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VIEWPOINT

Past, present and future of zebrafish in epilepsy research

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Animal models contribute greatly to our understanding of brain development and function as well as its dysfunction in neurological diseases. Epilepsy research is a very good example of how animal models can provide us with a mechanistic understanding of the genes, molecules, and pathophysiological processes involved in disease. Over the course of the last two decades, zebrafish came in as a new player in epilepsy research, with an expanding number of laboratories using this animal to understand epilepsy and to discover new strategies for preventing seizures. Yet, zebrafish as a model offers a lot more for epilepsy research. In this viewpoint, we aim to highlight some key contributions of zebrafish to epilepsy research, and we want to emphasize the great untapped potential of this animal model for expanding these contributions. We hope that our suggestions will trigger further discussions between clinicians and researchers with a common goal to understand and cure epilepsy.

Introduction

Epilepsy is the most common group of brain disorders affecting more than 50 million people worldwide [1]. Spontaneous and synchronous neuronal hyperactivity is the main hallmark of epilepsies, which can result in a variety of clinical features, ranging from a simple twitch of a few fingers to a generalized seizure with tonic–clonic manifestations, as well as other accompanying symptoms [2]. Despite the available therapies, approximately 30% of epilepsy patients still suffer from drug-resistant seizures [3]. This is partly because the current drugs against epilepsy mostly aim at suppressing seizures, rather than interfering with the underlying mechanisms [4,5]. Hence, investigating fundamental principles and alterations leading to epilepsy is crucial for developing mechanistic therapies that can help epilepsy patients.

Mammalian seizure models have contributed tremendously to our understanding of the mechanisms underlying epilepsy, complementing clinical research in human patients. The zebrafish seizure models came into play a lot later than mammalian models, but have already made significant contributions to our understanding of the epilepsies and to the development of treatments. With its ever-expanding genetic toolbox [6–9] for perturbing and monitoring neural or glial activity in the entire brain [10–13] during specific behaviors [14–17], together with available neural/glial transcriptome [18–20], and whole brain connectome [21,22] embedded in standardized atlases [14,22,23], zebrafish offers tremendous possibilities to investigate the principles underlying seizure generation in various epilepsy models [11–13,24–29]. We argue that

Abbreviations
Cx43, connexin 43; EAAT2, excitatory amino acid transporter 2; GABA, gamma-aminobutyric acid; PTZ, pentylenetetrazol.

combining such mechanistic investigations with the incredible advantage of zebrafish for designing high-throughput experiments [30–33] will further boost our understanding of genetic and environmental causes of epilepsy and will foster discoveries of novel antiseizure and epilepsy therapies. In this viewpoint article, we aim to summarize the contributions of zebrafish to various concepts in epilepsy research. Moreover, we also highlight some of the future opportunities that zebrafish can provide for a better mechanistic understanding of epilepsy and inspire novel therapies. We hope that this review will provide a framework for clinicians and researchers to make the most out of zebrafish seizure and epilepsy models.

Causes of epilepsy

Trauma, tumors, neurodegeneration, and stroke-induced epilepsies

Traumatic brain injury, tumors, neurodegeneration, encephalitis, and stroke are common causes of epilepsy [34–38] (Fig. 1). The epileptogenic process after such insults to brain tissue is often attributed to glutamate excitotoxicity [2] and loss of inhibitory interneurons followed by network reorganization and a net increase in excitability [36]. Moreover, several of such insult-related epilepsies are also thought to be associated with the disruption of the blood–brain barrier, altered cerebral blood flow, imbalanced microenvironment, and electrochemical milieu leading to impaired neural and glial function and neuroinflammation [36,37,39–41]. In line with these observations, the rodent models of post-traumatic epilepsy and postencephalitic epilepsy exhibit similar pathological findings, that is, reduced inhibition, neurolysis, gliosis, inflammation, disrupted blood–brain barrier, hyperexcitability, and spontaneous epileptiform neuronal discharges [42–44]. These studies also revealed changes in neural connectivity, reorganization of extracellular matrix, and metabolic disruptions in the brain after trauma [39,43,44].

Brain injury models are often used in adult zebrafish for studying neuroregenerative processes, inflammation, and scar tissue formation, due to its high regenerative capacity [45–49]. Similarly, the zebrafish has been a very popular vertebrate model for studying neurodegenerative diseases [20,50]. These studies successfully revealed the molecular and cellular processes following the brain injury and neurodegeneration [20,45–48,50]. Yet, there is still a great potential in investigating whether such brain injury or neurodegenerative disease models in zebrafish would lead to epileptogenesis by integrating functional recordings of neural network activity. A recent zebrafish study showed that brain trauma in the adult telencephalon can induce epileptogenesis with spontaneous seizures which was associated with neuroinflammation, increase in phosphorylated tau and mTOR, and blood–brain barrier damage [51], thus resembling processes observed in rodents. Since the exceptional regenerative capacity of the zebrafish brain leads to eventual healing of the brain tissue [45,46,52], it would be exciting to identify whether tissue regeneration can cure trauma-induced epilepsy. This would further validate stem cell-based therapies in non-regenerative species, including humans.

In analogy to rodent models of stroke and hypoxia-induced epilepsies via middle cerebral artery occlusion and cortical photothrombosis [53], zebrafish with their well-described cerebrovascular system [54,55] and accessible brains also presents a great potential for developing stroke-induced epilepsy models. In fact, there are currently several zebrafish stroke models readily available via genetic interventions [56], reduced oxygen levels [57], and photochemical thrombosis [58]. Yet, at this point, none of these studies investigated the long-term outcome of stroke, hypoxia, or the disruption of blood–brain barrier in the zebrafish. Hence, whether seizures would develop in these models is still an open question. All these findings suggest that there is a lot of untapped potential to investigate epileptogenesis in zebrafish brain injury, stroke, hypoxia, and blood–brain barrier disruption.

Febrile seizures

Febrile seizures often occur in children and are symptomatically treated by the administration of antiseizure drugs [59] (Fig. 1). Febrile seizures are thought to have little long-term consequences, but in some cases can lead to epileptogenesis later in life, which is a process that is insufficiently understood [60]. Rodent models of febrile seizures have been instrumental for identifying the neural basis of febrile seizures [61,62]. These studies revealed that prolonged febrile seizures did not lead to excessive neural death, and there was no obvious effect on neurogenesis [62]. However, prominent alterations of neural circuit connectivity and plasticity as well as alterations in the levels of interleukin-1beta, hyperpolarization-activated cyclic nucleotide-gated channels, and of endocannabinoid signaling were observed [61,62].

Zebrafish is a cold-blooded aquatic vertebrate, which can tolerate a large range of temperatures [63]. Hence, it is in principle a lot more straightforward to induce hyperthermia in zebrafish by controlling its bath temperature to study potential effects on seizure

