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Zebrafish as an emerging model for studying complex brain disorders

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Abstract

The zebrafish (*Danio rerio*) is rapidly becoming a popular model organism in pharmacogenetics and neuropharmacology. Both larval and adult zebrafish are currently used to increase our understanding of brain function, dysfunction, and their genetic and pharmacological modulation. Here we review the developing utility of zebrafish in the analysis of complex brain disorders (including, for example, depression, autism, psychoses, drug abuse and cognitive disorders), also covering zebrafish applications towards the goal of modeling major human neuropsychiatric and drug-induced syndromes. We argue that zebrafish models of complex brain disorders and drug-induced conditions have become a rapidly emerging critical field in translational neuropharmacology research.

Keywords

zebrafish; brain disorders; behavioral tests; translational research

The zebrafish

A small aquatic vertebrate, the zebrafish (*Danio rerio*) is rapidly becoming a new popular model organism in biomedical research (Figures 1 and 2)[1–5]. Major universities and research centers worldwide have established zebrafish facilities, and the US National Institutes of Health have recently constructed the world's biggest zebrafish research center, able to house 19,000 tanks and 100,000 fish. The utility of both adult and larval zebrafish in neuroscience has grown markedly in the last decades as it is a vertebrate species with high physiological and genetic homology to humans, as well as because of the ease of genetic manipulation and similar CNS morphology (Boxes 1 and 2, Figure 3a)[1–5]. The zebrafish genome is well characterized and its sequencing has just been completed by the UK Sanger Institute [6], which has further increased interest in this fish as a model organism in neuroscience and pharmacology (Figure 2). Possessing both rapid development and a relatively long lifespan

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(Box 1), zebrafish are currently used to model various human brain disorders (Table 1). The availability of multiple zebrafish strains (Table 2) is another important aspect of this species, enabling studies of strain differences in brain function, behavior and drug responses.

Box 1

Summary of zebrafish biology and its application to biomedical research

Natural habitat

South-East Asia (typically includes streams and rivers, silt-bottomed well-vegetated pools and rice paddies adjacent to streams shown in Figure 1b), see [132, 133] for details.

Life cycle

Several distinct stages, such as embryonic pre-hatching (0–72 hpf) and post-hatching (72–120 hpf); 'larval' (1–29 dpf; including a free-swimming larva from 5 dpf) to juvenile fish (30–89 dpf), adult zebrafish (90 dpf-2 years) and aging/aged zebrafish (from 2 years, Figure 1c). Zebrafish grow till death (4–5 years old) and remain sexually mature/active almost all this time[141]. Zebrafish are excellent breeders, and a single female can lay several hundred eggs each week. The embryonic development of zebrafish is very rapid (e.g., all major organs form within 1 dpf, and the fish hatch and start feeding within 3 dpf). Unlike mammals, zebrafish develop from fertilised eggs to adults in *transparent* eggs, which enables monitoring the developing embryos (and their organs), as well as manipulating these processes (e.g., by injecting drugs or genes) *in-vivo*. This feature empowers neurodevelopmental studies using zebrafish models (e.g., [72, 115, 142]), including neonatal drug exposure with subsequent analyses of neural abnormalities at various time windows through the adulthood. At the same time, expanded lifespan of zebrafish (e.g., ~4–5 years vs. 3 in mice) fosters translational studies of aging-related behavioral deficits using this model organism[110, 111].

Behaviors

Span multiple behavioral domains summarized in Table 1 (also see recent reviews[25, 84, 116, 127, 143] for details of behavioral phenotypes observed in larval and adult zebrafish).

Brain morphology

Strikingly similar between zebrafish and mammalian (rodent) models, including both general macro-organization of the brain, and cellular morphology (Figure 1a; see more details in zebrafish online atlas www.zebrafishbrain.org). The knowledge gained from these studies is very relevant to human brain functioning. For example, the involvement of the amygdala and habenula in zebrafish affective behaviors parallels human data on these structures. The habenula, a phylogenetically ancient group of nuclei in the epithalamus, regulates the release of serotonin and dopamine[122]. Because the habenular circuit is evolutionarily highly conserved, this enables a dissection of brain circuits in more experimentally amenable vertebrate systems[144]. Illustrating the parallels between brain substrates in zebrafish and mammals, and translational value of such research for studying pathological behavior, the habenula is hyper-activated in individuals with depression, in rodent models of this disorder[145], and in zebrafish exhibiting stress-related behaviors[122].

Brain neurochemistry

Highly conserved across vertebrate species. Zebrafish possess all major neuromediator systems, including neurotransmitter receptors, transporters, and enzymes of synthesis and metabolism, similar to those observed in humans and rodents[58, 59, 146].

Endocrine responses

Zebrafish display well-developed functional neuroendocrine systems, generally homologous to those established in mammals. Similar to humans, stress responses in zebrafish are mediated by cortisol activated by the cascade of hypothalamo-putuitary hormones and acting via glucocorticoid receptors[12, 13, 15, 147]. For example, zebrafish cortisol responses strongly parallel behavioral indices of stress, and may be modulated genetically and pharmacologically[12, 13, 42, 121].

Genetics

Zebrafish have 25 pairs of chromosomes containing >26,000 protein-coding genes[6]. The genetic homology of zebrafish (to mammals and humans) is relatively high, supporting the translational value of zebrafish models. For example, the nucleotide sequence of zebrafish genes shows approximately 70% homology with that of human genes, and 84% of genes known to be associated with human disease have zebrafish counterparts[6]. It is also important to consider the role of genome duplication in zebrafish, which occurred during teleost evolution, resulting in the presence of several copies of multiple genes. Despite this duplication, zebrafish have a very similar number of chromosomes as humans, rats and mice (23, 21 and 20 pairs, respectively), and mounting evidence supports generally conserved genetics and physiology of major brain processes and behavioral traits in rodents and zebrafish, and the duplicated CNS genes seem to mostly encode for proteins with similar (or substantially overlapping) functions and properties.

High-throughput screens (HTS)

Models used in biomedical discovery, testing live organisms and analyzing their responses in a very time-efficient data-dense objective manner, using powerful computer-aided methodologies, such as video-tracking tools. HTS, typically analyzing hundreds of compounds per day, are becoming critical for bioinnovation and biotechnology, as they enable quick inexpensive analyses of large libraries of genetic or pharmacological modifiers, rapidly identifying active compounds (hits) or candidate genes. Zebrafish are an excellent organism for HTP (Figure 3d)[8–10, 148], and costs of *in-vivo* screening of one drug in zebrafish are estimated to be ~$300, which is 500 times cheaper than a similar rat assay[143]. Comparative analysis of cost-efficiency of mouse and zebrafish studies, using an example of a 2-week chronic fluoxetine treatment (n = 15/group, based on 2012–2013 estimates from the Kalueff Laboratory), reveals a 5-fold saving in zebrafish (total $ 562) vs. mouse ($ 2790) experiments of similar design.

Zebrafish strains

See Table 2 for details.

