Review

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

Adam Michael Stewart, Daniel Desmond, Evan Kyzar, Siddharth Gaikwad, Andrew Roth, Russell Riehl, Christopher Collins, Louis Monnig, Jeremy Green, Allan V. Kalueff*

Department of Pharmacology and Neuroscience Program, Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA 70112, USA

ARTICLE INFO

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.

© 2011 Elsevier Inc. All rights reserved.

Contents

* Corresponding author at: Department of Pharmacology, Room SL-83, Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA 70112, USA.
Tel.: +1 504 988 3354.
E-mail address: avkalueff@gmail.com (A.V. Kalueff).

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]	Increased neurophysiological responses in larval [61,9] and adult [110] zebrafish
	Elevated brain *c-fos* expression in mice [122,123] and rats [24,145]	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]

Table 2
Summary of recent studies of epilepsy utilizing larval and adult zebrafish models.

Study	Treatment	References
Larval models		
Seizure behavior, brain *c-fos* assay	Pentylenetetrazole (immersion)	[8]
Expression and function of potassium channels in larval zebrafish	n/a	[30]
Synergistic activity of several convulsants	Pentylenetetrazole, domoic acid (immersion)	[153,154]
'Mind bomb' mutation	Spontaneous seizures	[61]
Knockdown of zebrafish *Lgi1*	Seizure-like phenotype, pentylenetetrazole (immersion)	[151]
Adult zebrafish models		
Seizure behavior assay	Kainate (injection)	[2]
Seizure behavior, cortisol and *c-fos* assays	Caffeine, pentylenetetrazole, picrotoxin (immersion)	[161]; Fig. 1A
Seizure behavioral assay	Strychnine (immersion)	Fig. 1B
Cerebral field potential recordings	Pentylenetetrazole (immersion)	[110]
Seizure behavior, cortisol and *c-fos* assays	1,3,5-Trinitroperhydro-1,3,5-triazine (immersion)	[160]

early-onset (e.g., pediatric) epilepsy [6], 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 [161].

Adult zebrafish are typically tested in observation tanks, where seizures are measured using a special scoring system [161,2,160,35] either manually, or using video-recording for automated analyses [161,160,35] (Fig. 1; Table 2, see [35] 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 [143]). Both *top*- and *side*-view recording of observation tanks are used for neurophe-notyping of seizure-like responses in adult zebrafish [161,61,160,35]. 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) [52], picrotoxin [56], pilocarpine [26], kainate [165] and caffeine [126]. 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 [153] (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 [160]. There are also some differences in seizure-like activity among the drugs. For example, PTZ and picrotoxin induce generalized motor seizures [161], whereas caffeine and kainate also evoke spasms (own observations) and clonus-like head-shaking convulsions [2], respectively. Fig. 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 [93,131], but with lower potency and toxicity. This drug has already been tested in other fish species, showing abnormal electrophysiological responses [135] and "weaving" seizure-like behavior [78]. 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, albeit causing non-significant 48-fold elevation of brain c-fos expression at 6 g/L (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 [138], suggested to represent an experimental model of absence-like epilepsy without overt motor seizures [138,95] Clearly, testing other penicillin-related (e.g., beta-lactam) compounds may help to further clarify such profiles.

