDA autoreceptors in nerve terminals, subtracting them from synaptic transmission. Complement releases glutamate from hippocampal synaptosomes but the incubation of synaptosomes with anti-GluN antibodies reduces, instead of increasing, the complement-mediated effect.

**Discussion and conclusion:** Our data unveil a cascade of events through which anti-GluN antibodies impair glutamatergic transmission in a complement-independent manner. Our results improve the comprehension of the cellular events accounting for disrupted synaptic plasticity in patients suffering of autoimmune anti-NMDA diseases.