Radiprodil, a selective GluN2B negative allosteric modulator, rescues audiogenic seizures in mice carrying the GluN2A(N615S) mutation

Ilaria Bertocchi[1], Lorenzo Cifarelli[2], Alessandra Oberto[1], Carola Eva[1], Rolf Sprengel[3], Mirza Rohman[4], and Pierandrea Muglia[5]

[1]Neuroscience Institute Cavalieri Ottolenghi
[2]University of Turin
[3]Max-Planck-Institute for Medical Research
[4]Sygnature Discovery Ltd
[5]GRIN Therapeutics Inc

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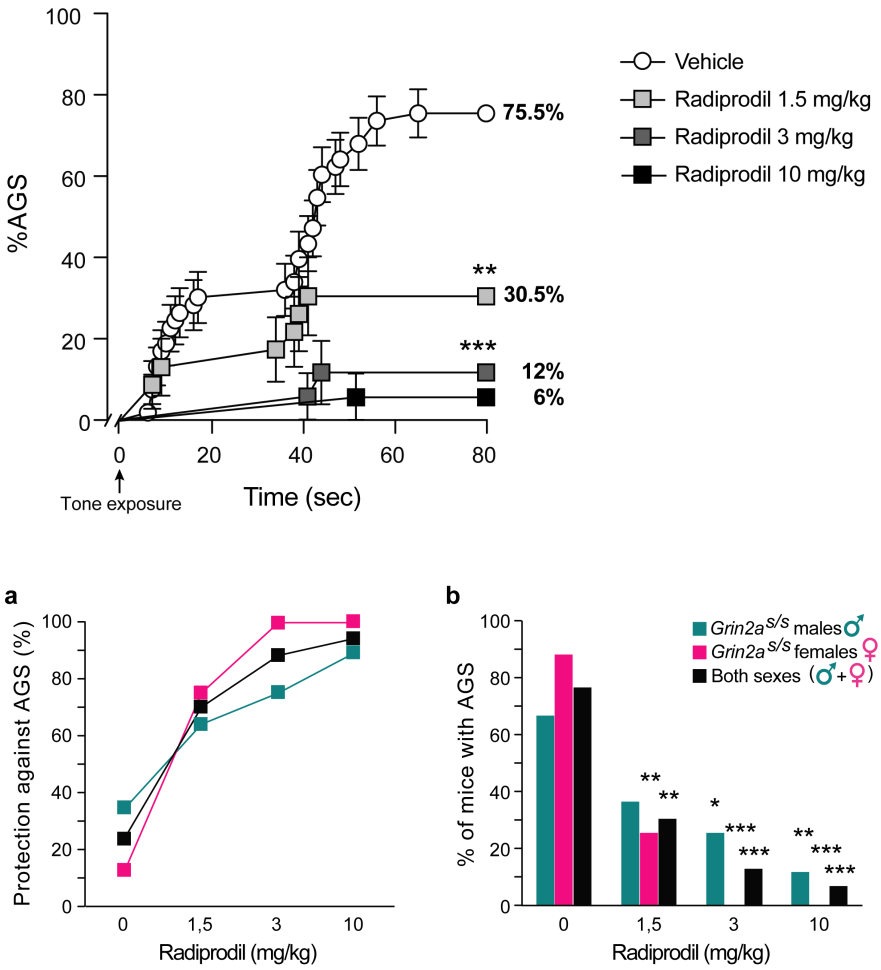
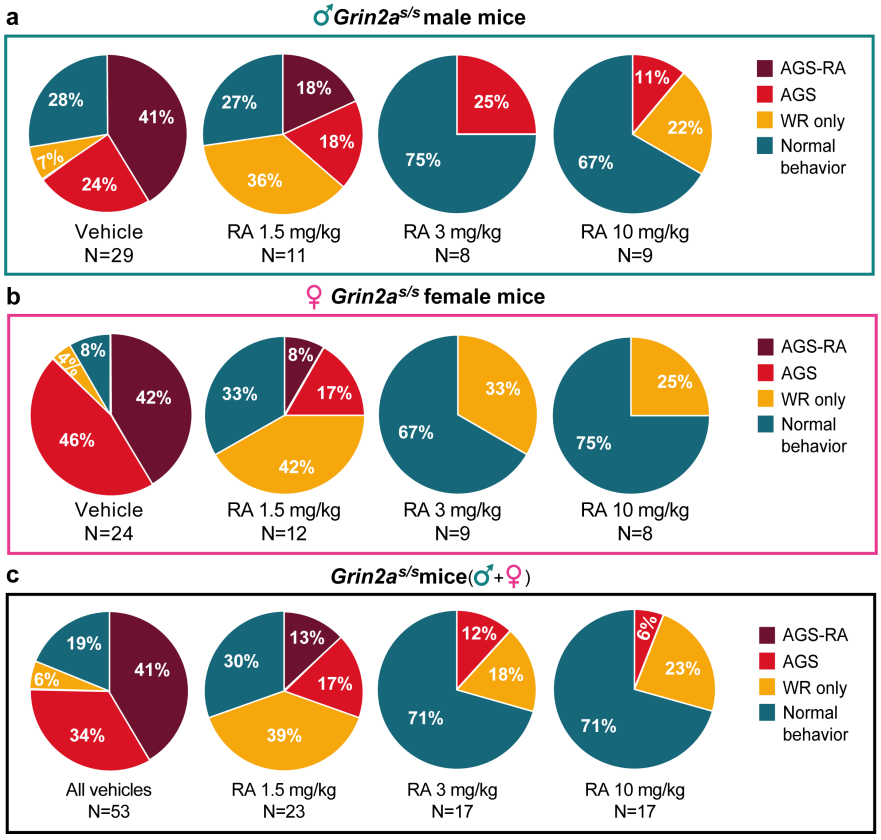
Abstract

