"Special Issue on Epilepsy & Comorbidities"

WAG/Rij rat model: a resource for the pharmacology of epileptogenesis and related neurological/psychiatric comorbidities

Antonio Leo [1,*], Carmen De Caro [1], Valentina Nesci [1], Martina Tallarico [1,2], Giovanna Mangano [1], Ernesto Palma [1], Michelangelo Iannone [2], Giovambattista De Sarro [1], Rita Citraro [1] and Emilio Russo [1]

[1] Science of Health Department, School of Medicine, University of Catanzaro, Italy.

[2] CNR, Section of Pharmacology, Institute of Neurological Sciences, Borgia, Italy.

* Correspondence: aleo@unicz.it; Tel.: +39 0961 3694191; Fax: +39 0961 3694192

ABSTRACT: The discovery of potential antiseizure drugs (ASDs) requires the use of experimental models that can also provide a unique chance for identifying new effective molecules able to prevent and/or cure epilepsy. Most of the preclinical knowledge on epileptogenesis derives from studies performed on post-insult models that are characterized by a recognizable first insult, a silent period lasting until the onset of the first seizure and a chronic period characterized by spontaneous recurrent seizures (SRSs). At odds, genetic models, in which the first insult remains to be identified, have been poorly investigated. Among the genetic models, the WAG/Rij rat was validated as a suitable experimental model of absence epileptogenesis with neuropsychiatric symptomatology, in which, according to our previous hypothesis on SRSs onset, genes could be considered the first 'insult' underlying all plastic modifications supporting the occurrences of absence seizures in this strain. In fact, in several genetic models, the initial insult could be described as the mutation leading to epilepsy that, to date, remains to be defined in this strain. The silent period ends at the occurrence of the first SRS, which is approximately at 2-3 months of age in these rats and after that time the chronic phase initiates, in which, absence seizures increase over time underlying likely further epileptogenic processes. In this review, we describe both the features of this experimental model and the effects of several pharmacological treatments against epileptogenesis and its related comorbidities including depressive-like symptoms and cognitive decline.

1. Introduction

Animal models of seizures/epilepsy remain a key tool in the identification of new drugs for the symptomatic management of epilepsy. Up to now, patients with epilepsy (PWE) have benefited by the successful translation of results come from animal models into the clinical practice. Furthermore, to date, it has widely been accepted that these models also represent a unique resource both to understand the mechanisms involved in epilepsy and to identify new effective therapies able to prevent and/or cure epilepsy and its related comorbidities [1,2]. Most of the preclinical studies on epileptogenesis have mainly been performed in post-brain insult models, however, genetic models deserve to be considered. As reported, these models could give rise to knowledge on the epileptogenic process, which could be exploited to discover drugs able to counteract epileptogenesis as well as to identify biomarkers of epilepsy [3,4]. Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats represent a well-validated genetic model of absence epilepsy, epileptogenesis and neuropsychiatric comorbidities, in which several studies aimed at understanding the pathological abnormalities involved into the epileptogenic process and/or to identify drugs able to prevent or cure this type of non-convulsive epilepsy [3,5-7]. EEG recordings, in this strain, have shown that WAG/Rij rats between 2 and 3 months of age, develop synchronous bilateral spike-wave discharges (SWDs; about 8 Hz, mean duration 5s) that increase in number age-dependently. Notably, at 3 months of age, 50% of the WAG/Rij display SWDs, whereas at 6 months of age 100% of the rats have mature SWDs (about 16-20 per hour) [3,8].

Interestingly, these rats have also been validated as a model of a chronic low-grade depression (dysthymia) that represent comorbidity of absence epilepsy. In details, it was reported that absence seizures are necessary for the occurrence of dysthymia in this strain [8,9]. Moreover, recently, it has also been observed that adult WAG/Rij rats present cognitive decline [10-12]. However, regarding this latter point, several issues need to be clarified. In details, as reported for dysthymia, it remains to be determined whether this cognitive decline is secondary or not to SWDs in this strain. In this review, we have briefly described both the features of this experimental model and the effects of several pharmacological treatments against epileptogenesis and its related comorbidities.

2. The genetically-programmed epileptogenic process in WAG/Rij rats

Epileptogenesis (latency period) is a chronic dynamic process that progressively alters neuronal excitability; this term indicates a cascade of events that appear in a specific period of time starting after or during the occurrence of an insult such as traumatic brain injury, infection, or genetic predisposition, and can continue after epilepsy diagnosis [13]. WAG/Rij rats are a validated and widely studied animal model of absence epilepsy and epileptogenesis. However, the mechanisms underlying the development of spontaneously recurrent seizures (SRS) in this strain remains to be clarified. In fact, to date, studies concerning the pathological mechanisms involved in epileptogenesis have been performed mainly in experimental models where an initial well-defined insult can be detected. At odds, the occurrence of epileptogenesis in genetic epilepsy is poorly investigated [14,15].

Epileptogenesis is defined as the occurrence as well as the structural and functional extension of brain tissue able of producing SRSs, leading to the occurrence of a chronic epileptic condition and/or its progression after the condition is established. The silent period refers to the time between the pathological brain insult and the onset of the first SRSs. The epileptogenic process, in this strain, occurs during lifetime, whereas the latent

phase or silent period ends at about 2-3 months of age when the first SWD (absence seizure) appear [3,16]. Therefore, this latent (silent) period offers a range of opportunity in which an appropriate treatment could prevent or modify epileptogenesis. Furthermore, the silent period can also offer the possibility to identify the biomarkers of epileptogenesis, which can have both diagnostic and predictive value. Based on this background, it is possible to affirm that, during any process of epileptogenesis, a potential susceptible time window for prevention and management of disease could exist.

In WAG/Rij rats, the silent period (up to the age of P50-P60) could be considered as the "*period*" indispensable for the SRS onset; moreover, bearing in mind that the number of absence seizures continue to increase between 2 and 6 months of age or even after [17,18], it might be theorized that during the epileptogenic process continuous pathological modifications occur [3]. Up to now, the mechanisms supporting the epileptogenic process, and the precise time window for its treatment, are still unclear. Despite, some changes including an augmented expression of NaV1.1 and 1.6 subtype Na$^+$ channels and reduced expression of hyperpolarization-activated cyclic nucleotide-gated potassium channel 1 (HCN1) were detected; however, it is debatable whether such modifications are the cause or are secondary to the epileptogenesis mechanisms [19].

WAG/Rij rats descend from a fully inbred strain, created in 1924 from outbred Wistar rats, sharing all autosomal genes. All individuals around 6 months of age show several hundred of spike-wave discharges (SWDs) per day, without distinctions between sexes [3,18]. However, number and/or duration of SWDs can be different between rats. Therefore, inheritance is improbable to be linked to a single gene locus; this is further sustained by the crucial role played by epigenetic factors [20-22]. To date, genes that control SWDs, in these rats, have only been detected on chromosomes 5 and 9 [23], whereas none specific gene mutation was detected for the SRS onset in this strain

[17]. Some genetically programmed factors could give rise to abnormal brain networks of hyperexcitation (*e.g.* cortico-thalamo-cortical network) leading to the development of SRS. However, such factors could also create new circuits able to produce SRS. Several studies, performed in WAG/Rij in rats, have affirmed that, in this strain, exists a focally increased excitability in the deep layers of the perioral region of somatosensory cortex, where the SWDs arise. Accordingly, seizures in WAG/Rij rats could have a bilateral focal origin [24-26]. Very recently, it has been described as the surgical removal of both foci, in these rats, totally abolished SWDs [24]. According to these evidence, it is possible to hypothesize that these focal epileptic areas could be genetically programmed to become hyperexcitable [25,27,28]. Subsequently, these areas could induce adaptive modifications into the cortico-thalamo-cortical network giving rise and maintaining these bilateral synchronous SWDs in this strain [3,6]. Accordingly, such modifications detected in the brain of WAG/Rij rats could be secondary of this unremitting stimulation; thus, the first seizure could be identified when the network, following adaptive changes, is able to express SWDs. In other words, genes could be the first 'insult' underlying all plastic modifications supporting the occurrences of SRSs in this strain. Accordingly, the genetic predisposition could lead to the appearance of pathological bilateral cortical epileptic foci that then non-genetically reorganize several brain networks in order to generate seizures as well as to what occurs throughout electrical kindling [3,29]. However, to date, it is still debated whether blocking these neuroadaptive modifications, before seizure onset, can prevent SRS development during lifetime. Theoretically, in WAG/Rij rats, it is possible to recognize three different connected phases in the epileptogenic process: 1) genetically-programmed brain modifications leading to the focal epileptic region(s); 2) involvement of other brain regions up to the occurrence of the first seizure and 3) the establishment of chronic SRS. To date, only the occurrence of the first seizure has been detected in a well-defined period, whereas the other time windows need to be identified.

