Epilepsy & Behavior 73 (2017) 214–235

Contents lists available at ScienceDirect

Epilepsy & Behavior

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

Review

The relevance of inter- and intrastrain differences in mice and rats and their implications for models of seizures and epilepsy

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ARTICLE INFO

Article history:
Received 9 March 2017
Revised 29 May 2017
Accepted 29 May 2017
Available online 23 June 2017

Keywords:
Genetic heterogeneity
Inbred rodent strains
Genetic background effects
Genetic mapping

ABSTRACT

It is becoming increasingly clear that the genetic background of mice and rats, even in inbred strains, can have a profound influence on measures of seizure susceptibility and epilepsy. These differences can be capitalized upon through genetic mapping studies to reveal genes important for seizures and epilepsy. However, strain background and particularly mixed genetic backgrounds of transgenic animals need careful consideration in both the selection of strains and in the interpretation of results and conclusions. For instance, mice with targeted deletions of genes involved in epilepsy can have profoundly disparate phenotypes depending on the background strain. In this review, we discuss findings related to how this genetic heterogeneity has and can be utilized in the epilepsy field to reveal novel insights into seizures and epilepsy. Moreover, we discuss how caution is needed in regards to rodent strain or even animal vendor choice, and how this can significantly influence seizure and epilepsy parameters in unexpected ways. This is particularly critical in decisions regarding the strain of choice used in generating mice with targeted deletions of genes. Finally, we discuss the role of environment (at vendor and/or laboratory) and epigenetic factors for inter- and intrastrain differences and how such differences can affect the expression of seizures and the animals' performance in behavioral tests that often accompany acute and chronic seizure testing.

1. Introduction

Similar to humans, genetic background plays an important role in modulating both seizure susceptibility and its neuropathological consequences in rodent models of seizures or epilepsy, a factor which has received inadequate consideration in prior studies [1]. Outbred strains of mice (e.g., Swiss, NMRI or CD-1) or rats (e.g., Wistar or Sprague–Dawley (SD)) have been widely used in models of seizures or epilepsy, but such outbred strains can increase seizure variability with a high intrastrain phenotypic variation due to genetic heterogeneity [2,3]. Genetic divergence between outbred subpopulations may arise from a number of mechanisms, including natural selection, mutation, unconscious experimenter selection, and genetic drift [2,3]. Such intrastrain differences may be an important reason for discrepancies between studies from different laboratories using outbred strains. However, outbred strains of rodents can have important contributions to experimental design, since in many ways they can model the genetic diversity observed in the human population. But the genetic variance imparted by the use of outbred strains also needs to be factored into the experimental interpretations, particularly as it may relate to the understanding of single gene effects being studied (i.e., targeted deletions of genes). Furthermore, apart from genetics, intrastrain and interstrain differences in models of seizures or epilepsy can also be due to the environmental conditions under which the animals are bred and maintained [4].

It is generally assumed that using inbred strains of mice or rats minimizes the effect of intrastrain differences because of genetic homogeneity

Abbreviations: AD, afterdischarge; ADD, afterdischarge duration; ADHD, attention deficit hyperactivity disorder; ADT, afterdischarge threshold; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASD, autism spectrum disorder; B6, C57BL/6; BLA, basolateral amygdala; CNS, central nervous system; CRL, Charles River; EST, electroshock seizure threshold; GAERS, Genetic Absence Epilepsy Rat from Strasbourg; GSP, Genetic Stability Program; GST, generalized seizure threshold; HAR, Harlan; HFO, high frequency oscillation; HL, Harlan Laboratories; HPD, hippocampal paroxysmal discharge; HVRS, high-voltage rhythmic spike; HVSW, high-voltage spike discharge; HW, Harlan–Winkelmann; IGS, International Genetic Standardization; MES, maximal electroshock seizure; mTLE, mesial temporal lobe epilepsy; NMDA, *N*-methyl-D-aspartate; PDZ, polarity complex component; PTZ, pentylenetetrazole; QTL, quantitative trait loci; SD, Sprague–Dawley; SE, status epilepticus; SNP, single-nucleotide polymorphism; SPWS, sharp waves; SRP, signal-recognition-particle; SRS, spontaneous recurrent seizures; SUDEP, sudden unexplained death in epilepsy; SWD, spike–wave discharge; TLE, temporal lobe epilepsy; TMEV, Theiler's murine encephalomyelitis virus.

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http://dx.doi.org/10.1016/j.yebeh.2017.05.040
1525-5050/

within inbred strains. However, the inbreeding that makes an inbred strain so useful can also result in genetic divergence between differing substrains of the same inbred strain. This genetic divergence is often unaccounted for in experiments, but may be a confounding factor when comparing studies that have utilized different inbred substrains [1]. Inbred mouse strains may contain hidden or "quiet" mutations, that have no discernable effect, but which may be uncovered during behavioral phenotyping [5,6]. Inbred strains are also subject to new mutations and to genetic drift during breeding [5]. Careful attention is needed in rodent colony management to prevent or limit genetic drift by reintroducing the parental strain systematically into breeding programs. Furthermore, variation of the environmental conditions under which the animals are bred and reared at a specific vendor may have marked effects on animal behavior once the rodents are used for experiments, not to mention variabilities from University animal facility to animal facility. In addition, genetically similar mice from different commercial vendors may exhibit differences in their gut microbiota composition, which can exert profound variation in animal models [7,8].

