Published in final edited form as:
Neurobiol Dis. 2010 October ; 40(1): 66–72. doi:10.1016/j.nbd.2010.05.016.

Use of zebrafish as a model to understand mechanisms of addiction and complex neurobehavioral phenotypes

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Abstract

Despite massive research efforts the exact pathogenesis and pathophysiology of addiction and neuropsychiatric disorders such as anxiety, schizophrenia and autism remains largely unknown. Animal models can serve as tools to understand the etiology and pathogenesis of these disorders. In recent years researchers are turning to zebrafish as it allows easy access to all developmental stages and imaging of pathological processes as well as automated behavioral quantification coupled with large scale screening and mutagenesis strategies. This review summarizes studies conducted over the last few years which demonstrate the relevance of the zebrafish model to human diseases including addiction and neuropsychiatric disorders.

Keywords

Zebrafish; addiction; anxiety; schizophrenia; autism

Introduction

Neuropsychiatric disorders have a relatively high prevalence in modern society and can affect individuals at all life stages. They often have severely disabling symptoms and represent a huge burden on individuals, families and society as a whole (Kessler et al., 2005). Neuropsychiatric disorders are genetically complex and there are no validated biological markers for distinguishing and characterizing the different disorders (Hyman, 2008). Despite immense research the precise risk factors and pathogenesis of neuropsychiatric disorders are still unknown. Almost no new therapeutic mechanisms or new drug targets have been identified in the last few decades (Nestler, 2009).

Human experimental neurobiology is mostly limited to non-invasive and indirect methods of investigation. An animal model gives us the opportunity to study how genetic and environmental factors can lead to the neuropsychiatric disorder by allowing us to manipulate molecules and confirm their role in the disease process.

The most commonly used animal models are small mammals (rats and mice). Although they have several advantages, these models are expensive to maintain and use in large-scale genetic or chemical screens and they are not readily accessible for studies at the embryonic

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stages. The small body size, large brood size, external development, and optical transparency of zebrafish allows us to overcome these limitations of mammalian models and provides a powerful tool kit, which includes large-scale genome mutagenesis and gene mapping, transgenesis, protein overexpression or knockdown, cell transplantation and chimeric embryo analysis, and chemical screens (Veldman and Lin, 2008), making this organism particularly well-suited to the molecular genetic analysis of vertebrate neurodevelopmental mechanisms relevant to neuropsychiatric disorders. It is possible to visualize complete neurotransmitter systems in the whole zebrafish brain at 5 days post fertilization when the fish already displays a sophisticated repertoire of behaviors (Panula et al., 2006). Magnetic Resonance imaging (MRI) on living zebrafish embryos for molecular screening or molecular tracking over time (Canaple et al., 2008) and Magnetic Resonance Histology (MRH) of the zebrafish brain have also been carried out (Ullmann et al., 2009). Therefore, the relative simplicity of the nervous system in fish larvae facilitate circuit analysis (Burgess and Granato, 2007).

An additional round of genome duplication occurred early in the evolution of teleosts but not all duplicated genes were retained by zebrafish. Current estimates for zebrafish in Zv8 zebrafish genome database is 24,000 protein coding genes, only about 20% more than human. While this can lead to uncovering redundant, species-specific information, duplicate genes can also be advantageous in studies of sub-functionalisation, since zebrafish co-orthologs represent selected expression patterns and developmental functions of mouse orthologs (Bergner, 2009; Key and Devine, 2003)

Zebrafish show similarity to humans in terms of the structure of the nervous system, which includes a fore-, mid-and hind-brain, including diencephalon, telencephalon (Mueller and Wullimann, 2009) and cerebellum and a peripheral nervous system with motor and sensory components, enteric and autonomic nervous systems. However, the telencephalon has only a rudimentary cortex. Although simplified compared to human behaviors, zebrafish do exhibit higher behaviors and integrated neural functions including memory, conditioned responses and social behaviors (for example, schooling) (Lieschke, 2007).

In the following sections we highlight various neuropsychiatric disorders including addiction, anxiety and depression, autism and schizophrenia, and discuss current efforts toward creating zebrafish models for these disorders (Tables 1 and 2). These disorders are covered in this review because of our interests as well as the presence of available data in zebrafish. As always, it is worth noting that not a single model is able to recapitulate all aspects of the complex human conditions.

