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Abstract

Due to their well-characterized neural development and high genetic homology to mammals, zebrafish (*Danio rerio*) have emerged as a powerful model organism in the field of biological psychiatry. Here, we discuss the molecular psychiatry of zebrafish, and its implications for translational neuroscience research and modeling CNS disorders. In particular, we outline recent genetic and technological developments allowing for *in-vivo* examinations, high-throughput screening and whole-brain analyses in larval and adult zebrafish. We also summarize the application of these molecular techniques to the understanding of neuropsychiatric disease, outlining the potential of zebrafish for modeling complex brain disorders, including attention-deficit/hyperactivity disorder (ADHD), aggression, post-traumatic stress and substance abuse. Critically evaluating the advantages and limitations of larval and adult fish tests, we suggest that zebrafish models become a rapidly emerging new field in modern biological psychiatry research.

Keywords

zebrafish; brain disorders; behavioral tests; translational research

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1. Introduction

Psychiatric disorders affect many brain and behavioral processes, including thoughts, feeling, sociability and mood. These complex human diseases also cause a significant burden to society, especially as the available drug therapies have not sufficiently progressed[1, 2]. The etiology of psychiatric disorders is multifaceted, and involves the interaction of multiple genetic and environmental factors.

Genetic mutations trigger psychiatric disorders by altering gene expression and disrupting normal physiological functions of the brain. Mutations linked to psychiatric disorders are often found in genes expressed during embryogenesis, and affect early brain development, cell division, migration, differentiation or survival, as well as axon path finding, dendritic architecture and wiring of neural circuits. For example, Rett syndrome is caused by mutations in the X-linked *MECP2* gene, encoding methyl CpG binding protein-2 that binds to methylated DNA and controls gene expression[3]. While *MECP2* is particularly critical during brain development, its mutations are also associated with other 'early-onset' developmental brain disorders, such as X-linked mental retardation and autism[3] (also see the substantial contribution of mutations in genes with higher expression in early fetal life to schizophrenia[4]). Environmental factors also play an important role in psychiatric disorders, leading to well-established gene × environment (G × E) interactions[5].

The notion that psychiatric disorders have fundamental underlying molecular mechanisms is critical for understanding complex brain illnesses, such as anxiety, depression, schizophrenia or addiction. The promise of such an approach is that once these mechanisms are identified and understood, the brain disorder can be treated, reversed or prevented[6, 7].

Clearly 'mainstream' today, molecular psychiatry had a long journey before being accepted as a new, dynamic field of translational neuroscience[7]. Utilizing various *in-vivo* tests and applying a wide range of model organisms (Fig. 1) is critical for uncovering novel mechanisms of brain disorders[8]. Zebrafish (*Danio rerio*) have long been underrepresented in experimental biological psychiatry, and only recently emerged as a model organism in this field[9–11]. It is therefore timely to discuss the molecular psychiatry of zebrafish and its implications for clinical as well as translational neuroscience research.

2. The utility of zebrafish for translational neuroscience

2.1. Understanding 'zebrafish psychiatry'

While zebrafish represent a powerful model for developmental neuroscience (Fig. 1), a combination of well-characterized neural development and several cutting-edge genetic tools also makes them an ideal species to study complex psychiatric disorders. Although similar manipulations are often available in other vertebrates (e.g., mice), the ease of generating large numbers of fish, their external fertilization and transparent embryonic/larval stages are particularly useful, also facilitating high-throughput screening and CNS imaging[10, 11]. Zebrafish also have an excellent potential to link genes to CNS disorders, because the nucleotide sequence of zebrafish genes is homologous (usually exceeding 70%) to corresponding human genes, and their function in these distantly related species is often

similar[12]. As zebrafish genes can be mutated with high efficiency, the strains (lines) carrying interesting mutations may be detected using phenotype screens, and the mutated genes can be efficiently identified genetically (see further). Once genes involved in particular functions are identified using such 'forward genetic' strategy, their biological role(s) may be thoroughly investigated using 'reverse genetic' methods, specifically developed for zebrafish (e.g., the 'transcription activator-like effector nuclease' TALEN system[13], allowing targeted mutation of pre-selected sequences). Thus, to the extent that a psychiatric disease can be modeled in any animal by the generation of a genetic mutant or by pharmacological manipulation, zebrafish present a useful, cost-effective and uniquely tractable system.

While aberrant neurotransmission is one of the main causes of psychiatric disorders[6, 7], vertebrate neurochemistry is generally highly conserved[14–17]. Zebrafish possess all primary neuromediator systems, including transmitters, receptors, transporters and the enzymes required for synthesis and metabolism of these mediators[10, 11]. The spatial and temporal distribution of these systems has been well characterized in both larval and adult zebrafish for glutamate, γ-aminobutyric acid (GABA), acetylcholine and the aminergic neurotransmitters dopamine (DA), noradrenaline (NA), serotonin (5-HT) and histamine (HA, Fig. 2B) – key mediators implicated in multiple psychiatric diseases.

2.2. Major neurotransmitter systems in zebrafish

Glutamate is the primary excitatory CNS neurotransmitter involved in schizophrenia, post-traumatic stress disorder (PTSD) and epilepsy. Glutamate signaling is visualized using *in situ* hybridization for vesicular glutamate transporters (*VGLUT1* and *VGLUT2*)[18], and glutamate metabotropic (mGluRs) and ionotropic (iGluRs) receptors, including three subtypes N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and kainate. *VGLUT2* is found in zebrafish embryos beginning at 20–24 h post fertilization (hpf) in Rohon Beard neurons and in the mediodorsal domain of the zebrafish spinal cord[19]. As the fish develops, there is an increase in existing areas of expression, as well as the formation of new domains throughout the spinal cord. By 4–5 days post fertilization (dpf), *VGLUT1* and *VGLUT2* are distributed throughout the majority of the midbrain, hindbrain and spinal cord[19]. Examination of the expression patterns of mGluRs in zebrafish reveals a similar pattern to that seen in rodents, with mGluR expression in larval and adult zebrafish found in the olfactory bulb, optic tectum, hypothalamus, cerebellum and retina[20]. Zebrafish also possess high-affinity excitatory amino acid transporters (EAATs), which regulate glutamate levels and prevent excitotoxicity. Four of the five EAAT-related genes are found in the zebrafish genome, including three EAAT1 paralogs, EAAT2, EAAT3, and two EAAT5 sequences[21].

GABA is the primary inhibitory neurotransmitter in the adult CNS, modulating neuronal excitability and also exerting its action during development. Altered GABA and glutamic acid decarboxylase (GAD, the enzyme producing GABA from glutamate) are seen in many CNS disorders, including ADHD, bipolar disorder and major depression[22–24]. Expression patterns of GABA are usually investigated by direct immunostaining for GABA or the two GAD isoforms (GAD65 and GAD67[25]), as well as by visualizing the ionotropic and

metabotropic $GABA_{A/C}$ and $GABA_B$ receptors[26]. In the embryonic zebrafish, GABA-immunoreactive (ir) cells can be detected very early during development. By 16 hfp, GABA-ir cells are found in the telencephalic nucleus, the nucleus of the tract of the postoptic commissure, the nucleus of the medial longitudinal fascicle, and throughout the hindbrain and spinal cord[27]. At 24 hpf, existing ir-cell clusters increase in cell numbers, and new clusters are located in the dorsomedial nucleus of the posterior commissure and the primordium of the hypothalamus. As the zebrafish develops, three identifiable GABAergic axonal projections include the supraoptic tract, the tract of the postoptic commissure and the medial longitudinal fascicle. By 3 dpf, the distribution of GABA-ergic neurons in the zebrafish brain is very similar to its mammalian counterparts at a corresponding developmental stage[28]. GABA-ir cells are widely distributed, locating in the olfactory bulb, subpallium, posterior preoptic area, the diencephalic basal plate, the central optic tectum, torus semicircularis, ventral mesencephalic tegmentum, valvula of the cerebellum and medulla oblongata[19, 28]. In adult zebrafish, GABA is broadly distributed. GABA-ergic expression in the telencephalon is very similar to embryonic zebrafish, with GABA-ir cells in the internal cellular layer of the olfactory bulb[29] and all regions of the subpallium, as well as the preoptic, pretectal, ventral, thalamic, hypothalamic, preglomerular and posterior tubercular nuclei, cerebellar corpus, valvus and vestibulolateral lobe[25, 26].