3. WAG/Rij rats and neuropsychiatric comorbidities

Neuropsychiatric comorbidities including anxiety, mood disorders, and cognitive impairment are common in PWE, representing a rising problem in clinical practice [30]. Furthermore, none of the currently available ASDs has shown the ability to counteract neuropsychiatric comorbidities that are often more harmful than seizures themselves, worsening quality of life in PWE. Moreover, it has also been observed that neuropsychiatric side effects may sometimes be a consequence of an ASDs treatment. Therefore, to date, the pharmacological treatment of neuropsychiatric comorbidities is another pressing clinical need in epilepsy management [30-32]. Therefore, in any PWE, the management of epilepsy should not only be limited to the achievement of seizure-free state but should also be able to counteract its related comorbidities [32,33].

In the past, comorbidities were considered as a complication of the epileptic disorder. However, recently, new clinical and preclinical evidence supported the existence of a particular bidirectional link between comorbidities and epilepsy, sharing common pathogenetic mechanisms. It was proven that not only PWE have a greater risk to develop a neuropsychiatric disorder, but also patients with primary neuropsychiatric disorders are at greater risk of developing epilepsy. Accordingly, in some conditions, comorbidities and seizures can be generated independently from the same underlying network disease [32,34,35]. To support this linkage, it has also been demonstrated as several drugs acting on the catecholaminergic system, such as antidepressant drugs, could be potential candidate for the prevention and/or treatment of several types of epilepsy and its related neuropsychiatric comorbidities [36-38]; similarly different ASDs such as valproate and carbamazepine are often used in the clinical management of mood disorders [32,39,40]. However, regarding antidepressant drugs, several controversies have been reported in the literature [37,41,42].

Moreover, studies have also highlighted the association among epilepsy, suicidality and psychiatric disorders supporting a common underlying etiology. Likewise, pharmacoepidemiologic studies, despite some methodological limitations, have also reported the increased risk of suicide for people taking AEDs [43,44]. Based on this background, the early detection of these pathological abnormalities could be exploited not only to discover potential antiepileptogenic and/or disease-modifying treatments but also to identify biomarkers of illnesses [4,32,45]. To this aim, experimental models represent a valid tool to study these pathological abnormalities underlying the bidirectional link between epileptogenesis and neuropsychiatric comorbidities. WAG/Rij rats are also a validated model of chronic low-grade depressive-like (dysthymia) comorbidity [3,8].

In details, WAG/Rij rats have shown an increased immobility time in the forced swimming test (FST) and a reduced sucrose consumption/preference (anhedonia) test [3,8,36,46]. This psychiatric symptomatology arises around the age of 3-4 months and worsens in parallel with the increase in SWDs [5,6,47], which in turn increase with age in the WAG/Rij rat model. By this, SWDs seems to be necessary for the appearance of depression-like symptoms, thus, as suggested, epilepsy and depression are directly interconnected in this experimental model [3,9,48].

Nevertheless, regarding this latter point, some controversies have been described. Briefly, it has been observed as fluoxetine at 5 mg/kg, acutely administered, induced a mild increase in SWDs in WAG/Rij rats [49]. At odds, other authors, reported as fluoxetine at the same dose for 15 days had antidepressant-like effects in this strain [50]. Moreover, a proabsence effect was described in adult WAG/Rij rats after 7 weeks of treatment with fluoxetine at 10 and 30 mg/kg/day [36].

Interestingly, to confirm this theory, it was also reported as a chronic treatment, started before absence seizures onset, with antidepressant drugs, such as fluoxetine, possesses both antiepileptogenic and antidepressant-like properties in WAG/Rij rats. Likewise, up to now, through a revision of literature, drugs that abolish the occurrence of absence seizures, with some exceptions (e.g. Levetiracetam and Zonisamide), were also capable of improving depressive-like symptomatology in WAG/Rij rats [3,6,51]. Depression and anxiety are considered two separate diseases; however, anxiety is present in as many as 90% of patients affected by depression. Regarding anxiety-like behaviour in WAG/Rij rats, it was recognized that only audiogenic susceptible rats exhibited anxiety-like symptoms in several validated tasks including open field arena, light-dark choice and elevated plus-maze [48,52]. Therefore, such evidence suggests a relationship between anxiety and predisposition to audiogenic seizures.

Furthermore, it has been documented that a single exposure to sound is sufficient to induce anxiety-like behaviour in the WAG/Rij rat model. Based on this evidence, anxiety-like behaviour is not a trait of WAG/Rij rats as a whole, but representing a characteristic of a sub-group (audiogenic) of these rats [8]. Recently, two studies have also described cognitive impairment, assessed in some memory tasks, in 6 and 13-month-old WAG/Rij rats [10,12]. However, these studies have not recognized whether cognitive decline, in this strain, is secondary or not to absence seizures. Therefore, this issue should be clarified in future studies. Interestingly, neuropsychiatric comorbidities can also occur in childhood absence epilepsy (CAE) that in the past was wrongly considered as a benign form of epilepsy [53-55]. Despite the underlying mechanisms involved in neuropsychiatric comorbidity in CAE remain still to be uncovered, it has been suggested that the same mechanisms leading to network abnormalities can also play a key role in the neuropsychiatric comorbidities onset in these patients [30]. Therefore, further studies are warranted in order to better clarify this hypothesis both in CAE and WAG/Rij rat model.

4. Efficacy of several drugs against the appearance of spontaneous recurrent seizures and related neuropsychiatric comorbidities in WAG/Rij rats

Blumenfeld et al. (2008) were the first to demonstrate as a pharmacological treatment possessed antiepileptogenic effects in a genetic absence seizure model [19]. In this section, we summarize the effects of several drug treatments on the development of absence seizures and related comorbidities (see Table 1, modified from Russo and Citraro, 2018 [6]).

4.1 Ethosuximide

Blumenfeld et al. performed their study with the anti-absence drug ethosuximide, which is a T-type Ca^{2+} channels blocker, orally delivered at 300 mg/kg/day. This pharmacological treatment was started before seizure onset; moreover, at this time point the development of central nervous system (CNS) is still incomplete. In details, in the first group of WAG/Rij rats, treatment started at P21 up to 5 months of age, whereas in the second group the treatment started at P21 and lasted until WAG/Rij rats were 8 months old. ETH was able to markedly suppress the number of SWDs, whereas it was not able to influence the mean duration of a single absence seizure; this effect was recorded up to 3 months after drug discontinuation [19]. Moreover, it has also been reported that this early treatment was able to modify the expression of Na^+ channels and hyperpolarization-activated cation channels (HCN1), which are apparently associated with the SWDs onset in WAG/Rij rats [56,57]. Interestingly, a recent retrospective clinical study reported as a treatment with ETH was linked to a higher rate of remission, in comparison to valproic acid, in CAE [58]. Furthermore, the antiepileptogenic effects of ETH, in this strain, was also confirmed, in another study, by Sarkisova et al. (2010) [9], where the SWDs onset was related to the appearance of depressive-like symptomatology. Likewise, despite some evident differences, the properties of ETH were also evaluated in other studies performed in WAG/Rij rats. In details, Russo et al. (2010) [59] started the oral treatment with ETH 80 mg/kg/day (300 mg/kg/day in the Blumenfeld et al. (2008) [19] study) at P42 (P21 in the Blumenfeld