Use of zebrafish to model addiction

The zebrafish model has been widely used to better understand the complex processes involved in the development of addiction. We (Guo, 2009) and others have previously reviewed studies of the psycho-stimulatory (Bretaud et al., 2007; Darland and Dowling, 2001; Gerlai et al., 2000; Lau et al., 2006; Lockwood et al., 2004; Ninkovic et al., 2006) and reinforcing (Bretaud et al., 2007; Darland and Dowling, 2001; Lau et al., 2006; Ninkovic et al., 2006) effects of alcohol and drugs of abuse in zebrafish.

Numerous studies in recent years have focused upon modeling alcohol addiction in zebrafish and the findings have striking parallels with the effect of ethanol in mammals. Acute alcohol exposure has been shown to result in significant changes in dopamine, serotonin and related metabolites in adult zebrafish brain (Chatterjee and Gerlai, 2009). Different strains of zebrafish have been found to show differences in response to a zebrafish shoal and predator while undergoing alcohol withdrawal and this is correlated with differences in neurochemical (including dopamine) responses (Gerlai et al., 2009). Withdrawal after

chronic exposure to ethanol in adult zebrafish has been profiled in terms of freezing bouts and erratic movements in the novel tank diving test and correlated with changes in whole body cortisol (Cachat et al., 2009).

In addition to alcohol, the effects of drugs of abuse including cocaine, amphetamine, and morphine have also been studied in zebrafish. Withdrawal symptoms following euphoria are part of the cycle of drug abuse for drugs like cocaine. In zebrafish, cocaine withdrawal produces an anxiety-like state which develops earlier in the female but is more robust and persistent in males and is accompanied by a decrease in dopamine transporter and an increase in dopamine levels (Lopez Patino et al., 2008b). Thus the zebrafish provides new opportunities to study genetic and endocrine gender-related factors involved in cocaine withdrawal symptoms. Studies in zebrafish have also shown that exposure to drugs such as morphine during development changes the expression level and localization of nociceptin receptors indicating that nociceptin (NOP) plays a role in development of dependence/addiction to drugs (Macho Sanchez-Simon and Rodriguez, 2009). Recently the mu-opioid receptor from zebrafish was shown to have a pharmacological profile similar to that of mammalian mu-opioid receptor (de Velasco et al., 2009). Additionally, the effects of a potent psychoactive drug Salvinorin-A have been documented in zebrafish. Salvinorin-A produces rewarding effects independently of its effects on zebrafish locomotor activity and these effects are mediated by kappa-opioid and cannabinoid receptors (Braida et al., 2007).

The above-mentioned studies have established a similarity between zebrafish and mammals in terms of their behavioral responses to alcohol and drugs of abuse and possibly also the underlying neural substrates. However, in order to exploit the strength of zebrafish to understand the underlying molecular and cellular mechanisms, assays amenable to high throughput screening need to be developed to reveal novel molecular and cellular insights.

Recent studies (Petzold et al., 2009) using larval zebrafish address the question of how genetic variation accounts for differential predisposition to nicotine dependence. Four-day old larval zebrafish show increased locomotor activity in response to a water stimulus and pretreatment with nicotine (2.5–50 μM) results in significantly greater locomotor activation. Zebrafish display sensitization due to prior nicotine exposure and the nicotine response is blocked by nicotinic receptor antagonists. This nicotine behavioral assay has been coupled with zebrafish forward genetic screens using gene-breaking transposon mutagenesis to identify mutants with defects in Chaperonin Containing Protein 8 (CCT8) and GABA-B receptor Subunit 1. These genes provide candidates for human association studies of predisposition to nicotine addiction.

The ethanol-modulated camouflage response of zebrafish, which is subjected to regulation by a variety of neurotransmitters and neuropeptides, has been used as a screening assay for understanding the biological effect of ethanol. A mutant which displays reduced camouflage response as well as behavioral sensitivity to ethanol has been identified to disrupt the evolutionarily conserved *adenylyl cyclase 5* (*ac5*) gene (Peng et al., 2009). The reduced sensitivity of the mutant to the stimulatory effects of ethanol can be mimicked in wildtype zebrafish by partial inhibition of phosphorylation of Extracellular regulated Kinase (ERK). These studies demonstrate critical roles of AC5 and ERK signaling in behavioral sensitivity to ethanol. It will be of importance to determine whether polymorphisms in the human counterparts of these genes may predispose individuals to alcoholism.