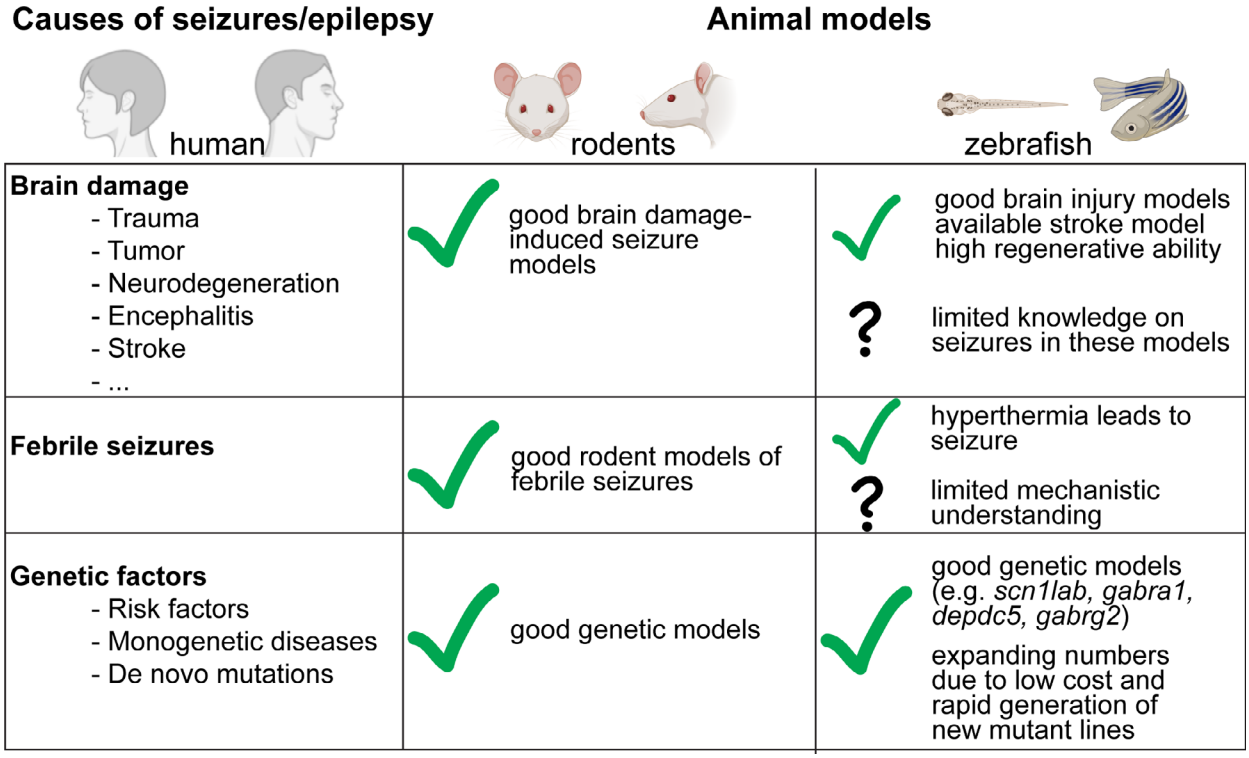

Fig. 1. Causes of epilepsy and commonly used animal models to study these conditions. Rodents have contributed extensively to our mechanistic understanding of different epilepsies and associated seizures. Over the last two decades, zebrafish has had growing popularity as an animal model for studying seizures, and the already broad contribution of zebrafish to epilepsy research, will most likely grow over the coming years. The introduction of trauma-induced zebrafish epilepsy models and especially the expansion of genetic zebrafish seizure models used as avatars in precision therapy are important factors.

generation. Indeed, a recent study showed that hyperthermia can induce seizures in zebrafish [64]. Also, a *de novo* mutation causing febrile seizures in humans was shown to elicit abnormal electrical activity in zebrafish brain [65]. Such hyperthermia-induced seizures in zebrafish can be potentially used for high-throughput screening approaches [30–33]. It is yet to be seen, whether zebrafish hyperthermia-induced seizures can reveal what biophysical and neural processes lead to febrile seizures, and whether early exposure to such seizures results in epileptogenesis at later developmental stages.

Genetic causes of epilepsies

Genetic polymorphisms or mutations in an individual may greatly affect the risk for epilepsy in all above-mentioned epilepsy etiologies and influence the outcome of antiseizure treatments [66,67]. Genetic mutations can also alone be the cause of epilepsy, in the case of monogenic epilepsy or epileptic encephalopathy [68,69] (Fig. 1). The latter, which is a heterogeneous group of severe epilepsies where the epileptic activity itself contributes to cognitive and behavioral deficits [68,70], includes among others the Dravet syndrome [30]. Most epileptic encephalopathies are clinically sporadic and commonly associated with *de novo* mutations [71,72], which are usually detected by DNA sequencing of gene panels or whole genome sequencing. During the analysis of sequencing results, o major challenge faced by clinical geneticists is to confirm the causality of novel genetic variants for the disease [67] to provide a personalized treatment. This is where research in genetic model organisms is crucial. Being able to identify whether the genetic variant is solely causative of the disease and to understand the molecular and functional impact of this mutation on the brain are crucial for the management of the patients. In this regard, zebrafish has been a model of choice to test the causative effect of human mutations on organ physio- and pathophysiology due to its highly conserved genome [73], widely available collections of mutants, well-optimized transient knockdown methods [74], transgenesis [75], genetic engineering

approaches [9,76], and its low maintenance cost in comparison with mammalian models [77].

The first and most widely studied zebrafish model of epilepsy is the Dravet model with a homozygous mutation in the *SCN1A* sodium channel orthologue gene *scn1lab* [78]. Various zebrafish models with *scn1lab* loss of function have been reported to exhibit hallmarks of epileptic seizures, including spontaneous electric discharge measured by local field potential, altered locomotor bouts, enhanced response to light stimuli, in addition to altered gene expression profiles, and premature death at larval stage [29,32,78–80]. Since the original discovery of the *scn1lab* zebrafish Dravet model, many mutant zebrafish lines have been generated for epilepsy-related genes [28,29,81–85]. Many of these genetic mutants displayed epilepsy-related phenotypes already in larval stages, which include altered spontaneous and/or sensory-driven brain activity, aberrant locomotion activity with or without neurodevelopmental defects, and early mortality. However, seizure phenotypes are not always apparent at larval stage but develop later in life. For instance, zebrafish mutants for the gamma-aminobutyric acid (GABA)-A receptor subunit *gabra1* gene, associated with juvenile myoclonic epilepsy, present light-induced seizures resulting in sudden unexpected death at around 1 month of age [28]. Interestingly, this study revealed progressive transcriptional changes prior to seizure onset unraveling novel molecular processes underlying the transition of the brain from a healthy to an epileptic stage [28]. Despite showing variability in the onset, severity, and underlying mechanisms of the disease, all these zebrafish mutant lines display common epilepsy-related phenotypes. Thus, they offer the possibility to perform comparative analysis to identify common and divergent pathophysiological mechanisms in epilepsy.

Due to its small size, the zebrafish larva is commonly used for high-throughput phenotypic drug screening [86]. For example, screens in *scn1lab* Dravet model identified novel molecules that reduce the enhanced locomotor activity associated with the mutation [78,80,87,88]. In fact, some of these molecules are now in clinical trials as antiseizure drug in humans [30], highlighting the great potential of this approach. It is, however, important to stress that different zebrafish epilepsy models may display very different locomotor behaviors, mimicking human patients suffering from different forms of epilepsy. It is also likely that the manifestation of seizures in zebrafish may depend on multiple factors such as age, mutation, and genetic background of the animals, as well as experimental settings, including light, temperature, physiological states (i.e., sleep), and even the size of behavioral arenas.

Thus, relying solely on increased locomotor activity or velocity as a high-throughput screening readout may miss certain types of seizures. In fact, simultaneous imaging of brain activity and locomotion (Fig. 2A,B, Movie S1) in head-restrained tail-free zebrafish larvae [26,89] can be very informative for revealing the relationship between different types of seizures and locomotor activity. Our analysis of *elavl3:GCaMP6s* zebrafish larvae expressing GCaMP6s pan-neuronally [11] revealed that during baseline, small locomotor bursts coincides very well with bursts of brain activity (Fig. 2D left). However, after the addition of the GABA-A receptor antagonist pentylenetetrazol (PTZ) to induce seizures [11], locomotor bursts coincide only with less than half of the brain activity bursts during the pre-ictal period (Fig. 2D middle). These results are in line with earlier observations [26] and highlight that at least in the PTZ-induced seizure model, monitoring locomotor activity alone might not be sufficient to follow abnormal pre-ictal brain activity. In fact, our observation and earlier studies [26] suggest that only those large bursts of abnormal pre-ictal neural activity (Fig. 2B) and generalized seizures (Fig. 2C) invading the brainstem lead to locomotor bursts. All these findings are in line with the idea that recording brain activity is crucial for accurately evaluating new zebrafish seizure models or all high-throughput locomotion-based screens, prior to making conclusions on the presence or absence of seizure-like activity. Especially while assessing novel genetic seizure models, a good alternative to recording of spontaneous seizures can be the use of simple sensory stimuli (i.e., light, vibrations). Using sensory stimulation can be a very powerful method to reliably assess neuronal hyperexcitability or even trigger seizure in a time-controlled manner. This approach also increases the robustness of high-throughput drug screening, as it was successfully demonstrated in the zebrafish Dravet model, *scn1lab* mutants [32,79].