DA and NA are the primary catecholamine neurotransmitters in zebrafish. DA plays a key role in modulating learning, memory, motor control, food intake, and motivation. Deficits of DA neurotransmission have been implicated in bipolar disorder, schizophrenia, ADHD and drug addiction. NA-ergic neurons serve an important function in the autonomic nervous system, but also modulate attention, arousal, reward, depression and schizophrenia. In zebrafish, the catecholaminergic signaling pathways have been characterized by visualizing the temporal and spatial expression of tyrosine hydroxylase (TH)[30, 31] or the *th* transcript[32] in all catecholaminergic neurons, the dopamine transporter (*dat*) for DA neurons and dopamine beta hydroxylase (*dbh*) for NA neurons[33, 34], and the three zebrafish DA receptors D1, D2 and D3 [35, 36]. Zebrafish DA-ergic neurons are first formed in the telencephalon (olfactory bulb and subpallium) and diencephalon (preoptic area, pretectum, ventral thalamus, posterior tuberculum, and hypothalamus) with projections that terminate locally[30, 33]. In adult zebrafish, *th1* and *th2* are differentially expressed, with *th1* expressed in the telencephalon, ventral thalamus and pretectum, *th2* more broadly distributed in the hypothalamus, and both genes strongly expressed in the posterior tuberculum and preoptic region[32]. There are some notable differences between zebrafish and mammals: for example, zebrafish lack DA-ergic cell groups in the mesencephalon[14, 33] and ventral midbrain[37], but possess an additional pretectal group[18]. The zebrafish NA system is very similar to mammals, with NA cell groups located in the locus coeruleus, medulla oblongata and area postrema[38, 39]. Zebrafish NA neurons can already be identified by 16 hpf, with few changes between larval and adult fish[40]. Projections from NA neurons are also conserved between zebrafish and mammals[37], with neurons in the locus coeruleus projecting to the telencephalon, diencephalon and mesencephalon, in the medulla projecting to the hindbrain and spinal cord, and the in the area postrema projecting to the same areas as the locus coeruleus[40].

HA also plays a key role in regulating diverse brain functions (e.g., higher cognitive functions, circadian rhythms, locomotor activity) and modulating other aminergic systems. The zebrafish HA-ergic system is very similar to other vertebrates, with L-histidine decarboxylase and three of the four HA receptors (H1, H2, and H3) found in the brain[41]. The first HA-ir neurons detected in zebrafish embryos are located in the ventral hypothalamus at ~85 hpf, and by 90 hpf the first ir-fibers can be detected in the dorsal telencephalon[42]. In adult zebrafish, HA-ir neurons are found in the ventrocaudal region of the hypothalamus surrounding the posterior recess[16] and innervate every major region in the brain (except the cerebellum)[14].

5-HT mediates excitatory and inhibitory neurotransmission, serving a critical role in aggression, anxiety, cognition, learning, memory, mood and sleep. Perturbation of 5-HT levels during development have been implicated in abnormal psychiatric behaviors, such as anxiety spectrum disorders, schizophrenia and depression[43]. Zebrafish express four 5-HT receptors (*htr1aa*, *htr1ab*, *htr1bd*, and *htr2c*)[44, 45], the spatial expression of which in the brain is primarily visualized by immuno-histochemistry and *in situ* hybridization[46]. More recently, transgenic strains expressing enhanced green fluorescent protein (eGFP) under control of the zebrafish raphe-specific gene *pet1* have also been developed[47]. 5-HT neurons are expressed in the spinal cord of embryonic zebrafish by 1 dpf, with additional clusters distributed throughout the brain (including the telencephalon and hindbrain) by 5 dpf [30, 48]. In adult zebrafish, 5-HT neurons are primarily located in the raphe nucleus with ascending projections terminating in the telencephalon, diencephalon, tegmentum, hindbrain and spinal cord[47]. Additional groups of anatomically distinct clusters of 5-HT-ir cells are also found in the pineal, pretectal area, posterior tuberculum and hypothalamus, reticular formation, area postrema and spinal cord[14, 47, 49]. 5-HT neurons have also been identified by the expression of other markers, such as three tryptophan hydroxylase genes (*tph1a*, *tph1b* and *tph2*)[50, 51], two 5-HT transporters genes (*serta* and *sertb*)[44, 52], and two vesicular monoamine transporter genes (*vmat1* and *vmat2*)[53]. Examination of the transporter genes permits more specific analysis of temporal and spatial expression pattern of 5-HT, since it can be unique, with *serta* expressed in the pineal, pretectal area, and raphe, and *sertb* in the retina, posterior tuberculum/hypothalamus and area postrema[54].

Acetylcholine plays a fundamental role in CNS functions (e.g., sleep, learning/memory and attention) and dysfunctions (e.g., schizophrenia, ADHD and depression). The zebrafish cholinergic system is generally similar to other vertebrates, with both muscarinic[55] and the full set of nicotinic receptors[56, 57]. The distribution of cholinergic neurons in the zebrafish brain has been primarily described by mapping the enzymes choline acetyltransferase (ChAT) and acetylcholinesterase (AChE). Cholinergic-ir neurons first appear immediately after hatching in the zebrafish embryo optic tectum. By 60 hpf, additional cells are seen in the preoptic area and tegmentum, and by the completion of the embryonic stage, additional reactivity is seen in the isthmic region and medulla oblongata[58]. Adult zebrafish display widespread distribution of both ChAT and AChE. AChE-ir neurons are found in the preoptic region, periventricular layer of the optic tectum, rostral tegmental, oculomotor, trochlear nuclei of the mesencephalic tegmentum, isthmic region, octaval nuclei and motor nuclei of the cranial nerves[59]. In contrast, ChAT-ir neurons are located in the pretectum and the

tectum, except the marginal layer, the mesencephalic tegmentum, isthmic region, in Purkinje and granule cells in the cerebellum, and throughout the medulla.

Finally, it is important to consider the fish-specific genome duplication that occurred around 450 million years ago[60]. In zebrafish, this duplication resulted in expressing more neurotransmitter receptors than other species. For example, zebrafish have an estimated 122 G-protein coupled receptors for biogenic amines (such as 5-HT, noradrenaline, dopamine, histamine, adrenaline and trace amines), compared to 57 in mouse and 44 in humans[61]. Importantly, not all zebrafish receptor paralogs have been identified and characterized. For example, to date only three 5-HT receptors have been cloned, despite the existence of seven distinct 5-HT receptor families in other animals[44]. Furthermore, the functional and pharmacological properties of zebrafish receptors have not been well studied[62], making it more difficult to translate information across species. One reasonably well characterized group is the alpha-2 adrenoceptors (adra2). The zebrafish genome contains five adra2 receptors, three of which (*adra2a*, *adra2b* and *adra2c*) are conserved with mammals[63]. However, the other two subtypes, *adra2da* and *adra2d*, are zebrafish-specific, whereas mammalian *adra2a-c* paralogs are not found in fish. Strikingly, despite fairly low sequence homology (~55% overall, rising to ~75% in the trans-membrane domains), human and zebrafish receptors show similar ligand-binding profiles[64]. For example, the adra2 agonist dexmedetomidine acts as a sedative in zebrafish and other species, an effect that can be inhibited by the adra2-specific antagonist atipamezole[65]. Thus, while the function of these receptors appears to be conserved across species[65], the increased number of neurotransmitter receptors in zebrafish (vs. mammals) means that their pharmacological and functional properties must be carefully examined before translating them to other species. In particular, the above-mentioned sub-functionalization of receptor subtypes implies that manipulating genes linked to psychiatric disorders may not recapitulate all symptoms of a disease, and that some novel drugs characterized in fish may have different pharmacological properties in humans (see further).

2.3. Visualizing zebrafish CNS signaling pathways

Imaging neural patterns and circuits is a key strategy in biopsychiatry research, and zebrafish offer a highly amenable experimental model for investigating the spatiotemporal distribution of neurotransmitter systems. In addition to conventional immunohistochemistry for neurotransmitter proteins or *in situ* hybridizations for the transcripts, recent genetic and technological developments permit *in-vivo* screening, high-throughput analyses and whole-brain assays in this model organism.

Larval zebrafish are particularly suited for translational models of psychiatric disease because they are small, undergo external development, and are transparent (until all major organs, including the brain, have fully developed)[10, 11]. This permits neurons in larval fish to be easily observed *in-vivo* and then ablated or manipulated. A wide range of genetic tools, developed or adapted to zebrafish to transiently alter gene expression and generate knockout disease models, includes antisense oligonucleotides (the morpholinos), zinc finger nucleases[66], TALEN[13] and ‘targeting induced local lesions in genomes’ (TILLING)[67]. Similar to invertebrate models, novel mutants or small molecules can then be rapidly

screened (in the thousands) using a combination of conventional fluorescent or confocal microscopy with automated fluidic screening platforms[68, 69].

Complementing multiple genetic methods, a suite of sophisticated tools also help elucidate the anatomical alterations associated with novel phenotypic alterations. These techniques include *in-vivo* monitoring of neural activity, functional analyses of specific neurons, and tracing of neural circuits. Genetically encoded calcium indicators (GECIs, such as the GCaMP reporter constructs) fluoresce when calcium is present, thereby serving as an indicator for action potential firing[70]. By driving expression with the Gal4/UAS[71] or transposon-based Tol2 systems[72] GECIs and optogenetic constructs, such as channelrhodopsin and halorhodopsin, can easily be inserted into the zebrafish genome to target specific cellular populations and perform functional analyses. With advanced microscope techniques (e.g., light-sheet microscopy), functional imaging of the entire brain of a larval zebrafish can be performed in less than 1.5 s[73], a task unattainable in other vertebrate models. In contrast to rodents, this procedure is also almost entirely non-invasive, and does not require attaching fiber optic cables. Albeit currently used in zebrafish to address fundamental questions (e.g., sensory processing[74] or motor control[75]), this optogenetic toolbox can be similarly employed in this organism to model altered neural activity associated with psychiatric disease in humans[76].

