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 an indispensable tool 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.

**Keywords:** Epilepsy, zebrafish, seizure, disease model, epileptogenesis, antiepileptic drugs, biomarkers
1. Introduction

Epilepsy is a common neurological disorder caused by an imbalance of excitatory and inhibitory processes [1-4]. In humans, it manifests in various types of seizures within several epilepsy syndromes [5, 6] with both genetic and environmental determinants [7-12]. 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 [13, 14] 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 [6]. Collectively, this emphasizes the growing importance of further innovative research using experimental models of epilepsy. As rodent models are expensive to maintain and difficult to modify genetically, lower organisms emerge as useful species for the initial screening of drugs or mutations related to epilepsy [16]. Although invertebrates provide important insights into epilepsy [16-18], the absence of a complex nervous system limits their application in modeling complex aspects of this disorder.

Addressing the need for novel experimental models of evoked-seizure behavior to study epilepsy [6, 16], zebrafish offer a reasonable compromise between physiological complexity and throughput [19-22] for such testing. Zebrafish have a fully characterized genome, and display significant physiological homology to mammals and humans (see [23-25] 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 [26] The evolutionary divergence between humans and fish, as well as the more primitive nature of zebrafish behavior, further complicates their predictive validity [27-29].

However, despite these limitations, zebrafish possess several key characteristics useful for studying epilepsy not offered by traditional models. For example, the faster development and longer lifespan of zebrafish, compared to rodents, makes them an ideal choice to model developmental trajectories (e.g., early toxicant exposure or aging) of epilepsy pathogenesis. Their ease of genetic manipulation has also lead to zebrafish being increasingly used to investigate the genetic aspects of epilepsy-related phenotypes [30-32], including the use of mutagenesis screens to identify gene mutations that confer seizure resistance [33, 34]. Moreover, zebrafish also possess a tight junction-based blood brain barrier that is similar to higher vertebrates, with substantial macromolecule permeability yielding a high sensitivity to drugs [35, 36]. 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 [20, 37-39]. Here, we will discuss the opportunities offered by zebrafish to study epilepsy.

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 [32, 40]. Brain electrical activity during experimental epilepsy can also be recorded to generate electro-encephalograms (EEG) [30]. For example, combining EEG recording in agar-immobilized larvae with large-scale mutagenesis screening identified zebrafish mutations that confer resistance to chemically induced seizures [30]. Other sophisticated methods include in vivo Ca\(^{2+}\)
imaging with genetically encoded indicators and extrinsic dyes, to visualize neural activity and networks during epilepsy [41]. 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 [22] for details). Thus, while larvae may be particularly useful for modeling early-onset (e.g., pediatric) epilepsy [42], other phenotypes (e.g., complex behaviors and biomarkers, see further) can be effectively modeled in adult zebrafish, and used to complement the strength of high-throughput larval screens [22].

Adult zebrafish are typically tested in observation tanks, where seizures are measured using a special scoring system [22, 43-45] either manually, or using video-recording for automated analyses [22, 44, 45] (Fig. 1; Table 2, see [45] for review). The ability to apply drugs via water immersion (rather than by injections, as in rodents) enables multiple fish to be simultaneously treated by adding the drug to system water to increase throughput (however, injections may also be performed [46]). Both top- and side-view recording of observation tanks are used for neurophenotyping of seizure-like responses in adult zebrafish [22, 30, 44, 45]. As shown in Table 2, typical endpoints relevant to epilepsy in zebrafish include hyperactive, spiral or circular swimming, rapid twitching, spasm-like body contractions, loss of body posture, paralysis (immobility) and death.

Several convulsant drugs, historically used in rodent models, include pentylenetetrazole (PTZ [47], picrotoxin [48], pilocarpine [49], kainate [50] and caffeine [51]. These drugs also evoke robust seizure-like responses in larval and adult zebrafish (Table 1), ranging from initial hyperactivity to convulsions and loss of posture/paralysis [52] (Table 2). The convulsant agent 1,3,5-trinitroperhydro-1,3,5-triazine (RDX) evokes similar seizure-like responses, including hyperactivity, spasms and corkscrew swimming [44]. There are also some differences in seizure-like activity among the drugs. For example, PTZ and picrotoxin induce generalized motor seizures [22], whereas caffeine and kainate also evoke spasms (own observations) and clonus-like head-shaking convulsions [43],
respectively. Figure 1A illustrates the effects of PTZ on adult zebrafish, showing robust seizure-like behaviors evoked by this convulsant agent (also note ‘jerky’ movements on representative locomotor traces).

