Title: Upholding WAG/Rij rats as a model of absence epileptogenesis: Hidden mechanisms and a new theory on seizure development

Authors: Emilio Russo Prof. Emilio Russo, PhD, Chair of Pharmacology Rita Citraro Andrew Constanti Antonio Leo Annika L*ü*ttjohann Gilles van Luijtelaar Giovambattista De Sarro

PII: S0149-7634(15)30367-5
DOI: http://dx.doi.org/doi:10.1016/j.neubiorev.2016.09.017
Reference: NBR 2603

To appear in:

Received date: 27-12-2015
Revised date: 19-9-2016

Please cite this article as: Russo, Emilio, Citraro, Rita, Constanti, Andrew, Leo, Antonio, L*ü*ttjohann, Annika, van Luijtelaar, Gilles, De Sarro, Giovambattista, Upholding WAG/Rij rats as a model of absence epileptogenesis: Hidden mechanisms and a new theory on seizure development.Neuroscience and Biobehavioral Reviews http://dx.doi.org/10.1016/j.neubiorev.2016.09.017

Upholding WAG/Rij rats as a model of absence epileptogenesis: hidden mechanisms and a new theory on seizure development

Emilio Russo[a,*], Rita Citraro[a], Andrew Constanti[b], Antonio Leo[a], Annika Lüttjohann[c,d], Gilles van Luijtelaar[c], Giovambattista De Sarro[a].

[a]Science of Health Department, School of Medicine, University of Catanzaro, Italy; [b]Department of Pharmacology, UCL School of Pharmacy, 29/39 Brunswick Square, London, United Kingdom; [c]Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, The Netherlands; [d]Institute of Physiology I, Westfälische Wilhelms University, Münster, Germany

*Author for correspondence:

Prof. Emilio Russo, PhD,

Chair of Pharmacology,

Department of Science of Health,

School of Medicine,

University of Catanzaro, Italy

Viale Europa-Germaneto; 88100 Catanzaro, ITALY.

Phone +39 0961 3694191; Fax +39 0961 3694192

e-mail: erusso@unicz.it

Highlights

- WAG/Rij rats should be considered a model of genetically determined epileptogenesis
- Genetically determined epileptogenesis might differ from post insult epileptogenesis
- Drug effects on seizure development might not be persistent
- No clear neuroinflammation has been proven during epileptogenesis in WAG/Rij rats

Abstract

The WAG/Rij rat model has recently gathered attention as a suitable animal model of absence epileptogenesis. This latter term has a broad definition encompassing any possible cause that determines the development of spontaneous seizures; however, most of, if not all, preclinical knowledge on epileptogenesis is confined to the study of post-brain insult models such as traumatic brain injury or post-status epilepticus models. WAG/Rij rats, but also synapsin 2 knockout, Kv7 current–deficient mice represent the first examples of genetic models where an efficacious antiepileptogenic treatment (ethosuximide) was started before seizure onset. In this review, we have critically reconsidered all articles published regarding WAG/Rij rats, from the perspective that the period before SWD onset is considered as the latent period. In our new theory on seizure development, it is proposed that genes might be considered as the initial 'insult' responsible for all plastic changes underpinning the development of spontaneous seizures. According to this idea, in WAG/Rij rats, genetic predisposition would lead to the development of abnormal bilateral cortical epileptic foci, which would then non-genetically stimulate the rest of the brain to rearrange networks in order to phenotypically develop seizures similarly to what happens during electrical kindling.

Keywords: Epileptogenesis; Spontaneous chronic absence seizures; Genetic animal model; Antiepileptic drugs; WAG/Rij rats

Index

1. Introduction

Wistar-Albino-Glaxo from Rijswijk (WAG/Rij) rats, a strain of Wistar origin, is a widely used and validated genetic model of generalized absence epilepsy (Coenen and Van Luijtelaar, 2003). All rats of this strain, older than 2-3 months, start to develop highly synchronous, bilateral spike-wave discharges (SWDs; 7-10 Hz) in the cortical electroencephalogram (EEG) accompanied by concomitant behavioral episodes of vibrissae twitching and accelerated breathing, head tilting, and, often, eye twitching as in human absence epilepsy (van Luijtelaar and Sitnikova, 2006; van Luijtelaar and Zobeiri, 2014). At an age of six months, all WAG/Rij rats show SWDs, on average 16-20 per hour (Coenen and Van Luijtelaar, 1987). Despite validation, the WAG/Rij rat phenotype differs in some aspects from human absence epilepsy: *i*) absence epilepsy is characteristically a childhood disease, which may disappear or transform into other types of epilepsy around or after puberty, while in WAG/Rij rats, SWDs appear after puberty (>P50) reaching a plateau at 6 months of age; *ii*) patients with typical absence epilepsy have SWDs of about 3Hz while in WAG/Rij rats, but also in GAERS (Genetic Absence Epilepsy Rats from Strasbourg) and in mouse absence models, seizure frequency is higher, varying between 7-11Hz (Drinkenburg et al., 1993; van Luijtelaar and Coenen, 1986).

The SWDs in the WAG/Rij rats are bilaterally symmetrical and generalized over the entire cortex (Midzianovskaia et al., 2001). Frontal regions show more, and sometimes larger, SWDs than occipital regions. Moreover, the waves are more clearly expressed in the occipital cortex (Midzianovskaia et al., 2001). The tendency for SWDs to occur during drowsiness and light slow-wave sleep closely corresponds to what is observed in humans (Drinkenburg et al., 1991; Smyk et al., 2012).

WAG/Rij rats, in addition to being a validated model of absence epilepsy, have been more recently validated as a model of a chronic low-grade depression (dysthymia) accompanying

the development of absence seizures (Sarkisova and van Luijtelaar, 2011); therefore, it can be considered as an animal model of absence epilepsy with depressive-like comorbidity. In this light, behavioral alterations are temporally linked to the development of absence epilepsy (Russo et al., 2011a) and disappearance of these symptoms accompanying the prevention of SWDs suggested that seizures might be necessary for the appearance of depressive-like behavior (Sarkisova et al., 2010); however, this latter point still remains to be completely clarified, considering that also some opposite results have been obtained (see Section 4).

Finally, WAG/Rij rats have been recently identified as a potential animal model of absence *epileptogenesis* (Blumenfeld et al., 2008; Giblin and Blumenfeld, 2010; White and Loscher, 2014). This review article aims at critically analyzing previously published articles in order to identify potential alterations in WAG/Rij rat brain, not previously considered, which might contribute to the epileptogenic process underlying the development of spontaneous absence seizures; furthermore, the actual experimental results of various antiepileptogenic treatments are critically reconsidered and a new hypothesis for a genetically-based absence epileptogenesis in these animals is proposed.

2. WAG/Rij rats as a model of absence epileptogenesis

This section of the review will discuss a new hypothesis on the perspective of a genetically-driven epileptogenesis and the possible involvement of some distinct brain areas in this process. Also, the current knowledge on the efficacy of some tested treatments (summarized in Figure 1) for the prevention of seizure development in this animal model will be critically reviewed.

2.1 Hypothesis for genetically-determined epileptogenesis

Epileptogenesis refers to the development as well as structural and functional extension of brain tissue capable of generating spontaneous seizures, resulting in the development of a

chronic epileptic condition and/or progression of epilepsy after the condition is established (Pitkanen and Engel, 2014). The *latency period* of epileptogenesis currently refers to the time between an initial brain insult and the appearance of the first spontaneous seizure (Pitkanen and Lukasiuk, 2011). Most of the currently available studies focus on identifiable brain insults such as traumatic brain injury or stroke, while the possibility that the process of epileptogenesis also occurs in genetic epilepsy still remains a possibility or at least a suggestion (Giblin and Blumenfeld, 2010; Goldberg and Coulter, 2013; Guerrini et al., 2014; Pitkanen and Engel, 2014).

Several mechanisms leading to the development of spontaneous seizures have been identified in animal models and suggested as potential therapeutic targets; however, this knowledge has been mainly obtained in epilepsy models where an initial well-defined insult can be identified (Goldberg and Coulter, 2013). It is clear that during any process of epileptogenesis, despite the cause, a possible sensitive time window for treatment and prevention might exist; for example, it has been demonstrated that in heterozygous Na^+ channel subunit ($Na_V1.1$) knock-out mice (a model of Dravet syndrome, or Severe Myoclonic Epilepsy of Infancy) seizures can only be elicited by hyperthermia after postnatal day 19 (P19), while between P14 and P18, there is less hippocampal hyperexcitability, indicating that during the period between P10, when this channel begins to be expressed, and P19, some kind of adaptive changes occur, suggestive of epileptogenesis (Guerrini et al., 2014; Liautard et al., 2013). Similarly, in WAG/Rij rats, the pre-seizure period (up to the age of P50-60) might be considered as the respective "*latent period*" necessary for the development of spontaneous absence seizures; furthermore, considering that the number of absence seizures keeps on increasing between 2 and 4-5 months of age or even after (Coppola and Moshe, 2012; van Luijtelaar and Zobeiri, 2014), it could be hypothesized that the underlying epileptogenic process implies continuous adaptive/plastic changes which are not necessarily dependent on seizures. This latter idea

arises from the assumption that before the appearance of the first seizure, some epileptogenic processes are already present. This does not exclude the possibility that the seizures generated by these processes can contribute to the overall epileptogenic process. In agreement with this notion, it was previously suggested that interictal spikes recorded on the EEG are both the result of cellular changes during epileptogenesis and the cause of further subsequent changes reinforcing the epileptic circuit (Lillis et al., 2015; Staley et al., 2005; Staley and Dudek, 2006).

WAG/Rij rats indeed undergo an epileptogenic process and this is supported by the fact that several brain alterations are observed before and after seizure onset and that several treatments have been shown to prevent/reduce seizure development (see following sections for details). To date, the mechanisms underlying the epileptogenic process in this animal model are unknown, as well as the exact sensitive time window for preventive treatments. Indeed, some alterations such as increased expression of $Na_v1.1$ and 1.6 subtype Na^+ channels and decreased expression of slow inward rectifier HCN1 channels (which parallel the development of spontaneous seizures; these channels were also recently found to have an N-terminal deletion of 37 amino acids in WAG/Rij rats, see section 3.3), have been identified, but it still remains to be clarified whether such alterations are the cause or a consequence of the epileptogenic process and seizures (Blumenfeld et al., 2008); on the other hand, the time window for intervention also appears very broad ranging, from P8 to P45 up to 5 months of age, and yet it is not possible to exclude that therapeutic intervention during another life period might have a beneficial long-term effect (see Section 4).

WAG/Rij rats are a fully inbred strain sharing all autosomal genes; all individuals exhibit spike-wave discharges (SWDs) on their EEGs suggesting a dominant transmission with no differences between sexes, similar to what has been found in GAERS (van Luijtelaar et al., 2014). Variability exists in the number and/or duration of SWDs between rats which supports

the idea that inheritance is unlikely to be due to a single gene locus; this is further supported by the influence played by the environment and epigenetic factors (Schridde et al., 2006; Schridde and van Luijtelaar, 2004; van Luijtelaar and Sitnikova, 2006). Finally, only quantitative trait loci on chromosomes 5 and 9 of WAG/Rij rats have been identified (Gauguier et al., 2004) and no identified specific gene mutation accounts for the development of spontaneous seizures in this model (van Luijtelaar and Sitnikova, 2006); this latter point creates a clear difference between the above-mentioned model of Dravet syndrome (with a single defined (loss-of-function) mutation on Na^+ channels) and WAG/Rij rats. Nevertheless, the same hypothetical mechanism can be suggested; namely, in genetic models, one or more factors may create (directly or indirectly; *i.e.* predominant loss of interneuron function) abnormal brain areas of hyperexcitation, which will not be initially easily identifiable but will drive a continuous stimulus to interconnected areas leading to the development of fully functional '*abnormal*' networks responsible for the phenotypic appearance of spontaneous seizures. In other words, defined areas of genetically-predetermined hyperexcitation will induce adaptive changes in an already expressed network (*e.g.* cortico-thalamo-cortical network) or generate new circuits with the ability to engender seizures.

In this light, it has been demonstrated and widely confirmed that the WAG/Rij rat model possesses a focal area(s) localized in the perioral facial region of the somatosensory cortex where SWDs are initiated. Therefore, it has been suggested that absence seizures in this strain may in fact, have a bi-lateral focal origin (Luttjohann et al., 2011; Meeren et al., 2002; van Luijtelaar and Sitnikova, 2006). In agreement with this, it has recently been demonstrated that the removal of only both foci completely abolishes SWDs (Scicchitano et al., 2015) while fMRI (functional magnetic resonance imaging) studies have confirmed the presence of bilateral activated regions in the somatosensory cortex (Nersesyan et al., 2004). Based on this background, we can then suppose that these focal epileptic areas may be genetically

predetermined to be hyperexcitable (D'Antuono et al., 2006; Kole et al., 2007; Luttjohann et al., 2011; Strauss et al., 2004) from a certain developmental age onwards. These areas might then trigger neuroadaptive changes into the cortico-thalamo-cortical network, which will then become responsive to the hyperexcitable stimulus generating seizures. In other words, the foci will be responsible for inducing the plastic changes into the circuit, phenotypically generating and initiating SWDs and driving and maintaining these bilateral synchronous SWDs in this absence model. It is most likely that similar processes occur in other rodent genetic models such as GAERS as well (David et al., 2008).

Based on this hypothesis, most of the changes observed in the brains of these rats might be a consequence of this continuous stimulation, and the onset of the first spontaneous seizure may represent the moment when the network has already reached the minimally necessary conformation to phenotypically express SWDs. Theoretically, this process might be similar to what happens during experimental electrical kindling stimulation of limbic regions where an initial *subthreshold* stimulus triggers over time, a larger neuronal response resulting in generalized seizures with increasing duration and intensity (Morimoto et al., 2004). Several mechanisms have been identified that may underlie this process, and it is clear that kindling causes functional plasticity in the stimulated focus while also recruiting other networks outside the stimulated area (Giblin and Blumenfeld, 2010; Morimoto et al., 2004). Accordingly, it is necessary to understand when this endogenous stimulation actually begins in WAG/Rij rats, since this will help to guide the search for the best moment for starting a treatment aimed at antiepileptogenesis. Furthermore, it is clear that when the first seizure appears, although the epileptic network might not be considered completely formed yet, it will become more difficult to restore the system to an initial non-hyperexcitable state. Thus, once a seizure is phenotypically characterized, the network should be already considered epileptic and while progression might be blocked, the alterations observed and the related

consequences may no longer be rescuable. Finally, the last question requiring an answer is whether blocking adaptive changes into this network before seizure onset, is really enough to prevent seizure development forever; in fact, if the kindling-like electrical activity of the focus is maintained over time, after drug suspension, this stimulation might lead again to the development of an epileptic phenotype. This latter point is further discussed below when reviewing drug effects (see Section 4).

Critically, we might therefore, theoretically identify three different (not necessarily separated) phases involved in the genetic epileptogenesis: 1) initial genetically-determined brain alteration leading to the establishment of the focal region(s); 2) beginning of focal activity, stimulating other brain areas with no SWDs up to the appearance of the first seizure and 3) from first seizure to the complete network maturation leading to chronic stable expression of SWDs (Figure 2). While the onset of the first seizure in this strain has been identified in a well-defined time window, all other time windows should also be identified. Furthermore, the identification of these milieus might also help in identifying possible biomarkers of the epileptogenesis process.

2.2 Brain regions of interest

It is widely agreed that SWDs in absence epilepsy are generated within the thalamo-cortical neuronal network (Huguenard and McCormick, 2007; Meeren et al., 2002; Pinault and O'Brien, 2005; Steriade, 1998). As mentioned above, a bilaterally present, hyperexcitable, focal area within the deep layers (V and VI) of the perioral somatosensory cortex (S1po) has been identified as the seizure onset zone (Meeren et al., 2002; Polack et al., 2007) or rather near in the S2 or insular cortex (Zheng et al., 2012) and the necessity of this focal area(s) for SWD generation has been validated by several independent research groups exploiting different experimental techniques (for review see (van Luijtelaar and Sitnikova, 2006). It can

be noted, that such a focal cortical onset zone, although not located within the somatosensory cortex, but rather in fronto-temporal cortical regions, has also been identified in children with absence epilepsy in several EEG, MEG (magnetoencephalogram) and EEG-fMRI studies (Holmes et al., 2004; Moeller et al., 2008; Westmijse et al., 2009).

While the presence of a cortical focus might be sufficient for the generation of localized SWD-like discharges, which have sometimes been noted in LFP (local field potential) recordings obtained in the somatosensory cortex of WAG/Rij and GAERS rats (Luttjohann and van Luijtelaar, 2012; Seidenbecher et al., 1998), it is not sufficient for the generation of "*full blown*", bilaterally synchronous SWDs. Rather, an intact cortico-thalamic network is essential for the generation of bilaterally synchronous SWDs. The initial strong excitatory activity originating in the somatosensory cortex, activates (via cortico-thalamic (TC) neurons) sensory thalamic nuclei including the ventral-postero-medial (VPM) and the posterior thalamic nucleus (Po), as well as via collaterals of the TC neurons, the inhibitory reticular thalamic nucleus (NRT). Activation of the NRT results in an increased GABAergic output from the NRT onto the TC relay neurons, which become slightly more hyperpolarized. Indeed, next to the local cortical hyperexcitation, an increased tonic inhibition of TC relays has been described as another key-element of absence seizure generation (Cope et al., 2009; D'Amore et al., 2015).

As a result of this hyperpolarization, TC neurons of the VPM and Po respond in a burst-like pattern towards the cortical input (Crunelli and Leresche, 2002; McCormick and Bal, 1997; (Polack et al., 2009). Since TC neurons of the VPM and Po project to the somatosensory cortex, their activity "*reactivates*" the cortical focus and via these nuclei, provides a reverberation circuit, the cortico-thalamo-cortical network, relevant for SWD generation and maintenance. Recent network analytical studies (Luttjohann et al., 2013; Luttjohann and van Luijtelaar, 2012) demonstrated that the cortex displays a unique coupling profile with each of

the above mentioned nuclei and even subparts of the nucleus (*i.e.* the cortex was found to interact differently with the caudal and rostral part of the NRT) during SWDs and in a short period immediately prior to SWD onset, where the network already seems to prepare towards SWD generation. Based on these findings, different functional contributions of the different thalamic nuclei for SWD preparation, generation and maintenance, as well as a temporal SWD generation scenario was proposed (for review see(Luttjohann and van Luijtelaar, 2015)).

Considering the occurrence of SWDs is related to the state of vigilance (with the majority of SWDs occurring during drowsiness and light slow-wave sleep)(Drinkenburg et al., 1991; Smyk et al., 2012), next to the above described thalamic nuclei, the intralaminar thalamic nuclei (known to be involved in the regulation of arousal), have also been proposed to be involved in SWD generation and maintenance (Gorji et al., 2011; Seidenbecher and Pape, 2001). Like the Po, these nuclei not only interact with the cortical focus, but also possess a widespread projection to other cortical areas, which makes them suitable candidates to be involved in SWD generalization.

Last but not least, structures outside the CTC loop, have also been shown to possess modulatory capacities on the expression of SWDs. Two structures will be mentioned here: the substantia nigra pars reticulata (SNr), where several pharmacological manipulations as well as electrical stimulation have been shown to modulate SWD activity (Deransart et al., 1998; Feddersen et al., 2007). Also, drug injection into the hippocampus modulates SWDs (Tolmacheva and van Luijtelaar, 2007).

Overall, several brain structures appear to interact during absence seizures and all of them might be susceptible to adaptive changes during epileptogenesis. It is likely that in the beginning of the "*latent period*", the network is still non-epileptic and that plastic changes due to some unknown reasons will modify at least some of the intrinsic properties of the system in

order to generate absence seizures. In the following sections, the current knowledge about alterations in several areas of the cortico-thalamo-cortical network in the WAG/Rij rats that could contribute to epileptogenesis will be summarized along with a discussion of what is understood about the effectiveness of various drug treatments against epileptogentic processes in these animals.

