# Metabotropic glutamate receptors as drug targets for the treatment of absence epilepsy

Richard Teke Ngomba[1*] and Gilles van Luijtelaar[2]

*Addresses*

[1]*School of Pharmacy in College of Science, University of Lincoln, Lincoln, LN6 7TS, UK*

[2]*Donders Centre of Cognition, Radboud University, Nijmegen, the Netherlands*

**Corresponding Author: Richard T. Ngomba,* richardngomba@hotmail.com

*(rngomba@lincoln.ac.uk)*

## Short title

mGlu receptors and absence epilepsy

## Keywords

Absence seizures; SWDs; mGlu PAM; cortex; thalamus; WAG/Rij rats; genetic models

## Abstract

Metabotropic glutamate (mGlu) receptors are expressed in key regions of the cortex and the thalamus and are known to regulate spike and wave discharges (SWDs), the electroclinical hallmarks of absence seizures. Recent preclinical studies have highlighted the therapeutic potential of selective group I and III mGlu receptor subtype allosteric modulators, which can suppress pathological SWDs. Of particular interest are positive allosteric modulators (PAMs) for mGlu5 receptors, as they currently show the most promise as novel anti-absence epilepsy drugs. The rational design of novel selective positive and negative allosteric mGlu modulators, especially for the mGlu5 receptor, has been made possible following the recent crystallographic structure determination of group I mGlu receptors. Our current knowledge of the role of different mGlu receptor subtypes in absence epilepsy is outlined in this article.

Highlights:

- mGlu receptors are located within the cortico-thalamo-cortical system, which generates spike-wave discharges typical of absence epilepsies.
- mGlu receptors of all three subgroups are involved in the control of SWDs, although selective PAMs and NAMs are not available for all receptor subtypes.
- The pharmacological profile of a mGlu5 receptor PAM has a good preclinical anti-absence profile.
- The mGlu7 receptor seems to be an attractive target for putative anti-absence drugs.

## Introduction

Absence epilepsy is non-convulsive and is characterized by a sudden decrease in responsiveness, accompanied by staring and the simultaneous appearance of highly stereotypical bilateral symmetrical network activity in the form of spike-and-wave discharges (SWDs) in the EEG. The main anatomical structure where these SWDs are generated is the extensive cortico-thalamo-cortical network which includes the reticular thalamic nucleus (nRT) [1*,2, 3*,4]. The frontal cortex has been identified as the major initiation site for SWDs in some patients [5], although other cortical locations including the temporal, occipital and parietal lobe can also be initiation sites [6]. In a widely used and validated genetic absence animal model, the WAG/Rij strain of rat, the peri-oral region of the somatosensory cortex (S1po) has been identified as the SWD initiation site [5]. These WAG/Rij rats are an inbred strain that shows an age-dependent increase in the probability of developing spontaneous SWDs. Following their development, SWDs increase in both frequency and mean duration. SWDs occur mainly during passive wakefulness, in an otherwise motionless animal, not during deep slow wave sleep. During SWDs, animals do not make overt behavioural responses to obtain food pellets, as they would do normally suggesting that responsiveness is reduced.

Many classical and newer antiepileptic drugs have been tested in this and in a similar absence model, the GAERS (Genetic absence epilepsy rats from Strasbourg) . Based on the outcomes of such experiments, it has been concluded that these animal models provide a good prediction of the efficacy of drugs and the possible off-target effects that will be observed in humans with absence seizures [3].

The glutamatergic and GABAergic systems are involved in controlling excitation and inhibition respectively, in the cortex and thalamus. Aside from the highly excitable site in the cortex that initiates SWDs in WAG/Rij rats and in GAERS, increased thalamic tonic

GABAergic inhibition has also been demonstrated in some genetic absence epilepsy models [7*, 8*]. The fact that metabotropic glutamate (mGlu) receptors are expressed in different regional circuit cell types within cortico-thalamo-cortical networks, and that they modulate synaptic transmission [9*,10, 11] (see also Figure 1), suggest a potential role for mGlu receptors in regulating SWDs.

The mGlu receptors are classified into three main groups (I, II and III), and pre-clinical studies to date have shown that ligands for the different receptor subtypes in each group have anti-absence properties [12*, 13-15, 16*, 17-19].

Furthermore, interest in identifying new mGlu receptor ligands with anti-absence properties has been encouraged by the discovery of distinct allosteric binding sites in the crystallographic structure of transmembrane domains in group I mGlu receptors (mGlu1 and mGlu5 receptors), permitting a rational design of specific compounds [20*,21*]. Here, we focus on the role of mGlu receptors and their allosteric modulators as studied in the WAG/Rij rat model of absence epilepsy [3].

## Localisation and modulation of mGlu receptors in the cortico-thalamo-cortical circuitry

The classification of mGlu receptors into groups is based on their pharmacological properties and amino acid sequence homology profile [12,22,23]. These receptors are coupled to different G proteins and they modulate slow postsynaptic neuronal responses, either through the presynaptic or postsynaptic machinery or through modulating astrocytes function [9-12, 24] (see also Figure 1).

**Group, I mGlu receptors**

mGlu1 and mGlu5 receptor subtypes are members of group I and they are coupled to Gq/ $G_{11}$ proteins, which upon activation trigger polyphosphoinositide hydrolysis leading to the production of inositol-1,4,5-trisphosphate and diacylglycerol. These receptors are also able to regulate the activity of different types of $Ca^{2+}$ and $K^{+}$ channels [22,25,26] (see also Table 1). Data from molecular studies show that they are localized on postsynaptic dendrites of thalamic neurons and on GABAergic interneurons in the cortex [27-30]. Glial cells expressing mGlu5 receptors may also modulate synapses within the thalamus, particularly on inputs to the somatosensory ventrobasal (VB) thalamus [31] (see also Figure 1).