Penicillin is another pro-convulsant agent that blocks gamma-aminobutyric acid (GABA) A receptors, similar to PTZ and picrotoxin [53, 54], but with lower potency and toxicity. This drug has already been tested in other fish species, showing abnormal electrophysiological responses [55] and “weaving” seizure-like behavior [56]. While the drug seemed to only slightly increase swimming activity in zebrafish at high doses (4-12 g/L, 20-min water immersion), it did not provoke overt spasms, circling or corkscrew swimming in a wide dose range (1.2-12 g/L) tested (data not shown). The doses of penicillin tested were relatively high, and approximately 10 times higher than active doses of PTZ. The lack of overt penicillin seizures in zebrafish is surprising, although this profile is somewhat similar to effects evoked by this drug in rodents [57], suggested to represent an experimental model of absence-like epilepsy without overt motor seizures [57, 58].

Strychnine is a potent convulsant neurotoxin that inhibits glycine and acetylcholine receptors, and has been a popular agent in modeling epilepsy in rodents [59-61]. This drug also affects zebrafish, inducing spasms, bursts of hyperactivity and circular swimming in adult animals (Fig. 1B) and fast bilateral contractions in larvae [62]. Strychnine is toxic in zebrafish, and therefore low doses and short pre-treatment time are generally needed to avoid mortality (own systematic observations). While the drug at a non-toxic dose tested did not affect distance traveled, velocity or immobility endpoints, it induced several seizure-like behaviors (Fig. 1B), as well as ‘jerky’ locomotion in 71±13% (P<0.001, U-test) and frequent ‘wavering’ in 79±11% (P< 0.0005, U-test) of fish. Overall, the ability of various convulsants to evoke robust seizures in zebrafish is important, as this parallels rodent data on pharmacogenic seizures and clinical findings on epilepsy in humans.

2.2.Genetic zebrafish models of epilepsy
Genetic factors play an important role in epilepsy [63-65], and have been extensively investigated in zebrafish models, including mutants showing spontaneous seizures [30-32] or hypersensitivity to convulsant drugs [66]. For example, mutation of the ubiquitin E3 ligase gene in developing larvae disrupts Notch signaling in the mind bomb (mib) zebrafish, leading to their seizure-like behavior and down-regulation of GABA-ergic genes [30, 31]. Likewise, inhibition of potassium voltage-gated KCNQ3 channels using antisense morpholino oligonucleotides affects the excitability regulation of central neurons, causing seizure behaviors in freely swimming zebrafish larvae [32]. The morpholino-induced knockdown of the epilepsy susceptibility gene la (lgi1a) evoked a similar seizure-like phenotype in larval zebrafish, with low-dose morphants showing hypersensitivity to PTZ seizures, and high-dose morphants exhibiting hyperactivity and seizure-like behavior [66]. Collectively, this demonstrates the growing utility of zebrafish models for studying genetic and pharmacological modulation of epilepsy-related phenotypes. The question which remains, however, is how to make this screening more efficient? We will attempt to address this important question further.

3. Strategies for further research

3.1. Modeling pathogenesis across different domains

Since seizures and epilepsy represent related but distinct phenomena that are part of several different syndromes [67-70], complex multi-domain approaches are necessary to understand their pathogenesis. For example, seizures can be caused by brain trauma, tumors, ischemia or stroke. Zebrafish-based models of stroke have already been reported in the literature [71], and assessing seizure-related responses in such models may be relevant to modeling the clinical picture of organic epilepsy.

Comparison between various species is also important for uncovering mechanisms underlying brain pathogenesis [72]. For example, paralleling zebrafish seizure profiles with those of goldfish,
guppies, medaka fish and other well-characterized aquatic species may enable a better dissection of observed phenotypes, also widening the spectrum of models available to study epilepsy. While complementary use of zebrafish and other aquatic models are an important strategy for translational research in this field, it represents an integral part of a more global cross-species modeling - from fish to rodents, primates and humans - for uncovering evolutionarily conserved mechanisms of epileptogenesis.