3. Potential mechanisms of epileptogenesis: role of neurotransmitters and other processes

WAG/Rij rats have long been studied as a model of absence epilepsy and for this reason, several potential neurotransmitter-based and other mechanisms of epileptogenesis have been investigated as reviewed below (Table 1). Historically, such experiments mainly followed two basic protocols; symptomatic WAG/Rij rats, older than 5 months were usually compared with 1) age-matched control rats (*i.e.* Wistar or ACI rats, a fully inbred strain of rats) and or 2) presymptomatic WAG/Rij rats 2 to 3 months old or younger, or a combination of both protocols. A statistically significant interaction between strains and age is then considered as an indication that a certain dependent variable might be involved in epileptogenesis. For the purpose of this review, we have included all relevant data also including pharmacological manipulation; however, we focus on any kind of difference encountered before and after seizure appearance, considering that alterations observed in old rats in comparison to age-matched controls might be a *consequence* of seizures more than a cause or an epiphenomenon. Finally, despite some alterations that can be identified in the pre-seizure period, no studies after long-term antiepileptogenic treatments have been performed except some on ion channels (see sections 3.3. and 4) and on mGlu5Rs (D'Amore et al., 2016).

3.1 GABA

The involvement of γ-aminobutyric acid (GABA) in the occurrence and control of SWDs in absence epilepsy is widely recognized (Crunelli and Leresche, 2002). GABA-mediated mechanisms have been extensively studied within the CTC system, and there is evidence that both $GABA_A$ and $GABA_B$ receptors are involved in the generation of SWDs (Snead, 1995; van Luijtelaar and Sitnikova, 2006). GABAergic neurons in the NRT and their projections to the specific relay nuclei of the thalamus are involved in the development/regulation of absence seizures (Hosford et al., 1997; Snead, 1992; Snead et al., 1992). Interestingly, it has been suggested that the balance between $GABA_A(Cl^-)$ and $GABA_B(K^+)$-mediated conductances is essential for the control of SWDs (Kaminski et al., 2001; Marescaux et al., 1992b; Vergnes et al., 1984). The peak frequency of SWDs might be mainly determined by favoring the balance between $GABA_A$ and $GABA_B$ conductances towards $GABA_B$ neurotransmission (Bouwman et al., 2007a; Destexhe and Sejnowski, 2003). Different studies indicate that in TC neurons during absence seizures, (1) $GABA_A$-mediated events are recruited with each SWD, (2) SWD-related activity can be evoked with no significant contribution of $GABA_B$ receptors, and (3) blockade of $GABA_A$ receptors *potentiates* SWD-related activity, presumably through an indirect effect mediated by $GABA_B$ receptors (Staak and Pape, 2001).

$GABA_A$ receptors are of chief importance in the pathogenesis of absence epilepsy because of their apparent role in the synchronization and desynchronization of CTC circuitry. Altered $GABA_A$ receptor function has been described in the cerebral cortex and thalamus of WAG/Rij rats and may contribute to abnormal brain states of absence epilepsy (D'Antuono et al., 2006; Luhmann et al., 1995). In WAG/Rij rats, GABAergic inhibition is *reduced* in upper-layer frontal cortical neurons (Luhmann et al., 1995), and $GABA_A$ receptor-mediated fast hyperpolarizing inhibitory postsynaptic potentials (IPSPs) show decreased peak conductances in deeper-layer neurons (D'Antuono et al., 2006). WAG/Rij rats in comparison to Wistar rats,

exhibited a significant reduction in the efficiency of intracortical GABAergic inhibition concomitant with hyperexcitability (Luhmann et al., 1995) and interestingly, many cortical areas are lacking parvalbumin-containing interneurons.

It has been shown that the properties of GABAergic synaptic transmission in intralaminar thalamic nucleus and NRT neurons are altered in GAERS, another established absence epilepsy model, at a developmental stage prior to seizure onset, suggesting that alterations in the fine tuning of $GABA_A$ receptor-mediated inhibition in thalamic networks may be significant for absence epileptogenesis (Bessaih et al., 2006). The '*gain*' of intrathalamic GABAergic inhibition plays a critical role for CTC oscillations, particularly in relation to SWD generation. However, the local enhancement of GABAergic inhibition in the NRT (Aker et al., 2006) and in the perioral region of the somatosensory cortex (Citraro et al., 2006a; D'Amore et al., 2015) results in a decrease in SWDs, suggesting that there is little GABAergic inhibition in the cortex.

Human absence epilepsy is associated with mutations in the genes encoding the α_5, β_3, or γ_2 subunits of the $GABA_A$ receptor (Hirose, 2014) and the amount of the α_3 subunit in the NRT has been shown to be reduced in WAG/Rij rats as compared to non-epileptic control rats (Gauguier et al., 2004; Liu et al., 2007); instead, $GABA_A$ receptors are preserved in the neocortex of WAG/Rij epileptic rats, which are defective in several $GABA_B$ receptor isoforms (Liu et al., 2007; Merlo et al., 2007).

It has been reported that the somatosensory cortex of epileptic WAG/Rij rats, that plays a major role in the generation of SWDs, exhibits alterations in $GABA_B$ receptor subunit expression and localization (Crunelli and Leresche, 2002; Inaba et al., 2009; Merlo et al., 2007). These alterations may lead to hyperexcitability by down-regulating the function of (inhibitory) presynaptic $GABA_B$ receptors in neocortical networks.

The cortical hyperexcitability in WAG/Rij rats, measured by extra- and intracellularly recorded synaptic responses, is probably due to a decrease in GABA-mediated inhibition leading to the hypothesis that the GABA effects on SWDs may depend on regional processes and are not homogeneous within the brain (Luhmann et al., 1995). Interestingly, Merlo et al. indicate that these molecular changes are not seen in young (about 60days old) WAG/Rij rats that do not yet present SWDs (Merlo et al., 2007). In WAG/Rij rats, but also in GAERS, bilateral injections of the $GABA_A$ receptor agonist muscimol or the GABA reuptake inhibitor tiagabine into the medial part of the ventral lateral thalamus, causes a dose-dependent increase in SWDs (Liu et al., 1991; Peeters et al., 1989b). However, the known therapeutic efficacy of benzodiazepines ($GABA_A$ receptor positive modulators) in generalized absence seizures has been difficult to reconcile with the consistent finding that experimentally, $GABA_A$ agonists make absence seizures *worse*, since benzodiazepines are known to augment $GABA_A$-mediated inhibition. Thus, administration of diazepam increases SWD activity dose-dependently in WAG/Rij rats (Peeters et al., 1990a).

Other systemically administered drugs that enhance GABA activity in the brain, such as VGB and tiagabine, aggravate absence seizures in GAERS and WAG/Rij rats (Bouwman et al., 2007b; Coenen et al., 1995; Russo et al., 2011b) and are also contraindicated in the treatment of human absence seizures (Glauser et al., 2013). In agreement, focal bilateral microinjections of allopregnanolone or ganaxolone, two neurosteroids acting as positive allosteric modulators of the $GABA_A$ receptor, into thalamic nuclei, also increase the incidence of absence epileptic seizures in WAG/Rij rats (Citraro et al., 2006a). Cortical and thalamic concentrations of the most potent endogenous positive modulators of $GABA_A$ receptors (allopregnanolone and 3α,5α-TH-DOC) were compared between pre- (2 months) and post-seizure (6 months) WAG/Rij rats. Significant age-dependent decreases in both neurosteroids were found in the thalamus but not in the cortex in comparison to non-epileptic control Wistar rats. This

alteration in neurosteroid concentration was accompanied by an increase in α_4 $GABA_A$ receptor subunit expression in some dorsal thalamic nuclei and an age-dependent increase in δ subunits. Both α_4 and δ subunits are found in extrasynaptic $GABA_A$ receptors, where they facilitate tonic inhibition. Therefore, it has been hypothesized that the age-dependent increase in α_4 and δ subunits increases tonic inhibition in thalamic relay neurons, which then contributes to the age-dependent increase in SWDs (Pisu et al., 2008). GABAergic neurotransmission clearly plays a relevant role during SWDs; in WAG/Rij rats, this neurotransmitter system is functionally altered in several areas and this may depend on an altered subunit composition of GABA receptors. The actual data, however, suggest that such changes mainly occur *after* seizure development and therefore, they might be a consequence of the adaptive changes due to the epileptogenic process more than a cause.

3.2 Glutamate

N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) and metabotropic-type glutamate receptors are involved in excitatory synaptic responses recorded from TC neurons (McCormick and von Krosigk, 1992). NMDA and AMPA glutamate receptors may also be involved in the mechanisms of initiation and maintenance of SWDs (D'Arcangelo et al., 2002; Filakovszky et al., 2001; Jakus et al., 2004; Kaminski et al., 2001; Peeters et al., 1994a). Cortical and/or thalamic excitation of the NRT through glutamatergic NMDA and AMPA receptors is essential for synchronous thalamic oscillations (Huguenard and McCormick, 2007). There are data concerning excitatory neurotransmission in experimental absence seizures suggesting involvement of glutamatergic pathways in the production of SWDs (Snead, 1995). Every glutamate receptor subtype and their possible contribution to absence generation will be discussed separately in the following subsections.

3.2.1 NMDA Receptors

An increase in synaptic excitability mediated by NMDA receptors in neurons of deep neocortical layers in epileptic WAG/Rij rats has been previously demonstrated (D'Antuono et al., 2006). Cortical application of NMDA receptor antagonists reduced field potential oscillations recorded in WAG/Rij cortico-thalamic brain slices although they persisted following application of an NMDA receptor antagonist to the thalamus (D'Arcangelo et al., 2002). Alterations in the morphological localization of NMDA receptors in the neocortex of WAG/Rij rats have also been reported (van de Bovenkamp-Janssen et al., 2006). WAG/Rij rats express less NR1 subunits of NMDA receptors in the somatosensory cortex in comparison to old non-epileptic ACI rats both at 3 and 6 months of age; considering the WAG/Rij strain, an age-dependent decrease was especially observed in cortical layers IV, V and VI, which contain the major input-output connections to the thalamus and NRT (van de Bovenkamp-Janssen et al., 2006). It was suggested that since 3-month-old WAG/Rij rats do not yet show full SWD activity, a low amount of NMDA and AMPA-GluR4 (see section 3.2.2) receptors might precede the development of SWD activity and perhaps even be the cause of it. A lower expression of the NR2B subunit of the NMDA receptors was found in both 2-month-old (not yet epileptic) and 6-month-old (epileptic) WAG/Rij rats in different layers of the somatosensory cortex in comparison to Wistar rats of the same age (Karimzadeh et al., 2013). This subunit also had a decreased expression in the hippocampal CA1 area in WAG/Rij rats (6 but not 2 months old) in comparison to age-matched control Wistar rats (Karimzadeh et al., 2013). A decrease of the NR2B subunit expression in the somatosensory cortex was observed in particular in young animals before the age of seizure onset. It has been shown that dysregulation of NMDA-mediated transmission by application of agonists or antagonists of NMDA receptors prevents the development of the cortico-thalamo-cortical rhythmic activity, which underlies SWDs in GAERS (Koerner et al., 1996).

Intracerebroventricular injections of NMDA increase the number of SWDs in WAG/Rij rats, whereas NMDA receptor antagonists decrease the number of SWDs in this strain (Peeters et al., 1994a; Peeters et al., 1989a; Peeters et al., 1990b).

Similarly to GABA, modulation of NMDA receptors is also able to modify SWD incidence. NMDA receptors are involved in the cortical hyperexcitability observed in this strain; however, the expression of both NR1 and NR2B subunits is lower in comparison to non-epileptic rats. This alteration is also observed before seizure appearance but it is maintained during life; therefore, it would seem that these alterations precede seizure development and are not influenced by seizures with the exception of the hippocampus, which is not affected before seizure development while it has a decreased expression at 6 months of age.

3.2.2 AMPA receptors

The AMPA receptor is also a member of the ion channel family of glutamate receptors mediating fast excitatory neurotransmission. It is a hetero-oligomer formed from GluA1, GluA2, GluA3 andGluA4 subunits (Citraro et al., 2014). AMPA receptor-mediated transmission may contribute to the enhancement of cortical excitability and, as a consequence, to SWD generation. van de Bovenkamp-Janssen et al. showed that AMPA-GluA4 subunit density is lower in WAG/Rij rats than in ACI rats in peri-oral somatosensory cortex (S1po) layers independently from age and seizure development. Also an age-dependent increase in the numerical density of AMPA-GluA4 subunits was found in the NRT of WAG/Rij rats. This increase continued during ageing after 3 months till at least 6 months. An increase in extrasomal AMPA-GluA4 receptors seemed to be correlated with the appearance of SWDs, since the size of the increase was larger in epileptic than in non-epileptic control rats (van de Bovenkamp-Janssen et al., 2006). Therefore, increased AMPA-GluA4 receptor density in the NRT of 6 months old WAG/Rij rats may increase the cortico-thalamic drive to the NRT,

promoting the synchronization of SWD activities. Other authors demonstrated that GluA2 subunits are not expressed around cell bodies in thalamic nuclei with the exception of the NRT, whereas, they are widely expressed in the two cortical sites examined (S1po and the forelimb region of the primary somatosensory cortex(S1FL))(Citraro et al., 2006b). High doses of intracerebroventricularly-administered AMPA produces a dose-dependent increase in SWDs (Peeters et al., 1994b; Russo et al., 2008). Citraro et al. found that non-competitive AMPA receptor antagonists, CFM-2 and THIQ-10c, showed different effects depending on the brain site of administration; in particular, when the two AMPA antagonists were administered into the thalamic nuclei ventralis postero-medialis (VPM) and ventralis postero-lateralis (VPL) or S1FL they did not modify the characteristic EEG recordings from WAG/Rij rats, whereas when administered into the NRT and S1po they induced a significant reduction of SWDs (Citraro et al., 2006b).

Despite these findings, AMPA antagonists administered systemically do not seem to be very effective drugs against absence seizures, only the highest dose showed a decrease in SWD incidence (Kaminski et al., 2001). AMPA receptors are altered in the CTC circuit; alterations in the subunit composition appear before seizure onset therefore suggesting that they might be involved in the epileptogenesis process. Furthermore, in contrast with what was observed for NMDA receptors, the appearance of absence seizures further alters AMPA receptor expression.

3.2.3 mGlu Receptors

Metabotropic glutamate receptors (mGluRs) form a family of eight G-protein-coupled receptor subtypes (mGlu1-8), which exert complex modulatory actions on glutamatergic neurotransmission. mGluRs are subdivided into three groups on the basis of structural homology, pharmacological profile and transduction pathways: group-I includes mGlu1 and -

5 receptors (discussed here), which are coupled to Gq proteins (linked to activation of phospholipase C (PLC); group-II (mGlu2 and -3) and group-III (mGlu4, -6, -7 and -8) receptors (discussed in Section 5) are coupled to Gi proteins (linked to inhibition of cAMP) in heterologous expression systems (Nicoletti et al., 2015). These receptors are strategically distributed at synapses of the cortico-thalamo-cortical network, which is the anatomical site of origin of SWDs underlying absence epilepsy (Ngomba et al., 2011b). mGluRs within the cortico-thalamo-cortical loops are particularly positioned to modulate rather than mediate synaptic transmission and are therefore potential targets for controlling SWDs (Ngomba et al., 2011b).

mGlu1 and mGlu5 receptors show a different pattern of distribution in the cortico-thalamo-cortical network, which suggests distinct rather than complementary functions of these two receptor subtypes (Ngomba et al., 2011b). mGlu1 receptors are highly expressed in thalamic relay neurons (Ngomba et al., 2011a; Ngomba et al., 2011b). Thus, mGlu1 receptors are critically localized to regulate the firing rate and oscillatory properties of thalamic relay neurons. Neurons of the NRT do not express mGlu1 receptor mRNA (Shigemoto et al., 1992), whereas in the cortex, mGlu1 receptors appear to be exclusively expressed postsynaptically on GABAergic interneurons (Stinehelfer et al., 2000). Immunoblotting analysis demonstrated lower levels of the thalamic mGlu1α receptors in epileptic 8-month old WAG/Rij rats in comparison to 2-month old (pre-seizure) WAG/Rij rats (Ngomba et al., 2011a). It has been reported that mGlu1 receptors are down-regulated in the ventral basal thalamus of symptomatic WAG/Rij rats, and that pharmacological potentiation of mGlu1 receptors (with the mGlu1 receptor enhancer SYN119) reduces SWDs in these rats, whereas treatment with the non-competitive mGlu1 receptor antagonist JNJ16259685, increased the incidence of SWDs (Ngomba et al., 2011a).

The downregulation of mGlu1 receptors in the thalamus only occurs in epileptic 8 months old WAG/Rij rats (Ngomba et al., 2011a). Therefore, mGlu1 receptors have a *protective* function against SWDs and a loss of this protective mechanism might contribute to the epileptic-prone phenotype of WAG/Rij rats while being a consequence of SWDs. Moreover, it has been shown that activation of mGlu5 receptors has an excitatory effect similar to that mediated by mGlu1 receptors and these receptors can participate in sensory responses of thalamic relay cells (Salt and Binns, 2000). Reduced expression and function of mGlu5 receptors in the thalamus has been observed in WAG/Rij rats both at 2 and 8 months of age, while an increased receptor expression in the sensorimotor cortex was described at the same ages (D'Amore et al., 2013). Reduced expression and function of mGlu5 receptors in the thalamus is a molecular determinant of the pathological phenotype of WAG/Rij rats, and pharmacological amplification of thalamic receptors is sufficient to reduce absence seizures. Changes in mGlu5 receptor expression are antecedent to the onset of epileptic phenotype, and, therefore, are not secondary to seizure activity being already visible in pre-seizure WAG/Rij rats (D'Amore et al., 2013). The enhanced expression of mGlu5 receptors in the cerebral cortex represents a compensatory protective mechanism against SWDs. Acute systemic treatment with positive allosteric modulators (PAMs) of mGlu1 and mGlu5 mGluRs (RO0711401 and VU030172, respectively) suppress SWDs in WAG/Rij rats (D'Amore et al., 2013; Ngomba et al., 2011a). Chronic treatment (twice per day for 10 days) induced tolerance within 48-72 hrs for RO0711401, but not for VU030172 (D'Amore et al., 2014).

Modulation of type-1 mGluRs has been suggested as a potential target for the treatment of absence seizures; this effect could be based on the dysregulation observed in the expression and function of these receptors along the CTC circuit since opposite effects of an orthosteric mGlu5R agonist had anti-absence action in the lethargic mice absence model (Chapman et al., 2000). In conclusion, mGlu1 and 5 are altered before seizure onset in several areas and

therefore participate in the generation of the epileptic network responsible for absence seizures; however, the increased expression of mGlu5 in the cortex seems to be a consequence of the seizures.

3.3 Ion channels

Different voltage-sensitive ion channels are highly expressed and involved in CTC brain areas responsible for SWD development. Several studies indicate a recruitment of the hyperpolarization-activated cation current (I_h) in TC neurons during SWDs (Kanyshkova et al., 2012). An increase in the expression of a channel isoform (hyperpolarization-activated and cyclic nucleotide-gated cation channel-1; HCN1) in TC neurons of epileptic WAG/Rij rats was associated with a decrease in cAMP responsiveness of I_h. This produces an impairment in control of the shift from burst to tonic firing, which, in turn, will prolong burst activity after recruitment of I_h during absence seizures (Bazyan and van Luijtelaar, 2013). Altered HCN channel expression and cAMP sensitivity of I_h has been shown in WAG/Rij rats in the postnatal age continuing into the chronic epilepsy state (Budde et al., 2005; Kuisle et al., 2006). The I_h-dependent changes in active and passive membrane properties become significant at 3 months of age and persist into adulthood(Budde et al., 2005). Although the resulting alterations of I_h current density, voltage-dependency, current kinetics, and cAMP-dependent modulation are mostly compensated in older animals (Kuisle et al., 2006), early HCN channel dysregulation has been suggested to contribute to the development of the epileptic phenotype (Blumenfeld et al., 2008; Kanyshkova et al., 2012) and therefore they might be relevant to absence epileptogenesis.

In WAG/Rij rats, HCN1 channel expression decreases, mainly in the dendrites of cortical layer V pyramidal neurons that occurs temporally before the developmental onset of SWDs, and at a cellular level plays a direct role in promoting dendritic Ca^{2+} electrogenesis and burst

firing. This loss of neocortical HCN1 function may contribute to an increased cortical excitability since there are substantially few HCN1 subunits in the combined complex of the I_h channel in the cortical zone containing the focal region in WAG/Rij rats in comparison to non-epileptic rats. Indeed, the density of HCN1 channels in the apical dendrites of the subgranular layers in the cortical onset zone determines the somatodendritic excitability and a decrease of these dendritic HCN1 channels is accompanied by an age-dependent increase of SWDs (Bazyan and van Luijtelaar, 2013; Strauss et al., 2004). Recently, it was demonstrated that HCN1 channels from the thalamus only (not in genomic DNA) of WAG/Rij rats have an N-terminal deletion of 37 amino acids; these channels expressed in Xenopus oocytes indicated a gain-of-function of about 2 fold which was further accompanied by a suppression in HCN2 and HCN4 currents (Wemhoner et al., 2015).