Strychnine is a potent convulsant neurotoxin that inhibits glycine and acetylcholine receptors, and has been a popular agent in modeling epilepsy in rodents [16,107,108]. This drug also affects zebrafish, inducing spasms, bursts of hyperactivity and circular swimming in adult animals (Fig. 1B) and fast bilateral contractions in larvae [58]. 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 $71 \pm 13\%$ ($P < 0.001$, U-test) and frequent 'wavering' in $79 \pm 11\%$ ($P < 0.0005$, 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 [140,46,55], and have been extensively investigated in zebrafish models, including mutants showing spontaneous seizures [61,60,30] or hypersensitivity to convulsant drugs [151]. For example, mutation of the ubiquitin E3 ligase gene in developing larvae disrupts Notch signaling in the *mind bomb* (*mib*[hi904]) zebrafish, leading to their seizure-like behavior and down-regulation of GABA-ergic genes [61,60]. 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 [30]. The morpholino-induced knockdown of the epilepsy susceptibility gene 1a (*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 [151]. 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 [121,43,12,103], 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 [53], 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 [129]. 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 [144,66,80]. New biomarkers are particularly needed in epilepsy research, where motor seizures and abnormal brain activity remain the only wildly accepted and validated markers [7,56,165,29,25]. In search of new markers, *c-fos* expression assays have been recently applied to epilepsy in larval [8] and adult [161,160] zebrafish. Early protooncogene *c-fos* is a marker of neuronal activation [17,59,28], and its application to experimental epilepsy was logical in zebrafish models [8,35]. In these studies, *c-fos* expression was measured in whole-brain assays, to globally characterize epilepsy-related brain hyperactivity in zebrafish [161,160]. However, given regional patterns of *c-fos* expression in epilepsy in rodents [24,145] and differential expression of *c-fos* in the zebrafish brain [81], further analyses of the regional distribution of *c-fos* expression during
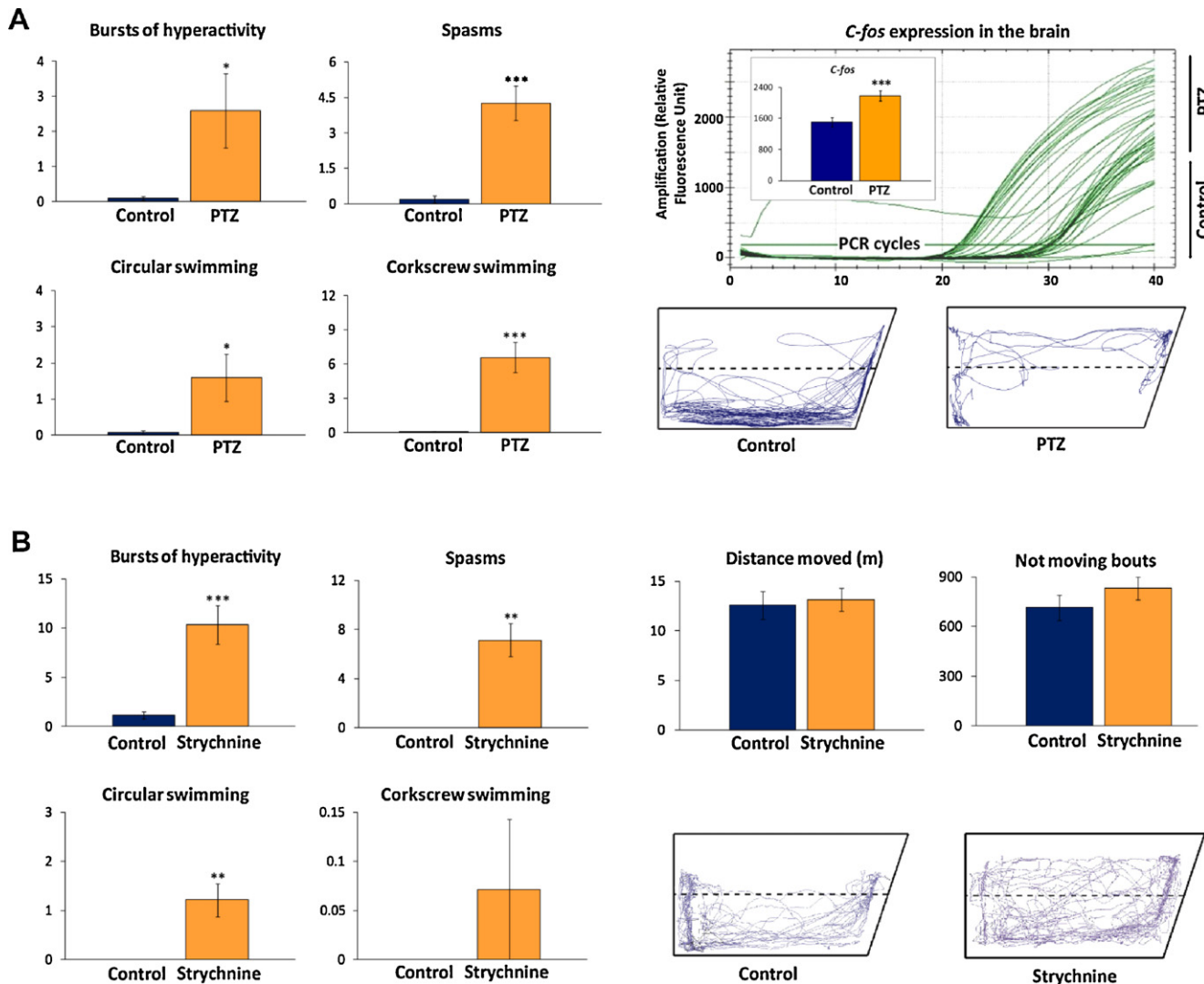
Fig. 1. Examples of behavioral effects evoked in adult (5–8 months old) 'wild type' short-fin zebrafish by acute exposure to convulstant drugs pentylenetetrazole (PTZ; A) and strychnine (B). *Panel A* shows that robust seizure-like behavioral responses to 1.5 g/L PTZ (20-min immersion in water, based on [162]) 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 [162], 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, 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.

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 [11,79].

Endocrine dysregulations have already been associated with clinical epilepsy [4,100,146,47]. While not considered a typical endophenotype of epilepsy, animal evidence parallels clinical observations linking epilepsy to glucocorticoid hormones [100,75,125,148] (see discussion of potential mechanisms in [161,116]). Bridging both seizure-like and whole-body cortisol phenotypes, zebrafish offer excellent opportunities for the discovery of novel corticoid biomarkers of epilepsy [161]. The ability of AEDs to suppress clinical epilepsy and endocrine deficits [47] 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 [132,39]), cytokines (e.g., IL-1, IL-6, TNF-alpha [114,84]), peripheral blood transcriptomes [23,85], oxidant and antioxidant mechanisms [36], and intracerebroventricularly administered lipopolysaccharides [74]

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 [83], while PTZ seizures in zebrafish inhibit adenosine deaminase, an enzyme already implicated in rodent epilepsy [128]. 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 [117,33] and quantitative trait loci (QTL) analyses. Recent studies have already shown the potential of zebrafish models to study QTLs associated with complex neurobehavioral traits [163,164], 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 [72], while drug-induced rodent seizures are accompanied by histone modifications in the *CREB* promoter [155]. Currently, efforts are underway to foster novel epigenetic treatment strategies using epigenetic modifiers, such as DNA methyltransferase- and histone deacetylase inhibitors [71,113], 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 [73,50]. Conditionally immortalized neurons genetically engineered to produce GABA have successfully suppressed seizure-like behavior when transplanted into the rat brain [152]. 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 pose a significant challenge in cell therapy [152]. 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 [61] for review). Zebrafish models also share a common sensitivity to traditional AEDs, illustrated by their ability to inhibit seizures in larval and adult screens [14,54,127,41], 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 in this field.

Due to low cost and high throughput, zebrafish screens may also help identify seizure-resistant individuals, thereby expanding the opportunities for studying resistance to epilepsy (see [137,157,87]

for discussion of this aspect in animal models). In line with this, kindling represents another key aspect of epileptogenesis [86,32], 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 [54]. 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 [31,21,45]. 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

Table 3
Selected seizure-related endpoints assessed in zebrafish models.