3.2. Thinking outside the phenotypic box: looking for new biomarkers

In addition to developing new models, the discovery of novel biomarkers of brain disorders is also vitally important [73-75]. New biomarkers are particularly needed in epilepsy research, where motor seizures and abnormal brain activity remain the only validated markers [16, 48, 50, 76, 77]. In search of new markers, $c$-fos expression assays have been recently applied to epilepsy in larval [40] and adult [22, 44] zebrafish. Early proto-oncogene $c$-fos is a marker of neuronal activation [78-80], and its application to experimental epilepsy was logical in zebrafish models [40, 45]. In these studies, $c$-fos expression was measured in whole-brain assays, to globally characterize epilepsy-related brain hyperactivity in zebrafish [22, 44]. However, given regional patterns of $c$-fos expression in epilepsy in rodents [81, 82] and differential expression of $c$-fos in the zebrafish brain [83], further analyses of the regional distribution of $c$-fos expression during experimental seizures will better characterize circuitry involved in these epilepsy-like states. In addition to $c$-fos, other similar genes (e.g., $c$-jun) can be assayed in zebrafish epilepsy, based on their role as markers of neuronal activation in other models [84, 85].

Endocrine dysregulations have already been associated with clinical epilepsy [86-89]. While not considered a typical endophenotype of epilepsy, animal evidence parallels clinical observations linking epilepsy to glucocorticoid hormones [87, 90-92] (see discussion of potential mechanisms in [22, 93]). Bridging both seizure-like and whole-body cortisol phenotypes, zebrafish offer excellent
opportunities for the discovery of novel corticoid biomarkers of epilepsy [22]. The ability of AEDs to suppress clinical epilepsy and endocrine deficits [89] strongly supports this notion, also raising the possibility that endocrine assays may become part of zebrafish AED screens (see further).

Other hormones (e.g., prolactin [94, 95]), cytokines (e.g., IL-1, IL-6, TNF-alpha [96, 97]), peripheral blood transcriptomes [98, 99], oxidant and antioxidant mechanisms [100], and Intracerebroventricularly administered lipopolysaccharides [101] implicated in human and rodent epilepsy may also be tested in zebrafish epilepsy models. Biochemical alterations induced in the brain by seizures and/or corrected by AEDs have also been reported in zebrafish. For example, the antiepileptic action of valproate correlates with reduced heat shock protein HSP70 mRNA [102], while PTZ seizures in zebrafish inhibit adenosine deaminase, an enzyme already implicated in rodent epilepsy [103]. Thus, the availability of inexpensive and highly sensitive zebrafish models offers excellent opportunities to identify and validate further molecular biomarkers of epilepsy.

The search for novel genetic markers of epilepsy in zebrafish is markedly enhanced by N-ethyl-N-nitrosourea (ENU)-induced mutagenesis [104, 105] and quantitative trait loci (QTL) analyses. Recent studies have already shown the potential of zebrafish models to study QTLs associated with complex neurobehavioral traits [106, 107], and similar approaches can be developed for epilepsy phenotypes. Epigenetic biomarkers of epilepsy must also be considered for zebrafish models. For example, increased DNA methylation in the promoter region of Reelin is associated with human epilepsy [108], while drug-induced rodent seizures are accompanied by histone modifications in the CREB promoter [109]. Currently, efforts are underway to foster novel epigenetic treatment strategies using epigenetic modifiers, such as DNA methyltransferase- and histone deacetylase inhibitors [110, 111], and using these approaches to target epigenetic regulation of zebrafish epilepsy may be particularly promising.
The potential application of cell therapies is another significant area of epilepsy research. For example, bilateral grafts of GABA-releasing cells in animals models of temporal lobe epilepsy have been shown to suppress seizures as well as prevent focal seizures from generalizing [112, 113]. Conditionally immortalized neurons genetically engineered to produce GABA have successfully suppressed seizure-like behavior when transplanted into the rat brain [114]. However, understanding and ultimately orchestrating tissue availability, graft survival, immunogenicity, and varying levels of cell migration, differentiation and integration into functional circuits within a brain microenvironment poses a significant challenge in cell therapy [114]. The rapid development of zebrafish and the ease of experimental manipulations make them a promising organism for developing models of cell therapies in epilepsy.

