

7-1-2017

Zebrafish Models in NeuroPsychopharmacology and CNS Drug Discovery

Kanza M. Khan

University of Southern Mississippi, kanza.khan@usm.edu

Adam D. Collier

University of Southern Mississippi, adam.d.collier@eagles.usm.edu

Darya A. Meshalkina

St. Petersburg State University

Elana V. Kysil

St. Petersburg State University

Sergey L. Khatsko

Ural Federal University

See next page for additional authors

Follow this and additional works at: https://aquila.usm.edu/fac_pubs



Part of the [Pharmacology, Toxicology and Environmental Health Commons](#)

Recommended Citation

Khan, K. M., Collier, A. D., Meshalkina, D. A., Kysil, E. V., Khatsko, S. L., Kolesnikova, T., Morzherin, Y. Y., Warnick, J. E., Kalueff, A. V., Echevarria, D. (2017). Zebrafish Models in NeuroPsychopharmacology and CNS Drug Discovery. *British Journal of Pharmacology*, 174(13), 1925-1944.
Available at: https://aquila.usm.edu/fac_pubs/14989

This Article is brought to you for free and open access by The Aquila Digital Community. It has been accepted for inclusion in Faculty Publications by an authorized administrator of The Aquila Digital Community. For more information, please contact Joshua.Cromwell@usm.edu.

Authors

Kanza M. Khan, Adam D. Collier, Darya A. Meshalkina, Elana V. Kysil, Sergey L. Khatsko, Tatyana Kolesnikova, Yury Yu Morzherin, Jason E. Warnick, Allan V. Kalueff, and David Echevarria

**Zebrafish models in neuropsychopharmacology
and CNS drug discovery**

Kanza M. Khan^a, Adam D. Collier^{a,b}, Darya A. Meshalkina^{b,c}, Elana V. Kysil^c,
Sergey L. Khatsko^d, Tatyana Kolesnikova^d, Yury Yu. Morzherin^d, Jason E. Warnick^{b,e},
Allan V. Kalueff^{b,c,d,f*}, and David J. Echevarria^{a,b*}

^aDepartment of Psychology, University of Southern Mississippi, Hattiesburg, MS, USA;

^bThe International Zebrafish Neuroscience Research Consortium (ZNRC), Slidell, LA, USA;

^cInstitute of Translational Biomedicine, St. Petersburg State University, St. Petersburg, Russia;

^dUral Federal University, Ekaterinburg, Russia;

^eDepartment of Behavioral Sciences, Arkansas Tech University, Russellville, AR, USA;

^fResearch Institute of Marine Drugs and Nutrition, College of Food Science and Technology, Guangdong Ocean University, Zhanjiang, Guangdong, China

*Co-corresponding authors:

Dr. Allan V. Kalueff, PhD, Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg, Russia

David J. Echevarria, PhD, Department of Psychology, University of Southern Mississippi, Hattiesburg, MS, USA

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bph.13754

Abstract

Despite high prevalence of neuropsychiatric disorders, their etiology and molecular mechanisms remain poorly understood. The zebrafish (*Danio rerio*) is increasingly utilized as a powerful animal model in neuropharmacology research and in-vivo drug screening. Collectively, this makes zebrafish a useful tool for drug discovery and the identification of disordered molecular pathways. Here, we discuss zebrafish models of selected human neuropsychiatric disorders and drug-induced phenotypes. Covering a broad range of brain disorders (from anxiety and psychoses to neurodegeneration), we also summarize recent developments in zebrafish genetics and small molecule screening, which markedly enhance the disease modeling and the discovery of novel drug targets.

Keywords: Zebrafish; Behavioral models; Toxicology models; Genetic Models; Preclinical study; Model organism

TARGETS	
Other protein targets^a	Transporters^c
<u>NAC</u>	<u>Vmat2</u>
<u>PSEN1</u>	Enzymes^d
<u>PSEN2</u>	<u>AChE</u>
Nuclear hormone receptors^b	<u>COX-2</u>
<u>GR</u>	

LIGANDS	
<u>ACTH</u>	<u>APP</u>
<u>CRH</u>	<u>GABA</u>
<u>KA</u>	<u>MB</u>
<u>NMDA</u>	

These Tables of Links list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan et al., 2016), and are permanently archived in The Concise Guide to PHARMACOLOGY 2015/16 (Alexander et al., 2015).

