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***In vivo* injection of anti-LGI1 antibodies into the rodent M1 cortex and hippocampus is ineffective in inducing seizures**

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Abstract

Autoimmune encephalitis (AIE) associated with antibodies directed against the leucine-rich glioma inactivated 1 (LGI1) protein is the second most common autoimmune encephalitis and is responsible for deleterious neocortical and limbic epileptic seizures. Previous studies demonstrated a pathogenic role of anti-LGI1 antibodies via alterations in the expression and function of Kv1 channels and AMPA receptors. However, the causal link between antibodies and epileptic seizures has never been demonstrated. Here, we attempted to determine the role of human anti-LGI1 autoantibodies in the genesis of seizures by analyzing the impact of their intracerebral injection in rodents. Acute and chronic injections were performed in rats and mice

in the hippocampus and primary motor cortex, the two main brain regions affected by the disease. Acute infusion of cerebrospinal fluid or serum IgG of anti-LGI1 AIE patients did not lead to the emergence of epileptic activities, as assessed by multisite electrophysiological recordings over a 10-hour period after injection. A chronic 14-day injection, coupled with continuous video-EEG monitoring, was not more effective. Overall, these results demonstrate that acute and chronic injections of cerebrospinal fluid or purified IgG from LGI1 patients are not able to generate epileptic activity by themselves in the different animal models tested.

Significance statement

Anti-LGI1 encephalitis is a frequent and severe autoimmune encephalitis. Several previous studies have shown a pathogenic role of anti-LGI1 antibodies, but their link with the emergence of seizures has never been demonstrated. To study the role of anti-LGI1 autoantibodies in the genesis of seizures, we performed acute and chronic injections of cerebrospinal fluid and purified serum IgG of anti-LGI1 encephalitis patients in rodents, targeting the two main brain regions affected by the disease, the hippocampus and primary motor cortex. Brain activities were monitored for 10 hours after acute injections and for 1 month after the beginning of chronic injections. Our results show that chronic and acute injections of anti-LGI1 antibodies were ineffective in inducing epileptic activity in rats and mice.

Abbreviations: AIE = autoimmune encephalitis; AMPAR = AMPA receptor; APC = associative parietal cortex; CSF = cerebrospinal fluid; D = day; ECoG = electrocorticogram; EEG = electroencephalogram; IgG = immunoglobulin G; LFP = local field potential; LGI1 = leucine-rich, glioma inactivated 1; M1 = primary motor cortex; NMDA-R = NMDA-receptor; REM sleep = rapid eye movement sleep; TDS = tonic-dystonic seizure;

Introduction

Autoimmune encephalitis (AIE) is a group of autoimmune syndromes that are responsible for an increasing number of unexplained drug-resistant epilepsies. (Dalmau et al., 2017; Goodfellow and Mackay, 2019; Husari and Dubey, 2019). The second most frequent AIE, which is caused by the production of antibodies directed against the leucine-rich glioma inactivated 1 protein (anti-LGI1 AIE), is associated with limbic encephalitis and tonic-dystonic motor seizures (TDS). The evolution of the disease, generally within months, is characterized by a progressive bilateral involvement of limbic and motor brain structures (Navarro et al., 2016) and a gradual increase in seizures frequency, possibly leading to life-threatening status epilepticus. An absence or delay in diagnosis and treatment can thus have devastating consequences and lead to irreversible lesions, such as hippocampal atrophy (Ghimire et al., 2020; Griffith et al., 2020; Liu et al., 2020; Ramanathan et al., 2021).

LGI1 is a 60 kDa-secreted glycoprotein, ubiquitously expressed in the central nervous system. It takes part in a trans-synaptic complex, including Kv1.1 voltage-dependent potassium channels and glutamatergic AMPA receptors (AMPA) (Fukata et al., 2021; Schulte et al., 2006; Yamagata et al., 2018). LGI1 is also enriched at the axon initial segment, where it co-localizes with Kv1.1 channels (Hivert et al., 2019; Seagar et al., 2017). LGI1 is highly conserved between human and rodents. Previous studies reported a specific labeling of human anti-LGI1 IgG on rodent wild-type hippocampal slices, suggesting that the human autoantibodies effectively react with the rodent protein (Extr met et al., 2022; Kornau et al., 2020; Ramberger et al., 2020). Anti-LGI1 antibodies have been shown to prevent fixation of LGI1 to its pre- and postsynaptic partners or to induce the internalization of the LGI1-associated protein complex (Ramberger et al., 2020), resulting in alterations of the expression and functioning of Kv1.1 channels and AMPAR (Extr met et al., 2022; Kornau et al., 2020; Ohkawa et al., 2013; Petit-Pedrol et al., 2018; Ramberger et al., 2020).

However, the pathophysiological mechanisms linking these abnormal molecular interactions to the emergence of seizures remain unclear. Several groups have attempted to develop *in vivo* animal models of anti-LGI1 AIE, especially through the passive transfer into the murine brain of total immunoglobulins or purified anti-LGI1 antibodies from patients. They observed a decrease in Kv1.1 and AMPAR expression, but none of these previous studies used electrophysiological measurements to assess the occurrence of seizure activities in the involved brain structures of interest (Petit-Pedrol et al., 2018; Ramberger et al., 2020).

Here, we sought to characterize in rodents the functional impact of intracerebral injections of anti-LGI1 antibodies from patients. Antibodies from either cerebrospinal fluid (CSF) or patient serum were injected into the hippocampus or primary motor cortex (M1), the two main target regions of the human disease (Navarro et al., 2016). Because action kinetics of autoantibodies are not known, we set up protocols to study its acute and chronic impact. To avoid species-dependent effects, the effects of intracerebral injections were examined in both rats and mice.

