Autoantibodies to the N-methyl-D-aspartate receptor and seizure susceptibility in mice

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Background and aims of study

Autoantibodies to neuronal surface proteins (Figure 1) are found in adult and paediatric seizure-related disorders such as NMDAR-Ab encephalitis and limbic encephalitis. Most patients present with seizures as well as other clinical features such as confusion, memory loss and movement disorder (Dalmau 2008, Irani 2010). Studies involving adult and paediatric epilepsy patients without encephalitis (Brenner 2013, Suleiman and Wright 2013) show CNS autoantibody positivity in 9-12%, NMDAR-Abs are one of the most commonly found. The aim of this study is to determine whether these antibodies are pathogenic and epileptogenic.

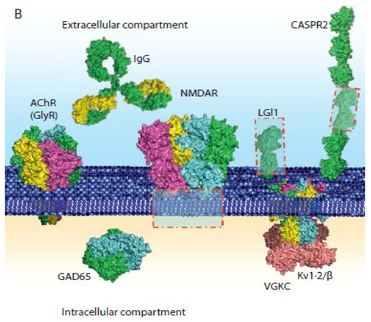


Figure 1. Neuronal surface antibody targets. From Vincent, Bien, Irani, Waters 2011.

Methods

Transmitter implantation

A 2 cm incision was made caudally over the skull surface. The transmitter was then placed beneath the skin at the left flank. The electrode leads with screws were positioned into drilled skull holes (Figure 2). Dental cement was used to hold the screws and wires in place. The EEG of the animals was continuously recorded using the telemetry system layout shown in Figure 3. The animals were allowed a minimum of 5 days to recover before ICV injections.

Figure 2. Transmitter placement and skull fixation of electrode leads.

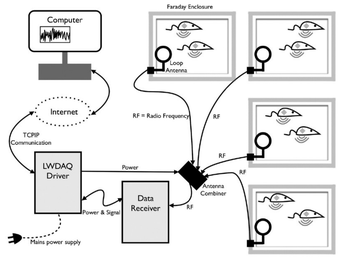
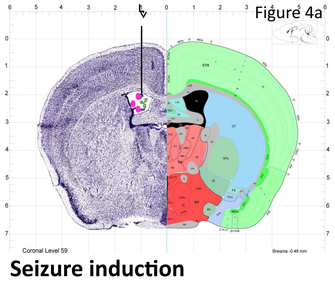


Figure 3. Main components of wireless telemetric system. From Changa, Hashemi, Walker 2011

Intracerebroventricular (ICV) injections

8 µl of purified IgG (9-25mg/ml) from the NMDAR-Ab patients (n=3) or healthy controls (HC) was injected at a depth of 1.85 mm into the lateral ventricle. 300 nl of fluorescent beads (Lumafluor Inc.) were injected with the IgG to verify the position of injection post-mortem (Figure 4).



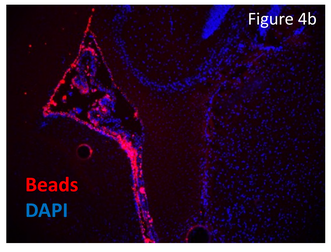
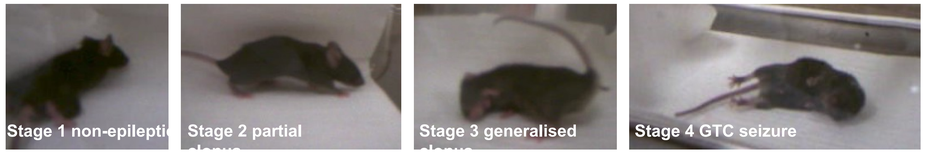


Figure 4. Schematic showing co-ordinates of the ICV injection on a mouse brain atlas (4a). Coronal brain section showing fluorescent beads lining ventricle confirming the site of IgG injection was correct (4b).

Seizure induction

A subthreshold i.p. dose of the proconvulsant Pentylenetetrazol (PTZ) was given 48h post ICV injection of IgG, mice were then observed for 60 mins. Severity staging, frequency of seizures, and total seizure score were recorded (Weirergraber 2006, Luttjohann2009).



Results

We performed EEG telemetry in 15 mice, 9 injected with NMDAR-Ab positive IgG and 6 with HC IgG. Animals were coded until completion of analysis.

- Following seizure induction NMDAR-Ab IgG injected mice displayed more frequent, and severe seizures compared to HC IgG injected mice. Data was analysed using Fisher's exact test and the Mann-Whitney U test (Figure 5).

