Epilepsy & Behavior 71 (2017) 250–273

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Review

The Wistar Audiogenic Rat (WAR) strain and its contributions to epileptology and related comorbidities: History and perspectives

Norberto Garcia-Cairasco [a,b,*], Eduardo H.L. Umeoka [a,b], José A. Cortes de Oliveira [a]

[a] Physiology Department, Ribeirão Preto School of Medicine, University of São Paulo, Brazil
[b] Neuroscience and Behavioral Sciences Department, Ribeirão Preto School of Medicine, University of São Paulo, Brazil

ARTICLE INFO

Available online 11 May 2017

Keywords:
WAR
Epilepsy
Neuroscience
Seizures
Audiogenic
Strain
Genetics
Comorbidities

ABSTRACT

In the context of modeling epilepsy and neuropsychiatric comorbidities, we review the *Wistar Audiogenic Rat* (WAR), first introduced to the neuroscience international community more than 25 years ago. The WAR strain is a genetically selected reflex model susceptible to audiogenic seizures (AS), acutely mimicking brainstem-dependent tonic–clonic seizures and chronically (by audiogenic kindling), temporal lobe epilepsy (TLE).
Seminal neuroethological, electrophysiological, cellular, and molecular protocols support the WAR strain as a suitable and reliable animal model to study the complexity and emergent functions typical of epileptogenic networks. Furthermore, since epilepsy comorbidities have emerged as a hot topic in epilepsy research, we discuss the use of WARs in fields such as neuropsychiatry, memory and learning, neuroplasticity, neuroendocrinology, and cardio-respiratory autonomic regulation. Last, but not least, we propose that this strain be used in "omics" studies, as well as with the most advanced molecular and computational modeling techniques.
Collectively, pioneering and recent findings reinforce the complexity associated with WAR alterations, consequent to the combination of their genetically-dependent background and seizure profile. To add to previous studies, we are currently developing more powerful behavioral, EEG, and molecular methods, combined with computational neuroscience/network modeling tools, to further increase the WAR strain's contributions to contemporary neuroscience in addition to increasing knowledge in a wide array of neuropsychiatric and other comorbidities, given shared neural networks.
During the many years that the WAR strain has been studied, a constantly expanding network of multidisciplinary collaborators has generated a growing research and knowledge network. Our current and major wish is to make the WARs available internationally to share our knowledge and to facilitate the planning and execution of multi-institutional projects, eagerly needed to contribute to paradigm shifts in epileptology.

This article is part of a Special Issue entitled "Genetic and Reflex Epilepsies, Audiogenic Seizures and Strains: From Experimental Models to the Clinic".

1. Introduction

When discussing the etiologies of the epilepsies, we readily discover how difficult it is to detect and classify the highly heterogeneous and multifactorial causes associated with these neurological and neuropsychiatric entities. In a search for causality, we also realize that the study of human subjects, with respect to epidemiology, history, diagnosis, and response to therapeutics, is quite challenging. Further, neurologists and neuropsychiatrists commonly differ in their views, nicely summarized by Kanner and Barry [1] with the question "why do neurologists and psychiatrists not talk to each other?". We may also extend this to "why do basic neuroscientists and clinical epileptologists not talk to each other?"

With regard to studying the cause–effect relationships of epilepsy and related comorbidities by means of experimental or even computational models, some attempts have been made recently to define the main features of human epilepsy in order to develop reliable experimental models [2]. These include, for example, the necessary criteria for a model of mesial TLE (MTLE), such as seizures with non-convulsive behavioral signs, hippocampal sclerosis, and focal electroencephalographic (EEG) activity [3]. Depaulis and Hamelin [3] experimentally demonstrated that among three different MTLE models, only those resulting from the induction of focal status epilepticus (SE) appear to model such characteristics of human MTLE.

