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FULL-LENGTH ORIGINAL RESEARCH

Human limbic encephalitis serum enhances hippocampal mossy fiber-CA3 pyramidal cell synaptic transmission

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Summary

Purpose: Limbic encephalitis (LE) is a central nervous system (CNS) disease characterized by subacute onset of memory loss and epileptic seizures. A well-recognized form of LE is associated with voltage-gated potassium channel complex antibodies (VGKC-Abs) in the patients' sera. We aimed to test the hypothesis that purified immunoglobulin G (IgG) from a VGKC-Ab LE serum would excite hippocampal CA3 pyramidal cells by reducing VGKC function at mossy-fiber (MF)-CA3 pyramidal cell synapses.

Methods: We compared the effects of LE and healthy control IgG by whole-cell patch-clamp and extracellular recordings from CA3 pyramidal cells of rat hippocampal acute slices.

Results: We found that the LE IgG induced epileptiform activity at a population level, since synaptic stimulation elicited multiple population spikes extracellularly recorded in the CA3 area. Moreover, the LE IgG increased the rate of tonic firing and strengthened the MF-evoked synaptic responses. The synaptic failure of evoked excitatory postsynaptic currents (EPSCs) was significantly lower in the presence of the LE IgG compared to the control IgG. This suggests that the LE IgG increased the release probability on MF-CA3 pyramidal cell synapses compared to the control IgG. Interestingly, α-dendrotoxin (120 nM), a selective Kv1.1, 1.2, and 1.6 subunit antagonist of VGKC, mimicked the LE IgG-mediated effects.

Conclusions: This is the first functional demonstration that LE IgGs reduce VGKC function at CNS synapses and increase cell excitability.

KEY WORDS: Limbic encephalitis, Synaptic transmission, Hippocampal mossy fiber, Leucine-rich glioma-inactivated gene-1 (Lgi1), K^+ channels, α-Dendrotoxin.

Limbic encephalitis (LE) is a central nervous system (CNS) autoimmune disease characterized by memory loss, psychologic disturbance, and epileptic seizures; it is usually associated with high signal in the hippocampal regions of the medial temporal lobe(s) on magnetic resonance imaging (MRI). Although traditionally found in patients with specific tumors (Dalmau & Rosenfeld, 2008), it is increasingly recognized in patients without tumors and with serum antibodies that immunoprecipitate α-dendrotoxin (α-DTX)–binding voltage-dependent K^+ channel (VGKC, subunits Kv1.1, Kv1.2, and Kv1.6) complexes extracted from mammalian brain tissue (Buckley et al., 2001; Thieben et al., 2004; Vincent et al., 2006). Immunotherapies that reduce the VGKC–antibody (Ab) levels lead to substantial clinical improvement, strongly suggesting that antibodies are causing this condition (Vincent et al., 2004). The sera of patients with LE strongly label mossy fibers (MFs), apparently colocalizing with Kv1.1 and also partly overlapping with Kv1.2 subunits, other hippocampal axon terminal areas, cerebellum, and to a less extent spinal cord (Kleopa et al., 2006). Because Kv1.1 channels play crucial roles in hippocampal excitability and nerve conduction (Dodson & Forsythe, 2004), it seems likely that these channels or associated proteins are the main target for the VGKC-Abs. Indeed, Kv1.1 knockout mice develop seizures (Smart et al., 1998; Rho et al., 1999) resembling both electrographic and behavioral features observed in rodent models of temporal lobe epilepsy (Wenzel et al., 2007). Hence, alteration of VGKC function at hippocampal MFs is likely an essential mechanism underlying the clinical phenotypes of LE, such as seizures, agitation, hallucinations, and memory impairment.

The aim of this study was to test the hypothesis that the immunoglobulin G (IgG) of the VGKC-Ab LE patient enhances pyramidal cell excitability and hippocampal MF neurotransmission by affecting the function of α-DTX-sensitive VGKCs.

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Material and Methods

Preparation of acute slices

All procedures involving animals were performed according to methods approved by the United Kingdom Home Office and The Animals (Scientific Procedures) Act, 1986. Every effort was made to minimize the number of animals used and their suffering. Male postnatal day 14–24 Sprague-Dawley rats were anesthetized with inhalation of isoflurane, decapitated, and their brain quickly removed and placed into ice-cold high-magnesium artificial cerebrospinal fluid (ACSF; composition in mM: 85 $NaCl_2$, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgCl_2$, 10 glucose, 75 sucrose) saturated with 95% O_2 and 5% CO_2, at pH 7.3. Horizontal sections (325 μm) were prepared consisting of the dorsal hippocampus and attached entorhinal cortex, which were allowed to recover in recording ACSF (same as in the preceding text, but 130 $NaCl_2$, 2 $CaCl_2$, 2 $MgCl_2$) at room temperature for at least 45 min. Next, slices were transferred to another storage containing the preceding ACSF and treated in either the LE or control IgG at room temperature for at least 2 h before and during the recording.

IgG preparation and application to the slices, immunohistochemistry

IgG was purified using protein G sepharose (Sigma-Aldrich, Poole, United Kingdom) from the plasma of one patient with highly increased VGKC antibodies (>5,084 pM at first testing; control values <100 pM). Control IgG was prepared from one healthy age-matched individual. The IgG preparations (5 mg/ml) were dialyzed against ACSF overnight at 4°C and diluted to 98 μg/ml for each electrophysiologic experiment. For immunohistochemical experiments, rat tissue was snap frozen in isopentane chilled with liquid nitrogen and sagittally sectioned. Tissue sections (10 μm) were consecutively incubated with 10% goat serum for 1 h, and patient/control IgG (diluted 1:100) for 2 h at room temperature. Bound IgG was detected with goat anti-human IgG 488 1:1,000 (Invitrogen, Paisley, United Kingdom).