Pathophysiological oscillations leading to absence seizures have also been shown to be dependent on the activity of (transient) T-type Ca^{2+}currents (Broicher et al., 2008; Schridde et al., 2006; Steriade et al., 1993). It has been established that the amplitude of the T-type Ca^{2+} current is increased in the NRT in both WAG/Rij and GAERS (Broicher et al., 2008; Tsakiridou et al., 1995). Also, immunocytochemical data show that the development of absence epilepsy in 6-month-old WAG/Rij rats is concomitant with an increase in presynaptic P/Q-typeCav2.1 channel expression in the NRT in comparison to presymptomatic (3 months old) WAG/Rij rats (van de Bovenkamp-Janssen et al., 2004). Therefore, the increase in the expression of Cav2.1 channels in the NRT of 6-month-old WAG/Rij rats correlates with the manifestation of SWD activity in this animal model. Other studies suggest the involvement of other Ca^{2+} channels in generalized absence epilepsy (Burgess and Noebels, 1999). In young WAG/Rij rats, an increased peak current density of L-type Ca^{2+} current in the dorsal part of the lateral geniculate nucleus (dLGN) TC neurons correlated with up-regulated mRNA and protein expression of a particular L-type Ca^{2+}channel (Cav1.3)(Kanyshkova et al., 2014).

In WAG/Rij rats, mRNA and protein expression of Na^+ channel genes Nav1.1 and Nav1.6 are upregulated in the perioral region of the primary somatosensory cortex (Klein et al., 2004). This upregulation is age-dependent (only in 5-6 months old rats, not in younger rats), and this corresponds with the age-dependent increase in SWDs. The age-dependent increase in SWDs is closely related to the age-dependent upregulation of certain types of Na^+ channels in the superficial layers of the perioral area of the somatosensory cortex (S1po) only. Overall, this increase seems to be subsequent to seizure appearance; therefore, this might be an adaptive change to seizures more than based on a genetic predisposition to overexpress these channels. Summarizing, several ion channels are altered in WAG/Rij rats; HCN1 seem to be involved in the pre-seizure phase also from a genetical point of view while Na^+ channels are also modulated by seizures.

3.4 Glia, neuroinflammation and the mTOR pathway

Numerous studies have shown that neuroinflammatory pathways contribute to the pathogenesis of epilepsy both in terms of epileptogenesis and the long-term consequences of seizures (Vezzani et al., 2013). Dutuit et al. (2000) demonstrated that expression of glial fibrillary acidic protein (GFAP), the first sign of reactive astrocytosis, and its mRNA levels were increased in the cortex and thalamus of both adult and young GAERS suggesting that reactive astrocytes are present before the development of absence seizures (Dutuit et al., 2000). Therefore, reactive astrocytes, already present before the onset of seizures, might contribute to the processes giving rise to epileptic seizures by modifying relevant neuronal structures. These findings suggest that there is an age-related early malfunction of the neuron-glia interactions in rats with genetic absence epilepsy. In addition, the potential role of astrocytic abnormalities in the expression of absence seizures is demonstrated by production of IL-1β (interleukin-1 beta), a prototypical inflammatory molecule, in activated astrocytes,

specifically in the somatosensory cortex of GAERS prior to seizure onset (Akin et al., 2011). In WAG/Rij rats, a deficit in glial cells and a lower glia-neuron index was found in the somatosensory cortex (Sitnikova et al., 2011); this impairment of glia-neuron interactions in this area may well underlie the pathological processes in the primary epileptic foci of absence epilepsy (Sitnikova et al., 2011).

Bacterial lipopolysaccharide (LPS), the biologically active cell wall component of gram-negative bacteria, binds to toll-like receptor 4 (TLR4) of the innate immune system and induces proinflammatory cytokine release from systemic immune cells (Vezzani and Granata, 2005). It has been reported that both peripheral and central LPS administration facilitates SWD generation in the WAG/Rij rat in parallel with increased brain and/or plasma cytokine levels (*e.g.* IL-1β, IL-6 and TNF-α [tumor necrosis factor alpha])(Kovacs et al., 2011; Kovacs et al., 2014; Kovacs et al., 2006; Russo et al., 2014a). Analysis of the brain and blood plasma levels of IL-1β and TNF-α of 2-, 4-, and 6-month-old WAG/Rij rats and of age-matched control ACI rats revealed higher levels of TNF-α in blood serum of the youngest (2 months) WAG/Rij rats while in the brain, the only significant difference was observed in rats of 4 months of age (van Luijtelaar et al., 2012). Overall therefore, neuroinflammation does not seem to actively participate in epileptogenesis in this model; however, neuroinflammatory modulators can regulate seizures and are probably regionally enrolled in the CTC circuit.

The role of cytokines and neuroinflammation in absence seizure generation is also supported by the acute anti-absence effects of non-steroidal anti-inflammatory drugs, both indomethacin and etoricoxib (Citraro et al., 2015b; Kovacs et al., 2014; Rimoli et al., 2009), even though no clear dose-response relationship was observed, suggesting that prostaglandins only partially contribute to absence seizures; however, etoricoxib possesses antiepileptogenic properties in this model (see above section 4 and Table 1).

Astrocytes in the adult brain are connected to each other via gap junction channels composed of connexin 43 (Cx43) and Cx30 (Nagy and Rash, 2000), allowing the intercellular exchange of ions, second messengers, metabolites, and amino acids. The gap junction blocker carbenoxolone reduces absence seizures in adult WAG/Rij rats (Gareri et al., 2005) although this drug is known to exert several other non-specific effects which may complicate interpretations (Connors, 2012). Several connexins are expressed in the brain of WAG/Rij rats, namely Cx30, 36, 43 and 45, but none was found to be altered in comparison to Wistar rats at the age of 6 months (Gareri et al., 2005).

The mammalian target of rapamycin (mTOR) signaling pathway, a conserved serine/threonine kinase and member of the phosphatidylinositol 3-kinase-related kinase (PIKK) family, has been recently indicated as a suitable drug target for the prevention of epileptogenesis in view of its activation during the epileptogenic phase of several epilepsy models and the ability of its specific inhibitor, rapamycin (RAP), to prevent the development of spontaneous seizures (Russo et al., 2012). Recently, the involvement of the mTOR pathway in absence seizures of WAG/Rij rats was demonstrated; chronic oral treatment with rapamycin (see Section 4), started prior to the onset of seizures, reduced the subsequent development of SWDs in adult WAG/Rij rats, thus displaying antiepileptogenic properties (Russo et al., 2013b). These effects were linked to the ability of mTOR to modulate neuroinflammation, which was studied in this strain following the pharmacological interaction between rapamycin and LPS (see below); furthermore, it has been demonstrated that WAG/Rij rats in comparison to Wistar rats, have higher levels of total mTOR in several brain areas, including the cortex, hippocampus and thalamus (Russo et al., 2014b). In a subsequent study it was reported that in the early phases of the inflammatory response induced by LPS, there is an interaction between AMPK (AMP-activated protein kinase) and AKT (protein kinase B) through the mTOR pathway (Russo et al., 2014a). Rapamycin

treatment, 30 min after LPS infusion in the WAG/Rij rat brain, regulated the LPS-induced neuroinflammatory processes in line with its effects against absence seizures and LPS-dependent sickness behavior. Based on the current knowledge, a clear involvement of glial cells in absence seizures has been demonstrated; however, neuroinflammation seems to be more a seizure companion than part of the epileptogenic process, probably contributing to plastic adaptive changes. On the other hand, mTOR appears to be constantly activated before and after seizures; indeed, both neuroinflammation and mTOR might represent good targets for the development of novel antiepileptogenic drugs.

3.5 Endogenous cannabinoids

Endogenous cannabinoids (eCBVs) are generated and released on demand following sustained postsynaptic neuron excitation (Alger and Kim, 2011) and they may be involved in the modulation of seizures in absence as well as other models of epilepsy (Fezza et al., 2014). Thus, in adult WAG/Rij rats, focal microinjections of the eCB anandamide into the thalamic nuclei or somatosensory cortical foci (S1po) reduced the number and duration of absence seizures, supporting the notion that a dysfunctional eCB '*tone*' could contribute to the generation and maintenance of absence oscillations in the TC network of these animals (Citraro et al., 2013a; Citraro et al., 2013b). In contrast, focal administration of the CB1R antagonist/inverse agonist SR141716 (rimonabant) had pro-convulsant effects and blocked the anti-absence effects of anandamide in this model (Citraro et al., 2013b). Accordingly, cannabinoid CB_1 receptors (CB_1Rs) are known to be downregulated in the NRT of WAG/Rij rats suggesting a generally lowered sensitivity to released eCBs (van Rijn et al., 2010). Furthermore, levels of AEA but not 2-arachidonoylglycerol (2-AG) in 2 months old WAG/Rij rats were lower than those observed in both Wistar and ACI rats only in the thalamus and not in the cortex (Citraro et al., 2013b). Interestingly, anandamide has also been shown to inhibit

T-type Ca^{2+} channels directly (Chemin et al., 2001) independent of CB_1Rs, which is akin to the known therapeutic effectiveness of the T-type Ca^{2+} channel blocker ethosuximide in absence epilepsy (Patsalos, 2005).

Overall, whether eCBs have any significant role to play in absence epileptogenesis remains to be tested; this could perhaps be examined with experiments aimed at elevating eCB tone using inhibitors of eCB reuptake (AM404, AM1172, UCM707) and/or degradation via the fatty acid amide hydrolase (FAAH; AM3506)(Beltramo et al., 1997; Godlewski et al., 2010). In view of the recent suggestion that dysregulated eCB signaling could also be a feature of major depressive disorder (MDD)(Ogawa and Kunugi, 2015), it would be of interest to see whether such experiments with WAG/Rij rats would influence epileptogenesis as well as the depression-like symptoms that co-exist in these animals (Sarkisova & van Luijtelaar, 2011).

4. Drug efficacy against the development of spontaneous absence seizures in WAG/Rij rats

The first paper demonstrating drug-dependent antiepileptogenic effects in a genetic absence model appeared in 2008 (Blumenfeld et al., 2008). The question was whether a pharmacological intervention at a certain point of development in a genetic absence epilepsy model would influence the course of epileptogenesis. This latter point is in keeping with the future possibility to determine whether a known genetic predisposition to develop epilepsy in patients can be used to prevent this phenomenon pharmacologically. Therefore, genetic models of epilepsy might be considered a different approach to the study of potential antiepileptogenic treatments in comparison to post-traumatic or induced models (*e.g.* traumatic brain injury or kindling). In this light, drugs effective in genetic models will not be necessarily efficacious in these latter models and furthermore, they will point to a different clinical area, namely genetic epilepsies.

Ethosuximide: In this first study by Blumenfeld et al., the classical anti-absence drug ethosuximide (ETH; a T-type Ca^{2+} channel blocker; 300 mg/kg/day) was administered through drinking water and treatment was started at P21 lasting up to 5 months of age in one group or continuously up to the age of 8 months in a second group of WAG/Rij rats. Therefore, drug treatment was started early after weaning and before seizure onset (P50-60); in any case, before complete central nervous system (CNS) development. Drug choice was based on the possibility that a "seizures beget seizures" process would be responsible for the time-dependent increase in absence seizures in this model; in fact, the dose was chosen according to its ability to completely suppress absence seizures in adult WAG/Rij rats. In this study, ETH treatment markedly suppressed the development of absence seizures; this effect was observed up to 3 months (8 months of age) following drug withdrawal. The data presented demonstrated an effect on the number but not on the mean duration of SWDs. Therefore, despite an effect of the treatment on the changes observed in the expression of Na^{+} channels and hyperpolarization-activated cation channels (HCN1), which accompanies the development of SWDs in this strain (see below Section 3.3) (Klein et al., 2004; Strauss et al., 2004), ETH treatment did not seem to influence the function of the circuit in the maintenance of a single seizure once it is started, but only their generation at the focal site(s).

Although no statistically significant difference in SWD incidence was found by Blumenfeld et al., between rats where ETH treatment was stopped and those where ETH was continued for several months (2nd group; continuous treatment up to 8 months), in the graphs presented, a 'rebound' effect appeared to be present when comparing the number of seizures/hour at 1 and 14 days to 30 days after ETH withdrawal. Furthermore, a time-dependent increase at 60 and 90 days after ETH suspension was noticeable. Others also found a gradual increase in SWD incidence in 4 months-treated rats, 2 months after the treatment had stopped (van Luijtelaar et al., 2013). All together, these data indicate that ETH effects might be transient, or

that ETH treatment suspended only temporarily, the age-dependent increase in SWD incidence. Longer-lasting studies after drug suspension should ideally be performed in order to determine whether the observed effects are permanent or only due to an incomplete maturation of the epileptogenic circuit, which requires seizures to become fully active. In this light, even a small residual number of SWDs might again start a remodeling process, even in adulthood when the brain is less plastic, leading to the development of the full epileptic phenotype. Indeed, a recent retrospective clinical study demonstrated that ETH is associated with a higher rate of remission than valproic acid in children with typical absence epilepsy (Berg et al., 2014).

The results of Blumenfeld et al. were later confirmed by another research group using an identical protocol (Sarkisova et al., 2010); in this case, the development of absence seizures was associated with the development of behavioral depression-like comorbidity, and when rats were followed up to 47 days after ETH withdrawal, a 74% seizure reduction was still observed at that age. To be noted in the Blumenfeld et al. study at 30 days, an ~85% seizure decrease (Figure 2) was observed while at 60 and 90 days after ETH suspension, about 50% and 60% reduction was seen respectively, in comparison to untreated controls as can be evidenced from their figures (Blumenfeld et al., 2008).

ETH effects against epileptogenesis in this model were further confirmed in other studies, despite some differences that were evident. In the first study (Russo et al., 2010), ETH treatment was started at 6 weeks of age (P42 vs P21 in the Blumenfeld et al. study) and the dose used was much lower (80 vs 300 mg/kg/day). The same route of administration was used and animals were treated up to the same final age of 5 months; EEG recordings were only obtained at 6 months of age (1 month after ETH withdrawal). ETH reduced seizure development by about 56% and a reduction in mean SWD duration was also observed in contrast to the results of Blumenfeld et al. van Luijtelaar et al. (2013) investigated whether a 2

months treatment with ETH, which either started at PN30 or at PN 90, affected SWDs at 6 months. They found that only a 4 months' treatment affected SWDs.

Levetiracetam, zonisamide, carbamazepine: The same protocol of administration for the antiepileptic drug (AED) levetiracetam (LEV; a selective ligand for the synaptic vesicle protein SV2A) was used and this drug also showed marked antiepileptogenic effects (about 60% reduction in the number of SWDs). Moreover, the rats chronically treated with ETH or LEV were, one month after suspension, acutely exposed to the same respective drug: surprisingly, while ETH was still effective in reducing the residual SWDs, LEV was nearly not as effective, suggesting that residual seizures after LEV treatment were refractory to the drug. Notably, LEV is not very effective in this animal model; acute doses of 50 and 100 mg/kg reduced SWDs by about 40% with no apparent dose-dependent relationship (Bouwman and van Rijn, 2004; Russo et al., 2010). As mentioned above, ETH effects, as well as those of LEV, were again studied by the same group in a subsequent article published in 2011; in this case, the authors also studied the effects of the AEDs zonisamide (ZNS) and carbamazepine (CBZ) further focusing on drug effects on the development of behavioral depression-like comorbidity (Russo et al., 2011a). The protocol of this second study differed from the previous, since rats started drug treatment at 1 month of age (P30 vs P42 in the previous study and P21 in the Blumenfeld study), drug treatment duration and dose of ETH and LEV were identical; however, in this case, rats at 6 months of age (1 month after treatment suspension) underwent the forced swimming test (FST) for the evaluation of depression-like behavior whereas EEG recordings were performed at 45 days after drug suspension. Under these experimental conditions, ETH reduced seizures by only about 31% while LEV by about 43%; for both drugs, a lower efficacy in comparison to the previous study was underlined (Russo et al., 2011a). In the same article, it was demonstrated that at 45 days after drug suspension, ZNS (40 mg/kg/day) also had antiepileptogenic effects (about

38% reduction) whereas CBZ (20 mg/kg/day) was not effective and no significant increase in SWD parameters was observed after the same treatment schedule. Based on all these studies, it can be concluded that only drugs effective against absence seizures might be able to block/reduce epileptogenesis in WAG/Rij rats.

In this latter study (Russo et al., 2011a), it was also evident that a reduction in the development of spontaneous seizures was not necessarily accompanied by an alteration in depression-like behavior and that different drugs might have differential effects on the two parameters (*i.e.* absence seizures and depressive-like behavior). This point still needs further clarification; in fact, subsequent studies have reported opposite effects. To be noted is a study of Kovacs et al. (2012). These authors administered clomipramine (20 mg/kg i.p.; a tricyclic antidepressant) in neonatal WAG/Rij rats between P8 and P21 (before weaning); SWD activity was found to be attenuated (about 60% reduction) at 8 months of age whereas, WAG/Rij rats showed signs of worsened anhedonia with reduced sucrose solution intake (this is in agreement with the common reported effects of neonatal clomipramine in rats) (Kovacs et al., 2012; Vogel et al., 1990). Accordingly, antiepileptogenic effects (maybe via REM sleep deprivation, since all tricyclic antidepressant drugs reduce REM sleep) did not seem to be associated with the development of depression-like comorbidity. From this study, another possibility arises suggesting that also short-term treatment very early in life might influence the development of absence seizures in this strain. While this point still needs to be clarified, it is already known that experimental febrile seizures (induced between P21 and P42) in this strain do not alter the development of absence seizures in adulthood (Ates et al., 2005); neonatal sensory deprivation however, does promote the development of SWDs (Sitnikova, 2011), and WAG/Rij rats fostered by Wistar dams have less SWDs than rats fostered by their epileptic mothers (Sitnikova et al., 2015). These data further support the idea that early short-term intervention might alter the development of absence seizures. Not surprisingly, a few

months after the first article published by Blumenfeld et al. (2008), another study demonstrated that WAG/Rij rats born from dams treated with ethanol starting from the first week of pregnancy up to 1 week after delivery had ~ 64% less SWDs than the control group at the age of 6 months. In this study, it was also suggested that ethanol would influence the formation of neuronal circuits and depending on the genetic background, this could lead either to development or worsening of epilepsy (as demonstrated in the same article for genetically epilepsy prone (GEPRs) rats) or to an inhibition of circuit reorganization, blocking the development of an epileptic neuronal network as might also be the case in WAG/Rij rats (Russo et al., 2008b).

Vigabatrin: Surprising effects were found when the AED vigabatrin (VGB; an inhibitor of GABA transaminase: GABA-T) was tested for potential antiepileptogenic effects in this model. Similarly to CBZ, VGB administered acutely in symptomatic WAG/Rij rats increased SWDs (Russo et al., 2011b); however, when the usual long-term treatment (starting at P30 ending at 5 months) with a dose of 100 mg/kg/day was performed, VGB reduced the development of spontaneous SWDs (~52%) at 1 month after suspension and this effect was accompanied by a reduction in immobility time in the FST. Therefore, it seems that a drug that when acutely administered aggravates SWDs, might, when administered long-term, have antiepileptogenic effects, supporting the notion that drugs with no clear anti-absence (or even pro-absence) effects might also possess potentially useful mechanisms of action which can modulate the epileptogenic process; in line with this finding, it was later demonstrated that VGB can inhibit the mTOR (mammalian target of rapamycin) signaling pathway (Zhang et al., 2013), which is known to be involved in the epileptogenic process (Russo et al., 2012a). Furthermore, a subsequent study in WAG/Rij rats demonstrated that rapamycin (RAP), an mTOR inhibitor, is also an effective antiepileptogenic treatment in WAG/Rij rats (Russo et al., 2013b).