Abbreviations:

AChE = acetylcholinesterase;
ACTH = adrenocorticotrophic hormone;
ALS = amyotrophic lateral sclerosis;
AD = Alzheimer's disease;
ApoE4 = apolipoprotein E ϵ 4;
APP = Amyloid beta A4 precursor protein;
BMAA = beta-methylamino-alanine;
CRH = corticotrophin-releasing hormone;
COX-2 = cyclooxygenase-2;
CPP = conditioned place preference;
dpf = days post fertilization;
GABA = gamma aminobutyric acid;
GR = glucocorticoid receptors;
HPA = hypothalamus-pituitary-adrenal;
HPI = hypothalamus-pituitary-interrenal;
HSR = heat shock stress response;
microCT = micro-computer tomography;
KA = kainic acid;
MB = methylene blue;
MO = morpholino-modified antisense oligonucleotide;
NAC = N-acetylcysteine;
NFT = neurofibrillary tangles;
NMDA = N-methyl-D-aspartate;
NMJ = neuromuscular junction;
OCT = optical coherence tomography;
PPI = pre-pulse inhibition;
PSEN1 = PRESENILIN1;
PSEN2 = PRESENILIN2;
PTSD = post-traumatic stress disorder;
PTZ = pentylenetetrazole;
RNAi = RNA interference;
SEA = Similarity Ensemble Approach;
SNRI = selective norepinephrine reuptake inhibitors;
SSRI = selective serotonin reuptake inhibitor;
UCMS = unpredictable chronic mild stressors;
Vmat2 = vesicular monoamine transporter 2;
WGD = whole-genome duplication

1. Introduction: Zebrafish as an emerging animal model

Widespread and debilitating, neuropsychiatric disorders have poorly understood mechanisms and often lack efficient therapies (Garakani, Mathew & Charney, 2006; Griebel & Holmes, 2013b). Identifying clinically relevant biomarkers, the underlying neurobiological mechanisms, as well as genetic and environmental factors of psychopathology, are critical steps in discovering efficacious treatments (Caspi & Moffitt, 2006; Nestler, 2013). While rodent models of human brain disorders have long been employed in this effort, they are often impeded by high-cost and experimental inefficiency (Cryan & Holmes, 2005).

The zebrafish (*Danio rerio*) has recently garnered attention as a powerful animal model for a wide range of human brain disorders (Kalueff, Echevarria & Stewart, 2014b; Kalueff, Stewart & Gerlai, 2014; Stewart et al., 2015b). Zebrafish is a small, low-cost and genetically tractable aquatic vertebrate species with a high degree of morphological, physiological and genetic homology to humans (Kalueff, Echevarria & Stewart, 2014a; Kalueff, Stewart & Gerlai, 2014). The zebrafish genome, fully sequenced, shows orthologues corresponding to ~82% of disease-related genes in humans (Howe et al., 2013). Gene expression databases (e.g., <http://zfin.org/>) and atlases of zebrafish brain are also available to explore the genomics and neuroanatomy of brain areas associated with neuropsychiatric disorders (Mueller & Wullimann, 2015; Ullmann, Cowin, Kurniawan & Collin, 2010; Wulliman, Rupp & Reichert, 2012).

Modeling human conditions in zebrafish empowers the discovery of potential therapeutic targets and their underlying molecular interactions (Table 1). For example, analyzing the efficacy of various compounds to ameliorate the amyotrophic lateral sclerosis (ALS)-like phenotype, a recent study found therapeutic potential of methylene blue (MB) in a mutant mTDP-43 zebrafish (Vaccaro et al., 2012). Likewise, the mTDP-43 mutant zebrafish presents with short, abnormally branched motor axons, increased oxidative stress and

aberrant escape response (Vaccaro et al., 2012). Administration of MB, a neuroprotective agent, corrects swimming and axonal phenotypes while reducing the endoplasmic reticulum (ER) stress that occurs as a result of an accumulation of unfolded mutant proteins (Vaccaro et al., 2013; Vaccaro et al., 2012). The identification of ER stress as a potential target for ALS drug treatment prompted further testing the efficacy of several related agents in a G93A mtSOD1 transgenic mouse model, which led to the identification and repositioning of guanabenz, an approved drug for hypertension, as a potential new treatment for ALS (Vaccaro et al., 2013). Clearly, the zebrafish mutant model played a critical role in the identification of new ALS treatment options.