Endpoints	Definition	Interpretation	Can be assessed		
			Manually (observer)	Automatically in 2D	Automatically in 3D
Bursts of hyperactivity	Bouts of darting-like erratic movements with rapid turning and uncoordinated high-velocity locomotion	Hyperarousal during early stages of seizures	+	−	+
"Twitching"	Spontaneous, rapid movements of body	Mild neurological deficits associated with seizures	+	−	+
Hyperlocomotion	Abnormally fast swimming endured for an extended period of time	Hyperlocomotion (extended hyperactivity) during early stages of seizures	+	−	+
Corkscrew swimming	Spiral swimming with an increased speed and in an uncoordinated direction	Significant neurological deficits associated with seizures	+	+	+
Circular swimming	Repetitive swimming in a circular direction	Significant neurological deficits associated with seizures	+	+	+
Abnormal body position	Contortion of the body	Uninstructed peripheral responses to seizures	+	−	+
Loss of posture	Loss of dorso-ventral balance	Severe neurological deficits associated with seizures	+	−	+
Loss of touch response	Loss of reflexive reaction to contact stimuli	Severe neurological deficits associated with seizures	+	+	+
Immobility	Cessation of movement ('not moving' bouts) except for respiratory and ocular motion	Severe neurological deficits associated with seizures	+	+	+
Death	Total immobility with the lack of eye/gill movements for >2 min	Epilepsy-related mortality response	+	−	−

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 [19]. While currently validated for non-epileptic zebrafish phenotypes [19,118], 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 [38,82]. Complementing 3D reconstructions of swim paths, movement pattern analysis is a promising approach for zebrafish phenotyping, using trajectory segmentation based on individual locomotion parameters (i.e., velocity or turn angle) to extract 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 markedly from bioinformatics-based tools. For example, similar to Mouse Genome Informatics database, the comprehensive Zebrafish Information Network (ZFIN) [136] 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 [32], and recent large-scale gene expression studies [61] 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 [115]. 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 [77] 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 [77,166]. 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 [76], a greater understanding of its etiology is needed to advance the development of new antiepileptic treatments [94]. Animal models are an invaluable tool for studying human

disorders, and the utility of zebrafish as a model for epilepsy research is growing rapidly [6,35]. 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 [150,57].

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 [61,110] – an application previously available only to larger, but 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 [150].

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 detection 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

References

[1] I. Abidin, et al., Penicillin induced epileptiform activity and EEG spectrum analysis of BDNF heterozygous mice: an in vivo electrophysiological study, Brain Res. Bull. 86 (3–4) (2011) 159–164.
[2] J.M. Alfaro, J. Ripoll-Gomez, J.S. Burgos, Kainate administered to adult zebrafish causes seizures similar to those in rodent models, Eur. J. Neurosci. 33 (7) (2011) 1252–1255.
[3] Y. Arai, et al., Effects of midazolam and phenobarbital on brain oxidative reactions induced by pentylenetetrazole in a convulsion model, Immunopharmacol. Immunotoxicol. (2011), in press.
[4] R.M. Arida, et al., Physical exercise in epilepsy: what kind of stressor is it? Epilepsy Behav. 16 (3) (2009) 381–387.
[5] M. Avoli, et al., Cellular and molecular mechanisms of epilepsy in the human brain, Prog. Neurobiol. 77 (3) (2005) 166–200.
[6] S.C. Baraban, Modeling Epilepsy and Seizures in Developing Zebrafish Larvae, in: A. Pitkänen, P.A. Schwartzkroin, S.L. Moshé (Eds.), Models of Seizures and Epilepsy, Academic Press, Burlington, 2006.
[7] S.C. Baraban, Emerging epilepsy models: insights from mice, flies, worms and fish, Curr. Opin. Neurol. 20 (2) (2007) 164–168.
[8] S.C. Baraban, et al., Pentylenetetrazole induced changes in zebrafish behavior: neural activity and c-fos expression, Neuroscience 131 (3) (2005) 759–768.
[9] S.C. Baraban, et al., A large-scale mutagenesis screen to identify seizure-resistant zebrafish, Epilepsia 48 (6) (2007) 1151–1157.
[10] W.B. Barbazuk, et al., The syntenic relationship of the zebrafish and human genomes, Genome Res. 10 (9) (2000) 1351–1358.
[11] J. Beer, et al., Expression of c-jun: junB, c-fos, fra-1 and fra-2 mRNA in the rat brain following seizure activity and axotomy, Brain Res. 794 (2) (1998) 255–266.
[12] E. Beghi, et al., Comment on epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE), Epilepsia 46 (10) (2005) 1698–1699 (author reply 1701-2).

[13] Z. Bencan, D. Sledge, E.D. Levin, Buspirone: chlordiazepoxide and diazepam effects in a zebrafish model of anxiety, Pharmacol. Biochem. Behav. 94 (1) (2009) 75–80.

[14] S. Berghmans, et al., Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants, Epilepsy Res. 75 (1) (2007) 18–28.

[15] J.A. Blake, et al., The Mouse Genome Database (MGD): premier model organism resource for mammalian genomics and genetics, Nucleic Acids Res. 39 (Database issue) (2011) D842–D848.

[16] L.C. Borges Fernandes, C. Campos Camara, B. Soto-Blanco, Anticonvulsant activity of extracts of Plectranthus barbatus leaves in mice, Evid. Based Complement. Altern. Med. 2012 (2011) 860153.

[17] E. Bullitt, Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat, J. Comp. Neurol. 296 (4) (1990) 517–530.

[18] H.A. Burgess, M. Granato, The neurogenetic frontier-lessons from misbehaving zebrafish, Brief. Funct. Genomic Proteomic 7 (6) (2008) 474–482.

[19] J.M. Cachat, et al., Three-dimensional neurophenotyping of adult zebrafish behavior, PLoS One 6 (3) (2011) e17597.