Background and Purpose: GRIN-related disorders are neurodevelopmental disorders caused by mutations in the N-methyl-D-aspartate receptor (NMDAR) subunit receptor GRIN genes. A large fraction of these mutations leads to gain of function (GoF) of the NMDAR. Patients present with a combination of symptoms that includes epilepsy, intellectual disability, behavioural and motor symptoms. Controlling seizures is a significant medical need in most patients with GRIN-related disorders. The aim of this study was to assess the therapeutic efficacy of radiprodil, a selective negative allosteric modulator of GluN2B-containing NMDARs, in counteracting audiogenic seizures (AGS) in a murine model carrying the GluN2A(N615S) mutation in homozygosity (Grin2aS/S mice). Experimental Approach: Grin2aS/S mice were acutely treated with radiprodil at different doses before the presentation of a high-frequency acoustic stimulus commonly used for AGS induction. Key Results: Radiprodil significantly and dose-dependently reduced the onset and severity of AGS in Grin2aS/S mice. Conclusion and Implications: Our data clearly indicates that radiprodil has the potential to control seizures in patients with GRIN2A GoF mutations, targeting the underlying pathophysiology of the disorder.





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Bertocchi Ilaria[1,2,3], Cifarelli Lorenzo[1,] Oberto Alessandra[1,2,3], Eva Carola Eugenia[1,2,3], Sprengel Rolf [4], Mirza Naheed Rohman[5,6], Muglia Pierandrea[5]

[1]Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Regione Gonzole 10, IT-10043 Orbassano (Turin), Italy
[2] Department of Neuroscience Rita Levi Montalcini, University of Turin, Via Cherasco 15, IT-10126 Turin, Italy.
[3]Neuroscience Institute of Turin, Regione Gonzole 10, Orbassano, 10043 Turin, Italy
[4] Max Planck Institute for Medical Research, Jahnstr. 29, 69120, Heidelberg, Germany
[5] GRIN Therapeutics Inc, Brussels, Belgium
[6] Sygnature Discovery, BioCity, Pennyfoot Street, Nottingham, NG1 1GR, UK

†**Correspondence:** ilaria.bertocchi@unito.it

Authors contribution
IB, CE, NRM and PG participated in research conception and design. IB and AO maintained the mouse lines. IB, AO and LC conducted experiments. LC performed the computational analysis. IB analyzed the data, performed the statistical analysis and prepared the figures. IB and PM wrote the manuscript. All authors contributed to the editing of the manuscript.