Sensitivity to pharmacological manipulations

Zebrafish are sensitive to all major classes of neurotropic drugs, including antipsychotics, mood stabilizers, anxiolytics, antidepressants, ethanol, sedatives/hypnotics, stimulants, hallucinogens, antiepileptics, anesthetic/analgesics and cognitive enhancers (see Table 3 for details).

Box 2

Molecular genetic tools for zebrafish research

Various ‘forward genetic’ tools exist to generate random mutations and discover novel genes involved in particular brain functions and behavior in zebrafish. For example, Ethyl-Nitroso Urea (ENU) chemical mutagenesis has been successfully utilized in large-

scale mutation screens in zebrafish[149], whereas viral vector-mediated insertional mutagenesis has also been developed to expedite the identification of the mutated genes[150]. The development of increasingly powerful forward genetic methods continues, including recent conditional gene-trapping and gene-breaking transposon-based approaches[151] and novel high-throughput large-scale *loss-of-function* screens using the 'Clustered regularly interspaced short palindromic repeats' (CRISPR) system[152, 153]. Numerous 'reverse genetic' methods are also available to functionally characterize a target zebrafish gene of interest by overexpressing or selectively targeting/knocking it out. Analogous to the classical antisense oligonucleotide-based gene knock-down in rodents, the morpholino is currently the most frequently used reverse genetic tool in zebrafish[154]. In addition, the classical 'Targeting Induced Local Lesions in Genomes' (TILLING) method, developed for *Arabidopsis*, has also been successfully adapted to zebrafish[155]. Most recently, an elegant, efficient and less labor-intensive gene targeting method has been designed using the 'Transcription activator-like effector nucleases' (TALEN) system[156, 157], likely to revolutionize targeting genes in zebrafish and other model organisms. Also importantly, a large number of genetic markers are available for laboratories still using classical linkage analysis-based mapping with zebrafish (e.g., [158, 159]). Collectively, this wide range of available molecular genetic tools contributed to the fact that zebrafish has become one of the favorite model organisms in genetics and neurogenetics[5, 160].

The close parallels between mammalian and zebrafish behavioral paradigms (Figure 3, Table 1) suggest the evolutionarily conserved nature of many behaviors (and deficits of their control) across species, implying face and construct validity of zebrafish models. Zebrafish are also very cost-efficient, easy to breed, and can be housed in large numbers in relatively small space (Figure 1a, Box 1). Therefore, they may represent an ideal species for medium- and high-throughput screens (HTS, Figure 2d) for genetic mutations and small molecules[7–10]. Here, we review recent successes and existing challenges in this field, and emphasize the developing utility of zebrafish for translational neuroscience, drug discovery and the search for novel candidate genes.

Zebrafish models of brain disorders

Numerous behavioral tests (Figure 3 and Table 1) illustrate how various common neurobehavioral disorders can be modeled or studied in zebrafish. Consider depression, one of the most widespread and severely debilitating brain disorders that affects ~20% of global population at some point during life[11]. Strongly implicated in clinical depression, various genetic factors, environmental stress and neurochemical disturbances seem to play a similar role in zebrafish phenotypes. For example, *gr-s357* zebrafish with a mutated glucocorticoid receptor gene display aberrant corticoid biofeedback, increased levels of glucocorticoids, and aberrant behaviors (reduced locomotion, impaired habituation, potentiated startle) that resemble phenotypes seen in clinical depression[12]. Interestingly, antidepressants (such as selective serotonin reuptake inhibitors, SSRIs) normalize some of the mutant phenotypes, paralleling known effects of these drugs in modifying glucocorticoid signaling and alleviating stress disorders in human patients, which also confirms the translational relevance of serotonergic modulation of zebrafish stress responses[12, 13].

In addition to genetic models, other factors, such as chronic stressors, commonly trigger affective pathogenesis in both clinical and animal studies[14]. For example, the chronic unpredictable stress (CUS) paradigm is a widely used model of experimental stress, in which rodents are subjected to a battery of chronic stressors, such as restraint, crowding, isolation, novelty, temperature change, light, noise and/or predator exposure[14]. Recent

studies have successfully applied CUS in zebrafish, which affects shoaling, exploration and anxiety behaviors, as well as alters brain proteome profiles and neurogenesis (the hallmark of affective disorders in rodent models)[15, 16]; they also show chronic stress-induced memory deficits and elevated cortisol levels [15, 16], paralleling depression-like states in humans and rodents.

Complementing genetic and experimental manipulations, pharmacological models are also widely used in brain research. For example, depression-like behaviors in humans and rodents can be evoked by reserpine, which depletes brain monoamines by irreversibly blocking vesicular monoamine transporter. The drug induces strong pro-depressant effects in humans, also causing hypoactivity, motor stereotypies, lethargy and anhedonia in rodents[17]. Reserpine treatment and related neurochemical and behavioral deficits are commonly used as a model of depression in rodents, but can also evoke depression-like behavior in zebrafish (including hypolocomotion and disrupted shoaling, resembling motor retardation and social withdrawal symptoms observed in clinical depression)[17]. Emphasizing the role of monoamine dysregulation in depression, these results also support the developing utility of zebrafish to model complex affective brain disorders. Autism spectrum disorder (ASD) represents another cluster of serious behavioral deficits, affecting ~1–2% of the general population. Although the prevalence of ASD is lower than depression or anxiety (which affect >10–15% of the adult population worldwide), autism causes an enormous amount of human suffering, which (if expressed in patient-years; i.e., the number of patients multiplied by the length of time for which the patient suffers from the disease) represents an urgent unmet medical need[18]. In addition to severe behavioral and cognitive impairments, ASD is characterized by high (~90%) heritability, representing one of the most heritable brain disorders in humans[19]. The use of zebrafish to model ASD is supported by several lines of evidence[20]. First, various models relevant to ASD-related social deficits (e.g., social interaction, social preference) have been adapted from rodent studies, and successfully applied to zebrafish paradigms (see examples in Figure 3e, f)[20]. Second, because of the excellent genetic tools developed for the zebrafish, this species is expected to be a useful model organism for human disorders with high heritability, which includes ASD[21]. For instance, the human 16p11.2 locus is tightly linked to ASD, and the homologous region in zebrafish spans genes important for brain development[21]. Likewise, the variants of the *MET* gene, which encodes a transmembrane receptor tyrosine kinase of the hepatocyte growth factor/scatter factor (HGF/SF), have been linked to greater autism risk in humans[22, 23]. Notably, in zebrafish, *met* genetic knock-down impairs cerebellar development and facial motor neuron migration[24]. As these genes are important for zebrafish brain development, and ASD is believed to be a disorder of neural development, these findings are likely relevant to ASD pathogenesis, and suggest strong translational relevance of zebrafish models (also see [25, 26]). Notably, zebrafish embryonic development has been thoroughly characterized because these fish remain practically transparent for the first 2 weeks of life, during which most developmental changes occur (Box 1). Thus, as a result of over four decades of developmental biology research with zebrafish, this species has become one of the most powerful vertebrate tools for embryologists, and may therefore offer uniquely efficient answers to the conundrum of the developmental abnormalities associated with ASD[20].