Material and methods

Patients

CSF and serum of patients were obtained from the Department of Neurology at the Pitié-Salpêtrière Hospital. The main patients' characteristics are summarized in Table 1. In anti-LGI1 AIE (LGI1 patients, $n = 3$), serum and CSF were collected before the start of immunotherapy and the presence of anti-LGI1 antibodies was assessed on HEK293 cells expressing LGI1 (FA1439-1003-1, EuroImmun). The group of control patients ($n = 3$) included one non-epileptic patient, with a negative autoantibody test, hospitalized for repeated discomfort of psychogenic origin. The two other controls were LGI1 patients, who were sampled 1 year after the complete

remission of their AIE. These two patients were free of neurological symptoms and tested negative for antibodies in serum and CSF. None of the control patients had neurodegenerative diseases, brain tumor or acute inflammatory pathology. Their CSF was normal in terms of cellularity, proteinorachia and glycorrhachia. Samples from different patients were used independently in order to be able to assess any specific patient-dependent effect and to avoid drawing general conclusions from effects caused by a specific patient profile. The protocol was sponsored by INSERM (C16-16, 20152482) and approved by a local ethics committee.

Purification of IgG from patient serum

Total immunoglobulin G (IgG) were extracted from the patients' serum and purified. IgG were isolated using protein A columns (PURE1A-KIT, Sigma-Aldrich). After IgG isolation, samples were dialyzed, normalized to a concentration of 1 mg/ml in PBS using Amicon 30kD ultrafiltration filters (UFC503096, Sigma-Aldrich), filtered, and stored at -80°C until use. The presence of anti-LGI1 antibodies was verified after purification by labeling on HEK293 cells expressing LGI1, revealed by fluorescent human anti-IgG (FA1439-1003-1, EuroImmun). Semi-quantitative estimation of anti-LGI1 antibodies was performed on the IgG purified from serum samples, through titration and labeling on HEK293 cells expressing LGI1 (FA1439-1003-1, EuroImmun). We diluted the IgG sample progressively by adding PBS such as the volume was multiplied by two between each staining. The titration ratio for each sample is reported in Table 1 and corresponds to the last dilution in which we could detect a staining on HEK cells.

Animals

Experiments were performed on 61 Sprague Dawley male rats, and 27 C57B6/6J mice, all aged between 8 to 16 weeks (Charles River Laboratories, France). The C57BL6J mouse strain was chosen as it is commonly used in previous in vitro and in vivo studies addressing the effects of

anti-LGI1 human auto-antibodies (Kornau et al., 2020; Petit-Pedrol et al., 2018; Ramberger et al., 2020). The Sprague Dawley rat strain is the one used to develop our LGI1 encephalitis seizure-like model, which recapitulates the electrical and behavioral hallmarks of patients (Baudin et al., 2022). Both rat and mice were used to verify that the absence of seizure was not species specific

The experiments detailed below complied with the European Union guidelines (Directive 2010/63/EU) and were approved by the French Ministry of Research, and the local Ethics Committee. The number of animals used in each experimental group is detailed in Tables 2 and 3.

Acute injections and multi-scale *in vivo* recordings in sedated rats

Rats were first anesthetized by inhalation of 4% isoflurane (Osalia) and maintained with 2% isoflurane throughout the surgery. Animals were intubated to perform artificial ventilation (room air, 80 cycles.min⁻¹, 2.6 ml.cycle⁻¹) and placed on a stereotaxic frame. The incision and pressure areas were regularly infiltrated with lidocaine (2%, Centravet). CSF or purified patient IgG was injected using a Hamilton[®] 1701 syringe with a 200 µm outer diameter needle at a rate of 0.1 µL/min. A volume of 0.5 and 2 µL were, respectively, injected in the left M1 and the left hippocampus. The localization of the injection sites was validated by injecting the fluorescent tetramethylrhodamine dextran-amine Fluoro-Ruby (Thermofisher) in the hippocampus ($n = 2$) and M1 ($n = 2$) (Fig. 1B). Unilateral injections in the hippocampus or cortex were preferred to bilateral injections to avoid traumatizing large brain volumes and to offer the possibility to detect focal seizures and determine their initiation site.

Multi-site electrocorticographic (ECoG) recordings were made using low-impedance (60 kΩ) silver electrodes placed on the dura over the right M1, left M1, and left associative parietal cortex (APC). A reference electrode was apposed to the right temporal muscle. ECoG

signals were amplified with a differential AC amplifier (model 1700; A-M Systems), filtered between 0.1 Hz and 1 kHz, and digitized at 3 kHz (CED 1401 Micro3; Spike2 software version 7.20, Cambridge Electronic Design). Local field potentials (LFP) were recorded using a multichannel linear probe of 16 electrodes separated by 250 μm (IrOx, 35 μm diameter, ATLAS Neuroengineering[®]) inserted in M1 or in the hippocampus, 200 μm in front of the injection site. LFP signals were amplified and filtered between 0.1 Hz and 1 kHz, using a Digital Lynx amplifier (NeuraLynx[®]), and digitized at 3.2 kHz. The stereotaxic coordinates for injection and recording sites are reported in the Extended Data Table 1-1. All coordinates were derived and adjusted from the rat brain atlas (Paxinos and Watson, 1997a). The experimental setup is schematically illustrated in Figure 1A.

After completion of surgery, isoflurane was gradually discontinued, and the animal was maintained in a sedated and analgesic state by repeated injections of sufentanil (3 $\mu\text{g}\cdot\text{kg}^{-1}$ i.p. every 30 minutes, Piramal), combined with repeated intramuscular injections of gallamine triethiodide (40 mg/2h, Sigma Aldrich) to allow for stable long-term recordings. Sufentanil sedation was chosen because it does not alter spontaneous activity and excitability of cortical cells (Altwegg-Boussac et al., 2014) and does not interfere with the induction and expression of focal and generalized seizures (Depaulis et al., 2016; Langlois et al., 2010). Heart rate and ECoG activity were continuously monitored to assess the depth of sedation. The stability of physiological parameters, i.e., end-tidal carbon dioxide concentration, oxygen saturation, and body temperature (37°C), was verified throughout the experiments (Altwegg-Boussac et al., 2017). At the end of the experiments, animals were euthanized by injection of a lethal dose of euthasol (0.6 $\text{ml}\cdot\text{kg}^{-1}$, TVM). Their brain were then extracted to check the position of the injection syringes and intracranial recording probes. Briefly, after fixation with 4% paraformaldehyde, brains were frozen with isopentane, cut in 20- μm cryostat sections and stained with safranin (RAL Diagnostics, Martillac, France) (Williams et al., 2016).