Another example of bringing laboratory findings to the bedside includes two modulators of hematopoietic stem cells (HSC) recently discovered in zebrafish (Zon, 2014) and then becoming therapies in patients (North et al., 2007). Original screening of nearly 2500 small molecules in zebrafish identified 35 ‘leads’ that up-regulate vital HSC genes, *runx1* and *c-myb*, ten of which modulate the prostaglandin pathway, implicating it in HSC regulation. One of these potent candidates, 16,16-dimethyl prostaglandin E2 (dmPGE2), was next tested in a mouse model, increasing the number of HSC grafted (North et al., 2007; Zon, 2014). Subsequent preclinical testing using primate blood model yielded successful results, allowing the drug to move to the approved Phase I clinical trial (Goessling et al., 2011). These studies have recently yielded positive results in leukemia patients and demonstrated the safety of the treatment, allowing it to move to Phase II testing (Cutler et al., 2013). Thus, the translatability of original zebrafish results was critical for the application of this drug in mice, and in humans (see other examples of translational approaches in Table 1).

Both larval and adult zebrafish are useful pre-clinical *in-vivo* models highly amenable to experimental, pharmacological and genetic manipulations (Barros, Alderton, Reynolds, Roach & Berghmans, 2008; Brennan, 2011; Bruni et al., 2016). Due to their transparency and

small size, larval zebrafish are particularly useful for optical manipulation and imaging of neural activity, as well as for large-scale high-throughput screens of molecular drug targets and candidate genes (Brennan, 2011; Stewart, Gerlai & Kalueff, 2015; Wyart & Del Bene, 2011). Together with recent developments in genome editing techniques (e.g., CRISPR/Cas) and automated 3D behavioral phenotyping, this makes zebrafish an ideal model to study genotype-phenotype and genotype-drug-phenotype relationships (Cachat et al., 2011b; Hwang et al., 2013; Kokel et al., 2010; Stewart et al., 2015a). Furthermore, zebrafish develop externally to the maternal organism, reach sexual maturity fast (in ~90 days), and live for ~4-5 years in the laboratory, allowing for direct and easy analyses of pathogenetic trajectories (Fonseka, Wen, Foster & Kennedy, 2016; Kalueff, Stewart & Gerlai, 2014). Complementing larval models, adult zebrafish exhibit complex behaviors (Kalueff et al., 2013a) relevant to cognition (Blaser & Vira, 2014; Gerlai, 2016), reward (Collier, Khan, Caramillo, Mohn & Echevarria, 2014; von Trotha, Vernier & Bally-Cuif, 2014), social behavior (Gerlai, 2014; Qin, Wong, Seguin & Gerlai, 2014) and affect (Gerlai, 2013; Jesuthasan, 2012; Wang et al., 2016a). Numerous experimental paradigms have been converted for aquatic models to investigate major behavioral phenotypes which are well-conserved in zebrafish and mammals (Renier et al., 2007; Stewart, Braubach, Spitsbergen, Gerlai & Kalueff, 2014a).

Rats and mice are currently the most commonly employed animals to study normal and abnormal brain functioning, with nearly 1/3 of all published neuroscience papers in 2015 utilizing rodent models, and <11% using other animal models, including zebrafish (Keifer & Summers, 2016). However, the rate of zebrafish publications is growing faster than any other model organisms, and experimental tools and resources for this organism are becoming increasingly available (Kalueff, Echevarria & Stewart, 2014b; Wyatt, Bartoszek & Yaksi, 2015). A new animal model that still requires validation across multiple domains, the zebrafish has a growing utility in high-throughput phenotyping, gene and drug screening, thus

becoming increasingly useful in neuropsychopharmacology and drug discovery research. Here, we highlight recent successes and challenges in this rapidly expanding field.