Increasing accessibility of genome sequencing-based diagnostics boosted our knowledge on the genetics of epilepsy and lead to a surge in personalized medicine or precision therapies tailored for specific seizure disorders [90]. Such personalized medicine requires rapid and economically viable investigation of disease-associated genes to identify potential therapies. As discussed above, zebrafish offers well-optimized genetic tools to recapitulate human mutations, as well as high-throughput and low costs drug screening platforms. Therefore, zebrafish is an optimal animal model that can serve as patient avatars for discovering precision drugs for a variety of diseases [91]. In fact, zebrafish-based precision therapies have already started saving patients' lives [92]. Over the coming years, it is very likely that we will see further precision therapies

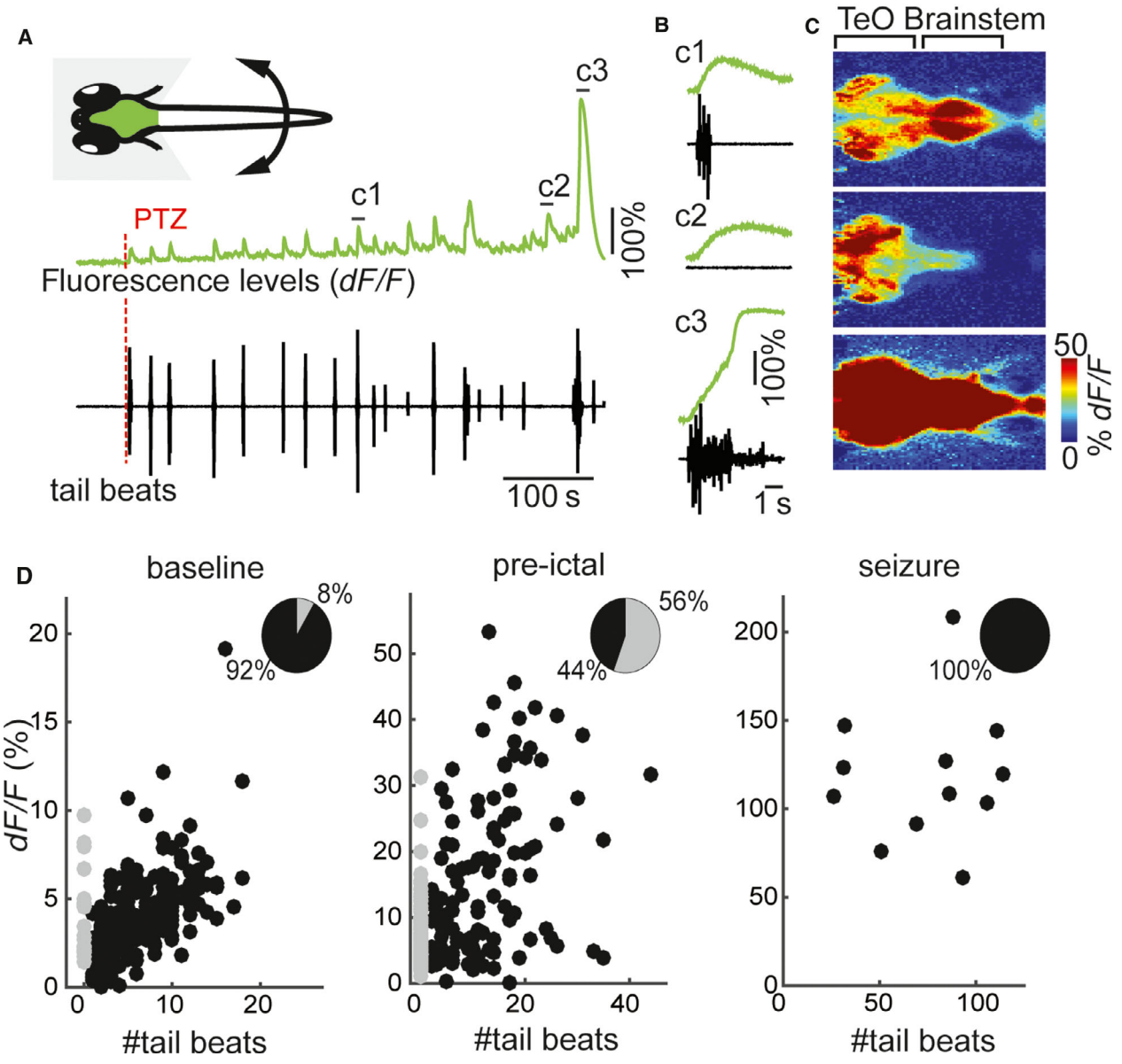

Fig. 2. Monitoring brain activity is crucial while evaluating zebrafish seizure models. (A) Simultaneous fluorescence calcium imaging of brain activity (green) and video recording of locomotor tail beats (black) in 7-day-old *elavl3:GCaMP6s* zebrafish larvae expressing GCaMP6s pan-neuronally, treated with PTZ to induce seizures [11]. Red dashed line marks the time point where PTZ reaches the animals. Scheme (top-left) represents a head-restrained zebrafish larva with a free tail to measure locomotor activity. (B) Examples of different calcium bursts (green) detected during pre-ictal (c1, c2) and ictal (c3) activity, and the locomotor tail beats (black) associated with these selected events (left). Note that pre-ictal calcium bursts 'c2' do not elicit any detectable tail beat. (C) Spatial distribution of neural activity across the zebrafish brain regions optic tectum (TeO) and brainstem, during pre-ictal (c1, c2) and ictal (c3) calcium bursts (right). Warm colors represent stronger neural activity. Note that the small signals in the brainstem during pre-ictal calcium bursts 'c2' do not elicit locomotor tail beats. (D) The relationship between the number of locomotor tail beats and the amplitude of detected calcium bursts during baseline, pre-ictal period, and generalized seizure. Black dots represent calcium bursts that coincide with locomotor tail beats. Gray dots represent calcium bursts that do not elicit any detectable locomotor tail beat. Pie charts represent the ratio of calcium bursts overlapping (black), and not overlapping (gray) with locomotor activity. Note that during baseline and generalized seizures almost all calcium bursts coincide with locomotor activity. However, half of the pre-ictal calcium bursts do not elicit any detectible locomotor activity. These results highlight the importance of measuring brain activity ideally across the entire brain, while evaluating zebrafish seizure models and associated high-throughput screens.

