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CRITICAL REVIEW

A complex systems view on the current hypotheses of epilepsy pharmacoresistance

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Abstract

Drug-resistant epilepsy remains to this day as a highly prevalent condition affecting around one-third of patients with epilepsy, despite all the research and the development of several new antiseizure medications (ASMs) over the last decades. Epilepsies are multifactorial complex diseases, commonly associated with psychiatric, neurological, and somatic comorbidities. Thus, to solve the puzzling problem of pharmacoresistance, the diagnosis and modeling of epilepsy and comorbidities need to change toward a complex system approach. In this review, we have summarized the sequence of events for the definition of epilepsies and comorbidities, the search for mechanisms, and the major hypotheses of pharmacoresistance, drawing attention to some of the many converging aspects between the proposed mechanisms, their supporting evidence, and comorbidities-related alterations. The use of systems biology applied to epileptology may lead to the discovery of new targets and the development of new ASMs, as may advance our understanding of the epilepsies and their comorbidities, providing much deeper insight on multidrug pharmacoresistance.

KEYWORDS
comorbidities, complexity, drug-resistance, emergent properties, epilepsy

Abbreviations: ASM, Antiseizure medication; ASP, Anticonvulsant Screening Program; CBD, cannabidiol; DMN, default mode network; DRE, drug-resistant epilepsy; ETSP, Epilepsy Therapy Screening Program; ILAE, International League Against Epilepsy; MES, maximal electroshock; NINDS, National Institute of Neurological Disorders and Stroke; PWE, patients with epilepsy; SE, status epilepticus.

1 | DEFINITIONS AND CHANGING CONCEPTS ON EPILEPSY, COMORBIDITIES, AND PHARMACORESISTANCE

Key Points

- A complex systems approach on epilepsies, drug resistance, and comorbidities are absolutely required to advance research, diagnosis, and therapy
- Comorbidities are an integral part of epilepsy
- Isolated, the hypothesized mechanisms of pharmacoresistance offers only a partial view of the issue
- There is a need for better, more complex, experimental models of epilepsy and drug-resistant epilepsy

Epilepsy is a highly prevalent chronic neurological disorder estimated to affect about 50 million people worldwide.[1] From ancient cultures' recognition of the epilepsies as brain-dependent phenomena to their contemporary treatment, executed with rational, non-supernatural views and tools, three generations of antiseizure medications (ASMs) have been developed.[2,3] However, despite the exponential increase of clinically available ASMs over the past decades, an estimated 30% of the patients are still unable to achieve sustained seizure freedom through pharmacological treatment.[3,4] Drug-resistant epilepsy (DRE) is defined as the failure to achieve sustained seizure freedom after the trials of two appropriately chosen and used antiepileptic drug schedules.[5] Indeed, DREs are conditions that severely affect the patient's quality of life and are correlated with an increased risk of premature death.[6,7]

Because of the obvious consequences of DRE, researchers have invested their efforts in the elucidation of the underlying mechanisms of pharmacoresistance in epilepsy, which led to the proposal of several mechanistic hypotheses (for comprehensive reviews, see Tang et al, 2017[8]; Löscher et al, 2020[9]; Janmohamed et al, 2020[10]; and Pérez-Pérez et al, 2019[11]). These hypotheses offer varying degrees of evidence and scientific plausibility, but, and this will be the main point of our review, isolated, none of them adequately explains the phenomena of multidrug resistance in the epilepsies.[8] But of course, the nervous system did not appear in a vacuum, it is the product of millions of years of evolution, as are the dysfunctional mechanisms which serve as the basis for the pathogenic mechanisms of epilepsy and its comorbidities. So why should any of these hypothesized mechanisms occur in isolation?

The absolute bottom line is that although we have hundreds of years of the search, with enormous amounts of strategies, for the causes of the epilepsies and their associated comorbidities (for a comprehensive review see Garcia-Cairasco et al, 2021[2]), and also a great variety of attempts to diagnose and to treat those complex neurological-neuropsychiatric entities, the number of people with them are still too high.

No matter what has been the rationale used in the development of every ASMs since the introduction of bromide as an antiseizure medication in 1857, the concrete situation is that their actual effectiveness continues to be too low, only 45.7% of adult patients with newly diagnosed epilepsy reach seizure freedom in the first ASM tried regimen.[12] The joint efforts of cell and molecular biologists, with contemporary pharmacological and computational tools, frequently used to make quite selective target predictions, through the use of simulations or docking protocols, among others, have not yet been enough to improve the overall effectiveness of newly released ASMs.[13] We think that in addition to genuine needs, coming from disease, patients, family, and science-medicine, undesirable commercial pressure has been a relevant factor for a sequence of problems in the path of developing new treatments.

Notably, there is no clear definition, right from the beginning, of epilepsies as multifactorial entities [see the more recent International League Against Epilepsy (ILAE) classifications in Fisher et al (2017)[14] and Scheffer et al (2017)[15]], an aspect that hinders the need to examine them through a complex systems approach. Although seen as a general situation, our limited knowledge in science and medicine makes the consequent definition of signs and symptoms (semiology) and the associated diagnosis much more challenging, with or without the aid of other patient´s exams (eg, EEG, CT scan, MRI, fMRI, MEG). In the cases in which the diagnosis of the seizures and epilepsies are inappropriate, it is probable that so will be the treatment. Even if we assume we had the ability, although with the mentioned constraints, to correctly classify the dozens of seizures and the so-called epileptic syndromes, there would still be the unclassified types, with unknown onset and etiology and often overlooked comorbidities.[14,15] How much, indeed, of the recognition of seizure differences and types correspond with our advancement in the production of new ASMs?