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Conflict of interest statement: PM is founder of GRIN Therapeutics that is developing radiprodil for GRIN related disorders. The other authors declare no conflicts of interest.

Keywords: knock-in mice, gain of function mutation, audiogenic seizures, dose-response curve

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Abstract

Background and Purpose: *GRIN*-related disorders are neurodevelopmental disorders caused by mutations in the N-methyl-D-aspartate receptor (NMDAR) subunit receptor *GRIN* genes. A large fraction of these mutations leads to gain of function (GoF) of the NMDAR.
Patients present with a combination of symptoms that includes epilepsy, intellectual disability, behavioural and motor symptoms. Controlling seizures is a significant medical need in most patients with *GRIN*-related disorders.
The aim of this study was to assess the therapeutic efficacy of radiprodil, a selective negative allosteric modulator of GluN2B-containing NMDARs, in counteracting audiogenic seizures (AGS) in a murine model carrying the GluN2A(N615S) mutation in homozygosity (*Grin2a*$^{S/S}$ mice).
Experimental Approach: *Grin2a*$^{S/S}$ mice were acutely treated with radiprodil at different doses before the presentation of a high-frequency acoustic stimulus commonly used for AGS induction.
Key Results: Radiprodil significantly and dose-dependently reduced the onset and severity of AGS in *Grin2a*$^{S/S}$ mice.

Conclusion and Implications: Our data clearly indicates that radiprodil has the potential to control seizures in patients with *GRIN2A* GoF mutations, targeting the underlying pathophysiology of the disorder.

What is already known

- Radiprodil is a selective negative allosteric modulator of GluN2B-containing NMDAR
- Radiprodil showed anticonvulsant effects in animal seizure models and patients with drug resistant infantile spasms

What this study adds

- This study shows that radiprodil is highly effective in controlling seizures in *Grin2a*$^{S/S}$ mice
- This indicates that radiprodil may control seizures in patients with *GRIN2A* GoF mutations

Clinical significance

- The results obtained in this study support the clinical use of radiprodil
- The ongoing clinical trial could be extended to patients having syndromes associated with *GRIN2A* mutations

1_Introduction

The N-methyl-D-aspartate receptor (NMDAR) is a heterotetrameric ion channel containing two copies of the essential subunit GluN1 and two accessory subunits from the GluN2(A-D) or the GluN3(A-B) family, all encoded by *GRIN* genes (Sprengel et al., 2001). The GluN1 subunit is expressed ubiquitously in the brain throughout development and its germ line knockout is lethal (Forrest et al., 1994). The subunits belonging to the GluN2 and GluN3 families are expressed differentially through development and across brain regions. They combine to form heterotetrameric channels imparting different pharmacological properties crucial to the receptor (Paoletti et al., 2013). The tightly controlled excitatory activity of NMDARs is critical to nervous system development and function and, consequently, it is largely implicated in neuropathology (Hansen et al., 2017; Hanson et al., 2023).

Mutations, mostly *de novo,* in the NMDAR subunits genes *GRIN1, GRIN2A, GRIN2B*, and *GRIN2D,* have been identified as responsible for recently described *GRIN*-related neurodevelopmental disorders (Benke et al., 2021; Chen et al., 2017; Fry et al., 2018; Guerrini et al., 2023; Lemke et al., 2014; Platzer and Lemke, 2019; Platzer et al., 2017; Strehlow et al., 2019).

A large fraction of these mutations leads to enhanced NMDAR activity and are defined as Gain of Function (GoF) mutations according to thorough characterization *in vitro* (Han et al., 2022; Myers et al 2023). *GRIN*-related disorder represents an area of clear unmet medical need, as current available therapies cannot control the highly disabling symptoms, and this include seizures that are often resistant to broad spectrum antiseizure medications (ASM). Seizures and the electroclinical activity have to be controlled, as their persistence during crucial periods of brain development may have long-term consequences (Löscher et al., 2020).