Electrophysiology and data analysis

Acute slices were placed in a recording chamber mounted on the stage of an upright microscope (Axioscope, Zeiss, Jena, Germany), superfused with recording ACSF, LE, or control sera and maintained at a temperature of 34°C (bubbled with 95% O_2, 5% CO_2). Whole-cell patch-clamp recordings were performed using an EPC9/2 amplifier (HEKA, Lambrecht/Pfalz, Germany) from visually identified CA3 pyramidal cells in current or voltage clamp mode (holding potential −65 mV). Borosilicate glass capillaries were used (4–7 MΩ) and filled with an intracellular solution containing: (in mM) 126 K-gluconate, 10 HEPES, 10 Na_2-phosphocreatine, 4 KCl, 4 Mg-ATP, 0.3 Na-GTP, pH 7.3 with KOH, osmolarity 280–290 mOsm. The series resistance was monitored online throughout the experiments to check for stability, and was compensated up to 60% to reduce voltage errors. The resting membrane potential was not corrected for the liquid junction potential. Spontaneous action potential firing was recorded in current clamp mode without applying any DC current. Membrane currents were amplified (10 mV/pA), filtered at 2.9 kHz, and digitized at 5 kHz. The currents were acquired online with ''Pulse'' software (HEKA) and analyzed offline with MiniAnalysis (Synaptosoft, Decatur, GA, U.S.A.), Pulsefit (HEKA), and Igor Pro 5.05 (Wavemetrics, Lake Oswego, OR, U.S.A.). The input resistance was calculated from the slope of a line fitted to the subthreshold range on a plot of the injected current versus the steady-state membrane voltage when a family of hyperpolarizing and depolarizing current injections was applied. Cell responses to current injection of 1 s duration ranging from 50–250 pA with an increment of 50 pA were measured at −65 mV to construct the plot of firing frequency versus intensity of stimulation. To study membrane fast and slow afterhyperpolarization (fAHP and sAHP), a depolarizing current step (respectively, 3 and 25 ms × 500–1,000 pA) was applied and the peak amplitude of the AHP after a single or three action potentials, respectively, was measured from the baseline (−65 mV). For minimal stimulation recordings, patch pipettes filled with ACSF were used as monopolar stimulation electrodes (intensities: 7–13 μA, 0.03 Hz) and were placed near the recorded neurons. Minimal stimulation was applied as detailed previously (Kerr & Capogna, 2007); namely, the stimulus intensity was gradually decreased until the amplitude of events was small, all-or-none, with numerous failures identified by the amplitude being threefold less than the standard deviation of the baseline current. The synaptic response once visually identified and measured using Pulsefit software, was defined as the point at which the amplitude could be visualized to deviate from background noise levels. A failure was recorded as a trial in which no event could be visually detected and the failure rate was calculated as the number of failures divided by the number of trials. The unitary event peak amplitude was visually delimited and then measured using Pulsefit. The amplitude values either included failures as 0 pA or excluded (where stated) and were averaged for 20 trials. When a paired-pulse protocol was used, the two stimuli were evoked with a 25-ms interval.

Extracellular field postsynaptic potentials (fEPSPs) and population spikes were evoked in response to extracellular stimulation of MFs using a monopolar stimulating electrode and recorded with a patch pipette filled with ACSF solution in the CA3 pyramidal cell layer or stratum lucidum. The intensity of the stimulation used was the one that evoked a submaximal response of the first extracellular response (about two-thirds of the peak amplitude of maximal response). Extracellular potentials were analyzed using the coastline bursting index (CBI) (Korn et al., 1987), which measures the total length of the waveform of multiple population spikes. Specifically, one cursor was set immediately

after the end of the stimulus artifact and another after the end of the synaptic response. The total coast length of the line between the two cursors was calculated with a user-defined program in Matlab (Mathworks, Natick, MA, U.S.A.). The following statistical tests performed with SPSS 17.0 (SPSS Inc., Chicago IL, U.S.A.) were used: unpaired or paired *t*-test, Wilcoxon test, and one way analysis of variance (ANOVA) with Bonferroni post hoc test. Values presented in the text and in figures represent the mean ± standard error of the mean (SEM).

Chemicals and drugs

All drugs or sera were superfused to the slices through the bath solution. Salts used for the patch pipette solution and ACSF were obtained from either VWR International or Sigma. Drugs were added at the following concentrations: α-dendrotoxin (α-DTX, 120 nM, Latoxan, Valence, France) and tetrodotoxin (TTX, 1 μM; Tocris Cookson Inc, Avonmouth, United Kingdom).

Results

Clinical presentation and immunoreactivity of LE IgGs in the rat hippocampus

We used purified IgG from a 57-year-old male patient. He experienced subacute onset of behavioral change and frequent, short-lived ''panic attacks'' in 2004. He subsequently developed impaired episodic and spatial memory and presented to the neurologists 5 months after symptom onset. They found increased medial temporal lobe signal on MRI (Fig. 1A), and confirmed that his ''panic attacks'' were complex partial seizures. Neuropsychometry revealed mild intellectual underfunctioning (Performance IQ 92, Verbal IQ 99), mild word-retrieval problems, and poor

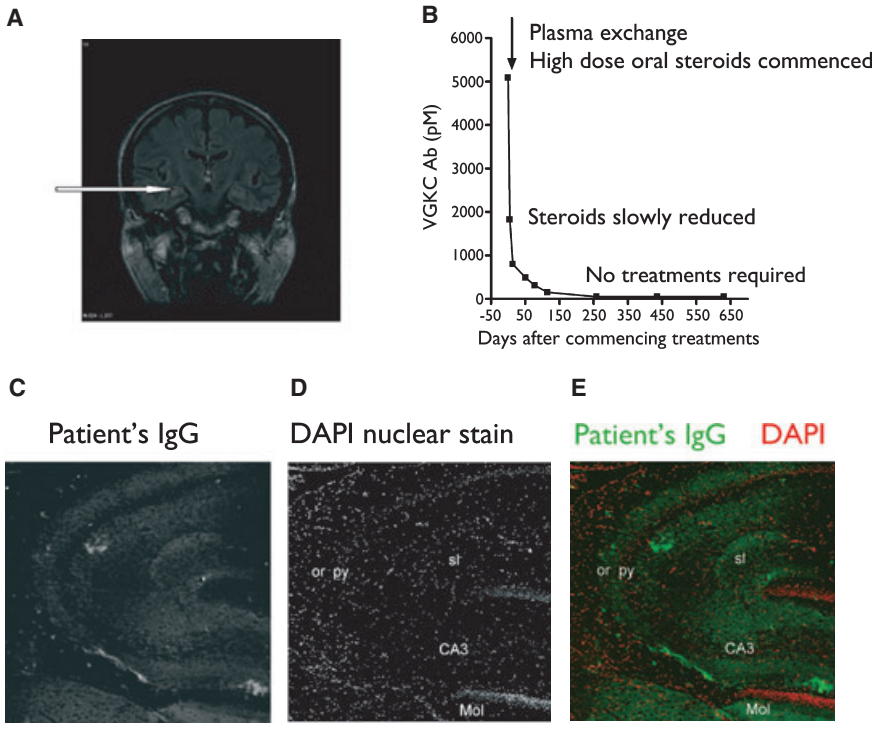

Figure 1.