Group I mGlu receptors are the most extensively studied group in the WAG/Rij rat model [11]: the expression of both receptor subtypes (mGlu1 and mGlu5) have been shown to be reduced in the thalamus as compared to non-epileptic ACI control rats [13, 14]. Similar results have been observed for mGlu1 expression in the laterodorsal thalamus in the same model [32]. Interestingly, mGlu5 receptor expression was up-regulated in the motor cortex and in the S1po region, again in comparison with age-matched ACI rats, without any change in mGlu5 receptor function. Overall, both receptors show reduced expression and activity in the cortico-thalamo-cortical network in the WAG/Rij rat [13, 14, 32].

Electroencephalographic (EEG) studies carried out following systemic treatment with the specific mGlu1 receptor positive allosteric modulator (PAM) RO0711401 showed a long-lasting (6 hours) dose-related reduction in frequency and duration of SWDs (the latter only occurring only after the highest dose). As expected, treatment with the mGlu1 negative allosteric modulator (NAM) JNJ16259685 increased the incidence of SWDs [13], (see also Table 1). The mGlu5 receptor PAM VU0360172 reduced the number of SWDs in a dose-dependent manner, without any behavioural changes, such as an increase or decrease in locomotor activity in the home cage. The reduction of SWDs was antagonized by the mGlu5

receptor NAM, MTEP. MTEP itself was without any effect on SWDs [13] (see also Table 1). The lack of behavioural effects is important since clinically useful anti-epileptic drugs should lack sedative or hypnotic effects. Intriguingly, MPEP significantly reduced the occurrence of SWDs in the lethargic mouse (lh/lh) model [33-35], a different genetic absence model, in which animals show an ataxic gait in addition to SWDs. Moreover, the mGlu1 receptor orthosteric antagonists AIDA and LY367385 reduced SWDs in the same model [34]. These results are in contrast with those obtained in WAG/Rij rats. It is not clear whether these differences could be due to the different species used or to differences in ligand-site activity on receptor subtypes.

Considering that absence epilepsy is a chronic disease requiring long-term treatment, the effects of chronic administration of both selective group I receptor subtype PAMs were tested to see whether tolerance developed to the suppressing effects on SWDs. WAG/Rij rats were treated with comparable doses of each compound (RO0711401 and VU0360172) for 10 days twice daily. The rats developed complete tolerance to RO0711401 after 2 days, while VU0360172 maintained its anti-SWD effects throughout this period and even after 48 hours from ceasing treatment [15] (see also Table 1). The mechanism for this tolerance remains unclear, but it limits the clinical usefulness of RO0711401.

Further studies have been carried out to determine which regions within the cortex or the thalamus were responsible for the effects observed following systemic injection of the mGlu1 or mGlu5 receptor PAMs. When either RO0711401 or VU0360172 were individually administered locally into the cortex or the thalamus, both proportionally reduced SWDs. However, when introduced into the thalamus, VU0360172 had a more pronounced efficacy than RO0711401 [16].

How do these PAMs reduce SWDs? Both mGlu1 and mGlu5 receptors are coupled to second messenger effectors, including PLC-beta 4; these may negatively regulate T-type calcium channels in the cortex and thalamus, reducing SWDs [36-39]. Of note, mutation of PLC-beta

4 is known to influence pathological cortico-thalamo-cortical rhythms associated with T-type channels [40].

As previously stated, in physiological conditions glutamate and GABA are in a critical balance within the cortico-thalamo-cortical network [1,12,16]. Coenen and collaborators have shown that systemic injection of tiagabine a GABA re-uptake inhibitor with high affinity at the GABA transporter (GAT-1) – upset the balance, leading to increased SWD frequency and duration in WAG/Rij rats [41]. When tiagabine was administered intra-cortically at the focal region, it reduced the number of SWDs, similarly to the effect produced by local infusion of VU0360172 [16]. Intra-cortical injection of VU0360172 in combination with tiagabine produced a slight prolongation of the SWD suppressive effect, suggesting that the modes of action of VU0360172 and tiagabine may be similar [16]. Opposite results were obtained when tiagabine was infused in the thalamus: it enhanced SWDs, most likely by increasing GABA levels perisynaptically at thalamic relay cells. Interestingly, VU0360172 lost its anti-absence action when it was infused in the thalamus in combination with tiagabine [16].

Regarding VU0360172, the group I mGlu5 receptor PAM, it produced neither tolerance nor overt effects on behaviour but had good efficacy in the cortex and thalamus. Interestingly, one of the drugs of choice in the treatment of absence epilepsy, ethosuximide, is also highly effective in the cortex, but less so in the thalamus [42]. This demonstrates the importance of targeting mGlu5 receptors for the development of novel anti-absence drugs.

**Group II mGlu receptors**

Group II (mGlu2 and mGlu3) receptors are coupled to Gi/Go proteins, which inhibit the activity of adenyl cyclase and voltage-sensitive $Ca^{2+}$ channels (VSCCs) [22,26]. Molecular analysis shows that mGlu3 receptors have a higher expression on GABAergic terminals of the nRT than at glutamatergic synapses. These receptors are also present on axons of cortical layer

VI [4]. mGlu3 receptors are expressed by glial cells, and mGlu2 receptor activity has been identified in astrocytes in the ventrobasal thalamic nuclei [9,28,43] (see also Figure 1).