Combining a zebrafish forward genetic screen with CPP and micro-array analysis lead to the discovery of the “no-addiction” or nad mutant which does not exhibit preference for the compartment paired with amphetamine (Cadet, 2009; Webb et al., 2009). Many of the genes involved in reward pathways and brain development and associated with neurogenic zones

of the adult brain responded inappropriately to amphetamine in the nad mutant, indicating that drugs of abuse can hijack the brain s natural reward system. In addition, the transcription factors identified in this study can be used to study the link between neurogenesis and addiction (Cadet, 2009; Webb et al., 2009).

Microarray analysis has been coupled with the Conditioned Place Preference (CPP) assay in adult zebrafish to study whether prolonged nicotine/ethanol exposure in zebrafish results in changes in behavior as well as changes in gene expression at the level of the brain (Kily et al., 2008). Using zebrafish as a model system, candidate molecules and pathways that underlie neuro-adaptation to both ethanol and nicotine were identified and these include glutamate receptors, benzodiazepine receptors and molecules associated with synaptic plasticity (Kily et al., 2008). It will be of importance to determine, using high throughput zebrafish technologies, if any genes and pathways discovered in the profiling study are causally related to the change of behavior.

Taken together, zebrafish show great promise as a model for understanding the molecular and cellular mechanisms underlying behavioral responses to addictive substances. Continued exploitation of this model organism shall provide valuable insights into addiction.

Use of zebrafish to study Autism

Just like with any animal model for autism spectrum disorder (ASD), the repertoire of zebrafish behaviors is limited and cannot recapitulate all aspects of human behaviors that are impaired in autism. However, zebrafish are a useful tool to study the *in vivo* function of genes implicated in autism, since the larvae develop externally and rapidly, large numbers of larvae can be generated and used for molecular and genetic screens and the larvae are transparent enabling observations of brain development in living embryos at single cell resolution. Indeed, reviews discussing the potential of the zebrafish system for modeling autism (Tropepe and Sive, 2003), the zebrafish homologs of genes implicated in autism and assays developed to measure social interaction in zebrafish (Guo, 2009) have been published previously. Zebrafish behavioral assays that could be used to study social behaviors affected in autism include so-called “measures of personality” including social distance, activity in open field under social isolation and distance from predator (Dadda et al., 2010); novelty induced responses (Blaser and Gerlai, 2006); inhibitory avoidance (Blank et al., 2009); social interaction (Delaney et al., 2002); Courtship behavior (Colman et al., 2009) and dominant–subordinate relationships (Larson et al., 2006). Unvarying, repetitive behavioral patterns with no obvious function (stereotypy) (Lopez-Patino et al., 2008a) and comorbid conditions in autism including aggression (Colman et al., 2009) and seizure disorders (Hortopan et al.) have been modeled in zebrafish.

Roughly one percent of cases of autism are associated with deletions within a single region of chromosome 16, which contains nearly 30 genes (Eichler and Zimmerman, 2008). Of these 30 genes, at least 25 have clear homologs in zebrafish (sfari.org). Using morpholino antisense oligonucleotides (Nasevicius and Ekker, 2000), which inhibit maturation of the mRNA corresponding to each gene, it may be possible to identify genes required for the formation of normal brain structure or neurons, and assess interactions between genes. Recently, zebrafish morpholino knockdown experiments provided the first insight into the important physiological roles of Sushi domain-containing protein 4 (SUSD4), which is deleted in patients with autism. SUSD4 is highly expressed in the central nervous system (CNS) in different species including human, mouse and zebrafish, and plays an essential role in zebrafish development (Tu et al., 2010). However, high-resolution analyses of the neuronal phenotypes were not carried out in the study.

Mutations in Neuroligin 3 and Neuroligin 4 genes in humans have been linked to mental retardation and autism (Talebizadeh et al., 2006) and neuroligins along with neurexins are involved in synaptic function and maturation (Boucard et al., 2005). Studies in zebrafish (Rissone et al., 2007; Rissone et al., 2010) have revealed that seven genes in the zebrafish neuroligin family are very similar to their human homologs suggesting that these genes are subjected to a very strong evolutionary pressure to preserve their function. Neuroligins are expressed throughout the nervous system of the zebrafish and this model system provides an excellent opportunity to further explore the role of neurexins and neuroligins in the development of ASD.