Chronic injection and long-term video-EEG recordings in freely moving rodents

Rats and mice were initially anesthetized by inhalation of 4% isoflurane and placed on a stereotaxic frame to implant chronic EEG electrodes and an intracerebral cannula. Analgesia, induced by subcutaneous injection of buprenorphine (0.05 mg.kg^{-1} ; Centravet), was initiated 40 minutes before isoflurane anesthesia. Anesthesia was maintained with 2% isoflurane to complete the surgical procedures and body temperature was stabilized at 37°C with a homoeothermic blanket.

EEG recordings in M1 and APC were performed using 200- μm diameter insulated stainless steel wire (A-M Systems[®]). For rats, a small screw was welded to the stainless steel wires (Fine Science Tools, 19010-00). In addition, an interlaced bipolar insulated stainless steel electrode was bilaterally inserted in the hippocampus. All electrodes were soldered to a 6-pin female connector. Stereotaxic coordinates are reported in the Extended Data Table 2-1. All coordinates were derived and adjusted from rat and mouse brain atlas (Paxinos and Watson, 1997a, 1997b). An Alzet[®] Model 1002 osmotic pump was placed subcutaneously on the animal's right flank and connected to an injection cannula (Alzet[®], Brain infusion kit) implanted in the right hippocampus to allow for a continuous injection of $84 \mu\text{L}$ for 14 days, at a flow rate of $0.25 \mu\text{L.h}^{-1}$. A reference electrode was placed over the cerebellum. Electrodes and cannula were fixed with surgical glue (Surgibond[®]) and immobilized in dental cement on the animal's skull.

After a recovery period of 3 days, implanted rats were placed under freely moving conditions in transparent custom made recording cages, with ad libitum access to food and water. Continuous recordings of 24 or 48 hours were performed every week with a complete video-EEG acquisition system. EEGs were amplified and digitized (Brainbox EEG-1166, Natus) at a sampling rate of 4096Hz, filtered between 0.1 and 300 Hz. Video was synchronized to the electrophysiological signal and recorded at 25 frames per second. At the end of the

recording period, animals were euthanized by injection of a lethal dose of euthasol (0.6 ml.kg⁻¹, TVM). Brains were then removed and processed following histological procedures described above to check the position of the electrodes and injection cannula.

The IgG diffusion within the hippocampus was assessed in 3 mice and 3 rats, euthanized 7 days after surgery, after infusion of purified IgG samples. Labeling was done with biotinylated anti-human-IgG antibodies (BA-3000, Vector Laboratories), followed by enzymatic revelation.

Analysis of electrophysiological signals

A visual reading of the recordings over a sliding window of 20 seconds was performed in order to evaluate the putative epileptic phenotype of rats and mice injected with human CSF and serum-purified IgG. Detection of epileptiform discharges or seizures was based on different criteria: an abrupt onset and termination, an amplitude threshold clearly different from the baseline activity (at least 3 times the SD), and/or abnormal changes in the background rhythm. In chronic recordings from freely-moving animals, myoclonus and other epileptic movements, whether or not associated with events on the EEG, were also looked for on video recordings. For acute injections in sedated rats, a 30-minute period was recorded before the injection. This served as a baseline against which the post-injection recordings were compared.

In acute experiments, a 15-minute window was selected before injection, as well as 2 hours and 5 hours after injection. Fast-Fourier transforms (FFT) were computed on those windows, between 1 and 30 Hz. Power-spectra were then binned in 1-Hz frequency bands, and compared between Control and LGI1 experiments with a two-tailed Mann-Whitney rank-sum test. This analysis was performed with a combination of Fieldtrip (release 20200919) (Oostenveld et al., 2011) and custom-developed scripts in MATLAB (The Mathworks Inc., version R2021b). M1 injection data and hippocampal injection data were analyzed independently. The statistical power was estimated for each frequency bin as the probability to

find a difference of $\pm 50\%$ knowing the mean and SD control values, using the “sampsizewr” built-in MATLAB function.

Results

Absence of seizure activity after acute intracerebral injection of CSF and purified serum IgG from LGI1 patients

To investigate the effect of anti-LGI1 antibodies on brain activity, we performed acute injections of CSF or purified serum IgG with anti-LGI1 antibodies into the hippocampus and M1 cortex of sedated rats, together with ECoG and LFP recordings (Fig. 1A, B). The different experimental configurations ($n = 42$) are detailed in Table 2.

Baseline ECoG activity was recorded for 30 minutes before injection. The ECoG profile appeared desynchronized and dominated by relatively fast, small-amplitude cortical waves (Fig. 1C, left), as classically observed under sufentanil sedation (Altwegg-Boussac et al., 2014). Recording duration after injection varied between 5 and 11 hours, with an average (\pm SD) of 7.2 ± 2.2 hours ($n = 42$ experiments). We first verified that the injection of CSF ($n = 14$ experiments) and purified serum IgG ($n = 6$ experiments) from control patients did not modify the background ECoG activity (data not shown). Given that the production of anti-LGI1 antibodies cause characteristic limbic and motor seizures in patients, we searched for a possible induction of epileptic seizures in experiments wherein anti-LGI1 antibodies-containing CSF or serum purified IgG was injected into the hippocampus ($n = 12$) or M1 cortex ($n = 14$). As illustrated in Figure 1C for an experiment in which recordings were performed in the M1 cortex before and after an injection of purified IgG serum in the same cortical site, we did not detect seizure-like events or inter-ictal activity at the different ECoG locations. Epileptic activity was also absent from LFP recordings (Fig. 1C), regardless of the injection site. The frequency

content of LFP activities after injection was similar between Control and LGI1 experiments, when all experiments were pooled together (Fig. 1C and 1D) as well as when serum IgG experiments and CSF experiments were analyzed separately (Extended data Fig. 1-1). CSF ($n = 24$) and purified IgG serum ($n = 18$) from LGI1 patients were similarly ineffective in inducing seizures.

Absence of seizures after chronic intracerebral injections of CSF and purified serum IgG from LGI1 patients

We next investigated if longer injection, over several days, of anti-LGI1 antibodies could be effective in inducing epileptic seizures. We thus used osmotic pump connected to an injection cannula implanted in the hippocampus for a continuous release of the antibody-containing solutions (PBS containing purified IgG or CSF) for two weeks. The different experimental configurations ($n = 36$) are listed in Table 3. After two weeks of injection, a continuous video-EEG monitoring of the animals was undertaken for another 14 days. Animals were first recorded at post-injection day 3 (D3) for 24h, and then once a week for 48 h (D7-8, D14-15, D21-22, D28-29), corresponding to a total of ~200 h of recordings per animal (Fig. 2A). In six animals ($n = 3$ rats and $n = 3$ mice), we ensured of the diffusion of human antibodies in the hippocampus by immuno-histochemical detection of human IgG 7 days after the start of the injection of purified serum IgG (Fig. 2B).