Rapamycin: The mTOR signaling pathway has recently gained attention in view of its demonstrated involvement in both genetic and acquired epilepsy syndromes (Citraro et al., 2016; Russo et al., 2012a). mTOR inhibitors have demonstrated consistent protective effects in various animal models of epileptogenesis, however, mTOR should not be considered as a universal target for any epileptogenic process (Citraro et al., 2016; Galanopoulou et al., 2012). In WAG/Rij rats, chronic oral treatment with RAP (1 mg/kg/day), started at P45 and continued for 17 weeks, decreased (about 52% reduction) the incidence of SWDs at 6 months of age (1 month after drug suspension); furthermore, the same rats were again EEG monitored at 10 months of age (5 months after suspension) and RAP effects were still maintained with a reduction of about 49% in comparison to their respective untreated controls. To be noted, RAP did not affect the mean duration of a single SWD. Moreover, this chronic RAP treatment also showed unexpected pro-depressant effects in both WAG/Rij rats and Wistar rats (non-epileptic controls) at the age of 6 months, whereas at the age of 10 months, no differences were noted (Russo et al., 2013b). In this and in a subsequent study by the same group, RAP effects were suggested to be also dependent on the modulation of neuroinflammation by the mTOR signaling pathway (see section 3.4)(Russo et al., 2014a). In agreement, RAP was also effective in reducing absence seizures in 6 months old WAG/Rij rats without a clear dose-dependent response, while RAP dose-dependently inhibited the increase in SWDs produced by intracerebral injection of the pro-inflammatory bacterial lipopolysaccharide (LPS) in this strain (Russo et al., 2014a; Russo et al., 2013b).

Etorixicob: The role of neuroinflammation in the epileptogenic process underlying the development of absence seizures in WAG/Rij rats is an important issue, but not yet conclusive and further studies are needed (see section 3.4); however, some of the drugs tested so far suggest the possibility of non-AEDs acting on neuroinflammatory cascades as potential antiepileptogenic treatments in this model. For example, etoricoxib, a selective

cyclooxygenase-2 (COX-2) inhibitor, prevented the development of SWDs (Citraro et al., 2015b). Etoricoxib (10 mg/kg/day) treatment was started at P45 and as usual, lasted up to 5 months of age; also in this study, EEG recordings were obtained at both 6 and 10 months of age (1 and 5 months after drug withdrawal). Etoricoxib significantly and persistently reduced the number of SWDs by ~50%. However, although the % seizure reduction was maintained, it should be noted that the total number of seizures increased proportionally both in treated and untreated rats, therefore, this might indicate that the etoricoxib effect is not necessarily permanent, similar to what was found for the effects of ETH (see above). Interestingly, etoricoxib non dose-dependently reduced absence seizures in 6 months-old WAG/Rij rats by ~50%; this effect was apparently all-or-none, since a dose of 5 mg/kg had no effects, whereas both 10 and 20 mg/kg had similar effects (Citraro et al., 2015b).

Statins: Based on the known neuroprotective effects and anti-inflammatory properties of some statins (Scicchitano et al., 2015a), Citraro et al. (2014b) studied the effects of some of the drugs of this class in the WAG/Rij model. All statins used were administered from P45 up to 5 months of age and EEG effects on SWDs were measured at both 6 and 10 months of age (1 and 5 months after drug withdrawal). Atorvastatin and pravastatin at the doses of 5 and 10 mg/kg/day, respectively, were not effective whereas, atorvastatin (10 mg/kg; SWD reduction ~57%), simvastatin (10 mg/kg/day; SWD reduction ~59%) and pravastatin (30 mg/kg/day; SWD reduction ~45%) were all effective in reducing the development of SWDs at 6 months of age and this reduction was still significant at 10 months of age. However, also in this case, the number of SWDs increased proportionally in treated and untreated rats between 6 and 10 months of age; therefore, as observed for etoricoxib but not for RAP, the antiseizure effects of statins might not be long-lasting (Citraro et al., 2014b). To be underlined, the observed effects were very similar between all statins and therefore, it could be considered a drug class effect linked to their known mechanism of action in inhibiting HMG-CoA (3-hydroxy-3-methyl-

glutaryl-CoA) reductase, involved in cholesterol biosynthesis. Furthermore, all statins at 6 months, reduced immobility time in the FST showing modulating properties on depressive-like comorbidity; however, this effect disappeared at 10 months of age. This latter observation suggests that statins' effects might be only temporary if we consider that depressive-like behavior is linked to the development of SWDs.

Antidepressants: It is an accepted view that psychiatric comorbidity should be considered part of the epileptogenic process and it is somehow related to seizure development or worsening (Brooks-Kayal et al., 2013; Cardamone et al., 2013; Sankar and Mazarati, 2012). Furthermore, the role of AEDs or drugs effective against psychiatric symptoms has only been infrequently studied in animal models of epileptogenesis, thus it is not yet possible to draw any firm conclusion on their effects on seizure development and/or psychiatric comorbidity. A recent study evaluated the effects of some drugs used for the treatment of psychiatric diseases in WAG/Rij rats for both epileptogenesis and depression-like comorbidity (Citraro et al., 2015a). In this study, all drugs were administered between P45 and 5 months of age; EEG was only recorded at 6 months of age together with immobility time in the FST. Two standard antidepressant drugs were used; i) fluoxetine, a selective serotonin reuptake inhibitor, at a dose of 10 mg/kg/day did not modify the development of absence seizures while administered at a dose of 30 mg/kg/day, it reduced them (~46%); likewise, ii) duloxetine, a dual-acting serotonin-noradrenaline reuptake inhibitor, both at 10 and 30 mg/kg/day, reduced the development of SWDs by ~20 and 37%, respectively. Of note, the effects on SWDs of both drugs were also studied after chronic 7 weeks treatment in 6 month-old rats and fluoxetine was pro-epileptic while duloxetine at a dose of 30 mg/kg/day only reduced absence seizures; therefore, the antiepileptogenic effects of these drugs seem not to be necessarily related to their anti-absence effects. Furthermore, opposite effects of both drugs were observed on depression-like comorbidity; duloxetine did not modify immobility time at either dose

whereas, fluoxetine at the lower dose (not effective as an antiepileptogenic treatment) was pro-depressant, and at the highest dose (effective as an antiepileptogenic treatment) it reduced immobility time. These controversial results on comorbidity are currently unexplainable and unfortunately considering all data published up to date, no final conclusions can be outlined. Notably, other mechanisms of action might have also contributed on the observed effects for these drugs; e.g. fluoxetine inhibits TREK channels which have been recently indicated as a suitable target for the development of drugs with antidepressant properties and possibly other neurological diseases (Borsotto et al., 2015). Nevertheless, overall, current data suggest that a link between absence seizures, epileptogenesis and depression-like behavior does exist, that needs to be further explored.

Indeed, these drugs acting on central serotonin and serotonin/noradrenaline systems may modulate the epileptogenic process and this is also supported by the results obtained in WAG/Rij rats after orally administering a milk whey protein rich in tryptophan (α-lactoalbumin; ALAC), which is known to increase tryptophan blood levels and possibly serotonin levels in the brain (Citraro et al., 2011). Despite the fact that the mechanism of action of ALAC has not yet been completely clarified, its administration at a dose of 250 mg/kg/day starting at P30 up to 5 months of age in WAG/Rij rats reduced the development of SWDs by ~28% (Russo et al., 2012b). This effect might be related to an increase in cerebral serotonin and indeed serotonin brain tissue levels correlated with SWD activity (Midzyanovskaya et al., 2006); however, it was also suggested that part of its action may be due to an interaction at the glycine binding site on NMDA receptors through the possible conversion of tryptophan to kynurenic acid (Russo et al., 2012b). Interestingly, a deficit of endogenous kynurenic acid has been found in the frontal cortex of symptomatic WAG/Rij rats, but not in younger WAG/Rij rats and in age-matched controls (Kaminski et al., 2003)(Kaminski et al., 2003).

Finally, in a different study, WAG/Rij rats were also treated from P45 to 5 months of age with three different antipsychotic drugs and EEG recordings obtained 1 month after withdrawal (6 months of age) together with immobility time in the FST. None of the drugs tested, namely haloperidol (1 mg/kg/day), risperidone (0.5 mg/kg/day) and quetiapine (10 mg/kg/day), modified the development of SWD seizures (a slight but not significant increase was noted with haloperidol and risperidone); furthermore, haloperidol and risperidone were pro-depressant while quetiapine had no effects on immobility time (Citraro et al., 2015a). Of note, after 7 weeks of oral treatment at the same doses in 6 month-old rats, only haloperidol and risperidone were pro-absence, while quetiapine did not modify the number of SWDs. Based on this observation, it could be argued that during long-term treatment, a continuous increase in SWDs was present in WAG/Rij rats up to 5 months of age and this might be responsible of the pro-depressant effects observed. Therefore, antipsychotics might not give rise to a permanent increase in SWDs but they would only aggravate depressive-like comorbidity (Citraro et al., 2015a). At odds, CBZ treatment did not modify immobility time in the FST (Russo et al., 2011a). Furthermore, it seems that drugs increasing SWDs during treatment do not induce a pro-absence effect leading to an increased number of SWDs after the end of treatment; namely, no pro-epileptogenic effects seem to be based on a possible increased seizure frequency in the long term.

In conclusion: overall, several drug treatments, if administered early, seem to be efficacious in preventing absence seizure development in this animal strain and in particular, the recently published results on ETH for childhood absence epilepsy are in agreement with the results obtained in WAG/Rij rats (Berg et al., 2014) about the potential disease-modifying effects of this AED. The age of drug intervention also needs to be reevaluated, considering that most studies were performed with long-term (about 4 months) drug treatments started mainly after

1 month of age; shorter periods with ETH were not effective, possibly earlier intervention might require shorter treatment periods as suggested by the effects on WAG/Rij rat seizure development after clomipramine administration earlier in life (P8) and for only 14 days (Kovacs et al., 2012). Finally, persistence of drug effects has not always been reassessed long after drug withdrawal and in some cases, drug effects were not convincingly maintained over time.

5. Potential adaptive changes appearing after seizure onset

5.1 Dopamine

Dopaminergic neurotransmission/modulation has been shown to participate in the control of absence seizures (Citraro et al., 2015a; de Bruin et al., 2000; Russo et al., 2013a) very likely through its action on the nigrostriatal pathway and control of the activity of GABAergic neurons in the substantia nigra (Deransart et al., 2000; Warter et al., 1988).

In WAG/Rij rats, a low dopaminergic reactivity of the nigrostriatal system and the lesser amounts of disinhibition of TC neurons contribute to the high incidence of SWDs (de Bruin et al., 2000). In agreement, dopamine antagonists enhance SWDs and dopamine agonists reduce SWD incidence (Midzianovskaya et al., 2001), which suggests that reduced dopaminergic activity in the nucleus accumbens (core) might enhance SWDs, and that ventral pathways of the basal ganglia system are involved in the modulation of absence seizures (Deransart et al., 2000). The anatomical pathways through which this occurs might be the projections from the nucleus accumbens (shell) and core of the ventral accumbens to various thalamic nuclei, including the NRT (Groenewegen et al., 1999).

In WAG/Rij rat's brain at 7 months of age in comparison to age-matched ACI rats, the density of dopamine D1-like and D2-like receptors is lower in the striatal and hippocampal subregions. D1-like receptor density is decreased in dorsal caudate-putamen and core of

nucleus accumbens, while D2-like receptor density is lower in dorsal caudate-putamen and hippocampus CA3. At odds, an increased density of D2-like receptors has been found in cortical areas: frontal (motor and somatosensory areas) and parietal cortices. Therefore, it has been proposed that the mesocortical dopaminergic system might be involved in the pathogenesis of SWDs (Birioukova et al., 2005).

Compared to non-epileptic Wistar rats, WAG/Rij rats show enhanced susceptibility to catalepsy and the lowered density of DA receptors found in the dorsal caudate-putamen of these rats has been considered as a neurochemical basis of this susceptibility. Enhanced susceptibility to catalepsy was found even in young (8-week-old) WAG/Rij rats in which SWDs could not yet be detected (Kuznetsova et al., 1996). This indicates that the dopaminergic deficiency appears prior to the development of absence seizures and therefore could not be attributed solely to a consequence of repeated absence seizures but directly associated with the appearance of absence seizures. It will be necessary to understand whether these alterations are confirmed in the pre-seizure period and whether they are also linked to the depressive-like behavior and epileptogenesis (Sarkisova and van Luijtelaar, 2011; Sarkisova et al., 2008); however, the lack of effects of antipsychotic drugs acting on dopamine receptors suggests that dopamine might not have a direct role in epileptogenesis and that all these observed differences might only be an adaptation to the underlying epileptogenic process or an epiphenomenon.

5.2 Glutamate receptors

5.2.1 Group-II (mGlu2/3 receptors)

Epileptic 6-month-old WAG/Rij rats have an increased expression of mGlu2/3 receptors in the ventrolateral portion of the somatosensory cortex, which includes the putative “triggering zone(s)” of SWDs (Meeren et al., 2002; Ngomba et al., 2005). The expression of these

receptors is also increased in ventrobasal thalamic nuclei and in the hippocampus, but not in the NRT. Instead, no changes in mGlu2/3 receptors expression and function were found in pre-seizure 2-months-old WAG/Rij rats. Pharmacological blockade of mGlu2/3 receptors with LY341495 reduces and treatment with a selective mGlu2/3 receptor agonist (LY379268) increases the incidence of SWDs in WAG/Rij rats (Ngomba et al., 2005). mGlu2/3 receptors are involved in absence seizures in this strain; the up-regulation of these receptors in the somatosensory cortex and other areas being evident only in adult epileptic rats indicates that this might be a consequence of repetitive seizures or an epiphenomenon.

5.2.2 Group-III (mGlu4, -6, -7 and -8 receptors)

Only mGlu4 receptors have been studied in WAG/Rij rats (Ngomba et al., 2008). Higher levels of presynaptic mGlu4 receptors at cortico-thalamic reticular synapses seem to be correlated with the appearance of SWDs; an increased mGlu4 receptor expression was observed only in the NRT of epileptic 6 months old WAG/Rij rats, but not in the NRT of young pre-symptomatic WAG/Rij rats. These mGlu4 receptors in the NRT are localized on excitatory cortical afferents. No changes in mGlu4 mRNA or protein levels were found in the somatosensory cortex and the selective mGlu4 receptor enhancer (PHCCC) increases the incidence of absence seizures (Ngomba et al., 2008). To date, no data exist on the role of the other group-III mGluRs in WAG/Rij rats. Similarly to mGlu2/3, mGlu 4 receptors also seem to be altered after the appearance of seizures and might not therefore contribute to the initial phases of epileptogenesis in this strain.

5.3 Endogenous opioids

Different biochemical studies have indicated a possible role of the endogenous opioid system in the genesis and/or control of absence epilepsy in WAG/Rij rats (Lason et al., 1992; Lason

et al., 1994b). The activity of the endogenous opioid system was studied by measuring the levels of proenkephalin (PENK; mainly interacting with μ and δ opioid receptors) and prodynorphin (PDYN; mainly interacting with κ opioid receptors) and their mRNA, as well as the density of opioid receptors (μ, δ and κ) in various brain areas of WAG/Rij rats at 3 and 6 months in comparison to ACI rats.

A higher level of PENK mRNA and PENK-derived peptide was found in the striatum but also in the thalamus and frontal cortex of 6 months old (symptomatic) in comparison to 3 months old WAG/Rij rats (indicated as pre-symptomatic at that time, although WAG/Rij rats have some seizures at this age) but also age-matched ACI control rats (Lason et al., 1992; Lason et al., 1994b). Thalamus and frontal cortex are essential for SWDs, therefore, PENK was suggested to participate in the symptomatology and/or pathogenesis of absence epilepsy in WAG/Rij rats (Lason et al., 1994b). Moreover, enhanced striatal PENK biosynthesis may lead to a more pronounced release of PENK-derived peptides and may influence other structures involved in the generation of SWDs (Lason et al., 1994b). It is known that the striatum is involved in the control of absence seizures, possibly via the dopaminergic system (Buzsaki et al., 1990). A low dopaminergic activity of nigrostriatal fibers may significantly contribute to increase SWDs (Buzsaki et al., 1990; Cools and Peeters, 1992), which is in agreement with later findings demonstrating that dopamine receptor antagonists increase SWDs (Birioukova et al., 2005; Citraro et al., 2015a; Midzianovskaia et al., 2001).

PENK-derived peptides exert their action through activation of both μ and δ opioid receptors, and it has been demonstrated that selective μ receptor agonists increase the number of SWDs, while selective δ receptor agonists have no effects on SWDs (Lason et al., 1994a). Different effects after activation of the μ and δ receptors may result from a diverse distribution of these opioid receptors in the rat brain, especially in the thalamus (Lason et al., 1994a); autoradiographic studies showed a high density of μ opioid receptors in structures primary

involved in the generation and propagation of SWDs, such as the thalamus, cortex and striatum of 6 months old WAG/Rij rats in comparison to 3 months old rats of the same strain as well as ACI rats (3 and 6 months old) (Mansour et al., 1987; Przewlocka et al., 1998). Instead, a low density of δ receptors has been found in thalamic nuclei and this may explain the lack of effect of the δ agonist and antagonist in WAG/Rij rats (Mansour et al., 1987). Therefore, epileptic activity in WAG/Rij rats involves μ opioid receptors, while δ receptors may not be involved.

Regarding prodynorphin (PDYN), an increased hippocampal level of PDYN mRNA has been found and an increase of PDYN-derived peptide (neoendorphin) in the striatum and hippocampus of epileptic 6 months old WAG/Rij rats in comparison to 3 months old WAG/Rij rats and ACI control rats (both at 3 and 6 months of age) (Lason et al., 1992; Lason et al., 1994b). Instead, a comparable age-related decrease in the PDYN mRNA levels was found in the frontal cortex and, to a lesser extent, in the striatum of both WAG/Rij and ACI rats (Lason et al., 1994b); this may be an independent additional factor facilitating the occurrence of SWDs, since the peptides derived from PDYN, interacting mainly with κ receptors, produce antiepileptic effects (Lason et al., 1994b; Przewlocka et al., 1995). Indeed, i.c.v. administration of U50,488H, which like PDYN-derived peptides, stimulates κ opioid receptors, decreased the number and mean duration of SWDs indicating that activation of the κ opioid receptor exerts an inhibitory effect on absence-like seizure activity in WAG/Rij rats (Przewlocka et al., 1995). κ receptor agonists not only act in a way opposite to μ receptor agonists (Lason et al., 1994a), but can also attenuate the μ agonist-induced increase of the number of SWDs; however, this receptor does not seem to be involved in the epileptogenesis process (Przewlocka et al., 1995).

Concluding, changes in the level of PENK and PDYN in several brain structures may be relevant for the occurrence of SWDs in WAG/Rij rats; however, their role in epileptogenesis

(above all for μ receptors) needs to be better defined since the above-summarized experiments were carried out comparing 3 months old *vs* 6 months old WAG/Rij rats and, therefore, results may be influenced by the fact that rats at 3 months already may have SWDs which might have affected the endogenous opioid system, although tendencies can be inferred from the age dependent differences.

5.4 Metalloproteinases

MMPs (matrix metalloproteinases, MMP1–MMP28) are zinc-dependent proteases which can degrade or modify the extracellular matrix components, involved in angiogenesis, neurogenesis, regeneration and synaptic plasticity but also in several pathological processes such as cerebral ischemia, neuroinflammation and epilepsy, many of which are associated with glutamate transmission dysfunction (Jourquin et al., 2003; Wilczynski et al., 2008).

MMP-9 might be involved in generalized absence epilepsy considering that an elevated activity was found in the WAG/Rij rat (Takacs et al., 2010). This elevation is correlated with diurnally occurring SWDs in WAG/Rij rats. Nevertheless, no cell death was associated with the emergence of absence seizures, which occur in WAG/Rij rats aged about 2-3 months and which are associated with an increase in MMP-9 activity.

In detail, in WAG/Rij rats, MMP-9 levels are significantly elevated 1) in brain areas implicated in SWD genesis; 2) at the age at which SWDs were first generated and 3) during diurnal changes of SWD genesis, in particular in areas involved in seizure generation. Both MMP-9 and its precursor pro-MMP-9 are enhanced during periods of high seizure activity in adult (6 months old) WAG/Rij animals, in comparison to young (6 weeks old) rats. At odds, the enzymatic activity of MMP-2 was largely reduced in adult rats (Takacs et al., 2010). Overall, the role of MMPs needs to be better investigated, but their alteration seems a consequence of seizures more than part of the epileptogenic process.

6. Other systems or processes

6.1 Acetylcholine

The occurrence of SWDs in WAG/Rij rats depends on the presence of combined cholinergic input from the brainstem and nucleus basalis of Meynert (NB) to both cortical and thalamic areas. Therefore, the NB plays a key role in neocortical arousal by directly activating the neocortex, including the somatosensory cortex (Baskerville et al., 1993), the region of origin of the SWDs (Meeren et al., 2002).