(**A**) Increased medial temporal lobe signal on MRI of brain 2 years after onset of seizures. (**B**) Serial estimations of antibodies to VGKC detected by immunoprecipitation of 125I-dendrotoxin-labeled VGKCs extracted from rabbit brain tissue. After the initial diagnosis, based on the clinical picture of LE (seizures, cognitive dysfunction, psychological disturbance, and high signal on MRI), the patient was treated with plasma exchange to reduce circulating antibodies and started on oral high dose corticosteroids. The antibodies dropped rapidly and normalized within 6 months. The steroids were withdrawn slowly during the next 10 months. After the treatment, the patient displayed only mild residual cognitive defects and no seizures. (**C**) Immunostaining of rat hippocampal area with the patient's IgG, purified from the plasma exchange plasma, detected with goat anti-human IgG. (**D**) Binding of 4',6-diamidino-2-phenylindole (DAPI) to stain for nuclei. (**E**) Merged image of (**C**) and (**D**). Patient's IgG binding (green) and neuronal nuclei (red) showing binding of IgG antibodies to the molecular layer, the hilus, the stratum lucidum, and the stratum radiatum (×10). This binding is typical of VGKC antibodies at similar high titers (Buckley et al., 2001; Kleopa et al., 2006). Or, stratum oriens; Py, stratum pyramidale; sl, stratum lacunosum moleculare; Mol, stratum moleculare.

performance on tests of executive function and speed and attention. He performed adequately on tests of verbal and nonverbal memory, but had evidence of temporally ungraded retrograde memory impairment. His VGKC-Abs were significantly elevated at 5,084 pmol (normal values <100 pM). Treatment with plasma exchange followed by oral prednisolone was associated with a marked decline in antibody titers to normal levels (Fig. 1B), cessation of seizures, and much improved cognitive function. Over 4 years later, the patient still has some mild residual memory impairment, but has had no further seizures.

We tested the immunoreactivity of the IgGs of this patient on frozen sections of adult rat brain. We found that the hippocampus and the cerebellum were the two areas prominently labeled, as previously reported for the sera of other LE patients (Kleopa et al., 2006). Specifically, the serum of our patient labeled the hippocampal axon terminal areas such as the molecular layer, the hilus, the stratum lucidum, and the stratum radiatum, whereas the immunoreactivity in the CA3 and CA1 stratum pyramidale or the granule cell layer was less evident (Fig. 1C–E). Because the clinical manifestations—that is, complex partial seizures and impaired episodic and spatial memory—impinge on typical hippocampal functions, we decided to focus on the functional effects of the patient serum on this area. We tested the CA3 area, since this region has a particularly low threshold for seizures (Schwartzkroin, 1994), and is directly involved in spatial memory (Singer & Frank, 2009).

LE IgG desynchronizes CA3 extracellular field potentials

We tested the effects of purified IgG from this patient with LE and VGKC-Abs (LE IgG) and from a healthy individual (control IgG) in rat acute hippocampal slices. First, we used extracellular stimulation of MFs and extracellular recording in the stratum lucidum of CA3 pyramidal cell layer to monitor the simultaneous activity of hundreds of pyramidal cells. Interestingly, we observed in some slices, but not all, a pronounced epileptiform activity consisting of multiple population spikes in acute slices treated with the LE IgG (Fig. 2). By contrast, slices treated with control IgG or ACSF produced a single population spike observed at short latency after the stimulation (Fig. 2). The analysis performed by using a CBI, which is used as a measure of the epileptiform activity (Korn et al., 1987), indicated that the extracellular response was significantly altered by the LE IgG ($p < 0.005$, $n = 10$, 13, and 12 for LE IgG, control IgG, or ACSF, respectively).

LE sera enhances CA3 pyramidal cell excitability

Next, we examined the action of LE IgG at the single cell level. The CA3 pyramidal cells were normally silent at