A robust and often observed behavioral feature of zebrafish is their propensity to form tight groups, a behavior called shoaling[27–29]. Shoaling may be induced and quantified in a variety of ways in the laboratory[27, 29–32]. The developmental trajectory and neurobiological correlates of this social behavior have also started to be investigated[31, 33–36]. Such studies are noteworthy because they will offer potential tools with which human brain disorders associated with abnormal social behavior may be examined. In addition to ASD, for example, schizophrenia is also characterized by significantly impaired social behavior[37].

Similarly, several neuropsychiatric conditions, such as depression and anxiety disorders, also include impaired or abnormal social behaviors[38, 39]. Thus, a laboratory species which offers vertebrate system biology with easy to induce and measure social behavior may have important translational relevance[3, 40].

The latter disorders are only some of many examples (see Table 1 for more details) showing how complex brain disorders may be successfully analyzed in zebrafish. In fact, mounting evidence suggests that various aspects of almost every brain disorder that can be modeled in rodents could also be modeled in zebrafish–perhaps, in a cheaper and more powerful manner.

Zebrafish models of drug-related disorders

Drug-induced disorders are an important area of biomedical research. One of the key examples is addiction, a widespread disorder commonly associated with drug abuse. Complementing traditional (rodent) models, zebrafish are valid translational models to study reward and drug abuse. Both larval and adult zebrafish show high sensitivity to various drugs of abuse (Figure 4, Table 3), as well as tolerance, clear preference (reward stimuli) for these agents, and withdrawal symptoms[41, 42]. For instance, the effects of alcohol in zebrafish have been studied for more than a dozen years, revealing numerous behavioral changes in fish that resemble those seen in rodents and humans[43–46]. Acute alcohol reduces zebrafish fear/anxiety at lower doses (increasing ‘top’ dwelling or reducing erratic movements, also showing stimulant effects, increasing zebrafish speed and aggression), while higher doses induce lethargy and sedation[43, 45]. These changes show a biphasic trajectory in zebrafish that is highly similar to that in rodents and humans[43]. Chronic alcohol exposure also demonstrates parallels between zebrafish and mammals[44, 47]. For example, after continuous alcohol exposure, when briefly challenged by a high acute dose, zebrafish show markedly diminished (or absent) behavioral responses to this challenge. Similarly, withdrawal from chronic alcohol exposure leads to several behavioral and physiological abnormalities that resemble anxiogenic withdrawal symptoms in humans or rodents[42, 43]. Notably, a variety of alcohol effects in humans extends to the social domain, where (in contrast to the common “knowledge” that alcohol facilitates social interaction) it impairs empathy, social problem-solving and the ability to interpret and respond to social signals[48]. Interestingly, a significant impairment in social behavior induced by acute alcohol exposure has also been reported in zebrafish, including reduced responding to conspecifics, as assessed by the increased distance between the focal and the stimulus fish[49]. These changes can be elicited and quantified in a single fish (viewing animated conspecific images moving on a computer screen) or in live freely-moving shoals[27–29, 49].

The effects of alcohol have also been investigated in the context of fetal alcohol syndrome[50, 51]. Its milder, but more prevalent form, fetal alcohol spectrum disorders (FASD) represents the largest preventable cause of mental retardation in children, with a huge societal cost and suffering. Modeling FASD in laboratory animals may lead to the discovery of more efficient diagnostic tools (biomarkers) and treatment options. FASD has been modeled in zebrafish by exposing them to low doses of alcohol for short periods of time during early embryonic development[52]. This exposure led to a striking lack of gross anatomical deformities, but caused significant behavioral anomalies in the adult fish, including reduced social cohesion in shoals[31] and increased distance to conspecific stimuli when the individual fish were tested alone[52], a behavioral impairment that parallels the social deficits seen in FASD children.

Although these examples suggest good face validity (similar appearance of alcohol effects on behavior in zebrafish and humans), the question of construct validity (similar underlying

biological mechanisms) is only starting to be addressed in zebrafish models. Nevertheless, the first studies already showed neurochemical changes that resemble those in humans, and also changes in the expression of genes whose sequences are homologous in zebrafish and humans[44, 53]. Here we use only one example to illustrate common underlying mechanisms, the dopaminergic system, in alcohol-induced behavioral changes. Alcohol modulates the dopaminergic system in various species[54–56], and, when administered acutely, dose-dependently elevates levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in zebrafish brain[57]. Chronic alcohol pre-treatment attenuates these acute alcohol-induced changes, demonstrating neuroadaptation in zebrafish dopaminergic system, similar to that found in mammals[44]. Recently, we have also shown that this alcohol-induced increase in dopamine and DOPAC levels correlates with the activity of brain tyrosine hydroxylase, but not monoamine oxidase (A Kalueff, unpublished observations, also see[53]). Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of dopamine, and zebrafish have two copies of the tyrosine hydroxylase gene (*th1* and *th2*, differentially expressed in the brain and the periphery, respectively[58]). Despite the differential expression of tyrosine hydroxylase, the levels of both *th1* and *th2* mRNA increased dose-dependently in response to alcohol in zebrafish larvae[53]. Monoamine oxidase (MAO) is the enzyme responsible for the breakdown of catecholamines, such as dopamine, into DOPAC. Unlike mammals, which have two forms of the *MAO* gene (*MAO-A, MAO-B*), only a single MAO gene has been identified in zebrafish[59]. Although zebrafish MAO (zMAO) resembles both MAO-A and MAO-B in humans, zMAO exhibits a higher amino acid sequence homology to human MAO-A[60]. Despite these differences, the biosynthesis pathway of dopamine and the enzymes involved, as well as the behavioral effects and the mechanisms of alcohol action studied so far, show significant similarities between zebrafish and mammals.

The effects of other drugs of abuse have also been investigated in zebrafish. Albeit considered as ‘dangerous’ drugs of abuse, hallucinogenic drugs often have low-to-mild addictive properties, and are of interest because of their strong psychotropic effects and potential for treating brain disorders, including depression, anxiety and post-traumatic stress[61, 62]. The growing clinical and preclinical interest in hallucinogenic drugs[63] also impacts zebrafish models. Potently affecting human and animal CNS, various hallucinogenic drugs (such as serotonergic psychedelic, glutamatergic dissociative, and cholinergic deliriant agents) have recently been screened in zebrafish[64]. These studies not only revealed prominent behavioral and physiological responses to these psychoactive drugs (similar to those in humans)[64], but also established striking parallels between the drugs’ pharmacological profiles in zebrafish, humans and rodents (Figure 4). Importantly, we need to know about both positive and negative effects of hallucinogenic drugs[61, 62]. Thus, we can learn from basic studies of hallucinogenic drug action in such animal models[62] and use zebrafish to identify novel promising drugs[65]. For instance, translational studies of therapeutic effects of ketamine (e.g., its putative fast-acting antidepressant profile) and ibogaine (e.g., its potential anti-addictive action) can be empowered by the fact that zebrafish are highly sensitive to these compounds, and represent an excellent tool for dissecting molecular pathways involved in pharmacological action of these drugs[66–68]. Likewise, targeting unwanted reward and pro-psychotic effects of hallucinogens, these drug-evoked behaviors in zebrafish can be useful to both study the molecular mechanisms of such action and to screen novel anti-addictive or antipsychotic agents (see[67, 69–71] for examples). Consider, for example, schizophrenic patients which are resistant to dopamine antagonists but may markedly benefit from drugs (e.g., clozapine) which act on other neurotransmitters (e.g., serotonin). With no antipsychotic drug currently free of serious side effects or providing complete resolution, we need to identify the mechanisms of these drugs and understand why certain compounds work on some patients and not others, and how their therapeutic effects can be maximized without combined side-effects. Drug candidates that would resolve the above issues can only be discovered through investigation into

hallucinogen-evoked mechanisms in animal models[63], including zebrafish HTS and biosensors[2, 8, 9, 65, 72], as well as sensitive behavioral tests such as discussed here (Figure 3 and Table 3).