The ascending activation from brainstem and basal forebrain weakens during drowsiness and light slow-wave sleep and then the neurons of the NRT may start to fire in the bursting mode, necessary for the production of and (via widespread ascending projections) synchronization across the thalamus and cortex, which can be registered as sleep spindles or paroxysmal SWDs (Buzsaki et al., 1988). It has been shown that mainly muscarinic cholinergic stimulation of the NB and, to a smaller extent, of the NRT, plays an important role in the inhibition of the CTC synchronous activity, typical for SWDs in WAG/Rij rats (Berdiev et al., 2007; Berdiev and van Luijtelaar, 2009). In particular, the administration of the cholinergic agonist carbachol in the NB suppressed SWD activity in WAG/Rij rats while the muscarinic antagonist scopolamine alone had no effect on SWDs, but antagonized the carbachol-induced decrease of cortical discharges (Berdiev and van Luijtelaar, 2009). Nevertheless, the selective destruction of cholinergic cells within the NB provokes an *increase* of SWDs in the cortical EEG of adult WAG/Rij rats (Berdiev et al., 2007), suggesting that the activity of the cholinergic system indeed inhibits SWDs. Despite these data suggesting an involvement of the cholinergic system in seizure modulation, no specific studies on the central distribution or function of cholinergic receptors have so far been

performed in this strain of rats. Considering the role of this system in the regulation of SWDs, epileptogenic alterations cannot be excluded, therefore further investigations are warranted.

6.2 Noradrenaline

Little is known about the role of noradrenaline (NA) transmission in absence epilepsy and epileptogenesis of WAG/Rij rats, though some rat strains with absence epilepsy have a congenital deficit of brain aminergic neurotransmitter systems (Buzsaki et al., 1990). It is known that drugs, which interact with β-noradrenergic transmission (salbutamol, isoprenaline, propranolol), do not affect absence seizures; instead, SWDs are reduced by the administration of the α1-agonist cirazoline or α2-antagonist yohimbine, but aggravated by the α1-antagonist prazosin and α2-agonist clonidine (Marescaux et al., 1992a; Micheletti et al., 1987).

Clonidine aggravates absence seizures in WAG/Rij rats; the administration of clonidine in a low dose in WAG/Rij rats results in EEG and behavioral sedation, associated with the reduction of noradrenergic neurotransmission (Sitnikova and van Luijtelaar, 2005). Further studies are therefore needed to clarify whether this system is involved in the epileptogenic process in this model.

6.3 Serotonin

There are a few studies investigating the role of serotonin (5-hydroxytryptamine, 5-HT) in absence seizures/epilepsy (Filakovszky et al., 1999; Gerber et al., 1998; Graf et al., 2004; Jakus et al., 2003; Midzyanovskaya et al., 2006; Sander et al., 2000; Sarkisova and van Luijtelaar, 2011); it has been suggested that 5HTergic neurotransmission participates in the modulation of the occurrence of SWDs in absence epilepsy through various different 5-HT receptors, by acting on the main components of the CTC loop. An increase in endogenous 5-HT produces a dual effect on SWDs; the activation of 5-HT1A receptors enhances

epileptiform activity, and the activation of 5-HT2C receptors inhibits the generation of SWDs of absence epilepsy in WAG/Rij rats (Filakovszky et al., 1999; Gerber et al., 1998; Graf et al., 2004; Jakus et al., 2003).

Extracellular and intracellular recordings revealed that 5-HT2 receptors are involved in 5-HT-induced inhibition of rhythmic burst firing of the thalamic neurons, which are closely related to the generation of SWDs and/or cortical spindle activity (Pape and McCormick, 1989). Thus, these inhibitory functions of 5-HT2 receptors on thalamic neurons seem to be responsible for the anti-absence activity of 5-HT2 agonists, although the precise mechanisms remain uncertain. In addition, a previous study using WAG/Rij rats showed that the 5-HT2B/2C agonist (mCPP) reduced the incidence of SWDs and these actions were antagonized by the selective 5-HT2C antagonist SB-242084 (Jakus et al., 2003). Despite a clear involvement of 5-HT neurotransmission in absence epilepsy, no specific studies exist in WAG/Rij rats regarding the distribution and function of its receptors, therefore, no conclusions can be drawn about whether 5-HT systems are involved in epileptogenesis.

7. Current limitations and future directions

WAG/Rij rats are currently considered a plausible model of absence epileptogenesis or more generally, a genetic animal model of epileptogenesis (Blumenfeld et al., 2008; Giblin and Blumenfeld, 2010; White and Loscher, 2014). This point is strongly supported by the recent clinical data demonstrating a potential antiepileptogenic effect of EHT in childhood absence epilepsy, similarly to its effects in this strain (Berg et al., 2014; Blumenfeld et al., 2008). Furthermore, the hypothesis that epileptogenesis occurs before two months of age, earlier than the development of spontaneous absence seizures, is also highly probable, while not yet completely demonstrated and studied.

Indeed, WAG/Rij rats have long been known and studied as an animal model of absence epilepsy and from this point of view much is known (van Luijtelaar, 2011; van Luijtelaar and Zobeiri, 2014) even though, as evidenced in this review, most of these data are not helpful to understand the process of *epileptogenesis* in this strain. Genetic analysis of this strain by quantitative trait loci (QTL) mapping demonstrated the polygenic control of SWD phenotypes, which was earlier hypothesized considering that WAG/Rij rats are a fully inbred strain from Wistar rats. Mendelian cross-breeding studies suggested that one dominant gene determines the occurrence and other genes modulate the number and duration of SWDs (Peeters et al., 1992); subsequently, Gauguier et al. identified the above mentioned QTLs, *T1swd/wag* on chromosome 5 controlling type-1 SWD average duration and *T2swd/wag* on chromosome 9 controlling the total duration of type-2 SWDs (Gauguier et al., 2004). Epigenetic changes convincingly contribute (both positively and negatively) to the development of absence seizures (see Section 2.1) even if only few studies are currently available and none of them has demonstrated the ability to completely suppress the development of spontaneous seizures. Further studies may thus aim at a better characterization of this aspect.; e.g. Repressor element 1-silencing transcription (REST) factor pathway negatively regulates the expression of several genes including these for HCN1-4 channels (Goldberg and Coulter, 2013; Roopra et al., 2012) which have been involved in the development of absence seizures in this strain (see Section 3.3) among others.

Overall, the clarification of the mechanisms involved during the epileptogenesis process (*e.g.* neuroinflammation, plastic changes) should be considered as most relevant, considering that these kind of studies will highlight both the best time-window for intervention and the best targets for drug(s); this latter point should also be considered in light of the concept of "network pharmacology" for epileptogenesis and the possibility to use contemporary drugs with different mechanisms of action to prevent the development of spontaneous seizures and

to reduce toxicity (Klee et al., 2015). To date, several drugs have already been tested and studied in this model, although all studies were based on a hypothetical period of intervention and drug-treatment duration and their choice was mostly not based on earlier evidenced specific targets. Indeed, several other drugs might be tested, but only by hypothesizing that a particular epileptogenic mechanism might be involved or on the basis of their effectiveness in other models (*i.e.* bumetanide, brivaracetam). Therefore, while these kind of experiments are also warranted, they appear to be secondary to the need to better study the epileptogenic process *per se* as mentioned above.

Similarly, a clinical approach cannot yet be hypothesized; the study by Berg et al. is undoubtedly encouraging by supporting animal data and *vice versa*; however, many differences exist and several aspects must be considered (Berg et al., 2014). First of all, the fact that WAG/Rij rats do not have seizure remission later in life as happens in childhood absence epilepsy and that children start drug treatment only after diagnosis (no data are available, to date, demonstrating that ETH started in rats after the appearance of the first seizure has antiepileptogenic effects) in contrast to the animal model protocol (treatment started at least 15 days before seizure development), does not permit us to easily consider the potential use of other drugs directly on patients. For example, while rapamycin was found to be very effective, it is obviously not yet advisable to start a clinical trial to demonstrate its effectiveness in childhood absence epilepsy. On the other hand, the major limitation for the treatment of patients who might be affected by genetic epilepsy is the current impossibility to predict the development of epilepsy before the appearance of the first seizure; therefore, it is currently not possible to start a treatment as early as we do in this animal model. In this light, the identification of a possible presymptomatic biomarker in this strain also represents a compelling need.

Finally, despite the fact that this rat strain only develops absence seizures, this might not necessarily mean that drugs efficacious in this model might not also be effective against epileptogenesis leading to other seizure types; for example, rapamycin and levetiracetam are both effective in this and other models of epileptogenesis. Furthermore, epileptogenesis mechanisms that might be identified in this model might also be present in other genetic animal models of epileptogenesis.

8. Conclusions

The lack of antiepileptogenic therapies is one of the major unmet needs on epilepsy research, despite the availability of many animal models (Pitkanen and Engel, 2014; Trinka and Brigo, 2014; White and Loscher, 2014). To this aim, animal models have been the major source of our current understanding and have led to the identification of potential targets for epilepsy development prevention and/or progression; however, none of the currently available models has been clinically validated (Kobow et al., 2012; Pitkanen and Lukasiuk, 2011; Pitkanen et al., 2013). New insights are now coming from so-called genetic animal models which are being reconsidered as potential models to study the pathophysiology of the *epileptogenic* process (differently from acquired/post-insult models) and the potential *antiepileptogenic* efficacy of drug treatments (Blumenfeld et al., 2008; Chugh et al., 2015; Liautard et al., 2013; Marguet et al., 2015). Our current knowledge suggests that some processes (*e.g.* neuroinflammation) might be in common in the epileptogenesis that occurs in post-insult models and genetic models; however, some important differences also exist (*e.g.* no clear neurodegeneration, neurogenesis) and also differences might well be observed between different genetic models.

In WAG/Rij rats, the first seizure appears about the age of 2 months; therefore, we have hypothesized that the period of life before seizure appearance in this strain, as well as other

genetic models, might be considered the corresponding *latent phase* (period) studied in post-insult models. Brain insults such as brain injury or stroke are clearly identifiable events, while in WAG/Rij rats, altered genes might be considered as the initial 'insult' leading to the development of spontaneous seizures. Indeed, this theory needs to be validated and further studies are warranted to better understand and identify differences between epileptogenic processes in genetic and post-insult models.

Starting from this background, we have reconsidered all published articles on WAG/Rij rats in order to identify potentially already known processes involved in epileptogenesis. It was clear from the beginning, that studies not focused on epileptogenesis could only give partial information. However, some interesting results on several systems have been published and they present the starting point for future investigations aimed at the study of epileptogenesis in this strain or for comparison with other genetic animal models. The major limitation found was related to the age when animals were studied, considering that in general, experiments were run in 1 month old animals or even worse, at 2 or 3 months of age; furthermore, if we believe that the epileptogenic process in genetic animals might start very early, even before birth, studies on rats younger than 1 month are completely missing.

Comparing WAG/Rij rats with what is known for post-insult models, we found that: 1) there is not yet a clear demonstration of an involvement of neuroinflammation in epileptogenesis; it cannot however, be excluded that local inflammatory processes are involved, as observed for GAERS (Akin et al., 2011). The currently published articles confirm that no major inflammatory processes are undergoing before and after seizure appearance; 2) no neurodegeneration is observed in any brain area of these rats before seizure onset as well as any other major brain abnormalities. Both these points clearly differentiate WAG/Rij rats from other post-insult models, while mTOR *hyper*activation seems to be a common link with the latter (Russo et al., 2012). Furthermore, several alterations have been identified in the

glutamatergic systems, which are, the most studied in this strain, while considering pre-seizure age. Finally, some relevant alterations are observed in the function of several ion channels, leading to hyperexcitability, which, together with possible changes in endocannabinoids, deserve further confirmatory and explanatory studies.

On the other hand, the recently published results on ETH in childhood absence epilepsy are in agreement with the antiepileptogenic effects of this drug observed in WAG/Rij rats (Berg et al., 2014), although the mechanism by which this is exerted is not yet clear. A variety of drug treatments have already been tested in this strain and while antiseizure efficacy was demonstrated for some of them, we critically observed that in some studies, drug effects were not persistent and seizures tended to reappear some time after drug withdrawal. Drug testing protocols for this strain and possibly other genetic models should therefore be structured to consider longer-lasting follow-up seizure detection after drug treatment suspension.

In conclusion, we propose that WAG/Rij rats and other genetic epilepsy models represent a unique opportunity to increase our knowledge on epileptogenesis; future studies should consider earlier life periods and should be aimed at the identification of early processes that could be targeted for future drug therapies.

Conflict of interest

There are no conflicts of interest to be disclosed.

References

Aker, R.G., Yananli, H.R., Gurbanova, A.A., Ozkaynakci, A.E., Ates, N., van Luijtelaar, G., Onat, F.Y., 2006. Amygdala kindling in the WAG/Rij rat model of absence epilepsy. Epilepsia 47, 33-40. 10.1111/j.1528-1167.2006.00367.x.

Akin, D., Ravizza, T., Maroso, M., Carcak, N., Eryigit, T., Vanzulli, I., Aker, R.G., Vezzani, A., Onat, F.Y., 2011. IL-1beta is induced in reactive astrocytes in the somatosensory cortex of rats with genetic absence epilepsy at the onset of spike-and-wave discharges, and contributes to their occurrence. Neurobiol Dis 44, 259-269. 10.1016/j.nbd.2011.05.015.

Alger, B.E., Kim, J., 2011. Supply and demand for endocannabinoids. Trends Neurosci 34, 304-315. 10.1016/j.tins.2011.03.003.

Baskerville, K.A., Chang, H.T., Herron, P., 1993. Topography of cholinergic afferents from the nucleus basalis of Meynert to representational areas of sensorimotor cortices in the rat. J Comp Neurol 335, 552-562. 10.1002/cne.903350407.

Bazyan, A.S., van Luijtelaar, G., 2013. Neurochemical and behavioral features in genetic absence epilepsy and in acutely induced absence seizures. ISRN Neurol 2013, 875834. 10.1155/2013/875834.

Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., Piomelli, D., 1997. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277, 1094-1097.

Berdiev, R.K., Chepurnov, S.A., Veening, J.G., Chepurnova, N.E., van Luijtelaar, G., 2007. The role of the nucleus basalis of Meynert and reticular thalamic nucleus in pathogenesis of genetically determined absence epilepsy in rats: a lesion study. Brain Res 1185, 266-274. 10.1016/j.brainres.2007.09.010.

Berdiev, R.K., van Luijtelaar, G., 2009. Cholinergic stimulation of the nucleus basalis of Meynert and reticular thalamic nucleus affects spike-and-wave discharges in WAG/Rij rats. Neurosci Lett 463, 249-253. 10.1016/j.neulet.2009.07.068.

Berg, A.T., Levy, S.R., Testa, F.M., Blumenfeld, H., 2014. Long-term seizure remission in childhood absence epilepsy: might initial treatment matter? Epilepsia 55, 551-557. 10.1111/epi.12551.

Bessaih, T., Bourgeais, L., Badiu, C.I., Carter, D.A., Toth, T.I., Ruano, D., Lambolez, B., Crunelli, V., Leresche, N., 2006. Nucleus-specific abnormalities of GABAergic synaptic transmission in a genetic model of absence seizures. J Neurophysiol 96, 3074-3081. 10.1152/jn.00682.2006.

Birioukova, L.M., Midzyanovskaya, I.S., Lensu, S., Tuomisto, L., van Luijtelaar, G., 2005. Distribution of D1-like and D2-like dopamine receptors in the brain of genetic epileptic WAG/Rij rats. Epilepsy Res 63, 89-96. 10.1016/j.eplepsyres.2004.12.001.

Blumenfeld, H., Klein, J.P., Schridde, U., Vestal, M., Rice, T., Khera, D.S., Bashyal, C., Giblin, K., Paul-Laughinghouse, C., Wang, F., Phadke, A., Mission, J., Agarwal, R.K., Englot, D.J., Motelow, J., Nersesyan, H., Waxman, S.G., Levin, A.R., 2008. Early treatment suppresses the development of spike-wave epilepsy in a rat model. Epilepsia 49, 400-409. 10.1111/j.1528-1167.2007.01458.x.

Borsotto, M., Veyssiere, J., Moha Ou Maati, H., Devader, C., Mazella, J., Heurteaux, C., 2015. Targeting two-pore domain K(+) channels TREK-1 and TASK-3 for the treatment of depression: a new therapeutic concept. Br J Pharmacol 172, 771-784. 10.1111/bph.12953.

Bouwman, B.M., Suffczynski, P., Lopes da Silva, F.H., Maris, E., van Rijn, C.M., 2007a. GABAergic mechanisms in absence epilepsy: a computational model of absence epilepsy simulating spike and wave discharges after vigabatrin in WAG/Rij rats. Eur J Neurosci 25, 2783-2790. 10.1111/j.1460-9568.2007.05533.x.

Bouwman, B.M., Suffczynski, P., Midzyanovskaya, I.S., Maris, E., van den Broek, P.L., van Rijn, C.M., 2007b. The effects of vigabatrin on spike and wave discharges in WAG/Rij rats. Epilepsy Res 76, 34-40. 10.1016/j.eplepsyres.2007.06.006.

Broicher, T., Kanyshkova, T., Meuth, P., Pape, H.C., Budde, T., 2008. Correlation of T-channel coding gene expression, IT, and the low threshold Ca2+ spike in the thalamus of a rat model of absence epilepsy. Mol Cell Neurosci 39, 384-399. 10.1016/j.mcn.2008.07.012.

Budde, T., Caputi, L., Kanyshkova, T., Staak, R., Abrahamczik, C., Munsch, T., Pape, H.C., 2005. Impaired regulation of thalamic pacemaker channels through an imbalance of subunit expression in absence epilepsy. J Neurosci 25, 9871-9882. 10.1523/JNEUROSCI.2590-05.2005.

Burgess, D.L., Noebels, J.L., 1999. Single gene defects in mice: the role of voltage-dependent calcium channels in absence models. Epilepsy Res 36, 111-122.

Buzsaki, G., Bickford, R.G., Ponomareff, G., Thal, L.J., Mandel, R., Gage, F.H., 1988. Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. J Neurosci 8, 4007-4026.

Buzsaki, G., Laszlovszky, I., Lajtha, A., Vadasz, C., 1990. Spike-and-wave neocortical patterns in rats: genetic and aminergic control. Neuroscience 38, 323-333.

Chapman, A.G., Nanan, K., Williams, M., Meldrum, B.S., 2000. 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 (SIB 1893). Neuropharmacology 39, 1567-1574.

Chemin, J., Monteil, A., Perez-Reyes, E., Nargeot, J., Lory, P., 2001. Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. EMBO J 20, 7033-7040. 10.1093/emboj/20.24.7033.

Chugh, D., Ali, I., Bakochi, A., Bahonjic, E., Etholm, L., Ekdahl, C.T., 2015. Alterations in Brain Inflammation, Synaptic Proteins, and Adult Hippocampal Neurogenesis during Epileptogenesis in Mice Lacking Synapsin2. PLoS One 10, e0132366. 10.1371/journal.pone.0132366.

Citraro, R., Aiello, R., Franco, V., De Sarro, G., Russo, E., 2014. Targeting alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors in epilepsy. Expert Opin Ther Targets 18, 319-334. 10.1517/14728222.2014.874416.

Citraro, R., Leo, A., De Fazio, P., De Sarro, G., Russo, E., 2015a. Antidepressants but not antipsychotics have antiepileptogenic effects with limited effects on comorbid depressive-like behaviour in the WAG/Rij rat model of absence epilepsy. Br J Pharmacol 172, 3177-3188. 10.1111/bph.13121.

Citraro, R., Leo, A., Marra, R., De Sarro, G., Russo, E., 2015b. Antiepileptogenic effects of the selective COX-2 inhibitor etoricoxib, on the development of spontaneous absence seizures in WAG/Rij rats. Brain Res Bull 113, 1-7. 10.1016/j.brainresbull.2015.02.004.

Citraro, R., Russo, E., Di Paola, E.D., Ibbadu, G.F., Gratteri, S., Marra, R., De Sarro, G., 2006a. Effects of some neurosteroids injected into some brain areas of WAG/Rij rats, an animal model of generalized absence epilepsy. Neuropharmacology 50, 1059-1071. 10.1016/j.neuropharm.2006.02.011.

Citraro, R., Russo, E., Gratteri, S., Di Paola, E.D., Ibbadu, G.F., Curinga, C., Gitto, R., Chimirri, A., Donato, G., De Sarro, G., 2006b. Effects of non-competitive AMPA receptor antagonists injected into some brain areas of WAG/Rij rats, an animal model of generalized absence epilepsy. Neuropharmacology 51, 1058-1067. 10.1016/j.neuropharm.2006.06.014.