Changes in cerebellar structure, and disrupted cerebellar gene expression has also been associated with ASD (Fatemi et al., 2008). The role of the Met (proto-oncogene associated with cellular metastatic cancer)/HGF (hepatocyte growth factor) signaling pathway in cerebellar development has been studied in zebrafish to understand the developmental basis of autism (Elsen et al., 2009). These studies in zebrafish revealed that Met signaling is critical for cerebellar morphogenesis, including normal growth and cell type specification, and plays an important role in hindbrain cell migration. These recent studies demonstrate that zebrafish is a viable model system for future exploration of the underlying molecular and cellular mechanisms of autism.

Use of zebrafish to study Fragile X Syndrome

Fragile X Syndrome (FraX) is a leading heritable cause of mental retardation that results from the loss of FMR1 (fragile X mental retardation) gene function. The zebrafish embryo has been established as a model for fmr1 loss-of-function analysis using a morpholino antisense oligonucleotide approach (Tucker et al., 2006). The zebrafish has genes orthologous to the human FMR1 gene family as well as fmr1 interacting proteins and can be used to examine phenotypes that are difficult or inaccessible to observation in other model organisms. Treatment with mGluR antagonist MPEP [2-methyl-6-(phenylethynyl)-pyridine] rescues the neurophysiological abnormalities and alleviates craniofacial defects associated with the loss of zebrafish Fmr1 and a model has been proposed for the interaction of Fmr1 and mGluR in control of axonal branching, guidance and fasciculation that has implications for the synaptic morphology component of fragile X syndrome (Tucker et al., 2006).

In support of this model loss-of-function transgenic zebrafish exhibiting abnormal neuron morphology and connectivity similar to those in human FraX have been generated using manmade miRNA transgenes directed against the fish fmr1 gene (Lin et al., 2006).

With the advance of such a miRNA-mediated loss of-fmr1-function zebrafish model, it will be possible to investigate the molecular, pathological and neurobehavioral changes that are common in human patients. Recently this approach has been extended with the generation of two independent fmr1 knockout alleles in the zebrafish using TILLING (targeted induced local lesions in genomes) (den Broeder et al., 2009). Surprisingly, these knockout zebrafish (with no detectable FMR1 protein) did not display any phenotype, which was in contrast to that of the morphant reported in the previous study. It remains to be resolved whether the difference is due to the morpholino s off-target effect (making the morphant phenotype a potential artifact), the morpholino s ability to target multiple *fmr1*-like genes (while the knockout only targeted a single gene), or alterations in the genetic background used in each study.

Use of zebrafish to study Schizophrenia

Schizophrenia is a devastating disorder caused by both genetic and environmental factors that disrupt brain development and function. It is distinguished as a neurodevelopmental disorder in part due to early cognitive impairments, behavioral dysfunction in childhood and adolescence, and abnormalities in central nervous system development with no neurodegenerative component (Lewis and Levitt, 2002). The zebrafish model system provides an opportunity to study both the genetic and developmental basis of schizophrenia as well as various pathological processes affecting neurogenesis, cell-fate determination and neuronal migration (Morris, 2009).

Neuropsychiatric disorders including schizophrenia are associated with disturbances in pre-pulse inhibition (PPI), which affects the individual s ability to filter out extraneous information from the environment (Braff et al., 2001). Larval zebrafish have been found to exhibit PPI which modulate the acoustic startle response in a manner similar to mammalian PPI. Larval PPI is disrupted by dopamine agonists and this disruption can be corrected by antipsychotic drugs similar to the mammalian situation. A zebrafish screen has isolated the *Ophelia* mutant, which has reduced PPI (Burgess and Granato, 2007) and further high-throughput zebrafish screens could help understand the genetic basis for defects in PPI observed in schizophrenia.

A study of the *in-vitro* effects of anti-psychotic drugs on zebrafish brain membranes has revealed that these drugs alter ectonucleotidase activity which can in turn modulate adenosine levels and affect the pathophysiology of schizophrenia (Seibt et al., 2009). Another study employing both *in-vitro* and *in-vivo* studies in zebrafish found that antipsychotic drugs produce alterations in acetylcholinesterase activity and this could reveal molecular mechanisms related to cholinergic signaling in schizophrenia (Seibt et al., 2009).