Furthermore, if we consider the multitude of factors associated, causally or not, with the expression of epilepsy in humans, there would be no reason to think that single, specifically directed treatments, should be considered as the best therapeutic strategy for the vast majority of patients. The presence of single-gene mutations, channelopathies, and other similar "narrow" genotypes, seems to be

a plausible justification for the search for selective therapies (pharmacological or not), which, in fact, is one of the main principles of contemporary precision medicine.[16] Indeed, most epilepsies bear multifactorial etiologies, being a result of both genetic (polygenic) and environmental factors. As such, patients with epilepsy (PWE) may be afflicted by a vast diversity of seizure patterns and associated comorbid conditions. In clinical practice, the apparent paradox between the reductionist approach to drug discovery and the complex nature of the epilepsies is managed by using multiple or add-on medications, with the additional issue of side effects, mostly because of pharmacological interactions and effects on unknown targets.[17] Finally, we need to add to this picture, both in the diagnosis and in the treatment of the epilepsies, the notorious presence of neuropsychiatric (and other) comorbidities, appearing before, simultaneously, or after the epileptic seizures.

This current halt of the progress in the development of new therapies is not new in neurology/neuropsychiatry/neurosciences since similar cases can be found, for instance, in other conditions, such as depression and Alzheimer's disease. For the latter, there has been, over several decades, huge investment and disappointment, in the production of anti-Alzheimer´s therapies with a failure rate of 99.6% in clinical trials, which could be attributed to persistence in a single set of hypotheses.[18] In the case of antidepressants, the search for any association between central[19,20] or peripheral[21] biomarkers of depression brought little improvement to patients' quality of life. Specifically, Insel (2017)[22] refers to the lack of improvement in our ability to decrease the number of depressive patients and suicidality, in spite of the high investment in that kind of research, as reasons why he proposes "digital phenotyping"[23] and the use of technology as a contemporary, more efficient alternative. What is important for the current discussion, is that the above-mentioned conditions, with their own challenges in diagnosis and treatment, are often comorbid in PWE. As it will be discussed further, most comorbidities share with epilepsy the same biological substrate and intersecting cellular and molecular pathways, in manners that any attempt to exam them isolated from each other would only provide a partial understanding of any of these conditions.

2 | DRUG SCREENING AND THE MODELING OF EPILEPSY AND DRUG RESISTANCE

Let's look for a moment to the strategies for the search for brain networks, cellular and molecular mechanisms of the epilepsies, and by consequence, of the expected ASMs. Since 1936, when the first preclinical models of epilepsy started being used in drug screening, thousands of substances have been assessed for their potential as ASMs.[24,25] In fact, from 1975 to 2017 over 32 000 potential ASMs have been tested as part of The Epilepsy Therapy Screening Program (ETSP) of the National Institute of Neurological Disorders and Stroke (NINDS).[26] This program proposes the use of a select number of rodent models of epilepsy with sufficient screening power and high throughput such as maximal electroshock (MES), 6 Hz electrical stimulation, corneal kindling, amygdala kindling, *Status Epilepticus* (SE) models with spontaneous recurrent seizures, among others.[26] The current ETSP was reformulated from what used to be known as the Anticonvulsant Screening Program (ASP). The renaming and the revision from the original ASP reflect the new emphasis of the program on the identification of novel antiseizure agents for the treatment of patients with DREs, as well as disease-modifying substances that could "ameliorate or even cure established epilepsy and its comorbidities."[26] These are commendable steps in the right direction; the current approach, however, still focuses mainly into models of temporal lobe epilepsy and do not reflect our current understanding of the epilepsies and their complexity.[2,27] Therefore, it will be interesting to see if the number of models of the ETSP can be increased over time to include not only new rodent models (or less frequently used, such as the genetic ones) but also new *in vitro* and *in silico* models, as well as non-rodent models such as Caenorhabditis elegans and zebrafish.

Regarding the experimental protocols used to study pharmacoresistance, preclinical models of DRE can be broadly categorized into two groups: models with the selection of "responder" and "nonresponder" subgroups of animals and *per se* drug-resistant models, where inherent resistance can be observed (for comprehensive reviews, see Löscher et al, 2020[9]; Campos et al, 2018[28]). The first of these approaches is built upon the paradigm proposed by Löscher et al (1993),[29] that standard ASMs such as phenobarbital and phenytoin can be used to treat amygdala-kindled or post-SE epileptic rats, which can, thereafter, be categorized into subgroups based on their treatment responsiveness.