Table 1. Summary of drugs effects against epileptogenesis and neurological/psychiatric comorbidities

Drugs	Doses and treatment	Effects on SWDs onset	Effects against neurological / psychiatric comorbiddities	Drugs effects duration after 5 months of discontinuation	Ref(s).
Ethosuximide	300 mg/kg/day (Started at P21 up to 5 months)	Decreased SWDs onset	NA	NA	[9,19]
	300 mg/kg/day (Started at P30 up to P150)	Decreased SWDs onset	Reduced depressive-like behaviour	NA	[60]
	80 mg/kg/day (Started at P42 up to 5 months)	Decreased SWDs onset	NA	NA	[9]
	80 mg/kg/day (Started at P30 up to 5 months)	Decreased SWDs onset	Reduced depressive-like behaviour	NA	
Levetiracetam	80 mg/kg/day (Started at P42 up to age of 5 months)	Decreased SWDs onset	Pro-depressant effects	NA	
Zonisamide	40 mg/kg/day (From P42 up to age of 5 months)	Decreased SWDs onset	None	NA	[51]
Carbamazepine	20 mg/kg/day (Started P42 up to age of 5 months)	None	None	None	
Clomipramine	20 mg/kg/i.p (Started P8 up to of P21)	Decreased SWDs onset	Pro-depressant effects	NA	[46]
Vigabatrin	100 mg/kg/day (Started P30 up to 5 months)	Decreased SWDs onset	Reduced depressive-like behaviour	NA	[61]
Perampanel	3 mg/kg/day (Started P30 up to 5 months)	Decreased SWDs onset	Reduced depressive-like behaviour None effects on anxiety-like behaviour and cognitive decline	Increased SWDs and depressive-like behaviour	[62]
Rapamycin	1 mg/kg/day (Started at P45 up to 5 months)	Decreased SWDs onset	Pro-depressant effects	Maintained	[63]
Etoricoxib	10 mg/kg/day (Started P45 up to 5 months)	Decreased SWDs onset	NA	NA	[64]
Atorvastatin	10 mg/kg/day (Started P45 up to 5 months)	Decreased SWDs onset	Reduced depressive-like behaviour	NA	
Simvastatin	10 mg/kg/day (Started P45 up to 5 months)	Decreased SWDs onset	Reduced depressive-like behaviour	NA	[65]
Pravastatin	30 mg/kg/day (Started P45 up to 5 months)	Decreased SWDs onset	Reduced depressive-like behaviour	NA	

Fingolimod	1 mg/kg/day (Started at P30 up to 5 months)	Decreased SWDs onset	Reduced depressive-like behaviour None effects on anxiety-like behaviour Improved cognitive performance	Increased SWDs and depressive-like behaviour Improved cognitive performance	[11]
Fluoxetine	10 and 30 mg/kg/day (Started at P45 up to 5 months)	None at 10 mg/kg/day Decreased SWDs onset at 30 mg/kg/day	Pro-depressant effects at 10 mg/kg/day Anti-depressant effects at 30 mg/kg/day	Increased SWD	
Duloxetine	10 and 30 mg/kg/day (Started at P45 up to 5 months)	Decreased SWDs onset	None	Increased SWD	[36]
Haloperidol	1 mg/kg/day (Started at P45 up to 5 months)	None	Pro-depressant effects	None	
Risperidone	0.5 mg/kg/day (Started at P45 up to 5 months)	None	Pro-depressant effects	None	
Quetiapine	10 mg/kg/day (Started at P45 up to 5 months)	None	None	None	
α-lactoalbumin	(Started at P30 up to 5 months)	Decreased SWDs onset	NA	NA	[66]

SRSs: Spikes Wave Discharged; NA: Not available

et al. (2008) [19] study) up to the same final age of 5 months. EEG studies, performed only at 6 months of age (1 month after drug discontinuation), show that ETH decreased SWDs occurrence (56% of reduction) as well as the mean single SWD duration in contrast to the results of Blumenfeld et al. (2008) [19]. Furthermore, van Luijtelaar et al. (2013) [60] have demonstrated that an ETH treatment period of at least 4 months is needed to have a full antiepileptogenic effect in WAG/Rij rats.

4.2 Levetiracetam, zonisamide and carbamazepine

Levetiracetam treatment, started at 1.5 months of age up to 5 months of age in WAG/Rij rats, has shown antiepileptogenic effects (about 60% reduction in the number of SWDs). Interestingly, WAG/Rij rats chronically administered with ETH or LEV were also, after 30 days of drug discontinuation, acutely treated with the respective drugs. Surprisingly, ETH was still effective in decreasing the remaining absence seizures, whereas LEV was almost ineffective, indicating that residual SWDs after this treatment were resistant to LEV [59]. The same authors again examined ETH and LEV properties in a successive article (dose of ETH and LEV were identical). In details, in this new study, the authors also investigated the effects of zonisamide (ZNS) and carbamazepine (CBZ) [51], starting drug treatment at 1 month of age (P30 vs. P42 in the previous study). The duration of treatment was the same as the previous studies. Interestingly, in this study, WAG/Rij rats at 6 months of age (1 month after drug discontinuation) were also subjected to forced swimming test (FST) to evaluate depression-like symptomatology. EEG recordings, obtained after 45 days of drug discontinuation, have reported that ETH decreased SWDs by only about 31%, whereas LEV by about 43%. Furthermore, it has also been observed that after drug discontinuation, ZNS (40 mg/kg/day) showed antiepileptogenic properties (about 38% reduction), at odds CBZ (20 mg/kg/day) did not present antiepileptogenic effects. In agreement with these evidence, it may be hypothesized that only drugs effective against absence seizures could be able to prevent epileptogenesis in this strain [51]. Regarding

the neuropsychiatric symptomatology, LEV increased immobility time in FST at 6 months of age, indicating a significant worsening of depressive-like symptomatology in WAG/Rij rats, whereas CBZ and ZNS were totally ineffective [51].

Accordingly, it has been suggested that a reduction in the occurrence of SRSs was not necessarily linked to a modification in depressive-like symptomatology. Thus, several drugs could have distinct effects on these two parameters (*i.e.,* absence seizures and depressive-like behaviour); therefore, this point remains to be clarified. Of note, Kovacs et al. (2012,) after treatment with clomipramine (20 mg/kg i.p.), starting from P8 to P21 in WAG/Rij rats, have reported decreasing in SWD activity (about 60% of reduction) at 8 months of age. However, a worsening of anhedonia was detected in these rats [46]. Accordingly, this study suggested that a short-term treatment started early in life could affect the appearance of SRSs in WAG/Rij rats (see below).

4.3 Vigabatrin
Similarly to CBZ, vigabatrin (VGB) when acutely injected, in epileptic WAG/Rij rats, augmented absence seizures [61]; at odds, a long-term treatment (100 mg/kg/day) started at P30 up to 5 months, was able to decrease, 1 month after treatment discontinuation, SWDs onset (~52% of reduction) in WAG/Rij rats. Moreover, this antiepileptogenic effect was linked to a decrease in immobility time in the FST. Based on this evidence, drugs that show poor anti-absence and/or pro-absence effects could also have potentially useful mechanisms of action regulating the epileptogenic process. A subsequent study has reported as VGB was able to inhibit the mTOR pathway [67], which, plays a crucial role in the epileptogenic process also in this strain [68,69].

