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Investigating the Effects of NMDAR and LGI1 Antibodies on Absence Seizures: Insights from Genetic Absence Epilepsy Rats and Acute Pharmacological Model of Absence Seizures

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ABSTRACT

Introduction: Childhood Absence Epilepsy, a subtype of genetic generealised epilepsy, is characterised by sudden and brief episodes of impaired consciousness. The Leucine-rich glioma-inactivated protein 1 (LGI1) and N-methyl-D-aspartate receptor (NMDAR) are key proteins involved in regulating neuronal excitability. In conditions like anti-LGI1 encephalitis and anti-NMDAR encephalitis, autoantibodies target and disrupt these proteins, causing memory deficits, behavioural changes, sleep disturbances, and epileptic seizures. However, the roles of LGI1 and NMDAR dysfunction in the pathophysiology of absence of seizures remain unclear. This study aims to investigate the effects of LGI1 and NMDAR antibodies on absence seizures using two experimental models: Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and a low-dose pentylenetetrazol (PTZ) model of absence seizures.

Methods: IgG purified from the peripheral blood of healthy controls (HC IgG), and patients with anti-NMDAR, and anti-LGI1 encephalitis, was administered intracerebroventricularly into GAERS and Wistar rats every other day for 11 days. Before and after antibody administration, electroencephalography (EEG) recordings were performed to analyse spontaneous spike-and-wave discharges (SWDs) in GAERS. In Wistar rats, after the completion of antibody infusions, PTZ was administered (35 mg/kg) on the 12 th day to induce absence seizures. The occurrence of PTZ-induced SWDs was quantified.

Results: NMDAR IgG significantly increased the duration and number of SWDs in GAERS compared to HC IgG. LGI1 IgG had no significant effect, suggesting a differential role of NMDAR and LGI1 antibodies in modulating SWD activity. Similarly, NMDAR IgG-treated Wistar rats showed increased susceptibility to PTZ-induced absence seizures, while LGI1 IgG did not cause significant changes in PTZ-induced SWDs.

Conclusion: These results reveal a distinct pro-epileptogenic effect of NMDAR antibodies in both genetic and pharmacological models of absence epilepsy, while LGI1 antibodies appear to have a negligible effect. These findings suggest a specific role for NMDAR dysfunction in absence seizure pathophysiology and support further investigation into antibody-mediated seizure mechanisms.

Keywords: Autoantibodies, childhood absence epilepsy, genetic absence epilepsy rat model, LGI1, NMDAR, PTZ, SWDs

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INTRODUCTION

Absence epilepsy presents with sudden and brief periods of impaired consciousness, often accompanied by spike-and-wave discharges (SWDs) on electroencephalography (EEG). These SWDs, typically 3-4 Hz oscillations, are generated through aberrant neurophysiological interactions within the corticotalamocortical (CTC) network (1). Moreover, recent clinical and preclinical studies have suggested a potential role of immune response and neuroinflammation in the pathophysiology of epilepsy and epileptogenesis, including childhood absence epilepsy (2–4). Notably, autoantibodies targeting neuronal surface antigens have been identified in both adults and children with epilepsy (5), suggests that this autoimmune response may have important therapeutic implications (6).

In the past 40 years, autoantibodies targeting neuronal surface antigens such as the N-methyl-D-aspartate receptor (NMDAR) and leucine-rich glioma-inactivated 1 (LGI1) have been implicated in autoimmune encephalitis, a condition frequently associated with various neurologic syndromes including seizures and neurobehavioral comorbidities (6). While seizures are very common in autoimmune encephalitis and can present in various forms, absence seizures are extremely rare, and our understanding of this phenomenon remains limited.

The specific contribution of these antibodies to seizure onset continues to be a focus of ongoing research. Both in vivo and in vitro studies provide growing evidence supporting the pathogenicity of these autoantibodies.