Unfortunately, existing *in vivo* characterization of preclinical models of *GRIN* mutations do not adequately reproduce the human pathology (Oyrer et al., 2018; Bertocchi et al., 2023). Recently, two knock-in mice with inserted different point mutations on the GluN2A subunit were described that show an epileptic phenotype and other symptoms akin to human *GRIN*-related disorder (Amador et al., 2020; Bertocchi et al., 2021). Accordingly, a careful analysis of human *GRIN*-related mutations and rare variants reported *GRIN2A* mutations as those most involved in epileptic disorders (XiangWei et al., 2018).

One of these *GRIN2A* knock-in mice, the *Grin2a*$^{S/S}$ mouse, is homozygous for the GluN2A(N615S) mutation, and exhibits high sensitivity to audiogenic seizures (AGS) (Bertocchi et al., 2021).

The asparagine (N) amino acid residue N615 of the GluN2A subunit is localized in the P-loop structure of the M2 membrane domain forming the ion pore, and is implicated in augmenting the voltage-dependent Mg^{2+} block of the channel pore (Burnashev et al., 1992). The NMDAR does not function uniquely in a ligand-dependent manner like other ionotropic receptors, since channel opening only occurs when the membrane is sufficiently depolarized to remove the Mg^{2+} ion inside the pore. Hence, the NMDAR is better described as a 'coincidence detector' of pre- and postsynaptic activity, which is an essential property relevant to synaptic plasticity and critical for brain development and cognitive function (Sjöström & Nelson, 2002).
In *Grin2a*$^{S/S}$ mice, the mutation induces a significant decrease in Mg^{2+} block but does not induce alterations in channel conductance or in the synaptic expression of GluN2A(N615S)-containing NMDARs (Bertocchi et al., 2021).This reduction of the Mg^{2+} block was more prominent at postnatal day (P)42 than P14, in accordance with the expression profile of *Grin2a* during development, which starts only after birth reaching its peak in adulthood (Monyer et al., 1994).
The GluN2A(N615S) mutation is analogous to those described in children with developmental and epileptic encephalopathies (DEEs) (Endele et al., 2010; Allen et al., 2016), thus the *Grin2a*$^{S/S}$ mouse represent a robust model for *GRIN*-related disorders (Bertocchi et al., 2021).
Radiprodil is a potent and selective negative allosteric modulator of the NMDAR GluN2B subunit. The efficacy of radiprodil to inhibit NMDAR currents is fully retained when tested using receptors containing GluN2B GoF mutations (Mullier et al., 2017) and showed anticonvulsant effect *in vivo* in both acute and sub-chronic seizure models (Auvin et al., 2020). Moreover, initial pediatric clinical experience with radiprodil showed that in three infants with drug resistant infantile spasms, radiprodil administered for up to 34 days was safe and well-tolerated and reduced spasms in all three infants, with one becoming spasm-free (Auvin et al., 2020).
More recently, radiprodil's inhibitory activity *in vitro* has also been confirmed in heterotrimeric NMDARs containing GluN2 GoF mutations (Han et al., 2022), suggesting that it would have efficacy also in rodents or humans with *GRIN2A* GoF mutations. Indeed, increasing evidence suggest that, in the cortex and hippocampus, heterotrimeric GluN1/GluN2A/GluN2B receptors constitute the largest portion of the NMDAR pool (Han et al., 2022). Accordingly, radiprodil extended the survival of mice carrying the GluN2A(S644G) mutation, which usually have lethal spontaneous seizures by P17 (Amador et al., 2020). Interestingly, *Grin2a*$^{S/S}$ mice express normal levels of GluN2A but increased levels of GluN2B protein, and their hippocampal long-term potentiation (LTP) is supported almost exclusively by GluN2B-containing NMDARs (Bertocchi et al., 2021).
Based on its mechanism of action and available data, radiprodil, targeting the underlying pathophysiology of *GRIN2A* GoF patients, has the potential to control seizures. To test this hypothesis, the present study aimed to demonstrate that radiprodil has the ability to control AGS in the *Grin2a*$^{S/S}$ mouse model.