2. Zebrafish CNS

The overall architecture, neuroanatomical features and cellular morphology of the zebrafish CNS are generally similar to those of mammals (Kalueff, Stewart & Gerlai, 2014). For example, the medial teleost pallium contains homologous structures to the mammalian amygdala (Martín, Gómez, Salas, Puerto & Rodríguez, 2011; Mueller, Dong, Berberoglu & Guo, 2011; Portavella, Torres & Salas, 2004; von Trotha, Vernier & Bally-Cuif, 2014) – the brain structure key for affective processing and emotionality in humans. The amygdala is pathologically hyperactivated in clinical anxiety (Rauch et al., 2000; Shin, Rauch & Pitman, 2006), social anxiety disorder (Furmark et al., 2004; Stein, Goldin, Sareen, Zorrilla & Brown, 2002), and drug abuse (Buffalari & See, 2010; Mead, Vasilaki, Spyraiki, Duka & Stephens, 1999). The zebrafish medial pallium shows increased Fos protein expression, a measure of neuronal activation, following both acute administration of D-amphetamine and during drug-seeking behavior in a conditioned place preference (CPP) assay (von Trotha, Vernier & Bally-Cuif, 2014), collectively supporting the role of zebrafish medial pallium as a homologous structure to the mammalian amygdala with evolutionarily conserved functions in modulating key behaviors.

Visualizing CNS activity through imaging methods is an important step to discern how the brain contributes to normal and abnormal behavior. The small size, and optical transparency of larval zebrafish allows for high resolution *in-vivo* imaging and manipulation of neural activity in behaviorally active animals (Orger & Portugues, 2016). For example, imaging neuronal activity of larval zebrafish behavior has been achieved by expressing a genetically encoded calcium indicator and recording whole-brain activity using light-sheet microscopy (Ahrens, Orger, Robson, Li & Keller, 2013). Optogenetic neuromodulation of the

transparent and genetically accessible larval zebrafish is particularly useful to investigate the neural circuitry underlying behaviors relevant to brain disorders (Knafo & Wyart, 2015). Neuronal excitation and inhibition of targeted neuronal populations has been successfully triggered in behaving larval zebrafish by expressing optogenetic actuators, including channelrhodopsin-2 and halorhodopsin (Arrenberg, Del Bene & Baier, 2009; Douglass, Kraves, Deisseroth, Schier & Engert, 2008). To date, optogenetic studies in zebrafish have largely focused on several simpler behaviors, such as escape (Douglass, Kraves, Deisseroth, Schier & Engert, 2008) and locomotion (Arrenberg, Del Bene & Baier, 2009; Ljunggren, Haupt, Ausborn, Ampatzis & El Manira, 2014), as well as sensory processing (Kubo, Hablitzel, Dal Maschio, Driever, Baier & Arrenberg, 2014). However, optogenetic neuromodulation in zebrafish help future research to create robust models of complex human neuropsychiatric disorders (Stewart et al., 2015b; Tye & Deisseroth, 2012). Furthermore, imaging neural activity in adult zebrafish is more challenging due to their larger and opaque brains. Recently applied in adult zebrafish, contrast-enhanced X-ray micro-computer tomography (microCT) with iodine as a contrasting agent provided 3D visualization of zebrafish brain anatomy in intact animals (Babaei, Hong, Yeung, Cheng & Lam, 2016). Optical coherence tomography (OCT) has also recently been used *in-vivo* in adult zebrafish to non-invasively generate real-time cross-sectional images at high resolution that and then reconstructed in 3D (Rao, Alex, Verma, Thampi & Gupta, 2009; Zhang & Yuan, 2015).

Neurochemistry is generally conserved across vertebrate species, as they share major neurotransmitters, receptors and transporters (Alsop & Vijayan, 2008; Panula et al., 2010; Panula et al., 2006). Thus, zebrafish are sensitive to major classes of pharmacological agents, such as psychostimulants (Ninkovic & Bally-Cuif, 2006), opiates (Lau, Bretau, Huang, Lin & Guo, 2006), ethanol (Tran, Chatterjee & Gerlai, 2015), hallucinogens (Stewart, Cachat, Gaikwad, Robinson, Gebhardt & Kalueff, 2013), anxiolytics (Bencan, Sledge & Levin,

