Pre-and postsynaptic effects of LGI1 autoantibodies in a murine model of limbic encephalitis

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Pre- and postsynaptic effects of LGI1 autoantibodies in a murine model of limbic encephalitis

This scientific commentary refers to 'LGI1 antibodies alter $K_v1.1$ and AMPA receptors changing synaptic excitability, plasticity and memory', by Petit-Pedrol *et al.* (doi:10.1093/brain/awy253).

Limbic encephalitis is a neurological disease characterized by psychiatric symptoms, episodic memory loss and seizures. A subset of autoimmune limbic encephalitis patients possess autoantibodies that immunoprecipitate a protein complex containing the dendrotoxin (DTx) target potassium channel (K_v1). In more than a third of limbic encephalitis patients who test positive in this assay, the immunoprecipitating autoantibodies are directed against LGI1 (leucine-rich glioma-inactivated 1), a secreted glycoprotein composed of a leucine-rich N-terminal domain (LRR) and a C-terminal β-propeller also known as the epitempin domain (EPTP). Mutations in LGI1 are linked to early-onset autosomal dominant lateral temporal lobe epilepsy (ADLTE). Haploinsufficiency seems to be the major consequence of these mutations since about 30% of the mutations are premature stops and most of the others lead to substitutions resulting in either protein instability or impaired LGI1 secretion. LGI1 directly binds the inactive metalloproteases and cell adhesion surface proteins ADAM22 and ADAM23 in complexes containing membrane-associated guanylate kinases (MAGUKs) and the cell adhesion molecules Caspr2 and contactin 1 (Fukata *et al.*, 2010; Ogawa *et al.*, 2010). It is therefore suggested that LGI1 mediates a trans-synaptic complex, linking—through ADAM proteins—presynaptic K_v1 channels and postsynaptic glutamate receptors of the AMPA subclass, and coordinating several aspects of synaptic development and function. The role of LGI1 in neuronal excitability has mostly been examined in epileptic $Lgi^{-/-}$ animals, where deletion of the *Lgi1* gene leads to spontaneous severe epileptic seizures (Chabrol *et al.*, 2010). Enhanced excitatory neurotransmission in $Lgi^{-/-}$ mice (Yu *et al.*, 2010) contrasts with the reported reduction in AMPA receptor function (Fukata *et al.*, 2010; Ohkawa *et al.*, 2013) and therefore raises questions over the molecular substrate of the increase in excitatory neurotransmission. Furthermore, the loss of AMPA receptor function in inhibitory interneurons cannot account for an overall increase in excitability since conditional *LGI1* knock-out in inhibitory parvalbumin interneurons does not confer an epileptic phenotype (Boillot *et al.*, 2014). In a recent report it has been shown that in $Lgi^{-/-}$ animals, the increase in neuronal excitability results from a massive decrease in axonal and presynaptic K_v1 channel expression (Seagar *et al.*, 2017). This downregulation presumably occurs earlier than the perturbation of AMPA receptor expression. These data suggest that LGI1 regulates action potential firing by setting the density of the axonal K_v1 channels that underlie DTx-sensitive D-type potassium current. *In vitro* electrophysiological recordings in acute hippocampal slices confirmed the pathogenic nature of limbic encephalitis-immunoglobulin G antibodies (LE-IgGs) and showed an increase in neuronal excitability (Lalic *et al.*, 2011), mimicking the DTx effect. At the molecular level, using a cell ELISA assay, Ohkawa *et al.* showed that limbic encephalitis anti-LGI1 autoantibodies inhibit binding of LGI1 to cells expressing ADAM22 and 23 and that these autoantibodies recognize both LRR and EPTP domains of LGI1. In parallel, a decrease in AMPA receptor expression levels was observed in autoantibody-treated hippocampal cultures (Ohkawa *et al.*, 2013). Hence, analyses of autoimmune and genetic disease mechanisms suggest that LGI1 normally restricts neuronal excitability, but its absence either due to a mutation or to antibody-mediated disruption causes increased excitability that results in seizures. In this issue of *Brain*, Petit-Pedrol and co-workers take a substantial step forward in establishing a murine limbic encephalitis model and in characterizing the physiological effect of LE-IgGs through the analysis of synaptic plasticity in hippocampal mouse brain slices and memory deficits in behaving animals after cerebroventricular antibody infusion (Petit-Pedrol *et al.*, 2018).