In symptomatic WAG/Rij rats, mGlu2/3 receptor expression in the S1po area is altered as compared with age-matched controls [17]. Only a few pharmacological studies have been carried out with orthosteric ligands for these receptors, one in WAG/Rij rats [17] and one in 1h/1h mice [45]. In WAG/Rij rats, blockade of the mGlu2/3 receptor with the antagonist LY341495 reduced the occurrence of SWDs in a dose-dependent manner, while activation of these receptors had opposite effects [17]. Intriguingly, activation of the mGlu2/3 receptor in 1h/1h mice decreased SWDs activity [45]. It is not known whether these contrasting results are due to (mGlu2 or mGlu3) receptor/ligand selectivity, specificity or model/species differences. The development of novel specific ligands for mGlu2 and mGlu3 receptors combined with genetic manipulation of these receptors might be relevant to unravelling the specific role of each subtype.

**Group III mGlu receptors**

Group III comprises mGlu4, mGlu6, mGlu7 and mGlu8 receptors, which are also coupled to Gi/Go proteins, similar to group II receptors. All except mGlu6 are expressed in the cortico-thalamo-cortical network. mGlu4 receptors are found on glutamatergic terminals in the nRT and in the VB, while mGlu7 and mGlu8 are present also on nRT neurons [9, 18, 46-50].

Group III mGlu receptors seem to be important in the mechanisms underlying the initiation of absence seizures. Different studies link the human mGlu4 receptor gene to genetic/idiopathic generalised epilepsies [51-53]. mGlu4 receptor expression is increased in the nRT of symptomatic WAG/Rij rats as compared with asymptomatic control rats; pharmacological enhancement of the activation of this receptor with the PAM PHCCC increased SWDs [7].

The other group III receptors (namely, mGlu7 and mGlu8) are implicated in the modulation of neuronal plasticity, learning, and memory [54,55], and are also involved in seizures and epilepsy [56]. mGlu7 knockout mice show increased susceptibility to chemically induced seizures [54]. The lack of specific subtype ligands for group III mGlu receptors has limited the studies of their individual roles in absence seizures.

The mGlu7 receptor has a very low affinity for glutamate, and this allows selective recruitment of the receptor during high levels of synaptic activity [57]. Conversely, prolonged agonist activation of the mGlu7 receptor can also activate a phospholipase C–mediated signalling pathway that enhances glutamate release [58].

Nevertheless, knockout mice in which PDZ-interaction with protein-interaction-C-kinase-1(PICK-1) signalling decoupled from the C-terminus of the mGlu7 receptor develop spontaneous absence-like epilepsy [56]. In addition, a study by Kyuyong and Huguenard has demonstrated that mGlu7 receptors are functionally present as autoreceptors on inhibitory synapses between nRT and thalamocortical neurons [18] (see also Table 1).

A recent study demonstrated that mGlu7 receptors are found on synapses projecting onto the nRT, and from the nRT onto the VB [19] (see also Figure 1). Blockade of mGlu7 receptor activity with the specific mGlu7 receptor NAM ADX71743 induced a lethargic condition similar to absence seizures with spindle and/or SWDs. The authors showed that the mGlu7 receptor regulates tonic modulation, particularly between GABAergic and glutamatergic synapses within the cortico-thalamo-cortical network [19].

In terms of the group III receptors, the properties and location of the mGlu7 receptor make it an attractive target in that mGlu7 receptor ligands might be beneficial for the development of anti-absence epilepsy drugs.

## Conclusion

Targeting subtype-selective metabotropic glutamate receptor is a potentially novel method for treating absence seizures given that many of these receptors are expressed in the thalamo-cortico-thalamic circuit and studies with agents that modulate receptor activity show promise in reducing SWD occurrence in animal models. The challenge will be to provide an alternative therapy to current medication for the ~40% of absence epilepsy patients who are resistant to current drugs [59]. Hypothetically, mGlu PAMs could be developed for sufferers of absence seizures refractory to conventional medications. Indeed, ligands for mGlu receptors have been under phase I and II clinical investigation for the treatment of CNS disorders [26,60*]. As an ultimate goal, the further design of subtype mGlu receptor PAMs with biased allosteric modulatory properties is relevant to delivering in vivo efficacy and the elimination of adverse effects.

We propose that designing mGlu receptor ligands with a modulatory effect on voltage-gated channels (primarily Cav3.1, the major T-channel in TC neurons) affecting neuronal electrical properties during seizures would lead to effective anti-absence therapy with reduced side effects associated with non-specific anti-absence medication, such as ethosuximide, which acts on $Na^+$ channels as well as T-channels [61,62].

## Funding

Dr. RT Ngomba is currently funded by (Epilepsy research UK) ERUK.

## Conflict of interest statement

Nothing declared.

## References and recommended reading

References of particular interest, have been highlighted as:

• of special interest

## Acknowledgements

The authors would like to thank Dr. Mark Wall for reading an earlier version of the manuscript and for scientific discussions. The Authors will also like to thank; Dr. Liz Mitchell, Dr. James Flint and Mr. Michael Latronico for critical reading of the manuscript.

## References and recommended reading


1. Blumenfeld H: **Cellular and network mechanisms of spike-wave seizures**. *Epilepsia* 2005, **46**:21-33.
    - Relevant manuscript describing cellular and molecular mechanisms contributing to the occurrence of spike-wave discharges

2. Meeren H, van Luijtelaar G, Lopes da Silva F, Coenen A: **Evolving concepts on the pathophysiology of absence seizures: the cortical focus theory**. *Arch Neurol* 2005, 62:371-376.