2_Methods

2.1 Animals

All animal experiments at the Neuroscience Institute Cavalieri Ottolenghi (NICO) in Orbassano (Turin) were conducted in accordance with the European Community Council Directive of 24 November 1986 (86/EEC) and approved by the University of Turin Ethical Committee for animal research and by the Italian Ministry of Health (licenses no. 209/2022-PR).
Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020).
Mice with the GluN2A(N615S) mutation (Bertocchi et al., 2021) (Grin2Atm2Rsp strain), available for purchase at the EMMA European Mutant Mice Archive (EM:09319:B6.129-Grin2atm2Rsp/Kctt, https://www.informatics.jax.org/allele/MGI:6406404), were bred and housed in a temperature (22 ±

1 °C) and humidity (50 ± 10%) controlled room, in groups of 2-4 per cage, with ad libitum access to food and water. Nesting paper materials were used for environmental enrichment and diurnal rhythm was maintained with a 12:12 h light-dark cycle (08:00 a.m. - 08:00 p.m.). The number of animals used has been estimated through the use of a statistical program for power analyzes (G*Power Version 3.1.2; Franz Faul, Università di Kiel, Germany). Precautions have been taken to reduce the number of animals used.

2.2 Experimental design and radiprodil administration

Male and female *Grin2a*$^{S/S}$ mice were treated with radiprodil by intraperitoneal injection (ip) at different doses (1.5, 3 or 10 mg/kg) 30 min before the tone induction. Each mouse was placed inside the sound-attenuating box (cubicle by Ugo Basile) 5 minutes before the presentation of a high-frequency acoustic stimulus (11 kHz, ~ 100dB).
As the ED50 of radiprodil in adult DBA/2 mice (also sensitive to AGS) is 2.1 mg/kg ip (Auvin et al., 2020), we tested first the dose at 3 mg/kg (N= 8,8 males; N= 7,9 females), and then the highest (10 mg/kg, N= 8,9 males; N= 8,8 females) and, lastly, the lowest dose (1.5 mg/kg, N= 13,11 males; N= 9,12 females). The mice were tested one at a time under the same conditions during the day.

2.3 Audiogenic seizure induction protocol

The AGS testing was conducted in a standardized sound-attenuating chamber (isolation cubicle by Ugo Basile), provided with a speaker for high-frequency acoustic stimulus (11 kHz, ~100 dB) and with an infrared camera for recording. The cubicle was connected with a laptop provided with the AnyMaze Software (Stoelting Co) to control the protocol induction. Immediately after drug treatment mice were left undisturbed in their home cage in the induction room for 25 minutes and, after this time window, they were gently introduced into the isolation cubicle for tone induction. After 5 minutes of acclimatation, the stimulus was presented until the onset of AGS or for a maximum of 80 s (4 repetition of 20 s tone, interrupted by 2-s breaks). After the tone presentation, in case of no AGS or AGS recovery (no death for respiratory arrest), mice were left undisturbed inside the cubicle for other 5 minutes.

2.4 Behavioral analysis

Video collected were analyzed by an investigator blinded to the mouse treatment. A typical complete response to the tone stimulus by a *Grin2a*$^{S/S}$ mutant mouse includes the following phases: 1) wild running (WR), 2) loss of posture with clonic seizures, tonic extension of limb extremities and 3) respiratory arrest (RA) (Bertocchi et al., 2021). Accordingly, the behaviors analyzed were falling into 4 categories:

- **only WR**: mice who experienced only wild running;
- **AGS**: mice who experienced WR and the above mentioned second phase but recover and were alive at the end of the induction protocol;
- **AGS-RA**: mice who experienced all the phases and died for respiratory arrest (AGS-RA);
- **normal behavior**: mice who didn't show any of the phases described above but react normally to the tone.

In the survival plots, mice that showed only WR, the first stage of audiogenic fit, were included in the AGS surviving mice group.

2.5 Data and Statistical analysis

Data were first recorded and analyzed in Excel (Microsoft), which was used also for the construction of the pie charts, whereas for the construction and analysis of survival plots, bar graphs and the dose response curve, Graphpad Prism software 9.0 (GraphPad, USA) was used. For the survival plots, results were considered statistically significant when $p \leq 0.05$ and the Log-rank (Mantel–Cox) test was used. Data are shown as mean ± SEM.

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2022). All animals tested were treated as independent values.