Fluorescent markers (e.g., GFP) can also be utilized to resolve the neuronal projections and formation of neural circuits in larval zebrafish. Using combinatorial expression of several fluorescent proteins to individually label neurons and their projections[77], Brainbow technology can be particularly useful for examining disease phenotypes related to miswired neural circuits (e.g., autism[78]). Briefly, Brainbow visualizes synaptic circuits by labeling neurons genetically, using Cre/lox recombination to create a stochastic choice of expression between several fluorescent proteins[79–81]. Originally developed in mice, this transgenic technology has been successfully applied to other model organisms, including zebrafish[77], to map multiple individual cells within a population and provide a large-scale dissection of CNS circuitry[80, 81]. A recent modification of Brainbow, the Zebrabow, has been introduced for in-vivo multicolor imaging in zebrafish[82]. Applying several new transgenic strains for ubiquitous or tissue-specific multicolor labeling, this approach maximizes color diversity by optimizing Cre activity, and can be particularly useful for long-term color-based anatomical and lineage analyses in a wide variety of zebrafish tissues, including neurons and glia[82].

Furthermore, while calcium and voltage sensors are fundamental to functional analyses in the larval zebrafish brain, they do not specifically measure neurotransmitter release or concentration (typically measured by cyclic voltammetry, HPLC or ELISA). However, more recent specific markers enable *in-vivo* measuring of neurotransmitters, based on placing a fluorescent label very close to the synapse, labeling the synaptic vesicle content or directly measuring extracellular neurotransmitters. Fluorescent false neurotransmitters (FFNs) are optical tracers that permit visualization of neurotransmitter release and uptake at presynaptic terminals[83]. Initially developed for the DA-ergic system, FFNs are fluorescent substrates for targeting vesicular monoamine transporter 2, carrying monoamine neurotransmitters to synaptic vesicles. FFN has also recently been extended to be a pH-responsive DAT

substrate, which is able to identify DA cell bodies and dendrites and measures DAT activity and DA release at individual synapses[84].

Several optical sensors have also been developed to measure excitatory synaptic activity in the brain. The glutamate optical sensor (EOS) is a fluorescent indicator made up of the glutamate-binding domain of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor and a small fluorescent molecule bound near the glutamate-binding pocket[85]. When bound to glutamate the EOS changes its fluorescence intensity. Intensity-based glutamate-sensing fluorescent reports (iGluSnFR) are a single-wavelength glutamate sensor that responds to glutamate *in situ* and therefore directly reports excitatory synaptic release[86]. This sensor can be genetically encoded and used in zebrafish to demonstrate the spatial organization of direction-sensitive synaptic activity in the optic tectum[86]. Finally, fluorescent indicators for acetylcholine volume transmission have also been created. Cell-based sensors of neurotransmitters (CNiFERs) are cultured cells that co-express M1 muscarinic receptors and the genetically encoded calcium indicator TN-XXL, and (when implanted into the brain) provide a fluorescent readout of acetylcholine levels[87].

Imaging neurotransmitter systems in the adult zebrafish brain is significantly more difficult due to its increased size and lack of transparency. While transparent zebrafish strains (e.g., *absolute*, *nacre*, and *casper*, Fig. 1A) exist[88], the brains are still relatively opaque making it difficult to image the various photo-proteins described above. As a result, studies have typically been limited to sectioning the brain. However, numerous methods may render a fixed brain transparent and, when imaged with long working distance objectives, permit the examination of neurotransmitters *in situ*[89–96]. These techniques use hydrophobic or hydrophilic chemical mixtures to remove lipids and reduce light scattering to visualize endogenous fluorescent markers in the brain.

2.4. Additional zebrafish neuroimaging tools

Several gene transcription factors, such as pair box 3 (Pax3), Fos and Orthopedia (Otp), represent a useful tool for visualizing CNS development and function in zebrafish. For example, Pax3 is a transcription factor that regulates cell proliferation, migration and neural development. Its gene is not only highly conserved (at the sequence level) between zebrafish and mammals, but also shows similar pattern of neural expression during embryogenesis[97–99]. Thus, analyses of *Pax3* expression in zebrafish can be particularly useful for imaging early CNS development[97, 98], which is relevant to modeling many neurodevelopmental disorders.

The homeodomain transcription factor Otp is an important regulator of neuroendocrine and dopaminergic cells in vertebrate species[100, 101]. *Otp* has two paralogs in zebrafish (*otpa* and *otpb*)[101], and genetic manipulations suppressing Otp deplete certain dopaminergic neurons, enabling a functional dissection of dopaminergic contribution to CNS circuitry in early larval zebrafish[102].

Likewise, an early proto-oncogene *c-fos* serves as a well-established marker of neuronal activation in various species, including humans[103], rodents[104, 105] and zebrafish[106]. For example, *c-fos* expression analyses of whole-brain zebrafish samples can be used to assess

neuronal activation globally (e.g., reflecting CNS excitation during epilepsy[106, 107] or following anxiogenic/anxiolytic or hallucinogenic treatments[108, 109]). Region-specific analyses of *c-fos* expression in zebrafish brain offer a more detailed functional mapping of neural circuits during various behavioral tests[110]. For instance, in the light-dark test, *c-fos* neuronal activity mapping implicated several brain regions, including several telencephalic areas (the teleost homologs of the mammalian amygdala and striatum), during zebrafish 'anxiogenic' light avoidance behavior[110]. Characterizing neuronal activity induced in zebrafish by acute amphetamine, drug-seeking or the light avoidance behavior, quantitative analyses of *c-fos* expression can also be combined with neuronal subtype-specific markers at single-cell resolution[111]. Showing the recruitment of the medial and the lateral pallium (structures homologous to the mammalian amygdala and hippocampus), these data suggests evolutionarily conserved function of amygdala-like structures in positive emotions and motivated behavior in zebrafish and mammals[111]. Collectively, this evidence strongly supports the utility of *c-fos* and other biomarkers in visualizing normal and aberrant CNS development and functioning.

Finally, with the increased use of zebrafish in disease genetics research, a quantitative voxel-based technique may help compare disease models with wild-type zebrafish. Magnetic resonance imaging (MRI) is an inherent 3D tool that facilitates investigations in morphology, connectivity and function of the brain. Due to its 3D nature, multiple datasets can be mapped to a standard coordinate space and registered to produce a model representative of a population (thus, limiting individual morphological variance and instead demonstrating the average morphology of the entire population). Data collected from MRI can include mean volumes of individual brain regions, the stereotaxic locations of individual structures and major fiber bundles, and functional activity. Importantly, MRI models can also serve as a canonical space for registration of other imaging datasets. For example, recent whole-brain immunostaining and light microscope imaging (e.g. SeeDB or Clarity), optical projection tomography and microCT can all be registered to the same stereotaxic space, collectively enabling quantitative analysis of a wide range of markers in zebrafish brain.

3. Zebrafish models of brain disorders

3.1. Bridging molecular events with behavior

Many psychiatric disorders are moderately or highly heritable, and have developmental trajectories[112, 113]. As behavior is controlled by activity within neural circuits, a major challenge is to map the genotype-phenotype relationship in order to understand how genetic variations affect neural circuits that control behavior. Various molecular tools have been effectively used for uncovering brain mechanisms in zebrafish. For example, in addition to traditional N-ethyl-N-nitrosourea (ENU)-induced[114] and viral-vector-mediated[115] mutagenesis, new sophisticated 'gene-breaking transposon' (GBT)[116] screens and 'clustered regularly interspaced short palindromic repeats' (CRISPR) approaches for generating targeted zebrafish mutants have been developed[117]. Combined with TILLING and TALEN tools, this makes the process of exploring genotype-phenotype relationships in zebrafish better targeted and less labor-intensive. With the establishment of translationally relevant

zebrafish behavioral assays[9–11] comes the ability to apply these molecular techniques to modeling psychiatric diseases. Here, we discuss how various zebrafish models can be utilized to target selected psychiatric disorders and their neurodevelopmental trajectories. ADHD, aggression, post-traumatic stress and drug abuse (Fig. 2) were selected as examples, reflecting a wide and diverse spectrum of psychiatric disorders that can be modeled using the zebrafish.

3.2. Modeling ADHD

Zebrafish have recently been utilized to investigate some aspects of ADHD (Table 1, Fig. 2A), a common psychiatric disorder affecting ~3–5% of children worldwide. ADHD symptoms (inattention, hyperactivity and impulsivity) persist into adulthood in ~50% of cases, reducing the patients' quality of life, academic performance and sociality[118, 119]. Altered DA, NA, 5-HT and glutamate neurotransmission in the prefrontal cortex, striatum, parietal cortex, vermis and the inferior lobes of the cerebellum is implicated in ADHD[120–123].