3.3. Screening for novel anti-epileptic drugs

As already mentioned, zebrafish seizures share similarities with rodent epilepsy, including progressive behavioral responses, altered brain electrical activity and sensitivity to major convulsant drugs (Table 2; see [30] for review). Zebrafish models also share a common sensitivity to traditional AEDs, illustrated by their ability to inhibit seizures in larval and adult screens [115-118], and evoke several other behavioral effects (Table 1). The fact that AEDs can be identified using zebrafish high-throughput screens within and outside of epilepsy paradigms makes this model organism a particularly promising tool for drug discovery.

Due to low cost and high throughput, zebrafish screens may help identify seizure-resistant individuals, thereby expanding the opportunities for studying resistance to epilepsy (see [6, 119, 120] for discussion of this aspect in animal models). In line with this, kindling represents another key aspect of epileptogenesis [121, 122], and zebrafish are ideally suited for evoking seizures by repeated challenges (e.g., subconvulsant doses of epileptogenic drugs) and/or screening AEDs. Administering varying doses of convulsants to the system water in zebrafish facilities makes the kindling protocols
particularly easy to perform in this species, and may also help detect seizure-insensitive mutants, thereby fostering the search of genetic biomarkers of seizure sensitivity and resistance.

Finally, in addition to screening pro- or anti-convulsant drugs, zebrafish models can help detect adverse effects of these compounds. For example, sensitive to the anticonvulsant effects of GBR12909, zebrafish also demonstrated its side effects (anti-arrhythmia) during parallel assessment of their cardiac activity [116]. Such ‘combined’ application of zebrafish seizure and cardiac screens offers an excellent example of how this model species can be used creatively for solving various, often unconventional, biomedical problems associated with epilepsy and its therapy.

**3.4. Utilizing sophisticated IT-based tools**

The development of computer technologies is rapidly advancing neuroscience research, fostering innovative modeling of epilepsy. For example, experimental modeling may benefit from more objective and time-efficient *automated* quantification of zebrafish seizures [123-125]. As already mentioned, zebrafish models of epilepsy have multiple endpoints (Table 3), some of which are difficult to assess automatically. While video-tracking has been broadly applied to zebrafish, multiple complex phenotypes cannot be examined using traditional 2D approaches. For example, seizure-like circling is impossible to analyze using the side-view camera. While top-view recording will assess circling, it will not detect the vertical position of zebrafish in the tank – an important response sensitive to various experimental manipulations. Typical for some drug- or mutation-induced seizures, corkscrew swimming is also unsuitable for 2D analyses (Table 3). Other relevant phenotypes, such as bursts of hyperactivity/erratic movements, are not assessed automatically because they occur in 3D space, and are based on human recognition prone to subjective interpretation and inter/intra-rater variability. Collectively, this indicates that novel 3D based analyses may improve and refine behavioral phenotyping in zebrafish [126]. While currently validated for non-epileptic zebrafish phenotypes [126, 127], these approaches may be used for both adult and larval
zebrafish, including detailed 3D-based cataloguing and automated recognition of their seizure-like responses. Movement pattern analysis is another method that formalizes and describes movement patterns in spatiotemporal data [128, 129]. Complementing 3D reconstructions of swim paths, movement pattern analysis is a useful approach for zebrafish phenotyping, using trajectory segmentation based on individual locomotion parameters (i.e., velocity or turn angle) to extracts local locomotion features, likely to be useful for the dissection of zebrafish epilepsy-related responses.

3.5. Utilizing bioinformatics- and omics-based tools

Epilepsy research using zebrafish models also benefits from bioinformatics-based tools. For example, similar to Mouse Genomic Informatics database, the comprehensive Zebrafish Information Network (ZFIN) [130] contains mounting genetic and phenotypic data, including several zebrafish strains with aberrant epilepsy-like responses. The value of genomic analyses of epilepsy in animal models is well recognized [122], and recent large-scale gene expression studies [30] strongly support the utility of these approaches in zebrafish. They are further enhanced by the growing availability of DNA microarray kits for zebrafish (e.g., Affymetrix (Santa Clara, USA), Roche Applied Science (Indianapolis, USA) and Agilent Technologies (Santa Clara, USA)) and open-access bioinformatics-based tools that link neural phenotypes with genomic profiles.