No animals died during the weeks of injection and the weeks of EEG-recording that followed. We did not observe any behavioral difference between the animals of the different groups during handling: no particular aggressiveness, motor hyperactivity or apathy. EEG activity of control ($n = 10$ mice and 6 rats) and LGI1 ($n = 14$ mice and 6 rats) animals was characterized by normal physiological activities during wakefulness (exploratory hippocampal theta waves) and sleep (slow delta waves during phases of deep sleep and theta activity during

rapid eye movement (REM) sleep) (Gottesmann, 1992) (Fig. 2C). Sudden and brief twitches of the animal's trunk were observed in all animals during REM sleep episodes.

We did not detect any epileptic seizures or paroxysmal epileptic abnormalities, such as spikes, poly-spikes or spike-waves on the EEGs of control animals or animals injected with anti-LGI1 autoantibodies after exhaustive analysis of the records. Overall, these results show that, like acute injections, chronic injections of CSF or purified total IgG did not induce abnormal epileptic activity that could be recorded on the EEG in both LGI1 and control groups of rats and mice.

Discussion

Several studies indicated pathogenic effects of anti-LGI1 antibodies through altered expression and function of Kv1 and AMPA-R channels (Extr met et al., 2022; Kornau et al., 2020; Ohkawa et al., 2013; Petit-Pedrol et al., 2018; Ramberger et al., 2020). However, the causal link between these deleterious molecular interactions and seizure activity has never been demonstrated. The effect of anti-LGI1 antibody injections *in vivo* has been previously examined 7 days after hippocampal injection (Ramberger et al., 2020) or after 14 days of chronic infusion into brain ventricles (Petit-Pedrol et al., 2018). However, while these experimental procedures led to a reduced expression of Kv1.1 and AMPA-R, as well as an increased excitability of hippocampal neurons, the authors did not use appropriate electrophysiological recordings to assess the presence or the absence of seizures.

We filled this gap by studying the effect of acute or chronic intracerebral infusion of anti-LGI1 autoantibodies, in rats and mice, using multisite electrophysiological recordings allowing direct detection of possible epileptic activity. In the acute injection protocol, we targeted the hippocampus and the M1 cortex, which are the two main regions affected in anti-

LGI1 AIE (Navarro et al., 2016). We found that injection of CSF or IgG purified from serum of anti-LGI1 patients into hippocampus or M1 in rats did not induce epileptiform pattern in both ECoG and LFP. Similar results were obtained with chronic hippocampal injections protocol. Upon diffusion of human IgG from anti-LGI1 patients, no epileptic abnormalities could be observed on the video-EEG, in mice and in rats chronically injected for 14 days.

The lack of epileptic phenotype could arise from multiple mechanisms. First, as the human disease evolves from several weeks to months, it is possible that the duration of antibodies injection was not sufficient to induce seizures and related electrophysiological and molecular defects. Indeed, previous studies reported a 10-15% decrease in Kv1.1 expression after two weeks of infusion of anti-LGI1 antibodies (Petit-Pedrol et al., 2018), whereas in LGI1^{-/-} mice, with frequent generalized seizures, Kv1.1 density is decreased by more than 50% compared to control mice (Seagar et al., 2017). In the most frequent AIE, the anti-NMDA-receptor (NMDA-R) AIE, patients typically exhibit rapid progression of neuropsychiatric manifestations that can lead to coma within days or weeks (Dalmau et al., 2017). After chronic injection of anti-NMDA-R AIE patients' antibodies into the hippocampus of mice *in vivo*, behavioral changes and memory deficits were observed (Planagumà et al., 2015; Taraschenko et al., 2019), as well as after 6 days an acute injection (Würdemann et al., 2016). However, no differences in anxiety and locomotion were detected between anti-NMDA-R and control animals (Planagumà et al., 2015). Moreover, mice were reported to be either seizure-free (Planagumà et al., 2015; Würdemann et al., 2016) or to exhibit non convulsive seizures (Taraschenko et al., 2019). Thus, anti-NMDA-R antibody injections in mice appears to result in an attenuated phenotype compared to AIE patients. This suggests that the passive antibody transfer method, crucial for demonstrating the pathogenicity of autoantibodies, does not appear suitable for reproducing the epileptic phenotype of AIE patients. In this context, our results,

reporting the absence of seizure during acute and chronic injections of anti-LGI1 antibodies, seem consistent with the recent literature.

Other factors can explain our negative results. Incubation of anti-LGI1 antibodies on organotypic hippocampal cultures for 3 days decreased the amount of GluA1 subunits of AMPA-R expressed at the synapse by over 50% (Ohkawa et al., 2013). In comparison, after 14 days of *in vivo* injection, GluA1 was only decreased by 10% compared to controls (Planagumà et al., 2015). This could be explained by a slow diffusion of antibodies in the brain, which would greatly reduce the access of autoantibodies to their antigenic targets, in contrast to isolated neuron cultures or brain slices where antibodies can directly access a large neuronal surface. Accordingly, real-time imaging techniques have been used to measure the diffusion properties of nonspecific fluorescently labeled IgG after injection into agarose or into the cortex of adult rats *in vivo* (Wolak et al., 2015). IgG diffusion was shown to be approximately 10-fold greater in agarose than in brain. The corresponding diffusion rate of antibodies in the brain was 6.10^{-8} cm²/s, which corresponds to 1 mm² in 41 hours. This slow diffusion could arise from the structural properties of the brain microenvironment, but also from specific IgG features, such as their size, shape and their electrical charge. In addition, nonspecific binding of IgG to brain proteins, especially *via* their Fc fraction, could slow down or stop its diffusion (Wolak et al., 2015). Specific binding, especially in case of autoantibodies which by definition have specific targets, could also slow diffusion. Therefore, the acute injection method followed by 5-10 h recording may be insufficient to study the effect of autoantibodies on neuronal activities because, (i) this duration would not allow the antibodies to reach a sufficiently large amount of autoantigens and, (ii) several days of chronic injection of anti-LGI1 antibodies are needed to affect significantly the amount of Kv1.1 (Petit-Pedrol et al., 2018).