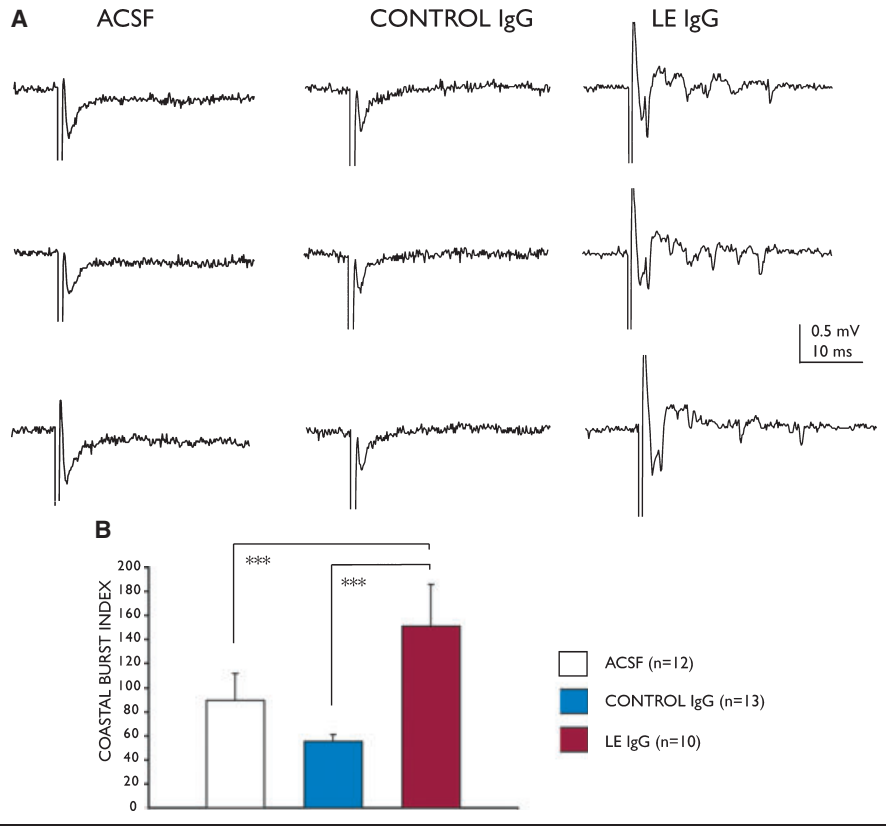

Figure 2.
LE IgG elicits epileptiform activity in the CA3 area of hippocampus. (**A**) Representative examples of extracellular potentials recorded in the stratum lucidum or CA3 pyramidal cell layer upon extracellular stimulation of MFs following the treatment of ASCF, control IgG, or LE IgG. (**B**) Pooled data illustrating coastal burst index. ***Represents significance between the groups, $p < 0.005$.
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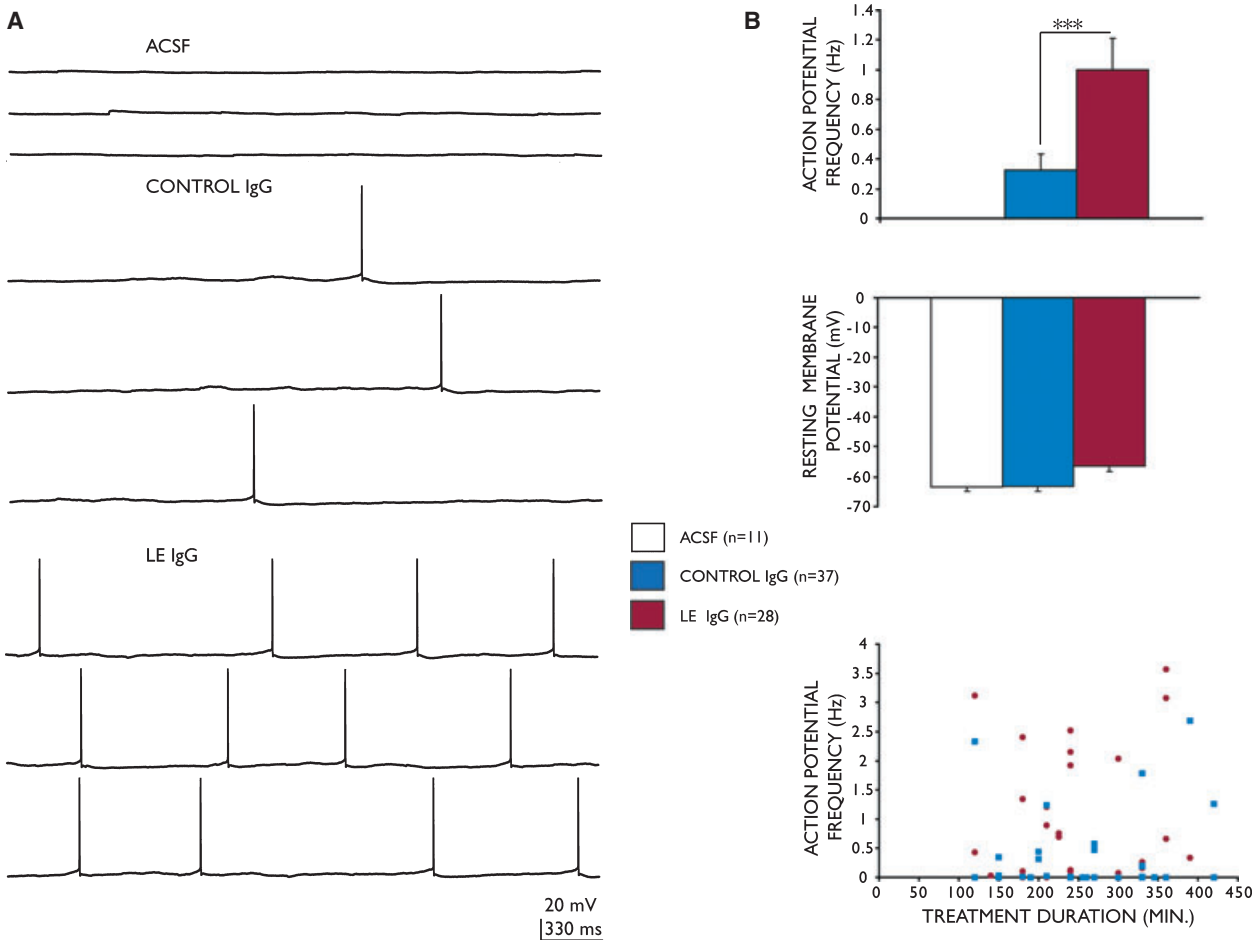

Figure 3.
LE IgG enhances CA3 pyramidal cell excitability. (**A**) Example of current clamp recordings showing action potential firing in CA3 pyramidal cells after being treated in ACSF (top), control IgG (middle), or LE IgG (bottom) at resting membrane potential −62, −62, and −59 mV, respectively. (**B**) Upper graphs: pooled current clamp data showing action potential frequency and resting membrane potential in response to three different conditions tested (*** denotes significance, $p < 0.005$, n = number of cells tested in this and following figures). (**B**) Lower scatterplot showing lack of correlation between the duration of the treatment of acute slices and the action potential frequency after an initial treatment of 2 h with the sera.

resting membrane potentials, and when treated with ACSF alone (Fig. 3A). The treatment of the acute slices with the LE-IgG elicited sustained action potential activity in the majority of cells tested (25 of 28, 89%) (Fig. 3A). The application of the control IgG produced action potential activity in 15 of 37 cells tested (40%). On average, the LE IgG evoked action potentials at a significantly higher frequency than those evoked by control IgG (0.99 ± 0.21 Hz versus 0.32 ± 0.11 Hz, $p < 0.05$). We also observed a trend toward more depolarized resting membrane potential in cells recorded from LE IgG–treated slices compared to control IgG or ACSF groups ($p = 0.068$, ANOVA) (Fig. 3B). The scatterplot of Fig. 3B shows that after an initial two hour period of treatment with LE IgG or control IgG (or ACSF), there was no correlation between the duration of the application of the LE IgG to the acute slices and the action potential frequency observed in the recorded cells. Interestingly, spontaneous burst activity was observed in cells tested with the LE IgG (n = 6 of 28 cells, Fig. 4A), and it was never present in naive slices. In these cells, the average number of bursts over 2 min was 2.3 ± 0.88, and the average number of action potentials in a burst was 26.3 ± 14.1 (Fig. 4B). Spontaneous burst activity was also observed in 2 of 37 cells from slices treated with the control IgG (two bursts for each cell with an average number of action potentials in a burst of 12 ± 3, Fig. 4B). We tested intrinsic membrane properties under current clamp conditions by injecting hyperpolarizing/depolarizing current pulses (see Material and Methods). Consistent with the marked increase in the occurrence of spontaneous firing in LE IgG–treated cells, we observed