Part of the "differentiation phase" of the ETSP workflow,[26] the lamotrigine-resistant kindling rat model, a per se drug-resistant model, was originally described by Postma et al (2000).[30] This model is based on the finding that exposure to lamotrigine in the electrical amygdala kindling model, produces poor drug responsiveness to lamotrigine, as well as other ASMs.[30] Similar effects also have been demonstrated to occur with carbamazepine.[31] A lamotrigine-resistant corneal kindling (60 Hz) mice model has recently been developed by Koneval et al (2018).[32] In

this protocol, mice exposed to lamotrigine during the kindling process became insensitive to lamotrigine, as well as carbamazepine, retigabine, and valproic acid.[32]

It is interesting to notice that despite their extensive use, genetic models of epilepsy are less frequently used in ASM screening programs and pharmacoresistance studies. In favor of them, we present here a couple of cases: first, the one of levetiracetam, one of the most important ASM of the second generation, which would not be released if the acute PTZ and MES models were the only tested. Indeed, levetiracetam was discovered in a test made in audiogenic susceptible mice.[33] Additional effects of levetiracetam were observed in two genetic rat models of epilepsy: GAERs (absence seizures) and WAG/Rij (audiogenic and absence seizures).[34] Furthermore, pioneer assays were made in Brazil with cannabidiol (CBD), with audiogenic seizures induced after barbiturates abstinence.[35] Further, CBD behavior as a potent ASM and antiepileptic drug has been confirmed internationally in genetic models, such as the Wistar Audiogenic Rat (WAR) strain, in our and other laboratories.[36–38]

It is unfortunate for the patients and families, but also for the clinicians/surgeons and the basic scientists that we still depend on a tunnel view that has plagued the last decades of epileptology, with separate concerns and selective solutions, ignoring the fact that we are dealing with phenomena that as mentioned above, if seen as complex challenges, therefore, they will also need complex solutions.[39,40] This is particularly the case when on top of the epilepsies' networks, we find overlapped the neuropsychiatric entities networks, among others, as comorbidities.[39] The solution to these "networks of networks" problem needs strongly non linear metrics, connectedness, computational neuroscience algorithms, among others, as tools.

3 | RECOGNITION THAT EPILEPSY AND COMORBIDITIES RESEARCH NEED COMPLEX SYSTEMS APPROACHES

The definition of complex systems and their consequent emergent properties can be applied to several issues in the fields of mathematics, physics, computer sciences, sociology, economy, and biology, as well as in medicine and neuroscience.[41,42] Indeed, in the particular case of the brain, we are dealing with a system with a large number of interacting components with nonlinear dynamics, self-organized to give emergence to different scales of complexity. Thus, as a complex system, brain function and stability are susceptible to chaotic effects, which entails that even minor alterations may ripple through different levels of its constitution (eg, molecular, cellular, tissue, organ, system, and organism) and may, therefore, result in unpredictable effects over time.[43]

In that scenario, there are also several reasons to examine epilepsy through the scope of a complex system problem. Epilepsies share properties of complex systems, such as the emergence of patterns that cannot be easily predicted by the description of their constituting elements.[39] These elements function at different scales of resolution and are structured in a hierarchical manner (Figure 1). Gene expression and regulation are required for protein synthesis and cellular processes, elements fundamental for the structuring of tissues, synaptic signaling and neuronal plasticity, conditions which, in turn, give rise to small and large networks and circuits of cells, interacting with each other, making possible the emergence of behaviors, and in the case of epilepsies and comorbid conditions, the emergence of dysfunction. This hierarchy, however, does not mean that any of these elements has higher importance than the others since none of them can naturally occur in isolation. The existence of feedback mechanisms also means that the association between these different hierarchies is often nonlinear in nature.[40] In effect, the complex patterns of interactions inside and between layers are responsible for many phenomena in epilepsy. A notable example is the lack of correspondence, and therefore, the consequent lack of predictability between etiology and the pattern of seizure expression. In fact, none of the numerous etiologies can predict with certainty the resulting seizure types.[39]

In our own experience, we began to discuss these issues when challenged by the need to make paradigm shifts, as a result of the attempt to remove the traditional view of the epilepsies as self-contained events in the so-called epileptic focus[44] and, more recently, with the recognition of connectedness between not only near but distant areas, the epileptogenic zone[45] or the epileptogenic network.[46,47]

We first demonstrated, for example, the behavioral sequences of epileptic seizures in acute and chronic preclinical models[38] and applied this methodology to the evaluation of seizures of patients with temporal and frontal lobe epilepsy. This quantitative semiology approach can be described through complex entities or behavioral clusters, built by probabilities of occurrence.[48–50] Briefly, the next following step, more than 12 years ago was to propose, based upon Kuhn's "The Structures of Scientific Revolutions,"[51] that a paradigm shift was needed, in the case of the epilepsies, first of all, using a "puzzle-solving strategy," to identify semiology and next, epileptogenic networks.[2,39,52]

Naturally, if the nervous system itself is a multiscale entity and is organized into different spatiotemporal and topological scales,[53] likewise, are brain networks. Neuronal networks are capable of generating complex patterns of synchronized activity, which are, in fact, essential for the normal functioning of the brain. In the case of pathologies, however, these patterns deviate from normality. In this sense, Default Mode Network (DMN) and other intrinsic connectivity network studies provide not only highly