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Highlights

- **NMDAR-IgG infusion increased the duration and frequency of SWDs in GAERS.**
- **NMDAR-IgG infusion increased susceptibility to PTZ-induced absence seizures.**
- **LGI1 antibodies produced a modest effect on SWD activity.**
- **NMDAR and LGI1 antibodies play distinct roles in absence seizure pathophysiology.**

For instance, anti-NMDAR antibodies that was the first antibody identified that targets an extracellular antigen, causes a selective reduction of NMDAR expression in vitro on the neuron membrane mediated by autoantibody cross-linking of the receptors and internalization (7,8). In vivo, the antibodies appear to disrupt the localization of NMDARs at inhibitory synapses by interfering with their interaction with EphrinB2 (9). Mechanistically, anti-LGI1 antibodies appear to disrupt the interaction between LGI1 and ADAM22 (a disintegrin and metalloproteinase domain-containing protein 22), impairing the signalling of both the presynaptic voltage-gated potassium channel complex (VGKCC) and the postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which could contribute to an increased susceptibility to seizures (10–12). LGI1 mutations and LGI1 autoantibodies have been associated with various forms of epilepsy, including autoimmune limbic encephalitis (13) and epilepsy syndromes (14,15). Preclinical studies showed that antibodies against LGI1 in patients with limbic encephalitis increase neuronal hyperexcitability (16), cause seizures in-vivo (17). The most likely underlying molecular mechanism involves a reduction in Kv1.1 channels, as well as decreasing total and synaptic AMPAR subunits in the hippocampus (12).

Although there is a well-established association between neuronal antibodies and epileptic activity, absence seizures occur extremely rarely in patients with autoimmune encephalitis (18) and the underlying reasons for this low incidence remain unexplored. This study aims to investigate the effects of human polyclonal NMDAR and LGI1 autoantibodies on SWDs in two distinct animal model of absence epilepsy: i) the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a well-validated and widely used genetic rat model that closely mimics the clinical, neurophysiological, and pharmacological aspects of absence seizures in humans exhibiting spontaneous SWDs (19); ii) the low-dose pentylenetetrazol (PTZ) rat model of absence seizures, an acute pharmacological model fulfils accepted criteria for an animal model of absence epilepsy (20). Understanding how autoantibodies influence SWDs in the presence or absence of a genetic predisposition could provide valuable insights into the autoimmune contributions to absence epilepsy and highlights potential targets for immunomodulatory therapies. The primary objective of this study is to determine whether the administration of human polyclonal autoantibodies against NMDAR and LGI1 affects the incidence of SWDs in two different animal models of absence epilepsy. This investigation aims to clarify the role of immune mechanisms in the pathophysiology of absence epilepsy and to evaluate their potential as therapeutic targets.

METHODS

Animals

Experiment was performed on adult (3-4 months old), 250–300 g male GAERS and Wistar rats from breeding colony of Acıbadem Mehmet Ali Aydınlar University, Laboratory Animal Application and Research Centre. Animals were housed under standard laboratory conditions with a 12-hour light/dark cycle and had unrestricted access to food and water. All procedures were approved by the Animal Ethics Committee of Istanbul University Faculty of Medicine (Ethics number: 1107593) and conducted in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Efforts were made to minimise animal use and to reduce pain and distress throughout the study.

Patient characteristics and purification of IgG

IgG purified from the peripheral blood of patients with anti-NMDAR encephalitis (n=3), anti-LGI1 encephalitis (n=4) and healthy controls (HC) (n=4) as previously described (11,21). Serum samples were collected within the first week of symptom onset and stored at -80°C. IgG was isolated from the collected serum samples by protein A-Sepharose columns, then dialysed with phosphate buffered saline solution (PBS) and filtered and sterilised. The purified IgG was confirmed by gel electrophoresis, and concentrations were measured using the Bradford method. Immunoreactivity of LGI1 antibodies was confirmed through cell-based assays and indirect immunohistochemistry, as reported in previous studies (22,23). Appropriate ethical approval was obtained from the Istanbul University Faculty of Medicine (Ethics number: 408/2013) and informed consent was collected from all participants.

Stereotaxic surgery

The rats were anaesthetised via intraperitoneal (i.p.) injection of ketamine (100 mg/kg, Ketasol 10%, RichterPharma ag, Wels, Austria) and xylazine (10 mg/kg, Rompun 20 mg/ml, Bayer, Germany) and subsequently positioned in a stereotaxic frame. A guide cannula (C313G, Plastic's One Inc., Roanoke, VA, USA) was implanted into the lateral ventricle (coordinates: AP: -0.8, ML: -1.5, V: -4.1 mm) (24), along with recording electrodes placed over the left frontal cortex (coordinates: AP: +2, ML: ±3.5 mm) and the occipital cortex (coordinates: AP: -6, ML: ±4 mm). The implants were then secured with dental cement, and the rats were given a one-week recovery period before starting antibody infusions.