Behavioural response to subthreshold PTZ	Healthy control IgG (n=6)	NMDAR-Ab positive IgG (n=9)
Stage 3a: Generalised clonus with no loss of postural control	3/6	9/9*
Stage 3b: Generalised clonus with loss of postural control	0/6	1/9
Stage 3c: Wild jumping/running	0/6	2/9
Stage 4: Lethal seizure	0/6	0/9

*p= 0.04

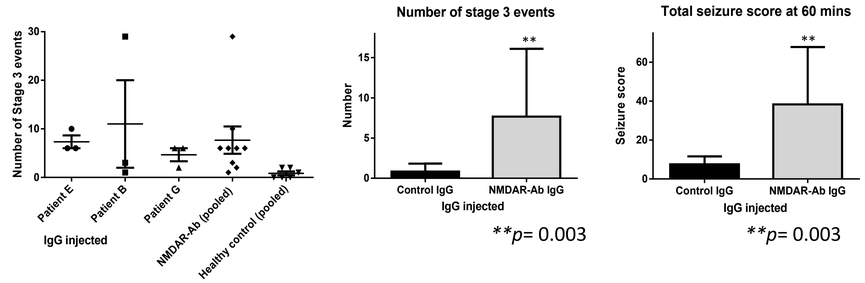


Figure 5. Analysis of observed seizures in 60 minutes following PTZ injection.

- In-vivo electrophysiology recordings were used to detect epileptiform activity (Figure 6). A library of signature events was created to analyse the EEG recordings of all mice (Figure 7). This analysis showed that recurrent spontaneous seizures were not seen post ICV injection of NMDAR-antibody positive IgG.

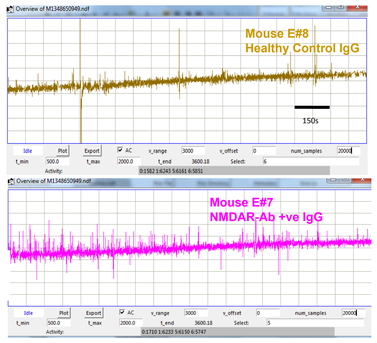


Figure 6. The increased epileptic activity seen in the NMDAR-Ab injected mice after PTZ is demonstrated in these example raw EEGs.

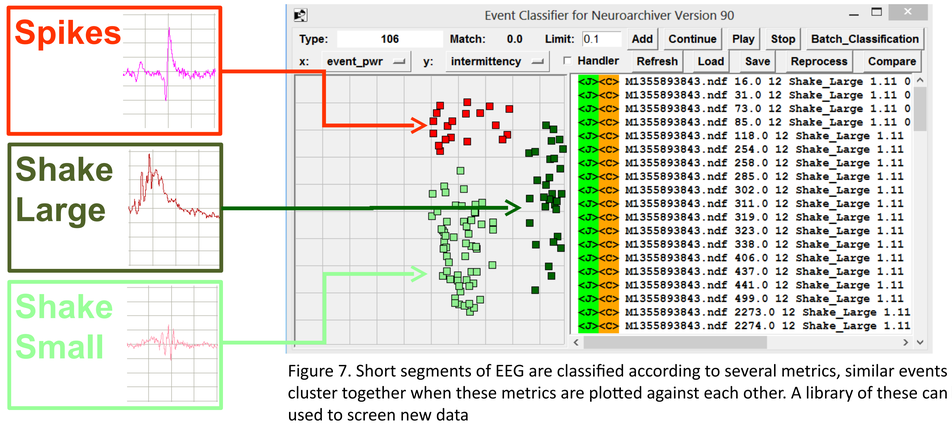
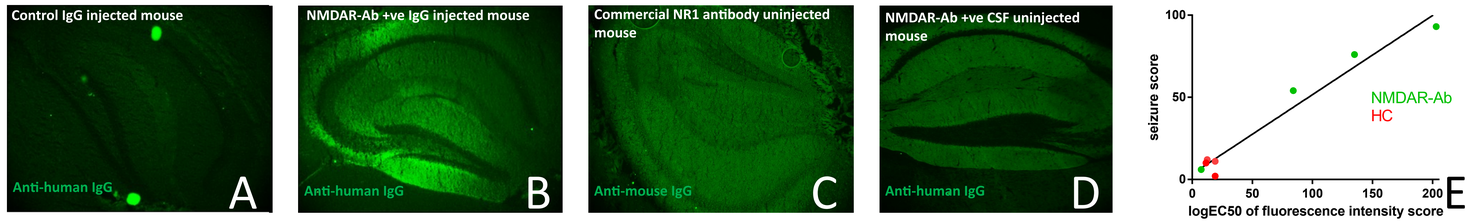


Figure 7. Short segments of EEG are classified according to several metrics, similar events cluster together when these metrics are plotted against each other. A library of these can be used to screen new data

- NMDAR-Ab IgG binds strongly to mouse hippocampus at 48 hours post injection (B). Control IgG does not remain detectable in the hippocampus (A). The binding pattern is similar to both commercial NR1 antibody (C) and NMDAR-Ab positive CSF staining on uninjected WT mouse sections (D). The fluorescence intensity of the IgG binding in injected mice is proportional to the severity of seizures following seizure induction (E).



Summary and future work

This study demonstrates that passive transfer of NMDAR-Ab +ve IgG increases their susceptibility to seizures. The NMDAR-Ab +ve IgG has been able to access the brain tissue and bind specifically to the NMDARs in *vivo*. Higher levels of bound IgG correlate with higher seizure scores, suggesting a dose response effect. Future work will concentrate on completing EEG and tissue analysis.