Closely related to the questions on the actions of hallucinogenic drugs is the utility of animal models for the analysis of psychoses[73–75]. For instance, hallucinogens in animal models successfully mimic the impaired habituation and disrupted pre-pulse inhibition characteristic of schizophrenic patients–two abnormalities potentially responsible for the thought disorder key to the symptoms of the disease[63]. A thorough understanding of the mediatory effects of these compounds is therefore critical, but will only be possible through further research, especially at the pre-clinical stage. Glutamatergic antagonists have long been known to evoke clinical psychoses. In zebrafish, the dissociative hallucinogens ketamine and dizocilpine (MK-801) produce various aberrant behaviors, which resemble those seen in humans and rodents treated with drugs that evoke psychoses, and include cognitive impairment, reduced social interaction, altered anxiety/activity and stereotypic circling[67, 70, 76] (also see Figure 4a for cross-species comparative analyses of doses). Notably, some of these changes can be reversed by antipsychotic drugs[71], collectively indicating the utility of larval and adult zebrafish to model psychoses and their mechanisms, also demonstrating the potential use of zebrafish in HTS for novel antipsychotic medication.

In addition to the drug-evoked responses discussed above, zebrafish models are very useful for studying selected neurotoxic conditions. For example, serotonin syndrome (SS) is a serious, potentially lethal disorder associated with elevated brain serotonergic function due to an overdose or combined application of several serotonergic drugs[77]. With the increased use of serotonergic medication, SS affects a large portion of general population and is becoming a major biomedical concern[78]. Thus, there is a widely recognized need for novel experimental models of SS[77, 78]. Characteristic behavioral, neuromuscular and autonomic responses are observed in rodents following administration of serotonergic drugs, representing useful experimental models of SS[77, 79]. Exposed to serotonergic agents and their combinations, zebrafish exhibit a characteristic top dwelling (surfacing behavior) and hypolocomotion, which positively correlate with brain serotonin and may therefore represent potential markers of SS-like states in zebrafish[78]. Thus, the zebrafish seems to offer great promise for HTS of novel serotonergic drugs, probing the SS pathogenetic mechanisms and the analysis of drug × gene interactions[78].

Critical evaluation and future directions

Although there are many human brain disorders whose modeling have been attempted, with some success, using traditional laboratory species (e.g., rats or mice), the zebrafish appears to have an excellent future in this line of research, and offers several important advantages. For example, genetic factors play a key role in pathogenesis of brain disorders[80–83], and the utility of experimental models in clinical neuroscience relies on the model's applicability to genetics research. Box 2 summarizes recent successes in applying molecular genetics to zebrafish neuroscience research.

The relatively sophisticated zebrafish behavioral repertoire[84], including its highly social nature and the fact that it is a diurnal species that relies heavily on vision (the same modality humans use most), make it an excellent tool for investigators interested in the analysis of brain function and dysfunction. In addition to the genetic tools available for the zebrafish (Box 2)[5], transgenic zebrafish fluorescence-based screens and optogenetic models enable visualization of biological processes in the body, and an unprecedented dissection of circuitry and cellular mechanisms of brain disorders[85, 86]. Zebrafish behavioral models are also characterized by reliance on vision as an important sensory modality. Because humans

are also highly 'visual', computer screens[4, 27, 28] and robotic 'fish'[87, 88] can be cost-effectively utilized to deliver environmental stimuli to zebrafish in a precise, consistent and automated manner (a method much less applicable to the classical laboratory species, such as nocturnal rodents; but see[89–91]). The fact that zebrafish behavioral stimuli can be applied using computer screens or robots is also important methodologically (e.g., providing standardized experimental stimulation vs. the rodent versions, which use less-controllable 'live' stimuli). Similarly, operant behavior may be studied in zebrafish in a manner it is traditionally assessed in rodents. Although zebrafish are unable to press the lever or use touchscreens (as rodents do[90, 91]), it is the matter of time before novel technologies are developed to study zebrafish operant behavior. For example, swimming from one designated area to another, detected by sensitive video-tracking systems, is a form of 'operant behavior', a response that can be paired with software-generated positive reinforcement (e.g., automated food delivery or dimming the lights in the tank).

However, like any model organism, the zebrafish also has its limitations. For example, a recognized limitation in zebrafish genetics is the paucity of well-characterized inbred strains[92]. While a growing number of zebrafish strains is becoming available (Box 1), many of them are outbred, have an unclear breeding history, or show only partial inbreeding with heterozygosity at a substantial percentage of their loci[92]. Because some zebrafish strains are recognized as excellent performers, and enable a good analysis of strain differences (Table 2), the situation is still not as good as with mouse models, where almost 100 well-characterized inbred strains (http://phenome.jax.org/) and hundreds of mutants (http://www.informatics.jax.org/) are available to researchers. Thus, future efforts may be needed to increase the number of available zebrafish strains and increase our understanding of the strain differences in fish neural phenotypes.

In general, no experimental animal model can target the entirety of any complex brain disorder observed clinically in human patients[93]. Thus, using animal models to target core component behaviors which form a part of the disease spectrum becomes a valid strategy of translational research in this field[94, 95], if phenotypes we assess are quantifiable, and have construct, predictive and face validity[96]. Many questions remain to be addressed in future zebrafish studies. For example, despite the fact that zebrafish are highly social shoaling animals, it is unclear if deficits in fish shoaling behaviors can be compared to complex human ASD-like phenotypes. Specifically, one of the deficits in ASD is alteration in attention processing which may lead to difficulties in interpreting facial expressions[97–99]. Whether fish shoaling requires similar attention processing has yet to be established. In addition to ASD, many other psychiatric disorders (including aggression, ADHD and schizophrenia) also involve deficits in attention and impulse control. Although this area is outside of the scope of this review, recent studies suggest the translational importance of attention- and impulse control-related phenotypes in zebrafish[100–103]. Other studies have shown the importance of social (kin) recognition in zebrafish shoaling[104], revealing the role of olfactory cues from the major histocompatibility complex (MHC) peptides, similar to the well-established role of MHC in social recognition in mammals[105–107].