In addition to genomic assays, bioinformatics-based tools become crucial for global analysis of pharmacological data, as the number of compounds tested in zebrafish models continues to grow. For example, a recent study exposed larval zebrafish to multiple psychoactive compounds to assess sleep/wake behavior. A behavioral cluster analysis performed on the matrix of pharmacological data visualized the effects of the drugs, predicting behavioral outcomes of similar compounds [131]. We argue that similar data-intensive “array”-like approaches will be particularly useful in the search for novel AEDs.
In addition to ZFIN, the recently developed Zebrafish Neurophenome Project (ZNP) database [132] represents another searchable open-access data repository, specifically dedicated to neurobehavioral and related physiological phenotypes in zebrafish. This dynamic database contains studies of genetic and pharmacological epilepsy models in zebrafish, enabling researchers to search published data and share their findings [132, 133]. ZNP also allows researchers to compare the effective doses and relative potency of various drugs previously tested in zebrafish, providing reference for designing pilot studies. Assisting its users to search and extract zebrafish information in a time-efficient manner, ZNP draws data from multiple sources (including journal articles, conference presentations, books, book chapters, theses and personal communications), providing a more comprehensive assessment of the literature on zebrafish epilepsy phenotypes.

4. Conclusion

As one third of epilepsy cases are refractory to current therapies [134], a greater understanding of its etiology is needed to advance the development of new treatments [135]. Animal models are an invaluable tool for studying human disorders, and the utility of zebrafish as a model for epilepsy research is growing rapidly [42, 45]. Complementing genetic and pharmacological screens, functional connectivity mapping is deciphering the neural mechanisms involved in epileptogenesis, including imaging of an entire zebrafish nervous system in vivo during seizures [41, 136].

Emerging bioinformatic and biotechnology tools provide further insight into epileptogenesis in zebrafish. In addition to Ca^{2+} imaging, advancements in electrophysiological recording through refined electrode design and implementation now allows for the electrographic evaluation of seizures in zebrafish [30, 137] – an application previously available only to larger, yet often unnecessarily complex, animal models. Combined with functional connectivity matrices and realistic simulations of neuronal networks, these approaches will help understand the mechanisms of neuronal interaction and activity propagation in zebrafish [41].
The continued integration and modeling of epileptogenesis across multiple domains is vital for elucidating the mechanisms of epilepsy and its treatment. Strategic directions for further research in this field, outlined above, include: 1) modeling epilepsy across behavioral, genetic/omic and electrophysiological domains; 2) identifying novel biomarkers of epilepsy; 3) enhancing screens for AEDs and epilepsy mutants; 4) improving behavioral detecting of epilepsy-related phenotypes; and 5) applying omics- and other bioinformatics-based tools to zebrafish epilepsy models. With the growing application of traditional and emerging phenotyping techniques to zebrafish models, the field of epilepsy research will continue to benefit immensely from the simpler, yet high-throughput, capacity offered by this species.

Acknowledgements

The study was supported by the Zebrafish Neuroscience Research Consortium (ZNRC), Tulane Neurophenotyping Platform (TNP), LA Board of Regents P-Fund grant and the Tulane School of Medicine Pilot Program grant. Funders had no involvement in the study design, data collection and analysis, or the preparation of this MS.
Figure 1. Examples of behavioral effects evoked in adult (5-8 months old) ‘wild type’ short-fin zebrafish by acute exposure to convulstant drugs pentylenetetrazole (PTZ; A) and strychnine (B). Panel A shows that robust seizure-like behaviors of 1.5 g/L PTZ (20-min immersion in water, based on [22]) are accompanied by increased expression of brain c-fos gene, which serves as a physiological marker of neuronal activation in the brain. In this experiment, PTZ significantly elevated c-fos expression by the end of 40 PCR cycles, compared to water-exposed control zebrafish (also significantly elevating cortisol levels [22], data not shown). Panel B shows typical behavioral responses of zebrafish to a non-toxic dose of 5 mg/L of strychnine (Sigma Aldrich, 5-min immersion in 0.01% dimethyl sulfoxide, DMSO). Note striking differences in zebrafish locomotor traces between controls and both convulsant drugs on both panels, inducing typical hyperactivity as well as jerky movements during testing (due to a short exposure period, c-fos and cortisol assays were not performed for strychnine study). Representative traces were selected as ‘median’ based on an independent evaluation and consensus of three observers. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. control, U-test.
Table 1. Examples of typical phenotypes related to epilepsy in humans, rodents and zebrafish models