An interesting example of using larval zebrafish to model this disorder involves a human gene *LATROPHILIN3 (LPHN3)*, identified as candidate ADHD gene by linkage analysis[124]. In zebrafish, its homolog *latrophilin3.1* (*lphn3.1*) plays a developmental role, and is expressed in differentiated neurons throughout the brain up to 6 dpf. The *lphn3.1* morphants display increased distance swum at 6 dpf (Fig. 2A)[125, 126] - the hyperactivity phenotype also maintained during the night, demonstrating a permanent increase in locomotion compared to control animals. Morphant larvae also show more bursts of accelerated swimming, indicative of motor impulsivity. Both hyperactivity and motor impulsivity can be rescued by the prototypical anti-ADHD drugs methylphenidate (MPH) and atomoxetine (Table 1). While acute 1-h treatment with either drug had no effect on control-injected larvae at the doses used, it rescued morphant behavior bringing locomotion back to control levels. The *lphn3.1* morphants also display fewer cells in the posterior tuberculum, a prominent group of DA-ergic neurons in the ventral diencephalon that control larval locomotion and project both anteriorly and posteriorly, but mainly to the spinal cord[101, 127]. Thus, despite the difficulty of fully modeling ADHD in zebrafish, research in these animals show an important role for *lphn3.1* in DA-ergic neuron development[128] (also see similar data in mice[129], proving the feasibility of translating findings from zebrafish to other species).

Exposure of zebrafish embryos to chemicals during development may also provide insights into possible environmental causes of ADHD. For example, prenatal exposure to perfluorooctane sulfonate has been linked to clinical adolescent ADHD[130], and treating zebrafish embryos with this agent during development elicits ADHD-like behaviors in 6-day old larvae (i.e., hyperactivity, motor impulsivity and hyper-responsiveness to startle)[131]. This phenotype is reminiscent of the behavior of *lphn3.1* larvae and strikingly, these behavioral changes can be rescued by dexamfetamine, implicating DA signaling in this phenotype. Zebrafish are also used to study the consequences of long-term exposure to MPH, displaying elevated DA, NE and 5-HT in the larval brain, and lasting behavioral changes (e.g., impaired predatory escape and learning)[132]. Such ease of conducting

embryonic manipulations and behavioral experiments in zebrafish strongly supports their potential for studying ADHD and related disorders.

3.3. Zebrafish models of aggression

Aggression, an adaptive animal behavior to monopolize resources, is essential for the organism's fitness and survival. However, pathological aggression is part of various brain disorders, and merits further studies *in-vivo* models. Although aggression has long been studied in fishes, the genetic and neurobiological tools available in zebrafish make them an ideal species to study aggression. Zebrafish display aggression as a series of stereotypic body postures (e.g., undulating body movements, short slaps of the caudal fin and bouts of swimming and biting directed against an opponent) which can easily be measured in the laboratory[133]. Aggressive incidents follow a highly structured pattern[134], from extending the fins to circling, chasing and attempting to bite, further progressing to more frequent chases, bites and attempts to strike the opponent. The fish which loses such as encounter becomes submissive, exhibiting postures that include fleeing, freezing and retreating[134, 135]. In laboratory zebrafish, aggression can be measured by recording the interaction of two fish in a tank, or the interaction of a single fish with its own reflection in the mirror exposure test (mirror-induced aggression, MIA; Fig. 2B)[133, 134].

Mounting evidence shows that zebrafish aggression has genetic a basis, and its moderate heritability suggests that environmental influences (e.g., maternal care, rearing conditions and environmental complexity) also contribute to this behavior. For example, aggression/boldness profiles in several wild-type zebrafish strains (TM1, SH and Nadia) show strain differences in MIA, supporting the genetic basis of aggression in zebrafish[136]. A group of 40 genes linked to aggression have been identified in fish, with differences in expression level depending on both the area of the brain analyzed and the sex[137]. HPLC analyses of adult zebrafish brains following an aggressive interaction reveal altered DA, 5-HT and their metabolites in the telencephalon, diencephalon and optic tectum. Other important brain areas include the preoptic area of the anterior hypothalamus[138] and the periventricular nucleus of the inferior hypothalamus[139]. Notably, similar brain areas have been linked to agonistic behavior in other vertebrates, highlighting the translatability of zebrafish research in this field. Like in other vertebrates, fish aggression is controlled by similar neurotransmitters, peptides and hormones, including DA, 5-HT, HA, 17α-ethinylestradiol and arginine vasopressin/vasotocin (AVP/AVT). For example, addition of estrogen to tank water alters zebrafish aggression, implicating certain pathways and circuits[133, 140], as reduced aggression in dominant male zebrafish correlates with altered 5-HT, DA, AVP levels and the expression of the somatostatin pathway genes[141].

A recently characterized novel aggressive *spiegeldanio* mutant zebrafish (*spd*, Fig. 1A)[142] harbors a mutation in the *fgf receptor 1a* gene, which disrupts the fibroblast growth factor (FGF) signaling in the brain. FGFs are a large family of secreted signaling molecules with important roles during development[143]. Homozygous *spd* mutants show unaltered locomotion but increased aggression, boldness and exploration (Fig. 2B), correlated with elevated breakdown of HA in the brain, and reduced neurotransmitter signaling in the hypothalamic periventricular nucleus. Importantly, pharmacological increase of HA rescues

all three behavioral alterations in *spd* mutants[139]. Although *spd* zebrafish are always more aggressive than wild-type siblings, their behavior differs between the laboratories (e.g., similar boldness but higher aggression in mutants raised in Germany vs. France)[139]. Taken together, these results demonstrate the importance of environmental influences on aggression, and that experimental modulation of selected target genes can strongly affect zebrafish aggressive phenotypes.

3.4. PTSD

Severe stress is the main cause of PTSD and other trauma/stress-related disorders (TSRDs). Zebrafish have recently been proposed as potential models of PTSD and TSRDs[144, 145]. The extensive, complex repertoire of behavioral responses of zebrafish to a wide variety of stress-evoking stimuli closely parallels that observed in mammalian models of PTSD (Fig. 2C, Table 1)[145]. For example, various PTSD-relevant phenotypes identified in zebrafish include robust anti-predatory responses[146, 147] and inhibitory or active avoidance conditioning[148]. Paralleling mammalian tests, an increasing number of zebrafish paradigms relevant to PTSD continue to be developed, based on fear conditioning[148–152] and exposure to aggressive conspecifics[134, 138], predators or other acute stressors[153–155] (Fig. 2). Moreover, the highly social nature of zebrafish[156–158] offers further potential to examine pathological social phenotypes, representing the serious adverse clinical effects of PTSD/TSRD. For instance, experimentally evoked disorganization in the social structure of zebrafish groups (shoals)[158–161] may represent an interesting PTSD-related ‘social withdrawal’ phenotype[145].

The physiology of zebrafish also supports their suitability for modeling PTSD and other stress-related CNS disorders[120, 121]. Zebrafish rapid development enables time- and cost-efficient modeling of the developmental and ‘temporal’ pathogenetic aspects of PTSD. Moreover, given the strong involvement of the neuroendocrine stress axis in PTSD[162], the cortisol-driven neuroendocrine axis of zebrafish makes them well-suited for studying glucocorticoid regulation in stress and PTSD[163, 164]. For example, zebrafish *gr*^{s357} mutants, possessing non-functional glucocorticoid receptors, have recently offered a model of glucocorticoid resistance to study the developmental effects of glucocorticoid signaling deficits on stress physiology and related behaviors[165, 166]. Furthermore, an extensive array of homologues of clinical biomarkers of PTSD has also been identified in zebrafish, including corticotropin releasing factor[167, 168], glucocorticoid receptor[169, 170], adrenocorticotropic hormone[171, 172], neuropeptide Y[173, 174], catechol O-methyltransferase[5, 175, 176] and monoamine oxidase A/B[171, 177]. Finally, the sensitivity of zebrafish sympathetic and parasympathetic responses (e.g., altered heart rate) to pharmacological manipulation has recently been established[178], offering the potential to study autonomic biomarkers of PTSD-relevant drug treatments. Overall, zebrafish emerge as a promising new model to complement traditional rodent models of PTSD[10, 144, 145].

3.5. Addiction, substance abuse

Zebrafish are increasingly utilized as a preclinical model to investigate molecular mechanisms of substance abuse. Drugs of abuse typically exert their effects by modulating DA pathways in the midbrain[179], and the ascending DA systems (VTA-NAc reward

pathway) in zebrafish share functional homology with those of mammals[180, 181]. Zebrafish express orthologs for many addiction-related human genes[182], including the majority of the DA-, 5-HT- and cholinergic receptor gene families. Zebrafish also display robust reward responses to translationally relevant doses of common drugs of abuse, including stimulants (cocaine[183], amphetamine[184], nicotine[185] and caffeine; Brock and Brennan, unpublished data), ethanol[185], opiates (morphine[186], fentanyl) and general anaesthetics (tetracaine and ketamine; Brock and Brennan, unpublished data). As drug-seeking behavior is evolutionarily conserved, zebrafish represent a powerful in-vivo model to assess the contribution of genetic factors across specific endophenotypes or drugs of abuse.