4.4 Perampanel
Recently, the effects of this selective non-competitive AMPA-receptor antagonist on the SWDs onset and related depressive-like symptomatology were assessed in WAG/Rij rats [62]. To date, the role of AMPA receptors (AMPAR) in epilepsy onset has widely been investigated [70-72]. Perampanel (PER) treatment, 3 mg/kg/day *per os,* started at P30 up to 5 months of age was able to decrease the occurrence of SWDs showing antiepileptogenic effects, 1 month after treatment discontinuation, in WAG/Rij rats. Unfortunately, this effect was not maintained at 10 months of age (5 months after treatment discontinuation). Furthermore, the antiepileptogenic effect was closely linked to antidepressant effects in WAG/Rij rats. This protective effect against dysthymia disappeared at 10 months of age. Finally, also this study supports the idea that absence epilepsy and depressive-like behaviour, in this strain, can share common pathogenic mechanisms [9].

4.5 Rapamycin
Recently, it has been demonstrated that the mTOR pathway is also involved in both genetic and acquired epilepsy onset [68,69]. mTOR inhibitors have shown neuroprotective and antiepileptogenic effects in several experimental models of epileptogenesis [68,73]. Particularly, in WAG/Rij rats, a long-term treatment (17 weeks) with rapamycin (RAP; 1 mg/kg/day *per os*), started at P45 before seizures onset, reduced (about 52%) the occurrence of SRSs at 6 months of age (1 month after drug withdrawal). Moreover, the effects of RAP were also maintained (SRSs reduction of about 49%) in the same rats at 10 months of age (5 months after withdrawal). However, RAP did not influence the mean duration of a single SWD. Interestingly, RAP treatment displayed pro-depressant properties in both WAG/Rij rats and Wistar rats at the age of 6 months, at odds, 5 months after RAP-withdrawal (10 months of age) no differences were observed [63]. The same authors suggested that RAP effects could be linked to the modulation of neuroinflammatory processes, which has also been investigated in a subsequent study performed on the same strain after an intracerebral injection of lipopolysaccharide (LPS). Briefly, RAP treatment started 30 minutes after LPS injection was able to modulate its pro-epileptic and pro-depressant effects by inhibiting the inflammatory response [74].

4.6 Etorixicob

To date, the role of inflammation as a potential cause involved in epileptogenesis in WAG/Rij rats remain unclear, thus further studies are needed. To support the notion, some drugs that act on the inflammatory signaling pathway, have antiepileptogenic and antiabsence effects in WAG/Rij rats [6,75]. Etoricoxib, a cyclooxygenase-2 (COX-2) inhibitor, was able to inhibit the occurrence and development of SWDs in WAG/Rij rats [64]. EEG recordings were performed at both 6 and 10 months of age (1 and 5 months after drug discontinuation) in WAG/Rij rats treated with etoricoxib at 10 mg/kg/day *per os* from P45 up to 5 months of age. This COX-2 inhibitor significantly decreased the onset of SWDs (∼45%; both number and total duration) in WAG/Rij rats at 6 months of age. Moreover, this effect was maintained at 10 months of age. However, the number of SWDs augmented proportionally in treated and control WAG/Rij rats between 6 and 10 months of age; by virtue of this, its antiseizure effects could not be long-lasting [64].

4.7 Statins

Several studies have reported neuroprotective and anti-inflammatory properties of some statins [65,76,77]. According to this, several statins were tested in WAG/Rij rats. All statins, tested in this strain, were administered for 17 weeks, starting from P45 up to 5 months of age. EEG recordings were performed in WAG/Rij rats both at 6 and 10 months of age (1 and 5 months after drug discontinuation). In details, 1 months after drugs discontinuation, atorvastatin (10 mg/kg/day), simvastatin (10 mg/kg/day) and pravastatin (30mg/kg/day) have decreased the development of SWDs by 57%, 59% and 45% respectively. Likewise, this reduction was still significant in WAG/Rij rats of 10 months of age. However, the number of SWDs augmented proportionally between 6 and 10 months of age. Based on this evidence, similarly to etoricoxib but not for RAP, the antiepileptic effects of statins appeared to be only transitory [65]. Regarding depressive-like behaviour, all statins at 6 months, decreased immobility time in the FST demonstrating antidepressant-like effects in WAG/Rij rats; this effect was not maintained after 5 months of drugs discontinuation. Accordingly, statins' properties could not be long-lasting considering that depressive comorbidity is related to the onset of SWDs.

4.8 Fingolimod

It has been documented that this sphingosine-1 phosphate receptor modulator has anti-inflammatory and neuroprotective effects [78-80]. This drug, approved for the relapsing-remitting multiple sclerosis management, has also demonstrated antiseizure and antiepileptogenic effects in experimental models [78,81-83]. Under this, the potential antiepileptogenic effects of this drug as well as its effects on neuropsychiatric comorbidities were assessed in WAG/Rij rats [11]. In details, Fingolimod treatment, from P30 up to 5 months of age at 1 mg/kg/day *per os*, was able to reduce SWDs (30% of reduction) as well as depressive symptomatology in WAG/Rij rats at 6 months of age (1 month after treatment discontinuation). However, similarly to other drugs tested, these effects were not maintained after 5 months of treatment withdrawal [11]. These temporary effects of fingolimod were accompanied by a transitory reduction of mTOR pathway activity. Interestingly, fingolimod has shown longer-lasting effects on cognitive performance in adult WAG/Rij rats, an effect related to augmented acetylation of lysine 8 of histone H4 (at both ages investigated) [11]. Based on these results, further studies should also clarify the role of epigenetics in the epileptogenic process in absence epilepsy [84].

4.9 Antidepressants

The effects of some antidepressants and antipsychotics drugs were investigated in WAG/Rij rats [36]. In this study, antidepressants were delivered from P45 up to 5 months of age. EEG study was only performed at 6 months of age together with FST. Fluoxetine is a selective serotonin reuptake inhibitor, when administered at 10 mg/kg/day *per os,* did not influence the development of SWDs, but when administered at 30 mg/kg/day *per os,* it was able to reduce SWDs

(~46% of reduction). Likewise, duloxetine, a serotonin-noradrenaline reuptake inhibitor, decreased the occurrence of absence seizures by ~20 and 37% at 10 and 30 mg/kg/day *per os*, respectively.

Furthermore, the antiseizure effects of both drugs were also investigated after short-term chronic treatment in WAG/Rij rats at 6 months of age. In details, after this short-term treatment, fluoxetine showed pro-epileptic effects, whereas duloxetine was able to decrease SWDs only at 30 mg/kg/day. By this, it has been hypothesized that the antiepileptogenic properties of both drugs could not be linked to their antiseizure effects. Regarding depressive-like comorbidity, duloxetine, at 10 and 30 mg/kg/day, did not affect immobility time, whereas fluoxetine at 10 mg/kg/day showed pro-depressant properties and at a higher dose it was able to decrease immobility time. Unfortunately, these controversial evidence were not entirely elucidated [36]. The role of serotonin and serotonin/noradrenaline systems, in the epileptogenic process in WAG/Rij rats, was also evaluated after orally treatment with a milk whey protein rich in tryptophan (α-lactoalbumin; ALAC) that has demonstrated ability to decrease SWDs, when administered at 250 mg/kg/day for 17 weeks, (28% of reduction) in WAG/Rij rats. The authors hypothesized that this effect could be linked to an enhancement of serotonin levels into the brain [66,85].

Of note, the effects observed after treatment with clomipramine, a tricyclic antidepressant drug, in newborn WAG/Rij rats [46]. In details, clomipramine at 20 mg/kg i.p. when administered from P8 up to P21 in neonatal WAG/Rij induced a worsening of depressive-like behaviour, whereas reduced SWDs (about 60%) in the same rats at 8 of age [46]. Therefore, antiepileptogenic effects did not seem related to the occurrence of depressive-like symptomatology. Moreover, according to this study, a short-term treatment starting early in life could modify the SWDs onset in WAG/Rij rats.