First, they confirmed that anti-LGI1 autoantibodies (LE-IgGs) target both LRR and EPTP domains of LGI1 and disrupt the interaction with ADAM22 and ADAM 23. Then, using Bassoon and PSD95 as pre- and postsynaptic markers, respectively, they investigated the expression level of K_v1 as well as AMPA receptors. Anti-LGI1 autoantibody infusion in living mice led to a decrease in total and synaptic hippocampal levels of the K_v1 potassium channel family member $K_v1.1$ as well as AMPA receptors at different time scales. They show that perturbation of K_v1 expression precedes AMPA receptor downregulation (occurring 13 days versus 18 days post-infusion, respectively; Fig. 1A). They also show that these modifications are reversible after Day 26, presumably due to antibody washout (dissociation/degradation). This reversibility is particularly important as systemic manifestations in limbic encephalitis patients are reversed after treatment.

Next, Petit-Pedrol and co-workers examined the effects of LE-IgGs on

synaptic transmission, synaptic plasticity and memory. First, they analysed excitatory post-synaptic currents (EPSCs) recorded in CA1 pyramidal neurons and evoked by minimal stimulation of the Schaffer collaterals. The EPSC failure rate was reduced in mice that received LE-IgGs compared to mice that received control IgGs, suggesting an increase in glutamate release compatible with the targeting of LE-IgG to presynaptic K_v1 channel-containing complexes. Then, they examined the effect of LE-IgG on the synaptic dynamics of another synaptic pathway (namely, the medial perforant path of the dentate gyrus that contacts granule cells), that is specifically enriched in LGI1 proteins. The paired-pulse ratio at this synapse was reduced in mice receiving LE-IgGs compared to mice receiving control IgGs, confirming the presynaptic excitability increase induced by LE-IgGs (Fig. 1B).

As limbic encephalitis is characterized by memory deficits, Petit-Pedrol *et al.* examined whether LE-IgGs affect synaptic plasticity. The magnitude of long-term synaptic potentiation (LTP) induced by theta burst stimulation (TBS) was reduced by half in mice treated with LE-IgGs compared to controls (Fig. 1C). While the reason for reduced LTP