Intracerebroventricular (ICV) infusions

Following a 1-week recovery period, each rat was placed in a plexiglass recording chamber and allowed to acclimate for 20 minutes. A 120-minute baseline EEG recording (from 9 a. m. to 11 a. m.) was then obtained from GAERS to verify the presence of typical absence seizures after the stereotaxic surgery. Recording from Wistar rats was also performed to confirm that the rats were non-epileptic. On the following day, an internal cannula, for intracerebroventricular (ICV) application was inserted into the guide cannula, extending 1 mm beyond the guide's tip. A total of 5 µL of purified IgG from either NMDAR, LGI1 antibody-positive patients, or HC was slowly injected into the right cerebral ventricle over a 20-minute period using a micro infusion pump (Legato® Dual syringe pump, KD Scientific, MA, USA). To ensure complete delivery of the antibodies into the ventricle, the internal cannula remained in place at the injection site for at least 10 minutes. The infusions were repeated once every two days for a total of ten days (Fig. 1a and 2a). By the end of the tenth day, each rat had received a total of 25 µL of the antibody pool, equivalent to approximately 150–225 µg of antibodies. To verify proper

ventricular placement, 5 µL of 1% methylene blue was injected into randomly selected rats via the internal cannula, and the dye's distribution within the ventricular system was examined to confirm the accuracy of the injection sites.

Low-dose PTZ model

The low-dose (subconvulsive dose) PTZ model is a validated model of human absence epilepsy (20). Systemic administration of PTZ with doses of 20–35 mg/kg has demonstrated the ability to consistently produce bilaterally synchronous SWDs with EEG and behavioural alterations resembling those observed in human absence seizures (25). In Wistar rats, after the completion of antibody infusions, PTZ was administered (s. c.) (35 mg/kg, Sigma, MA, USA) on the 12 th day to induce acute absence seizures (with bilaterally synchronous SWDs) on EEG. The occurrence of PTZ-induced SWDs was quantified via EEG for 120 minutes after the PTZ administration.

Electroencephalography acquisition and analysis of seizure patterns

Electroencephalography recordings were conducted for 120 minutes following each infusion. The cumulative seizure duration, number of seizures, and the duration of individual seizures were analysed and compared across the different groups. Electroencephalography signals were amplified using a BioAmp ML 136 amplifier with a band-pass filter range of 1–40 Hz, and the data were recorded and analysed using the Chart v. 8.1 software (PowerLab8S ADI Instruments, Oxfordshire, UK). The total time spent in seizures, latency, as well as the number and duration of individual seizures, were assessed. During both the baseline and post-administration recordings, only SWD complexes with a train of SWD activity (7–11 Hz for GAERS, 3–4 Hz for PTZ model) and an amplitude at least twice that of the background EEG, lasting for periods longer than 1 second, were analysed. The cumulative seizure duration, the number of seizures, and the duration of each individual seizure (duration of each SWD) were evaluated from the 120-min EEG recordings.

Histological Verification

Histological verification was performed to confirm the placement of the inserted cannula. Following the completion of the EEG recordings, each rat was anaesthetised, a needle was inserted into the guide cannula, and 10 µL of 1% methylene blue (Sigma, MA, USA) was injected into the lateral ventricle to confirm the proper placement of the ICV cannula. Before being removed, the needle was kept in place for at least 60 seconds. To confirm the exact location of the ICV injection sites, the brain was removed after decapitation and the dispersion of methylene blue traces was examined.

Data Analysis

Statistical analyses were conducted using GraphPad Prism version 10.4.2 (GraphPad Software LLC). The effects of IgG SWDs were assessed by evaluating: 1) cumulative seizure duration, 2) number of seizures, and 3) individual seizure duration, 4) latency to the first seizure (for PTZ model) and 5) time spent in seizure (for PTZ model). These parameters were compared using a two-way analysis of variance (ANOVA) with two factors, "treatment" and "time," followed by Tukey's multiple comparison test. For latency to the first seizure and time spent in seizure, data were analysed using one-way ANOVA followed by Bonferroni post hoc test. All data are presented as mean ± S. E. M., with *p <0.05 considered statistically significant.

RESULTS

The distinct effects of human polyclonal autoantibodies on absence seizures in GAERS rats

To evaluate the effects of human autoantibodies on absence seizures, GAERS rats received repeated ICV infusions of human IgG from HC (n=5), NMDAR antibody-positive patients (n=6) or LGI1 antibody-positive (n=5) patients. Electroencephalography recordings were performed during the infusions and the day after each infusion across an 11-day period (Fig. 1a), and the cumulative duration, number and the mean duration of individual SWDs were analysed.