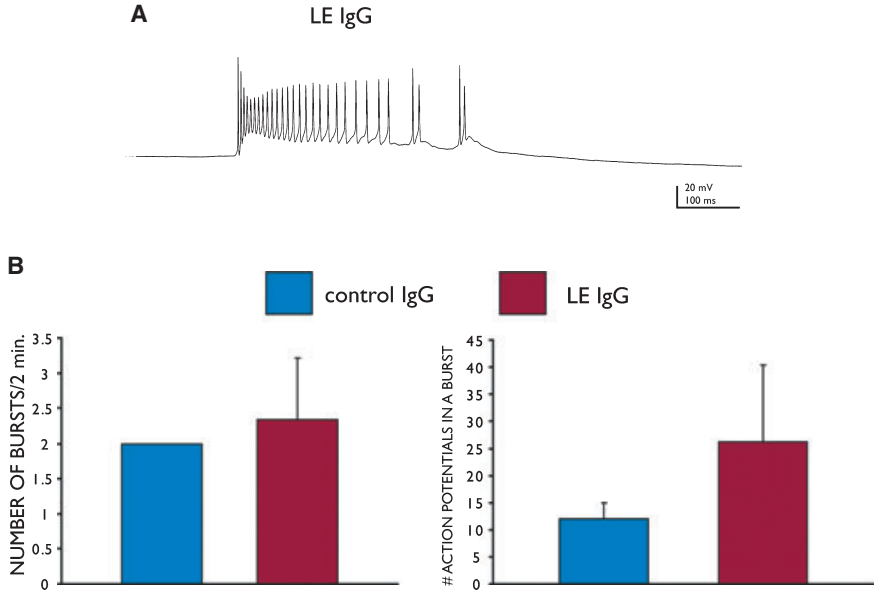

Figure 4.
LE IgG induces bursts firing in the CA3 pyramidal cell. (**A**) Example of current clamp recordings showing epileptiform activity in a CA3 pyramidal cell after being treated with LE IgG; the resting membrane potential was −60 mV. (**B**) Pooled data showing the average burst number over 2 min (left) and the average number of action potentials in a burst (right) occurring in control IgG (n = 2) or LE IgG (n = 6).
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that the frequency of action potentials elicited by depolarizing current pulses was enhanced in this group ($p < 0.05$, ANOVA, Fig. 5A). The input resistance was not significantly different in cells from control, control IgG, or LE IgG groups, although there was a trend toward higher values in the latter group (Fig. 5B). Furthermore, the peak amplitude of fAHP and sAHP occurring after a single and burst of action potential, respectively, was comparable in cells recorded from the three groups (Fig. 5C,D).

LE IgG enhances evoked MF neurotransmission

Are the effects elicited by the LE IgG caused by changes in cellular intrinsic excitability or rather due to modifications of synaptic transmission? Whole-cell recordings from CA3 pyramidal cells were performed and minimal stimulation protocol of MF was applied; the MF is one of the main excitatory inputs onto CA3 pyramidal cells in addition to the associational/commissural fibres and the perforant path (Lisman, 1999). Two stimuli (25 ms apart) evoked unitary excitatory postsynaptic currents (EPSC1 and EPSC2), as illustrated by examples of traces in one cell in Fig. 6A. By using this protocol we evaluated the probability of transmitter release (Kerr & Capogna, 2007). Postsynaptic responses recorded in the presence of LE IgG displayed a significant reduction of synaptic failure rate for EPSC1 (6.38 ± 2.2%, $p < 0.005$, n = 9) and EPSC2 (0.99 ± 0.09%, $p < 0.005$, n = 9) compared to the control IgG, where the failure rate for EPSC1 was 36.7 ± 6.5% and 21.2 ± 6.38% for EPSC2 (n = 13), and to ACSF where the failure rate for EPSC1 was 33.6 ± 5.82% and 18.1 ± 3.85% for EPSC2 (Fig. 6B). Notably, the failure rates for the second-pulse EPSC2 were generally lower than those of the first-pulse responses EPSC1 in all three conditions. Consistent with the effects on the failure rate, the EPSCs peak amplitude measured without failures was not altered in either condition (Fig. 6B). On average, EPSC1 was 50.1 ± 5.4 pA and EPSC2 was 67.5 ± 7.2 pA in control, 59.6 ± 8.5 pA and 75.8 ± 7.3 pA after the treatment with the LE IgG, and 50.6 ± 5.2 pA and 68.1 ± 7.5 pA after the treatment with the control IgG. Conversely, the EPSC amplitudes analyzed, including failures, were significantly changed in cells from acute slices treated with the LE IgG. On average, EPSC1 was 34.3 ± 5.9 pA and EPSC2 was 56.64 ± 8.29 pA in control (ACSF alone), 55.9 ± 8.2 pA and 74.7 ± 6.7 pA after the treatment with the LE IgG, 33.7 ± 6.0 pA and 50.8 ± 5.9 pA after the treatment with the control IgG; $p < 0.05$ for EPSC1, and $p < 0.05$ for EPSC2. The data suggest that the LE IgG increases the probability of action potential–dependent evoked release on MF-CA3 synapses compared to the control IgG and ACSF.

α-DTX mimics the actions of LE IgG

It is noteworthy that LE-IgG immunoreactivity colocalizes with Kv1.1, Kv1.2, and Kv1.4 subunits in the hippocampus; namely it is mostly expressed in subfields with excitatory axon terminals (Wang et al., 1994; Monaghan et al., 2001). Furthermore, LE IgGs immunoprecipitate Shaker-type, α-DTX-sensitive K^+ channels, particularly the Kv1.1-containing subunit (Kleopa et al., 2006), although it is now clear that many patients' sera do not bind directly to these channels but to Lgi1 (see Discussion).

Therefore, we tested the action of a selective VGKC 1.1, 1.2, and 1.6 subunit antagonist such as α-DTX (Robertson et al., 1996) on the excitability and MF-synaptic transmission of CA3 pyramidal cells. The bath application of this toxin at 120 nM caused a significant increase in action potential frequency (0.73 ± 0.31 Hz, n = 6), remarkably similar to, although more robust than, the effect that we