When discussing strain effects on expression of seizures and epilepsy, it is important to consider the commonly misapplied distinction of wild-type rodents [9]. Multiple studies have demonstrated that some inbred strains of so-called wild-type mice, even if they never show spontaneous seizures, can be much more easily induced to have seizures than other wild-type strains. Clearly, mice are only wild-type with respect to a specific genetic locus and according to a specific user-defined assay for the structure or function of that locus in a controlled laboratory environment [9]. Every "wild-type" mouse carries multiple genetic differences (mutations or polymorphisms) that distinguish it from mice of other strains, although only some of these differences produce phenotypes that are obvious to a scientist, and many fewer that are relevant to phenotypes related to seizures and epilepsy. Nonetheless, a large body of evidence has accrued to document the strong influence of genetic variation on susceptibility to seizures or epilepsy, especially in rodent models.

In this review, it is not possible to discuss all the innumerous studies that have demonstrated inter- and intrastrain strain effects on expression of seizures and epilepsy in laboratory mice and rats. Rather, we will illustrate the impact of such strain effects by reviewing a series of experiments that were performed by our groups in the last ~25 years in a variety of models of seizures and epilepsy. Furthermore, some important studies from other groups will be highlighted. This review will not deal with rodent models of genetic epilepsies, such as spontaneously occurring mutants or genetically engineered rodents (including knockout and transgenic strains or lines) that are widely used as models of epilepsy. Our aim is to emphasize that even in the absence of engineered mutations, different inbred strains, and even substrains of the same inbred strain or sublines of the same substrain, can vary drastically in their susceptibility to induction of seizures or epilepsy. However, these genetic differences present opportunities to identify biological factors (i.e., genes) that are relevant to elucidating mechanisms of seizures and epilepsy. Thus, the use of different strain backgrounds, when studying epilepsy mutations, enhances the modeling of epilepsy as a complex genetic disease [10] and facilitates insight into the pathophysiology of epilepsy and for potential treatments. Another important issue that we will discuss is that, in addition to genetic inter- and intrastrain differences in rodents, differences in housing and handling of the animals, both at the vendor and in the laboratory, may have a marked impact on the expression of seizures and associated behavioral alterations. Importantly, some of these factors may have effects that are strain-specific. Lastly, we will briefly discuss age and sex differences in the expression of seizures and epilepsy in rodents and how this may affect inter- and intrastrain differences, whereas we will not discuss species (mouse vs. rat) differences that are of documented importance when interpreting data from rodent models of seizures or epilepsy.

2. Intrastrain differences in mice and rats

As described in the introduction, genetic intrastrain differences can occur in both outbred and inbred strains of mice and rats. Furthermore, epigenetic differences may occur in response to the environment under which the animals are born and raised (see Section 6).

For generating seizure or epilepsy models in rats, outbred strains such as Wistar or SD are often used. Outbred rat strains are known to be genetically heterogeneous populations with a high intrastrain phenotypic variation [2,3,11,12]. In contrast to inbred strains, outbred strains (or stocks) are "genetically undefined" in the sense that each animal is genetically unique and the alleles it carries are unknown until they are analyzed [3]. Like other outbred strains, SD and Wistar rats are randomly outbred; hence allelic variations can occur across separate colonies. Thus, stock names such as SD or Wistar can be misleading because stocks with the same name from different breeders may have different genetic and/or phenotypic characteristics [3]. The phenotypic characteristics of an outbred stock can change relatively fast as a result of random genetic drift in gene frequency, selective breeding (say for large litter size, body weight, blood pressure, etc.) and/or genetic contamination, which may go undetected because genetic quality control is rarely done [3]. As a consequence, outbred rat strains from different vendors may have little in common with each other besides their names and similarities in pelage [2,11]. Lack of standards and the unreliability of stock names hinder scientific reproducibility [3]. Genetic divergence between outbred subpopulations may arise from a number of processes, including mutation, natural selection, unconscious selection, and random genetic drift [13]. By far the most common source of genetic divergence among outbred subpopulations is random genetic drift, especially in small isolated populations. Genetic drift over time, and the resultant genetic divergence between colonies, is the inevitable result of breeding stocks and strains in isolation without reintroduction of founder stocks to the breeding program. This may also occur within the same large subsidiary of a breeder when subpopulations of outbred rodents are maintained in different barriers or breeding colonies over the long-term, eventually resulting in genetic drift and genetic divergence between barriers [14]. In addition to germline genetic differences between outbred SD or Wistar rats from different vendors or locations of the same vendor, intrastrain differences can also be due to housing and handling conditions at the vendor during development of the animals through presumed epigenetic modifications or early life stress effects on brain development [4,15–19].

To minimize inbreeding, Charles River Laboratories have adopted the International Genetic Standardization (IGS) Program for SD and Wistar rats. The aim of the IGS Program is to standardize multiple production colonies of these animals that are geographically separated. This insures that each colony has the same range of genetic variation. This is accomplished by monitoring heterozygosity and actively managing genetic drift that both could otherwise lead to colony divergence in outbred strains [13]. However, to our knowledge, other vendors have not adopted similar programs in rats (Jackson Laboratory has established a Genetic Stability Program (GSP) for mice). In any event, investigators need to consider the issue of intrastrain differences when planning and performing a study, and preferably use rats from the same stock and barrier for all experiments within a study. Furthermore, intrastrain differences may form an important bias when comparing results from different laboratories. On the other hand, once characterized, such differences provide a chance to study the impact of genetic and environmental factors on seizure susceptibility, epileptogenesis, and the relationship between behavior and epilepsy and vice versa.

The potential problems associated with outbred stocks of rats also apply to outbred mice [20]. However, genetic and epigenetic differences may also occur between inbred lines of mice from different vendors or different barriers of the same vendor, as shown by our experiments with pilocarpine in the C57BL/6 (B6) mouse strain [21], the most widely used inbred mouse strain in biomedical research. There continues to be