The lack of seizures triggered after chronic 14-day injections, as well as the relatively small decrease in Kv1.1 and AMPA-R (Petit-Pedrol et al., 2018), could therefore be due to too

short injection durations, as it has been shown that longer injection leads to larger decreases of Kv1.1 and AMPA-R (Petit-Pedrol et al., 2018). However, the effectiveness of an infusion longer than 14 days may be limited by the instability of the antibodies, which are at ~37°C in a subcutaneous pump with a half-life time of about 15 to 30 days. (Mankarious et al., 1988). The use of a refillable pump could allow the antibody solution to be replenished and prevent the antibodies from remaining at room temperature for too long (Tan et al., 2011). An alternative approach could be the immunization of the animals against the LGI1 protein, so that they produce autoantibodies themselves. Active opening of the blood-brain barrier would also be required to allow antibodies to access the central nervous system, as done in models of experimental allergic encephalomyelitis (Reboldi et al., 2009) or Sydenham's chorea (Platt et al., 2017).

The lack of seizure induction in the various *in vivo* antibody injection methods we have performed suggests that passive delivery of human antibodies *in vivo* in rodents is not an appropriate method for modeling the epileptic symptoms of AIE. The development of animal models, wherein ion channels downstream to LGI1 will be impaired with specific inhibitory toxins could provide a promising approach to overcome these limitations. An interesting target could be Kv1 channels, whose expression and function are known to be impaired by down-regulation of LGI1 (Extrémet et al., 2022; Kornau et al., 2020; Lugarà et al., 2020; Petit-Pedrol et al., 2018; Ramberger et al., 2020; Seagar et al., 2017; Zhou et al., 2018). Indeed, the reduction of Kv1 currents due to loss of LGI1 function is associated with increases in neuronal excitability and activity, in both genetic and autoimmune models (Baudin et al., 2021), and their pharmacological blockade has been shown to contribute to LGI1-related seizures in a recent animal model (Baudin et al., 2022).

Author Contributions:

PB, SM, SC and VN designed research,

PB and DR performed research,

PB contributed unpublished reagents/analytic tools

PB analyzed data,

PB, SM, SC and VN wrote the paper.

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Conflict of Interest

V.N. reports personal fees from UCB, Liva Nova, and Eisai, outside the submitted work.

The other authors declare that they have no competing interests.

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Figures and legends

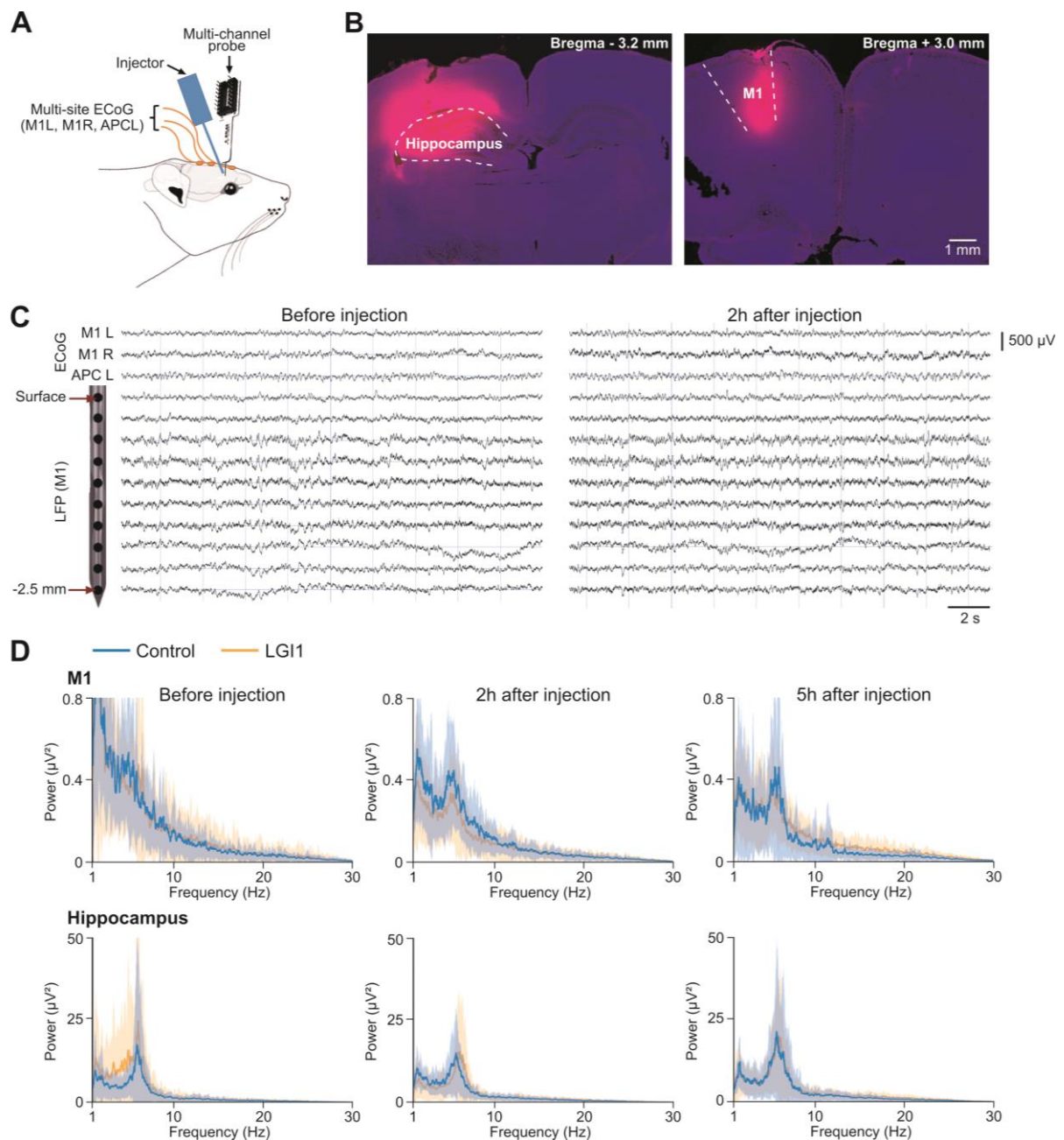


Figure 1. Acute injections of CSF and purified serum IgG. (A) Schematic of the experimental setup for acute injection and simultaneous electrophysiological recording from the sedated rat. (B) Control experiments for the location of the injection sites. Coronal brain slices at the indicated coordinates, showing the hippocampal (top) and M1 cortical (bottom) injection sites, as revealed by Fluoro-Ruby injections (in pink on the figure), at the exact same