Disrupted in schizophrenia 1 (*disc1*), a well-documented schizophrenia-susceptibility gene has recently been characterized in zebrafish cranial neural crest (CNC). Disc1 is a potent regulator of *sox10* which is an oligodendrocyte related schizophrenia susceptibility gene. Understanding the basic functions of Disc1 in transcriptional regulation, cell migration and cell differentiation in the zebrafish neural crest might give insight into the cellular processes that, when disrupted, predispose individuals to mental illness (Drerup et al., 2009).

Other recent findings in the zebrafish embryo reveal neurodevelopmental connections that may exist between key candidate schizophrenia susceptibility genes like DISC1 (Drerup et al., 2009), NudE-Like (NDEL1/NUDEL) (Drerup et al., 2007), NRG1 (Wood et al., 2009), OLIG2 and ERBB4 and suggest that disc1 and nrg1 function in common or related pathways controlling development of oligodendrocytes and neurons from olig2-expressing precursor cells (Wood et al., 2009). Future studies will continue to exploit the potential of zebrafish to elucidate further the roles of Disc1 and Neuregulin-ErbB signaling pathways in early brain development and to generate new experimental systems in which neurodevelopmental mechanisms and genetic pathways of relevance to schizophrenia can be dissected.

R1117X, a mutation in the gene encoding the synaptic protein SHANK3 identified in families of schizophrenia patients has been validated in zebrafish. Knockdown of zSHANK3 orthologous genes in zebrafish produced behavioral and differentiation defects (Gauthier et al., 2010).

Use of zebrafish to study Stress, Fear, and Anxiety

Anxiety disorders and phobias represent a large unmet medical need in the human population (Garner, 2008). Zebrafish have a single glucocorticoid and mineralocorticoid receptor which have been cloned and sequenced and the zebrafish corticoid signaling pathway which is involved in stress is similar to that of mammals (Alsop and Vijayan, 2008), (Denver, 2009).

At the behavioral level, fear reactions such as jumping, erratic movements and increased shoal cohesion can be elicited in adult zebrafish by exposing them to alarm substance extracted from the skin of zebrafish (Waldman, 1982), (Speedie and Gerlai, 2008) and also by synthetic substances such as Hypoxanthine 3-N Oxide (Parra et al., 2009). This zebrafish alarm response provides the opportunity to analyze fear at multiple levels from genes to circuits (Jesuthasan and Mathuru, 2008). Combining this assay with a genetic screen could allow the discovery of mutations leading to enhanced or reduced fear and could also be used to screen chemicals which alter the fear response.

Zebrafish have been observed to exhibit anti-predatory responses including erratic movements and freezing when exposed to animated images or live Indian leaf fish on the basis of visual cues alone. These responses can be quantified by automated methods (Gerlai, 2010). Both direct and visual contact with predators has been shown to increase whole body cortisol in zebrafish (Barcellos et al., 2007).

When exposed to novelty (new tank) zebrafish dive to the bottom and do not initially explore the environment. In the wild this response could help zebrafish escape from predators. The Novel tank diving response (Bencan and Levin, 2008) has been developed in recent years to characterize the fear/anxiety response in zebrafish (Bencan et al., 2009; Bergner, 2009). Fish spending more time at the bottom of the tank and freezing or swimming slowly or exhibiting increased erratic movements are considered to be fearful or anxious. Pre-treatment with anxiolytic substances like nicotine decreases bottom-dwelling (Levin et al., 2007). Acute exposure of adult zebrafish to substances like ethanol, nicotine, fluoxetine or diazepam results in anxiolytic effects in this assay (Bencan and Levin, 2008; Bencan et al., 2009; Egan et al., 2009), while exposure to alarm substance or caffeine had anxiogenic effects. Chronic ethanol and fluoxetine treatment improve habituation to novelty in zebrafish while anxiogenic agents like pentylenetetrazole and alarm pheromone attenuate habituation (Wong et al., 2010). Thus, zebrafish novel tank diving response can serve as an inexpensive, high-throughput model for screening anxiolytic drugs.