The first systematic screens relevant to addiction, examining mutant alleles affecting catecholaminergic neurons[187], showed that DA/hypothalamic neuronal development is regulated by forebrain embryonic zinc finger-like protein (*fezl*)[188], the mutation of which in zebrafish reduces opiate (but not food) reward[189]. Subsequent studies performed conditioned place preference (CPP, Fig. 2D) screens of ENU-mutagenized F_2 adult fish for cocaine and amphetamine reward[183], and found a number of families showing heritable reduced reward sensitivity[184]. For instance, the *nad*$^{+/-}$ mutant zebrafish show insensitivity to the rewarding effects of amphetamine[190]. Microarray analysis, followed by qPCR confirmation, identified multiple genes that were up/down-regulated in the *nad* mutant, particularly relating to neurogenesis and brain development. Similar approaches have been applied to assess drug-induced reward and to identify novel genes and molecular pathways involved in zebrafish reward[184, 189, 191–194]. For example, mutants heterozygous for a nonsense mutation in the AChE (*ache*+/−) gene[184] display reduced amphetamine-induced reward in a modified CPP paradigm, demonstrating the conservation of cholinergic regulation of reward pathways.

Although sensitivity to the rewarding effects of drugs is a fundamental aspect of vulnerability to addiction, addiction itself is characterized by the progression from occasional or transient to habitual drug taking, compulsive drug seeking and a tendency to relapse even after prolonged abstinence[195]. Drug-seeking despite adverse consequences, and reinstatement of drug seeking following extinction, are both commonly used to model these behaviors in laboratory species[196]. Recent studies demonstrate persistent drug-seeking despite punishment[197] and relapse[198] in zebrafish chronically exposed to either nicotine or ethanol. Microarray analysis in this model reveals shared changes in gene expression, consistent with conservation of neuroadaptations and the establishment of dependence in zebrafish[197]. Collectively, these findings show that genetic and neural pathways are well conserved between mammals and fish, and that fish employ similar molecular adaptations following chronic drug exposure[199].

In addition to classical screening techniques in adult fish to identify strains with aberrant drug responsivity, more targeted approaches help identify specific genes involved in drug sensitivity. Fluorescently tagged GBT mutagenesis has been used to examine molecular influences on a measure of nicotine addiction, behavioral sensitization[116]. This insertional method represents a leap forward from classic mutagenesis, enabling both visual identification of carriers and rapid identification of the disrupted gene. In a screen of GBT mutant strains for nicotine behavioral sensitization, 5-dpf larvae were exposed to nicotine

acutely or 8 h following previous administration of this drug. Acute exposure caused an overt locomotor response, significantly accentuated in the larvae pre-treated with nicotine. GBT-mutagenized families with diminished acute and sensitized responses to nicotine, identified in this screen, included the *bdav* mutants with a truncation in chaperonin-containing protein 8 (*ccb8*, linked to nicotinic acetylcholine receptor nAChR), and the *hbog* zebrafish with a mutant ortholog of the GABA-B receptor (*gabbr1.2*)[59, 116]. Furthermore, the behavioral phenotypes in these strains were reverted by cre-mediated recombination, revealing specific loci in zebrafish that may lead to translational targets for the treatment of tobacco addiction[116].

3.6. Alcohol

Alcohol is the drug with the longest history of use and abuse in human society. Although legally accepted and culturally tolerated, it is a dangerous drug, and alcohol-related disorders present an enormous burden for the patient and society[200]. Alcohol is a complex drug, affecting multiple mechanisms and behaviors in a dose- and use-dependent manner. This complexity is one of the reasons why effective therapies are still lacking, and alcohol-related disorders remain a serious medical concern. In general, findings from zebrafish alcohol research can by evaluated by three main criteria of a translationally acceptable model – face, construct and predictive validity. Addressing face validity, recent studies suggest that zebrafish respond to alcohol in a manner highly homologous to that of humans[133] (Fig. 2E). For example, acute administration of alcohol makes fish agitated at intermediate doses, but lethargic at higher doses[201]. Zebrafish exhibit motor impairment in response to high doses of acute alcohol (e.g., 1%), but show elevated aggression in response to intermediate (e.g., 0.25–0.50%) doses[133]. Acute alcohol also impairs zebrafish response to social stimuli in a linear dose-dependent manner (Fig. 2E), a reaction that is strikingly similar to that in humans[133, 202]. Chronic exposure to alcohol evokes tolerance in zebrafish, paralleling clinical findings[203]. Withdrawal from alcohol also leads to responses (e.g., elevated motor activity, anxiety, incoordination and reduced shoaling), phenotypically similar in zebrafish and humans[203, 204] (Fig. 2E). Likewise, embryonic alcohol exposure in zebrafish further parallels the clinical effects of embryonic alcohol exposure, including the social and learning impairment observed in fetal alcohol spectrum disorders (FASD)[10, 205].

Mounting evidence also suggests significant construct validity of zebrafish alcohol models. For example, following acute or chronic alcohol treatment, zebrafish show similar neurochemical alterations in the brain, compared to humans. Acute alcohol induces a rapid increase in brain DA, which is blunted after chronic alcohol exposure[203]. Likewise, withdrawal from alcohol increases brain DA levels[203], whereas chronic alcohol exposure alters the expression of nearly 2000 genes in zebrafish, many of which have been implicated in human alcoholism[206].

One of the greatest challenges for modeling the effects of alcohol in zebrafish is establishing predictive validity – the extent to which drugs (with particular known effects on human CNS) act in the same way in the zebrafish system. While the model system is expected to show efficacy of human drugs in the predicted manner, only a limited number of compounds expected to mediate the effects of alcohol has been assessed in zebrafish. However,

zebrafish have largely been shown to respond in a predictable manner to such compounds, paralleling clinical effects and suggesting a conservation of the main neurotransmitter signaling pathways between zebrafish and humans. Overall, while future studies are needed to further assess their face, construct and predictive validity as models of alcohol-related disorders, zebrafish already shows a significant potential in this field.

4. Current challenges

The advantages and limitations of zebrafish in biopsychiatry research have recently been discussed in the literature[9–11, 174]. However, despite recent progress in zebrafish neurobiology, many important questions remain to be addressed. For example, the logical step in the development of zebrafish models with translational relevance to human addiction is to apply systematic screening of mutagenized families, to characterize genetic risk factors associated with drug abuse. Humans with addiction and other linked psychiatric disorders (e.g., compulsive gambling, obsessive compulsive disorder, Tourette's syndrome or ADHD) often display deficits in general cognitive or executive function[207–210] (which becomes relevant to studying the molecular basis of cognitive dysfunction linked to addiction). Emerging as a key endophenotype for cocaine addiction[211, 212], trait impulsivity represents several behavioral constructs, including inability/unwillingness to wait, choice of smaller but more immediate (over larger, delayed) rewards, and motor impulsivity (failure to inhibit a pre-potent response)[213]. In compulsive drug use in rodents, animals with high levels of motor impulsivity (assessed by premature responses on a test of sustained attention, the five-choice serial reaction time task; 5-CSRTT[214]) also show compulsive cocaine seeking[211] and increased relapse after extinction training[215]. This task has recently been adapted for zebrafish[216], resulting in a fully automated model[217], validated pharmacologically by showing dose-dependent reductions in fish impulsivity following atomoxetine[218], similar to rodents[219]. Collectively, this provides the exciting prospect of using three-generation screens (discussed above) or insertional transposon technologies to study impulsivity, thus fostering the search for the molecular basis of psychiatric disorders linked to impulsivity and other reward-related traits.

Likewise, the ability to develop novel zebrafish models of ADHD is also interesting and translationally relevant. However, the suitability of zebrafish models (e.g., mutants lacking *lphn3.1* function) as an ADHD-like model must be further established by testing other behaviors, such as performance in the 5-CSRTT[217]. Although such a complex task will require a stable *lphn3.1* line to be established, this approach would be ideal to reinforce the utility of *lphn3.1* and other mutant fish as a tool to study the neurobiology of ADHD.

Because the formation, position and function of neurotransmitter signaling pathways sometimes differ between zebrafish and other vertebrates, comparative studies become important. For example, as already mentioned, similar to other teleosts, zebrafish have undergone a partial genome duplication, and some genes found in a single copy on the mammalian chromosome have two copies in the zebrafish chromosome[220]. Such genetic redundancy can make it more difficult to analyze a disease gene's function (e.g., if the effect of a mutated gene is masked by the unaltered 'sister' gene). While the function of some receptors and transporters is conserved across species[17, 65], the increased spectrum of

neurotransmitter signaling-related molecules in zebrafish (vs. mammals) suggests that their functional properties merit further scrutiny in genetic and pharmacological studies. However, this redundancy can also present an opportunity (e.g., permitting the analysis of the effects of genes whose null mutations lead to developmental lethality if in a single copy, a common problem in mouse knockout studies).