Several important neuropsychiatric domains were not discussed here in-depth because of their coverage by recent comprehensive literature elsewhere. For example, aggressive behavior is another important area in translational biological psychiatry research. Mounting evidence shows that zebrafish display a rich repertoire of aggressive behavior, the neural mechanisms of which can now be dissected to understand the core pathogenesis of aggression[108, 109]. Likewise, aging-related psychiatric disorders and cognitive decline are receiving growing recognition in clinical psychiatry, and have recently been successfully modeled in zebrafish[110, 111]. Bidirectional sensitivity of cognitive phenotypes to various pharmacological modulators[70, 110–112] further supports the value of zebrafish in this

research. The growing interest in using zebrafish to study pathogenic overlap between behavioral and metabolic disorders[113] is also encouraging, and merits further analyses. The utility of zebrafish for substance abuse research is also receiving wide recognition[41, 114], revealing overt reward responses to drugs of abuse[69, 102, 114–117] and conserved gene expression and adaptive (compulsive and relapse) behavior to chronic exposure to these drugs[118, 119].

Furthermore, there is a growing understanding of zebrafish affective (emotionality-related) phenotypes[84], which may be directly relevant to modeling anxiety, fear/panic, post-traumatic stress disorder (PTSD) and other stress-related human brain disorders (see Table 1 and [120–127] for details). Anxiety-related disorders are the most widespread psychiatric disorders[11]. Combined with analyses of neuroendocrine biomarkers (Box 1), genomic/proteomic profiling[128, 129] and dissection of the circuitry underlying fish affective behavior[122, 124, 130], zebrafish studies continue to provide important insights into core, evolutionarily conserved pathobiology of these stress-related disorders.

Concluding remarks

There is an urgent need for novel biobehavioral assays using alternative model organisms, especially those species with sufficient physiological complexity, similarity to humans, and HTS capacity, such as zebrafish[5, 40, 85, 131]. Although scientists traditionally compare laboratory species in terms of what they are better for, the fact that a novel species is added to the repertoire of biomedical tools is important *per se*. For example, it allows a comparative approach to brain disorders, that otherwise would not be possible. This is critical because comparative studies can uncover evolutionarily conserved functions, mechanisms and targets, which represent the most important ‘core’ aspects of the disease or function being studied. This approach markedly increases our ability to translate our findings from laboratory animal model to human disease.

In summary, zebrafish models are becoming an important tool expected to advance neuroscience and neurogenetics. Zebrafish sensitivity to all major neurotropic drugs (Table 3) and the ability to respond to them in a similar manner as humans (Figure 4) support their utility for pharmacological research. Representing an ideal organism for disease modeling and HTS, as well as possessing high physiological and genetic homology to humans, zebrafish prove increasingly useful in translational biomedical research, and are well suited to meet the rapidly growing challenges of this field.

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Highlights

- The zebrafish is a popular novel model in pharmacogenetics and neuropharmacology
- Both larval and adult zebrafish models contribute to studying complex brain disorders
- Zebrafish are highly sensitive to major classes of neurotropic drugs active clinically
- Zebrafish models emerge as a useful tool for genetic screening and drug discovery

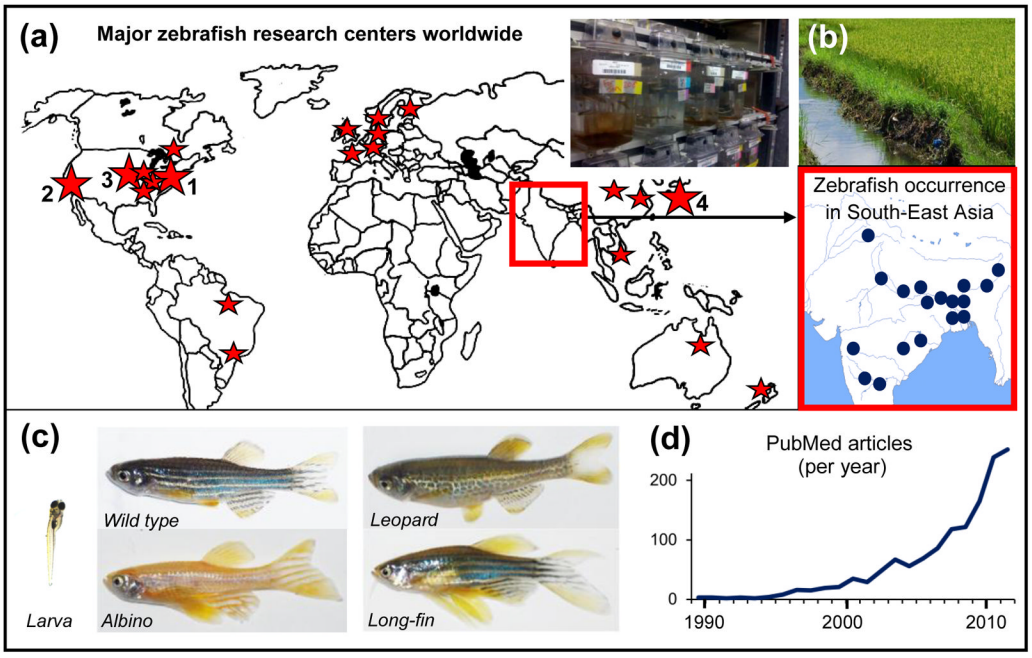


Figure 1. Zebrafish in laboratory research and natural environments

Panel (a) shows major zebrafish research centers established worldwide (red stars), including the National Institutes of Health (1), University of Oregon (2) and Washington University (3) in USA, and RIKEN Institute (4) in Japan. Inset – a typical rack housing hundreds of zebrafish in a research facility. (b) Typical habitat of zebrafish in the wild (shallow waters, e.g., rice fields) in various regions of South-East Asia (see [132, 133] for details). (c) Larval and adult zebrafish (including several common color variants, also see Table 2 for zebrafish strain information). (d) The growing number of published zebrafish models (assessed in Pubmed in September 2013, using terms “zebrafish” and “behavior”).