To date, this issue remains still unclear. However, it has been demonstrated that exposure to recurrent experimental febrile seizures (between P21 and P42) in WAG/Rij rats did not alter the appearance of SWDs later in life [86]. At odds, neonatal sensory deprivation endorses the appearance of SWDs [20], and WAG/Rij rats fostered by Wistar dams shown a smaller amount of SWDs in comparison to rats fostered by their biological dams [22,87]. Based on these two studies, it is possible to support the hypothesis that interventions, before P21, could counteract SWDs onset. Likewise, WAG/Rij rats born from dams drinking ethyl alcohol from the first week of pregnancy up to the 1st week after treatment did not fully develop SWDs in adulthood (~ 64% of reduction) [88]. Finally, this evidence strongly upholds the hypothesis that early short-term treatment could change the occurrence of SWDs.

4.10 Antipsychotics
Citraro et al. (2015a) have also tested haloperidol (1 mg/kg/day), risperidone (0.5 mg/kg/day) and quetiapine (10 mg/kg/day) in WAG/Rij rats, starting treatment at P45 up to 5 months of age. EEG recordings together with FST (immobility time) were evaluated 1 month after drugs withdrawal (6 months of age). None of these antipsychotic drugs influenced the occurrence of SWDs; moreover, haloperidol and risperidone showed pro-depressant effects, whereas quetiapine did not have effects on immobility time [36]. Furthermore, after short-term chronic treatment (7 weeks), haloperidol and risperidone demonstrated pro-epileptic properties, while quetiapine did not change the number of SWDs in WAG/Rij rats after drug discontinuation (1 month). Accordingly, it is possible to hypothesize that during long-term treatment, a constant augment in SWDs was present in these rats and this could be responsible for the pro-depressant properties detected. Antipsychotics drugs could not produce a constant increase in SWDs, but they could only worsen depressive-like symptoms in this strain [36].

Finally, several drugs, when early administered, seem to be able to prevent SWDs onset in WAG/Rij rats. Interestingly, the published effects on ETH in CAE are in agreement with the results detected in WAG/Rij rats [58,89] about its disease-modifying properties. The time to start drug treatment in this strain should be identified. The effectiveness of several drugs has been evaluated after a long-term (about 4 months) treatment, which was started generally after 1 month of age. However, pharmacological treatments much longer than 4 months could overcome the period of epileptogenesis. Thus, the reducing in SWDs could be linked to the combined anti-epileptogenic and antiseizure properties. At odds, shorter periods with ETH were not able to reduce SWDs; possibly earlier interventions could involve shorter treatment periods as supported by the properties on WAG/Rij rat' SWDs onset following clomipramine treatment (started at P8 up to P21) [90]. Furthermore, drug effects were not examined long after drug discontinuation, and for different drugs, effects were not maintained over time.

5. Conclusion

Despite the availability of several animal models of epilepsy, which have been fundamental in ASDs discovery, the lack of antiepileptogenic therapies remains an unmet need in the epilepsy field [1,91,92]. To date, these models represent a unique opportunity to address this issue; however, up to now, none of these models has been clinically validated [1,92,93]. New insights could come from genetic animal models that are now considered as a potential tool to investigate both the pathophysiology of the epileptogenic process (together with acquired/post-insult models) and the potential antiepileptogenic effect of several drugs [19,94,95].

WAG/Rij rats, as above reported, represent a validated genetic animal model of absence epileptogenesis with neuropsychiatric comorbidities [1,3,19]. There is a bidirectional link between epilepsy and neurological/psychiatric symptomatology in this strain as well as in CAE. To date, neurological/psychiatric comorbidities represent a severe problem in PWE. As

reported, comorbidities sometimes can be more harmful than seizures themselves [32,34]. The "face validity" of this animal model was also supported by the potential antiepileptogenic effect of ETH both in childhood absence epilepsy and this strain [19,58].

Interestingly, in WAG/Rij rats and CAE, behaviour and epilepsy could share common mechanisms [9,30,54]. Up to now, (see above) several drugs have been tested in this experimental model. Overall, some of these drugs have been efficacious to prevent seizures onset as well as neuropsychiatric comorbidities [3]. Interestingly, among drugs tested, ETH has shown antiepileptogenic effects; in agreement with its disease-modifying effects observed in CAE [19,58]. However, in these rats, it remains to be understood if a drug treatment, limited before seizures onset, was able to counteract the epileptogenic process preventing SRSs development. Many of these studies were performed after long-term treatment, started before seizure onset. Thus it is difficult to discern if the effect observed was linked to an antiepileptogenic and/or antiepileptic drug's action [3]. To date, only Kovacs et al. (2012) [46] reported an early treatment between P8 and P21 was able to decrease SWDs activity in adult WAG/Rij rats.

Furthermore, several of these drugs have only shown transitory effects after treatment discontinuation. Therefore, even though the WAG/Rij rat is an animal model of absence seizures, several useful information against epileptogenesis and its related comorbidities could be derived from its use. Such information could also be useful to investigate epileptogenesis and comorbidities in other models including post insult epilepsy models. However, to improve our knowledge in the epileptogenic process and its treatment, future studies should shed light on the real value of this strain. Notably, these studies should identify the pathogenic mechanisms underlying epileptogenesis with the aim to define the right time window better to start a potential antiepileptogenic treatment. In fact, in genetic animal models, in comparison to post insult models, the lack of a well-defined "first insult" that can

reflect the beginning of the epileptogenesis process complicates its definition [3].

Author Contributions

Every author of the manuscript has substantially contributed to the scientific process leading up to the writing of the paper.

Conflict of interest

The authors declare no conflict of interest.

References

1. Löscher W. Fit for purpose application of currently existing animal models in the discovery of novel epilepsy therapies. *Epilepsy Res*. 2016;126:157-184. https://doi.org/10.1016/j.eplepsyres.2016.05.016

2. Löscher W. Animal Models of Seizures and Epilepsy: Past, Present, and Future Role for the Discovery of Antiseizure Drugs. *Neurochem Res*. 2017;42(7):1873-1888. https://doi.org/10.1007/s11064-017-2222-z

3. Russo E, Citraro R, Constanti A, Leo A, Lüttjohann A, van Luijtelaar G, et al. Upholding WAG/Rij rats as a model of absence epileptogenesis: Hidden mechanisms and a new theory on seizure development. *Neurosci Biobehav Rev*. 2016;71:388-408. https://doi.org/10.1016/j.neubiorev.2016.09.017

4. Sueri C, Gasparini S, Balestrini S, Labate A, Gambardella A, Russo E, et al. Diagnostic Biomarkers of Epilepsy. *Curr Pharm Biotechnol*. 2018;19(6):440-450. https://doi.org/10.2174/1389201019666180713095251

5. Coenen AML, Van Luijtelaar ELJM. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet*. 2003;33(6):635-655. https://doi.org/10.1023/A:1026179013847

6. Russo E, Citraro R. Pharmacology of epileptogenesis and related comorbidities in the WAG/Rij rat model of genetic absence epilepsy. *J Neurosci Methods*. 2018;310:54-62. https://doi.org/10.1016/j.jneumeth.2018.05.020

7. van Luijtelaar G, Zobeiri M. Progress and outlooks in a genetic absence epilepsy model (WAG/Rij). *Curr Med Chem*. 2014;21(6):704-721. https://doi.org/10.2174/0929867320666131119152913

8. Sarkisova K, van Luijtelaar G. The WAG/Rij strain: a genetic animal model of absence epilepsy with comorbidity of depression [corrected]. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010;35(4):854-876. https://doi.org/10.1016/j.pnpbp.2010.11.010

9. Sarkisova KY, Kuznetsova GD, Kulikov MA, van Luijtelaar G. Spike-wave discharges are necessary for the expression of behavioral depression-like symptoms. *Epilepsia*. 2010;51(1):146. https://doi.org/10.1111/j.1528-1167.2009.02260.x

10. Karson A, Utkan T, Balcı F, Arıcıoğlu F, Ateş N. Age-dependent decline in learning and memory performances of WAG/Rij rat model of absence epilepsy. *Behavioral and Brain Functions*. 2012;8(1):51. https://doi.org/10.1186/1744-9081-8-51