volume and coordinates as the control and anti-LGI1 antibody-containing CSF and purified serum injected. Blue labeling is DAPI labeling of cell nuclei. (C) ECoG activities and local field potentials recorded before (left) and after (right) an acute injection of purified IgG from LGI1 patients into the M1 cortex. ECoG activity was collected from the left (L) and right (R) M1 cortices and the left associative parietal cortex (APC L). A multichannel electrode was inserted in M1 L, 200 μm anterior to the injection site to record local field potentials (LFP) from the different cortical layers. Note the absence of epileptiform activity or seizures on the post-injection recordings. (D) Frequency power (mean \pm SD) of intracerebral LFP recordings after injection in M1 (top) and hippocampus (bottom), in Control (blue) and LGI1 (orange) conditions. Electrophysiological signals were analyzed before injection (left), 2 hours after injection (middle), and 5 hours after injection (right). The electrode closest to the injection site was selected for the analysis. No significant difference was found between Control (M1, $n = 8$; Hippocampus, $n = 8$) and LGI1 (M1, $n = 14$; Hippocampus, $n = 12$) experiments (two-tailed Mann-Whitney rank-sum test for each time period, on data binned in frequency bands of 1 Hz width). The statistical power estimated for each frequency bin was on average 0.55 ± 0.15 . Frequency content of LFP activities are displayed separately between CSF and serum experiments in the Extended Data Figure 1-1. Stereotaxic coordinates used for the acute experiments are reported in the Extended Data Table 1-1.

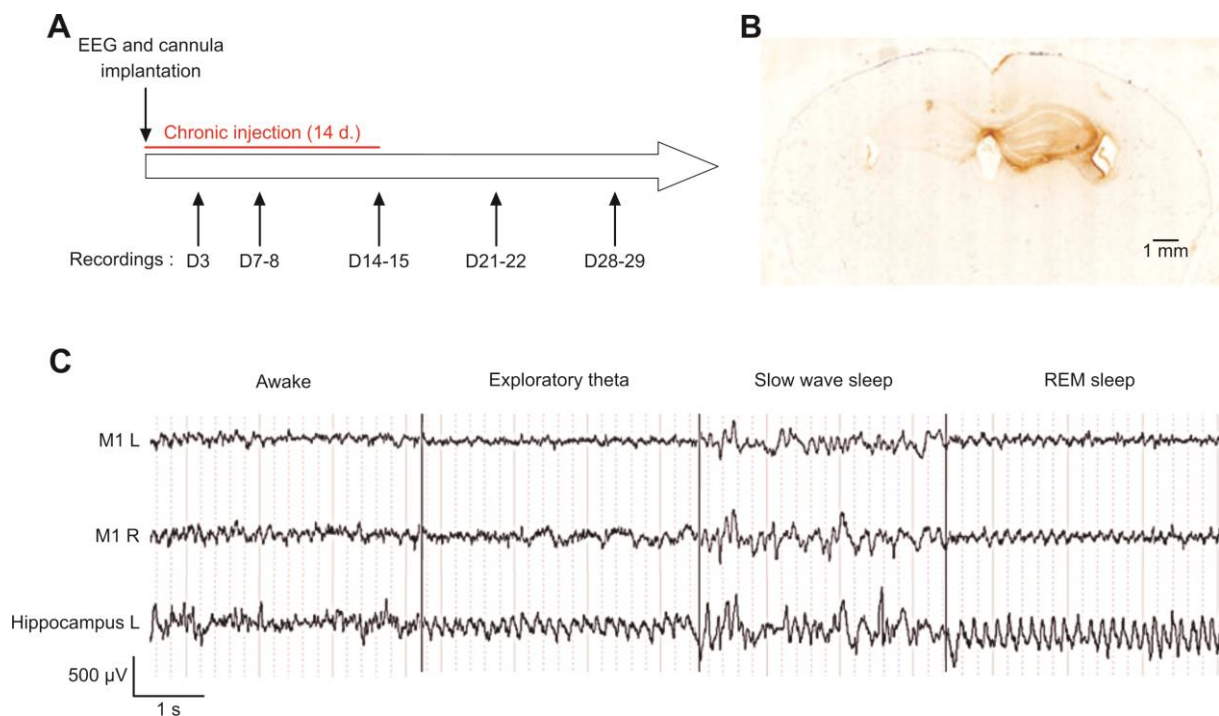


Figure 2. Chronic injections of CSF and purified serum IgG. (A) Experimental design. Rats and mice were implanted with an injection cannula in the left hippocampus, in addition to left and right primary motor cortex (M1L, M1 R) and bilateral hippocampus electrodes for long-term EEG monitoring of brain activity. The post-injection days of recording are indicated on the schema. (B) Representative staining of human IgG on coronal section of a mouse after 7 days of unilateral injection with patient-derived LGI1 serum antibodies to demonstrate the distribution in the hippocampus.. Slices were incubated with peroxidase-coupled anti-human IgG followed by DAB staining. (C) Typical EEG activity recorded in the different states of vigilance in controls ($n = 10$ mice and 6 rats) and animals injected with LGI1 antibodies ($n = 10$ mice and 6 rats). . Note the absence of epileptiform activity or seizures. Examples of recordings of Control and LGI1 awake mice at different time points are shown in the Extended Data Figure 2-1. Stereotaxic coordinates used for the chronic injection experiments are reported in the Extended Data Table 2-1.

