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

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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].

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Conflict of interest statement

Nothing declared.

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References of particular interest, have been highlighted as:

• of special interest

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

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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.

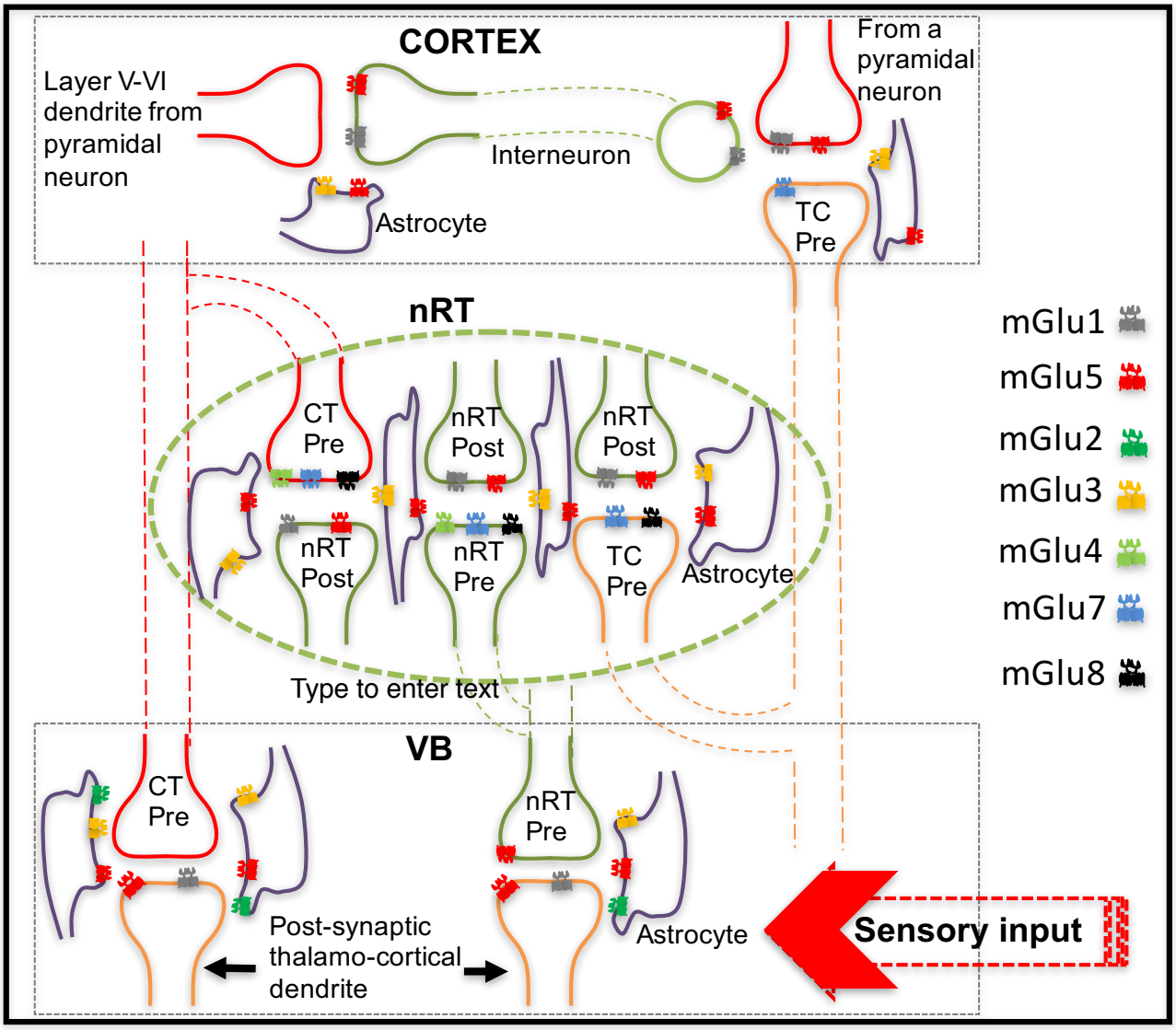

CORTEX
Layer V-VI dendrite from pyramidal neuron
Interneuron
From a pyramidal neuron
Astrocyte
TC Pre
nRT
CT Pre
nRT Post
nRT Post
nRT Post
nRT Pre
TC Pre
Astrocyte
mGlu1
mGlu5
mGlu2
mGlu3
mGlu4
mGlu7
mGlu8
Type to enter text
VB
CT Pre
nRT Pre
Post-synaptic thalamo-cortical dendrite
Astrocyte
Sensory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

Figure_1

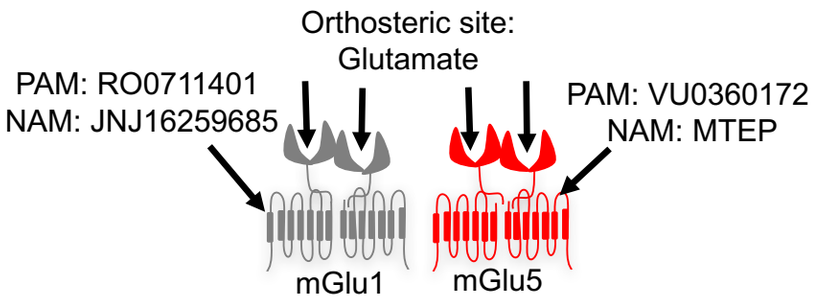
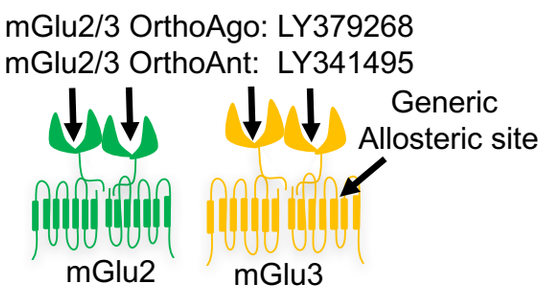
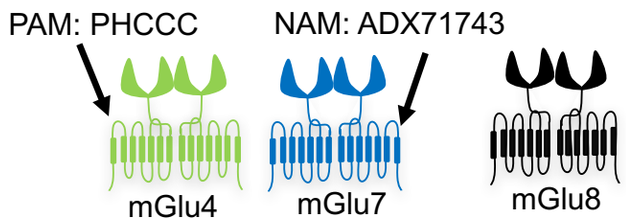
	Group I	Group II	Group III
Receptor subtypes and ligand sites of action			