Citraro, R., Russo, E., Ngomba, R.T., Nicoletti, F., Scicchitano, F., Whalley, B.J., Calignano, A., De Sarro, G., 2013a. CB1 agonists, locally applied to the cortico-thalamic circuit of rats

with genetic absence epilepsy, reduce epileptic manifestations. Epilepsy Res 106, 74-82. 10.1016/j.eplepsyres.2013.06.004.

Citraro, R., Russo, E., Scicchitano, F., van Rijn, C.M., Cosco, D., Avagliano, C., Russo, R., D'Agostino, G., Petrosino, S., Guida, F., Gatta, L., van Luijtelaar, G., Maione, S., Di Marzo, V., Calignano, A., De Sarro, G., 2013b. Antiepileptic action of N-palmitoylethanolamine through CB1 and PPAR-alpha receptor activation in a genetic model of absence epilepsy. Neuropharmacology 69, 115-126. 10.1016/j.neuropharm.2012.11.017.

Coenen, A.M., Blezer, E.H., van Luijtelaar, E.L., 1995. Effects of the GABA-uptake inhibitor tiagabine on electroencephalogram, spike-wave discharges and behaviour of rats. Epilepsy Res 21, 89-94.

Coenen, A.M., Van Luijtelaar, E.L., 2003. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. Behav Genet 33, 635-655.

Connors, B.W., 2012. Tales of a dirty drug: carbenoxolone, gap junctions, and seizures. Epilepsy Curr 12, 66-68. 10.5698/1535-7511-12.2.66.

Cools, A.R., Peeters, B.W., 1992. Differences in spike-wave discharges in two rat selection lines characterized by opposite dopaminergic activities. Neurosci Lett 134, 253-256.

Cope, D.W., Di Giovanni, G., Fyson, S.J., Orban, G., Errington, A.C., Lorincz, M.L., Gould, T.M., Carter, D.A., Crunelli, V., 2009. Enhanced tonic GABAA inhibition in typical absence epilepsy. Nat Med 15, 1392-1398. 10.1038/nm.2058.

Coppola, A., Moshe, S.L., 2012. Animal models. Handb Clin Neurol 107, 63-98. 10.1016/B978-0-444-52898-8.00004-5.

Crunelli, V., Leresche, N., 2002. Childhood absence epilepsy: genes, channels, neurons and networks. Nat Rev Neurosci 3, 371-382. 10.1038/nrn811.

D'Amore, V., Raaijmakers, R.H., Santolini, I., van Rijn, C.M., Ngomba, R.T., Nicoletti, F., van Luijtelaar, G., 2016. The anti-absence effect of mGlu5 receptor amplification with

VU0360172 is maintained during and after antiepileptogenesis. Pharmacol Biochem Behav 146-147, 50-59. 10.1016/j.pbb.2016.05.004.

D'Amore, V., Santolini, I., Celli, R., Lionetto, L., De Fusco, A., Simmaco, M., van Rijn, C.M., Vieira, E., Stauffer, S.R., Conn, P.J., Bosco, P., Nicoletti, F., van Luijtelaar, G., Ngomba, R.T., 2014. Head-to head comparison of mGlu1 and mGlu5 receptor activation in chronic treatment of absence epilepsy in WAG/Rij rats. Neuropharmacology 85, 91-103. 10.1016/j.neuropharm.2014.05.005.

D'Amore, V., Santolini, I., van Rijn, C.M., Biagioni, F., Molinaro, G., Prete, A., Conn, P.J., Lindsley, C.W., Zhou, Y., Vinson, P.N., Rodriguez, A.L., Jones, C.K., Stauffer, S.R., Nicoletti, F., van Luijtelaar, G., Ngomba, R.T., 2013. Potentiation of mGlu5 receptors with the novel enhancer, VU0360172, reduces spontaneous absence seizures in WAG/Rij rats. Neuropharmacology 66, 330-338. 10.1016/j.neuropharm.2012.05.044.

D'Amore, V., von Randow, C., Nicoletti, F., Ngomba, R.T., van Luijtelaar, G., 2015. 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 56, 1141-1151. 10.1111/epi.13024.

D'Antuono, M., Inaba, Y., Biagini, G., D'Arcangelo, G., Tancredi, V., Avoli, M., 2006. Synaptic hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures. Genes Brain Behav 5, 73-84. 10.1111/j.1601-183X.2005.00146.x.

D'Arcangelo, G., D'Antuono, M., Biagini, G., Warren, R., Tancredi, V., Avoli, M., 2002. Thalamocortical oscillations in a genetic model of absence seizures. Eur J Neurosci 16, 2383-2393.

David, O., Guillemain, I., Saillet, S., Reyt, S., Deransart, C., Segebarth, C., Depaulis, A., 2008. Identifying neural drivers with functional MRI: an electrophysiological validation. PLoS Biol 6, 2683-2697. 10.1371/journal.pbio.0060315.

de Bruin, N.M., van Luijtelaar, E.L., Jansen, S.J., Cools, A.R., Ellenbroek, B.A., 2000. Dopamine characteristics in different rat genotypes: the relation to absence epilepsy. Neurosci Res 38, 165-173.

Deransart, C., Riban, V., Le, B., Marescaux, C., Depaulis, A., 2000. Dopamine in the striatum modulates seizures in a genetic model of absence epilepsy in the rat. Neuroscience 100, 335-344.

Deransart, C., Vercueil, L., Marescaux, C., Depaulis, A., 1998. The role of basal ganglia in the control of generalized absence seizures. Epilepsy Res 32, 213-223.

Destexhe, A., Sejnowski, T.J., 2003. Interactions between membrane conductances underlying thalamocortical slow-wave oscillations. Physiol Rev 83, 1401-1453. 10.1152/physrev.00012.2003.

Drinkenburg, W.H., Coenen, A.M., Vossen, J.M., Van Luijtelaar, E.L., 1991. Spike-wave discharges and sleep-wake states in rats with absence epilepsy. Epilepsy Res 9, 218-224.

Drinkenburg, W.H., van Luijtelaar, E.L., van Schaijk, W.J., Coenen, A.M., 1993. Aberrant transients in the EEG of epileptic rats: a spectral analytical approach. Physiol Behav 54, 779-783.

Dutuit, M., Didier-Bazes, M., Vergnes, M., Mutin, M., Conjard, A., Akaoka, H., Belin, M.F., Touret, M., 2000. Specific alteration in the expression of glial fibrillary acidic protein, glutamate dehydrogenase, and glutamine synthetase in rats with genetic absence epilepsy. Glia 32, 15-24.

Feddersen, B., Vercueil, L., Noachtar, S., David, O., Depaulis, A., Deransart, C., 2007. Controlling seizures is not controlling epilepsy: a parametric study of deep brain stimulation for epilepsy. Neurobiol Dis 27, 292-300. 10.1016/j.nbd.2007.05.005.

Fezza, F., Marrone, M.C., Avvisati, R., Di Tommaso, M., Lanuti, M., Rapino, C., Mercuri, N.B., Maccarrone, M., Marinelli, S., 2014. Distinct modulation of the endocannabinoid

system upon kainic acid-induced in vivo seizures and in vitro epileptiform bursting. Mol Cell Neurosci 62, 1-9. 10.1016/j.mcn.2014.07.003.

Filakovszky, J., Gerber, K., Bagdy, G., 1999. A serotonin-1A receptor agonist and an N-methyl-D-aspartate receptor antagonist oppose each others effects in a genetic rat epilepsy model. Neurosci Lett 261, 89-92.

Filakovszky, J., Kantor, S., Halasz, P., Bagdy, G., 2001. 8-OH-DPAT and MK-801 affect epileptic activity independently of vigilance. Neurochem Int 38, 551-556.

Gareri, P., Condorelli, D., Belluardo, N., Citraro, R., Barresi, V., Trovato-Salinaro, A., Mudo, G., Ibbadu, G.F., Russo, E., De Sarro, G., 2005. Antiabsence effects of carbenoxolone in two genetic animal models of absence epilepsy (WAG/Rij rats and lh/lh mice). Neuropharmacology 49, 551-563. 10.1016/j.neuropharm.2005.04.012.

Gauguier, D., van Luijtelaar, G., Bihoreau, M.T., Wilder, S.P., Godfrey, R.F., Vossen, J., Coenen, A., Cox, R.D., 2004. Chromosomal mapping of genetic loci controlling absence epilepsy phenotypes in the WAG/Rij rat. Epilepsia 45, 908-915. 10.1111/j.0013-9580.2004.13104.x.

Gerber, K., Filakovszky, J., Halasz, P., Bagdy, G., 1998. The 5-HT1A agonist 8-OH-DPAT increases the number of spike-wave discharges in a genetic rat model of absence epilepsy. Brain Res 807, 243-245.

Giblin, K.A., Blumenfeld, H., 2010. Is epilepsy a preventable disorder? New evidence from animal models. Neuroscientist 16, 253-275. 10.1177/1073858409354385.

Glauser, T., Ben-Menachem, E., Bourgeois, B., Cnaan, A., Guerreiro, C., Kalviainen, R., Mattson, R., French, J.A., Perucca, E., Tomson, T., Guidelines, I.S.o.A., 2013. Updated ILAE evidence review of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. Epilepsia 54, 551-563. 10.1111/epi.12074.

Godlewski, G., Alapafuja, S.O., Batkai, S., Nikas, S.P., Cinar, R., Offertaler, L., Osei-Hyiaman, D., Liu, J., Mukhopadhyay, B., Harvey-White, J., Tam, J., Pacak, K., Blankman, J.L., Cravatt, B.F., Makriyannis, A., Kunos, G., 2010. Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. Chem Biol 17, 1256-1266. 10.1016/j.chembiol.2010.08.013.

Goldberg, E.M., Coulter, D.A., 2013. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. Nat Rev Neurosci 14, 337-349. 10.1038/nrn3482.

Gorji, A., Mittag, C., Shahabi, P., Seidenbecher, T., Pape, H.C., 2011. Seizure-related activity of intralaminar thalamic neurons in a genetic model of absence epilepsy. Neurobiol Dis 43, 266-274. 10.1016/j.nbd.2011.03.019.

Graf, M., Jakus, R., Kantor, S., Levay, G., Bagdy, G., 2004. Selective 5-HT1A and 5-HT7 antagonists decrease epileptic activity in the WAG/Rij rat model of absence epilepsy. Neurosci Lett 359, 45-48. 10.1016/j.neulet.2004.01.072.

Groenewegen, H.J., Galis-de Graaf, Y., Smeets, W.J., 1999. Integration and segregation of limbic cortico-striatal loops at the thalamic level: an experimental tracing study in rats. J Chem Neuroanat 16, 167-185.

Guerrini, R., Marini, C., Mantegazza, M., 2014. Genetic epilepsy syndromes without structural brain abnormalities: clinical features and experimental models. Neurotherapeutics 11, 269-285. 10.1007/s13311-014-0267-0.

Hirose, S., 2014. Mutant GABA(A) receptor subunits in genetic (idiopathic) epilepsy. Prog Brain Res 213, 55-85. 10.1016/B978-0-444-63326-2.00003-X.

Holmes, M.D., Brown, M., Tucker, D.M., 2004. Are "generalized" seizures truly generalized? Evidence of localized mesial frontal and frontopolar discharges in absence. Epilepsia 45, 1568-1579. 10.1111/j.0013-9580.2004.23204.x.

Hosford, D.A., Wang, Y., Cao, Z., 1997. Differential effects mediated by GABAA receptors in thalamic nuclei in lh/lh model of absence seizures. Epilepsy Res 27, 55-65.

Huguenard, J.R., McCormick, D.A., 2007. Thalamic synchrony and dynamic regulation of global forebrain oscillations. Trends Neurosci 30, 350-356. 10.1016/j.tins.2007.05.007.

Inaba, Y., D'Antuono, M., Bertazzoni, G., Biagini, G., Avoli, M., 2009. Diminished presynaptic GABA(B) receptor function in the neocortex of a genetic model of absence epilepsy. Neurosignals 17, 121-131. 10.1159/000197864.

Jakus, R., Graf, M., Ando, R.D., Balogh, B., Gacsalyi, I., Levay, G., Kantor, S., Bagdy, G., 2004. Effect of two noncompetitive AMPA receptor antagonists GYKI 52466 and GYKI 53405 on vigilance, behavior and spike-wave discharges in a genetic rat model of absence epilepsy. Brain Res 1008, 236-244. 10.1016/j.brainres.2004.01.087.

Jakus, R., Graf, M., Juhasz, G., Gerber, K., Levay, G., Halasz, P., Bagdy, G., 2003. 5-HT2C receptors inhibit and 5-HT1A receptors activate the generation of spike-wave discharges in a genetic rat model of absence epilepsy. Exp Neurol 184, 964-972. 10.1016/S0014-4886(03)00352-2.

Jourquin, J., Tremblay, E., Decanis, N., Charton, G., Hanessian, S., Chollet, A.M., Le Diguardher, T., Khrestchatisky, M., Rivera, S., 2003. Neuronal activity-dependent increase of net matrix metalloproteinase activity is associated with MMP-9 neurotoxicity after kainate. Eur J Neurosci 18, 1507-1517.

Kaminski, R.M., Van Rijn, C.M., Turski, W.A., Czuczwar, S.J., Van Luijtelaar, G., 2001. AMPA and GABA(B) receptor antagonists and their interaction in rats with a genetic form of absence epilepsy. Eur J Pharmacol 430, 251-259.

Kaminski, R.M., Zielinska, E., Dekundy, A., van Luijtelaar, G., Turski, W., 2003. Deficit of endogenous kynurenic acid in the frontal cortex of rats with a genetic form of absence epilepsy. Pol J Pharmacol 55, 741-746.

Kanyshkova, T., Ehling, P., Cerina, M., Meuth, P., Zobeiri, M., Meuth, S.G., Pape, H.C., Budde, T., 2014. Regionally specific expression of high-voltage-activated calcium channels in thalamic nuclei of epileptic and non-epileptic rats. Mol Cell Neurosci 61, 110-122. 10.1016/j.mcn.2014.06.005.

Kanyshkova, T., Meuth, P., Bista, P., Liu, Z., Ehling, P., Caputi, L., Doengi, M., Chetkovich, D.M., Pape, H.C., Budde, T., 2012. Differential regulation of HCN channel isoform expression in thalamic neurons of epileptic and non-epileptic rat strains. Neurobiol Dis 45, 450-461. 10.1016/j.nbd.2011.08.032.

Karimzadeh, F., Soleimani, M., Mehdizadeh, M., Jafarian, M., Mohamadpour, M., Kazemi, H., Joghataei, M.T., Gorji, A., 2013. Diminution of the NMDA receptor NR2B subunit in cortical and subcortical areas of WAG/Rij rats. Synapse 67, 839-846. 10.1002/syn.21687.

Klee, R., Tollner, K., Rankovic, V., Romermann, K., Schidlitzki, A., Bankstahl, M., Loscher, W., 2015. Network pharmacology for antiepileptogenesis: Tolerability of multitargeted drug combinations in nonepileptic vs. post-status epilepticus mice. Epilepsy Res 118, 34-48. 10.1016/j.eplepsyres.2015.11.003.

Klein, J.P., Khera, D.S., Nersesyan, H., Kimchi, E.Y., Waxman, S.G., Blumenfeld, H., 2004. Dysregulation of sodium channel expression in cortical neurons in a rodent model of absence epilepsy. Brain Res 1000, 102-109. 10.1016/j.brainres.2003.11.051.

Kobow, K., Auvin, S., Jensen, F., Loscher, W., Mody, I., Potschka, H., Prince, D., Sierra, A., Simonato, M., Pitkanen, A., Nehlig, A., Rho, J.M., 2012. Finding a better drug for epilepsy: antiepileptogenesis targets. Epilepsia 53, 1868-1876. 10.1111/j.1528-1167.2012.03716.x.

Koerner, C., Danober, L., Boehrer, A., Marescaux, C., Vergnes, M., 1996. Thalamic NMDA transmission in a genetic model of absence epilepsy in rats. Epilepsy Res 25, 11-19.

Kole, M.H., Brauer, A.U., Stuart, G.J., 2007. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. J Physiol 578, 507-525. 10.1113/jphysiol.2006.122028.

Kovacs, Z., Czurko, A., Kekesi, K.A., Juhasz, G., 2011. Intracerebroventricularly administered lipopolysaccharide enhances spike-wave discharges in freely moving WAG/Rij rats. Brain Res Bull 85, 410-416. 10.1016/j.brainresbull.2011.05.003.

Kovacs, Z., Dobolyi, A., Juhasz, G., Kekesi, K.A., 2014. Lipopolysaccharide induced increase in seizure activity in two animal models of absence epilepsy WAG/Rij and GAERS rats and Long Evans rats. Brain Res Bull 104, 7-18. 10.1016/j.brainresbull.2014.03.003.

Kovacs, Z., Kekesi, K.A., Szilagyi, N., Abraham, I., Szekacs, D., Kiraly, N., Papp, E., Csaszar, I., Szego, E., Barabas, K., Peterfy, H., Erdei, A., Bartfai, T., Juhasz, G., 2006. Facilitation of spike-wave discharge activity by lipopolysaccharides in Wistar Albino Glaxo/Rijswijk rats. Neuroscience 140, 731-742. 10.1016/j.neuroscience.2006.02.023.

Kuisle, M., Wanaverbecq, N., Brewster, A.L., Frere, S.G., Pinault, D., Baram, T.Z., Luthi, A., 2006. Functional stabilization of weakened thalamic pacemaker channel regulation in rat absence epilepsy. J Physiol 575, 83-100. 10.1113/jphysiol.2006.110486.

Kuznetsova, G.D., Petrova, E.V., Coenen, A.M., Van Luijtelaar, E.L., 1996. Generalized absence epilepsy and catalepsy in rats. Physiol Behav 60, 1165-1169.

Lason, W., Przewlocka, B., Coenen, A., Przewlocki, R., Van Luijtelaar, G., 1994a. Effects of mu and delta opioid receptor agonists and antagonists on absence epilepsy in WAG/Rij rats. Neuropharmacology 33, 161-166.

Lason, W., Przewlocka, B., Van Luijtelaar, E.L., Coenen, A.M., Przewlocki, R., 1992. Endogenous opioid peptides in brain and pituitary of rats with absence epilepsy. Neuropeptides 21, 147-152.

Lason, W., Przewlocka, B., Van Luijtelaar, G., Coenen, A., 1994b. Proenkephalin and prodynorphin mRNA level in brain of rats with absence epilepsy. Neuropeptides 27, 343-347.

Liautard, C., Scalmani, P., Carriero, G., de Curtis, M., Franceschetti, S., Mantegazza, M., 2013. Hippocampal hyperexcitability and specific epileptiform activity in a mouse model of Dravet syndrome. Epilepsia 54, 1251-1261. 10.1111/epi.12213.

Lillis, K.P., Wang, Z., Mail, M., Zhao, G.Q., Berdichevsky, Y., Bacskai, B., Staley, K.J., 2015. Evolution of Network Synchronization during Early Epileptogenesis Parallels Synaptic Circuit Alterations. J Neurosci 35, 9920-9934. 10.1523/JNEUROSCI.4007-14.2015.

Liu, X.B., Coble, J., van Luijtelaar, G., Jones, E.G., 2007. Reticular nucleus-specific changes in alpha3 subunit protein at GABA synapses in genetically epilepsy-prone rats. Proc Natl Acad Sci U S A 104, 12512-12517. 10.1073/pnas.0705320104.

Liu, Z., Vergnes, M., Depaulis, A., Marescaux, C., 1991. Evidence for a critical role of GABAergic transmission within the thalamus in the genesis and control of absence seizures in the rat. Brain Res 545, 1-7.

Luhmann, H.J., Mittmann, T., van Luijtelaar, G., Heinemann, U., 1995. Impairment of intracortical GABAergic inhibition in a rat model of absence epilepsy. Epilepsy Res 22, 43-51.

Luttjohann, A., Schoffelen, J.M., van Luijtelaar, G., 2013. Peri-ictal network dynamics of spike-wave discharges: phase and spectral characteristics. Exp Neurol 239, 235-247. 10.1016/j.expneurol.2012.10.021.

Luttjohann, A., van Luijtelaar, G., 2012. The dynamics of cortico-thalamo-cortical interactions at the transition from pre-ictal to ictal LFPs in absence epilepsy. Neurobiol Dis 47, 49-60. 10.1016/j.nbd.2012.03.023.