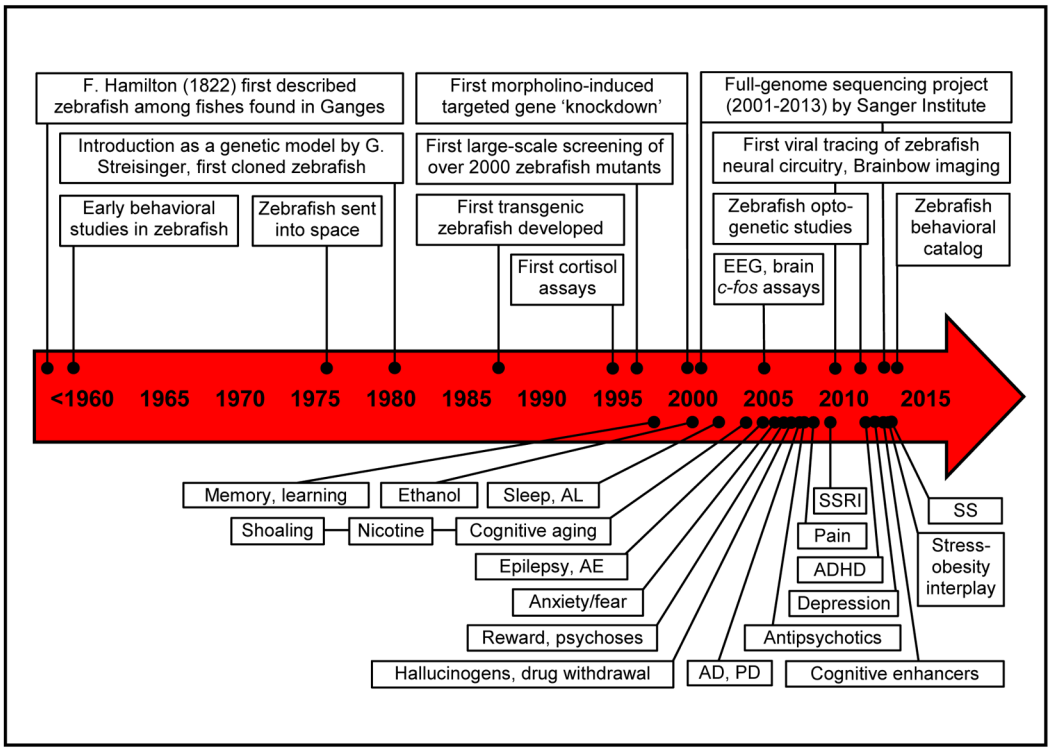
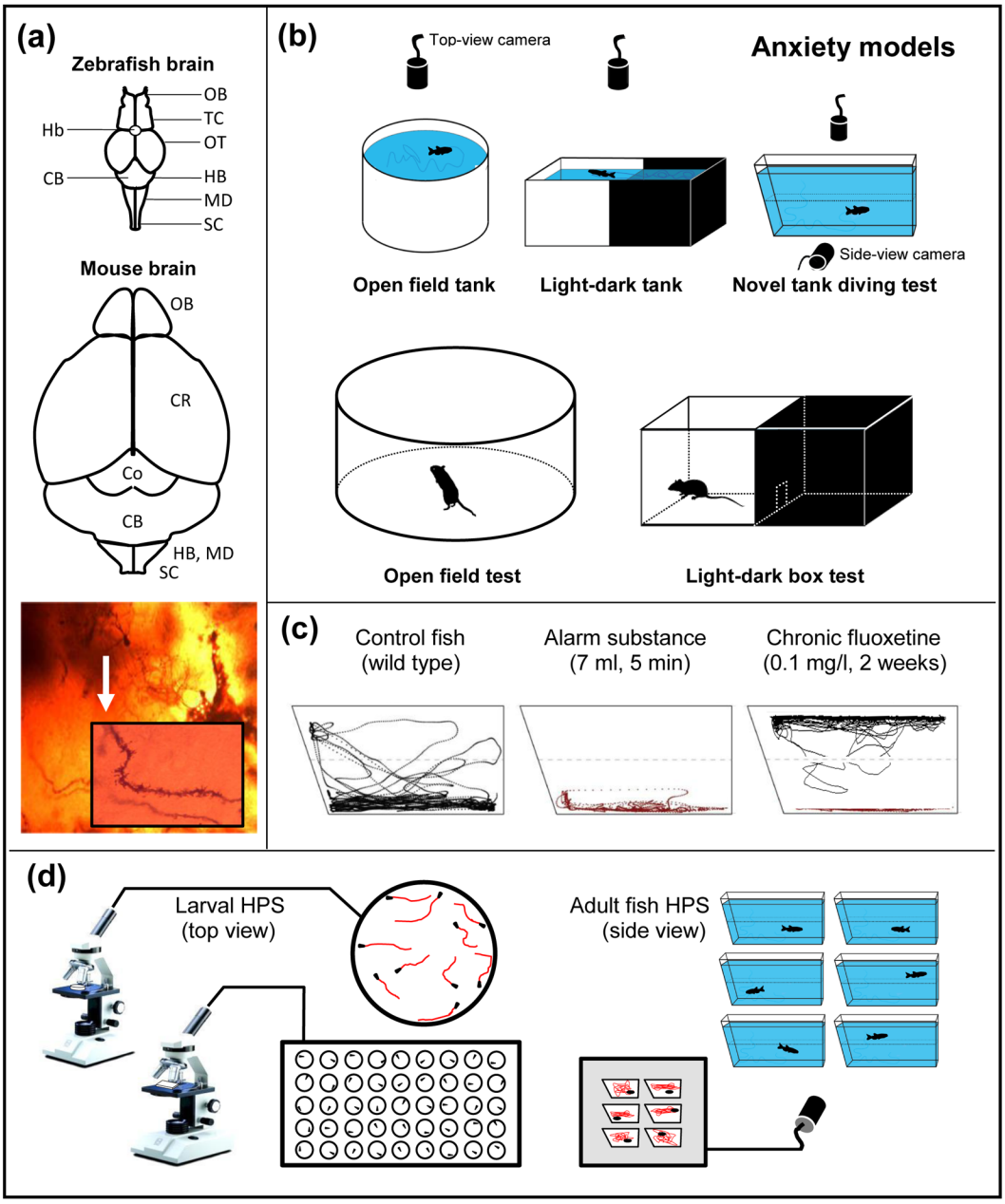


Figure 2. The timeline of the developing utility of zebrafish models in neuroscience and neuropharmacology research

SSRI – selective serotonin reuptake inhibitors, ADHD – attention deficit/hyperactivity disorder, AD – Alzheimer's disease, PD – Parkinson's disease, AL – anxiolytic drugs, AE – antiepileptic drugs, SS – serotonin syndrome (serotonin toxicity), see Tables 1 and 3 for details (note that toxicology studies were not included here).