	LGI1-1	LGI1-2	LGI1-3	Ctrl-1	Ctrl-2	Ctrl-3
Age	21	65	75	20	22	67
Sex	F	M	M	M	F	M
Inclusion criteria	Anti-LGI1 Abs in serum and CSF	Anti-LGI1 Abs in serum and CSF	Anti-LGI1 Abs in serum and CSF	Repeated discomfort of non-epileptic origin	Patient LGI1-1 after remission	Patient LGI-2 after remission
TDS (n. per hour)	yes (17)	yes (23)	yes (7)	no	no	no
Mesial temporal seizures	yes	no	no	no	no	no
Hyponatremia	yes	no	no	no	no	no
CSF (cellularity, proteinorachia, glycorachia)	normal	normal	normal	normal	normal	normal
Serum anti-LGI1 Ab titration ratio	1/32	1/64	1/32	-	-	-

Table 1. Main characteristics of the included patients. Three patients were included in the LGI1 group (LGI1-1, LGI1-2 and LGI1-3), and three in the control group (Ctrl-1, Ctrl-2, and Ctrl-3). The serum anti-LGI1 Ab titration ratio corresponds to the last dilution of the samples in which a staining on HEK cells was detected. Abs: antibodies; CSF: cerebrospinal fluid; Ctrl: control; F: female; M: male; n.: number; TDS: tonic-dystonic seizure

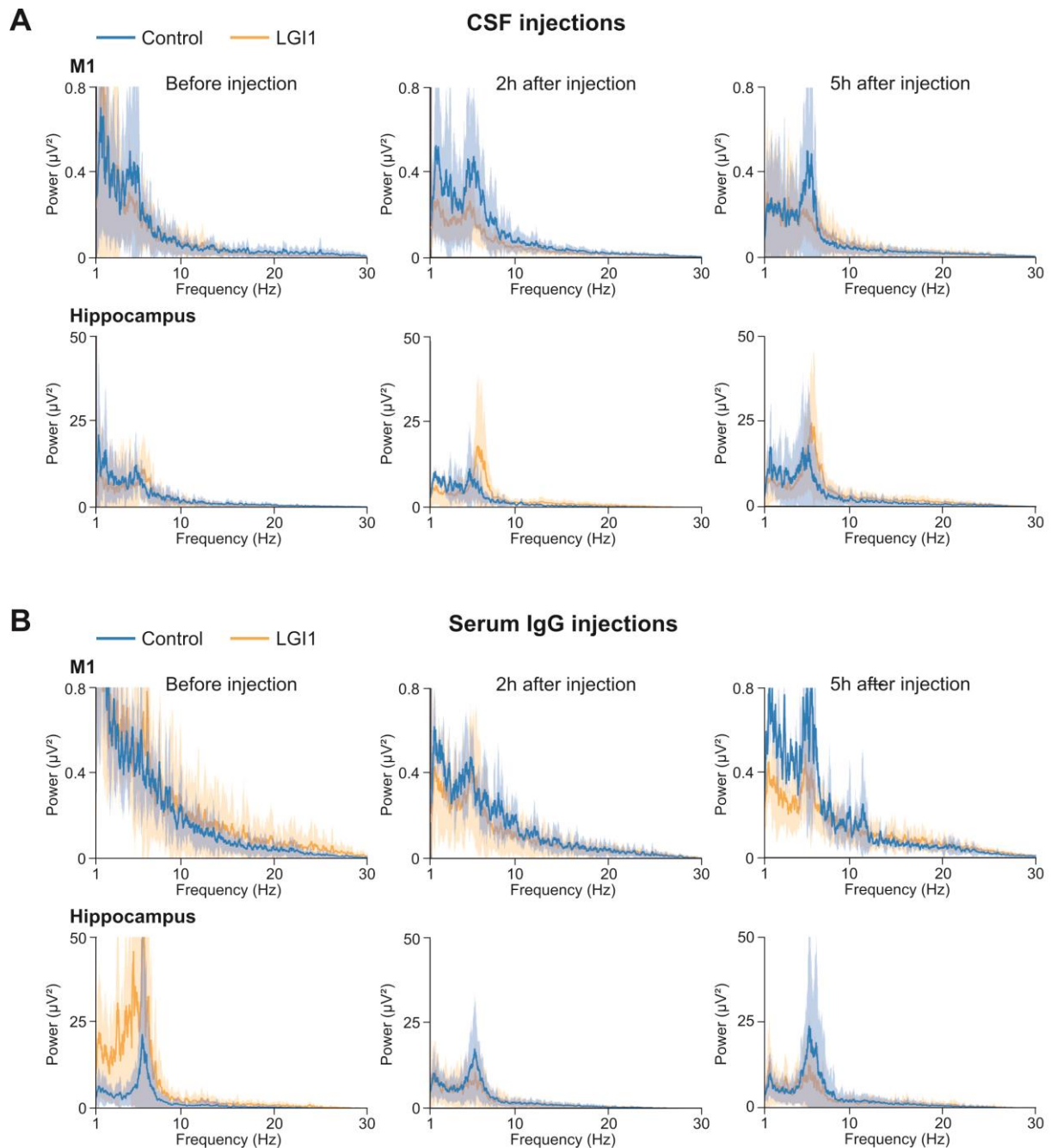
	LGI1 CSF	Control CSF	LGI1 patient-derived serum IgG	Control patient-derived serum IgG
M1	LGI1-1 : $n = 3$	Ctrl-1 : $n = 2$	LGI1-1 : $n = 2$	Ctrl-1 : $n = 1$
	LGI1-2 : $n = 2$	Ctrl-2 : $n = 1$	LGI1-2 : $n = 2$	Ctrl-2 : $n = 1$
	LGI1-3 : $n = 3$	Ctrl-3 : $n = 2$	LGI1-3 : $n = 2$	Ctrl-3 : $n = 1$
Hippocampus	LGI1-1 : $n = 2$	Ctrl-1 : $n = 2$	LGI1-1 : $n = 2$	Ctrl-1 : $n = 1$
	LGI1-2 : $n = 2$	Ctrl-2 : $n = 1$	LGI1-2 : $n = 2$	Ctrl-2 : $n = 1$
	LGI1-3 : $n = 2$	Ctrl-3 : $n = 2$	LGI1-3 : $n = 2$	Ctrl-3 : $n = 1$

Table 2. Experimental configurations for acute injection of CSF or purified total IgG. All these experiments were performed on rats.