From an evolutionary perspective, there are also important physiological differences between mammals and zebrafish. For example, fish are poikiloterms and mammals are homeothermic animals, and substantial differences exist in metabolism and organ systems between species[221]. Likewise, there are also well-known differences in brain development between fish and mammals, which will be only briefly mentioned here. For instance, instead of the laminar cortex, telencephalic nuclei in teleost fishes have evolved as the pallium, containing similar but simpler neural circuits, compared with the mammalian cortex, which also has some additional circuits[222] (note, however, recent data showing that emotions and motivated behavior in zebrafish converge on an amygdala-like structure, similar to their mammalian counterparts[111]). Another interesting example is the habenula - a key relay nucleus connecting the forebrain with the brainstem and modulating monoaminergic mechanisms of behavior and cognition[223]. Although the development of its circuits appears to be conserved phylogenetically, some species differences in habenular asymmetry exist between fish and mammals[223] (note, however, mounting evidence showing that the zebrafish habenula, like in humans[224], plays an important role in the regulation of affective behavior and aversive memories[152, 225–227]). While not within the scope of this review, such physiological and neurobiological differences between species must be considered critically when translating zebrafish responses into human disorders[221]. Together with some genetic differences between species[12] and poorly characterized protein function differences (stemming from divergent evolution), this represents a serious current challenge for zebrafish molecular psychiatry.

Finally, perhaps the biggest challenge in multidisciplinary zebrafish studies is the behavioral models and tools[9, 10]. Despite a rapidly growing research in this field, the zebrafish is still a new model in behavioral neuroscience[11]. Will zebrafish analyses keep pace with the rapid advancement of molecular and neurobiological tools developed for this species? While the answer to this question is unclear, recent evidence offers grounds for optimism. For example, the rapidly increasing computer processing power, coupled with constantly improving 2D and 3D video-recording and imaging tools, generates behavioral data with ever increasing quality, precision and spatiotemporal resolution. Simultaneous video-tracking of a large number of fish (either swimming in separate compartments; Fig. 2B or moving together in a group/shoal)[10, 160] now became a reality. As behavioral quantification becomes more precise, so does the delivery of stimuli that induce the behavioral responses. Because both stimulus delivery and behavioral response quantification are now increasingly computerized and standardized, the behavioral paradigms in zebrafish become more automated and scalable. Thus, running such multiple tasks in parallel makes even slow learning protocols high-throughput and efficient. Enhancing high-throughput screening of mutants and drugs that affect complex behavioral responses, such phenomics-based approaches in zebrafish foster further experimental modeling of human brain disorders.

5. Conclusion

Uncovering the molecular processes that underlie the formation of neural circuits and their activity to control the behavior, is key to the understanding and treatment of psychiatric diseases[6, 7]. Recognizing the importance of zebrafish to molecular psychiatry is the critical step in our continued use of this model organism to study neurobiological mechanisms of human brain disorders. In addition to the growing availability of genetic and pharmacological assays and probes in zebrafish, there is also an urgent need for new behavioral assays and imaging neural pathways and circuits implicated in various pathological states in this species[228, 229].

The availability of genetic, electrophysiological and optogenetic tools, as well as multiple mutant and transgenic strains, physiological biomarkers and robust protocols to measure behavior, collectively supports zebrafish potential for modeling psychiatric disorders. As both larval and adult zebrafish are now rapidly becoming key model organisms in neuroscience research, they represent a useful novel species for translational molecular psychiatry and CNS drug discovery.

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Abbreviations

5-CSRTT	5 choice serial reaction time task
5-HT	5-hydroxytryptamine (serotonin)
AChE	Acetylcholinesterase
ADD/ADHD	Attention deficit disorder and attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AP	Area postrema
CNiFERs	Cell-based sensors of neurotransmitters
ChAT	Choline acetyltransferase
dpf	Days post fertilization
EAATs	High-affinity excitatory amino acid transporters
EOS	Glutamate optical sensor
FFN	Fluorescent false neurotransmitters
GABA	γ:-Aminobutyric acid

GAD	Glutamic acid decarboxylase
GECIs	Genetically encoded calcium indicators
GFP	Green fluorescent protein
hpf	Hours post fertilization
iGluR	glutamate ionotropic receptor
iGluSnFR	Intensity-based glutamate-sensing fluorescent reports
ir	Immunoreactive
LO	Locus coeruleus
mGluR	Glutamate metabotropic receptor
MO	Medulla oblongata
NMDA	N-methyl-D-aspartate
Pax3	‘Paired box’, 3 (gene)
TILLING	Targeting induced local lesions in genomes
TALEN	Transcription activator-like effector nuclease
VGLUT	Vesicular glutamate transporter

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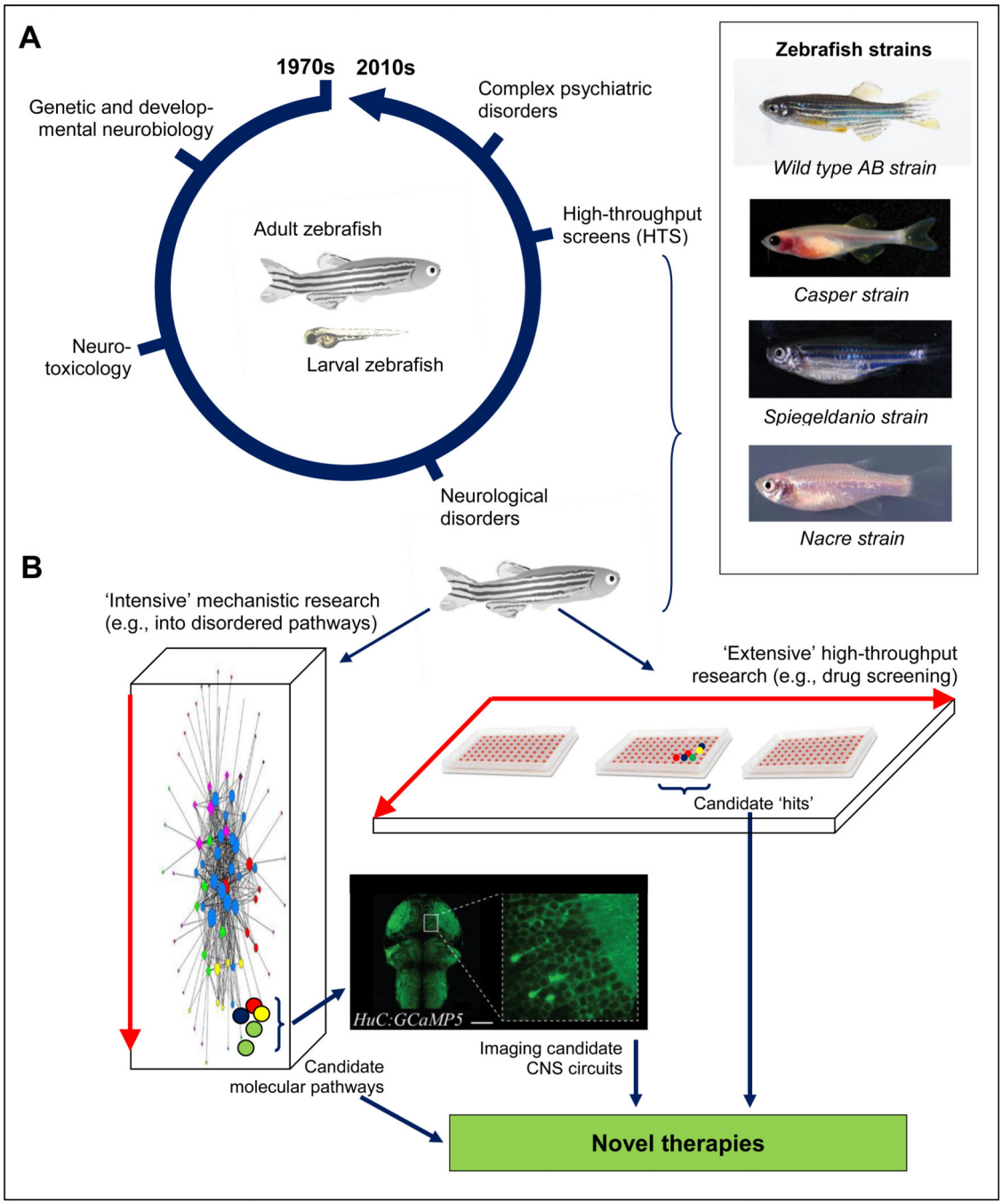