N-methyl-D-aspartate receptor antibodies significantly increase SWD activity in GAERS rats

In GAERS rats, NMDAR IgG infusion significantly increased the cumulative SWD duration after the NMDAR antibody infusion when compared to HC IgG infused group, highlighting its acute effect on SWD activity (Fig. 1. b and c). A two-way repeated-measures ANOVA revealed a significant interaction between treatment group and time for cumulative SWD duration (Fig. 1b; $F(20,130)=2.361$, $p < 0.01$). Post-hoc analysis using Tukey's multiple comparison test showed that NMDAR IgG significantly increased cumulative SWD duration on Days 2, 5, and 9 compared to control IgG ($p < 0.05$). Similarly, the number of SWDs was significantly higher in the NMDAR IgG group on Days 2 (Fig. 1c; two-way ANOVA $F(20,138)=1.717$, $p < 0.05$; Tukey's multiple comparison test $p < 0.01$). The mean duration of individual SWDs was also elevated in the NMDAR IgG group on Day 5 the day after the second infusion on Day 4 (Fig. 1d, $p < 0.05$); however, this effect did not persist in subsequent days.

LGI1 antibodies produced a modest effect on SWD activity

In contrast, LGI1 IgG produced a modest reduction in cumulative SWD duration compared to control IgG, though these differences did not reach statistical significance on most days (Fig. 1b and c, $p > 0.05$). However, on Day 11, a significant decrease in cumulative SWD duration was observed (Fig. 1b, $p < 0.05$, Tukey's multiple comparison test). The number of SWDs did not show a significant difference on most days, except for a notable increase on Day 9, the day following the fourth antibody infusion (Fig. 1b; $p < 0.05$). Interestingly, the mean duration of SWDs in GAERS rats injected with LGI1 IgG was significantly elevated on Day 3, the day after the first infusion (Fig. 1d, $p < 0.05$); however, this effect did not persist in subsequent days.

Acute effects after first ICV infusion

Analysis of the acute effects following the first ICV infusion (Day 2) over a 120-minute period post-injection revealed rapid and differential effects (Fig. 1. e–g). A time-course analysis following the first antibody infusion revealed distinct effects of LGI1 and NMDAR autoantibodies on cumulative SWD duration in GAERS (Fig. 1e). A two-way ANOVA followed by Tukey's multiple comparison test demonstrated a significant reduction in SWD duration over time in the LGI1 IgG group compared to HC IgG group. This reduction became more prominent from 40 minutes post-infusion and became significantly decreased at 80 minutes post injection period (Fig. 1e, $p<0.05$). In contrast, the NMDAR IgG group showed relatively higher SWD activity compared to both LGI1 IgG and control groups, but this was not statistically significant. These results suggest that LGI1 autoantibodies may acutely suppress absence seizures in GAERS, while NMDAR autoantibodies do not exert a similar modulatory effect during the same period. The number of SWDs followed a similar pattern (Fig. 1f), with LGI1 IgG decreasing and NMDAR IgG increasing SWDs in the early post-infusion period. The mean duration of individual SWDs remained relatively stable across groups, with no significant differences between groups (Fig. 1g).