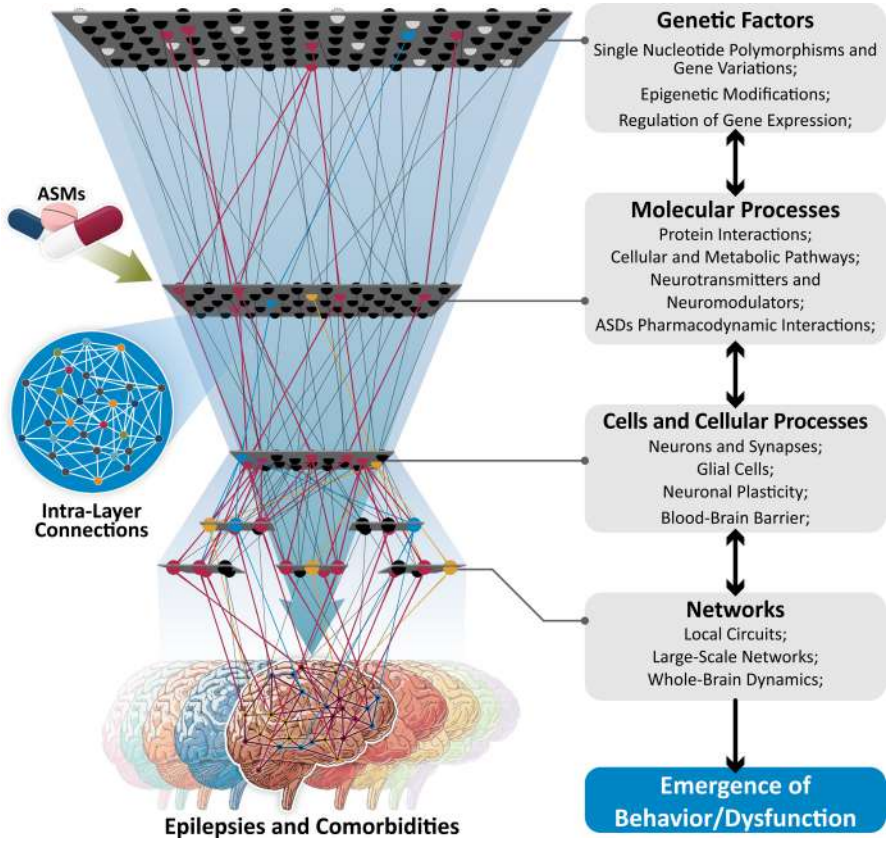

FIGURE 1 Schematic representation of the brain of a patient (or experimental model) with epilepsy and comorbidities, depicting the multiple scales of complexity, from genes to the emergence of behavior and dysfunction. Pathogenic alterations associated with epilepsies and comorbidities are represented as colored nodes and connecting lines of networks. Despite stratification into layers, nodes may establish intra- and inter-layer connections, with unidirectional and bidirectional modes of association. In fact, the organization of networks can follow different topologies and complexity rules

discriminative power in the identification of patients suffering from neurological and psychiatric disorders, such as major depression,[54] Alzheimer's disease,[55] and migraine[56] but also contribute to a better understanding of the underlying pathological mechanisms and neural circuits activity of these conditions. Notably, in epilepsy, during the occurrence of seizures, the physiologically dynamics of networks collapse into more synchronized oscillatory modes of activity with lower dimensions.[57] In this state, the synchronized patterns of activity may share some resemblance to the normal brain rhythms that would otherwise be present, at physiological states, in the structures that participate in the epileptogenic network (eg, hippocampal sharp-wave ripples, corticothalamic spindles, and cortical delta waves).[58]

4 | COMPLEXITY ABOUT DIAGNOSIS AND TREATMENT OF EPILEPSY AND ASSOCIATED COMORBIDITIES

Over the past decades, great effort has been made toward understanding epilepsy as a condition whose effects might be considered beyond the recurrence of seizures, therefore including its neurobiological, cognitive, psychological, and social aspects as part of its definition.[59] Epilepsies and neurological, psychiatric, and somatic disorders present diverse mechanisms of association (Figure 2A).[60] For example, the incidence of many comorbidities, such as cerebrovascular accidents, meningiomas, and brain neoplasms, is much higher in PWE when compared with the overall population, and present causative association, promoting molecular, structural, and functional alterations related to the onset of epilepsy (ie, epileptogenesis).[60,61] Conversely, epilepsies may serve as etiological factors leading to the development of comorbidities, either directly, through seizures (eg, aspiration pneumonia and seizure-related injuries), or indirectly as a side effect of the antiseizure treatment.[60] Other comorbidities share more complex patterns of association with shared risk factors (eg, single- nucleotide polymorphisms in pleiotropic genes) linking epilepsies and neurological and psychiatric disorders.[62]

Finally, there are strong bidirectional causal interactions between epilepsies and comorbidities.[63] Indeed, conditions such as migraine and depression are examples of bidirectionally associated comorbidities that can both precede or be preceded by the onset of epilepsy and are also associated with a poor response to pharmacological treatment and epilepsy surgery outcome.[63] Depression is one of the most common neuropsychiatric comorbidities to affect PWE, with a lifelong prevalence estimated to be somewhere between 10% to 24.9% in a population-based study but may reach a much higher prevalence at specialized epilepsy centers, where difficult-to-control epilepsy patients are more common.[64] Pathogenic mechanisms of depression have been previously proposed as being