3. van Luijtelaar G, Zobeiri M: **Progress and outlooks in a genetic absence epilepsy model (WAG/Rij).** *Curr Med Chem* 2014, **21**:704-21.
    - Recent review of the WAG/Rij absence model

4. van Luijtelaar G, Behr C, Avoli M: **Is there such a thing as "generalized" epilepsy?** *Adv Exp Med Biol* 2014, **813**:81-91.

5. Holmes MD, Brown M, Tucker DM. **Are "generalized" seizures truly generalized? Evidence of localized mesial frontal and frontopolar discharges in absence.** *Epilepsia* 2004, **45**:1568-1579.

6. Gupta D, Ossenblok P, van Luijtelaar G. **Space-time network connectivity and cortical activations preceding spike-wave discharges in human absence epilepsy: a MEG study.** *Med Biol Eng Comput.* 2011;**49**:555-565.

7. Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH: Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J Neurosci 2002, 22:1480-1495.
    • Seminal paper in which a focal cortical origin for spike-wave discharges was demonstrated in an often used and well-validated genetic absence model

8. Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V: **Enhanced tonic GABAA inhibition in typical absence epilepsy.** *Nat Med*. **2009**, 15:1392-1398
- The paper emphasizes that next to a cortical highly excitable initiation site of SWDs, increased thalamic tonic inhibition is property of 3 genetic absence models

9. Ngomba RT, Ferraguti F, Badura A, Citraro R, Santolini I, Battaglia G, Bruno V, De Sarro G, Simonyi A, van Luijtelaar G, Nicoletti F: **Positive allosteric modulation of metabotropic glutamate 4 (mGlu4) receptors enhances spontaneous and evoked absence seizures**. *Neuropharmacology* 2008, **54**:344-354.

- Relevant paper proposing mGlu4 receptor localization in the reticular thalamic nuclei by Electron microscopy and in situ hybridization: backbone for the further studies on Group III in absence epilepsy

10. Ferraguti F, Shigemoto R: **Metabotropic glutamate receptors.** *Cell Tissue Res* 2006, **326**:483-504.

11. Copeland CS, Wall TM, Sims RE, Neale SA, Nisenbaum E, Parri HR, Salt TE: **Astrocytes modulate thalamic sensory processing via mGlu2 receptor activation.** *Neuropharmacology* 2017, **121**:100-110.

12. Ngomba RT, Santolini I, Salt TE, Ferraguti F, Battaglia G, Nicoletti F, van Luijtelaar G: **Metabotropic glutamate receptors in the thalamocortical network: strategic targets for the treatment of absence epilepsy**. *Epilepsia* 2011, **52**:1211-1222.
- Earlier review on mGluRs elucidating a role of mGlu receptors in absence epilepsy

13. Ngomba RT, Santolini I, Biagioni F, Molinaro G, Simonyi A, van Rijn CM, D'Amore V, Mastroiacovo F, Olivieri G, Gradini R, Ferraguti F, Battaglia G, Bruno V, Puliti A, van Luijtelaar G, Nicoletti F: **Protective role for type-1 metabotropic glutamate receptors against spike and wave discharges in the WAG/Rij rat model of absence epilepsy**. *Neuropharmacology* 2011, **60**:1281-1291.

14. D'Amore V, Santolini I, van Rijn CM, Biagioni F, Molinaro G, Prete A, Conn PJ, Lindsley CW, Zhou Y, Vinson PN, Rodriguez AL, Jones CK, Stauffer SR, Nicoletti F, van Luijtelaar G, Ngomba RT: **Potentiation of mGlu5 receptors with the novel enhancer, VU0360172, reduces spontaneous absence seizures in WAG/Rij rats.** *Neuropharmacology* 2013, **66**:330-338.

15. D'Amore V, Santolini I, Celli R, Lionetto L, De Fusco A, Simmaco M, van Rijn CM, Vieira E, Stauffer SR, Conn PJ, Bosco P, Nicoletti F, van Luijtelaar G, Ngomba RT: **Head-to-head comparison of mGlu1 and mGlu5 receptor activation in chronic treatment of absence epilepsy in WAG/Rij rats.** *Neuropharmacology* 2014, **85**:91-103.

16. D'Amore V, von Randow C, Nicoletti F, Ngomba RT, van Luijtelaar G: **Anti-absence activity of mGlu1 and mGlu5 receptor enhancers and their interaction with a GABA reuptake inhibitor: Effect of local infusions in the somatosensory cortex and thalamus**. *Epilepsia* 2015, **56**:1141-1151.
- Relevant paper showing that the group I PAMs is very effective in cortex and thalamus.

17. Ngomba RT, Biagioni F, Casciato S, Willems-van Bree E, Battaglia G, Bruno V, Nicoletti F, van Luijtelaar EL: **The preferential mGlu2/3 receptor antagonist, LY341495 reduces the frequency of spike-wave discharges in the WAG/Rij rat model of absence epilepsy.** *Neuropharmacology* 2005, **49**:89-103

18. Kyuyoung CL, Huguenard JR: **Modulation of short-term plasticity in the corticothalamic circuit by group III metabotropic glutamate receptors**. *J Neurosci* 2014, **34**:675-687.