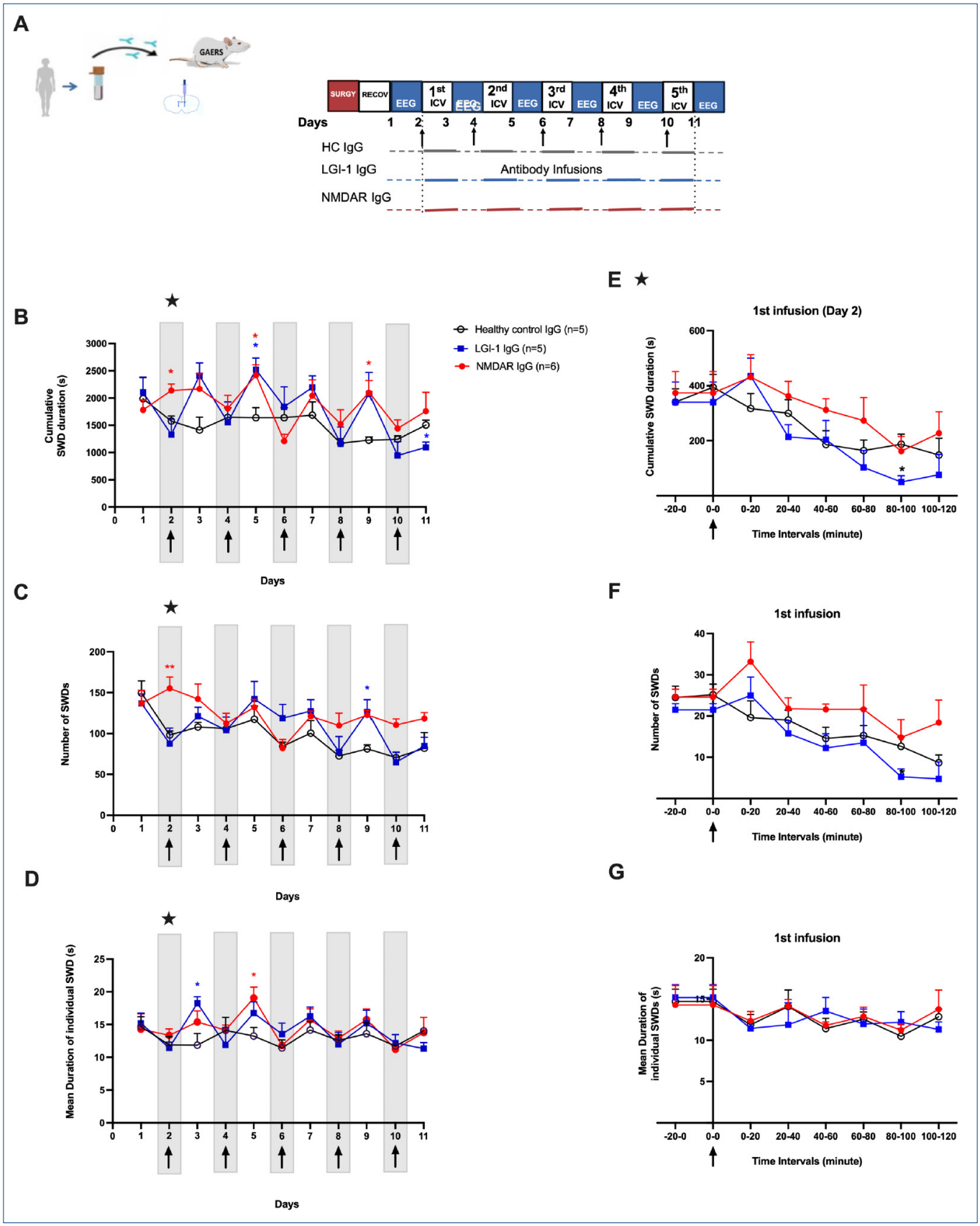

Figure 1. a–g. Effect of human polyclonal LGI1 and NMDAR autoantibodies on SWDs in the GAERS model of absence epilepsy. Schematic timeline and experimental protocol **(a).** GAERS rats were implanted for EEG recordings and received five ICV infusions indicated by clear boxes (Days 2, 4, 6, 8, 10) of human IgG from either healthy control (black), LGI1-positive patients (blue), or NMDAR-positive patients (red). EEG recordings were performed for 120 minutes before each infusion, immediately after, and on the following day (indicated by blue boxes on Days 1, 3, 5, 7, 9, and 11). The cumulative SWD duration **(b)**, number of SWDs **(c)**, the mean duration of individual SWDs **(d)** over a 120-min recording period. Grey shaded areas indicate infusion days. The black star highlights the time point for which 20-minute interval data are shown in panels E–G. Acute effects after the first antibody infusion on cumulative SWD duration **(e),** number of SWDs **(f)** and mean duration of individual SWDs **(g).** Data are presented as mean ± SEM. Statistical analysis was performed using two-way ANOVA with Tukey's multiple comparisons test. *p <0.05, ** p<0.01 compared to healthy control IgG.