11. Leo A, Citraro R, Amodio N, De Sarro C, Cantafio MEG, Constanti A, et al. Fingolimod Exerts only Temporary Antiepileptogenic Effects but Longer-Lasting Positive Effects on Behavior in the WAG/Rij Rat Absence Epilepsy Model. *Neurotherapeutics*. 2017;14(4):1134. https://doi.org/10.1007/s13311-017-0550-y

12. Jafarian M, Karimzadeh F, Alipour F, Attari F, Lotfinia AA, Speckmann EJ, et al. Cognitive impairments and neuronal injury in different brain regions of a genetic rat model of absence epilepsy. *Neuroscience*. 2015;298:161. https://doi.org/10.1016/j.neuroscience.2015.04.033

13. Pitkänen A, Lukasiuk K, Dudek FE, Staley KJ. Epileptogenesis. *Cold Spring Harb Perspect Med*. 2015;5(10):a022822. https://doi.org/10.1101/cshperspect.a022822

14. Terrone G, Pauletti A, Pascente R, Vezzani A. Preventing epileptogenesis: A realistic goal? *Pharmacol Res*. 2016;110:96-100. https://doi.org/10.1016/j.phrs.2016.05.009

15. Pitkänen A, Löscher W, Vezzani A, Becker AJ, Simonato M, Lukasiuk K, et al. Advances in the development of biomarkers for epilepsy. *The Lancet Neurology*. 2016;15(8):843-856. https://doi.org/10.1016/S1474-4422(16)00112-5

16. Pitkänen A, Engel J. Past and present definitions of epileptogenesis and its biomarkers. *Neurotherapeutics*. 2014;11(2):231-241. https://doi.org/10.1007/s13311-014-0257-2

17. van Luijtelaar G, Sitnikova E. Global and focal aspects of absence epilepsy: the contribution of genetic models. *Neurosci Biobehav Rev*. 2006;30(7):983-1003. https://doi.org/10.1016/j.neubiorev.2006.03.002

18. van Luijtelaar G, Onat FY, Gallagher MJ. Animal models of absence epilepsies: what do they model and do sex and sex hormones matter? *Neurobiol Dis*. 2014;72 Pt B:167-179. https://doi.org/10.1016/j.nbd.2014.08.014

19. Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, et al. Early treatment suppresses the development of spike-wave epilepsy in a rat model. *Epilepsia*. 2008;49(3):400-409. https://doi.org/10.1111/j.1528-1167.2007.01458.x

20. Sitnikova E. Neonatal sensory deprivation promotes development of absence seizures in adult rats with genetic predisposition to epilepsy. *Brain Res*. 2010;1377:109-118. https://doi.org/10.1016/j.brainres.2010.12.067

21. Sarkisova KY, Gabova AV. Maternal care exerts disease-modifying effects on genetic absence epilepsy and comorbid depression. *Genes Brain Behav*. 2018;17(7):e12477. https://doi.org/10.1111/gbb.12477

22. Sitnikova E, Rutskova EM, Raevsky VV. Reduction of epileptic spike-wave activity in WAG/Rij rats fostered by Wistar dams. *Brain Res*. 2015;1594:305-309. https://doi.org/10.1016/j.brainres.2014.10.067

23. Gauguier D, van Luijtelaar G, Bihoreau MT, Wilder SP, Godfrey RF, Vossen J, et al. Chromosomal mapping of genetic loci controlling absence epilepsy phenotypes in the WAG/Rij rat. *Epilepsia*. 2004;45(8):908-915. https://doi.org/10.1111/j.0013-9580.2004.13104.x

24. Scicchitano F, van Rijn CM, van Luijtelaar G. Unilateral and Bilateral Cortical Resection: Effects on Spike-Wave Discharges in a Genetic Absence Epilepsy Model. *PLoS ONE*. 2015;10(8):e0133594. https://doi.org/10.1371/journal.pone.0133594

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

26. Meeren HKM, Pijn JPM, Van Luijtelaar ELJM, Coenen AML, da Silva FHL. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci*. 2002;22(4):1480-1495. https://doi.org/10.1523/JNEUROSCI.22-04-01480.2002

27. D'Antuono M, Inaba Y, Biagini G, D'Arcangelo G, Tancredi V, Avoli M. Synaptic hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures. *Genes Brain Behav*. 2006;5(1):73-84. https://doi.org/10.1111/j.1601-183X.2005.00146.x

28. Kole MHP, Bräuer AU, Stuart GJ. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. *J Physiol (Lond)*. 2006;578(Pt 2):507-525. https://doi.org/10.1113/jphysiol.2006.122028

29. van Luijtelaar G, Sitnikova E, Littjohann A. On the origin and suddenness of absences in genetic absence models. *Clin EEG Neurosci*. 2011;42(2):83-97. https://doi.org/10.1177/155005941104200209

30. Salpekar JA, Mula M. Common psychiatric comorbidities in epilepsy: How big of a problem is it? *Epilepsy & Behavior*. 2018. https://doi.org/10.1016/j.yebeh.2018.07.023

31. Chen B, Choi H, Hirsch LJ, Legge A, Buchsbaum R, Detyniecki K. Cross-sensitivity of psychiatric and behavioral side effects with antiepileptic drug use. *Seizure*. 2018;62:38-42. https://doi.org/10.1016/j.seizure.2018.09.014

32. Kanner AM. Management of psychiatric and neurological comorbidities in epilepsy. *Nature Reviews Neurology*. 2016;12(2):106-116. https://doi.org/10.1038/nrneurol.2015.243

33. Trinka E. Ideal characteristics of an antiepileptic drug: how do these impact treatment decisions for individual patients? *Acta Neurol Scand, Suppl*. 2012;(194):10-18. https://doi.org/10.1111/ane.12015

34. Mula M. Depression in epilepsy. *Curr Opin Neurol*. 2017;30(2):180-186. https://doi.org/10.1097/WCO.0000000000000431

35. Rayner G. The Contribution of Cognitive Networks to Depression in Epilepsy. *Epilepsy Currents*. 2017;17(2):78-83. https://doi.org/10.5698/1535-7511.17.2.78

36. Citraro R, Leo A, De Fazio P, De Sarro G, Russo E. 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*. 2015;172(12):3177-3188. https://doi.org/10.1111/bph.13121

37. Hamid H, Kanner AM. Should antidepressant drugs of the selective serotonin reuptake inhibitor family be tested as antiepileptic drugs? *Epilepsy & Behavior*. 2013;26(3):261-265. https://doi.org/10.1016/j.yebeh.2012.10.009

38. Ahern TH, Javors MA, Eagles DA, Martillotti J, Mitchell HA, Liles LC, et al. The effects of chronic norepinephrine transporter inactivation on seizure susceptibility in mice. *Neuropsychopharmacology*. 2005;31(4):730-738. https://doi.org/10.1038/sj.npp.1300847

39. Korczyn AD, Schachter SC, Brodie MJ, Dalal SS, Engel J, Guekht A, et al. Epilepsy, cognition, and neuropsychiatry (Epilepsy, Brain, and Mind, part 2). *Epilepsy & Behavior*. 2013;28(2):283-302. https://doi.org/10.1016/j.yebeh.2013.03.012

40. Kanner AM, Mazarati A, Koepp M. Biomarkers of epileptogenesis: psychiatric comorbidities (?). *Neurotherapeutics*. 2014;11(2):358-372. https://doi.org/10.1007/s13311-014-0271-4

41. Cardamone L, Salzberg MR, Koe AS, Ozturk E, O'Brien TJ, Jones NC. Chronic antidepressant treatment accelerates kindling epileptogenesis in rats. *Neurobiol Dis*. 2013;63:194-200. https://doi.org/10.1016/j.nbd.2013.11.020

42. Li C, Silva J, Ozturk E, Dezsi G, O'Brien TJ, Renoir T, et al. Chronic fluoxetine treatment accelerates kindling epileptogenesis in mice independently of 5-HT2A receptors. *Epilepsia*. 2018;59(7):e114-e119. https://doi.org/10.1111/epi.14435