2.6 Materials

Radiprodil (2-[4-(4-Fluoro-benzyl)-piperidin-1-yl]-2-oxo-N-(2- oxo-2,3-dihydro-benzooxazol-6-yl)-acetamide, was provided by GRIN Therapeutics Inc, Brussels, Belgium. DMSO, NaCl and methylcellulose were obtained from Sigma-Aldrich. Drug solutions were freshly prepared on the day of the experiment in 5% (w/v) DMSO and 1.0% (w/v) methylcellulose in 0.9 % saline.

2.7 Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.

3_Results

3.1 Radiprodil protects *Grin2a*$^{S/S}$ mice against AGS

Adult (8–13 weeks old) males and females *Grin2a*$^{S/S}$ mice, homozygous for the mutation, were used for the experimentation, since both sexes proved to be equally sensitive to audiogenic seizures (AGS) (Bertocchi et al., 2021).

As highly vulnerable to AGS, when exposed to an audiogenic stimulus (11 kHz, ~100 dB), most of *Grin2a*$^{S/S}$ mutants treated with the vehicle responded after tone onset with a stereotypic response, composed of wild running (WR) followed by clonic seizures, tonic extension of limb extremities and, in most severe cases, death by respiratory arrest (RA).

A single dose of radiprodil, administered intraperitoneally 30 minutes before exposure to tone, was shown to significantly decrease the incidence of AGS in *Grin2a*$^{S/S}$ mutant mice (**Figure 1**). The percentage of AGS-susceptible *Grin2a*$^{S/S}$ mice was reduced in a dose-dependent manner, going from a rate of 75.5% in vehicle-treated mice to only 6% in mice treated with the highest dose of radiprodil used in this study (10 mg/kg). Already very low doses of radiprodil (1.5 mg/kg) were effective in reducing the initial susceptibility by half. Furthermore, treated mice that continued to be sensitive to AGS, showed increasing seizure onset latency along with increasing doses of radiprodil (**Figure 1**).

3.2 The rescue effect of radiprodil is dose-dependent and differ between sexes

The protective effect of radiprodil differed in magnitude between male and female *Grin2a*$^{S/S}$ mice (**Figure 2**). The incidence of AGS decreased in a dose-dependent manner in both groups but, as shown by the dose-response curve in **Figure 2a**, radiprodil effectiveness was higher in *Grin2a*$^{S/S}$ females, with a significant AGS protection already at low doses (1.5 mg/kg), that reached a full rescue (100%) at higher doses (3 and 10 mg/kg). In *Grin2a*$^{S/S}$ males, who showed in this study a lower susceptibility to AGS (65%) than their female littermates (88%), only the higher doses of radiprodil were able to significantly protect from AGS, whereas the protection achieved with the lowest dose didn't reach the significance as for females (**Figure 2b**).

3.3 Sex-dependent differences of radiprodil in rescuing the typical stages of the audiogenic fit

Radiprodil at increasing doses demonstrated strong efficacy in counteracting AGS in both male (**Figure 3a**) and female *Grin2a*$^{S/S}$ mice (**Figure 3b**). However, as mentioned above, a sex-dependent

difference in AGS susceptibility, as well as in the radiprodil dose-dependent rescue effect, is evident. It must be said that both sexes injected with the vehicle only were equally susceptible to the most severe form of AGS, which culminates with RA and death of the animal (41 vs 42%, **Figure 3a and b**). However, control *Grin2a*$^{S/S}$ males showed lower susceptibility to AGS with recovery compared to their female counterparts (26% against 46%, **Figure 3a and b**, respectively). In turn, the total susceptibility to AGS (AGS with recovery and AGS-RA) between sexes resulted different, even if not significantly (**Figure 2**), with 20% more *Grin2a*$^{S/S}$ males showing 'normal' behavior during the tone presentation compared to females (**Figure 3a and b**).
Moreover, in *Grin2a*$^{S/S}$ males, a low dose of radiprodil (1.5 mg/kg) was not able to significantly reduce AGS (**Figure 2b**), but it was able to halves the incidence of AGS-RA (**Figure 3a**). A powerful protective effect is visible at the dose of 3 mg/kg, with a complete rescue of AGS-RA, but with a quarter of males (25%) still presenting AGS (with recovery). The highest dose of 10 mg/kg was further able to reduce this percentage by more than half (to 11%, **Figure 3a**), leaving a small portion of male mice still susceptible to the wild-running stage (WR), the earliest audiogenic fit phase consisting 'only' in a 'crazy' run.
Conversely, in females, already low doses of radiprodil were highly effective in reducing both AGS with and without RA (from 42% to 8% and from 46% to 17%, respectively, **Figure 3b**) and higher doses completely abolish the incidence of AGS in all the *Grin2a*$^{S/S}$ females tested, leaving just a fraction manifesting WR (from 33% at 3mg/kg to 25% at 10 mg/kg, **Figure 3b**).

