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.

Keywords: epileptogenesis; absence seizures; comorbidity; depression; 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, 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 \cite{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 \cite{17,18}, it might be theorized that during the epileptogenic process continuous pathological modifications occur \cite{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 \cite{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 \cite{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 \cite{20-22}. To date, genes that control SWDs, in these rats, have only been detected on chromosomes 5 and 9 \cite{23}, whereas none specific gene mutation was detected for the SRS onset in this strain \cite{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 \cite{24-26}. Very recently, it has been described as the surgical removal of both foci, in these rats, totally abolished SWDs \cite{24}. According to these evidence, it is possible to hypothesize that these focal epileptic areas could be genetically programmed to become hyperexcitable \cite{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 \cite{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 \cite{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, 8, 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

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Doses and treatment</th>
<th>Effects on SWDs onset</th>
<th>Effects against neurological / psychiatric comorbidities</th>
<th>Drugs effects duration after 5 months of discontinuation</th>
<th>Ref(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethosuximide</strong></td>
<td>300 mg/kg/day (Started at P21 up to 5 months)</td>
<td>Decreased SWDs onset</td>
<td>NA</td>
<td>NA</td>
<td>[9,19]</td>
</tr>
<tr>
<td></td>
<td>300 mg/kg/day (Started at P30 up to P150)</td>
<td></td>
<td>Reduced depressive-like behaviour</td>
<td>NA</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg/day (Started at P42 up to 5 months)</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg/day (Started at P30 up to 5 months)</td>
<td></td>
<td>Reduced depressive-like behaviour</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Levetiracetam</strong></td>
<td>80 mg/kg/day (Started at P42 up to age of 5 months)</td>
<td>Decreased SWDs onset</td>
<td>Pro-depressant effects</td>
<td>NA</td>
<td>[51]</td>
</tr>
<tr>
<td><strong>Zonisamide</strong></td>
<td>40 mg/kg/day (From P42 up to age of 5 months)</td>
<td>Decreased SWDs onset</td>
<td>None</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Carbamazepine</strong></td>
<td>20 mg/kg/day (Started P42 up to age of 5 months)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td><strong>Clomipramine</strong></td>
<td>20 mg/kg/i.p (Started P8 up to of P21)</td>
<td>Decreased SWDs onset</td>
<td>Pro-depressant effects</td>
<td>NA</td>
<td>[46]</td>
</tr>
<tr>
<td><strong>Vigabatrin</strong></td>
<td>100 mg/kg/day (Started P30 up to 5 months)</td>
<td>Decreased SWDs onset</td>
<td>Reduced depressive-like behaviour</td>
<td>NA</td>
<td>[61]</td>
</tr>
<tr>
<td><strong>Perampanel</strong></td>
<td>3 mg/kg/day (Started P30 up to 5 months)</td>
<td>Decreased SWDs onset</td>
<td>Reduced depressive-like behaviour None effects on anxiety-like behaviour and cognitive decline</td>
<td>Increased SWDs and depressive-like behaviour</td>
<td>[62]</td>
</tr>
<tr>
<td><strong>Rapamycin</strong></td>
<td>1 mg/kg/day (Started at P45up to 5 months)</td>
<td>Decreased SWDs onset</td>
<td>Pro-depressant effects</td>
<td>Maintained</td>
<td>[63]</td>
</tr>
<tr>
<td><strong>Etoricoxib</strong></td>
<td>10 mg/kg/day (Started P45 up to 5 months)</td>
<td>Decreased SWDs onset</td>
<td>NA</td>
<td>NA</td>
<td>[64]</td>
</tr>
<tr>
<td><strong>Atorvastatin</strong></td>
<td>10 mg/kg/day (Started P45 up to 5 months)</td>
<td></td>
<td>Reduced depressive-like behaviour</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Simvastatin</strong></td>
<td>10 mg/kg/day (Started P45 up to 5 months)</td>
<td></td>
<td>Reduced depressive-like behaviour</td>
<td>NA</td>
<td>[65]</td>
</tr>
<tr>
<td><strong>Pravastatin</strong></td>
<td>30 mg/kg/day (Started P45 up to 5 months)</td>
<td></td>
<td>Reduced depressive-like behaviour</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>
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

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

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].
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 Etoricoxib
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 month 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 

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 showed 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


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