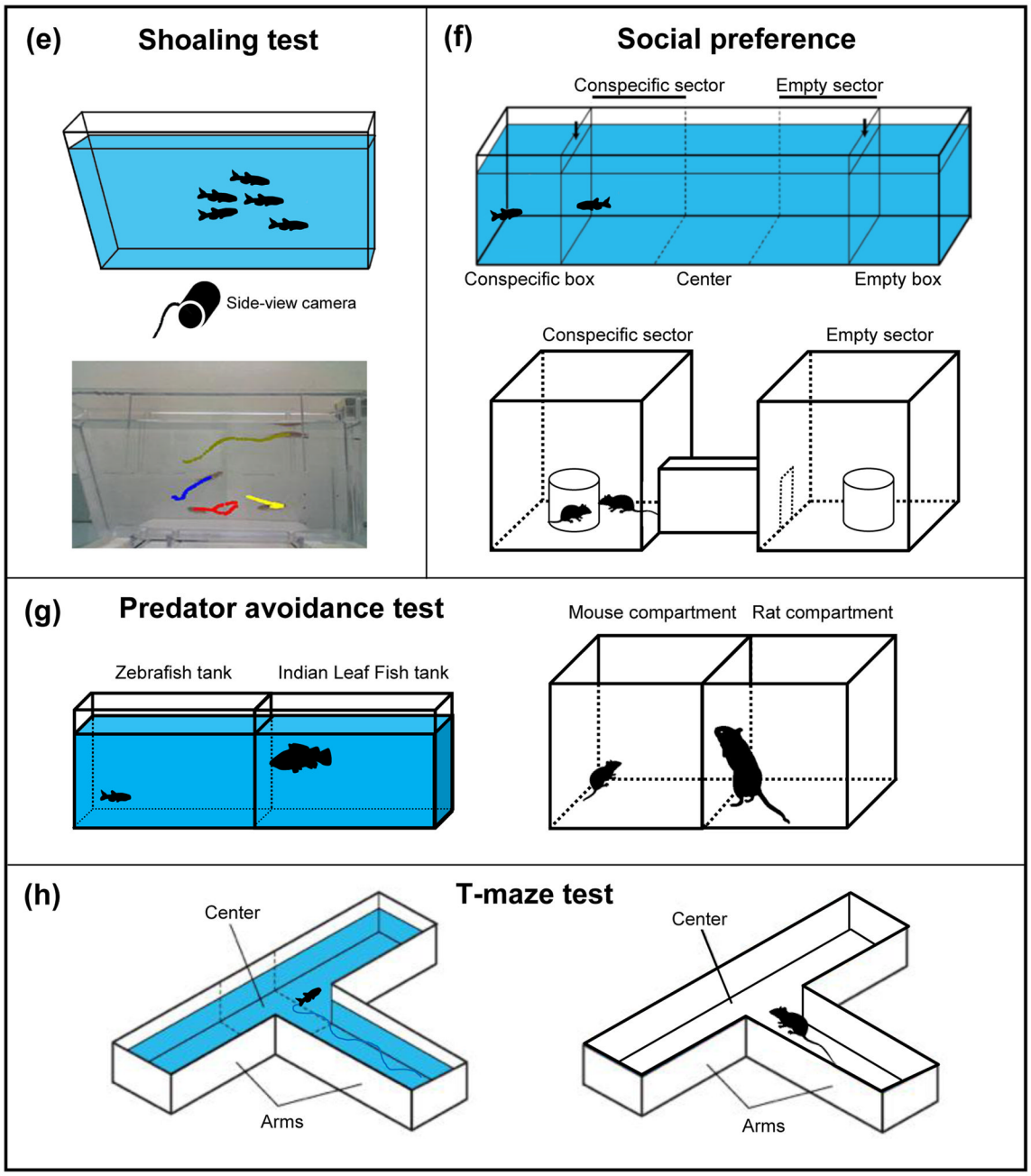


Figure 3. Comparison of zebrafish and mouse experimental models

Panel (a) shows the similarity of zebrafish and mouse brain morphology (OB – olfactory bulbs, TC – telencephalon, OT – optic tectum, Hb – habenula, CB – cerebellum, HB – hind brain, MD – medulla, SC – spinal cord, CR – cortex, Co – colliculi). Bottom inset: Golgi staining of zebrafish neurons (I) and their dendritic spines (II), showing similarity of zebrafish neuronal morphology to rodent neurons (photos by R. Mervis' laboratory; Tampa, FL). Panel (b) shows how major zebrafish neurobehavioral tests of exploration, anxiety and locomotion parallel those traditionally used in rodents (adapted from [66, 121, 134–136]), combined with automated video-tracking using top-view (rodents) or top/side view cameras (zebrafish). Note predominantly 2D nature of rodent locomotion (X,Z plane) vs. 3D locomotion of zebrafish in X,Y,Z coordinates. Panel (c) shows typical anxiety-like

behaviors observed in zebrafish in the novel tank diving test (including anxiety evoked by alarm substance acute 5-min exposure and reduced anxiety produced by a chronic 2-week 0.1 mg/l fluoxetine anxiolytic treatment, see Table 1 for explanation of behavioral signs of anxiety in zebrafish), an aquatic paradigm similar to rodent open field test (b). Note that alarm substance exposure test in zebrafish is similar to the cat odor rodent task. Panel (d) illustrates principles of high-throughput screens (HTS) using larval and adult zebrafish. Panels (e-h) show examples of zebrafish social and cognitive behavior tests (adapted from 66, 68, 136, [137]. Panel (e) illustrates the shoaling test's typical set up (top) and application of video-tracking tools to quantify zebrafish group behavior (bottom; photo from a collaborative project between Noldus IT, Netherlands and the Kalueff Laboratory). Panel (f) shows the aquatic social preference test (top) and its similarity to the mouse sociability test (bottom). Panel (g) shows behavioral similarity between zebrafish predator avoidance (e.g., Indian Leaf Fish, *Nandus nandus*) and the rat exposure mouse test. Panel (h) illustrates parallels between aquatic and rodent cognitive tasks, such as the T-maze test.