When given a choice between Light and Dark compartments adult zebrafish show a significant preference for the dark and avoidance of the light compartment. (Maximino et al., 2010b; Serra et al., 1999). This response is only seen if the tanks are open at the top and only the walls and floor are light/dark. Those zebrafish which show higher avoidance of the light compartment display a characteristic freezing response if confined to the light side and this response may be a reliable behavioral measure of anxiety (Blaser et al., 2010). Avoidance of the light compartment did not show intra-or intersession habituation (Maximino et al., 2010a). The light/dark preference task in zebrafish can be used to investigate anxiety-related behavior in a simple, high throughput manner (Maximino et al., 2010b).

Concluding remarks

Similar to humans, zebrafish (both larvae and adult) gather information about the environment by means of specialized sensory organs including eye, olfactory system and ear (zebrafish possess an additional sensory organ the lateral line). This information is processed

by the nervous system and can result in a large repertoire of behaviors. To date zebrafish, in particular larval zebrafish that are especially amenable for screening and cellular level analysis, have been successfully used as a model system to elucidate genetic (Table 2), molecular and behavioral (Table 1) mechanisms underlying several neuropsychiatric disorders including addiction (Cachat et al., 2009;Cadet, 2009), aggression (Larson et al., 2006), depression (Roger et al., 2010), anxiety (Bencan et al., 2009;Blaser et al., 2010), schizophrenia (Wood et al., 2009) and autism (Elsen et al., 2009). Zebrafish possess homologs of several genes implicated in these disorders (Renier et al., 2007). Zebrafish are ideal organisms for translational research as they can be used to study mechanisms at the genetic, cellular and developmental level (Bergner, 2009). Improved behavioral phenotyping and development of mutant and transgenic lines of zebrafish will allow researchers to model these neuropsychiatric disorders with greater accuracy (Bergner, 2009). A High-throughput, behavior-based approach to neuroactive small molecule discovery is uniquely possible in zebrafish (Kokel et al., 2010) and behavioral profiling using zebrafish has revealed conserved functions of psychotropic molecules and predicted the mechanisms of action of poorly characterized compounds (Rihel et al., 2010).

Human neuropsychiatric disorders can involve several genes and symptom clusters and a particular mutant zebrafish strain may represent only a sub-population of individuals diagnosed with the disorder. Any potential treatment may need to be tested on several strains of zebrafish using several different behavioral assays.

All these findings suggest that our understanding of the genetic, cellular and developmental mechanisms underlying addiction and neuropsychiatric disorders can be vastly improved by using zebrafish as a genetically tractable model system.

Acknowledgments

The research in our laboratory is supported by grants from the National Institutes of Health.

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Table 1

ZF disease models

Neuropsychiatric disorder	Example of ZF model/assay	Phenotype and/or studies of disease pathogenesis	References
Addiction			
Alcohol dependence	Adult ZF brain neurotransmitter levels	Increased dopamine and serotonin levels	Gerlai, Chatterjee et al. 2009
	Microarray analysis of brain samples	Changes in gene expression following conditioning	Kily, Cowe et al. 2008
	Adult ZF brain AchE	Increased AchE levels	Rico, Rosenberg et al. 2007
	Video recording of locomotor activity in adult ZF	Increased swim speed, Increased time in upper zone of novel tank	Gerlai, Lahav et al. 2000
	fan mutant	Reduced melanocyte response to ethanol	Peng, Wagle et al. 2009
	Video recording of locomotor activity in larval ZF	Retarded transition in activity from dark to light	MacPhail, Brooks et al. 2009
	Video recording of locomotor activity in larval ZF	Increased swim speed, thigmotaxis, melanocyte dispersion	Lockwood, Bjerke et al. 2000
Alcohol withdrawal	Novel tank diving test	Freezing bouts and erratic movements	Cachat, Canavello et al. 2009
Cocaine dependence	*dum, gts, jpy* mutants	Reduced cocaine-induced CPP	Darland, T. & Dowling, J. E. 2001
Cocaine withdrawal	Video recording of locomotor activity in adult ZF	Hyperactivity	Lopez Patino, Yu et al. 2008
Morphine dependence	Nociceptin receptor expression in embryos	Decreased nociceptin receptor expression	Macho Sanchez-Simon and Rodriguez 2009
	tof mutant	Reduced morphine-induced CPP	Lau, Bretaud et al. 2006, Bretaud, Li et al 2007
Morphine withdrawal	Novel tank diving test	Freezing bouts and erratic movements	Cachat, Canavello et al. 2009
Amphetamine dependence	*nad* mutant	Reduced amphetamine-induced CPP	Webb, Norton et al. 2009
	ache/+ mutant	Reduced amphetamine-induced CPP	Ninkovic, Folchert et al. 2006
Nicotine dependence	*bdav/cct8* and *hbog/gabbr1.2*	Altered locomotor sensitization to nicotine	Petzold, Balciunas et al. 2009
	Microarray analysis of brain samples	Changes in gene expression following conditioning	Kily, Cowe et al. 2008
Diazepam withdrawal	Novel tank diving test	Freezing bouts and erratic movements	Cachat, Canavello et al. 2009
Salvinorin A dependence	Locomotor activity and CPP	Preference for Salvinorin compartment	Braida, Limonta et al. 2007
Autism	MO knockdown of *SUSD4*	Developmental abnormalities	Tu, Cohen et al. 2010
	MO knockdown of *Met/HGF*	Altered Cerebeller growth and cell type specification	Elsen, Choi et al. 2009
Fragile X Syndrome	fmr1 knockout using TILLING	No obvious phenotype	den Broeder, van der Linde et al. 2009
	MO knockdown of FMR-1	Abnormal axonal branching and craniofacial abnormalities	Tucker, Richards et al. 2006
	miRNA transgenes against fmr1 gene	Abnormal neuronal morphology and connectivity	Lin, Chang et al. 2006