Luttjohann, A., van Luijtelaar, G., 2015. Dynamics of networks during absence seizure's on- and offset in rodents and man. Front Physiol 6, 16. 10.3389/fphys.2015.00016.

Luttjohann, A., Zhang, S., de Peijper, R., van Luijtelaar, G., 2011. Electrical stimulation of the epileptic focus in absence epileptic WAG/Rij rats: assessment of local and network excitability. Neuroscience 188, 125-134. 10.1016/j.neuroscience.2011.04.038.

Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., Watson, S.J., 1987. Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. J Neurosci 7, 2445-2464.

Marescaux, C., Vergnes, M., Depaulis, A., 1992a. Neurotransmission in rats' spontaneous generalized nonconvulsive epilepsy. Epilepsy Res Suppl 8, 335-343.

Marescaux, C., Vergnes, M., Liu, Z., Depaulis, A., Bernasconi, R., 1992b. GABAB receptor involvement in the control of genetic absence seizures in rats. Epilepsy Res Suppl 9, 131-138; discussion 138-139.

Marguet, S.L., Le-Schulte, V.T., Merseburg, A., Neu, A., Eichler, R., Jakovcevski, I., Ivanov, A., Hanganu-Opatz, I.L., Bernard, C., Morellini, F., Isbrandt, D., 2015. Treatment during a vulnerable developmental period rescues a genetic epilepsy. Nat Med 21, 1436-1444. 10.1038/nm.3987.

McCormick, D.A., von Krosigk, M., 1992. Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. Proc Natl Acad Sci U S A 89, 2774-2778.

Meeren, H.K., Pijn, J.P., Van Luijtelaar, E.L., Coenen, A.M., Lopes da Silva, F.H., 2002. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J Neurosci 22, 1480-1495.

Merlo, D., Mollinari, C., Inaba, Y., Cardinale, A., Rinaldi, A.M., D'Antuono, M., D'Arcangelo, G., Tancredi, V., Ragsdale, D., Avoli, M., 2007. Reduced GABAB receptor subunit expression and paired-pulse depression in a genetic model of absence seizures. Neurobiol Dis 25, 631-641. 10.1016/j.nbd.2006.11.005.

Micheletti, G., Warter, J.M., Marescaux, C., Depaulis, A., Tranchant, C., Rumbach, L., Vergnes, M., 1987. Effects of drugs affecting noradrenergic neurotransmission in rats with spontaneous petit mal-like seizures. Eur J Pharmacol 135, 397-402.

Midzianovskaia, I.S., Kuznetsova, G.D., Coenen, A.M., Spiridonov, A.M., van Luijtelaar, E.L., 2001. Electrophysiological and pharmacological characteristics of two types of spike-wave discharges in WAG/Rij rats. Brain Res 911, 62-70.

Midzyanovskaya, I.S., Kuznetsova, G.D., van Luijtelaar, E.L., van Rijn, C.M., Tuomisto, L., Macdonald, E., 2006. The brain 5HTergic response to an acute sound stress in rats with generalized (absence and audiogenic) epilepsy. Brain Res Bull 69, 631-638. 10.1016/j.brainresbull.2006.03.008.

Moeller, F., Siebner, H.R., Wolff, S., Muhle, H., Granert, O., Jansen, O., Stephani, U., Siniatchkin, M., 2008. Simultaneous EEG-fMRI in drug-naive children with newly diagnosed absence epilepsy. Epilepsia 49, 1510-1519. 10.1111/j.1528-1167.2008.01626.x.

Morimoto, K., Fahnestock, M., Racine, R.J., 2004. Kindling and status epilepticus models of epilepsy: rewiring the brain. Prog Neurobiol 73, 1-60. 10.1016/j.pneurobio.2004.03.009.

Nagy, J.I., Rash, J.E., 2000. Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS. Brain Res Brain Res Rev 32, 29-44.

Nersesyan, H., Hyder, F., Rothman, D.L., Blumenfeld, H., 2004. Dynamic fMRI and EEG recordings during spike-wave seizures and generalized tonic-clonic seizures in WAG/Rij rats. J Cereb Blood Flow Metab 24, 589-599. 10.1097/01.WCB.0000117688.98763.23.

Ngomba, R.T., Biagioni, F., Casciato, S., Willems-van Bree, E., Battaglia, G., Bruno, V., Nicoletti, F., van Luijtelaar, E.L., 2005. The preferential mGlu2/3 receptor antagonist, LY341495, reduces the frequency of spike-wave discharges in the WAG/Rij rat model of absence epilepsy. Neuropharmacology 49 Suppl 1, 89-103. 10.1016/j.neuropharm.2005.05.019.

Ngomba, R.T., Ferraguti, F., Badura, A., Citraro, R., Santolini, I., Battaglia, G., Bruno, V., De Sarro, G., Simonyi, A., van Luijtelaar, G., Nicoletti, F., 2008. Positive allosteric modulation of metabotropic glutamate 4 (mGlu4) receptors enhances spontaneous and evoked absence seizures. Neuropharmacology 54, 344-354. 10.1016/j.neuropharm.2007.10.004.

Ngomba, R.T., Santolini, I., Biagioni, F., Molinaro, G., Simonyi, A., van Rijn, C.M., D'Amore, V., Mastroiacovo, F., Olivieri, G., Gradini, R., Ferraguti, F., Battaglia, G., Bruno, V., Puliti, A., van Luijtelaar, G., Nicoletti, F., 2011a. Protective role for type-1 metabotropic glutamate receptors against spike and wave discharges in the WAG/Rij rat model of absence epilepsy. Neuropharmacology 60, 1281-1291. 10.1016/j.neuropharm.2011.01.007.

Ngomba, R.T., Santolini, I., Salt, T.E., Ferraguti, F., Battaglia, G., Nicoletti, F., van Luijtelaar, G., 2011b. Metabotropic glutamate receptors in the thalamocortical network: strategic targets for the treatment of absence epilepsy. Epilepsia 52, 1211-1222. 10.1111/j.1528-1167.2011.03082.x.

Nicoletti, F., Bruno, V., Ngomba, R.T., Gradini, R., Battaglia, G., 2015. Metabotropic glutamate receptors as drug targets: what's new? Curr Opin Pharmacol 20, 89-94. 10.1016/j.coph.2014.12.002.

Ogawa, S., Kunugi, H., 2015. Inhibitors of Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase: New Targets for Future Antidepressants. Curr Neuropharmacol 13, 760-775.

Pape, H.C., McCormick, D.A., 1989. Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. Nature 340, 715-718. 10.1038/340715a0.

Patsalos, P.N., 2005. Properties of antiepileptic drugs in the treatment of idiopathic generalized epilepsies. Epilepsia 46 Suppl 9, 140-148. 10.1111/j.1528-1167.2005.00326.x.

Peeters, B.W., Kerbusch, J.M., Coenen, A.M., Vossen, J.M., van Luijtelaar, E.L., 1992. Genetics of spike-wave discharges in the electroencephalogram (EEG) of the WAG/Rij inbred rat strain: a classical mendelian crossbreeding study. Behav Genet 22, 361-368.

Peeters, B.W., Ramakers, G.M., Ellenbroek, B.A., Vossen, J.M., Coenen, A.M., 1994a. Interactions between NMDA and nonNMDA receptors in nonconvulsive epilepsy in the WAG/Rij inbred strain. Brain Res Bull 33, 715-718.

Peeters, B.W., Ramakers, G.M., Vossen, J.M., Coenen, A.M., 1994b. The WAG/Rij rat model for nonconvulsive absence epilepsy: involvement of nonNMDA receptors. Brain Res Bull 33, 709-713.

Peeters, B.W., Van Rijn, C.M., Nutt, D.J., Titulaer, M.N., Vossen, J.M., Coenen, A.M., 1990a. Diazepam and Ro 15-1788 increase absence epilepsy in WAG/Rij rats chronically exposed to diazepam. Eur J Pharmacol 178, 111-114.

Peeters, B.W., Van Rijn, C.M., Van Luijtelaar, E.L., Coenen, A.M., 1989a. Antiepileptic and behavioural actions of MK-801 in an animal model of spontaneous absence epilepsy. Epilepsy Res 3, 178-181.

Peeters, B.W., van Rijn, C.M., Vossen, J.M., Coenen, A.M., 1989b. Effects of GABA-ergic agents on spontaneous non-convulsive epilepsy, EEG and behaviour, in the WAG/RIJ inbred strain of rats. Life Sci 45, 1171-1176.

Peeters, B.W., van Rijn, C.M., Vossen, J.M., Coenen, A.M., 1990b. Involvement of NMDA receptors in non-convulsive epilepsy in WAG/Rij rats. Life Sci 47, 523-529.

Pinault, D., O'Brien, T.J., 2005. Cellular and network mechanisms of genetically-determined absence seizures. Thalamus Relat Syst 3, 181-203. 10.1017/S1472928807000209.

Pisu, M.G., Mostallino, M.C., Dore, R., Mura, M.L., Maciocco, E., Russo, E., De Sarro, G., Serra, M., 2008. Neuroactive steroids and GABAA receptor plasticity in the brain of the

WAG/Rij rat, a model of absence epilepsy. J Neurochem 106, 2502-2514. 10.1111/j.1471-4159.2008.05538.x.

Pitkanen, A., Engel, J., Jr., 2014. Past and present definitions of epileptogenesis and its biomarkers. Neurotherapeutics 11, 231-241. 10.1007/s13311-014-0257-2.

Pitkanen, A., Lukasiuk, K., 2011. Mechanisms of epileptogenesis and potential treatment targets. Lancet Neurol 10, 173-186. 10.1016/S1474-4422(10)70310-0.

Pitkanen, A., Nehlig, A., Brooks-Kayal, A.R., Dudek, F.E., Friedman, D., Galanopoulou, A.S., Jensen, F.E., Kaminski, R.M., Kapur, J., Klitgaard, H., Loscher, W., Mody, I., Schmidt, D., 2013. Issues related to development of antiepileptogenic therapies. Epilepsia 54 Suppl 4, 35-43. 10.1111/epi.12297.

Polack, P.O., Guillemain, I., Hu, E., Deransart, C., Depaulis, A., Charpier, S., 2007. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. J Neurosci 27, 6590-6599. 10.1523/JNEUROSCI.0753-07.2007.

Polack, P.O., Mahon, S., Chavez, M., Charpier, S., 2009. Inactivation of the somatosensory cortex prevents paroxysmal oscillations in cortical and related thalamic neurons in a genetic model of absence epilepsy. Cereb Cortex 19, 2078-2091. 10.1093/cercor/bhn237.

Przewlocka, B., Lason, W., Machelska, H., van Luijtelaar, G., Coenen, A., Przewlocki, R., 1995. Kappa opioid receptor agonists suppress absence seizures in WAG/Rij rats. Neurosci Lett 186, 131-134.

Przewlocka, B., Lason, W., Turchan, J., de Bruin, N., van Luijtelaar, G., Przewlocki, R., Coenen, A., 1998. Anatomical and functional aspects of mu opioid receptors in epileptic WAG/Rij rats. Epilepsy Res 29, 167-173.

Rimoli, M.G., Russo, E., Cataldi, M., Citraro, R., Ambrosino, P., Melisi, D., Curcio, A., De Lucia, S., Patrignani, P., De Sarro, G., Abignente, E., 2009. T-type channel blocking properties and antiabsence activity of two imidazo[1,2-b]pyridazine derivatives structurally

related to indomethacin. Neuropharmacology 56, 637-646. 10.1016/j.neuropharm.2008.11.003.

Roopra, A., Dingledine, R., Hsieh, J., 2012. Epigenetics and epilepsy. Epilepsia 53 Suppl 9, 2-10. 10.1111/epi.12030.

Russo, E., Andreozzi, F., Iuliano, R., Dattilo, V., Procopio, T., Fiume, G., Mimmi, S., Perrotti, N., Citraro, R., Sesti, G., Constanti, A., De Sarro, G., 2014a. Early molecular and behavioral response to lipopolysaccharide in the WAG/Rij rat model of absence epilepsy and depressive-like behavior, involves interplay between AMPK, AKT/mTOR pathways and neuroinflammatory cytokine release. Brain Behav Immun 42, 157-168. 10.1016/j.bbi.2014.06.016.

Russo, E., Citraro, R., Constanti, A., De Sarro, G., 2012. The mTOR signaling pathway in the brain: focus on epilepsy and epileptogenesis. Mol Neurobiol 46, 662-681. 10.1007/s12035-012-8314-5.

Russo, E., Citraro, R., Davoli, A., Gallelli, L., Di Paola, E.D., De Sarro, G., 2013a. Ameliorating effects of aripiprazole on cognitive functions and depressive-like behavior in a genetic rat model of absence epilepsy and mild-depression comorbidity. Neuropharmacology 64, 371-379. 10.1016/j.neuropharm.2012.06.039.

Russo, E., Citraro, R., De Fazio, S., Marra, R., Gitto, R., Chimirri, A., De Sarro, G., Di Paola, E.D., 2008. Enhancement of anti-absence effects of ethosuximide by low doses of a noncompetitive alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist in a genetic animal model of absence epilepsy. Epilepsy Behav 13, 295-299. 10.1016/j.yebeh.2008.04.018.

Russo, E., Citraro, R., Donato, G., Camastra, C., Iuliano, R., Cuzzocrea, S., Constanti, A., De Sarro, G., 2013b. mTOR inhibition modulates epileptogenesis, seizures and depressive

behavior in a genetic rat model of absence epilepsy. Neuropharmacology 69, 25-36. 10.1016/j.neuropharm.2012.09.019.

Russo, E., Citraro, R., Scicchitano, F., De Fazio, S., Perrotta, I., Di Paola, E.D., Constanti, A., De Sarro, G., 2011a. Effects of early long-term treatment with antiepileptic drugs on development of seizures and depressive-like behavior in a rat genetic absence epilepsy model. Epilepsia 52, 1341-1350. 10.1111/j.1528-1167.2011.03112.x.

Russo, E., Citraro, R., Scicchitano, F., Urzino, A., Marra, R., Rispoli, V., De Sarro, G., 2011b. Vigabatrin has antiepileptogenic and antidepressant effects in an animal model of epilepsy and depression comorbidity. Behav Brain Res 225, 373-376. 10.1016/j.bbr.2011.07.030.

Russo, E., Follesa, P., Citraro, R., Camastra, C., Donato, A., Isola, D., Constanti, A., De Sarro, G., Donato, G., 2014b. The mTOR signaling pathway and neuronal stem/progenitor cell proliferation in the hippocampus are altered during the development of absence epilepsy in a genetic animal model. Neurol Sci 35, 1793-1799. 10.1007/s10072-014-1842-1.

Salt, T.E., Binns, K.E., 2000. Contributions of mGlu1 and mGlu5 receptors to interactions with N-methyl-D-aspartate receptor-mediated responses and nociceptive sensory responses of rat thalamic neurons. Neuroscience 100, 375-380.

Sander, T., Berlin, W., Ostapowicz, A., Samochowiec, J., Gscheidel, N., Hoehe, M.R., 2000. Variation of the genes encoding the human glutamate EAAT2, serotonin and dopamine transporters and Susceptibility to idiopathic generalized epilepsy. Epilepsy Res 41, 75-81.

Sarkisova, K., van Luijtelaar, G., 2011. The WAG/Rij strain: a genetic animal model of absence epilepsy with comorbidity of depression [corrected]. Prog Neuropsychopharmacol Biol Psychiatry 35, 854-876. 10.1016/j.pnpbp.2010.11.010.

Sarkisova, K.Y., Kulikov, M.A., Midzyanovskaya, I.S., Folomkina, A.A., 2008. Dopamine-dependent nature of depression-like behavior in WAG/Rij rats with genetic absence epilepsy. Neurosci Behav Physiol 38, 119-128. 10.1007/s11055-008-0017-z.

Sarkisova, K.Y., Kuznetsova, G.D., Kulikov, M.A., van Luijtelaar, G., 2010. Spike-wave discharges are necessary for the expression of behavioral depression-like symptoms. Epilepsia 51, 146-160. 10.1111/j.1528-1167.2009.02260.x.

Schridde, U., Strauss, U., Brauer, A.U., van Luijtelaar, G., 2006. Environmental manipulations early in development alter seizure activity, Ih and HCN1 protein expression later in life. Eur J Neurosci 23, 3346-3358. 10.1111/j.1460-9568.2006.04865.x.

Schridde, U., van Luijtelaar, G., 2004. The influence of strain and housing on two types of spike-wave discharges in rats. Genes Brain Behav 3, 1-7.

Scicchitano, F., van Rijn, C.M., van Luijtelaar, G., 2015. Unilateral and Bilateral Cortical Resection: Effects on Spike-Wave Discharges in a Genetic Absence Epilepsy Model. PLoS One 10, e0133594. 10.1371/journal.pone.0133594.

Seidenbecher, T., Pape, H.C., 2001. Contribution of intralaminar thalamic nuclei to spike-and-wave-discharges during spontaneous seizures in a genetic rat model of absence epilepsy. Eur J Neurosci 13, 1537-1546.

Seidenbecher, T., Staak, R., Pape, H.C., 1998. Relations between cortical and thalamic cellular activities during absence seizures in rats. Eur J Neurosci 10, 1103-1112.

Shigemoto, R., Nakanishi, S., Mizuno, N., 1992. 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 322, 121-135. 10.1002/cne.903220110.

Sitnikova, E., Kulikova, S., Birioukova, L., Raevsky, V.V., 2011. Cellular neuropathology of absence epilepsy in the neocortex: a population of glial cells rather than neurons is impaired in genetic rat model. Acta Neurobiol Exp (Wars) 71, 263-268.

Sitnikova, E., van Luijtelaar, G., 2005. Reduction of adrenergic neurotransmission with clonidine aggravates spike-wave seizures and alters activity in the cortex and the thalamus in WAG/Rij rats. Brain Res Bull 64, 533-540. 10.1016/j.brainresbull.2004.11.004.

Smyk, M.K., Coenen, A., Lewandowski, M.H., van Luijtelaar, G., 2012. Internal desynchronization facilitates seizures. Epilepsia 53, 1511-1518. 10.1111/j.1528-1167.2012.03577.x.

Snead, O.C., 3rd, 1992. Evidence for GABAB-mediated mechanisms in experimental generalized absence seizures. Eur J Pharmacol 213, 343-349.

Snead, O.C., 3rd, 1995. Basic mechanisms of generalized absence seizures. Ann Neurol 37, 146-157. 10.1002/ana.410370204.

Snead, O.C., 3rd, Depaulis, A., Banerjee, P.K., Hechler, V., Vergnes, M., 1992. The GABAA receptor complex in experimental absence seizures in rat: an autoradiographic study. Neurosci Lett 140, 9-12.

Staak, R., Pape, H.C., 2001. Contribution of GABA(A) and GABA(B) receptors to thalamic neuronal activity during spontaneous absence seizures in rats. J Neurosci 21, 1378-1384.

Staley, K., Hellier, J.L., Dudek, F.E., 2005. Do interictal spikes drive epileptogenesis? Neuroscientist 11, 272-276. 10.1177/1073858405278239.

Staley, K.J., Dudek, F.E., 2006. Interictal spikes and epileptogenesis. Epilepsy Curr 6, 199-202. 10.1111/j.1535-7511.2006.00145.x.

Steriade, M., 1998. Corticothalamic networks, oscillations, and plasticity. Adv Neurol 77, 105-134.

Steriade, M., McCormick, D.A., Sejnowski, T.J., 1993. Thalamocortical oscillations in the sleeping and aroused brain. Science 262, 679-685.

Stinehelfer, S., Vruwink, M., Burette, A., 2000. Immunolocalization of mGluR1alpha in specific populations of local circuit neurons in the cerebral cortex. Brain Res 861, 37-44.

Strauss, U., Kole, M.H., Brauer, A.U., Pahnke, J., Bajorat, R., Rolfs, A., Nitsch, R., Deisz, R.A., 2004. An impaired neocortical Ih is associated with enhanced excitability and absence epilepsy. Eur J Neurosci 19, 3048-3058. 10.1111/j.0953-816X.2004.03392.x.

Takacs, E., Nyilas, R., Szepesi, Z., Baracskay, P., Karlsen, B., Rosvold, T., Bjorkum, A.A., Czurko, A., Kovacs, Z., Kekesi, A.K., Juhasz, G., 2010. Matrix metalloproteinase-9 activity increased by two different types of epileptic seizures that do not induce neuronal death: a possible role in homeostatic synaptic plasticity. Neurochem Int 56, 799-809. 10.1016/j.neuint.2010.03.003.