19. Tassin V, Girard B, Chotte A, Fontanaud P, Rigault D, Kalinichev M, Perroy J, Acher F, Fagni L, Bertaso F: **Phasic and Tonic mGlu7 Receptor Activity Modulates the Thalamocortical Network.** *Front Neural Circuits* 2016, **10**:31.

20. Doré AS, Okrasa K, Patel JC, Serrano-Vega M, Bennett K, Cooke RM, Errey JC, Jazayeri A, Khan S, Tehan B, Weir M, Wiggin GR, Marshall FH: **Structure of class C GPCR metabotropic glutamate receptor 5 transmembrane domain**. *Nature* 2014, **31**:557-562.
- Important paper pointing to the identification of the crystallography structure of the mGlu5 receptor relevant for drug design and discovery

21. Wu H, Wang C, Gregory KJ, Han GW, Cho HP, Xia Y, Niswender CM, Katritch V, Meiler J, Cherezov V, Conn PJ, Stevens RC: **Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator**. *Science* 2014, **344**:58-64.
- Important paper pointing to the identification of the crystallography structure of the mGlu1 receptor relevant for drug design and discovery

22. Conn PJ, Pin JP: **Pharmacology and functions of metabotropic glutamate receptors**. *Annu Rev Pharmacol Toxicol* 1997, **37**:205-237.

23. Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, Wroblewski JT, Pin JP: **Metabotropic glutamate receptors: from the workbench to the bedside**. *Neuropharmacology* 2011, **60**:1017-1041.

24. Nakanishi S, Nakajima Y, Masu M, Ueda Y, Nakahara K, Watanabe D, Yamaguchi S, Kawabata S, Okada M: **Glutamate receptors: brain function and signal transduction**. *Brain Res Brain Res Rev* 1998, **26**:230-5.

25. Hermans E, Challiss RA: **Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors**. *Biochem J* 2001 **359**:465-484.

26. Niswender CM, Conn PJ: **Metabotropic glutamate receptors: physiology, pharmacology, and disease.** *Annu Rev Pharmacol Toxicol* 2010, **50**:295-322.

27. Shigemoto R, Nakanishi S, Mizuno N: **Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in adult and developing rat.** *J Comp Neurol* 1992 **322:**121-135.

28. Godwin DW, Van Horn SC, Eriir A, Sesma M, Romano C, Sherman SM:

**Ultrastructural localization suggests that retinal and cortical inputs access different metabotropic glutamate receptors in the lateral geniculate nucleus**. *J Neurosci* 1996, **16**:8181-8192.

29. Martin LJ, Blackstone CD, Huganir RL, Price DL: **Cellular localization of a metabotropic glutamate receptor in rat brain.** *Neuron* 1992, **9**:259-270.

*30.* Liu XB, Muñoz A, Jones EG: **Changes in subcellular localization of metabotropic glutamate receptor subtypes during postnatal development of mouse thalamus**. *J Comp Neurol* 1998, **395**:450-465.

31. Parri HR, Gould TM, Crunelli V: **Sensory and cortical activation of distinct glial cell subtypes in the somatosensory thalamus of young rats**. *Eur J Neurosci* **2010**, 32:29-40.

32. Karimzadeh F, Modarres Mousavi SM, Ghadiri T, Jafarian M, Soleimani M, Sadeghi SM, Mesgari M, Joghataei MT, Gorji A: **The Modulatory Effect of Metabotropic Glutamate Receptor Type-1α on Spike-Wave Discharges in WAG/Rij Rats.** *Mol Neurobiol.* 2017, **54**:846-854.

33. Chapman AG, Nanan K, Williams M, Meldrum BS: **Anticonvulsant activity of two metabotropic glutamate group I antagonists selective for the mGlu5 receptor: 2-methyl-6-(phenylethynyl)-pyridine (MPEP), and (E)-6-methyl-2-styryl-pyridine.** *Neuropharmacology* 2000, **39**:1567-1574

34. Chapman AG, Yip PK, Yap JS, Quinn LP, Tang E, Harris JR, Meldrum BS: **Anticonvulsant actions of LY 367385 ((+)-2-methyl-4-carboxyphenylglycine) and AIDA ((RS)-1-aminoindan-1,5-dicarboxylic acid)**. *Eur J Pharmacol* 1999, **368**:17-24.

35. Burgess DL, Jones JM, Meisler MH, Noebels JL: **Mutation of the Ca2+ channel beta subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse**. *Cell* 1997, **88**:385-392

36. McCool BA, Pin JP, Harpold MM, Brust PF, Stauderman KA, Lovinger DM: **Rat group I metabotropic glutamate receptors inhibit neuronal Ca2+ channels via multiple signal transduction pathways in HEK 293 cells**. *J Neurophysiol* 1998, **79**:379-391.

37. Kammermeier PJ, Xiao B, Tu JC, Worley PF, Ikeda SR: **Homer proteins regulate coupling of group I metabotropic glutamate receptors to N-type calcium and M-type potassium channels**. *J Neurosci* 2000, **20**:7238-7245.

38. Ferraguti F, Crepaldi L, Nicoletti F: **Metabotropic glutamate 1 receptor: current concepts and perspectives**. *Pharmacol Rev* 2008, **60**:536-581.

39. Beqollari D, Kammermeier PJ: **The interaction between mGluR1 and the calcium channel $Cav_{2.1}$ preserves coupling in the presence of long Homer proteins**. *Neuropharmacology* 2013, **66**:302-310.