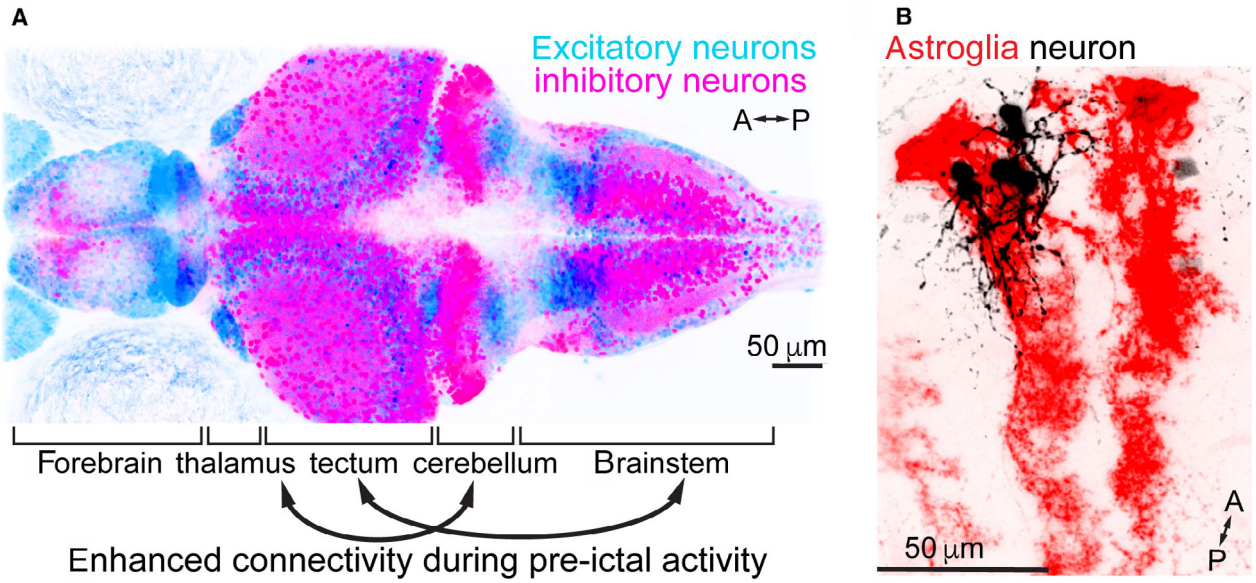

Fig. 3. Visualizing and perturbing diverse neuronal and astroglial populations of zebrafish brain. (A) Confocal microscopy image of a 7-day-old zebrafish larvae. Excitatory neurons are labeled by *Tg(vglut2a:dsRED)* [140] (cyan). Inhibitory neurons are labeling by *Tg(gad1:GFP)* [98] (magenta) transgenic expression. Note that the distribution of excitatory and inhibitory neurons across the brain regions are not homogenous. Arrows highlight brain regions with increased synchrony during pre-ictal activity in the PTZ-induced seizure model. (B) Confocal microscopy image of a 4-week-old old juvenile zebrafish. Scattered groups of astroglia (red) are labeled by *Tg(GFAP:Gal4)nw7;Tg (UAS:ChR2-mCherry)* [11]. Individual neurons (black) are filled by neurobiotin using a patch-clamp electrode. Note that neuronal and astroglial processes are in close proximity. Astroglia-neuron interactions play important roles in regulating neuronal excitability during seizure generation and propagation, by controlling extracellular glutamate via glutamate transporters and potassium levels via gap junctions.

developed using zebrafish, especially for those patients suffering from genetic epilepsies.

It is also important to highlight that the functional and mechanistic studies are missing for several new genetic seizure models of zebrafish as well as for newly identified antiseizure drugs. Thus, the next challenge will be to perform comparative functional studies using these mutant lines and identify both neuronal and glial correlates of hyperexcitability and seizure generation, and the action mechanisms of novel antiseizure drugs. Understanding the common and divergent neuronal or glial causes leading to seizures and the molecular/cellular pathways underlying the action of new antiseizure drugs will be crucial to design personalized therapies in the context of both genetic and nongenetic forms of epilepsy.

Mechanisms of seizure generation and seizure spread

The role of inhibition in seizures

Generation of epileptic seizures can be seen as a transition of the brain activity and connectivity from a balanced into an unbalanced state with excessive synchrony [93]. Breakdown of the inhibition is one of the main hypotheses for the triggering of seizures in several different forms of epilepsy [94–96]. Hence, similar to rodent seizure models, suppressing inhibition in the zebrafish brain leads to generation of seizures, both in pharmacologic [11–13,24–27] and in genetic perturbations of GABA receptors [28]. In fact, a recent study showed that the zebrafish brain regions rich in GABAergic neurons (Fig. 3A) display symptoms of synchronous hyperactivity earlier than other parts of the brain, even before full blown generalized seizures [11]. There are several transgenic zebrafish lines that effectively label and allow control of large groups of inhibitory neuron populations such as dlx4/6 [97] or gad1b [98,99] across the entire brain (Fig. 3A). Moreover, histological studies clearly demonstrated the presence of markers of several mammalian inhibitory interneuron markers (parvalbumin, calbindin, calretinin) in the zebrafish brain [100–102]. Investigating the activity of these inhibitory neuron populations across the entire brain before and during seizures, and revealing their cellular and molecular alterations during the epileptogenic processes, will open new avenues. An example was beautifully demonstrated

in a recent study, which unravels a whole new set of gene alterations in a zebrafish mutant lacking the *gabra1* gene, a GABA-A receptor subunit [28].

Spreading of seizures through hubs in the brain

Another well-accepted hypothesis with respect to spreading of the seizures is the concept of epileptogenic hubs, also known as choke points [103]. Such epileptogenic hubs are important for spreading of seizures from a focal point toward the entire brain, leading to generalized seizures. Excitingly, interfering with pathological hubs was also shown to be effective in preventing seizure propagation [104,105]. Two potential choke points in the mammalian brain that can interfere with seizure generation are the thalamus [104,106] and the cerebellum [107,108]. In fact, the anatomy and the connectivity of these brain regions are well conserved in vertebrates including in zebrafish [109,110]. Moreover, both of these regions were shown to exhibit excessive synchronous neural activity preceding generalized seizures in a pharmacologically induced zebrafish seizure model [11,13] (Fig. 3A). Several transgenic zebrafish lines are readily available for labeling and manipulating the thalamus [111] and the cerebellum [112,113]. Hence, future studies investigating the role of thalamic and cerebellar pathways in seizures can shed light on how or whether the stimulation of these brain regions can interfere with seizure generation and propagation.

The role of glia in promoting and preventing seizures

The role of glia and glial dysfunction in epilepsy is an expanding field [114,115]. Several mutations in glia-associated genes are linked with epilepsy [116]. Moreover, multiple aspects of glial biology from metabolism [117,118] to signaling [119–121], gap junctions [122–124], and even the immune responses [125–127] are attributed to the manifestation of epileptic seizures. All these results also highlight the potential of targeting glia biology as a potential alternative therapy against epileptic seizures [128,129]. Major glial types of the vertebrate brain are present in zebrafish, and they serve similar functions in regulating neural activity, neural development and the brain's immune response [52,130–132] (Fig. 3B). Accumulating evidence in zebrafish seizure models also highlight the role of glia in epilepsy. For example, a recent study showed that several astroglial marker genes are differentially expressed in a zebrafish model of Dravet syndrome [29]. Moreover, an earlier study revealed that zebrafish astroglia express gap junction protein connexin 43 (Cx43) similar to mammals and they exhibit highly synchronized astroglial calcium signals preceding epileptic seizures [11]. A computational model further showed how such gap junction coupled astroglial networks can contribute to the propagation of seizures [133]. Despite the correlated glial and neuronal activity during generalized seizures, glial calcium signals during 'the preictal state' preceding the seizures were shown to be anticorrelated with neural activity. This is indeed in line with the function of glial glutamate transporter excitatory amino acid transporter 2 (EAAT2) balancing excess glutamate and thus dampening excessive neural activity. In fact, mutations in EAAT2 in human patients [116,134] and in rodents [135] are directly associated with epileptic seizures. Not surprisingly, EAAT2 is proposed to be an interesting target for epilepsy [129,136]. Given the high-throughput nature of zebrafish for discovering antiseizure drugs [30–33,78–80,87,88], identifying new molecules to modulate glial glutamate transporters can help clearing excess glutamate and potentially dampen seizures. Similar approaches might also be interesting for targeting astroglial gap junction Cx43, given the important role of gap junctions in epilepsy and seizure generation [122–124].

Microglia have also been shown to play an important role for regulating neural excitability and inflammatory response in epilepsy [127,137]. Two recent zebrafish studies demonstrated how microglia can directly control the excitability of neurons *in vivo* [138], and how calcium is important for recruiting microglia to the site of brain injury [139]. All this evidence suggests that investigating microglial function and dynamics in trauma-induced or other seizure models in zebrafish can be a promising approach to better elucidate the role of microglia in seizure control. Several tools for interfering with microglial function are readily available in zebrafish [131]. It is now time to look further into the cellular and functional alterations of microglia during genetic or pharmacological seizure models of zebrafish. It will be interesting to investigate how perturbing microglial function can interfere with seizure generation and spread.

Conclusions

The number of research laboratories using zebrafish for investigating molecular, cellular, and functional processes underlying epileptic seizures is increasing rapidly. An obvious advantage of zebrafish is its amenability for high-throughput genetic and chemical screens to identify genetic pathways and molecules that can be used for epilepsy therapies. Yet, this small and transparent vertebrate offers so much more than that. As we discussed in this viewpoint, over the course of the last 20 years, research in zebrafish is experiencing a huge boost. The development of imaging technologies has

given unprecedented detail about the brain, vasculature, behavior, organ development, and even gene expression in living animals. We also now better understand the aspects of zebrafish brain development and function that relate to the human brain and its diseases, and what are the differences. In fact, knowing such differences can also lead to direct benefit for other important research fields, as it is for the case of the amazing capacity of zebrafish brain regeneration after injury. We argue that the plethora of recent studies using zebrafish makes a very good case for the immense potential of this small vertebrate model for epilepsy research. It is also important to highlight that the conservation of neural and developmental mechanisms leading to epileptic seizures both in zebrafish and in rodent models support the idea that such mechanisms are more likely to be common across all vertebrates, including humans.