Tolmacheva, E.A., van Luijtelaar, G., 2007. Absence seizures are reduced by the enhancement of GABA-ergic inhibition in the hippocampus in WAG/Rij rats. Neurosci Lett 416, 17-21. 10.1016/j.neulet.2007.01.038.

Trinka, E., Brigo, F., 2014. Antiepileptogenesis in humans: disappointing clinical evidence and ways to move forward. Curr Opin Neurol 27, 227-235. 10.1097/WCO.0000000000000067.

Tsakiridou, E., Bertollini, L., de Curtis, M., Avanzini, G., Pape, H.C., 1995. Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. J Neurosci 15, 3110-3117.

van de Bovenkamp-Janssen, M.C., Scheenen, W.J., Kuijpers-Kwant, F.J., Kozicz, T., Veening, J.G., van Luijtelaar, E.L., McEnery, M.W., Roubos, E.W., 2004. Differential expression of high voltage-activated Ca2+ channel types in the rostral reticular thalamic nucleus of the absence epileptic WAG/Rij rat. J Neurobiol 58, 467-478. 10.1002/neu.10291.

van de Bovenkamp-Janssen, M.C., van der Kloet, J.C., van Luijtelaar, G., Roubos, E.W., 2006. NMDA-NR1 and AMPA-GluR4 receptor subunit immunoreactivities in the absence epileptic WAG/Rij rat. Epilepsy Res 69, 119-128. 10.1016/j.eplepsyres.2006.01.003.

van Luijtelaar, E.L., Coenen, A.M., 1986. Two types of electrocortical paroxysms in an inbred strain of rats. Neurosci Lett 70, 393-397.

van Luijtelaar, G., 2011. The prevention of behavioral consequences of idiopathic generalized epilepsy: evidence from rodent models. Neurosci Lett 497, 177-184. 10.1016/j.neulet.2011.02.034.

van Luijtelaar, G., Lyashenko, S., Vastyanov, R., Verbeek, G., Oleinik, A., van Rijn, C., Volokhova, G., Shandra, A., Coenen, A., Godlevsky, L., 2012. Cytokines and Absence Seizures in a Genetic Rat Model. Neurophysiology 43, 478-486. 10.1007/s11062-012-9252-6.

van Luijtelaar, G., Mishra, A.M., Edelbroek, P., Coman, D., Frankenmolen, N., Schaapsmeerders, P., Covolato, G., Danielson, N., Niermann, H., Janeczko, K., Kiemeneij, A., Burinov, J., Bashyal, C., Coquillette, M., Luttjohann, A., Hyder, F., Blumenfeld, H., van Rijn, C.M., 2013. Anti-epileptogenesis: Electrophysiology, diffusion tensor imaging and behavior in a genetic absence model. Neurobiol Dis 60, 126-138. 10.1016/j.nbd.2013.08.013.

van Luijtelaar, G., Onat, F.Y., Gallagher, M.J., 2014. Animal models of absence epilepsies: what do they model and do sex and sex hormones matter? Neurobiol Dis 72 Pt B, 167-179. 10.1016/j.nbd.2014.08.014.

van Luijtelaar, G., Sitnikova, E., 2006. Global and focal aspects of absence epilepsy: the contribution of genetic models. Neurosci Biobehav Rev 30, 983-1003. 10.1016/j.neubiorev.2006.03.002.

van Luijtelaar, G., Zobeiri, M., 2014. Progress and outlooks in a genetic absence epilepsy model (WAG/Rij). Curr Med Chem 21, 704-721.

van Rijn, C.M., Gaetani, S., Santolini, I., Badura, A., Gabova, A., Fu, J., Watanabe, M., Cuomo, V., van Luijtelaar, G., Nicoletti, F., Ngomba, R.T., 2010. WAG/Rij rats show a reduced expression of CB(1) receptors in thalamic nuclei and respond to the CB(1) receptor agonist, R(+)WIN55,212-2, with a reduced incidence of spike-wave discharges. Epilepsia 51, 1511-1521. 10.1111/j.1528-1167.2009.02510.x.

Vergnes, M., Marescaux, C., Micheletti, G., Depaulis, A., Rumbach, L., Warter, J.M., 1984. Enhancement of spike and wave discharges by GABAmimetic drugs in rats with spontaneous petit-mal-like epilepsy. Neurosci Lett 44, 91-94.

Vezzani, A., Friedman, A., Dingledine, R.J., 2013. The role of inflammation in epileptogenesis. Neuropharmacology 69, 16-24. 10.1016/j.neuropharm.2012.04.004.

Vezzani, A., Granata, T., 2005. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia 46, 1724-1743. 10.1111/j.1528-1167.2005.00298.x.

Warter, J.M., Vergnes, M., Depaulis, A., Tranchant, C., Rumbach, L., Micheletti, G., Marescaux, C., 1988. Effects of drugs affecting dopaminergic neurotransmission in rats with spontaneous petit mal-like seizures. Neuropharmacology 27, 269-274.

Wemhoner, K., Kanyshkova, T., Silbernagel, N., Fernandez-Orth, J., Bittner, S., Kiper, A.K., Rinne, S., Netter, M.F., Meuth, S.G., Budde, T., Decher, N., 2015. An N-terminal deletion variant of HCN1 in the epileptic WAG/Rij strain modulates HCN current densities. Front Mol Neurosci 8, 63. 10.3389/fnmol.2015.00063.

Westmijse, I., Ossenblok, P., Gunning, B., van Luijtelaar, G., 2009. Onset and propagation of spike and slow wave discharges in human absence epilepsy: A MEG study. Epilepsia 50, 2538-2548. 10.1111/j.1528-1167.2009.02162.x.

White, H.S., Loscher, W., 2014. Searching for the ideal antiepileptogenic agent in experimental models: single treatment versus combinatorial treatment strategies. Neurotherapeutics 11, 373-384. 10.1007/s13311-013-0250-1.

Wilczynski, G.M., Konopacki, F.A., Wilczek, E., Lasiecka, Z., Gorlewicz, A., Michaluk, P., Wawrzyniak, M., Malinowska, M., Okulski, P., Kolodziej, L.R., Konopka, W., Duniec, K., Mioduszewska, B., Nikolaev, E., Walczak, A., Owczarek, D., Gorecki, D.C., Zuschratter, W., Ottersen, O.P., Kaczmarek, L., 2008. Important role of matrix metalloproteinase 9 in epileptogenesis. J Cell Biol 180, 1021-1035. 10.1083/jcb.200708213.

Zheng, T.W., O'Brien, T.J., Morris, M.J., Reid, C.A., Jovanovska, V., O'Brien, P., van Raay, L., Gandrathi, A.K., Pinault, D., 2012. Rhythmic neuronal activity in S2 somatosensory and insular cortices contribute to the initiation of absence-related spike-and-wave discharges. Epilepsia 53, 1948-1958. 10.1111/j.1528-1167.2012.03720.x.

Figure Legends

Figure 1. Summary of drug effects on the development of absence seizures in WAG/Rij rats (see section 4). Data represent the percent variation on the development (incidence) of absence seizures in comparison to controls (normalized to 1) as reported in every selected study. Only studies where the effects of drugs were evaluated at one month after drug suspension were included. The number reported after each drug name is the dose used in the study expressed in mg/kg/day. Data were obtained from the following studies: 1 = Citraro et al., 2014b; 2 = Russo et al., 2011a; 3 = Citraro et al., 2015a; 4 = Russo et al., 2012b; 5 = Russo et al., 2010; 6 = Blumenfeld et al., 2008; 7 = Citraro et al., 2015b; 8 = Russo et al., 2013b; 9 = Russo et al., 2011b. ALAC = alpha-lactoalbumin.

Figure 2. Schematic representation of the possible epileptogenic process in WAG/Rij rats. Genetically-determined epileptogenesis similarly to post-brain insult epileptogenesis, might have a latent phase (period) temporally interrupted by the appearance of the first seizure. The length of this latent phase is not yet defined and proposing that the altered gene(s) might be considered as the initial 'insult', then the initial insult is not necessarily time-limited. In this light, we might hypothesize three different (not necessarily separated) phases: 1) initial brain alteration leading to the establishment of the focal region(s); 2) beginning of focal activity, stimulating other brain areas with no SWDs up to the appearance of the first seizure and 3) from first seizure to the complete network maturation, leading to chronic stable expression of SWDs (for more details see section 2.1).

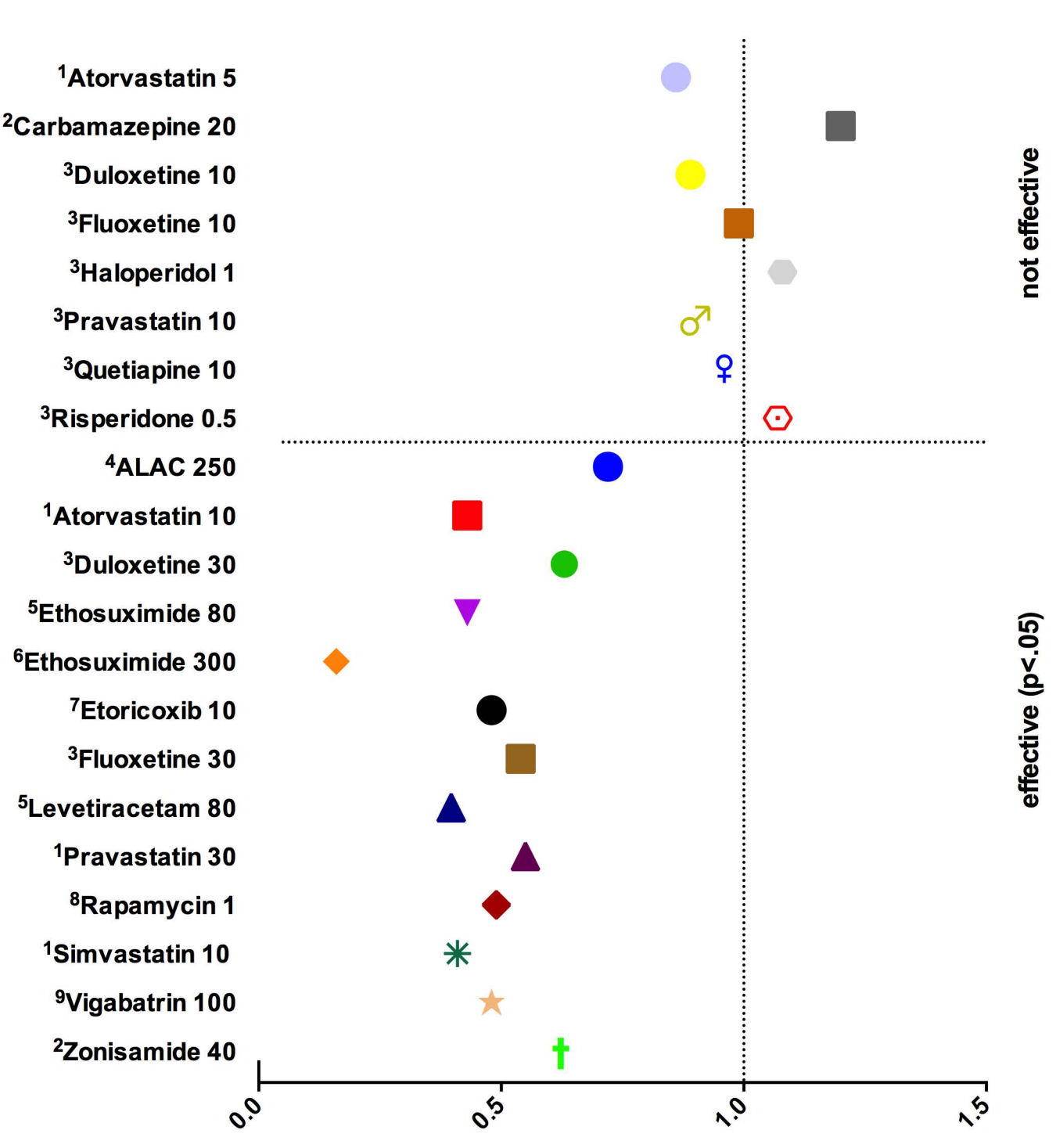
Table

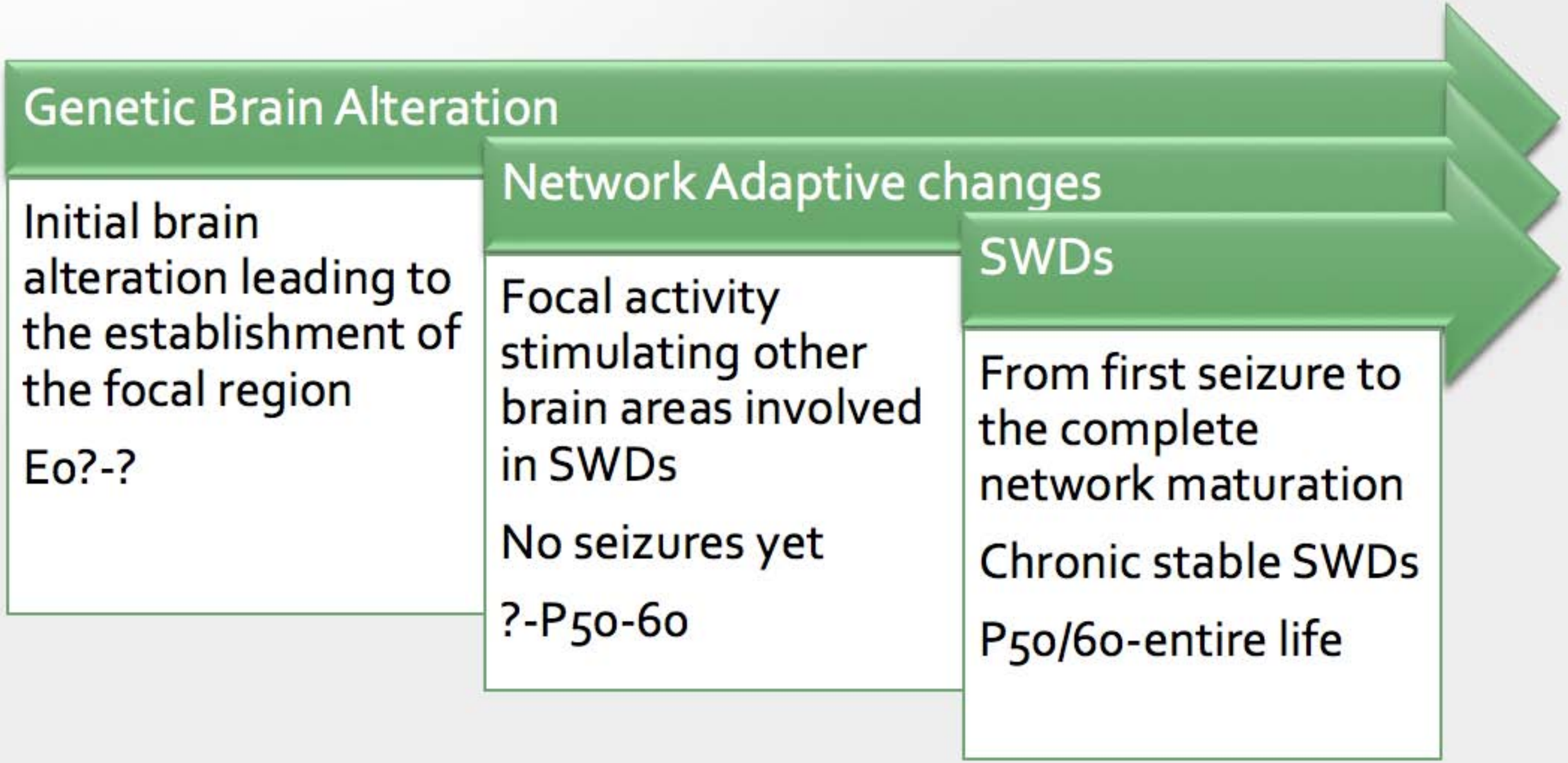
Table 1 Summary of known alterations relevant to epileptogenesis in WAG/Rij rats

System	Alteration	Ref
GABA	Decreased allopregnanolone and 3α,5α-TH-DOC in the cortex	(Pisu et al., 2008)
	Increase in α_4 $GABA_A$ receptor subunit expression in some dorsal thalamic nuclei and an age-dependent increase in δ subunits	
Glutamate	Reduced expression of NR1 subunits of NMDA receptors in the somatosensory cortex in comparison to non-epileptic ACI rats both at 3 and 6 months of age	(van de Bovenkamp-Janssen et al., 2006)
	Lower expression of the NR2B subunit of the NMDA receptors in both 2-month-old and 6-month-old WAG/Rij rats in different layers of the somatosensory cortex in comparison to Wistar rats of the same age	(Karimzadeh et al., 2013)
	AMPA-GluA4 subunit density is lower in WAG/Rij rats than in ACI rats in S1po layers independently from age and seizure development	(van de Bovenkamp-Janssen et al., 2006)
	Age-dependent increase in the numerical density of AMPA-GluA4 subunits was found in the NRT of WAG/Rij rats	(van de Bovenkamp-Janssen et al., 2006)
	GluA2 subunits are not expressed around cell bodies in thalamic nuclei with the exception of the NRT, whereas, they are widely expressed in the two cortical sites examined (S1po and the forelimb region of the primary somatosensory cortex	(Citraro et al., 2006b)
	Reduced expression and function of mGlu5 receptors in the thalamus has been observed in pre-epileptic WAG/Rij rats, while an increased receptor expression in the sensorimotor cortex was described	(D'Amore et al., 2013)
Ion Channels	Altered HCN channel expression and cAMP sensitivity of I_h has been shown in WAG/Rij rats in the postnatal age continuing into the chronic epilepsy state; HCN1 channel expression decreases, mainly in the dendrites of cortical layer V pyramidal neurons that occurs temporally before the developmental onset of SWDs, and at a cellular level plays a direct role in promoting dendritic Ca^{2+} electrogenesis and burst firing	(Budde et al., 2005; Kuisle et al., 2006)

System	Alteration	Ref
	In young WAG/Rij rats an increased peak current density of L-type Ca^{2+} current in the dorsal part of the lateral geniculate nucleus TC neurons correlated with up-regulated mRNA and protein expression of a particular L-type Ca^{2+} channel (Cav1.3)	(Kanyshkova et al., 2014)
Cytokines	The brain and blood plasma levels of IL-1β and TNF-α of 2-, 4-, and 6-month-old WAG/Rij rats and of age-matched control ACI rats revealed higher levels of TNF-α in blood serum of the youngest (2 months) WAG/Rij rats while in the brain, the only significant difference was observed in rats of 4 months of age	(van Luijtelaar et al., 2012)
mTOR	WAG/Rij rats have higher levels of total mTOR in several brain areas, including the cortex, hippocampus and thalamus in comparison to Wistar rats	(Russo et al., 2014b)
Endocannabinoids	Levels of anandamide but not 2-arachidonoylglycerol in 2 months old WAG/Rij rats are lower than those observed in both Wistar and ACI rats only in the thalamus and not in the cortex	(Citraro et al., 2013b)

3α,5α-TH-DOC = 3α,5α-tetrahydrodeoxycorticosterone; S1po = peri-oral region of the somatosensory cortex; NRT = Nucleus Reticularis Thalami; HCN1 = hyperpolarization-activated cation channels; TC = Thalamo-Cortical; IL-1β = Interleukin-1β; TNF-α = Tumor necrosis factor-α; mTOR = mammalian target of rapamycin.


[1]Atorvastatin 5
[2]Carbamazepine 20
[3]Duloxetine 10
[3]Fluoxetine 10
[3]Haloperidol 1
[3]Pravastatin 10
[3]Quetiapine 10
[3]Risperidone 0.5
[4]ALAC 250
[1]Atorvastatin 10
[3]Duloxetine 30
[5]Ethosuximide 80
[6]Ethosuximide 300
[7]Etoricoxib 10
[3]Fluoxetine 30
[5]Levetiracetam 80
[1]Pravastatin 30
[8]Rapamycin 1
[1]Simvastatin 10
[9]Vigabatrin 100
[2]Zonisamide 40
0.0
0.5
1.0
1.5
not effective
effective (p<.05)


Genetic Brain Alteration
Initial brain alteration leading to the establishment of the focal region
E0?-?
Network Adaptive changes
Focal activity stimulating other brain areas involved in SWDs
No seizures yet
?-P50-60
SWDs
From first seizure to the complete network maturation
Chronic stable SWDs
P50/60-entire life
Phase I
Phase II
Phase III