<table>
<thead>
<tr>
<th>Clinical epilepsy</th>
<th>Rodent models</th>
<th>Zebrafish models</th>
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<tbody>
<tr>
<td><strong>Neurophysiological symptoms</strong></td>
<td></td>
<td></td>
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<tr>
<td>Brain hyperactivity</td>
<td>Increased neurophysiological responses in mice [138, 139] and rats [50, 58] Elevated brain c-fos expression in mice [141, 142] and rats [81, 82]</td>
<td>Increased neurophysiological responses in larval [30, 34] and adult [140] zebrafish Elevated brain c-fos expression in larval [40] and adult zebrafish [22]</td>
</tr>
<tr>
<td><strong>Behavioral symptoms</strong></td>
<td></td>
<td></td>
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<tr>
<td>Convulsions/seizures</td>
<td>Convulsive seizures in mice [143, 144] and rats [47, 48]</td>
<td>Hyperactivity/seizure behavior in larval [34, 66] and adult [22, 44] zebrafish (see Table 2 for details)</td>
</tr>
<tr>
<td>Behavioral impairments</td>
<td>Loss of posture in mice [145, 146] and rats [147, 148]; non-motor, absence-like epilepsy in mice [13, 149] and rats [57, 58]</td>
<td>Immobility with the loss of body posture and insensitivity to touch [45] (see Table 3 for details)</td>
</tr>
<tr>
<td><strong>Sensitivity to selected antiepileptic drugs</strong></td>
<td></td>
<td></td>
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<tr>
<td>Barbiturates</td>
<td>Anticonvulsant in rodents [150-153]</td>
<td>Sedative in larvae [154] and adult zebrafish [21]</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Anticonvulsant in rodents [150, 152, 155]</td>
<td>Anticonvulsant in larvae [115], anxiolytic in adult zebrafish [156, 157]</td>
</tr>
<tr>
<td>Carboxamides</td>
<td>Anticonvulsant in rodents [153, 158]</td>
<td>Anticonvulsant in larvae [115]; alter brain biochemistry in adult zebrafish [117]</td>
</tr>
<tr>
<td>Fatty acids (valproic acid, vigabatrin, pregabide, tiagabine)</td>
<td>Anticonvulsant in rodents [152, 155]</td>
<td>Anticonvulsant in larvae [115, 159] and adult zebrafish [102], also improved learning [102]</td>
</tr>
<tr>
<td>GABA analogs (gabapentin, pregabalin)</td>
<td>Anticonvulsant in rodents [151, 158]</td>
<td>Anticonvulsant in larvae [115]</td>
</tr>
<tr>
<td>Hydantoins</td>
<td>Anticonvulsant in rodents [162] [163]</td>
<td>Anticonvulsant in larvae [115]; alter brain biochemistry in adult zebrafish [117]</td>
</tr>
<tr>
<td>Pyrrolidines</td>
<td>Anticonvulsant in rodents [164, 165]</td>
<td>Anticonvulsant in larvae [115]</td>
</tr>
<tr>
<td>Succinimides</td>
<td>Anticonvulsant in rodents [151, 152]</td>
<td>Anticonvulsant in larvae [115, 166]</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>Protective role in absence epilepsy in rodents [167]</td>
<td>Anticonvulsant in larvae [115]</td>
</tr>
<tr>
<td>Triazines (lamotrigine)</td>
<td>Anticonvulsant in rodents [168] [163]</td>
<td>Anticonvulsant in larvae [115]</td>
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</table>
Table 2. Summary of recent studies of epilepsy utilizing larval and adult zebrafish models