[20] J. Cachat, et al., Measuring behavioral and endocrine responses to novelty stress in adult zebrafish, Nat. Protocols 5 (11) (2010) 1786–1799.

[21] J.M. Cachat, et al., Video-aided analysis of zebrafish locomotion and anxiety-related behavioral responses, in: A.V. Kalueff, J. Cachat (Eds.), Zebrafish Neurobehavioral Protocols, Humana Press, New York, 2010.

[22] J. Cachat, et al., Modeling withdrawal syndrome in zebrafish, Behav. Brain Res. 208 (2) (2010) 371–376.

[23] L.P. Cacheaux, et al., Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis, J. Neurosci. 29 (28) (2009) 8927–8935.

[24] M. Carballo-Quintas, et al., A study of neurotoxic biomarkers: c-fos and GFAP after acute exposure to GSM radiation at 900 MHz in the picrotoxin model of rat brains, Neurotoxicology 32 (4) (2011) 478–494.

[25] S. Carmody, L. Brennan, Effects of pentylenetetrazole-induced seizures on metabolomic profiles of rat brain, Neurochem. Int. 56 (2) (2010) 340–344.

[26] E.A. Cavalheiro, The pilocarpine model of epilepsy, Ital. J. Neurol. Sci. 16 (1–2) (1995) 33–37.

[27] C. Chakraborty, et al., Zebrafish: a complete animal model for in vivo drug discovery and development, Curr. Drug Metab. 10 (2) (2009) 116–124.

[28] R.K. Chan, et al., A comparison of two immediate-early genes: c-fos and NGFI-B, as markers for functional activation in stress-related neuroendocrine circuitry, J. Neurosci. 13 (12) (1993) 5126–5138.

[29] A.B. Chatt, J.S. Ebersole, Comparisons between strychnine and penicillin epileptogenesis suggest that propagating epileptiform abnormalities require the potentiation of thalamocortical circuitry in neocortical layer 4, Exp. Neurol. 100 (2) (1988) 365–380.

[30] S.W. Chege, et al., Expression and function of KCNQ channels in larval zebrafish, Dev. Neurobiol. (2011), in press.

[31] S. Chen, et al., Automated analysis of zebrafish images for phenotypic changes in drug discovery, J. Neurosci. Methods 200 (2) (2011) 229–236.

[32] A.J. Cole, S. Koh, Y. Zheng, Are seizures harmful: what can we learn from animal models? Prog. Brain Res. 135 (2002) 13–23.

[33] E. de Bruijn, E. Cuppen, H. Feitsma, Highly efficient ENU mutagenesis in zebrafish, Methods Mol. Biol. 546 (2009) 3–12.

[34] S. DePrato Primeaux, et al., Experimentally induced attenuation of neuropeptide-Y gene expression in transgenic mice increases mortality rate following seizures, Neurosci. Lett. 287 (1) (2000) 61–64.

[35] D. Desmond, et al., Assessing epilepsy-related behavioral phenotypes in adult zebrafish, in: A.V. Kalueff, A.M. Stewart (Eds.), Zebrafish Protocols for Neurobehavioral Research, Humana Press, New York, 2012.

[36] M.O. Dillioglugil, et al., Effect of pentylenetetrazole and sound stimulation induced single and repeated convulsive seizures on the MDA: GSH and NO levels, and SOD activities in rat liver and kidney tissues, Brain Res. Bull. 83 (6) (2010) 356–359.

[37] C.A. Dlugos, R.A. Rabin, Ethanol effects on three strains of zebrafish: model system for genetic investigations, Pharmacol. Biochem. Behav. 74 (2) (2003) 471–480.

[38] S. Dodge, R. Weibel, E. Forootan, Revealing the physics of movement: comparing the similarity of movement characteristics of different types of moving objects, Comput. Environ. Urban Syst. 33 (6) (2009) 419–434.

[39] H.E. Edwards, W.M. Burnham, N.J. MacLusky, Partial and generalized seizures affect reproductive physiology differentially in the male rat, Epilepsia 40 (11) (1999) 1490–1498.

[40] B.P. Eliceiri, A.M. Gonzalez, A. Baird, Zebrafish model of the blood–brain barrier: morphological and permeability studies, Methods Mol. Biol. 686 (2011) 371–378.

[41] Evotec, Zebrafish Screening, ADMET and Zebrafish Screening, Hamburg, 2011.

[42] Evotec, Zebrafish Screening, Evotec, Inc., Hamburg, Germany, 2011.

[43] R.S. Fisher, et al., Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE), Epilepsia 46 (4) (2005) 470–472.

[44] W. Fleisher, et al., Comparative study of trauma-related phenomena in subjects with pseudoseizures and subjects with epilepsy, Am. J. Psychiatry 159 (4) (2002) 660–663.

[45] E. Fontaine, et al., Automated visual tracking for studying the ontogeny of zebrafish swimming, J. Exp. Biol. 211 (Pt 8) (2008) 1305–1316.

[46] W.N. Frankel, Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats, Trends Genet. 25 (8) (2009) 361–367.

[47] C.A. Galimberti, et al., Seizure frequency and cortisol and dehydroepiandrosterone sulfate (DHEAS) levels in women with epilepsy receiving antiepileptic drug treatment, Epilepsia 46 (4) (2005) 517–523.

[48] A.L. George Jr., Molecular basis of inherited epilepsy, Arch. Neurol. 61 (4) (2004) 473–478.

[49] R. Gerlai, A small fish with a big future: zebrafish in behavioral neuroscience, Rev. Neurosci. 22 (1) (2011) 3–4.

[50] M. Gernert, et al., Genetically engineered GABA-producing cells demonstrate anticonvulsant effects and long-term transgene expression when transplanted into the central piriform cortex of rats, Exp. Neurol. 176 (1) (2002) 183–192.