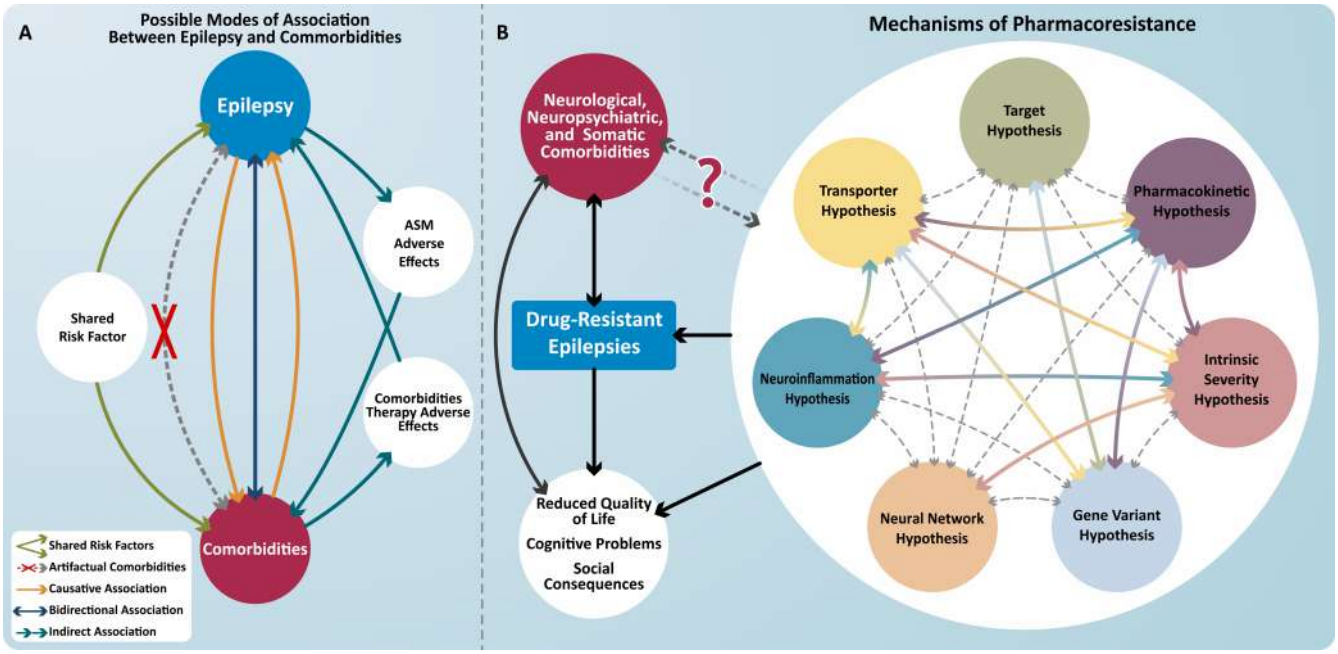

FIGURE 2 (A) Possible modes of association between epilepsy and comorbidities. Each arrow's color represents a different mechanism in which epilepsy and comorbidities might be associated. Grey dashed arrow represents artifactual comorbidities, in which there is no causal association between conditions. (B) Association between different comorbidities (neurological, neuropsychiatric, somatic), drug-resistant epilepsies (DREs) and the mechanisms of pharmacoresistance. The current hypotheses of pharmacoresistance share several converging aspects and linking evidence that when placed together, create a much more complete framework of ideas than any of them isolated. Although several links between DREs and some comorbidities have already been established, it remains unclear how these comorbidities could be interfering with the mechanisms of pharmacoresistance. The end-result, however, is clear: Patients with DREs and comorbidities suffer with reduced quality of life, cognitive problems, and several social consequences (eg, educational, professional, romantic). Arrows with a solid line represents known association between connecting nodes. Arrows with dashed line represents unestablished or inexistent association between connecting nodes

implicated in the epileptogenic process through several mechanisms, which can be categorized as endocrine abnormalities, structural and functional abnormalities of cortical and subcortical structures, neurotransmitter abnormalities, and immunological abnormalities (for review, see Kanner (2012)[65]). Likewise, migraine is a highly prevalent neurological comorbidity in PWE, affecting around 26% of patients.[66] The co-occurrence of epilepsy and migraine has important prognostic implications, being associated with treatment failure and should influence drug choice, as some ASMs, such as topiramate and valproic acid, have for example, analgesic value.[67] Common genetic alterations, neurotransmitter disturbances, ion channel dysfunctions, and increased cortical excitability are some of the proposed pathogenic mechanisms responsible for the interaction of both conditions.[63]

Adding further complexity to the subject, both psychotropic drugs and ASMs are known for interfering in epilepsy and comorbid neuropsychiatric disorders, respectively.[67] Selective serotonin reuptake inhibitors and serotonin–norepinephrine reuptake inhibitors, drugs used as first-line therapy for primary depression and anxiety disorders, respectively, are associated with reduced seizure frequency among PWE affected by these comorbidities.[68,69] Conversely, the anxiolytic alprazolam and the antidepressant bupropion (immediate-release form) were associated with an increase in seizure incidence. The antipsychotics clozapine, olanzapine, and clomipramine, the latter an antidepressant also indicated for the treatment of obsessive-compulsive disorder, were also associated with a higher seizure incidence.[69]

5 | DEFINITIONS OF INTRACTABLE OR PHARMACORESISTANT EPILEPSIES AND COMORBIDITIES: PLAYING WITH MULTIPLE HYPOTHESES IN COMPLEX SYSTEMS AND EMERGENT PROPERTIES SCENARIO

After presenting the clear complexity associated to the origin, diagnosis, and search for mechanisms of the epilepsies and associated comorbidities, let us now take a

look at pharmacoresistance issues, naturally a consequence to the previous collection of entities, in fact a challenging puzzle.