Discussion

GRIN-related disorders are severe neurodevelopmental syndromes for which there is not yet effective treatments. The research and development of new treatments is made difficult by the complex and multifaceted nature of these syndromes, regardless of the type of *GRIN* mutation (Xie et al., 2023). Taking into account the causal association of *GRIN* variants with epilepsy, ASM targeting NMDARs are of considerable medical interest. However, given "classical" non-selective NMDAR channel blockers engender side effects limiting their therapeutic index, more selective NMDAR modulators could lead to the desired therapeutic effects with fewer side effects, thus having greater therapeutic potential in various disorders, including syndromes associated with GoF *GRIN* mutations.
Here, we tested the efficacy of different doses of radiprodil, a novel negative modulator selective for GluN2B-containing NMDARs, in counteracting the epileptic phenotype caused by a GoF *Grin2a* mutation in *Grin2a*$^{S/S}$ mice. The *Grin2a*$^{S/S}$ mouse, carrying the GluN2A(N615S) mutation in homozygosity, is a model of *GRIN*-dependent DEEs and is highly vulnerable to AGS. The high vulnerability to AGS is particularly striking, given that the genetic background of these mice (C57BL/6) is resistant to AGS relative to other mouse strains (Heinrichs and Seyfried, 2006).
GluN2A subunit expression starts only after birth and increases during postnatal development to progressively 'replace' GluN2B in an activity-dependent manner in different brain areas related to cognition (Liu et al., 2004; Bellone and Nicoll, 2007). GluN2B, on the other hand, is highly expressed since embryonic development and in the 'excitable' immature brain (Monyer et al., 1994).
Despite this so called 'developmental switch', the expression levels of GluN2B remain high even after brain circuits have matured, being far more abundant than GluN2A in the forebrain and continuing to have a significant impact on synaptic activity into adulthood (Frank et al., 2016; Wong et al., 2021).
Interestingly, as in other epilepsy models (Yang et al., 2006; Ghasemi and Schachter, 2011), the GluN2B subunit is upregulated in adult *Grin2a*$^{S/S}$ mice, and mediates hippocampal LTP in these mutants, a role usually subserved by the GluN2A subunit in adulthood (Bertocchi et al., 2021). Therefore, with respects to hippocampal LTP, the adult *Grin2a*$^{S/S}$ mouse is similar to young wild-type mice in which the GluN2B subunit mediates hippocampal LTP (Köhr et al., 2003).
It has previously been shown that inhibition of GluN2B by a selective antagonist has anticonvulsive and neuroprotective effects in rodent models of epileptic seizures (Mareš et al., 2021; Mullier et al., 2017).

Accordingly, in this study, we have demonstrated a significant dose-dependent anticonvulsant effect of radiprodil in adult *Grin2a*$^{S/S}$ mice. The fact that radiprodil, which selectively targets GluN2B-containing NMDA receptors, restored AGS induced by a mutation present on the GluN2A subunit, suggests that this compound is able to restore neuronal function and reduce the excitation provoked by the GluN2A(N615S) mutation, and could therefore be effective in counteracting seizures in various epileptic syndromes where there is a *GRIN* GoF mutation. This makes sense, given that it is increasingly recognized that many NMDARs in cortical and hippocampal areas are triheteromeric, containing both GluN2A and GluN2B subunits alongside two essential GluN1 subunits (Han et al., 2022). Given there are various non-seizure clinical symptoms in *GRIN* related disorders, our findings suggest that GluN2B selective drugs like radiprodil might be effective in alleviating neurological symptoms as well as seizures (Benke et al., 2021). Whilst we have not explicitly tested for these other symptoms in the current study, our profiling of *Grin2a*$^{S/S}$ mice show that non-seizure symptoms (e.g., muscle tone, hippocampal associative learning, attention, and hyperactivity) are prominent (Bertocchi et al., 2021).