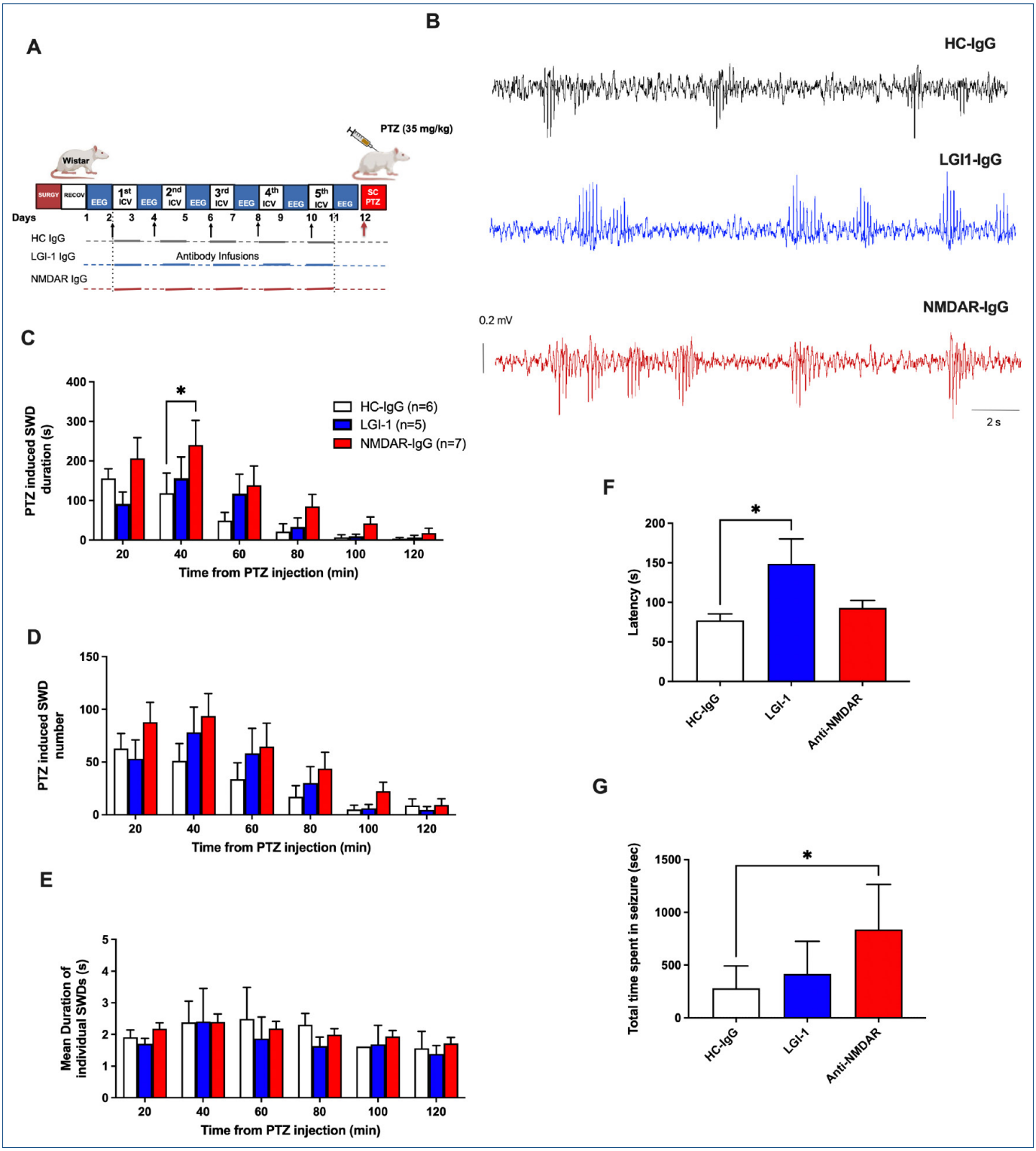

Figure 2. a–g. Effects of human polyclonal LGI1 and NMDAR autoantibodies on PTZ induced SWDs in Wistar rats. Experimental protocol **(a)**. Non-epileptic Wistar rats received five ICV infusions indicated by clear boxes (Days 2, 4, 6, 8, 10) of human IgG from either healthy control (black), LGI1-positive patients (blue), or NMDAR-positive patients (red). EEG recordings were performed for 120 minutes before each infusion, immediately after, and on the following day (indicated by blue boxes on Days 1, 3, 5, 7, 9, and 11). On Day 12, EEG was recorded following subcutaneous injection of PTZ (35 mg/kg). Representative EEG traces from each group 10 min after PTZ administration showed typical 2–3 sec SWDs **(b)**. The cumulative duration **(c)**, the number **(d)** and the mean duration **(e)** of PTZ induced SWDs over a 120-minute period, binned every 20 minutes. Latency to the first seizure was significantly prolonged in the LGI1 group **(f)**. Total time spent in seizures during the 120-minute recording period was significantly greater in the NMDAR group **(g)** (data are presented as mean ± SEM. *p <0.05 compared to healthy control IgG).