Indeed, to achieve a "paradigm shift" that addresses the urgent need for knowledge of underlying basic mechanisms and the detection of therapeutic targets, animal and computational models are required that

* Corresponding author at: Physiology Department, Ribeirão Preto School of Medicine, University of São Paulo, Avenida Bandeirantes, 3900, CEP 14049-900 Ribeirão Preto, SP, Brazil.
E-mail address: ngcairas@usp.br (N. Garcia-Cairasco).

http://dx.doi.org/10.1016/j.yebeh.2017.04.001
1525-5050/

mimic the necessary clinical features under more controlled conditions, facilitated by clinicians proposing testable hypotheses to basic neuroscientists for evaluation in their models [2,3].

With respect to experimental models, we need to choose between those based upon electrical, chemical or sensory stimuli (or combinations), as well as those produced with known genetic background, usually by inbreeding or genetic engineering, such as transgenics and knockouts [4–8]. Computational and network tools can model the complexity of the associated neural substrates [9,10].

This special issue of *Epilepsy & Behavior* is based on the international symposium "Audiogenic Epilepsies: From Experimental Models to the Clinic", held in September 2014 in Salamanca, Spain, where internationally recognized colleagues discussed their experience with genetically-developed models, especially audiogenic, in mice, rats, and hamsters (http://gredos.usal.es/jspui/bitstream/10366/125169/1/Programme%20book%20audiogenic%20epilepsy%20congress%202014.pdf).

In this paper, we first present selected data from different laboratories worldwide, including data that complements several articles in this special issue, based on several audiogenic seizure-susceptible strains, with comments on their historical development, as well as their main contributions to the epileptology literature from the perspectives of behavior, EEG, and cellular and molecular mechanisms. Next, we describe how studies with the WAR strain have contributed both to the neuroscience and the epileptology–neuropsychiatry research communities in elucidating the complexity of the epilepsies and their comorbidities. Whenever possible, we contrast WAR-related challenges, data, and perspectives with those of other models. We agree with Insel [11], who posed the question "animal models or model animals?", and who wisely recognized it is preferable to use the terms *"model organisms"* and *"model animals"* than models that try to mimic precisely the human condition, because we clearly know the former's limitations and their planned specific uses.

Making WARs available internationally is one of our highest priorities, because this will help in the design of multicenter protocols and facilitate the development of new knowledge and technology, including computational tools, for increasing the contribution of WARs to epileptology and, more broadly, to neuroscience.

2. Historical and comparative research priorities on the studies of the audiogenic seizure strains with emphasis on WARs

It is believed that in 1924, Studenzov [12] observed the first murine audiogenic seizure in Ivan Pavlov's laboratory when studying conditioning to sound in mice. Unfortunately, these observations were not known to the general scientific community because they were not published in English, though they are referred to in the literature [12–15].

Since then, in addition to known mouse strains such as the Frings [16–18] and the DBA/2 [19,20], several audiogenic rat strains were developed: the *Krushinsky–Molodkina* (K–M) strain in Russia [13], the *Genetically Epilepsy-Prone Rats* (GEPR) in the USA [21–23], the *P77PMC* in China [24], the *Wistar Albine Glaxo/Rijswijk* (WAG/Rij) in the Netherlands [25], the *Wistar Audiogenic Susceptible* (WAS) rats in France [26], the previously mentioned WAR strain in Brazil [4], and, more recently, the *Genetic Audiogenic Seizure-Prone Hamster* (GASH:Sal) in Spain [27–29].

There are several controversial aspects concerning the application of results produced with these audiogenic strains to human epileptology. For example, in this special issue, Wolf [30] reviews clinical aspects of reflex epilepsies and points out the difficulties in using results from audiogenic strains to understand these epilepsies. Audiogenic seizure strains do not fully mimic audiogenic seizures in humans. For example, Wolf [30] mentions that seizures in humans triggered from a simple acoustic stimulus are practically non-existent. Reflex auditory epilepsies are classified as idiopathic generalized, mostly with genetic origin, and are typically triggered by complex sounds or music with appropriate combinations of melody, harmony, and rhythm, and with associated impact on memory and cognition [31,32].