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>References</th>
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<tr>
<td><strong>Larval models</strong></td>
<td></td>
<td></td>
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<tr>
<td>Seizure behavior, brain <em>c-fos</em> assay</td>
<td>Pentylentetrazole (immersion)</td>
<td>[40]</td>
</tr>
<tr>
<td>Expression and function of potassium channels in larval zebrafish</td>
<td>n/a</td>
<td>[32]</td>
</tr>
<tr>
<td>Synergistic activity of several convulsants</td>
<td>Pentylentetrazole, domoic acid (immersion)</td>
<td>[52, 169]</td>
</tr>
<tr>
<td>‘Mind bomb’ mutation</td>
<td>Spontaneous seizures</td>
<td>[30]</td>
</tr>
<tr>
<td>Knockdown of zebrafish <em>Lgi1</em></td>
<td>Seizure-like phenotype, pentylentetrazole (immersion)</td>
<td>[66]</td>
</tr>
<tr>
<td><strong>Adult zebrafish models</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizure behavior assay</td>
<td>Kainate (injection)</td>
<td>[43]</td>
</tr>
<tr>
<td>Seizure behavior, cortisol and <em>c-fos</em> assays</td>
<td>Caffeine, pentylentetrazole, picrotoxin (immersion)</td>
<td>[22]   Fig. 1A</td>
</tr>
<tr>
<td>Seizure behavioral assay</td>
<td>Strychinine</td>
<td>Fig. 1B</td>
</tr>
<tr>
<td>Cerebral field potential recordings</td>
<td>Pentylentetrazole (immersion)</td>
<td>[137]</td>
</tr>
<tr>
<td>Seizure behavior, cortisol and <em>c-fos</em> assays</td>
<td>1,3,5-trinitroperhydro-1,3,5-triazine (immersion)</td>
<td>[44]</td>
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Table 3. Selected seizure-related endpoints assessed in zebrafish models

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>Definition</th>
<th>Interpretation</th>
<th>Can be assessed:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursts of hyperactivity</td>
<td>Bouts of erratic movements with rapid turning and uncoordinated high-velocity locomotion</td>
<td>Hyperarousal during early stages of seizures</td>
<td>+    -    +</td>
</tr>
<tr>
<td>“Twitching”</td>
<td>Spontaneous, rapid movements of body</td>
<td>Mild neurological deficits associated with seizures</td>
<td>+    -    +</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>Abnormally fast swimming endured for an extended period of time</td>
<td>Hyperlocomotion during early stages of seizures</td>
<td>+    -    +</td>
</tr>
<tr>
<td>Corkscrew swimming</td>
<td>Spiral swimming with an increased speed and in an uncoordinated direction</td>
<td>Significant neurological deficits associated with seizures</td>
<td>+    +    +</td>
</tr>
<tr>
<td>Circular swimming</td>
<td>Repetitive swimming in a circular direction</td>
<td>Significant neurological deficits associated with seizures</td>
<td>+    +    +</td>
</tr>
<tr>
<td>Abnormal body position</td>
<td>Contortion of the body</td>
<td>Uninstructed peripheral responses to seizure</td>
<td>+    -    +</td>
</tr>
<tr>
<td>Loss of posture</td>
<td>Loss of dorso-ventral balance</td>
<td>Severe neurological deficits associated with seizures</td>
<td>+    -    +</td>
</tr>
<tr>
<td>Loss of touch response</td>
<td>Loss of reflexive reaction to contact stimuli</td>
<td>Severe neurological deficits associated with seizures</td>
<td>+    +    +</td>
</tr>
<tr>
<td>Immobility</td>
<td>Cessation of movement (‘not moving’ bouts) except for respiratory and ocular motion</td>
<td>Severe neurological deficits associated with seizures</td>
<td>+    +    +</td>
</tr>
<tr>
<td>Death</td>
<td>Total immobility with the lack of eye/gill movements for &gt;2 min</td>
<td>Epilepsy-related mortality response</td>
<td>+    -    -</td>
</tr>
</tbody>
</table>