2009), antidepressants (Stewart et al., 2014) and antipsychotics (Bruni et al., 2016). The spatial and temporal distribution of major neurotransmitter systems in zebrafish is also similar to that of mammals, and has been well described in zebrafish for glutamate, gamma aminobutyric acid (GABA), acetylcholine, dopamine, serotonin, norepinephrine and histamine (Stewart et al., 2015b). For instance, the major pathways and receptor subtypes of the dopamine system are all present in zebrafish, with the exception of the D₅ receptor (Maximino & Herculano, 2010; Panula et al., 2010; Panula et al., 2006). The amino acid sequence recently compared between zebrafish and humans for D₁-D₄ receptors shows 100% amino acid homology in the binding site for D₁ and D₃, and 85-95% for D₂ and D₄ receptors (Ek et al., 2016). Consequently, pharmacological agents that act on the dopamine system produce similar phenotypes, as dopamine antagonists or depletors impair locomotion (Giacomini, Rose, Kobayashi & Guo, 2006; Kyzar et al., 2014) and agonists predictably increase zebrafish locomotion (Irons, Kelly, Hunter, Macphail & Padilla, 2013), paralleling similar effects in rodents (Akhisaroglu, Kurtuncu, Manev & Uz, 2005; Mobini, Chiang, Ho, Bradshaw & Szabadi, 2000). The dopamine agonist apomorphine produces a U-shaped dose-response relationship for distance traveled in larval zebrafish, with low-doses increasing time in center (anxiolytic effect) and high-doses increasing thigmotaxis (anxiogenic effect) (Ek et al., 2016). Strikingly paralleling similar drug action in rats (Ek et al., 2016), these findings further support the translational value of neuropharmacological studies in zebrafish.

Mounting evidence implicates alterations of the neuroendocrine system in various brain disorders, including depression (Herbert, 2013; Holsboer, 2001), anxiety (Hek et al., 2013; Korte, 2001), addiction (Keedwell, Poon, Papadopoulos, Marshall & Checkley, 2001; Lovallo, 2006) and Alzheimer's disease (AD) (Belanoff, Gross, Yager & Schatzberg, 2001; Wahbeh, Kishiyama, Zajdel & Oken, 2008). Activation of the neuroendocrine hypothalamus-pituitary-interrenal (HPI) axis of zebrafish releases cortisol that acts on glucocorticoid

receptors, similar to the hypothalamus-pituitary-adrenal (HPA) axis in humans (Alsop & Vijayan, 2009; Griffiths, Schoonheim, Ziv, Voelker, Baier & Gahtan, 2012b; Pavlidis, Theodoridi & Tsalafouta, 2015). The zebrafish neuroendocrine system can be easily modulated by experimental, pharmacological and genetic manipulations, and fish cortisol can be sampled using various invasive and non-invasive methods (Canavello et al., 2011; Félix, Faustino, Cabral & Oliveira, 2013; Pavlidis, Sundvik, Chen & Panula, 2011). For example, genetic mutation of the glucocorticoid receptors (GR) gene in adult gr^{s357} mutant zebrafish disrupts negative feedback and cortisol signaling by abolishing the transcriptional activity of GR upon cortisol binding (Ziv et al., 2013). This elevates blood cortisol levels and evokes aberrant phenotypes, such as freezing, reduced exploration, impaired habituation and potentiated startle, most of which can be rescued in gr^{s357} mutants by a selective serotonin reuptake inhibitor (SSRI) fluoxetine. This also emphasizes the high degree of evolutionary conservation between the neuroendocrine system and its modulation between zebrafish and humans (Griffiths, Schoonheim, Ziv, Voelker, Baier & Gahtan, 2012b; Ziv et al., 2013).

3. Zebrafish models of major CNS disorders

A clear advantage of non-human animals (like zebrafish) for modeling brain disorders is, as already mentioned, their amenability to experimental, genetic and pharmacological manipulations. Furthermore, the behavioral phenotypes, genetic factors and pharmacological sensitivity of zebrafish often show a high degree of similarity to those reported in rodent models of brain disorders and in human clinical populations (see further).

3.1. Depression and anxiety

Stress is a common risk factor for developing affective disorders, including Major Depressive Disorder (Lucassen, Oomen, Schouten, Encinas & Fitzsimons, 2016; Strüber, Strüber & Roth, 2014) and anxiety (Bystritsky, 2006). In mammals, stress response is mainly mediated by the interplay of the hypothalamus, the pituitary and the adrenal glands,

collectively forming the HPA axis (Smith & Vale, 2006). Prolonged stress and hyperactivation of the HPA axis have the potential to lower GR expression, ultimately reducing the ability to adapt and cope with stress-events (Howell, Kutiyanawalla & Pillai, 2011) and thereby triggering depression (Zhou, Zhu, Wu, Luo, Chang & Zhu, 2011).