Autoantibody effects on PTZ-induced absence-like seizures in Wistar rats

To determine whether genetic predisposition influences the previously observed effects of LGI1 and NMDAR autoantibodies in GAERS, we utilized the low-dose PTZ model of absence seizures in non-epileptic Wistar rats. Wistar rats were infused with human IgG from HC (n=6), LGI1-positive patients (n=5) or NMDAR-positive patients (n=7) over five sessions, followed by low-dose PTZ (35 mg/kg, s.c.) on Day 12

(Fig. 2a). Representative EEG recordings revealed that both LGI1 and NMDAR IgG treated Wistar rats exhibited bilateral synchronous SWDs on EEG accompanied by behavioural arrest with vibrissal and facial twitching in response to PTZ, as shown in Fig. 2b. During the polyclonal antibody infusions over 10 days, no spontaneous seizure activity was observed in Wistar rats, consistent with previous findings (11). On Day 12, administration of low-dose PTZ (35 mg/kg, s.c.) induced 2–3 second SWDs starting 2–5 minutes post-injection, peaking around 20–40 minutes and ceasing within 80–120 minutes (Fig. 2c).

The quantification of the EEG data demonstrated a significant increase in the cumulative SWD duration at 40 minutes post-PTZ injection in the NMDAR IgG group compared to HC IgG ($p < 0.05$, two-way ANOVA with Tukey's post hoc test; Fig. 2c). While the number of SWDs (Fig. 2d) and mean duration of individual SWDs (Fig. 2e) were not significantly different between groups, a trend toward increased seizure burden was observed in the NMDAR group. Latency to the first SWD event was significantly longer in the LGI1 IgG (148.8±29.31 seconds, n=5) group compared to rats that received HC IgG (77±30.12 seconds, n=6) as determined by one-way ANOVA followed by Bonferroni post hoc test (Fig. 2f, $p < 0.05$), suggesting a potential modulatory or protective effect. In contrast, total time spent in seizures during the 120-min EEG recording period, following PTZ injection was significantly higher in the NMDAR IgG group compared to HC IgG ($p<0.05$, One-way ANOVA, treatment effect $F(2,13)=4.168$, One-way ANOVA with Bonferroni post hoc test, $p < 0.05$; Fig. 2g), indicating an exacerbation of seizure activity. Together, these findings suggest that NMDAR autoantibodies enhance seizure susceptibility and severity in the low-dose PTZ model, while LGI1 antibodies may exert a mild delaying effect on seizure onset without significantly altering total seizure burden.

DISCUSSION

In this study, we investigated the effects of patient-derived polyclonal LGI1 and NMDAR IgG on absence seizures in two different rodent models: GAERS model and the low-dose PTZ model in Wistar rats. Our aim was to determine whether these autoantibodies—known to be associated with autoimmune encephalitis and diverse seizure types—have the capacity to influence SWDs, and whether the presence of a genetic predisposition alters their effects.

NMDAR autoantibodies enhance seizure susceptibility and severity in both GAERS and low-dose PTZ model. These findings suggest that NMDAR autoantibodies may modulate CTC network excitability early in the treatment timeline, though the effects did not appear to accumulate across subsequent infusions in GAERS. The transient nature of these changes could reflect compensatory mechanisms within the network or limited antibody penetration/stability after each ICV injection.

The observed pattern aligns with the hypothesis that NMDAR mediated modulation of reticular thalamic nucleus (RTN) excitability can influence absence seizure dynamics (26). RTN which forms a thin sheath of inhibitory GABAergic interneurons, receives excitation from cortex and thalamus and plays a pivotal role in SWDs (27). Cortical and/or thalamic excitation of RTN, through glutamatergic AMPA receptors (AMPARs) and NMDARs is essential for synchronous thalamic oscillations (27,28). The thalamic relay neurones receive feed-forward inhibition (FFI) from the RTN inhibitory interneurons, which do not extend from the thalamus. In this sense, the RTN is crucial for controlling the thalamic relay cells' excitability (28,29). Prior studies, including in stargazer (*stg*) mice—a model of absence epilepsy (30,31) linked to AMPAR dysfunction (32)—emphasises the effect of glutamatergic synapse disruption in the CTC network on the development of absence seizures. (28). Research has demonstrated that patient anti-NMDAR antibodies can specifically mediate the internalisation of surface NMDARs, resulting in a loss in glutamate synaptic function and the surface density of synaptic NMDAR clusters (7). All of these results point to the possibility that thalamic FFI dysfunction, caused by the weakened NMDAR mediated input to inhibitory RTN neurones within the CTC circuitry could contribute to absence seizures.