Interestingly, the efficacy of radiprodil in protecting *Grin2a*$^{S/S}$ mice from AGS was somewhat sex-dependent. In addition to the different susceptibility of males versus females to AGS, all doses of radiprodil showed greater efficacy in counteracting AGS in females compared to males. This may be due to inherent sex differences in physiology and response to drug treatment (Soldin and Mattison, 2009).

This unexpected sex-dependent effect could be due in part to the difference (even if not significant) in AGS susceptibility observed between males and females in vehicle-treated *Grin2a*$^{S/S}$ mice, with the males being more resistant. The same difference did not emerge in our previous study (Bertocchi et al., 2021), in which we described 100% penetrance of AGS-RA in all mice tested, without priming. This inconsistency is probably due to the different set-up and conditions: the intensity of the tone produced by two speakers used previously was most likely higher than that produced by the unique speaker in the current study (not exceeding 100 dB). AGS are the predominant reflex epilepsies in rodents and susceptibility is strongly associated with the interaction of genetics and environment (Faingold 2012). Housing conditions and even the most subtle differences in the "trigger" can influence the variability of AGS susceptibility, engendering variability between studies. Clearly, laboratory conditions and the setup used to induce AGS should be standardized as much as possible, to allow comparison of results in the same animal model by different research groups.

Nonetheless, the results of this study indicate that radiprodil effectively counteracts AGS in *Grin2a*$^{S/S}$ mice and may therefore have anticonvulsant activity in patients with analogous GoF mutations, indicating it might be effective in a broader range of patient carrying *GRIN* GoF mutations beyond those with *GRIN2B* mutations.

In conclusion, our data demonstrate the efficacy of radiprodil in protecting *Grin2a*$^{S/S}$ mice from AGS. Acute treatment with radiprodil resulted in a strong attenuation of AGS and total suppression of AGS-induced RA already at low doses, especially in female *Grin2a*$^{S/S}$ mice. Interestingly, although it may be just a coincidence, the two human patients described in the literature carrying an analogous mutation, in the very same amino acid position, are female (Endele et al., 2010; Allen et al., 2016).

Radiprodil available data *in vitro* and *in vivo* together with the anticonvulsant effect in the *Grin2a*$^{S/S}$ mouse here presented indicates its potential therapeutic effect in patients with *GRIN2A GoF* mutations.

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Figure legends

Figure 1. Survival plot showing the incidence of AGS in all *Grin2a*$^{S/S}$ mice tested, with males and females pulled together. The incidence of AGS dropped from 75.5% of the vehicle-treated group (N=53) to 30.5% of the group treated with radiprodil at the lower dose (1.5 mg/kg N=23), to 12% of the medium dose (3 mg/kg N=17) and to 6% of the group treated with the higher dose (10 mg/kg, N=17) [**p <0.01 and ***p <0.001 by Log-rank (Mantel–Cox) test].

Figure 2. a) Dose-dependent protection against AGS in the *Grin2a*$^{S/S}$ mouse model (black curve). Green and pink curves represent the same result when only male and female *Grin2a*$^{S/S}$ mice are considered, respectively. **b)** A sex-dependent difference in AGS incidence (not significant) can be observed in vehicle-treated mice (at 0) and also in the efficacy of radiprodil in rescuing AGS in males and females at different doses [*p <0.05, **p <0.01 and ***p <0.001 versus dose 0, by Log-rank (Mantel–Cox) test].

Figure 3. Pie charts showing the efficacy of increasing doses of radiprodil in counteracting the different phases of a typical audiogenic fit in order of severity: from normal behavior in blue, to wild running (WR) only in yellow, to AGS (audiogenic seizure) with recovery in red and AGS-RA (AGS followed by respiratory arrest) in brown-red, in **a)** males *Grin2a*$^{S/S}$ mice; **b)** females *Grin2a*$^{S/S}$ mice and **c)** male and female *Grin2a*$^{S/S}$ mice pulled together.