	LGI1 CSF	Control CSF	LGI1 patient-derived serum IgG	Control patient-derived serum IgG
Mice	LGI1-1 : $n = 2$ LGI1-2 : $n = 2$ LGI1-3 : $n = 2$	Ctrl-1 : $n = 2$ Ctrl-2 : $n = 2$ Ctrl-3 : $n = 2$	LGI1-1 : $n = 3$ LGI1-2 : $n = 3$ LGI1-3 : $n = 2$	Ctrl-1 : $n = 2$ Ctrl-2 : $n = 1$ Ctrl-3 : $n = 1$
Rat	LGI1-1 : $n = 2$ LGI1-2 : $n = 2$ LGI1-3 : $n = 2$	Ctrl-1 : $n = 2$ Ctrl-2 : $n = 2$ Ctrl-3 : $n = 2$	NA	NA

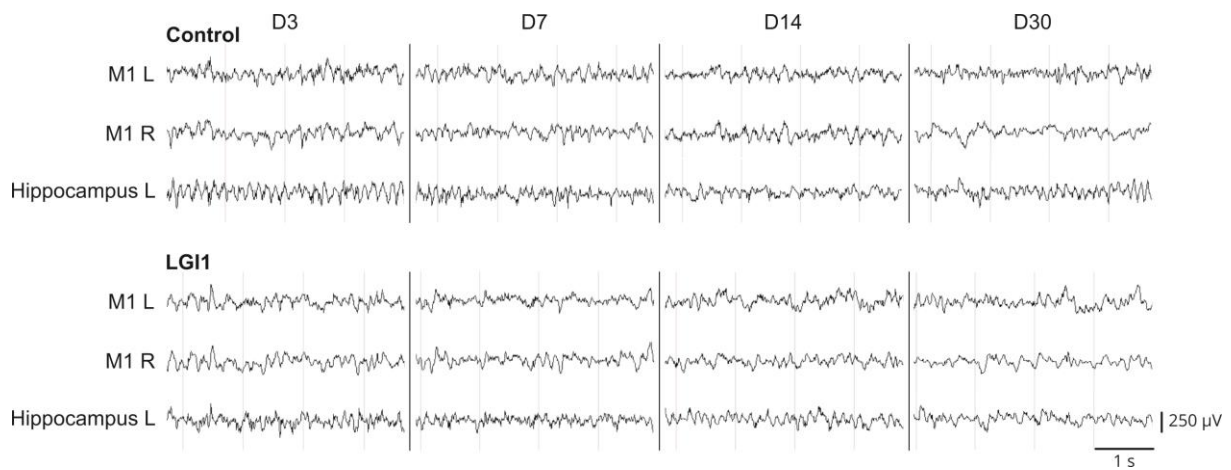
Table 3. Experimental configurations for chronic hippocampal injection of CSF or purified total IgG in rats and mice. NA: not available.

Extended Data



Extended Data Figure 1-1. Frequency content of LFP activities after acute injection of CSF (A) and serum IgG (B) from LGI1 and control patients in rat. (A) Frequency power (mean \pm SD) of intracerebral LFP recordings after CSF injection in M1 (top) and hippocampus (bottom), in Control (blue) and LGI1 (orange) conditions. Electrophysiological signals were analyzed before injection (left), 2 hours after injection (middle), and 5 hours after injection (right). The electrode closest to the injection site was selected for the analysis. No significant difference

was found between Control (M1, $n = 5$; Hippocampus, $n = 5$) and LGI1 (M1, $n = 8$; Hippocampus, $n = 6$) experiments (two-tailed Mann-Whitney rank-sum test for each time period, on data binned in frequency bands of 1 Hz width). **(B)** Same analysis as the one presented in **(A)**, but after serum IgG injection. No significant difference was found between Control (M1, $n = 3$; Hippocampus, $n = 3$) and LGI1 (M1, $n = 6$; Hippocampus, $n = 6$) experiments (two-tailed Mann-Whitney rank-sum test for each time period, on data binned in frequency bands of 1 Hz width).



Extended Data Figure 2-1. Examples of recordings of Control (A) and LGI1 (B) awake mice at different time points: day 3, 7, 14 and 30 after injection (D3, D7, D14 and D30). Note the absence of epileptiform activity or seizures. M1 L: left motor cortex; M1 R: right motor cortex.

Hippocampal injection experiments			
Brain structure	Antero-posterior	Medio-lateral	Depth
Right and left M1 cortex: ECoG	3	+/-2.6	-
Left APC: ECoG	-3.6	3.5	-
Left hippocampus: multichannel probe	-3.4	2.5	Between 0 and -3.75 (16 electrodes)
Left hippocampus: injection site	-3.6	2.5	-3
M1 cortex injection experiments			
Brain structure	Antero-posterior	Medio-lateral	Depth
Left M1 cortex: multichannel probe	3	2.6	Between 0 and -3.75 (16 electrodes)
Left M1 cortex: injection site	2.6	2.6	-1
Right M1 cortex: ECoG	3	-2.6	-
Left APC: ECoG	-3.6	3.5	-

Extended Data Table 1-1. Coordinates used for injection and electrode placement, in the acute injection experiments. All coordinates are expressed in millimeter from bregma, and were derived and adjusted from Paxinos and Watson rat brain atlas (Paxinos and Watson, 1997a). APC: associative parietal cortex; ECoG: electrocorticogram; M1 cortex: primary motor cortex

Rat experiments			
Brain structure	Antero-posterior	Medio-lateral	Depth
Right and left M1 cortex: EEG	3	+/-2.6	-
Left anterior hippocampus: chronic injection cannula	-2	1.5	-3
Left posterior hippocampus: intracranial electrode	-3.4	2.5	-3
Cerebellum: reference	-10	0	-
Mice experiments			
Brain structure	Antero-posterior	Medio-lateral	Depth
Right and left M1 cortex: EEG	1.8	-1.8	-
Left anterior hippocampus: chronic injection cannula	-1	-0.4	-2.3
Left posterior hippocampus: intracranial electrode	-1.8	-1.8	-2.3
Cerebellum: reference	-6.5	0	-

Extended Data Table 2-1. Coordinates used for injection and electrode placement, in the chronic injection experiments. All coordinates are expressed in millimeter from bregma, and were derived and adjusted from Paxinos and Watson rat and mouse brain atlas (Paxinos and Watson, 1997a, 1997b).