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Review

Perspectives of zebrafish models of epilepsy: What, how and where next?

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ABSTRACT

Epilepsy is a complex brain disorder with multiple underlying causes and poorly understood pathogenetic mechanisms. Animal models have been indispensable tools in experimental epilepsy research. Zebrafish (*Danio rerio*) are rapidly emerging as a promising model organism to study various brain disorders. Seizure-like behavioral and neurophysiological responses can be evoked in larval and adult zebrafish by various pharmacological and genetic manipulations, collectively emphasizing the growing utility of this model for studying epilepsy. Here, we discuss recent developments in using zebrafish models to study the seizure-like behavior involved in epilepsy, outlining current challenges and strategies for further translational research in this field.

Contents

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1. Introduction

Epilepsy is a common neurological disorder caused by an imbalance of excitatory and inhibitory processes [5,111,48,133]. In humans, it manifests in various types of seizures within several epilepsy syndromes [130,137] with both genetic and environmental determinants [124,109,149,104,70,44]. Animal models have long been used to study epilepsy, revealing striking similarities between experimental seizures and clinical phenotypes (Table 1). Genetic factors have also been explored in animal models, including multiple selectively bred [158,119] and genetically modified (knockout or transgenic) [15] strains with seizure-related profiles.

Despite the progress in this field, we still need better treatments and increased understanding of mechanisms of epilepsy in humans. The lack of novel antiepileptic drugs (AEDs) represents a challenge, requiring screening of multiple new compounds and pathways relevant to epilepsy [137]. Collectively, this emphasizes the growing importance of further innovative research using experimental models of epilepsy. As rodent models are expensive to maintain and more difficult to modify genetically, lower organisms emerge as useful species for the initial screening of drugs or mutations related to epilepsy [7]. Although invertebrates provide important insights into epilepsy [7,96,105], the absence of a complex nervous system limits their application in modeling intricate aspects of this disorder.

Addressing the need for novel experimental models of study seizure behavior and epilepsy [137,7], zebrafish offer a reasonable compromise between physiological complexity and throughput [20,141,142,161] for such testing. Zebrafish have a fully characterized genome, and display significant physiological homology to mammals, including humans (see [162,10,112] for review). The availability of both larval and adult zebrafish is also beneficial, enabling the investigation of a wider spectrum of epilepsy-related phenomena throughout the ontogenesis. However, it should be noted that both models are not without their limitations. For example, the smaller size of zebrafish also limits their use in assessing certain epilepsy interventions applicable to other animal models, such as deep brain stimulation [168]. The evolutionary divergence between humans and fish, as well as the more primitive nature of zebrafish behavior, further complicates their predictive validity [68,49,101].

However, despite these limitations, zebrafish possess several key characteristics useful for studying epilepsy and not offered by traditional models. For example, the faster development and longer lifespan of zebrafish, compared to mice, makes them an ideal choice to model developmental trajectories (e.g., early toxicant exposure or aging) of epilepsy pathogenesis. The ease of genetic manipulation has also lead to zebrafish being increasingly used to investigate the genetic aspects of epilepsy-related phenotypes [61,60,30], including high-throughput screens to identify gene mutations that confer seizure resistance [62,9]. Moreover, zebrafish also possess a tight junction-based blood–brain barrier, with substantial macromolecule permeability, yielding a high sensitivity to drugs [65,40]. The robustness of their phenotypes (exhibited through overt and easily quantified behavioral endpoints) and ease of treatment (e.g., immersion) further emphasizes the high-throughput nature of zebrafish [141,27,18,37]. Here, we will discuss the opportunities offered by zebrafish to the field of epilepsy research.

2. Experimental models of epilepsy using zebrafish

2.1. Pharmacological models

Recent studies have focused on behavior and brain activity in genetically modified or pharmacologically treated zebrafish. In larval models, animals (~5–7 dpf) are typically placed in multiple wells and monitored using video-tracking software, simultaneously recorded by a *top-view* camera [30,8]. Brain electrical activity during experimental epilepsy can also be recorded to generate electro-encephalograms (EEG) [61]. For example, combining EEG recording in agar-immobilized larvae with large-scale mutagenesis screening identified zebrafish mutations that confer resistance to chemically induced seizures [61]. Other sophisticated methods include *in vivo* Ca^{2+} imaging with genetically encoded indicators and extrinsic dyes, to visualize neural activity and networks during epilepsy [150]. Although larval zebrafish are crucial to modeling epilepsy (Table 2), they possess somewhat underdeveloped neural and endocrine systems, small body size and simple locomotor responses (see [161] for details). Thus, while larvae may be particularly useful for modeling

Table 1
Examples of typical phenotypes related to epilepsy in humans, rodents and zebrafish models.

Clinical epilepsy	Rodent models	Zebrafish models
Neurophysiological symptoms		
Brain hyperactivity	Increased neurophysiological responses in mice [69,1] and rats [165,95] Elevated brain *c-fos* expression in mice [122,123] and rats [24,145]	Increased neurophysiological responses in larval [61,9] and adult [110] zebrafish Elevated brain *c-fos* expression in larval [8] and adult zebrafish [161]
Behavioral symptoms		
Convulsions/seizures	Convulsive seizures in mice [156,97] and rats [52,56]	Hyperactivity/seizure behavior in larval [9,151] and adult [161,160] zebrafish (see Table 2 for details)
Behavioral impairments	Loss of posture in mice [106,34] and rats [102,63]; non-motor, absence-like epilepsy in mice [158,88] and rats [138,95]	Immobility with the loss of body posture and insensitivity to touch [35] (see Table 3 for details)
Sensitivity to selected antiepileptic drugs		
Barbiturates	Anticonvulsant in rodents [3,159,92,91]	Sedative in larval [167] and adult zebrafish [142]
Benzodiazepines	Anticonvulsant in rodents [3,92,147]	Anticonvulsant in larvae [14], anxiolytic in adult zebrafish [13,22]
Carboxamides	Anticonvulsant in rodents [91,90]	Anticonvulsant in larval [14]; alter brain biochemistry in adult zebrafish [127]
Fatty acids (valproic acid, vigabatrin, progabide, tiagabine)	Anticonvulsant in rodents [92,147]	Anticonvulsant in larval [14,64] and adult zebrafish [83], also improved learning [83]
Fructose derivatives (topiramate)	Anticonvulsant in rodents [91,134,67]	Anticonvulsant in larvae [14]
GABA analogs (gabapentin, pregabalin)	Anticonvulsant in rodents [159,90]	Anticonvulsant in larvae [14]
Hydantoins	Anticonvulsant in rodents [89] [139]	Anticonvulsant in larvae [14]; alter brain biochemistry in adult zebrafish [127]
Pyrrolidines	Anticonvulsant in rodents [98,99]	Anticonvulsant in larvae [14]
Succinimides	Anticonvulsant in rodents [159,92]	Anticonvulsant in larvae [14,42]
Sulfonamides	Protective role in absence epilepsy in rodents [120]	Anticonvulsant in larvae [14]
Triazines (lamotrigine)	Anticonvulsant in rodents [51,139]	Anticonvulsant in larvae [14]