The consensus definition of DRE determined by ILAE is derived from the observation that a majority of adult patients will achieve seizure freedom after the first or second ASMs tried regimes, with a pronounced decrease in the probability of achieving seizure freedom in successive trials.[5,12] This definition was created, therefore, not to be used as an unequivocal diagnosis, but as a testable hypothesis, allowing the early identification of difficult-to-control epilepsy patients by medical practitioners at all health care levels, prompting the referral of these patients to specialized centers for evaluation.[5]

According to the definition, to identify DRE, it is required to ensure that the ASM schedules were appropriately chosen and appropriately used.[5] Confounding factors such as poor adherence to the treatment, misdiagnosis of seizure type and etiology, psychogenic nonepileptic seizures, incorrect ASMs dosing, drug–drug interaction, and lifestyle problems (eg, tobacco and alcohol consumption, sleep deprivation, stress, and drug abuse) may lead to the incorrect diagnosis of drug-resistance.[70–72] While pseudorefractory epilepsy is itself a problem, the misdiagnosis in those cases may lead to tragic implications. When pharmacotherapy fails, alternative or complementary therapeutic approaches, with potential risks and side effects, should be employed in order to maximize the patient's quality of life. Some of these approaches include invasive procedures, such as resective and disconnective surgeries, which involve the removal or functional disconnection of non-eloquent regions of the brain, regions implicated in generating seizures (ie, the epileptic focus/epileptic zone) but that also may serve important functions outside of seizures.[61,71] These are the reasons why the evaluation and discernment between actual and apparent drug-resistance has to be performed by specialized multidisciplinary teams in epilepsy centers.[70]

As aforementioned, there are several hypothesized mechanisms of pharmacoresistance already proposed. In Table 1, we have summarized the main concepts of some of the most prominent hypotheses, their supporting evidence, and some converging aspects that link these different hypotheses. But despite the existence of supporting evidence, there are controversies and an overall lack of translation of these findings into clinical practice.[9,73] A growing consensus is that these hypotheses have limitations, and the mechanisms proposed are not always adequate to explain multidrug pharmacoresistance, if not considered as part of larger integrated phenomena.[8–11]

While the mechanisms proposed in the transporter,[8–11,74–83] pharmacokinetic,[8–11,84–89] target,[8–11,90–97] neural networks,[8–11,52,98–103] and intrinsic severity hypotheses[8–11,81,86,104] may offer a more direct association between alterations and drug resistance, mechanisms proposed in the gene variant[8–11,73,82,96,105–108] and neuroinflammatory[8–11,78,109–111] hypotheses offer a more integrative view, that could serve as the basis for the emergence of the other mechanisms (Table 1; Figure 2B).[2,8,9] Epigenetic changes associated with DRE have been previously identified and are likely another important regulatory component involved in the control of other mechanisms associated with drug resistance, but currently, there is not much evidence to support this hypothesis, the reason why it was omitted in Table 1.[9,11,112] The combination of those hypotheses makes it possible to build a much better and more cohesive framework of ideas, which may allow a new approach to the problem of DRE. Although it is fair to expect that not every aspect has equal contribution to the condition of every patient, it is, however, important to understand that all factors are associated in a way, even if not directly.[8,9,52]

As mentioned in the preceding topic, the presence of several comorbidities in PWE has been previously identified as relevant prognostic factors, associated with a poor long-term epileptological outcome. Psychiatric comorbidities have been identified as important predictors of pharmacoresistance.[113] A cohort study with data obtained from the Calgary Comprehensive Epilepsy Program database (Canadian population) has found that epilepsy patients with depression have a significantly higher chance of failing to achieve 1-year seizure freedom.[114] Nogueira et al (2017)[115] found that medial temporal lobe epilepsy patients with concurrent mood and anxiety disorder were about four times more likely to have pharmacoresistant epilepsy than psychiatric asymptomatic PWE. A prospective study (5-10-year follow-up) reported that comorbid migraine had a negative effect on the prognosis of epilepsy, in which patients that were diagnosed with both epilepsy and migraine had a higher incidence of intractable epilepsy and a significantly lower probability of being seizure-free over 10 years when compared with the epilepsy without migraine group.[116] A cohort study performed in 13 Italian epilepsy centers reported an inverse association between the degree of seizure control and psychiatric comorbidities, as well as endocrine/metabolic, and respiratory disorders.[117] Taken together, these reports present a strong association between comorbidities and DRE (Figure 2B). Although some mechanisms linking DRE and comorbidities, notably depression and migraine,[63,65] have already been proposed, further research in the field is yet required in order to elucidate the pathological mechanisms of association of comorbidities and the actual phenomena of pharmacoresistance in epilepsy.

TABLE 1 Hypothesized mechanisms of pharmacoresistance: main concepts, supporting evidence and converging aspects