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Conflict of interests

The authors declare no conflict of interest.

Author contributions

EY and NJ-Y conceptualized the study; CDV contributed to methodology and data; EY, AJ, and NJ-Y wrote the manuscript; all authors reviewed and edited; EY and NJ-Y contributed to funding acquisition and supervision.

Peer Review

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[Correction added on 24 March 2021, after first online publication: URL for peer review history has been corrected.]

References

1 WHO (2019) Epilepsy. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/epilepsy
2 Louis ED, Mayer SA & Rowland LP (2015) Epilepsy. Wolters Kluwer Health, Philadelphia, PA.
3 Kwan P, Schachter SC & Brodie MJ (2011) Drug-resistant epilepsy. *N Engl J Med* **365**, 919–966.
4 Libor VN, Nebieridze N, Tamar C & Jana V (2013) Anti-seizure medications and estradiol for neuroprotection in epilepsy: the 2013 update. *Recent Pat CNS Drug Discov* **8**, 24–41.
5 French JA & Perucca E (2020) Time to start calling things by their own names? The case for antiseizure medicines. *Epilepsy Curr* **20**, 69–72.
6 Kawakami K, Asakawa K, Hibi M, Itoh M, Muto A & Wada H (2016) Gal4 driver transgenic zebrafish: powerful tools to study developmental biology, organogenesis, and neuroscience. *Adv Genet* **95**, 65–87.
7 Scott EK, Mason L, Arrenberg AB, Ziv L, Gosse NJ, Xiao T, Chi NC, Asakawa K, Kawakami K & Baier H (2007) Targeting neural circuitry in zebrafish using GAL4 enhancer trapping. *Nat Methods* **4**, 323–326.
8 Marquart GD, Tabor KM, Brown M, Strykowski JL, Varshney GK, LaFave MC, Mueller T, Burgess SM, Higashijima S & Burgess HA (2015) A 3D searchable database of transgenic zebrafish Gal4 and Cre lines for functional neuroanatomy studies. *Front Neural Circuits* **9**, 78.
9 Wyatt C, Bartoszek EM & Yaksi E (2015) Methods for studying the zebrafish brain: past, present and future. *Eur J Neurosci* **42**, 1746–1763.
10 Ahrens MB, Li JM, Orger MB, Robson DN, Schier AF, Engert F & Portugues R (2012) Brain-wide neuronal dynamics during motor adaptation in zebrafish. *Nature* **485**, 471–477.
11 Diaz Verdugo C, Myren-Svelstad S, Aydin E, Van Hoeymissen E, Deneubourg C, Vanderhaeghe S, Vancraeynest J, Pelgrims R, Cosacak MI, Muto A *et al.* (2019) Glia-neuron interactions underlie state transitions to generalized seizures. *Nat Commun* **10**, 3830.
12 Liu J & Baraban SC (2019) Network properties revealed during multi-scale calcium imaging of seizure activity in Zebrafish. *eneuro* **6**.
13 Rosch RE, Hunter PR, Baldeweg T, Friston KJ & Meyer MP (2018) Calcium imaging and dynamic causal modelling reveal brain-wide changes in effective connectivity and synaptic dynamics during epileptic seizures. *PLoS Comput Biol* **14**, e1006375.
14 Randlett O, Wee CL, Naumann EA, Nnaemeka O, Schoppik D, Fitzgerald JE, Portugues R, Lacoste AM, Riegler C, Engert F *et al.* (2015) Whole-brain activity

mapping onto a zebrafish brain atlas. *Nat Methods* **12**, 1039–1046.
15 Thyme SB, Pieper LM, Li EH, Pandey S, Wang Y, Morris NS, Sha C, Choi JW, Herrera KJ, Soucy ER *et al.* (2019) Phenotypic landscape of schizophrenia-associated genes defines candidates and their shared functions. *Cell* **177**, 478–491.e20.
16 Dreosti E, Lopes G, Kampff AR & Wilson SW (2015) Development of social behavior in young zebrafish. *Front Neural Circuits* **9**, 39.
17 Palumbo F, Serneels B, Pelgrims R & Yaksi E (2020) The Zebrafish dorsolateral Habenula is required for updating learned behaviors. *Cell Rep* **32**, 108054.
18 Lange C, Rost F, Machate A, Reinhardt S, Lesche M, Weber A, Kuscha V, Dahl A, Rulands S & Brand M (2020) Single cell sequencing of radial glia progeny reveals the diversity of newborn neurons in the adult zebrafish brain. *Development* **147**.
19 Raj B, Wagner DE, McKenna A, Pandey S, Klein AM, Shendure J, Gagnon JA & Schier AF (2018) Simultaneous single-cell profiling of lineages and cell types in the vertebrate brain. *Nat Biotechnol* **36**, 442–450.
20 Cosacak MI, Bhattarai P, Reinhardt S, Petzold A, Dahl A, Zhang Y & Kizil C (2019) Single-cell transcriptomics analyses of neural stem cell heterogeneity and contextual plasticity in a Zebrafish brain model of amyloid toxicity. *Cell Rep* **27**, 1307–1318.e3.
21 Hildebrand DGC, Cicconet M, Torres RM, Choi W, Quan TM, Moon J, Wetzel AW, Scott Champion A, Graham BJ, Randlett O *et al.* (2017) Whole-brain serial-section electron microscopy in larval zebrafish. *Nature* **545**, 345–349.
22 Kunst M, Laurell E, Mokayes N, Kramer A, Kubo F, Fernandes AM, Forster D, Dal Maschio M & Baier H (2019) A cellular-resolution atlas of the larval Zebrafish brain. *Neuron* **103**, 21–38.e5.
23 Tabor KM, Marquart GD, Hurt C, Smith TS, Geoca AK, Bhandiwad AA, Subedi A, Sinclair JL, Rose HM, Polys NF *et al.* (2019) Brain-wide cellular resolution imaging of Cre transgenic zebrafish lines for functional circuit-mapping. *Elife* **8**.
24 Afrikanova T, Serruys AS, Buenafe OE, Clinckers R, Smolders I, de Witte PA, Crawford AD & Esguerra CV (2013) Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs. *PLoS One* **8**, e54166.
25 Baraban SC, Taylor MR, Castro PA & Baier H (2005) Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. *Neuroscience* **131**, 759–768.
26 Turrini L, Fornetto C, Marchetto G, Mullenbroich MC, Tiso N, Vettori A, Resta F, Masi A, Mannaioni G, Pavone FS *et al.* (2017) Optical mapping of neuronal activity during seizures in zebrafish. *Sci Rep* **7**, 3025.
27 Baxendale S, Holdsworth CJ, Meza Santoscoy PL, Harrison MR, Fox J, Parkin CA, Ingham PW & Cunliffe VT (2012) Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures. *Dis Model Mech* **5**, 773–784.
28 Samarut É, Swaminathan A, Riché R, Liao M, Hassan-Abdi R, Renault S, Allard M, Dufour L, Cossette P, Soussi-Yanicostas N *et al.* (2018) γ-Aminobutyric acid receptor alpha 1 subunit loss of function causes genetic generalized epilepsy by impairing inhibitory network neurodevelopment. *Epilepsia* **59**, 2061–2074.
29 Tiraboschi E, Martina S, van der Ent W, Grzyb K, Gawel K, Cordero-Maldonado ML, Poovathingal SK, Heintz S, Satheesh SV, Brattespe J *et al.* (2020) New insights into the early mechanisms of epileptogenesis in a zebrafish model of Dravet syndrome. *Epilepsia* **61**, 549–560.
30 Griffin A, Hamling KR, Hong S, Anvar M, Lee LP & Baraban SC (2018) Preclinical animal models for Dravet syndrome: seizure phenotypes, comorbidities and drug screening. *Front Pharmacol* **9**, 573.
31 Cunliffe VT, Baines RA, Giachello CN, Lin WH, Morgan A, Reuber M, Russell C, Walker MC & Williams RS (2015) Epilepsy research methods update: Understanding the causes of epileptic seizures and identifying new treatments using non-mammalian model organisms. *Seizure* **24**, 44–51.
32 Eimon PM, Ghannad-Rezaie M, De Rienzo G, Allalou A, Wu Y, Gao M, Roy A, Skolnick J & Yanik MF (2018) Brain activity patterns in high-throughput electrophysiology screen predict both drug efficacies and side effects. *Nat Commun* **9**, 219.
33 Ibhazehiebo K, Gavrilovici C, de la Hoz CL, Ma SC, Rehak R, Kaushik G, Meza Santoscoy PL, Scott L, Nath N, Kim DY *et al.* (2018) A novel metabolism-based phenotypic drug discovery platform in zebrafish uncovers HDACs 1 and 3 as a potential combined anti-seizure drug target. *Brain* **141**, 744–761.
34 Lowenstein DH (2009) Epilepsy after head injury: an overview. *Epilepsia* **50** (Suppl 2), 4–9.
35 Webster KM, Sun M, Crack P, O'Brien TJ, Shultz SR & Semple BD (2017) Inflammation in epileptogenesis after traumatic brain injury. *J Neuroinflammation* **14**, 10.
36 Politsky JM (2017) Brain tumor-related epilepsy: a current review of the etiologic basis and diagnostic and treatment approaches. *Curr Neurol Neurosci Rep* **17**, 70.
37 Shamji MF, Fric-Shamji EC & Benoit BG (2009) Brain tumors and epilepsy: pathophysiology of peritumoral changes. *Neurosurg Rev* **32**, 275–284; discussion 284–6.
38 Doria JW & Forgacs PB (2019) Incidence, implications, and management of seizures following ischemic and hemorrhagic stroke. *Curr Neurol Neurosci Rep* **19**, 37.