[51] D.P. Getova, A.S. Mihaylova, A study of the effects of lamotrigine on mice using two convulsive tests, Folia Med. (Plovdiv) 53 (2) (2011) 57–62.

[52] O. Giorgi, et al., Anticonvulsant effect of felbamate in the pentylenetetrazole kindling model of epilepsy in the rat, Naunyn. Schmiedebergs Arch. Pharmacol. 354 (2) (1996) 173–178.

[53] E. Gjini, et al., Zebrafish Tie-2 shares a redundant role with Tie-1 in heart development and regulates vessel integrity, Dis. Model. Mech. 4 (1) (2011) 57–66.

[54] P. Goldsmith, et al., GBR12909 possesses anticonvulsant activity in zebrafish and rodent models of generalized epilepsy but cardiac ion channel effects limit its clinical utility, Pharmacology 79 (4) (2007) 250–258.

[55] C.A. Gurnett, P. Hedera, New ideas in epilepsy genetics: novel epilepsy genes, copy number alterations, and gene regulation, Arch. Neurol. 64 (3) (2007) 324–328.

[56] C. Hamani, L.E. Mello, Spontaneous recurrent seizures and neuropathology in the chronic phase of the pilocarpine and picrotoxin model epilepsy, Neurol. Res. 24 (2) (2002) 199–209.

[57] S. Higashijima, et al., Imaging neuronal activity during zebrafish behavior with a genetically encoded calcium indicator, J. Neurophysiol. 90 (6) (2003) 3986–3997.

[58] H. Hirata, et al., Zebrafish bandoneon mutants display behavioral defects due to a mutation in the glycine receptor beta-subunit, Proc. Natl. Acad. Sci. U. S. A. 102 (23) (2005) 8345–8350.

[59] G.E. Hoffman, M.S. Smith, J.G. Verbalis, c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems, Front. Neuroendocrinol. 14 (3) (1993) 173–213.

[60] G.A. Hortopan, S.C. Baraban, Aberrant expression of genes necessary for neuronal development and notch signaling in an epileptic mind bomb zebrafish, Dev. Dyn. 240 (8) (2011) 1964–1976.

[61] G.A. Hortopan, M.T. Dinday, S.C. Baraban, Spontaneous seizures and altered gene expression in GABA signaling pathways in a mind bomb mutant zebrafish, J. Neurosci. 30 (41) (2010) 13718–13728.

[62] G.A. Hortopan, M.T. Dinday, S.C. Baraban, Zebrafish as a model for studying genetic aspects of epilepsy, Dis. Model. Mech. 3 (3–4) (2010) 144–148.

[63] L.T. Huang, et al., Pentylenetetrazol-induced recurrent seizures in rat pups: time course on spatial learning and long-term effects, Epilepsia 43 (6) (2002) 567–573.

[64] J.S. Isenberg, et al., Modulation of angiogenesis by dithiolethione-modified NSAIDs and valproic acid, Br. J. Pharmacol. 151 (1) (2007) 63–72.

[65] J.Y. Jeong, et al., Functional and developmental analysis of the blood–brain barrier in zebrafish, Brain Res. Bull. 75 (5) (2008) 619–628.

[66] A.V. Kalueff, M.V. Schmidt, Novel experimental models and paradigms for neuropsychiatric disorders: editorial, Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (6) (2011) 1355–1356.

[67] R.M. Kaminski, M. Banerjee, M.A. Rogawski, Topiramate selectively protects against seizures induced by ATPA: a GluR5 kainate receptor agonist, Neuropharmacology 46 (8) (2004) 1097–1104.

[68] G. Kari, U. Rodeck, A.P. Dicker, Zebrafish: an emerging model system for human disease and drug discovery, Clin. Pharmacol. Ther. 82 (1) (2007) 70–80.

[69] M. Ketzef, et al., Compensatory network alterations upon onset of epilepsy in synapsin triple knock-out mice, Neuroscience 189 (2011) 108–122.

[70] M.J. Kjeldsen, et al., Genetic and environmental factors in epilepsy: a population-based study of 11900 Danish twin pairs, Epilepsy Res. 44 (2–3) (2001) 167–178.

[71] K. Kobow, I. Blumcke, The methylation hypothesis: do epigenetic chromatin modifications play a role in epileptogenesis? Epilepsia 52 (Suppl. 4) (2011) 15–19.

[72] K. Kobow, et al., Increased reelin promoter methylation is associated with granule cell dispersion in human temporal lobe epilepsy, J. Neuropathol. Exp. Neurol. 68 (4) (2009) 356–364.

[73] M. Kokaia, et al., Seizure suppression in kindling epilepsy by intracerebral implants of GABA- but not by noradrenaline-releasing polymer matrices, Exp. Brain Res. 100 (3) (1994) 385–394.

[74] Z. Kovacs, et al., Intracerebroventricularly administered lipopolysaccharide enhances spike-wave discharges in freely moving WAG/Rij rats, Brain Res. Bull. 85 (6) (2011) 410–416.

[75] G. Kumar, et al., The acceleration of amygdala kindling epileptogenesis by chronic low-dose corticosterone involves both mineralocorticoid and glucocorticoid receptors, Psychoneuroendocrinology 32 (7) (2007) 834–842.

[76] P. Kwan, M.J. Brodie, Early identification of refractory epilepsy, N. Engl. J. Med. 342 (5) (2000) 314–319.

[77] E. Kyzar et al., The Zebrafish Neurophenome Database (ZND): a new open-access online resource for zebrafish neurophenotypic data, Zebrafish, in press.

[78] P.R. Laming, D.J. Rooney, J. Ferguson, Epileptogenesis is associated with heightened arousal responses in fish, Physiol. Behav. 40 (5) (1987) 617–624.