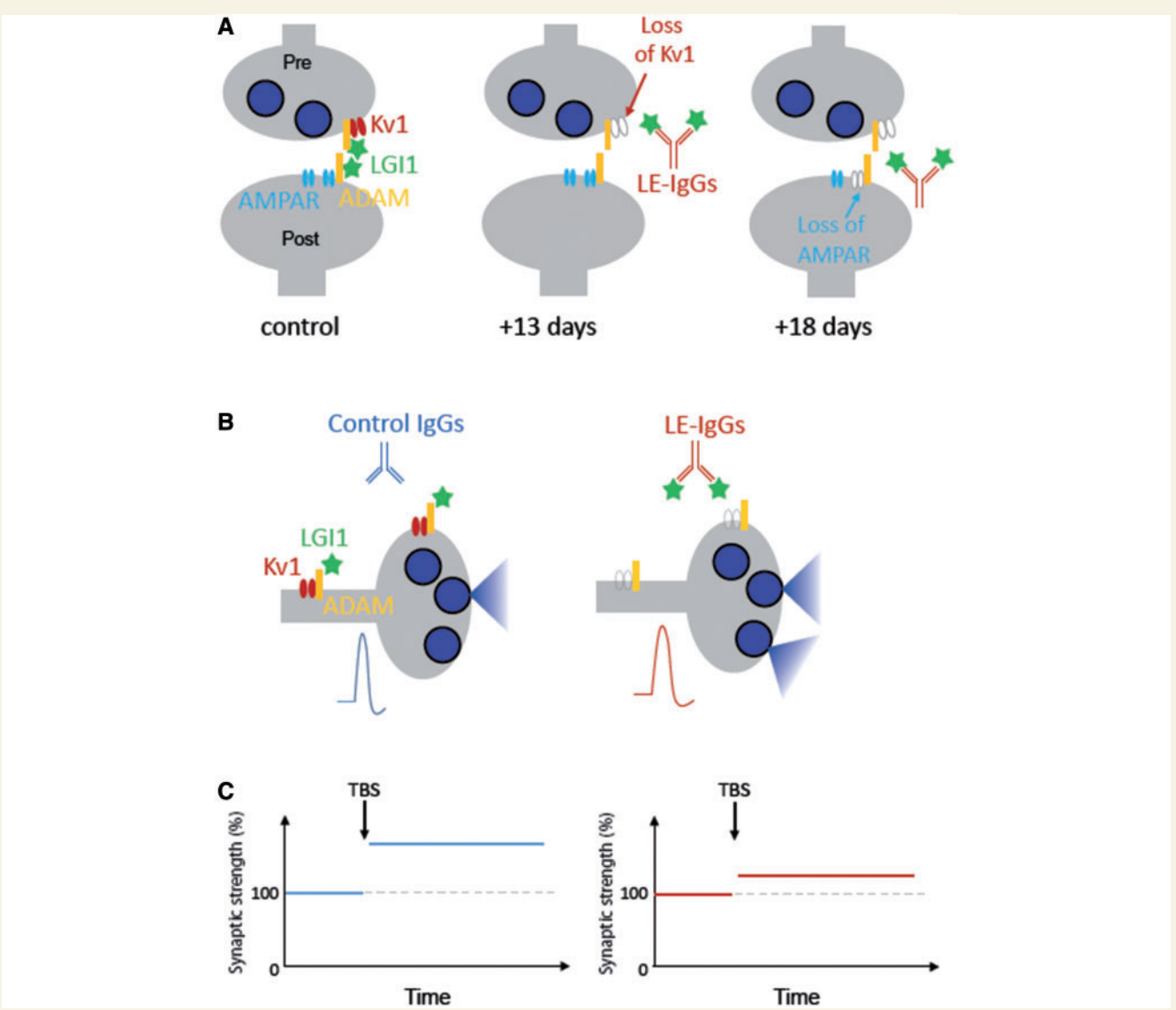

Figure 1 Effects of anti-LGI1 antibodies. (**A**) Time course of the reduction in K_v1 channels and AMPA receptors following infusion of LE-IgGs. *Left*: control. *Middle*: 13 days after LE-IgG infusion, presynaptic K_v1 channels are suppressed. *Right*: at +18 days, the number of AMPA receptors is reduced. (**B**) Impact of LE-IgGs on presynaptic release. *Left*: in the presence of control IgGs, presynaptic K_v1 channels are functional and reduce the duration of the presynaptic action potential, thus limiting glutamate release. *Right*: in the presence of LE-IgGs, the absence of presynaptic K_v1 channels broadens the presynaptic action potential and increases presynaptic release. (**C**) Reduced LTP in the presence of LE-IgGs. Compared to the control situation (*left*), the magnitude of LTP in response to theta burst stimulation (TBS) was reduced by about half in the presence of LE-IgGs (*right*).

Glossary

ADAM: 'A disintegrin and metalloproteinase' protein, a member of a family of metalloproteinase and cell adhesion molecules.

Caspr2: Contactin associated protein 2, a cell adhesion molecule important in voltage-gated potassium channel localization and a target of the autoimmune response in a subset of limbic encephalitis patients.

Haploinsufficiency: The condition where only one copy of a wild-type allele is available at a genetic locus. In patients with ADLTE, a single allele gives rise to functional LGI1 and the copy number of the wild-type protein is significantly diminished.

MAGUK: Membrane-associated guanylate kinase homologues. These are intracellular multimodular proteins containing an inactive guanylate kinase-like domain, an SH3 domain and several PDZ domains. They provide specialized sub-membrane scaffolds in junctional regions.

was not specifically examined, it did not result from the impairment of presynaptic K_v1 channels since blockade of these channels with DTx did not reduce LTP induced in the presence of control IgGs. Many reasons for this decrease in synaptic potentiation can, however, be envisaged. As Petit-Pedrol *et al.* propose, it may result from perturbation in AMPA receptor turnover at glutamatergic synapses. In fact, reduced LTP may be due to insufficient AMPA receptor incorporation during LTP resulting from disruption of the trans-synaptic linker function of LGI1. Alternatively, the reduced LTP observed in mice treated with LE-IgGs may result from spontaneously occurring epileptiform discharges of the type observed in limbic encephalitis patients. Indeed, even very brief episodes of epileptiform activity are known to induce NMDA receptor-dependent LTP that may occlude induction of additional synaptic potentiation by conventional protocols (Debanne *et al.*, 2006).

Finally, Petit-Pedrol and co-workers examined whether LE-IgGs induce memory deficits. For that they used the novel object recognition test. This is used to probe recognition memory deficits and involves comparing the time that a mouse spends exploring a new object versus familiar objects. If the mouse has normal recognition memory, it will spend more time exploring the novel object than the familiar objects. Using their murine model of limbic encephalitis, Petit-Pedrol *et al.* found that mice that received LE-IgGs had a much lower memory index than mice that received control IgGs. However, this difference in memory performance disappeared about 30 days after the infusion of LE-IgGs ended. Importantly, the impaired memory performance in mice treated with LE-IgGs was not due to reduced locomotor activity or to enhanced anxiety, as both of these remained comparable in the two groups.