39 Kim SY, Porter BE, Friedman A & Kaufer D (2016) A potential role for glia-derived extracellular matrix remodeling in postinjury epilepsy. *J Neurosci Res* **94**, 794–803.

40 Robel S (2017) Astroglial scarring and seizures: a cell biological perspective on epilepsy. *Neuroscientist* **23**, 152–168.

41 Vezzani A, Fujinami RS, White HS, Preux PM, Blumcke I, Sander JW & Loscher W (2016) Infections, inflammation and epilepsy. *Acta Neuropathol* **131**, 211–234.

42 Brady RD, Casillas-Espinosa PM, Agoston DV, Bertram EH, Kamnaksh A, Semple BD & Shultz SR (2019) Modelling traumatic brain injury and posttraumatic epilepsy in rodents. *Neurobiol Dis* **123**, 8–19.

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

44 Ostergard T, Sweet J, Kusyk D, Herring E & Miller J (2016) Animal models of post-traumatic epilepsy. *J Neurosci Methods* **272**, 50–55.

45 Kyritsis N, Kizil C & Brand M (2014) Neuroinflammation and central nervous system regeneration in vertebrates. *Trends Cell Biol* **24**, 128–135.

46 Diotel N, Lübke L, Strähle U & Rastegar S (2020) Common and distinct features of adult neurogenesis and regeneration in the telencephalon of Zebrafish and mammals. *Front Neurosci* **14**.

47 Kyritsis N, Kizil C, Zocher S, Kroehne V, Kaslin J, Freudenreich D, Iltzsche A & Brand M (2012) Acute inflammation initiates the regenerative response in the adult Zebrafish brain. *Science* **338**, 1353–1356.

48 Kroehne V, Freudenreich D, Hans S, Kaslin J & Brand M (2011) Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. *Development* **138**, 4831–4841.

49 Barbosa JS, Sanchez-Gonzalez R, Di Giaimo R, Baumgart EV, Theis FJ, Götz M & Ninkovic J (2015) Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain. *Science* **348**, 789–793.

50 Bhattarai P, Thomas AK, Cosacak MI, Papadimitriou C, Mashkaryan V, Froc C, Reinhardt S, Kurth T, Dahl A, Zhang Y *et al.* (2016) IL4/STAT6 Signaling activates neural stem cell proliferation and neurogenesis upon amyloid-β42 aggregation in adult zebrafish brain. *Cell Rep* **17**, 941–948.

51 Cho SJ, Park E, Telliyan T, Baker A & Reid AY (2020) Zebrafish model of posttraumatic epilepsy. *Epilepsia* **61**, 1774–1785.

52 Jurisch-Yaksi N, Yaksi E & Kizil C (2020) Radial glia in the zebrafish brain: functional, structural, and physiological comparison with the mammalian glia. *Glia* **68**, 2451–2470.

53 Leo A, De Caro C, Nesci V, Tallarico M, De Sarro G, Russo E & Citraro R (2020) Modeling poststroke epilepsy and preclinical development of drugs for poststroke epilepsy. *Epilepsy Behav* **104**, 106472.

54 Walcott BP & Peterson RT (2014) Zebrafish models of cerebrovascular disease. *J Cereb Blood Flow Metab* **34**, 571–577.

55 Kugler EC, van Lessen M, Daetwyler S, Chhabria K, Savage AM, Silva V, Plant K, MacDonald RB, Huisken J, Wilkinson RN *et al.* (2019) Cerebrovascular endothelial cells form transient Notch-dependent cystic structures in zebrafish. *EMBO Rep* **20**, e47047.

56 Crilly S, Njegic A, Parry-Jones AR, Allan SM & Kasher PR (2019) Using Zebrafish larvae to study the pathological consequences of hemorrhagic stroke. *J Vis Exp*.

57 Das T, Soren K, Yerasi M, Kumar A & Chakravarty S (2019) Revealing sex-specific molecular changes in hypoxia-ischemia induced neural damage and subsequent recovery using zebrafish model. *Neurosci Lett* **712**, 134492.

58 Chen J, He J, Ni R, Yang Q, Zhang Y & Luo L (2019) Cerebrovascular injuries induce lymphatic invasion into brain parenchyma to guide vascular regeneration in Zebrafish. *Dev Cell* **49**, 697–710.e5.

59 Laino D, Mencaroni E & Esposito S (2018) Management of pediatric febrile seizures. *Int J Environ Res Public Health* **15**.

60 Dube CM, Brewster AL, Richichi C, Zha Q & Baram TZ (2007) Fever, febrile seizures and epilepsy. *Trends Neurosci* **30**, 490–496.

61 Dube CM, Brewster AL & Baram TZ (2009) Febrile seizures: mechanisms and relationship to epilepsy. *Brain Dev* **31**, 366–371.

62 Bender RA, Dube C & Baram TZ (2004) Febrile seizures and mechanisms of epileptogenesis: insights from an animal model. *Adv Exp Med Biol* **548**, 213–225.

63 Engeszer RE, Patterson LB, Rao AA & Parichy DM (2007) Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish* **4**, 21–40.

64 Hunt RF, Hortopan GA, Gillespie A & Baraban SC (2012) A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors. *Exp Neurol* **237**, 199–206.

65 Suls A, Jaehn JA, Kecskes A, Weber Y, Weckhuysen S, Craiu DC, Siekierska A, Djemie T, Afrikanova T, Gormley P *et al.* (2013) *De novo* loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. *Am J Hum Genet* **93**, 967–975.