[79] P. Lanaud, et al., Temporal and spatial patterns of expression of c-fos: zif/268, c-jun and jun-B mRNAs in rat brain following seizures evoked focally from the deep prepiriform cortex, Exp. Neurol. 119 (1) (1993) 20–31.

[80] J.L. LaPorte, et al., Qui non proficit, deficit: experimental models for 'integrative' research of affective disorders, J. Affect. Disord. 121 (1–2) (2010) 1–9.

[81] B.Y. Lau, et al., Identification of a brain center whose activity discriminates a choice behavior in zebrafish, Proc. Natl. Acad. Sci. U. S. A. 108 (6) (2011) 2581–2586.

[82] P. Laube, Progress in movement pattern analysis, in: B. Gottfried, H. Aghajan (Eds.), Behaviour Monitoring and Interpretation – Ambient Assisted Living, IOS Press, 2009.

[83] Y. Lee, et al., Improvement of pentylenetetrazol-induced learning deficits by valproic acid in the adult zebrafish, Eur. J. Pharmacol. 643 (2–3) (2010) 225–231.

[84] G. Li, et al., Cytokines and epilepsy, Seizure 20 (3) (2011) 249–256.

[85] D.Z. Liu, et al., Brain and blood microRNA expression profiling of ischemic stroke: intracerebral hemorrhage: and kainate seizures, J. Cereb. Blood Flow Metab. 30 (1) (2010) 92–101.

[86] W. Loscher, Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy, Epilepsy Res. 50 (1–2) (2002) 105–123.

[87] W. Loscher, Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs, Seizure 20 (5) (2011) 359–368.

[88] A. Ludwig, et al., Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2, EMBO J. 22 (2) (2003) 216–224.

[89] K. Lukawski, et al., Influence of ethacrynic acid on the anticonvulsant activity of conventional antiepileptic drugs in the mouse maximal electroshock seizure model, Pharmacol. Rep. 62 (5) (2010) 808–813.

[90] J.J. Luszczki, M.M. Andres, S.J. Czuczwar, Synergistic interaction of gabapentin and oxcarbazepine in the mouse maximal electroshock seizure model—an isobolographic analysis, Eur. J. Pharmacol. 515 (1–3) (2005) 54–61.

[91] J.J. Luszczki, et al., Furosemide potentiates the anticonvulsant action of valproate in the mouse maximal electroshock seizure model, Epilepsy Res. 76 (1) (2007) 66–72.

[92] J.J. Luszczki, et al., Effects of WIN 55,212-2 mesylate (a synthetic cannabinoid) on the protective action of clonazepam, ethosuximide, phenobarbital and valproate against pentylenetetrazole-induced clonic seizures in mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (8) (2011) 1870–1876.

[93] R.L. Macdonald, J.L. Barker, Pentylenetetrazol and penicillin are selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurones, Nature 267 (5613) (1977) 720–721.

[94] R. Mani, J. Pollard, M.A. Dichter, Human clinical trails in antiepileptogenesis, Neurosci. Lett. 497 (3) (2011) 251–256.

[95] C. Marescaux, M. Vergnes, A. Depaulis, Genetic absence epilepsy in rats from Strasbourg—a review, J. Neural Transm. Suppl. 35 (1992) 37–69.

[96] R. Marley, R.A. Baines, Increased persistent Na+ current contributes to seizure in the slamdance bang-sensitive Drosophila mutant, J. Neurophysiol. 106 (1) (2011) 18–29.

[97] M. Maroso, et al., Interleukin-1beta biosynthesis inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice, Neurotherapeutics 8 (2) (2011) 304–315.

[98] A. Matagne, et al., Anti-convulsive and anti-epileptic properties of brivaracetam (ucb 34714): a high-affinity ligand for the synaptic vesicle protein, SV2A, Br. J. Pharmacol. 154 (8) (2008) 1662–1671.

[99] A. Matagne, et al., Profile of the new pyrrolidone derivative seletracetam (ucb 44212) in animal models of epilepsy, Eur. J. Pharmacol. 614 (1–3) (2009) 30–37.

[100] A.M. Mazarati, et al., Elevated plasma corticosterone level and depressive behavior in experimental temporal lobe epilepsy, Neurobiol. Dis. 34 (3) (2009) 457–461.

[101] B.D. Metscher, P.E. Ahlberg, Zebrafish in context: uses of a laboratory model in comparative studies, Dev. Biol. 210 (1) (1999) 1–14.

[102] I.M. Najm, et al., Temporal changes in proton MRS metabolites after kainic acid-induced seizures in rat brain, Epilepsia 38 (1) (1997) 87–94.

[103] M.B. Normark, J. Erdal, T.W. Kjaer, Video electroencephalography monitoring differentiates between epileptic and non-epileptic seizures, Dan. Med. Bull. 58 (9) (2011) A4305.

[104] R. Ottman, et al., Relations of genetic and environmental factors in the etiology of epilepsy, Ann. Neurol. 39 (4) (1996) 442–449.

[105] R. Pandey, et al., Baccoside A suppresses epileptic-like seizure/convulsion in Caenorhabditis elegans, Seizure 19 (7) (2010) 439–442.

[106] D. Papandrea, et al., Dissociation of seizure traits in inbred strains of mice using the flurothyl kindling model of epileptogenesis, Exp. Neurol. 215 (1) (2009) 60–68.

[107] M.S. Patil, et al., Anticonvulsant activity of aqueous root extract of Ficus religiosa, J. Ethnopharmacol. 133 (1) (2011) 92–96.

[108] M.E. Pesce, et al., Progesterone and testosterone modulate the convulsant actions of pentylenetetrazol and strychnine in mice, Pharmacol. Toxicol. 87 (3) (2000) 116–119.

[109] H.A. Phillips, et al., Autosomal dominant nocturnal frontal-lobe epilepsy: genetic heterogeneity and evidence for a second locus at 15q24, Am. J. Hum. Genet. 63 (4) (1998) 1108–1116.