Depression has been extensively modeled in rodents (Deussing, 2006; Krishnan & Nestler, 2011) utilizing early-life (Fumagalli, Molteni, Racagni & Riva, 2007) and adulthood stresses (Seligman, Rosellini & Kozak, 1975) as well as pharmacological interventions (Barr & Markou, 2005), selective breeding or genetic engineering (Deussing, 2006). Several hallmark depression symptoms (e.g., low self-esteem, and depressed mood) are difficult to evaluate in animals, as they do not clearly display a sense of self (Deussing, 2006). In contrast, evaluation of other phenotypes, including anhedonia, comorbid anxiety or sleep- and neuroendocrine disturbances, can be easily modeled in animals (Porsolt, 2000; Seligman & Beagley, 1975). In zebrafish, depressive-like states can be evoked by a battery of unpredictable chronic mild stressors (UCMS) applied for an extended period of time (Fulcher, Tran, Shams, Chatterjee & Gerlai, 2016; Marcon et al., 2016; Piato et al., 2011). Adult fish exposed to 7-14-day UCMS exhibit reduced locomotion, altered shoaling behavior and body coloration (Gerlai, Lahav, Guo & Rosenthal, 2000). Applied to zebrafish raised in social isolation for 5 months, UCMS increases anxiety-like behaviors in the novel tank test, and reduces body weight and whole-brain dopamine and serotonin metabolite 5-HIAA levels, compared to zebrafish raised in groups (Fulcher, Tran, Shams, Chatterjee & Gerlai, 2016). The effects of UCMS on exploratory and group/shoaling behaviors are reversed by fluoxetine (an SSRI) and bromazepam, a benzodiazepine anxiolytic (Marcon et al., 2016). In addition, several key pro-inflammatory molecules, such as tumor necrosis factor (TNF- α), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2), are differentially regulated in the zebrafish following 7 days of UCMS (Marcon et al., 2016). COX-2 transcription is greater in

individuals with recurrent depressive disorder, and is hypothesized to negatively affect cognitive functioning, emotionality and synaptic homeostasis (Galecki, Talarowska, Bobińska & Szemraj, 2014). Treatment with psychotropic drugs (fluoxetine, bromazepam and nortriptyline) reduces the expression of IL-6 and TNF- α (Marcon et al., 2016), highlighting the sensitivity of this model to established, clinically active antidepressants. Other pharmacological interventions, such as the administration of reserpine, produce depressive-like responses in zebrafish, including social withdrawal, motor retardation and elevated cortisol, that parallel clinical symptoms of depression (Nguyen, Stewart & Kalueff, 2014). Finally, several genetic models empower studying depression in the zebrafish. For instance, larval zebrafish with mutant glucocorticoid receptors (gr/s357) display heightened physiological responses (e.g., higher whole body cortisol levels) and dysfunctional HPI axis (Griffiths, Schoonheim, Ziv, Voelker, Baier & Gahtan, 2012a), similar to the effect observed in humans.

Anxiety disorders are debilitating psychiatric diseases with a lifetime prevalence of ~30%, higher than any other mental disorder (Kessler, 2007; Kessler, Chiu, Demler, Merikangas & Walters, 2005). There are several types of anxiety disorders, including panic disorder, post-traumatic stress disorder (PTSD), generalized anxiety disorder and specific phobias (American Psychiatric Association, 2013). The hallmark symptom of anxiety disorders is an overwhelming and exaggerated sense of worry in response to perceived threats (American Psychiatric Association, 2013), dramatically lowering patients' quality of life and work productivity (ADAA, 2016). The first line of treatment for anxiety disorders is typically a regimen of SSRIs or cognitive behavioral therapy (Bystritsky, 2006). Patients who do not respond to these treatments are then given selective norepinephrine reuptake inhibitors (SNRIs) or tricyclic antidepressants (Bystritsky, 2006). However, SNRIs or tricyclics increase the risk for tolerance and dependence (Otto, Pollack, Sachs, Reiter, Meltzer-Brody &

Rosenbaum, 1993), thereby limiting their use. Furthermore, although many treatments for anxiety disorders exist, approximately 30% of patients show no improvement (Brown, Schulberg, Madonia, Shear & Houck, 1996). This necessitates the identification and development of treatments that are devoid of the limitations in efficacy and tolerance (Griebel & Holmes, 2013a).