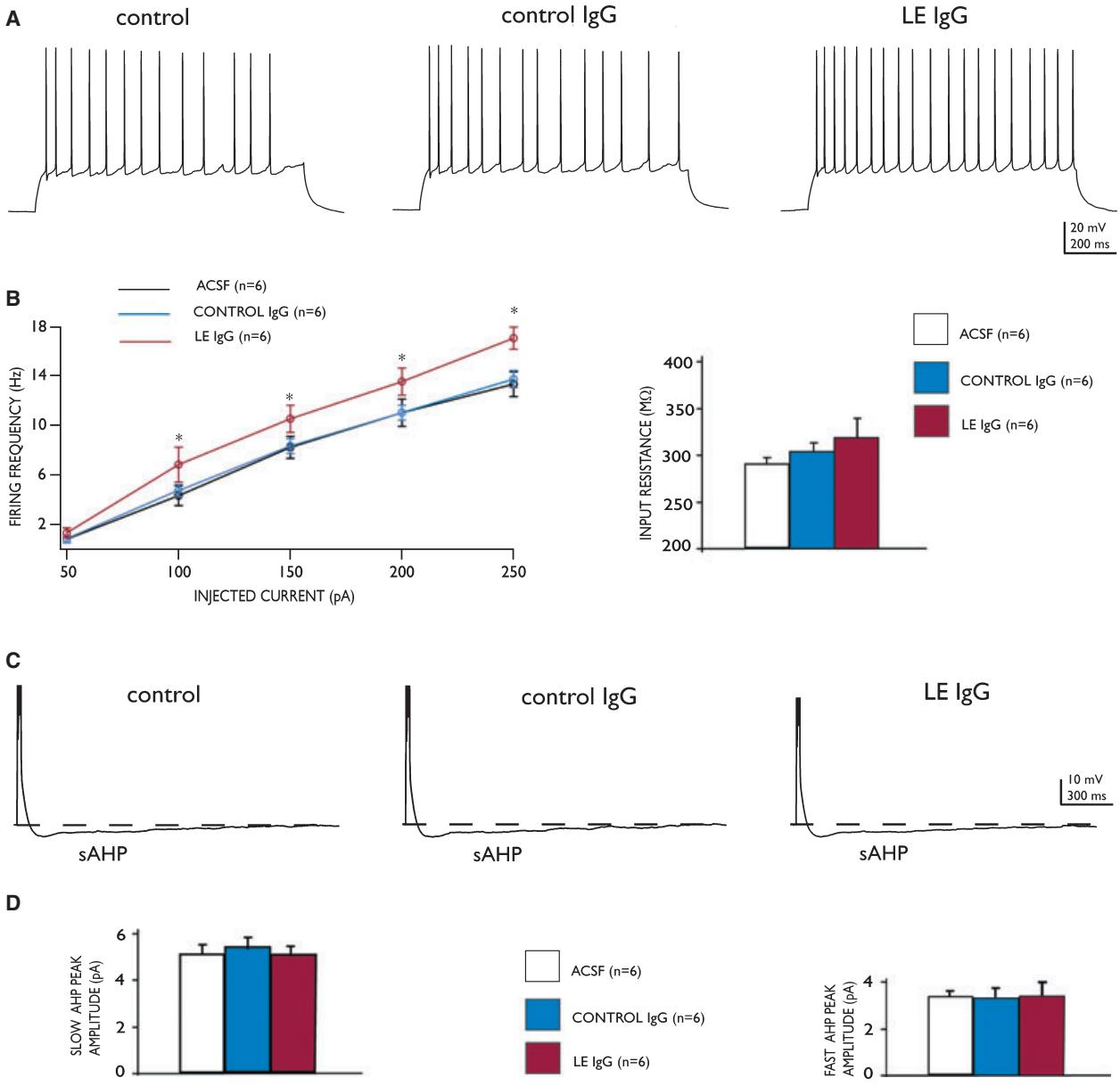

Figure 5.
Intrinsic properties of CA3 pyramidal cells recorded from control, control IgG, and LE IgG slices. (**A**) Typical responses of pyramidal neurons from three different groups to a depolarizing current step (250 pA × 1 s duration) from a resting membrane potential of −65 mV. (**B**) Frequency/intensity relationships (left graph) display a significant increase in firing after stimulation with current pulses (range 100–250 pA × 1 s) in cells recorded from the LE IgG group (* denotes significance, ANOVA $p < 0.05$). (**C**) Representative sAHPs occurring in pyramidal neurons from three different groups after a burst of three action potentials evoked from a resting membrane potential of −65 mV. Action potentials are cut; each trace is the average of five sweeps. (**D**) Average sAHP (left diagram) and fAHP (right diagram) in control, control IgG, and LE IgG; ANOVA $p > 0.05$ for all comparisons.
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observed after the treatment with the LE IgG (Fig. 7). We observed burst activity in six cells with an average number of bursts over 2 min of 10.5 ± 2.5, and with an average number of action potentials in a burst of 3.6 ± 0.4. The resting membrane potential tended to be depolarized by α-DTX, but not significantly different from control values ($p = 0.056$), similar to treatment with the LE IgG.

Interestingly, α-DTX also mimicked the action of the LE IgG on evoked synaptic currents recorded from CA3 pyramidal cells. First, α-DTX significantly decreased the

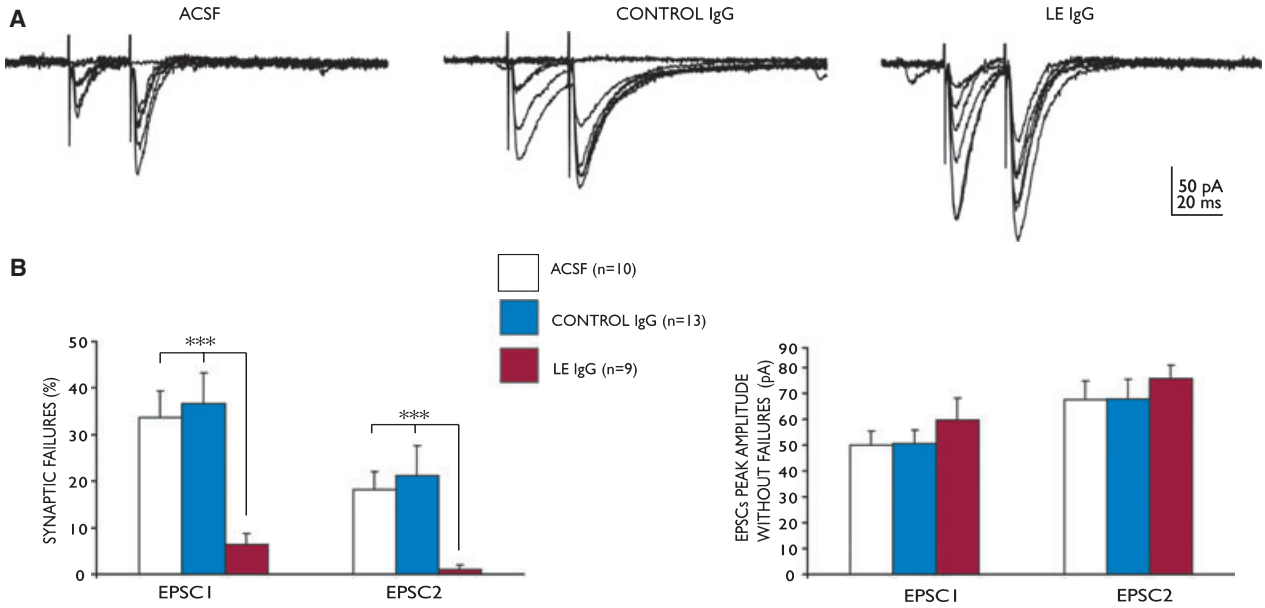

Figure 6.
Action of LE IgG on evoked synaptic transmission. (**A**) Whole-cell recording from CA3 pyramidal cell and minimal stimulation of MF applying two stimuli evoked unitary EPSCs as shown by one cell example. (**B**) Pooled data for synaptic failures and EPSCs peak amplitude without failures in ACSF, control IgG, and LE IgG. ***Denotes significance, $p < 0.005$.

number of synaptic failures of EPSC1 (from 32.67 ± 4.88% to 18.3 ± 5.1%), and EPSC2 (from 18.04 ± 3.25% to 4.3 ± 1.6%, $p < 0.005$, $n = 12$) (Fig. 8A). This effect resulted in changes of the EPSCs amplitude when analyzed including the failures (from 42.17 ± 7.25% and 70.93 ± 12.0% for EPSC1 and EPSC2, respectively to 48.78 ± 5.84% and 80.8 ± 11.9%), but not when analyzed without the failures (Fig. 8).