43. Hesdorffer DC, Hauser WA, Olafsson E, Ludvigsson P, Kjartansson O. Depression and suicide attempt as risk factors for incident unprovoked seizures. *Ann Neurol*. 2005;59(1):35-41. https://doi.org/10.1002/ana.20685

44. Mula M, Hesdorffer DC. Suicidal behavior and antiepileptic drugs in epilepsy: analysis of the emerging evidence. *Drug Healthc Patient Saf*. 2011;3:15-20. https://doi.org/10.2147/DHPS.S13070

45. Rudzinski LA, Meador KJ. Epilepsy and neuropsychological comorbidities. *Continuum (Minneap Minn)*. 2013;19(3 Epilepsy):682-696. https://doi.org/10.1212/01.CON.0000431382.06438.cd

46. Kovács Z, Czurkó A, Kékesi KA, Juhász G. Neonatal tricyclic antidepressant clomipramine treatment reduces the spike-wave discharge activity of the adult WAG/Rij rat. *Brain Research Bulletin*. 2012;89(3-4):102-107. https://doi.org/10.1016/j.brainresbull.2012.07.010

47. Midzyanovskaya IS, Shatskova AB, Sarkisova KY, van Luijtelaar G, Tuomisto L, Kuznetsova GD. Convulsive and nonconvulsive epilepsy in rats: effects on behavioral response to novelty stress. *Epilepsy & Behavior*. 2005;6(4):543-551. https://doi.org/10.1016/j.yebeh.2005.03.005

48. Sarkisova KY, Kulikov MA. Behavioral characteristics of WAG/Rij rats susceptible and non-susceptible to audiogenic seizures. *Behav Brain Res*. 2006;166(1):9-18. https://doi.org/10.1016/j.bbr.2005.07.024

49. Jakus R, Graf M, Juhasz G, Gerber K, Levay G, Halasz P, et al. 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*. 2004;184(2):964-972. https://doi.org/10.1016/S0014-4886(03)00352-2

50. Sarkisova KI, Folomkina AA. [Effect of selective serotonin reuptake inhibitor fluoxetine on symptoms of depression-like behavior in WAG/Rij rats]. *Zh Vyssh Nerv Deiat Im I P Pavlova*. 2010;60(1):98-108.

51. Russo E, Citraro R, Scicchitano F, De Fazio S, Perrotta I, Perrota I, et al. 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*. 2011;52(7):1341-1350. https://doi.org/10.1111/j.1528-1167.2011.03112.x

52. Midzyanovskaya I, Kopilov M, Fedotova E, Kuznetsova G, Tuomisto L. Dual effect of pyrilamine on absence seizures in WAG/Rij rats. *Inflamm Res*. 2005;54 Suppl 1:S40-41. https://doi.org/10.1007/s00011-004-0418-6

53. Caplan R, Siddarth P, Stahl L, Lanphier E, Vona P, Gurbani S, et al. Childhood absence epilepsy: behavioral, cognitive, and linguistic comorbidities. *Epilepsia*. 2008;49(11):1838-1846. https://doi.org/10.1111/j.1528-1167.2008.01680.x

54. Besag F, Caplan R, Aldenkamp A, Dunn DW, Gobbi G, Sillanpää M. Psychiatric and Behavioural Disorders in Children with Epilepsy (ILAE Task Force Report): Behavioural effects of epilepsy surgery. *Epileptic Disord*. 2016. https://doi.org/10.1684/epd.2016.0818

55. Verrotti A, Matricardi S, Rinaldi VE, Prezioso G, Coppola G. Neuropsychological impairment in childhood absence epilepsy: Review of the literature. *J Neurol Sci*. 2015;359(1-2):59-66. https://doi.org/10.1016/j.jns.2015.10.035

56. Strauss U, Kole MHP, Bräuer AU, Pahnke J, Bajorat R, Rolfs A, et al. An impaired neocortical Ih is associated with enhanced excitability and absence epilepsy. *Eur J Neurosci*. 2004;19(11):3048-3058. https://doi.org/10.1111/j.0953-816X.2004.03392.x

57. Klein JP, Khera DS, Nersesyan H, Kimchi EY, Waxman SG, Blumenfeld H. Dysregulation of sodium channel expression in cortical neurons in a rodent model of absence epilepsy. *Brain Res*. 2004;1000(1-2):102-109. https://doi.org/10.1016/j.brainres.2003.11.051

58. Berg AT, Levy SR, Testa FM, Blumenfeld H. Long-term seizure remission in childhood absence epilepsy: might initial treatment matter? *Epilepsia*. 2014;55(4):551-557. https://doi.org/10.1111/epi.12551

59. Russo E, Citraro R, Scicchitano F, De Fazio S, Di Paola ED, Constanti A, et al. Comparison of the antiepileptogenic effects of an early long-term treatment with ethosuximide or levetiracetam in a genetic animal model of absence epilepsy. *Epilepsia*. 2010;51(8):1560-1569. https://doi.org/10.1111/j.1528-1167.2009.02400.x

60. van Luijtelaar G, Mishra AM, Edelbroek P, Coman D, Frankenmolen N, Schaapsmeerders P, et al. Anti-epileptogenesis: Electrophysiology, diffusion tensor imaging and behavior in a genetic absence model. *Neurobiol Dis*. 2013;60:126-138. https://doi.org/10.1016/j.nbd.2013.08.013

61. Russo E, Citraro R, Scicchitano F, Urzino A, Marra R, Rispoli V, et al. Vigabatrin has antiepileptogenic and antidepressant effects in an animal model of epilepsy and depression comorbidity. *Behav Brain Res*. 2011;225(1):373-376. https://doi.org/10.1016/j.bbr.2011.07.030

62. Citraro R, Leo A, Franco V, Marchiselli R, Perucca E, De Sarro G, et al. Perampanel effects in the WAG/Rij rat model of epileptogenesis, absence epilepsy, and comorbid depressive-like behavior. *Epilepsia*. 2016;58(2):231-238. https://doi.org/10.1111/epi.13629

63. Russo E, Citraro R, Donato G, Camastra C, Iuliano R, Cuzzocrea S, et al. mTOR inhibition modulates epileptogenesis, seizures and depressive behavior in a genetic rat model of absence epilepsy. *Neuropharmacology*. 2013;69:25-36. https://doi.org/10.1016/j.neuropharm.2012.09.019

64. Citraro R, Leo A, Marra R, De Sarro G, Russo E. Antiepileptogenic effects of the selective COX-2 inhibitor etoricoxib, on the development of spontaneous absence seizures in WAG/Rij rats. *Brain Research Bulletin*. 2015;113:1-7. https://doi.org/10.1016/j.brainresbull.2015.02.004

65. Citraro R, Chimirri S, Aiello R, Gallelli L, Trimboli F, Britti D, et al. Protective effects of some statins on epileptogenesis and depressive-like behavior in WAG/Rij rats, a genetic animal model of absence epilepsy. *Epilepsia*. 2014;55(8):1284-1291. https://doi.org/10.1111/epi.12686

66. Russo E, Scicchitano F, Citraro R, Aiello R, Camastra C, Mainardi P, et al. Protective activity of α-lactoalbumin (ALAC), a whey protein rich in tryptophan, in rodent models of epileptogenesis. *Neuroscience*. 2012;226:282-288. https://doi.org/10.1016/j.neuroscience.2012.09.021

67. Zhang B, McDaniel SS, Rensing NR, Wong M. Vigabatrin inhibits seizures and mTOR pathway activation in a mouse model of tuberous sclerosis complex. *PLoS ONE*. 2013;8(2):e57445. https://doi.org/10.1371/journal.pone.0057445

68. Citraro R, Leo A, Constanti A, Russo E, De Sarro G. mTOR pathway inhibition as a new therapeutic strategy in epilepsy and epileptogenesis. *Pharmacol Res*. 2016;107:333-343. https://doi.org/10.1016/j.phrs.2016.03.039