While animal strains susceptible to audiogenic seizures may not model a clinically-relevant form of audiogenic epilepsy in humans, they are nonetheless useful because they are genetically selected animals, with a known activation trigger and a clearly described phenotype, expressed either after acute acoustic stimulation (tonic–clonic seizures) or after chronic audiogenic stimulation (kindling), which itself is used as a model of TLE [33]. Further, depending on the strain, there is extensive information available about their behavior, EEG, and cellular and molecular aspects from laboratories worldwide. Therefore, these strains represent "*model animals*" rather than "*animal models*", after Insel [11], and we need to understand the limitations of these models on the one hand and what clinically germane issues they do model, on the other hand, such as seizure type, EEG correlates, brain regions/circuits involved, developmental aspects, and sex- and aging-related features. In this way, results/conclusions may be applicable even to the more complex human condition.

Initially, in this review we sought to qualitatively illustrate and contrast the contributions made by different laboratories working on genetic strains with susceptibility to audiogenic seizures. Therefore, we searched for publications in PubMed using appropriate terms to identify all relevant papers for three different audiogenic strains. We found 45 articles using the WAR strain developed in Brazil [4,36–38] with the terms [WAR], [Garcia-Cairasco N], [Doretto MC], and [Dutra Moraes MF]. We found 162 articles with the USA-developed strain, the Sprague–Dawley derived Genetically Epilepsy-Prone Rat [21,37], using the terms [GEPR] and [Genetically Epilepsy-Prone Rat]. Finally, we found 184 articles with the Europe-developed WAG/Rij rats [25] using the terms [WAG/Rij rats], [seizure], [epilepsy], and [absence].

After exporting the PubMed search results to the reference manager Zotero (https://www.zotero.org/download/), we inserted the block of text including title, abstract, and keywords (but without any authors' names) of all selected articles from each strain (WAR, GEPR, and WAG/Rij) in the notepad of Wordle (http://www.wordle.net/), which allowed us to make qualitative comparisons, represented visually as word clouds, between the three strains (Fig. 1), where the graphical depiction is based on word frequency (the most frequent words are displayed as the tallest). This qualitative (but not quantitative) method visually highlights the apparent research themes for publications involving the three strains. Based on the word clouds, it is obvious that the WARs are an audiogenic Wistar-derived strain of genetically-selected rats, and that their seizures mimic both tonic–clonic (acute) and limbic (chronic/kindled) seizures (Fig. 1, top). For the WAR strain, the predominant words in descending frequency (using only one word for cases of repeated words, such as *Wistar* and *wistars*) are Wistar, seizures, audiogenic, rats, WAR, animals, stimulation, acoustic, kindling, model, epilepsy, limbic, strain, and tonic–clonic; whereas the corresponding list for the GEPRs strain (Fig. 1, up-middle), also derived from the Sprague–Dawley line, are AGS, GEPR, seizure, genetically, GEPR-9s, IC, epilepsy-prone, neurons, colliculus, audiogenic, epilepsy, Sprague–Dawley, and neuronal. For the WAG/Rij strain (Fig. 1, down-middle), also Wistar-derived, but mostly a model of absence seizures mixed with audiogenic susceptibility, the predominant words in descending frequency are WAG-Rij, rat, absence, seizures, SWDs, epilepsy, discharges, SWD, Wistar, animals, spike–wave, cortex, model, and epileptic.

We further contrasted word clouds based on publications of these three audiogenic strains with those using another experimental non-audiogenic model, the classical amygdala electrical stimulation kindling model [38–40] (Fig. 1, bottom), using the PubMed search terms [amygdala] and [electrical kindling]. The predominant words for this non-audiogenic model, in descending frequency, are kindling, amygdala, seizures, rat, electrical, kindled, stimulation, neurologic, male, and animals.

This quite simple qualitative data analysis for the WAR, GEPR, and WAG/Rij strains can be readily applied to hundreds of articles reporting other audiogenic genetic or non-genetic models, and demonstrates a very conserved expression of features and methods that represent specific hallmarks or signatures of these strains when compared to the traditional amygdala kindling limbic seizure model. At present and in the future, mathematical or computational algorithms (probably as simple