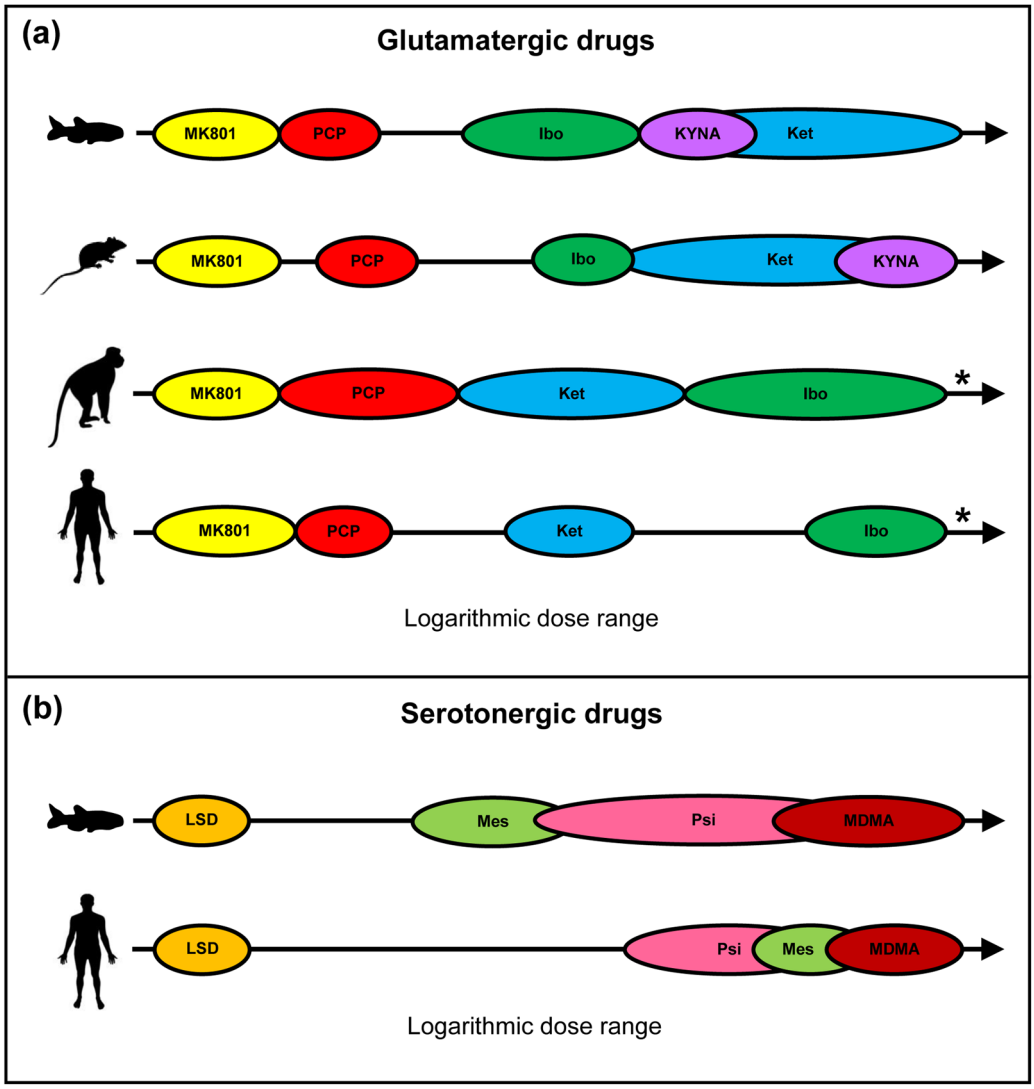


Figure 4. Comparative analysis of relative potencies of effective acute behavioral doses of selected psychotropic drugs in zebrafish and other species

Panel (a) shows glutamatergic antagonists dizocilpine (MK801), phencyclidine (PCP), ibogaine (Ibo), ketamine (Ket) and kynurenic acid (KYNA); 'Normalized' logarithmic dose range is 0.1–20 mg/L for zebrafish[138]; 0.05–200 mg/kg for mice; 0.003–25 mg/kg for non-human primates; 0.01–15 mg/kg for humans[139]. *Data for KYNA not available. Panel (b) shows zebrafish and human data for selected serotonergic drugs, including lysergic acid diethylamide (LSD), mescaline (Mes), psilocybin (Psi), and 3,4-methylenedioxymethamphetamine (MDMA); 'Normalized' logarithmic dose range is 0.1–120 mg/L for zebrafish and 0.0001–4 mg/kg for humans[140]. Collectively, these data show similar ranking of drugs' activity across various species, illustrating translational value of zebrafish models for screening clinically active neurotropic drugs.

Table 1

Selected key neurophenotypic domains and associated disorders modeled in zebrafish

(also see timeline in Figure 2, and zebrafish behavior catalog in [84]).

Domain/disorder	Zebrafish phenotypes	References
Anxiety/fear-related behavior	Reduction of exploration (especially in the top part of novel tanks), increased avoidance, erratic behavior and freezing, elevated cortisol and brain *c-fos*; highly sensitive to anxiolytic and anxiogenic agents	4, 123–125, 161–164
Mood/depression	Reduced activity (motor retardation) accompanied with chronic elevation of cortisol; responses can be reversed by antidepressant treatment	12, 17, 127
Cognitive behavior	Excellent short-term and long-term memory tested in various memory and learning tasks; sensitive to amnestic and promnestic agents	111, 112, 165, 166
Social behavior	Robust social behavior; sensitive to drugs (e.g., increasing or reducing shoaling responses)	27–29, 32
Psychoses	Hyperactivity, impaired cognitive processes (startle, pre-pulse inhibition) by pro-psychotic agents; rescued by antipsychotic drugs	8, 9, 167
Attention deficit hyperactivity (ADHD)	Impulsive hyperactive locomotion rescued by anti-ADHD agents	168
Reward-related behavior	Robust preference for rewarding stimuli, including food and abused substances	41, 69, 115–117, 169
Pain	Robust pain responses to nociceptive stimuli, and their inhibition with analgesic drugs	170–172
Epilepsy	Hyperactivity and seizure-like behavior, brain spikes (EEG) and *c-fos* up-regulation; rescued by anti-epileptics	173, 174
Neurodegeneration	Decline in locomotion, accompanied by characteristic biomolecular and cellular markers of neurodegeneration	1, 146, 175
Serotonin syndrome (SS)	Characteristic surfacing behavior in response to drugs (and their combination) known to evoke clinical SS	78
Eating disorders	Increased or reduced eating behavior (similar to anorexia and obesity); highly sensitive to drugs affecting appetite and mediating metabolic phenotypes	113, 176
Sleep disorders	Robust circadian rhythms bidirectionally sensitive to sleep-modulating drugs	110, 177–183

Table 2

Selected zebrafish strains commonly used in biomedical research.

Strain	Details	Behavioral phenotypes
Strains		
AB	Commonly used 'high-performance' strain, developed by G. Streisinger	Active strain, sensitive to various experimental (genetic and pharmacological) manipulations
Casper	Mutant strain translucent throughout adulthood due to a lack of melanocytes and reflective cells	Display active locomotor phenotype and some differences to developmental drug treatment
Ekkwill (EKW)	Derived from Ekkwill breeders (FL)	Active strain, sensitive to various experimental manipulations
Nadia	Domesticated strain derived from a wild-caught zebrafish	More anxious zebrafish
Tubingen (Tu)	Short-fin wild type strain, commonly used in neurobehavioral tests. Utilized for genome sequencing project by Sanger Institute	Active, sensitive to various genetic and pharmacological manipulations
Wild Indian Karyotype (WIK)	Derived from wild-caught Indian zebrafish, used for genome mapping	Highly anxious zebrafish
Wild-caught	Zebrafish caught in the wild in India	Highly anxious zebrafish
Color variants		
Long-fin variant	Contain spontaneous mutation causing long fins (Figure 1c)	More anxious and sensitive to anxiogenic stimuli
Leopard color variant	Contain spontaneous mutation causing spotting in adult fish (Figure 1c)	More anxious and sensitive to anxiogenic stimuli
Mutants		
naddne^{3256}	An N-ethyl-N-nitrosourea-induced mutant used to study the rewarding effects of amphetamine	Fails to respond to amphetamine
jpy	Increased number of mitotic cells "Jumpy" fish exhibiting cocaine sensitivity	Fails to respond to cocaine

Table 3

Documented sensitivity of zebrafish models to major classes of neurotropic drugs

Drugs	Larval models	Adult zebrafish
Antipsychotics	8, 9, 167, 184	71
Mood stabilizers	72	
Anxiolytics	72, 148	46, 120, 123, 163, 185126, 186
Antidepressants		187, 188
Ethanol	53, 189	31, 42, 52, 187, 190–192
Sedative/hypnotics	72, 148	193
Stimulants	2	69, 187
Hallucinogens	142	68, 69, 136, 140, 194
Antiepileptics	72, 148, 189	193, 195
Anesthetics/Analgesics		196
Cognitive enhancers	112	197