66 Myers CT & Mefford HC (2015) Advancing epilepsy genetics in the genomic era. *Genome Med* **7**, 91.

67 Poduri A, Sheidley BR, Shostak S & Ottman R (2014) Genetic testing in the epilepsies—developments and dilemmas. *Nat Rev Neurol* **10**, 293–299.
68 Epi KC, Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, Epstein MP, Glauser T *et al.* (2013) *De novo* mutations in epileptic encephalopathies. *Nature* **501**, 217–221.
69 Myers KA, Johnstone DL & Dyment DA (2019) Epilepsy genetics: Current knowledge, applications, and future directions. *Clin Genet* **95**, 95–111.
70 Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, Hirsch E, Jain S, Mathern GW, Moshe SL *et al.* (2017) ILAE classification of the epilepsies: position paper of the ILAE commission for classification and terminology. *Epilepsia* **58**, 512–521.
71 Heyne HO, Singh T, Stamberger H, Abou Jamra R, Caglayan H, Craiu D, De Jonghe P, Guerrini R, Helbig KL, Koeleman BPC *et al.* (2018) *De novo* variants in neurodevelopmental disorders with epilepsy. *Nat Genet* **50**, 1048–1053.
72 Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C & De Jonghe P (2001) *De novo* mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Human Genet* **68**, 1327–1332.
73 Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L *et al.* (2013) The zebrafish reference genome sequence and its relationship to the human genome. *Nature* **496**, 498–503.
74 Stainier DYR, Raz E, Lawson ND, Ekker SC, Burdine RD, Eisen JS, Ingham PW, Schulte-Merker S, Yelon D, Weinstein BM *et al.* (2017) Guidelines for morpholino use in zebrafish. *PLoS Genet* **13**, e1007000.
75 Kawakami K, Takeda H, Kawakami N, Kobayashi M, Matsuda N & Mishina M (2004) A Transposon-mediated gene trap approach identifies developmentally regulated genes in Zebrafish. *Dev Cell* **7**, 133–144.
76 Li M, Zhao L, Page-McCaw PS & Chen W (2016) Zebrafish genome engineering using the CRISPR–Cas9 system. *Trends Genet* **32**, 815–827.
77 Lieschke GJ & Currie PD (2007) Animal models of human disease: Zebrafish swim into view. *Nat Rev Genet* **8**, 353–367.
78 Baraban SC, Dinday MT & Hortopan GA (2013) Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. *Nat Commun* **4**, 2410.
79 Ghannad-Rezaie M, Eimon PM, Wu Y & Yanik MF (2019) Engineering brain activity patterns by neuromodulator polytherapy for treatment of disorders. *Nat Commun* **10**, 2620.
80 Zhang Y, Kecskés A, Copmans D, Langlois M, Crawford AD, Ceulemans B, Lagae L, de Witte PAM & Esguerra CV (2015) Pharmacological characterization of an antisense knockdown zebrafish model of Dravet syndrome: inhibition of epileptic seizures by the serotonin agonist fenfluramine. *PLoS One* **10**, e0125898.
81 Siekierska A, Stamberger H, Deconinck T, Oprescu SN, Partoens M, Zhang Y, Sourbron J, Adriaenssens E, Mullen P, Wiencek P *et al.* (2019) Biallelic VARS variants cause developmental encephalopathy with microcephaly that is recapitulated in vars knockout zebrafish. *Nat Commun* **10**, 708.
82 Perenthaler E, Nikoncuk A, Yousefi S, Berdowski WM, Alsagob M, Capo I, van der Linde HC, van den Berg P, Jacobs EH, Putar D *et al.* (2020) Loss of UGP2 in brain leads to a severe epileptic encephalopathy, emphasizing that bi-allelic isoform-specific start-loss mutations of essential genes can cause genetic diseases. *Acta Neuropathol* **139**, 415–442.
83 Liao M, Kundap U, Rosch RE, Burrows DRW, Meyer MP, Ouled Amar Bencheikh B, Cossette P & Samarut É (2019) Targeted knockout of GABA-A receptor gamma 2 subunit provokes transient light-induced reflex seizures in zebrafish larvae. *Dis Models Mech* **12**, dmm040782.
84 Swaminathan A, Hassan-Abdi R, Renault S, Siekierska A, Riché R, Liao M, de Witte PAM, Yanicostas C, Soussi-Yanicostas N, Drapeau P *et al.* (2018) Non-canonical mTOR-independent role of DEPDC5 in regulating GABAergic network development. *Curr Biol* **28**, 1924–1937.e5.
85 Mahmood F, Fu S, Cooke J, Wilson SW, Cooper JD & Russell C (2013) A zebrafish model of CLN2 disease is deficient in tripeptidyl peptidase 1 and displays progressive neurodegeneration accompanied by a reduction in proliferation. *Brain* **136**, 1488–1507.
86 MacRae CA & Peterson RT (2015) Zebrafish as tools for drug discovery. *Nat Rev Drug Discov* **14**, 721–731.
87 Dinday MT & Baraban SC (2015) Large-scale phenotype-based antiepileptic drug screening in a Zebrafish model of Dravet syndrome. *eneuro* **2**, ENEURO.0068-15.2015
88 Griffin A, Hamling KR, Knupp K, Hong S, Lee LP & Baraban SC (2017) Clemizole and modulators of serotonin signalling suppress seizures in Dravet syndrome. *Brain* **140**, 669–683.
89 Vendrell-Llopis N & Yaksi E (2015) Evolutionary conserved brainstem circuits encode category, concentration and mixtures of taste. *Sci Rep* **5**, 17825.
90 Epi PMC (2015) A roadmap for precision medicine in the epilepsies. *Lancet Neurol* **14**, 1219–1228.
91 Costa B, Estrada MF, Mendes RV & Fior R (2020) Zebrafish avatars towards personalized medicine-a comparative review between avatar models. *Cells* **9**, 293.
92 Li D, March ME, Gutierrez-Uzquiza A, Kao C, Seiler C, Pinto E, Matsuoka LS, Battig MR, Bhoj EJ,