Expression and canonic transduction Pathways	• Group I mGlu receptors are predominantly expressed post-synaptically and mGlu5 are also on astrocytes	• Group II mGlu receptors are both expressed pre and post-synaptically. mGlu3 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 • PLC stimulation • Stimulation of adenylyl cyclase • MAP kinase phosphorylation	• Associated with Gi/o class G proteins • Inhibition of adenylyl cyclase • Activation of K^+ channels • Inhibition of Ca^{2+} channels	• Associated with Gi/o class G proteins • Inhibition of adenylyl cyclase • Activation of K^+ channels • 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] • Orthosteric antagonists LY367385 and AIDA reduces SWDs in Ih/Ih mice [31,32, 33] • VU0360172 reduces SWDs in WAG/Rij rats: MTEP antagonizes the effect of VU0360172 in WAG/Rij rats [22]. • MPEP mGlu5 NAM with an mGlu4 NAM component reduces SWDs in Ih/Ih mice [31] • Development of tolerance: VU0360172 (NON after 10 days), while RO0711401 did develop after 2 days [24] • 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] • 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] • ADX71743 produces lethargic effects similar to SWDs in WT mice [44].Decoupling if PICK1 from mGlu7 causes SWDs [51]
		OrthoAgo = Orthosteric agonist OrthoAnt = Orthosteric antagonist	PAM = positive allosteric modulator NAM = negative allosteric modulator

Table_1