One of the problems with developing new treatments has been the identification of biochemical targets, genetic variants or mechanism of action for the onset of the disorder (Bystritsky, 2006; Griebel & Holmes, 2013a; Insel et al., 2011), beckoning the need for animal models. The zebrafish model is particularly amenable to high-throughput anxiolytic drug screens (Lundegaard et al., 2015). The larval zebrafish hatches from its chorion within 3 days post fertilization (dpf), and are able to inflate their swim bladder by 5 dpf and produce a broad range of behaviors (Richendrfer, Pelkowski, Colwill & Creton, 2012), see Fig. 1. For instance, staying near the periphery of the arena (thigmotaxis) reflects anxiety-like behavior, and is heightened following exposure to anxiogenic stimuli or drugs (Stewart, Gaikwad, Kyzar, Green, Roth & Kalueff, 2012). In adult fish, measures of anxiety include a latency to explore the top, or higher tendency to remain in the bottom (Stewart, Gaikwad, Kyzar, Green, Roth & Kalueff, 2012) in the novel tank test (Fig 2). In the light-dark test, allowed to freely explore brightly light and dark arenas, zebrafish spend more time in the dark (scototaxis) - an anxiety-like response which can be bidirectionally influenced by anxiolytic or anxiogenic treatments (Kalueff et al., 2013b). Genetic models of anxiety in zebrafish are also available, including the knockdown of vesicular monoamine transporter 2 (*Vmat2*) which produces an anxiety-like profile with social withdrawal and reduced exploration (Wang et al., 2016b).

In the effort to identify new treatments for anxiety and related disorders, there has also been a call to repurpose available drugs for novel applications (Lundegaard et al., 2015). This method of drug discovery has the advantage of reducing uncertainty regarding

pharmacokinetic issues or safety of the drug (Ashburn & Thor, 2004; Insel et al., 2011), thereby allowing for a more rapid drug screen and testing. For instance, N-acetylcysteine (NAC), a common mucolytic agent and antidote for paracetamol overdose, has shown promise in the treatment of several neuropsychiatric disorders (Berk, Malhi, Gray & Dean, 2013). NAC plays a role in maintaining oxidative balance germane to anxiety, and has been shown to modulate central glutamatergic pathways (Dean, Giorlando & Berk, 2011). While a growing body of evidence supports the role of glutamate in the anxiety response, there is a clear deficit of approved glutamatergic anxiolytics (Cortese & Phan, 2005). NAC administration to adult zebrafish prevents stress-induced anxiety (Mocelin et al., 2015), which is in line with previous reports of its clinical efficacy in depressed patients (Berk, Malhi, Gray & Dean, 2013). In another example of drug repositioning, potential anxiolytic targets were identified using traditional cancer treatments, as cAMP mediated anxiety in the zebrafish via crosstalk of the RAS-MAPK pathway (Lundegaard et al., 2015). The heightened anxiety-like response is attenuated by exposure to MEK inhibitors, anti-cancer treatment (Lundegaard et al., 2015), suggesting the MEK crosstalk as potential alternative target for treatments of anxiety as well.

3.2. *Epilepsy*

Epilepsy, which affects approximately 50 million people worldwide (WHO, 2016), is characterized by recurrent convulsions/seizures, behavioral impairments, pathological neural activity and endocrine dysfunction (Andrea Galimberti et al., 2005; Engel, 2013; Green et al., 2012; Zhang & Liu, 2008). Epilepsy can be modeled in larval and adult zebrafish (primarily by administration of convulsant drugs and genetic modifications) and evaluated by various behavioral and physiological endpoints (Cunliffe, 2015; Desmond et al., 2012; Wong et al., 2010). Characteristic behaviors for epilepsy-like states in adult zebrafish are hyperactivity, erratic swimming, loss of body posture, spasm-like corkscrew swimming (Desmond et al.,