Wenger TL *et al.* (2019) ARAF recurrent mutation causes central conducting lymphatic anomaly treatable with a MEK inhibitor. *Nat Med* **25**, 1116–1122.
93 van Diessen E, Diederen SJ, Braun KP, Jansen FE & Stam CJ (2013) Functional and structural brain networks in epilepsy: what have we learned? *Epilepsia* **54**, 1855–1865.
94 Trevelyan AJ, Sussillo D & Yuste R (2007) Feedforward inhibition contributes to the control of epileptiform propagation speed. *J Neurosci* **27**, 3383–3387.
95 Schevon CA, Weiss SA, McKhann G Jr, Goodman RR, Yuste R, Emerson RG & Trevelyan AJ (2012) Evidence of an inhibitory restraint of seizure activity in humans. *Nat Commun* **3**, 1060.
96 Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC & Rubenstein JL (2005) Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nat Neurosci* **8**, 1059–1068.
97 Zhu P, Narita Y, Bundschuh ST, Fajardo O, Scharer YP, Chattopadhyaya B, Bouldoires EA, Stepien AE, Deisseroth K, Arber S *et al.* (2009) Optogenetic dissection of neuronal circuits in zebrafish using viral gene transfer and the tet System. *Front Neural Circuits* **3**, 21.
98 Satou C, Kimura Y, Hirata H, Suster ML, Kawakami K & Higashijima S (2013) Transgenic tools to characterize neuronal properties of discrete populations of zebrafish neurons. *Development* **140**, 3927–3931.
99 Kermen F, Lal P, Faturos NG & Yaksi E (2020) Interhemispheric connections between olfactory bulbs improve odor detection. *PLoS Biol* **18**, e3000701.
100 von Trotha JW, Vernier P & Bally-Cuif L (2014) Emotions and motivated behavior converge on an amygdala-like structure in the zebrafish. *Eur J Neurosci* **40**, 3302–3315.
101 Castro A, Becerra M, Manso MJ & Anadon R (2006) Calretinin immunoreactivity in the brain of the zebrafish, Danio rerio: distribution and comparison with some neuropeptides and neurotransmitter-synthesizing enzymes. I. Olfactory organ and forebrain. *J Comp Neurol* **494**, 435–459.
102 Mueller T, Dong Z, Berberoglu MA & Guo S (2011) The dorsal pallium in zebrafish, Danio rerio (Cyprinidae, Teleostei). *Brain Res* **1381**, 95–105.
103 Paz JT & Huguenard JR (2015) Microcircuits and their interactions in epilepsy: is the focus out of focus? *Nat Neurosci* **18**, 351–359.
104 Paz JT, Davidson TJ, Frechette ES, Delord B, Parada I, Peng K, Deisseroth K & Huguenard JR (2013) Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. *Nat Neurosci* **16**, 64–70.
105 Varotto G, Tassi L, Franceschetti S, Spreafico R & Panzica F (2012) Epileptogenic networks of type II focal cortical dysplasia: a stereo-EEG study. *NeuroImage* **61**, 591–598.
106 Fogerson PM & Huguenard JR (2016) Tapping the brakes: cellular and synaptic mechanisms that regulate thalamic oscillations. *Neuron* **92**, 687–704.
107 Miterko LN, Baker KB, Beckinghausen J, Bradnam LV, Cheng MY, Cooperrider J, DeLong MR, Gornati SV, Hallett M, Heck DH *et al.* (2019) Consensus paper: experimental neurostimulation of the cerebellum. *Cerebellum* **18**, 1064–1097.
108 Kros L, Eelkman Rooda OHJ, De Zeeuw CI & Hoebeek FE (2015) Controlling cerebellar output to treat refractory epilepsy. *Trends Neurosci* **38**, 787–799.
109 Hibi M & Shimizu T (2012) Development of the cerebellum and cerebellar neural circuits. *Dev Neurobiol* **72**, 282–301.
110 Scholpp S & Lumsden A (2010) Building a bridal chamber: development of the thalamus. *Trends Neurosci* **33**, 373–380.
111 Mohamed GA, Cheng RK, Ho J, Krishnan S, Mohammad F, Claridge-Chang A & Jesuthasan S (2017) Optical inhibition of larval zebrafish behaviour with anion channelrhodopsins. *BMC Biol* **15**, 103.
112 Takeuchi M, Matsuda K, Yamaguchi S, Asakawa K, Miyasaka N, Lal P, Yoshihara Y, Koga A, Kawakami K, Shimizu T *et al.* (2015) Establishment of Gal4 transgenic zebrafish lines for analysis of development of cerebellar neural circuitry. *Dev Biol* **397**, 1–17.
113 Chang W, Pedroni A, Hohendorf V, Giacomello S, Hibi M, Koster RW & Ampatzis K (2020) Functionally distinct Purkinje cell types show temporal precision in encoding locomotion. *Proc Natl Acad Sci USA* **117**, 17330–17337.
114 Steinhauser C, Grunnet M & Carmignoto G (2016) Crucial role of astrocytes in temporal lobe epilepsy. *Neuroscience* **323**, 157–169.
115 Wetherington J, Serrano G & Dingledine R (2008) Astrocytes in the epileptic brain. *Neuron* **58**, 168–178.
116 Guella I, McKenzie MB, Evans DM, Buerki SE, Toyota EB, Van Allen MI, Adam S, Boelman C, Bolbocean C, Candido T *et al.* (2017) De novo mutations in YWHAG cause early-onset epilepsy. *Am J Human Genet* **101**, 300–310.
117 Boison D & Steinhauser C (2018) Epilepsy and astrocyte energy metabolism. *Glia* **66**, 1235–1243.
118 Chan F, Lax NZ, Voss CM, Aldana BI, Whyte S, Jenkins A, Nicholson C, Nichols S, Tilley E, Powell Z *et al.* (2019) The role of astrocytes in seizure generation: insights from a novel *in vitro* seizure model based on mitochondrial dysfunction. *Brain* **142**, 391–411.
119 Seifert G & Steinhauser C (2013) Neuron-astrocyte signaling and epilepsy. *Exp Neurol* **244**, 4–10.

120 Peterson AR & Binder DK (2019) Regulation of synaptosomal GLT-1 and GLAST during epileptogenesis. *Neuroscience* **411**, 185–201.
121 Carmignoto G & Haydon PG (2012) Astrocyte calcium signaling and epilepsy. *Glia* **60**, 1227–1233.
122 Bedner P, Dupper A, Huttmann K, Muller J, Herde MK, Dublin P, Deshpande T, Schramm J, Haussler U, Haas CA *et al.* (2015) Astrocyte uncoupling as a cause of human temporal lobe epilepsy. *Brain* **138**, 1208–1222.
123 Steinhauser C, Seifert G & Bedner P (2012) Astrocyte dysfunction in temporal lobe epilepsy: K+ channels and gap junction coupling. *Glia* **60**, 1192–1202.
124 Deshpande T, Li T, Herde MK, Becker A, Vatter H, Schwarz MK, Henneberger C, Steinhauser C & Bedner P (2017) Subcellular reorganization and altered phosphorylation of the astrocytic gap junction protein connexin43 in human and experimental temporal lobe epilepsy. *Glia* **65**, 1809–1820.
125 Morin-Brureau M, Milior G, Royer J, Chali F, Le Duigou C, Savary E, Blugeon C, Jourdren L, Akbar D, Dupont S *et al.* (2018) Microglial phenotypes in the human epileptic temporal lobe. *Brain* **141**, 3343–3360.
126 Aronica E, Ravizza T, Zurolo E & Vezzani A (2012) Astrocyte immune responses in epilepsy. *Glia* **60**, 1258–1268.
127 Zhao X, Liao Y, Morgan S, Mathur R, Feustel P, Mazurkiewicz J, Qian J, Chang J, Mathern GW, Adamo MA *et al.* (2018) Noninflammatory changes of microglia are sufficient to cause epilepsy. *Cell Rep* **22**, 2080–2093.
128 Hubbard JA, Hsu MS, Fiacco TA & Binder DK (2013) Glial cell changes in epilepsy: overview of the clinical problem and therapeutic opportunities. *Neurochem Int* **63**, 638–651.
129 Pajarillo E, Rizor A, Lee J, Aschner M & Lee E (2019) The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: potential targets for neurotherapeutics. *Neuropharmacology* **161**, 107559.
130 Lyons DA & Talbot WS (2014) Glial cell development and function in zebrafish. *Cold Spring Harbor Perspect Biol* **7**, a020586.
131 Sieger D & Peri F (2013) Animal models for studying microglia: the first, the popular, and the new. *Glia* **61**, 3–9.
132 Chen J, Poskanzer KE, Freeman MR & Monk KR (2020) Live-imaging of astrocyte morphogenesis and function in zebrafish neural circuits. *Nat Neurosci* **23**, 1297–1306.
133 Rosch RE & Dulla CG (2020) A tale of two networks-glial contributions to generalized seizures. *Epilepsy Curr* **20**, 108–110.
134 Myers CT, McMahon JM, Schneider AL, Petrovski S, Allen AS, Carvill GL, Zemel M, Saykally JE, LaCroix AJ, Heinzen EL *et al.* (2016) *De novo* mutations in SLC1A2 and CACNA1A are important causes of epileptic encephalopathies. *Am J Human Genet* **99**, 287–298.
135 Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T *et al.* (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* **276**, 1699.
136 Soni N, Reddy BV & Kumar P (2014) GLT-1 transporter: an effective pharmacological target for various neurological disorders. *Pharmacol Biochem Behav* **127**, 70–81.
137 Eyo UB, Murugan M & Wu LJ (2017) Microglia-neuron communication in epilepsy. *Glia* **65**, 5–18.
138 Li Y, Du XF, Liu CS, Wen ZL & Du JL (2012) Reciprocal regulation between resting microglial dynamics and neuronal activity *in vivo*. *Dev Cell*. **23**, 1189–1202.
139 Sieger D, Moritz C, Ziegenhals T, Prykhozhij S & Peri F (2012) Long-range Ca2+ waves transmit brain-damage signals to microglia. *Dev Cell* **22**, 1138–1148.
140 Miyasaka N, Morimoto K, Tsubokawa T, Higashijima S, Okamoto H & Yoshihara Y (2009) From the olfactory bulb to higher brain centers: genetic visualization of secondary olfactory pathways in Zebrafish. *J Neurosci* **29**, 4756–4767.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Movie S1. Simultaneous recording of brain activity and locomotor behavior during seizures. Fluorescence calcium imaging of 7 days old *elavl3:GCaMP6s* zebrafish larvae expressing GCaMP6s pan-neuronally (top). Zebrafish larvae was treated with PTZ to induce seizures. Average brain activity (middle, green) and locomotor tail beat angle (bottom, black) were recorded simultaneously by using a sensitive video camera (100 Hz).