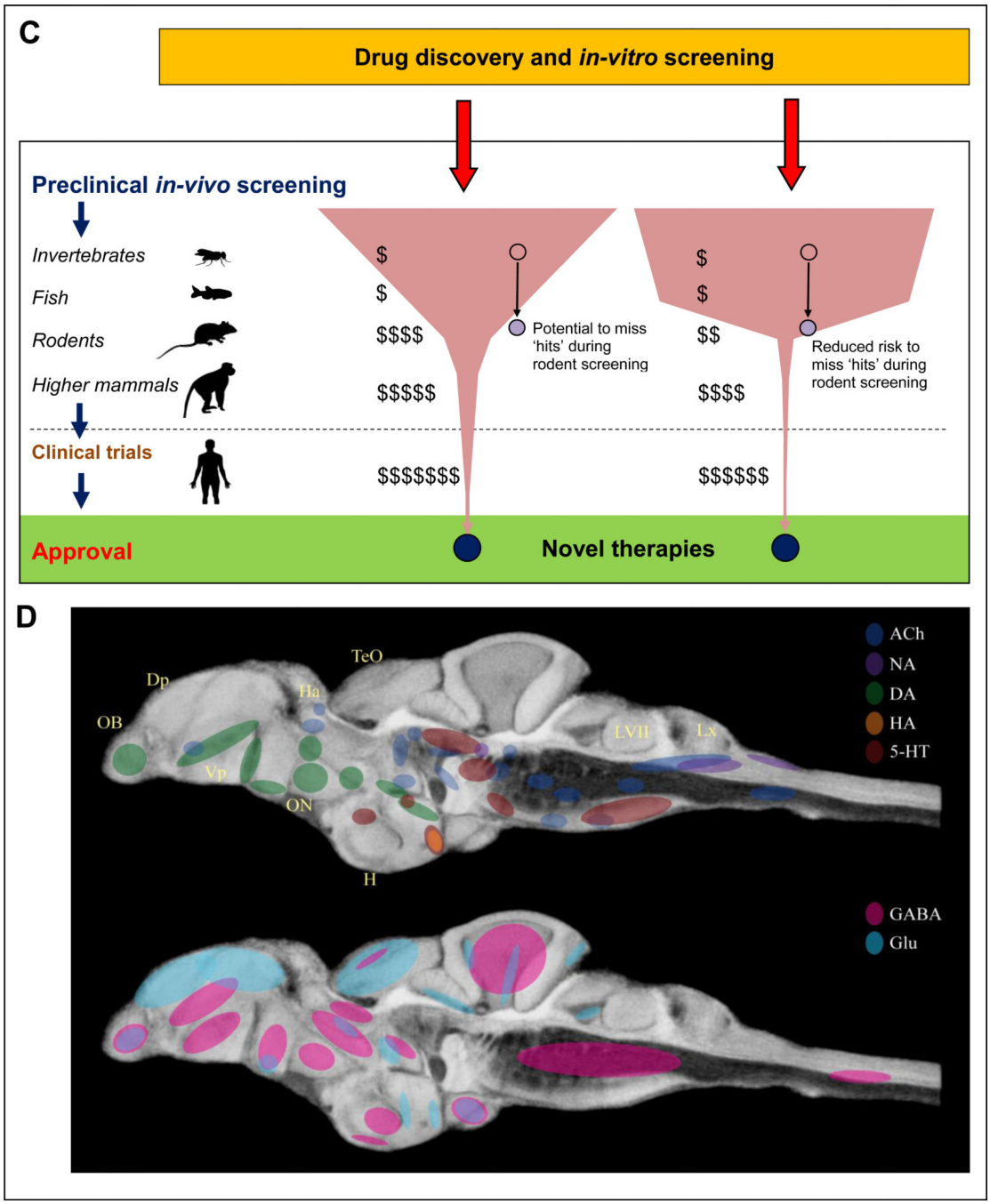


Figure 1. A brief summary of zebrafish experimental models in neuroscience research

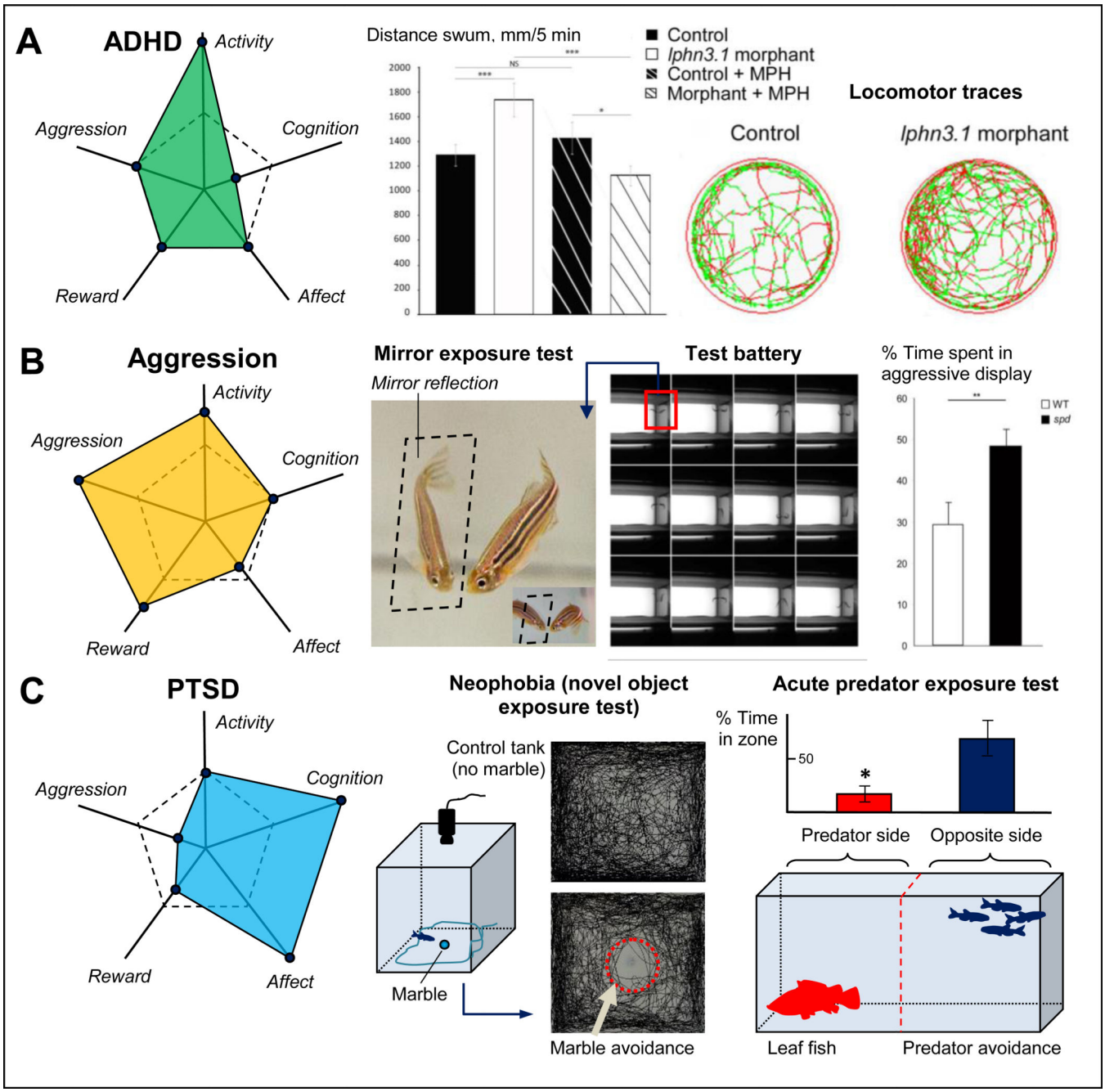
Panel A shows the evolving nature of zebrafish models in the last 50 years, initially used mainly for basic genetic and neurodevelopmental studies, but more recently applied to developing in-vivo models for complex brain disorders, such as autism, depression and psychoses. Inset: selected zebrafish strains useful in biological psychiatry research (top to bottom: adult wild type zebrafish, *casper*, *spiegeldanio and nacre* mutants); photos courtesy of the Kalueff (ZENEREI Institute, USA), the Norton (University of Leicester, UK), the

Parichy (University of Washington, USA) laboratories and Carolina Biological Supply Company (Burlington, USA).

Panel B illustrates two research strategies which can both be applied to zebrafish models. As a vertebrate species amenable to *in-vivo* analyses and with high genetic/physiological homology to humans, zebrafish are ideal for 'intensive', mechanistically driven neuroscience research into conserved, core molecular pathways or neural circuits (photo). Due to their small size, ease of maintenance and short generation time, zebrafish also represent an excellent model for 'extensive' biomedical research, including low-cost high-throughput screening for small molecules or genetic mutations. Both strategies can lead to the development of novel therapies for major psychiatric disorders. Photo: visualizing zebrafish signaling as an example of intensive, pathway-oriented mechanistic research (the left image shows expression patterns of genetically encoded calcium indicator; zebrafish at 5 dpf show pan-neuronal expression of HuC:Gal4; UAS:GCaMP5, a zoomed-in image on the right shows a single cell resolution: note that some cells are activated, as indicated by the increased fluorescence; scale bar 100 μm).

Panel C illustrates the benefits of including inexpensive zebrafish models into preclinical screening batteries. A substantial fiscal saving (shown as $) can be achieved by narrowing screening to potentially active compounds using fish (rather than mice) as the first vertebrate model organism in the screening pipeline. In addition, the risks of missing promising 'hits' are lower because the subsequent screening in mammals will be 'confirmatory', and based on more valid data generated from zebrafish (rather than invertebrate or in-vitro) tests.