[110] R. Pineda, C.E. Beattie, C.W. Hall, Recording the adult zebrafish cerebral field potential during pentylenetetrazole seizures, J. Neurosci. Methods 200 (1) (2011) 20–28.

[111] A. Pitkanen, K. Lukasiuk, Molecular and cellular basis of epileptogenesis in symptomatic epilepsy, Epilepsy Behav. 14 (Suppl. 1) (2009) 16–25.

[112] J.H. Postlethwait, et al., Zebrafish comparative genomics and the origins of vertebrate chromosomes, Genome Res. 10 (12) (2000) 1890–1902.

[113] I.A. Qureshi, M.F. Mehler, Epigenetic mechanisms underlying human epileptic disorders and the process of epileptogenesis, Neurobiol. Dis. 39 (1) (2010) 53–60.

[114] R.S. Rao, A. Prakash, B. Medhi, Role of different cytokines and seizure susceptibility: a new dimension towards epilepsy research, Indian J. Exp. Biol. 47 (8) (2009) 625–634.

[115] J. Rihel, et al., Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation, Science 327 (5963) (2010) 348–351.

[116] A.J. Roberts, J.C. Crabbe, L.D. Keith, Type I corticosteroid receptors modulate PTZ-induced convulsions of withdrawal seizure prone mice, Brain Res. 626 (1–2) (1993) 143–148.

[117] N. Rohner, et al., Enhancing the efficiency of N-ethyl-N-nitrosourea-induced mutagenesis in the zebrafish, Zebrafish 8 (3) (2011) 119–123.

[118] D.B. Rosemberg, et al., Differences in spatio-temporal behavior of zebrafish in the open tank paradigm after a short-period confinement into dark and bright environments, PLoS One 6 (5) (2011) e19397.

[119] D. Runke, et al., Relation between startle reactivity and sucrose avidity in two rat strains bred for differential seizure susceptibility, Exp. Neurol. 229 (2) (2011) 259–263.

[120] E. Russo, et al., Effects of early long-term treatment with antiepileptic drugs on development of seizures and depressive-like behavior in a rat genetic absence epilepsy model, Epilepsia 52 (7) (2011) 1341–1350.

[121] M. Salinsky, et al., Psychogenic nonepileptic seizures in US veterans, Neurology 77 (10) (2011) 945–950.

[122] A.R. Salomons, et al., Behavioural habituation to novelty and brain area specific immediate early gene expression in female mice of two inbred strains, Behav. Brain Res. 215 (1) (2010) 95–101.

[123] A.R. Salomons, et al., Identifying emotional adaptation: behavioural habituation to novelty and immediate early gene expression in two inbred mouse strains, Genes Brain Behav. 9 (1) (2010) 1–10.

[124] I.E. Scheffer, S.F. Berkovic, Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes, Brain 120 (Pt 3) (1997) 479–490.

[125] U. Schridde, G. van Luijtelaar, Corticosterone increases spike-wave discharges in a dose- and time-dependent manner in WAG/Rij rats, Pharmacol. Biochem. Behav. 78 (2) (2004) 369–375.

[126] T.W. Seale, et al., Coincidence of seizure susceptibility to caffeine and to the benzodiazepine inverse agonist: DMCM, in SWR and CBA inbred mice, Pharmacol. Biochem. Behav. 26 (2) (1987) 381–387.

[127] A.M. Siebel, et al., In vitro effects of antiepileptic drugs on acetylcholinesterase and ectonucleotidase activities in zebrafish (Danio rerio) brain, Toxicol. In Vitro 24 (4) (2010) 1279–1284.

[128] A.M. Siebel, et al., PTZ-induced seizures inhibit adenosine deamination in adult zebrafish brain membranes, Brain Res. Bull. 86 (5–6) (2011) 385–389.

[129] I.A. Signore, et al., Zebrafish and medaka: model organisms for a comparative developmental approach of brain asymmetry, Philos. Trans. R. Soc. Lond. B: Biol. Sci. 364 (1519) (2009) 991–1003.

[130] M. Sillanpaa, S. Shinnar, Long-term mortality in childhood-onset epilepsy, N. Engl. J. Med. 363 (26) (2010) 2522–2529.

[131] M.A. Simmonds, Classification of some GABA antagonists with regard to site of action and potency in slices of rat cuneate nucleus, Eur. J. Pharmacol. 80 (4) (1982) 347–358.

[132] A. Siniscalchi, et al., Serum prolactin levels in repetitive temporal epileptic seizures, Eur. Rev. Med. Pharmacol. Sci. 12 (6) (2008) 365–368.

[133] R.S. Sloviter, The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy, Ann. Neurol. 35 (6) (1994) 640–654.

[134] N. Sood, et al., Nicotine reversal of anticonvulsant action of topiramate in kainic acid-induced seizure model in mice, Nicotine Tob. Res. 13 (11) (2011) 1084–1089.

[135] M.E. Spira, M.V. Bennett, Penicillin induced seizure activity in the hatchet fish, Brain Res. 43 (1) (1972) 235–241.

[136] J. Sprague, et al., The Zebrafish Information Network: the zebrafish model organism database provides expanded support for genotypes and phenotypes, Nucleic Acids Res. 36 (Database issue) (2008) D768–D772.

[137] J.P. Stables, et al., Models for epilepsy and epileptogenesis: report from the NIH workshop, Bethesda, Maryland, Epilepsia 43 (11) (2002) 1410–1420.

[138] J. Stankiewicz, et al., Effects of repeated systemic penicillin injections on non-convulsive and convulsive epileptic seizures in the rat, Acta Neurobiol. Exp. (Wars) 55 (4) (1995) 281–287.