The paper by Petit-Pedrol and co-workers provides stimulating and innovative results regarding the perturbation of synaptic function, plasticity and memory by LE-IgGs. However, several issues remain unresolved. First, it is still not clear whether the downregulation of AMPA receptors results from a direct LGI1-dependent alteration or from a homeostatic process downstream to the increased network activity in LE-IgG-treated mice. Second, the precise origin of the reduced LTP in LE-IgG-treated mice has not been established in this study: future studies should attempt to distinguish between an alteration in AMPA receptor recycling and the partial occlusion of LTP by uncontrolled epileptic seizures. At the molecular level, it will be important to examine whether LE-IgGs disrupt LGI1 dimerization, and whether both anti-LRR and anti-EPTP antibodies are pathogenic and if they induce different effects. It also remains to be determined whether there are antigenic hotspots inside the LRR or the EPTP regions. No doubt all of these questions will be answered in the near future.

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Competing interests

The authors report no competing interests.

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Listening for the rhythm of a conscious brain

This scientific commentary refers to 'Robust EEG-based cross-site and cross-protocol classification of states of consciousness', by Engemann *et al.* (doi:10.1093/brain/awy251).

The question of whether someone is conscious of themselves and their environment may initially appear to be a philosophical thought experiment. However, for many families and clinicians throughout the world, it is a challenge with profound implications for decision-making after severe brain injury. The current standard approach to answering the question of consciousness in the clinic is to observe the patient's behaviour for evidence of purposeful action, such as tracking a moving object. However, because of inequalities in healthcare provision both between and within countries, many patients never receive standardized behavioural assessment. Consequently, misdiagnoses of so-called prolonged disorders of consciousness (PDOC) occur at alarmingly high rates: ~40% when standardized assessment is not available and ~30% when a standardized assessment is not conducted longitudinally (Wannez *et al.*, 2017). With the recent confirmation from the UK's Supreme Court that withdrawal of clinically assisted nutrition and hydration from individuals with PDOC may occur without the involvement of the courts (UK Supreme Court, 2018), access to accurate diagnostic tools is all the more imperative. In this issue of *Brain*, Engemann and co-workers present results from a trans-European collaboration that offer hope for accurate, accessible, and objective diagnoses of consciousness in this challenging patient group (Engemann *et al.*, 2018).

Over the past decade, researchers have identified an array of markers within the EEG of patients with PDOC that differ between those who are conscious (i.e. the minimally conscious state) and those who are entirely unconscious [i.e. the vegetative state, also known as unresponsive wakefulness syndrome (UWS)]. These markers may be task-based changes in the EEG that occur in response to stimuli, such as sounds or verbal instructions, or task-free features of the patient's EEG at rest. Engemann *et al.* (2018) apply advances in machine learning to the largest ever dataset of PDOC brain data and demonstrate that a combination of theoretically- and empirically-motivated EEG markers can accurately diagnose patients' levels of consciousness.

To test the efficacy of a machine learning method, researchers first require a set of training data for which they and their algorithm know the 'truth'—i.e. which data correspond to patients who are conscious by standardized assessment, and which belong to those who are not conscious. The researchers then apply the trained model to previously unseen test data from one patient in order to estimate their 'true' diagnosis. Crucially, the model described by Engemann *et al.* (2018) accurately diagnoses patients even when the training and test data were recorded in different countries, with different EEG equipment, and different EEG protocols. The success of this model, therefore, creates the real possibility for an objective online tool that families and clinicians across the world could use with locally recorded EEG data, and that could consequently improve diagnostic accuracy and subsequent decision-making. Indeed, through a range of stress tests, the authors demonstrate that their model performs well with only a few minutes of data recorded from 16 electrodes; fewer than the 19-channel EEG montage typically available in clinical EEG protocols.

While the diagnostic accuracy of the model is impressive, there is nevertheless potential for the model to misdiagnose patients due to fluctuations in levels of consciousness over time. Indeed, clinicians must conduct multiple behavioural assessments of consciousness before they can achieve a stable and accurate diagnosis (Wannez *et al.*, 2017). Equally, the level of consciousness that is evident in a patient's EEG is likely to fluctuate both within and across days, and may therefore require multiple EEG sessions before a stable diagnosis can be reached. Importantly, as the diagnostic model described by Engemann *et al.*, 2018 is robust with brief EEG recordings from a range of EEG systems and protocols, these necessarily longitudinal assessments of EEG may be more tractable now than ever before.

The authors report that power in the alpha band of the EEG (8–12 Hz) was consistently the most informative feature used by the models for accurate diagnosis. Alpha oscillations are the most prominent rhythm in the