Panel D summarizes major neurotransmitter systems in the adult zebrafish brain: ACh - acetylcholine (dark blue), NA - noradrenergic (purple), DA - dopamine (green), HA - histamine (orange), 5-HT - serotonin (red); GABA - γ-aminobutyric acid (pink), Glu - glutamate (light blue). The highlighted brain regions include olfactory bulb (OB), dorsal pallium (Dp), ventral pallium (Vp), habenula (Ha), optic nerve (ON), facial lobe (LVII) and vagal lobe (LX). Highlighted areas have been overlaid over a minimum deformation model of the adult zebrafish brain.



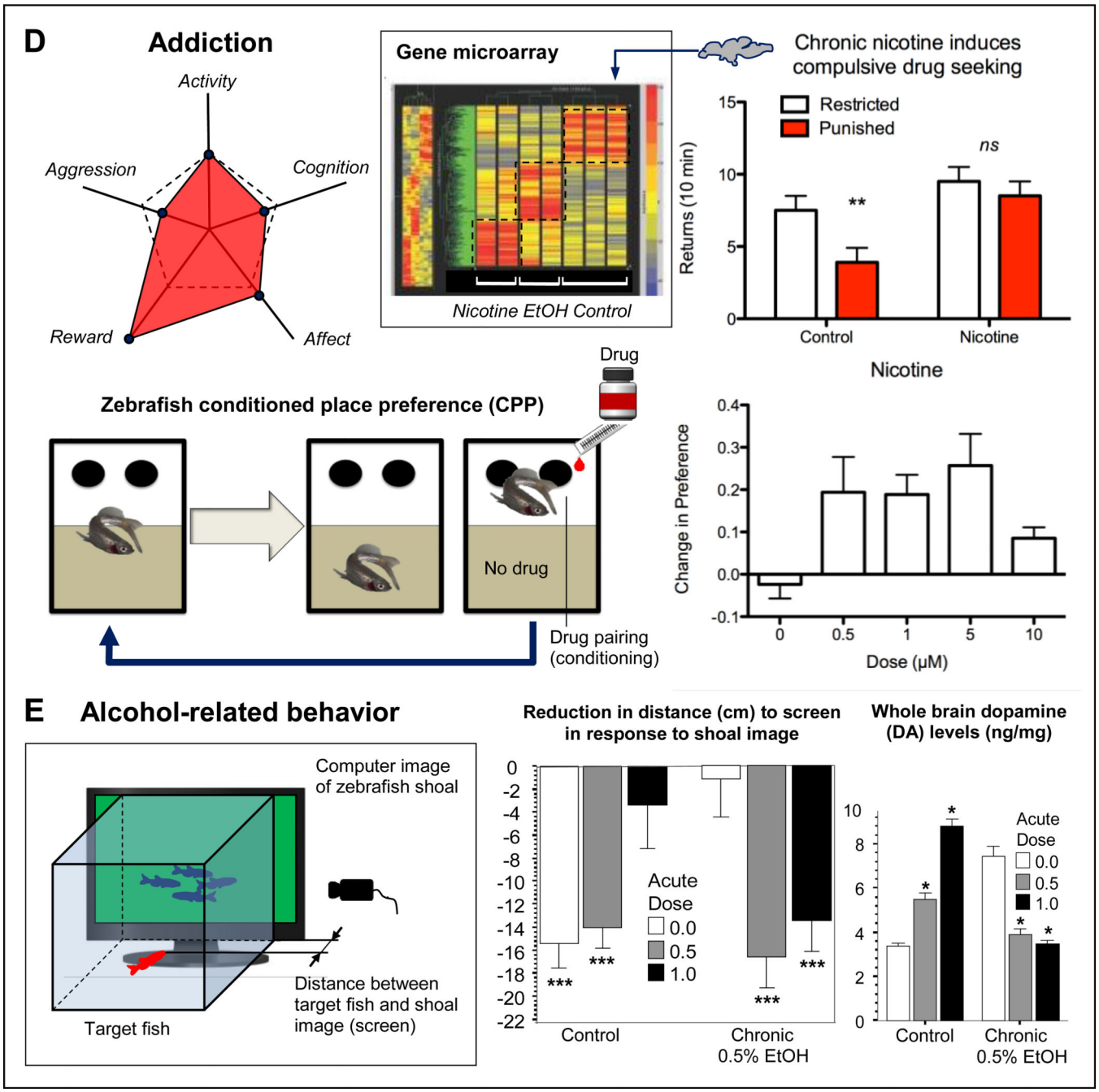


Figure 2. Neurobehavioral phenotypes in zebrafish relevant to modeling a diverse group of psychiatric disorders (also see Table 1)

Axis represent five major phenotypic domains of brain disorders, and include motor activity, cognition, affect/emotionality, reward and aggression. A dashed- line pentagon represents 'normal' healthy phenotype for each respective axis/domains.

Panel A shows typical zebrafish responses relevant to ADHD (modified from [125, 126]). The bar diagram shows total distance swum in 5 min for control larvae and *lphn3.1* morphants with and without methylphenidate (MPH) treatment. The right panel shows representative locomotion traces (recorded from the top view camera) in 6-dpf control and *lphn3.1*

morphant zebrafish (green color indicates swimming with normal speed, red color indicates bursts of 'impulsive' swimming with high-acceleration).

Panel B illustrates zebrafish mirror-induced aggression (MIA) responses in the mirror exposure test, and typical patterns of zebrafish aggressive confrontations (modified from [139]). Note that MIA is particularly simple to record and quantify[161], making it also suitable for high-throughput screening (MIA images in the middle: courtesy of the Robison laboratory[161], University of Idaho, USA).

Panel C illustrates zebrafish stress- and PTSD-related aversive behaviors, including neophobia (e.g., acute 5-min exposure to the unfamiliar blue marble in the novel object test) and predator avoidance (acute 5-min exposure to a predator fish). Note that zebrafish avoid a blue marble at the bottom of the tank (swim traces in the open field test recorded by Noldus Ethovision XT8 from the top view), as well as display robust aversion of a live predator (e.g., Leaf fish), clearly spending more time in the opposite side of the tank.

Panel D demonstrates zebrafish reward-related addiction behaviors (in the conditioned place preference model, CPP) and molecular alterations in zebrafish brain following exposure to a substance of abuse. In the CPP, fish are initially tested for their preference for both sides of the apparatus, each of which contains a discriminative stimulus (i.e., spots vs. no spots). They are then conditioned with drug to the least-preferred side over a number of sessions. Finally, their preference is assessed again in a probe trial, where the rewarding value of the drug can be ascertained by calculating the change in preference for the least-preferred side following conditioning (in addition to water immersion, also note the use of i.p. drug injections in some zebrafish CPP studies[184, 190]). The bottom right bar diagram also shows a classic dose-response curve for nicotine in adult fish (also note that mutant heterozygous for a nonsense mutation for the acetylcholinesterase gene (*ache*^{sb55}/+) do not show place preference for 6 μM nicotine; Brock et al., unpublished observations). Top right bar diagram shows fish that display persistent compulsive drug seeking (i.e., drug seeking despite adverse consequences/punishment) following chronic exposure to ethanol or nicotine, also demonstrating long-lasting drug-specific and conserved changes in gene expression[197] (brain genes' microarray, inset).

Panel E shows the reduction in zebrafish social behavior (e.g., shorter distance between a test fish and moving shoaling images delivered on a computer screen; inset). This reduction in hoaling response is abolished by acute administration of a high concentration of alcohol, which was inactive in fish chronically pre-treated with alcohol. Withdrawal from alcohol chronic exposure also abolishes the shoaling response. Note that alcohol-induced behavioral changes in zebrafish are closely paralleled by alterations in DA levels quantified from whole brain samples using HPLC (modified from [203, 230]).

Table 1

Examples of a diverse group of psychiatric disorders (Fig. 2) which can be modeled in zebrafish (see [135] for a comprehensive catalog of zebrafish normal and pathological behaviors).

Selected disorders	Zebrafish phenotypes	References
Attention deficit hyperactivity disorder (ADHD)	Impulsive swimming with hyperactive locomotion and impulsivity-like bursts of accelerated swimming, which can be reduced by anti-ADHD drugs (Fig. 2A)	125, 126, 217, 218, 231
Aggression	Social deficits (disrupted shoaling behavior), increased aggressive behaviors (follows, chases and biting), mirror-induced aggression, MIA (Fig. 2B)	135, 139, 161
Post-traumatic stress disorder (PTSD)	Reduced exploration following stress, increased avoidance (e.g., neophobia; Fig. 2C), erratic behavior and freezing, elevated cortisol and brain *c-fos* (all highly sensitive to a wide range of anxiolytic and anxiogenic agents), social deficits (e.g., disrupted shoaling behavior)	144, 146, 147, 232
Substance abuse, addiction (reward-related behavior)	Robust conditioned preference for commonly abused substances, compulsive drug seeking (i.e., despite adverse consequences; Fig 2D), withdrawal syndrome, stimulus-induced relapse following extinction, altered social behavior (Fig. 2E)	197, 198, 201, 202, 205, 233–235