[139] L. Stankova, H. Kubova, P. Mares, Anticonvulsant action of lamotrigine during ontogenesis in rats, Epilepsy Res. 13 (1) (1992) 17–22.

[140] O.K. Steinlein, Genetic mechanisms that underlie epilepsy, Nat. Rev. Neurosci. 5 (5) (2004) 400–408.

[141] A. Stewart, et al., The developing utility of zebrafish in modeling neurobehavioral disorders, Int. J. Comp. Psychol. 23 (1) (2010) 104–121.

[142] A. Stewart, et al., Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models, Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (6) (2010) 1421–1431.

[143] A. Stewart, et al., Intraperitoneal injection as a method of psychotropic drug delivery in adult zebrafish, in: A.V. Kalueff, J. Cachat (Eds.), Zebrafish Neurobehavioral Protocols, Humana Press, New York, 2010.

[144] A. Stewart, et al., Experimental models for anxiolytic drug discovery in the era of omes and omics, Expert Opin. Drug Discov. 6 (1–15) (2011).

[145] J. Szyndler, et al., Mapping of c-Fos expression in the rat brain during the evolution of pentylenetetrazol-kindled seizures, Epilepsy Behav. 16 (2) (2009) 216–224.

[146] T.R. Taher, et al., Chronic low-dose corticosterone supplementation enhances acquired epileptogenesis in the rat amygdala kindling model of TLE, Neuropsychopharmacology 30 (9) (2005) 1610–1616.

[147] K. Takechi, T. Ishikawa, C. Kamei, Epileptogenic activity induced by teicoplanin and effects of some antiepileptics in mice, J. Pharmacol. Sci. 107 (4) (2008) 428–433.

[148] M. Talmi, et al., Synergistic action of corticosterone on kainic acid-induced electrophysiological alterations in the hippocampus, Brain Res. 704 (1) (1995) 97–102.

[149] N.C. Tan, S.F. Berkovic, The Epilepsy Genetic Association Database (epiGAD): analysis of 165 genetic association studies, 1996–2008, Epilepsia 51 (4) (2010) 686–689.

[150] L. Tao, J.D. Lauderdale, A.T. Sornborger, Mapping functional connectivity between neuronal ensembles with larval zebrafish transgenic for a ratiometric calcium indicator, Front. Neural Circuits 5 (2011) 2.

[151] Y. Teng, et al., Knockdown of zebrafish Lgi1a results in abnormal development: brain defects and a seizure-like behavioral phenotype, Hum. Mol. Genet. 19 (22) (2010) 4409–4420.

[152] K.W. Thompson, L.M. Suchomelova, Transplants of cells engineered to produce GABA suppress spontaneous seizures, Epilepsia 45 (1) (2004) 4–12.

[153] J.A. Tiedeken, J.S. Ramsdell, DDT exposure of zebrafish embryos enhances seizure susceptibility: relationship to fetal p,p′-DDE burden and domoic acid exposure of California sea lions, Environ. Health Perspect. 117 (1) (2009) 68–73.

[154] J.A. Tiedeken, J.S. Ramsdell, Zebrafish seizure model identifies p,p-DDE as the dominant contaminant of fetal California sea lions that accounts for synergistic activity with domoic acid, Environ. Health Perspect. 118 (4) (2010) 545–551.

[155] N.M. Tsankova, A. Kumar, E.J. Nestler, Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures, J. Neurosci. 24 (24) (2004) 5603–5610.

[156] D. Vohora, et al., Effect of combined treatment of thioperamide with some antiepileptic drugs on methionine–sulfoximine induced convulsions in mice, Indian J. Exp. Biol. 48 (8) (2010) 858–860.

[157] H.A. Volk, W. Loscher, Multidrug resistance in epilepsy: rats with drug-resistant seizures exhibit enhanced brain expression of P-glycoprotein compared with rats with drug-responsive seizures, Brain 128 (Pt 6) (2005) 1358–1368.

[158] J.L. Wagnon, et al., Etiology of a genetically complex seizure disorder in Celf4 mutant mice, Genes Brain Behav. 10 (7) (2011) 765–777.

[159] Y. Watanabe, et al., Effects of antiepileptics on behavioral and electroencephalographic seizure induced by pentetrazol in mice, J. Pharmacol. Sci. 112 (3) (2010) 282–289.

[160] L.R. Williams, et al., Behavioral and physiological effects of RDX on adult zebrafish, Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 155 (1) (2012) 33–38.

[161] K. Wong, et al., Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish, Brain Res. 1348 (2010) 209–215.

[162] I.G. Woods, et al., A comparative map of the zebrafish genome, Genome Res. 10 (12) (2000) 1903–1914.

[163] D. Wright, et al., QTL analysis of behavioral and morphological differentiation between wild and laboratory zebrafish (*Danio rerio*), Behav. Genet. 36 (2) (2006) 271–284.

[164] D. Wright, R.K. Butlin, O. Carlborg, Epistatic regulation of behavioural and morphological traits in the zebrafish (*Danio rerio*), Behav. Genet. 36 (6) (2006) 914–922.

[165] L. Zagrean, et al., EEG study of kainate-induced epilepsy in non-anaesthetized freely moving rats, Rom. J. Physiol. 30 (1–2) (1993) 115–118.

[166] I. Zapolsky, et al., Utilizing the Zebrafish Neurophenome Project (ZNP) Database for analyses of complex neurophenotypes in zebrafish models, in: A.V. Kalueff, A.M. Stewart (Eds.), Zebrafish Protocols for Neurobehavioral Research, Humana Press, New York, 2012.

[167] I.V. Zhdanova, et al., Melatonin promotes sleep-like state in zebrafish, Brain Res. 903 (1–2) (2001) 263–268.

[168] X.L. Zhong, et al., Deep brain stimulation for epilepsy in clinical practice and in animal models, Brain Res. Bull. 85 (3–4) (2011) 81–88.