Hypothesis [References]	Main concepts	Supporting evidence	Converging aspects (Associated hypotheses)
Transporter hypothesis [8-11,74-83]	Drug resistance originates from the increased or altered expression of efflux transporters across the blood-brain barrier (BBB), leading to decreased availability of ASMs at their site of action	P-Glycoprotein (P-gp or MDR) expression is markedly increased in the brain of patients with DRE. Several ASMs are substrate for transport by P-gp and other efflux transport. Increased P-gp expression is associated with phenytoin and phenobarbital resistance in the amygdala electric kindling model and a model of spontaneous recurrent seizures, respectively. Overexpression of P-gp is highly localized to the epileptogenic networks in patients with DRE. Other efflux transporters, such as multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP) are also associated with DRE and have altered expression in different pathological conditions of the CNS	Higher seizure frequency is positively correlated to increased P-gp expression (intrinsic severity hypothesis). DRE is associated with polymorphisms of ABCB1 and ABCC2 genes, which encode P-gp and MRP2, respectively (gene variant hypothesis)
Pharmacokinetic hypothesis [8-11,84-89]	Peripheral overexpression of efflux transporters, in organs, such as the liver, intestine, and kidney and increased drug metabolism, increase the clearance of ASMs, therefore, reducing ASMs plasma levels and availability to cross the BBB, resulting in refractoriness to treatment	In two case studies, subtherapeutic plasma levels of phenytoin and phenobarbital were found, despite constant iv administration. P-gp was also found to be overexpressed in resected brain tissue of these patients. Plasma concentration of free phenytoin has positive correlation with the patient's responsiveness to the therapy. Several Cytochrome P450 (CYP) enzymes, known to be involved in ASMs degradation are expressed in endothelial cells of the brain and have been found to be overexpressed in patients with DRE	ABCB1 gene polymorphism C3435T, associated with decreased intestinal P-gp expression and activity and polymorphic cytochrome P450 CYP2C9, have predictive value for phenytoin blood concentrations (gene variant hypothesis). Hemodynamic shear stress, such as the one caused by changes in cerebral blood perfusion during the ictal state, has been demonstrated do affect the expressions of several CYP enzymes (eg, CYP3A4, CYP2C9, CYP2C19, CYP1A1, CYP1B1, CYP2A6, CYP2B6, CYP2E1, CYP2J2) and multidrug transporters (e.g, P-gp, MRP5, MRP1) (intrinsic severity hypothesis)

(Continues)

TABLE 1 (Continued)

Hypothesis [References]	Main concepts	Supporting evidence	Converging aspects (Associated hypotheses)
Target hypothesis [8-11,90-97]	Acquired molecular level alterations to the structure and functionality of ASMs targets (ie, changes in potential-dependent ion channels, neurotransmitter receptors), such as changes in transcription, alternative splicing, and altered posttranslational modifications could result in decreased drug sensitivity	Carbamazepine's use-dependent blockade of sodium voltage-gated channels is lost in carbamazepine-resistant patients, as demonstrated by patch-clamp recordings of human hippocampal neurons. In the same study, similar effects were also observed in a rat model of spontaneous recurrent seizures (SRS) after pilocarpine induced *Status Epilepticus* (SE). Pilocarpine induced SE has also been demonstrated to alter the mRNA expression of $GABA_A$ receptors in rat hippocampal neurons. $GABA_A$ receptors subunit expression was also found to be altered in the hippocampus of phenobarbital-refractory rats with SRS. Alteration of $GABA_B$ subunits mRNA expression has also been identified in the resected tissue of temporal lobe epilepsy patients with DRE. Patients with comorbid anxiety and/or depression showed increased expression of the mRNA encoding the γ2-subunit and reduced $GABA_B$ activation, despite elevated binding	Polymorphism of the gene SCN2A (IVS7-32A>G), which encodes de sodium channel $Na_V1.2$ has been associated with DRE (gene variant hypothesis). Polymorphism of the subunit gamma 2 of $GABA_A$ receptor predicts susceptibility to pharmacoresistance in Idiopathic Generalized Epilepsy (gene variant hypothesis)
Neural Network Hypothesis [8-11,52,98-103]	Epilepsy-related structural alterations, such as axonal sprouting, synaptic reorganization, aberrant neurogenesis, gliosis, and neurodegeneration can lead to the state of drug resistance through the formation of an abnormal neural network. These structural changes could not only contribute to a reduced inhibitory effect of the endogenous antiepileptic system, but also prevent ASMs from reaching their targets.	Altered gene expression of genes involved in cytoskeleton, synaptic plasticity, cellular reorganization, and growth cone function have been identified in the brain of DRE patients. Some of these genes, also involved in the development of the nervous system, are kept active during the epileptogenic process; Hippocampal mossy fiber sprouting and hippocampal sclerosis are common findings in TLE, that can also be found in experimental models being associated with DRE. Cortical malformations such as focal cortical dysplasia are common etiologies in patients with DRE. Epilepsy and several neuropsychiatric comorbidities have network dysfunctions as central aspect of their pathophysiology	Excitotoxicity may occur as a result of the high release of glutamate during seizures (intrinsic severity hypothesis)

TABLE 1 (Continued)

Hypothesis [References]	Main concepts	Supporting evidence	Converging aspects (Associated hypotheses)
Intrinsic severity hypothesis [8-11,81,86,104]	Epilepsy-related neurobiological factors contribute to define epilepsy in a range from mild to severe and determine its response to pharmacological treatment.	Frequent seizures are related to neurobiological factors associated to drug resistance	Seizure frequency, and seizure-related alterations are associated with the expression of efflux transporters and CYP enzymes (see transporter and pharmacokinetic hypotheses)
Gene variant hypothesis [8-11,73,82,96,105-108]	Endogenous variance in genes involved in the pharmacodynamics and pharmacokinetics of ASMs or genes associated with the epileptic phenotype could be the source of drug resistance.	Non-synonymous single-nucleotide polymorphisms (SNPs) can lead to changes in conformation, binding affinity and can even result in truncated forms of the encoded protein. Synonymous SNPs, which do not change the amino acid sequence, may affect messenger RNA splicing, stability, and structure, therefore affecting gene expression and protein function. Next-generation sequencing has been demonstrated as an effective diagnostic tool which allows the identification of specific genetic alterations. Some of these variations are "actionable," and can help direct drug choice. Genes associated with DRE have been recently reviewed by Cárdenas-Rodriguez et al (2020).[107]	SNPs of genes also associated with the transporter, pharmacokinetic, and target hypotheses (see mentioned topics above)
Neuroinflammation hypothesis [8-11,78,109-111]	Neuroinflammatory factors can induce BBB dysfunction and overexpression of efflux transporters resulting in loss of responsiveness to ASMs.	There are known links between inflammatory processes and epilepsy and epileptogenesis. Inflammation in the brain is associated with the loss of tight junctions between endothelial cells and induction of abnormal angiogenesis. Dysfunction of BBB and increased permeability to macromolecules, such as albumin can reduce the response to ASMs due to drug-binding. Inflammatory mediators, such as COX-2 and IL-1β, released by astrocytes and neurons as a result of hyperexcitability during the ictal state, may induce increased P-gp expression in astrocytes and endothelial cells	The neuroinflammatory hypothesis could be viewed as a possible underlying mechanism of the transporter and pharmacokinetic hypotheses (see mentioned topics above). Neuroinflammatory processes and mediators can lead to a state of hyperexcitability (Intrinsic severity hypothesis)