40. Cheong E, Zheng Y, Lee K, Lee J, Kim S, Sanati M, Lee S, Kim YS, Shin HS: **Deletion of phospholipase C beta4 in thalamocortical relay nucleus leads to absence seizures**. *Proc Natl Acad Sci* 2009, **106**:21912-21917

41. Coenen AM, Blezer EH, van Luijtelaar EL: **Effects of the GABA-uptake inhibitor tiagabine on electroencephalogram, spike-wave discharges, and behaviour of rats**. *Epilepsy Res* 1995, **21**:89-94.

42. van Luijtelaar G, D'Amore V, Santolini I, Ngomba R. **Is there a future for mGlu5-positive allosteric modulators in absence epilepsy? A comparison with Ethosuximide.** In: The receptors, Vol 31, eds: Ngomba RT, Di Giovanni G, Battaglia G, Nicoletti F, Eds. Springer, 2017, pp 207-224.
    - Shows the need for new anti-absence drugs

43. Ohishi H, Shigemoto R, Nakanishi S, Mizuno N: **Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an in situ hybridization study**. *J Comp Neurol* 1993, **335**:252-266.

44. Tamaru Y, Nomura S, Mizuno N, Shigemoto R: **Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: differential location relative to pre- and postsynaptic sites.** *Neuroscience* 2001, **106**:481-503

45. Moldrich RX, Jeffrey M, Talebi A, Beart PM, Chapman AG, Meldrum BS: **Anti-epileptic activity of group II metabotropic glutamate receptor agonists (--)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268) and (--)-2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY389795).** *Neuropharmacology* 2001, **41**:8-18.

46. Bradley SR, Rees HD, Yi H, Levey AI, Conn PJ: **Distribution and developmental regulation of metabotropic glutamate receptor 7a in rat brain**. *J Neurochem* 1998, **71**:636-645.

47. Bradley SR, Standaert DG, Rhodes KJ, Rees HD, Testa CM, Levey AI, Conn PJ: **Immunohistochemical localization of subtype 4a metabotropic glutamate receptors in the rat and mouse basal ganglia**. *J Comp Neurol* 1999, **407**:33-46.

48. Turner JP, Salt TE: **Group II and III metabotropic glutamate receptors and the control of the nucleus reticularis thalami input to rat thalamocortical neurons in vitro**. *Neuroscience* 2003, **122**:459-469.

49. Turner JP, Salt TE: **Group III metabotropic glutamate receptors control corticothalamic synaptic transmission in the rat thalamus in vitro**. *J Physiol* 1999, **519**:481-491.

50. Corti C, Aldegheri L, Somogyi P, Ferraguti F: **Distribution and synaptic localisation of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS**. *Neuroscience* 2002, **110**:403-420.

51. Wong CG, Scherer SW, Snead OC 3rd, Hampson DR: **Localization of the human mGluR4 gene within an epilepsy susceptibility locus (1)**. *Brain Res Mol Brain Res* 2001,**87**:109-116

52. Izzi C, Barbon A, Toliat MR, Heils A, Becker C, Nürnberg P, Sander T, Barlati S: **Candidate gene analysis of the human metabotropic glutamate receptor type 4(GRM4) in patients with juvenile myoclonic epilepsy**. *Am J Med Genet B Neuropsychiatr Genet* 2003, **123B**:59-63.

53. Muhle H, von Spiczak S, Gaus V, Kara S, Helbig I, Hampe J, Franke A, Weber Y, Lerche H, Kleefuss-Lie AA, Elger CE, Schreiber S, Stephani U, Sander T: **Role of GRM4 in idiopathic generalized epilepsies analysed by genetic association and sequence analysis**. *Epilepsy Res* 2010, **89**:319-326.

54. Sansig G, Bushell TJ, Clarke VR, Rozov A, Burnashev N, Portet C, Gasparini F, Schmutz M, Klebs K, Shigemoto R, Flor PJ, Kuhn R, Knoepfel T, Schroeder M, Hampson DR, Collett VJ, Zhang C, Duvoisin RM, Collingridge GL, van Der Putten H: **Increased seizure susceptibility in mice lacking metabotropic glutamate receptor** 7. *J Neurosci* 2001, **21**:8734-8745.

55. Gerlai R, Adams B, Fitch T, Chaney S, Baez M: **Performance deficits of mGluR8 knockout mice in learning tasks: the effects of null mutation and the background genotype.** *Neuropharmacology* 2002, **43**:235-249.

56. Bertaso F, Zhang C, Scheschonka A, de Bock F, Fontanaud P, Marin P, Huganir RL, Betz H, Bockaert J, Fagni L, Lerner-Natoli M: **PICK1 uncoupling from mGluR7a causes absence-like seizures.** *Nat Neurosci* 2008, **11**:940-948.

57. Okamoto N, Hori S, Akazawa C, Hayashi Y, Shigemoto R, Mizuno N, Nakanishi S: **Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction**. *J Biol Chem* 1994, **269**:1231-1266.

58. Martín R, Durroux T, Ciruela F, Torres M, Pin JP, Sánchez-Prieto J: **The metabotropic glutamate receptor mGlu7 activates phospholipase C, translocates munc-13-1 protein, and potentiates glutamate release at cerebrocortical nerve terminals.** *J Biol Chem* 2010, **285**:17907-17917.

59. Tenney JR, Glauser TA: **The current state of absence epilepsy: can we have your attention?** *Epilepsy Curr* 2013, **13**:135-140.
    - Relevant opinion paper, pleading for more attention to various types of absence epilepsy including the development of new anti-absence drugs

60. Nicoletti F, Bruno V, Ngomba RT, Gradini R, Battaglia G. **Metabotropic glutamate receptors as drug targets: what's new?** *Curr Opin Pharmacol* 2015, **20**:89-94.