While NMDAR autoantibodies markedly enhanced absence seizure susceptibility in both GAERS and PTZ models, LGI1 IgG exhibited a more subtle and distinct profile. In GAERS, LGI1 antibodies did not significantly alter SWDs, suggesting a limited or delayed modulatory impact in this genetic model of absence epilepsy. In the PTZ model, LGI1 IgG appeared to modestly delay seizure onset without significantly affecting the total seizure burden, indicating a potential modulatory role in seizure threshold rather than severity or duration. These differential effects are likely rooted in the unique biological functions of LGI1 within neuronal networks. LGI1 plays a key role in regulating neuronal excitability through its influence on Kv1.1 potassium channels and AMPAR expression. During neural development and into adulthood, LGI1 contributes to synaptic stabilisation and excitability modulation by interacting with its transmembrane partners ADAM22 and ADAM23 (10,12,14). The lack of a pronounced effect in our GAERS experiments may reflect a ceiling effect in a highly excitable genetically predisposed network, or possibly that LGI1 related mechanisms are not central to the initiation or propagation of SWDs in this context.

Alternatively, the mild delay in seizure onset observed in the PTZ model may point to a more subtle influence of LGI1 IgG on early excitability dynamics, potentially through modulation of AMPAR-mediated excitatory signalling. Given LGI1's role in maintaining synaptic homeostasis, these effects could represent an alteration in excitability thresholds rather than a direct pro– or anti-seizure effect. Altogether, these findings support the hypothesis that LGI1 autoantibodies may contribute to network instability in a context-dependent manner, potentially playing a more prominent role in focal epilepsies or conditions involving synaptic plasticity rather than generalised absence epilepsy.

Notably, while genetic mutations of the *Kcna1* gene encoding Kv1.1 channels and the *Cacna1a* gene encoding the voltage-gated calcium channels, associated with absence epilepsy, induce severe seizures in rodents, coexistence of these two mutations ameliorate seizure severity (33). In a single study, serum IgG of LGI1 antibody positive encephalitis patients has been shown to reduce calcium currents of cultured primary hippocampal neurones (34) further emphasising the intricate interplay between voltage-gated calcium and potassium channels. Further investigation is warranted to clarify the conditions under which LGI1 IgG might exert more substantial effects and to explore the therapeutic implications of modulating this pathway. To further elucidate the role of LGI1 in absence seizures, future studies should explore its specific molecular and circuit-level effects using region-targeted manipulations, electrophysiological recordings, and analyses of downstream targets such as AMPARs, Kv1.1 channels, and ADAM22/23 interactions, particularly within CTC circuitry.

While our findings provide novel insights into the differential effects of NMDAR and LGI1 antibodies on absence seizures, several limitations should be acknowledged. First, the sample size was limited due to the complexity of antibody purification and stereotaxic administration, which may reduce statistical power in detecting subtle effects, particularly for LGI1 IgG. Second, the study utilised passive transfer of patient IgG without direct confirmation of target engagement or receptor internalisation in brain tissue, which could be addressed in future work using immunohistochemistry or Western blotting. Moreover,

exploring the specific molecular and circuit-level effects through region-targeted manipulations, electrophysiological recordings, and analyses of downstream targets—such as AMPARs, Kv1.1 channels, and ADAM22/23 interactions—particularly within the CTC circuitry would provide deeper mechanistic insights. Third, behavioural assessments beyond EEG were not included, so potential cognitive or motor side effects of antibody exposure remain unexplored. Lastly, while two models of absence epilepsy were employed, these may not fully recapitulate the complexity of human absence epilepsy or encephalitis-related seizure phenotypes. Future studies incorporating chronic antibody exposure by using osmotic pumps, employing monoclonal antibodies, and conducting cellular-level analyses could help overcome these limitations.

In conclusion, this study demonstrates that NMDAR autoantibodies, but not LGI1 autoantibodies, exacerbate absence seizure activity in both genetic (GAERS) and pharmacologically induced (PTZ) models. These results highlight a specific contribution of NMDAR dysfunction to the pathophysiology of absence seizures and support the relevance of antibody-mediated synaptic alterations in seizure susceptibility. Understanding these mechanisms may guide future research into autoimmune contributions to generalised epilepsies and inform the development of targeted therapeutic strategies.

Ethics Committee Approval: This study has been approved by the Animal Ethics Committee of Istanbul University (Ethics number: 1107593).

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[#]H.Y.K. and Ş.A.P. contributed equally to the study.

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