Neuropsychiatric disorder	Example of ZF model/assay	Phenotype and/or studies of disease pathogenesis	References
Schizophrenia	Knockdown of disc-1	Altered cranial neural crest migration and differentiation	Drerup, Wiora et al. 2009
	Knockdown of nrg-1	Loss of olig2-positive cerebellar neurones	Wood, Bonath et al. 2009
	ophelia mutant	Reduced Prepulse inhibition	Burgess and Granao 2007
Fear/Anxiety	Light/dark preference task	Avoid light compartment	Maximino, Marques de Brito et al. 2010
	Expose adult ZF to predators or images of predators	Erratic movements and freezing	Gerlai 2010
	Expose adult ZF to alarm substance	Jumping, erratic movements, increased shoal cohesion	Speedie and Gerlai 2008
	Expose adult ZF to Hypoxanthine 3-N oxide	Increased jumping and erratic movements	Parra, Adrian et al. 2009
	Direct and indirect contact with predators	Increased whole body cortisol	Barcellos, Ritter et al. 2007
	Expose adult ZF to novel tank	Increased bottom-dwelling	Levin, Bencan et al. 2007
Aggression	Pair-house male ZF after separation	Chases and bites by dominant male and retreats by subordinate male	Larson, O'Malley et al. 2006

ZF = zebrafish, AchE = acetylcholinesterase, MO = morpholino, CPP = conditioned place preference, TILLING = targeted induced local lesions in genomes

Table 2

Examples of neuropsychiatric disease-related genes studied in ZF

Disease	Gene	Technique used	Reference
Drug dependency (ethanol)	*fan*	ENU mutagenesis, cloning	Peng, Wagle et al. 2009
Drug dependency (amphetamine)	*calcineurin B*	microarray analysis	Kily, Cowe et al. 2008
Autism	*SUSD4*	MO knockdown	Tu, Cohen et al. 2010
	neuroligin	studied expression patterns	Rissone, A., L. Sangiorgio, et al. 2010
	neurexin	studied ZF homologs	Rissone, A., M. Monopoli, et al. 2007
	Met/HGF	MO knockdown	Elsen, Choi et al. 2009
Fragile X Syndrome	*FMR-1*	TILLING	den Broeder, van der Linde et al. 2009
		MO knockdown	Tucker, Richards et al. 2006
		miRNA transgenes	Lin, Chang et al. 2006
Schizophrenia	*disc-1*	MO knockdown	Drerup, Wiora et al. 2009
	nrg1	MO knockdown	Wood, Bonath et al. 2009
	ndel1a, ndel1b	studied expression patterns	Drerup, C. M., S. C. Ahlgren, et al. 2007
	ophelia	mutagenesis	Burgess and Granato 2007

ZF = zebrafish, MO = morpholino, TILLING = targeted induced local lesions in genomes