- Important review the demonstrates clinical expectations of mGlu receptor on CNS conditions and the relevance of mGlu receptor subtypes

61. Leresche N, Parri HR, Erdemli G, Guyon A, Turner JP, Williams SR, Asprodini E, Crunelli V: **On the action of the anti-absence drug ethosuximide in the rat and cat thalamus**. *J Neurosci* 1998, **18**:4842-4853

62. Talley EM, Cribbs LL, Lee JH, Daud A, Perez-Reyes E, Bayliss DA: **Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels.** *J Neurosci* 1999, **19**:1895-1911.

## Figure and table legends

Figure_1

Diagram showing synaptic localization of mGlu receptor subtypes in the modulation of absence seizures. In red, dendritic spine from the pyramidal cell (e.g. layer IV), cortico-thalamic (CT) glutamatergic projection into the ventrobasal thalamus with collateral onto the reticular thalamic nucleus (nRT). Glutamatergic thalamic relay neuron (TC) sends projection (in orange) to the deep layers of the cortex (e.g. layer VI) and collateral onto the nRT. GABAergic nRT neuron in green, sending projection to the VB and collateral back onto the nRT. The synapses are mostly surrounded by astrocytic processes (in purple) expressing distinct mGlu receptor subtypes (principally mGlu3 and mGlu5) as indicated by the different colours. The sensory afferents at the thalamus are depicted as a large red arrow. mGlu1 and mGlu5 receptors (also present on astrocytes) are located perisynaptically at excitatory synapses. Group II receptors (mGlu2 and 3) are present in both cortical and thalamic synaptic terminals. mGlu3 receptors are expressed in astrocytes, and the activity of mGlu2 has recently been identified on astrocytes by a combination of electrophysiological and pharmacological methods. Subtypes of mGlu4, 7 and 8 are usually localized presynaptically at active regions.

Table_1

A summary of mGlu receptor subtypes, transduction mechanisms and site of action of specific subtype receptor ligands and their effect on models of absence epilepsy.