Discussion

Our study provides the first evidence that the LE IgG affects the function of VGKCs at CNS synapses. Using extracellular recordings, we detected epileptiform activity in the CA3 area of the hippocampus after exposure to the LE IgG, and this action is likely to contribute to memory impairment observed in LE patients (Kapur & Brooks, 1999). At a single cell level, we found that the LE IgG promoted moderate firing activity of CA3 pyramidal cells, which are normally silent, evoked burst activity, increased the frequency of firing elicited by depolarizing current pulses without changing AHPs, and significantly enhanced MF-EPSCs. We studied hippocampal MF-CA3 synapses, since they are targeted by LE IgG (Kleopa et al., 2006; Vincent et al., 2006), and they are widely studied as model synapses of the mammalian CNS (Henze et al., 2000; McBain, 2008). It is remarkable that relatively mild effects of the LE IgG observed at the single cell level appear to be sufficient to induce population epileptiform activity. This is consistent with previous literature indicating that only modest changes in single cell physiology of CA3 pyramidal cells can be sufficient to generate robust changes detected at the population level in Kv1.1 knockout mice (Smart et al., 1998). In these mice, CA3 pyramidal cells had the propensity to generate multiple action potentials; these effects probably contribute to the limbic and tonic components of epileptic phenotype (Smart et al., 1998; Rho et al., 1999; Lopantsev et al., 2003).

It is also important to remark that in our experiments, control IgG did produce some tonic firing in about 40% of CA3 pyramidal cells tested, and burst firing in a minority of cells tested, although these effects were significantly less robust than those elicited by LE IgG. Unlike LE IgG, however, the control IgG lacked any effect on MF-evoked synaptic transmission. The presence of some detectable actions by control IgG on cells' excitability highlights the importance of including control IgG groups in this type of study. The effects induced by LE IgG were mimicked by α-DTX, a Kv1.1, Kv1.2, and Kv 1.6 blockers, consistent with the previous literature showing increased excitability through a voltage-dependent mechanism by this toxin (Halliwell et al., 1986; Guan et al., 2007). Strikingly α-DTX produced more frequent and stronger bursts than the LE IgG, but that would be expected as the concentration of the specific VGKC-Abs in the purified IgG would be very low (approximately 40 pM). We suggest that the LE IgG and α-DTX