69. Leo A, Constanti A, Coppola A, Citraro R, De Sarro G, Russo E. mTOR Signaling in Epilepsy and Epileptogenesis. Molecules to Medicine with mTOR. 2016. 1 p. https://doi.org/10.1016/b978-0-12-802733-2.00006-2

70. Leo A, Giovannini G, Russo E, Meletti S. The role of AMPA receptors and their antagonists in status epilepticus. *Epilepsia*. 2018;59(6):1098-1108. https://doi.org/10.1111/epi.14082

71. Citraro R, Aiello R, Franco V, De Sarro G, Russo E. Targeting α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors in epilepsy. *Expert Opin Ther Targets*. 2014;18(3):319-334. https://doi.org/10.1517/14728222.2014.874416

72. Russo E, Gitto R, Citraro R, Chimirri A, De Sarro G. New AMPA antagonists in epilepsy. *Expert Opin Investig Drugs*. 2012;21(9):1371-1389. https://doi.org/10.1517/13543784.2012.705277

73. Galanopoulou AS, Gorter JA, Cepeda C. Finding a better drug for epilepsy: the mTOR pathway as an antiepileptogenic target. *Epilepsia*. 2012;53(7):1119-1130. https://doi.org/10.1111/j.1528-1167.2012.03506.x

74. Russo E, Follesa P, Citraro R, Camastra C, Donato A, Isola D, et al. 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*. 2014;35(11):1793-1799. https://doi.org/10.1007/s10072-014-1842-1

75. Rimoli MG, Russo E, Cataldi M, Citraro R, Ambrosino P, Melisi D, et al. T-type channel blocking properties and antiabsence activity of two imidazo[1,2-b]pyridazine derivatives structurally related to indomethacin. *Neuropharmacology*. 2008;56(3):637-646. https://doi.org/10.1016/j.neuropharm.2008.11.003

76. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nat Rev Drug Discov*. 2005;4(12):977-987. https://doi.org/10.1038/nrd1901

77. Rajanikant GK, Zemke D, Kassab M, Majid A. The therapeutic potential of statins in neurological disorders. *Curr Med Chem*. 2007;14(1):103-112. https://doi.org/10.2174/092986707779313462

78. Leo A, Citraro R, Marra R, Palma E, Di Donato Paola E, Constanti A, et al. The Sphingosine 1-Phosphate Signaling Pathway in Epilepsy: A Possible Role for the Immunomodulator Drug Fingolimod in Epilepsy Treatment. *CNS Neurol Disord Drug Targets*. 2016;16(3):311-325. https://doi.org/10.2174/1871527315666161104163031

79. Prager B, Spampinato SF, Ransohoff RM. Sphingosine 1-phosphate signaling at the blood-brain barrier. *Trends Mol Med*. 2015;21(6):354-363. https://doi.org/10.1016/j.molmed.2015.03.006

80. Wei Y, Yemisci M, Kim H-H, Yung LM, Shin HK, Hwang S-K, et al. Fingolimod provides long-term protection in rodent models of cerebral ischemia. *Ann Neurol*. 2011;69(1):119-129. https://doi.org/10.1002/ana.22186

81. Pitsch J, Kuehn JC, Gnatkovsky V, Müller JA, van Loo KMJ, de Curtis M, et al. Anti-epileptogenic and Anti-convulsive Effects of Fingolimod in Experimental Temporal Lobe Epilepsy. *Mol Neurobiol*. 2018;. https://doi.org/10.1007/s12035-018-1181-y

82. Gol M, Ghorbanian D, Hassanzadeh S, Javan M, Mirnajafi-Zadeh J, Ghasemi-Kasman M. Fingolimod enhances myelin repair of hippocampus in pentylenetetrazol-induced kindling model. *Eur J Pharm Sci*. 2017;96:72-83. https://doi.org/10.1016/j.ejps.2016.09.016

83. Gao F, Liu Y, Li X, Wang Y, Wei D, Jiang W. Fingolimod (FTY720) inhibits neuroinflammation and attenuates spontaneous convulsions in lithium-pilocarpine induced status epilepticus in rat model. *Pharmacol Biochem Behav*. 2012;103(2):187-196. https://doi.org/10.1016/j.pbb.2012.08.025

84. Citraro R, Leo A, Santoro M, D'agostino G, Constanti A, Russo E. Role of Histone Deacetylases (HDACs) in Epilepsy and Epileptogenesis. *Curr Pharm Des*. 2017;23(37):5546-5562. https://doi.org/10.2174/1381612823666171024130001

85. Citraro R, Scicchitano F, De Fazio S, Raggio R, Mainardi P, Perucca E, et al. Preclinical activity profile of α-lactoalbumin, a whey protein rich in tryptophan, in rodent models of seizures and epilepsy. *Epilepsy Res*. 2011;95(1-2):60-69. https://doi.org/10.1016/j.eplepsyres.2011.02.013

86. Ateş N, Akman O, Karson A. The effects of the immature rat model of febrile seizures on the occurrence of later generalized tonic-clonic and absence epilepsy. *Brain Res Dev Brain Res*. 2004;154(1):137-140. https://doi.org/10.1016/j.devbrainres.2004.10.001

87. Sarkisova KY, Gabova AV, Kulikov MA, Fedosova EA, Shatskova AB, Morosov AA. Rearing by foster Wistar mother with high level of maternal care counteracts the development of genetic absence epilepsy and comorbid depression in WAG/Rij rats. *Dokl Biol Sci*. 2017;473(1):39-42. https://doi.org/10.1134/S0012496617020077

88. Russo E, Citraro R, De Fazio S, Torcasio G, De Sarro G, Di Paola ED. Effects of ethanol on the development of genetically determined epilepsies in rats. *Int J Dev Neurosci*. 2008;26(7):739-744. https://doi.org/10.1016/j.ijdevneu.2008.07.002

89. Dezsi G, Ozturk E, Stanic D, Powell KL, Blumenfeld H, O'Brien TJ, et al. Ethosuximide reduces epileptogenesis and behavioral comorbidity in the GAERS model of genetic generalized epilepsy. *Epilepsia*. 2013;54(4):635-643. https://doi.org/10.1111/epi.12118

90. Kovács Z, Czurkó A, Kékesi KA, Juhász G. Clomipramine increases the incidence and duration of spike-wave discharges in freely moving WAG/Rij rats. *Epilepsy Res*. 2010;90(1-2):167-170. https://doi.org/10.1016/j.eplepsyres.2010.02.011

91. Pitkänen A, Nehlig A, Brooks-Kayal AR, Dudek FE, Friedman D, Galanopoulou AS, et al. Issues related to development of antiepileptogenic therapies. *Epilepsia*. 2013;54 Suppl 4:35-43. https://doi.org/10.1111/epi.12297

92. Pitkänen A, Lukasiuk K. Mechanisms of epileptogenesis and potential treatment targets. *The Lancet Neurology*. 2011;10(2):173-186. https://doi.org/10.1016/S1474-4422(10)70310-0

93. Kobow K, Auvin S, Jensen F, Löscher W, Mody I, Potschka H, et al. Finding a better drug for epilepsy: antiepileptogenesis targets. *Epilepsia*. 2012;53(11):1868-1876. https://doi.org/10.1111/j.1528-1167.2012.03716.x

94. Liautard C, Scalmani P, Carriero G, de Curtis M, Franceschetti S, Mantegazza M. Hippocampal hyperexcitability and specific epileptiform activity in a mouse model of Dravet syndrome. *Epilepsia*. 2013;54(7):1251-1261. https://doi.org/10.1111/epi.12213

95. Marguet SL, Le-Schulte VTQ, Merseburg A, Neu A, Eichler R, Jakovcevski I, et al. Treatment during a vulnerable developmental period rescues a genetic epilepsy. *Nat Med*. 2015;21(12):1436-1444. https://doi.org/10.1038/nm.3987