6 | CONCLUSIONS AND FUTURE CHALLENGES

To finish our discussion, we would like to stress, as commented in at the beginning of this review, that our proposals for an integrative view, with complex systems approach[39,40] of pharmacoresistance hypotheses, cannot be separated from the search for mechanisms, the definition and detailed diagnosis of the epilepsies and associated comorbidities, as well as their treatment. This is in absolute agreement, with the report of the workshop "Accelerating the Development of Therapies for Antiepileptogenesis (AEG) and Disease Modification (DM)" (Galanopoulou et al, 2021)[118] sponsored by the National Institute of Neurological Disease and Stroke (NINDS) "with the additional goal of informing the National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program as it develops new preclinical workflows to identify potential AEG and DM therapies." The workshop was organized into subgroups and the Preclinical Science subgroup "recognized several opportunities to advance the field forward, including the diversity of animal models, new tools to probe targets and biomarkers, and increasing knowledge about the mechanisms underlying epileptogenesis and comorbidities" and additionally highlighted:

> ...A significant gap is the difficulty in translating and validating preclinical discoveries to the clinic as well as the need to de-risk AEG/DM research. Looking forward, the Working Group proposed as high priority areas of research the development and validation of clinically relevant tools to identify, monitor, and regulate in vivo targets, processes, and networks involved in ictogenesis, epileptogenesis, and comorbidities, as well as develop infrastructure and strategies to validate and translate preclinical findings into the clinic. The engagement of the broader research community, as well as of other stakeholders, including expert patients, caregivers, or consumer organizations, into refining of research strategies and tools, data and expertise sharing, and enabling big data analyses was deemed essential in these efforts...

Finally,[118] still in the complex systems arena, the concepts of systems biology and systems pharmacology, as applied to epileptology,[17,119] particularly to ASM design, will be an avenue to shed light into the more efficient treatment of the epilepsies. It is, however, important to note that, even though rapidly evolving, the field of systems biology is still young and much of its methodologies and techniques are still in development. Its implementation will therefore require substantial investment in the research and development of new computational tools, but also in the qualification of individuals to work with such computational resources. Fortunately, several existing methods in systems biology and machine learning such as functional gene-gene interaction and weighted gene co-expression network analysis, principal component analysis, multidimensional scaling, and hierarchical cluster analysis, are already being used in epilepsy and other fields.[120] Exactly revealing a promising scenario, a recent study by Mirza et al (2021),[121] in fact, the product of the ILAE Consortium on Complex Epilepsies, is a notable example of such an approach. Using Genome-Wide Association Study (GWAS) summary statistics data and drugs' activity data on the function and abundance of proteins, they have developed a method to predict the antiseizure efficacy of drugs already used to treat other conditions, and have identified promising candidate molecules.[121] It is noteworthy that the authors validated the antiseizure effect of four out of five top candidate molecules, using the DBA/2 mouse model of audiogenic seizures,[122,123] a genetic model of epileptic seizures (see two recent comprehensive reviews on our experience in this field with the WAR strain in Garcia-Cairasco et al (2017)[38] and Lazarini et al (2021)[37]. It is important to highlight here that the WAR strain has been accepted recently by the Rat Resource and Research Center (RRRC) after a Material Transfer Agreement with the University of São Paulo and offered as the #697 donated strain to be available internationally to interested scientists. The RRRC is located at the University of Missouri and is supported by funding from the National Institutes of Health (P40 OD011062).

The results of the study by Mirza et al (2021)[121] demonstrate the efficacy of this approach and support our proposal of the inclusion of genetic models in ASM screening protocols. The use of the above-mentioned tools, applied to the study of the networks of interactions between the mechanisms of pharmacoresistance and comorbidities-related alterations may lead to the discovery of new targets and the development of new ASMs as well as advance our understanding of the epilepsies and their comorbidities.

For a comprehensive view on historical aspects, with old and new challenges, conflicts, and convergences for the research in both epilepsies and neuropsychiatric comorbidities, see Garcia-Cairasco et al, (2021).[2] Moreover, the concepts of precision medicine, as applied to psychiatry and of digital phenotyping,[23] initially and strongly proposed for a new psychiatry,[124] defined by Nesse (2019)[125] as evolutionary Medicine/Psychiatry, would be welcomed avenues of technological gain to the search not only for

mechanisms, for particularly for better diagnosis, follow-up and treatment of neuropsychiatric comorbidities associated with the epilepsies, with clear impact in the resolution of the still challenging pharmacoresistance issues.

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CONFLICT OF INTERESTS

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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