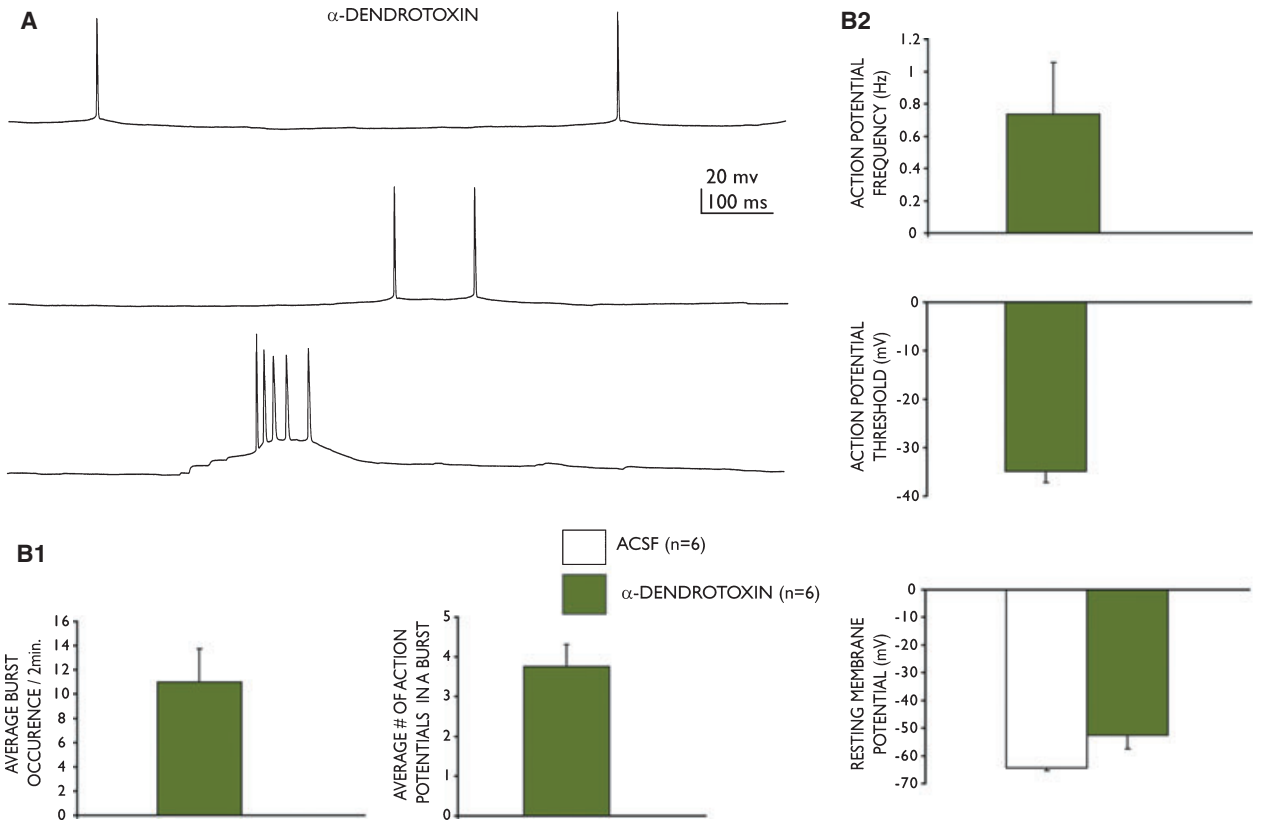

Figure 7.
α-Dendrotoxin (α-DTX) increases CA3 pyramidal cell excitability. (**A**) Example of current clamp recordings showing action potential firing in CA3 pyramidal cell after the application of α-DTX. Note that CA3 pyramidal cells in ACSF are normally silent. Resting membrane potential was −62 mV. (**B1**) Pooled current clamp data showing burst activity (**B2**) action potential frequency, action potential threshold, and resting membrane potential during the application of α-DTX.
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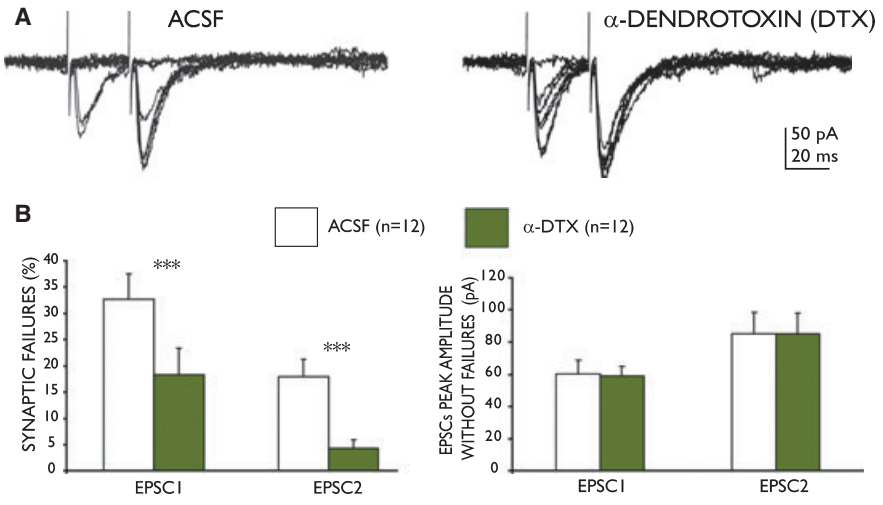

Figure 8.
Action of α-DTX on evoked synaptic transmission. (**A**) Whole-cell recordings from CA3 pyramidal cell in response to minimal stimulation of MFs to evoke unitary EPSCs, as shown in one cell example (top). (**B**) Pooled data showing synaptic failures and EPSCs without failures. ***Denotes significance, $p < 0.005$.
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share similar mechanisms of action; namely they functionally affect VGKCs expressing Kv1.1, Kv1.2, and/or Kv1.6 at both presynaptic/postsynaptic sites of CA3 pyramidal cells (Johnston et al., 2000; Dodson & Forsythe, 2004). Moreover, our finding that the LE IgG increased the evoked release probability of MFs is consistent with direct

recording from hippocampal MF boutons, which exhibit a fast inactivating current likely to be mediated by Kv1.1–Kv1.4 subunits (Geiger & Jonas, 2000). Whether the LE IgG also increased the cell's excitability, affecting other channel function or kinetics remains to be investigated. Alteration of MFs has been documented in animal models of several other neurologic diseases including temporal lobe epilepsy, Alzheimer's disease, and many psychiatric disorders including schizophrenia (Kobayashi, 2009).

We limited our study to one patient only. This was partly because of the availability of large volumes of plasma for purification of the IgG, but also because this 57-year-old man had a pure LE with no other neurologic defects, and the dramatic response to immunotherapies with decrease in VGKC antibodies strongly suggests that these antibodies were related to his cognitive disorder. Much of the previous evidence indicates that antibodies to ion channels and receptors can cause peripheral neurologic diseases such as myasthenia gravis and the Lambert Eaton myasthenic syndrome (Vincent et al., 2006). VGKC antibodies were first reported in acquired neuromyotonia, a peripheral nerve hyperexcitability syndrome, in the 1990s (Shillito et al., 1995; Hart et al., 1997). They were shown to be associated with an IgG-mediated increase in quantal release in mice and repetitive action potentials in cultured dorsal root ganglion cells (Shillito et al., 1995). Further studies showed that, when applied for several hours, neuromyotonia IgGs reduced K^+ channel current amplitudes (Sonoda et al., 1996; Nagado et al., 1999), most likely by increasing the channel turnover (Tomimitsu et al., 2004); no effects were seen at short incubations at room temperature. We found that under the conditions of our slice preparations, we were able to see an effect of the IgG after 2 h. The much higher antibody levels found in patients with LE compared to those with neuromyotonia, and the difference in specificity for different VGKCs, since neuromyotonia IgG shows little colocalization with Kv1.1 (Kleopa et al., 2006), may explain this difference.

However, during the course of these studies it became clear that many of the VGKC-Abs in patients with LE and high titers (unlike the majority of neuromyotonia sera) bind not directly to the VGKCs themselves but to other proteins, such as leucine-rich glioma-inactivated gene-1 (Lgi1) and contactin-associated protein-like 2 (Caspr2), that are tightly complexed with the α-DTX-binding–VGKCs in the rabbit brain extracts that are used for measuring "VGKC" Abs (Lai et al., 2010; Irani et al., 2010). The patient whose IgG we used here was strongly positive for Lgi1 antibodies (Irani et al., 2010). Lgi1 is found both presynaptically in excitatory synapses where it reduces Kv1.1 inactivation, and postsynaptically where it reduces *N*-methyl-D-aspartate (NMDA) receptor currents (Caleo, 2009). Mutations in Lgi1 are associated with an autosomal dominant form of lateral temporal lobe epilepsy (Morante-Redolat et al., 2002) and Lgi1 knockout mice develop epilepsy (Fukata et al., 2010). Furthermore, Lgi1 antibodies are present in >80% of patients with LE (Irani et al., 2010). It will be interesting to study the longer term effects of these "VGKC" antibodies on acute slices or organotypic slice cultures (Gähwiler et al., 1997) and by injection in vivo.

In conclusion, our study establishes that LE IgG enhances CA3 pyramidal cell excitability and desynchronizes the excitatory input coming from MF onto these cells, and that this is likely to be due to an effect on α-DTX-sensitive VGKCs. Details of the LE IgG mechanisms will need to be addressed in future studies, perhaps by using expression cellular systems that are more accessible than MF-CA3 synapses in situ. It will be important to determine whether LE IgGs reduce the number of functional VGKCs or rather affect channel kinetics, although under the conditions that we use (room temperature, short incubation), a direct effect is more likely. Hopefully future studies will clarify how binding of LE IgG to Lgi1 interferes with VGKC function. It could be by inducing an alteration of the VGKC conformation, by directly interfering with the ion pore, or by involving some type of intracellular signaling pathway. Whatever the exact nature of LE IgG action, our results suggest that drugs acting specifically as openers of VGKC might help to protect the hippocampus from immune-mediated damage.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure

None of the authors has any conflict of interest to disclose.

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