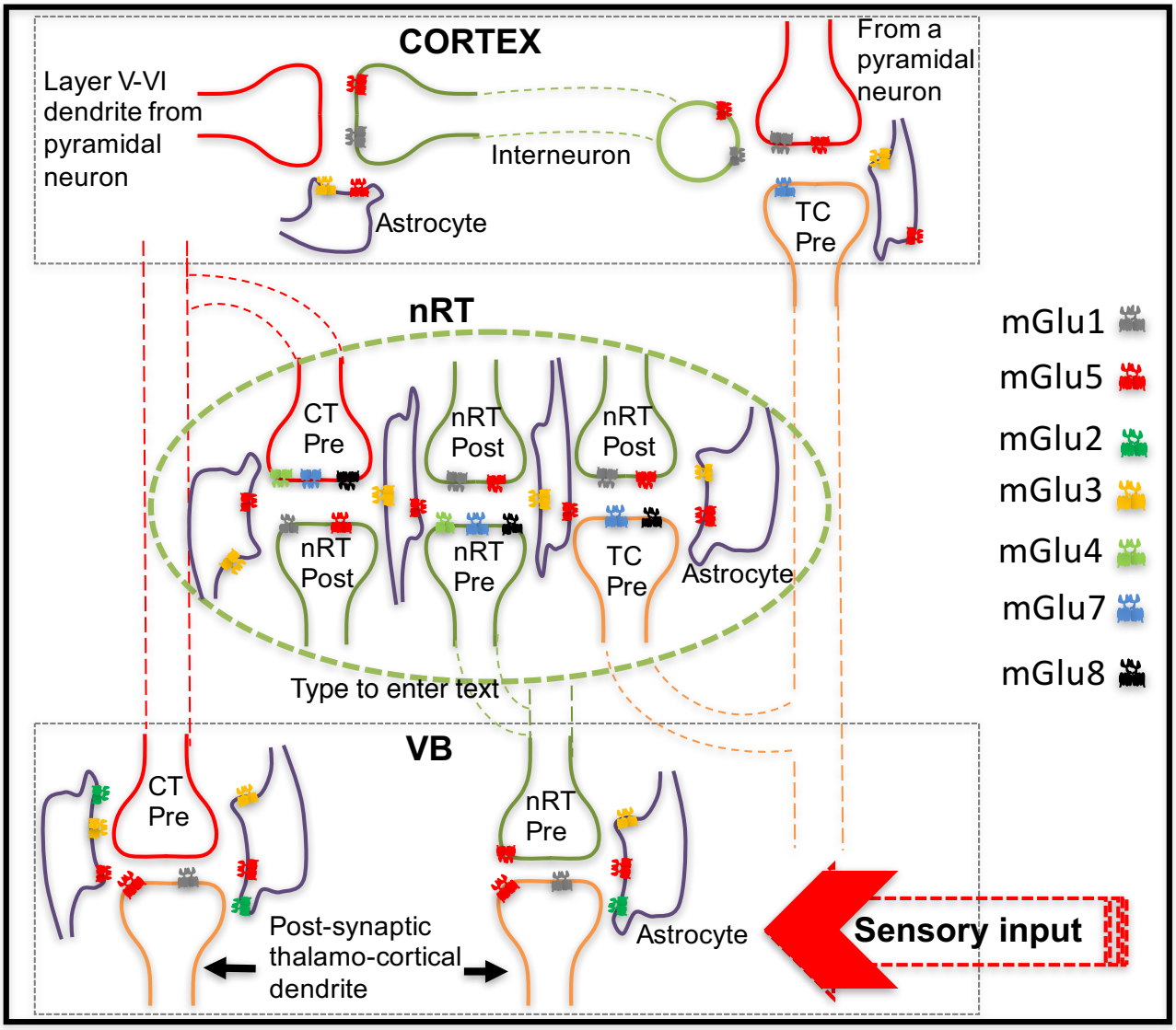

CORTEX
Layer V-VI dendrite from pyramidal neuron
Interneuron
From a pyramidal neuron
Astrocyte
TC Pre
nRT
CT Pre
nRT Post
nRT Post
nRT Post
nRT Pre
TC Pre
Astrocyte
mGlu1
mGlu5
mGlu2
mGlu3
mGlu4
mGlu7
mGlu8
Type to enter text
VB
CT Pre
nRT Pre
Post-synaptic thalamo-cortical dendrite
Astrocyte
Sensory input

Figure_1

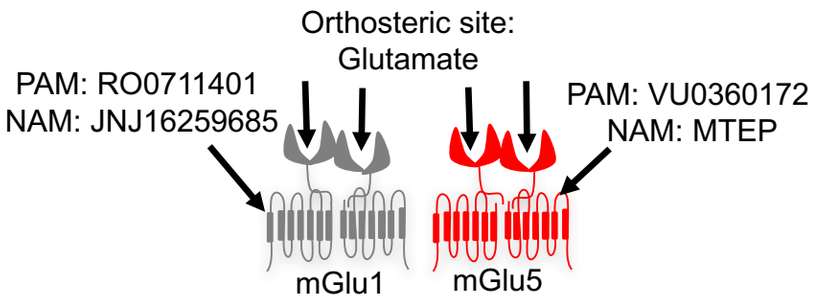
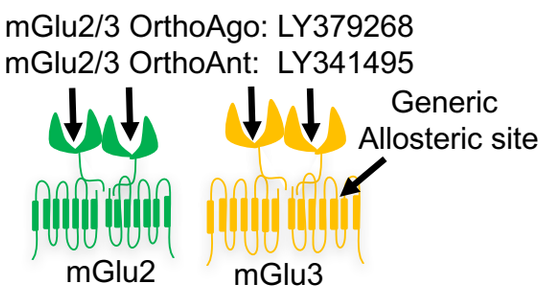
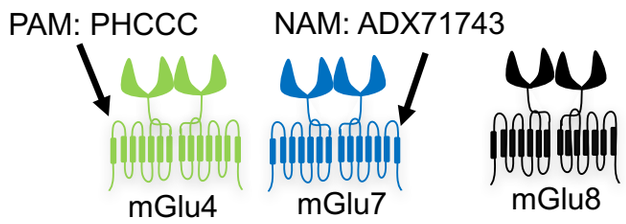
| | Group I | Group II | Group III |
|---|---|---|---|
| **Receptor subtypes and ligand sites of action** |  |  |  |
| **Expression and canonic transduction Pathways** | • Group I mGlu receptors are predominantly expressed post-synaptically and mGlu5 are also on astrocytes | • Group II mGlu receptors are both expressed pre and post-synaptically. mGlu3 are on astrocytes and recently mGlu2 astrocytic activity has been identified in the thalamus | • Group III mGlu receptors are predominantly expressed presynaptically |
| | • Associated with $G_q/_{11}$ class G proteins<br>• PLC stimulation<br>• Stimulation of adenylyl cyclase<br>• MAP kinase phosphorylation | • Associated with Gi/o class G proteins<br>• Inhibition of adenylyl cyclase<br>• Activation of $K^+$ channels<br>• Inhibition of $Ca^{2+}$ channels | • Associated with Gi/o class G proteins<br>• Inhibition of adenylyl cyclase<br>• Activation of $K^+$ channels<br>• Inhibition of $Ca^{2+}$ channels |
| **Ligand effects related to absence epilepsy** | • RO0711401 reduces SWDs (WAG/Rij rats) and JNJ16259685 increases SWDs (WAG/Rij rats) [21]<br>• Orthosteric antagonists LY367385 and AIDA reduces SWDs in Ih/Ih mice [31,32, 33]<br>• VU0360172 reduces SWDs in WAG/Rij rats: MTEP antagonizes the effect of VU0360172 in WAG/Rij rats [22].<br>• MPEP mGlu5 NAM with an mGlu4 NAM component reduces SWDs in Ih/Ih mice [31]<br>• Development of tolerance: VU0360172 (NON after 10 days), while RO0711401 did develop after 2 days [24]<br>• Local infusion of either RO0711401 or VU0360172 suppressed SWDs independently both in the cortex and thalamus [13] | • LY341495 an orthosteric antagonist reduces SWDs in WAG/Rij rats [15]<br>• LY379268 an orthosteric agonist increases SWDs in WAG/Rij rats [15]. LY341495 and LY379268 both reduces SWDs in Ih/Ih mice [41] | • PHCCC enhances SWDs in WAG/Rij rats [6]<br>• ADX71743 produces lethargic effects similar to SWDs in WT mice [44].Decoupling if PICK1 from mGlu7 causes SWDs [51] |
| | | OrthoAgo = Orthosteric agonist<br>OrthoAnt = Orthosteric antagonist | PAM = positive allosteric modulator<br>NAM = negative allosteric modulator |

Table_1