2012) and electrical discharges in the CNS (Baraban, Taylor, Castro & Baier, 2005; Zdebik, Mahmood, Stanescu, Kleta, Bockenhauer & Russell, 2013). Experimental seizures in zebrafish can be induced by acute caffeine (250 mg/L), pentylenetetrazole (PTZ, 2.5 g/L) and picrotoxin (100 mg/L), causing hyperactivity, circular/corkscrew swimming, spasms and elevated whole-body cortisol levels (Wong et al., 2010). These symptoms are suppressed by antiepileptic drugs in both larval and adult zebrafish (Green et al., 2012), enabling the discovery of more efficacious treatments for epilepsy (Alfaro, Ripoll- Gómez & Burgos, 2011). For instance, PTZ administration not only evokes characteristic seizures, but is also accompanied by the rapid transcription of *c-fos* and *npas4* (Cunliffe et al., 2015), paralleling responses observed in seizure onset in mammals (Cunliffe et al., 2015; Loebrich & Nedivi, 2009). Finally, various genetic techniques enable the greater exploration of function for specific candidate genes (Cunliffe, 2015; Mahmood, Fu, Cooke, Wilson, Cooper & Russell, 2013; Teng et al., 2010) or anti-epileptic treatments using high-throughput and rapid screening in zebrafish (Baraban, Dinday & Hortopan, 2013; Baxendale et al., 2012; Cunliffe, 2015).

3.3. Psychosis

Psychosis manifests as disturbances in cognition, affect, motor activity and social behavior (American Psychiatric Association, 2013), and is often accompanied by aberrant glutamatergic signaling (Merritt, McGuire & Egerton, 2013; Schobel et al., 2013). Glutamate N-methyl-D-aspartate (NMDA) receptor antagonists phencyclidine and ketamine produce psychotic symptoms in healthy volunteers, and worsen the positive, negative and cognitive symptoms of patients with schizophrenia (Merritt, McGuire & Egerton, 2013). MK-801 is a potent NMDA antagonist used to model schizophrenia in rodents, zebrafish and other animal models (Moghaddam & Jackson, 2003; Swain, Sigstad & Scalzo, 2004). Likewise, pre-pulse inhibition (PPI) is the attenuation of startle response when a weak non-startling response is

presented before the startling stimulus (Swerdlow, Geyer & Braff, 2001). Schizophrenia patients show impaired PPI (Braff, Geyer & Swerdlow, 2001) which can be rescued by antipsychotic therapy (Geyer, Krebs-Thomson, Braff & Swerdlow, 2001; Kumari, Soni & Sharma, 1999). PPI is reliably reproduced in larval zebrafish, including genetic mutants with reduced PPI currently available (Burgess & Granato, 2007). Overall, the similarity in neural pathways and startle response in the zebrafish demonstrate their utility as an unbiased platform for the discovery of regulatory genes and drugs for antipsychotic treatment.

3.4. Alzheimer's Disease

AD is a progressive neurodegenerative disease resulting in cognitive deficits, delusions, hallucinations and mood and behavior changes (Voisin & Vellas, 2009). One of the hallmark AD symptoms is the development of neurofibrillary tangles (NFT) and amyloid beta plaques (Newman, Verdile, Martins & Lardelli, 2011). There are two broad classes of AD: sporadic AD (developing at age >65), and familial AD, developing much earlier (Rossor, Iversen, Reynolds, Mountjoy & Roth, 1984). Sporadic AD accounts for more than 95% of all AD cases (Newman, Verdile, Martins & Lardelli, 2011), and is linked to the apolipoprotein E ϵ 4 allele (ApoE4) (Selkoe, 2001). The zebrafish orthologue of this gene is *apoE* (Babin, Thisse, Durliat, Andre, Akimenko & Thisse, 1997). Early-onset familial AD (fAD) is hereditary and has been linked to mutations in the *PRESENILIN1* (*PSEN1*), *PRESENILIN2* (*PSEN2*) and *AMYLOID BETA A4 PRECURSOR PROTEIN* (*APP*) genes, orthologous to the zebrafish *psen1*, *psen2*, *appa* and *appb* genes (Newman, Verdile, Martins & Lardelli, 2011). The injection of transcription-blocking morpholinos for *psen1* disrupts notch signaling and results in aberrant somite formation (Campbell et al., 2006; Nornes, Casper, Esther, Ey & Lardelli, 2003; Nornes, Newman, Wells, Verdile, Martins & Lardelli, 2009). *Psen2* blocking produces notch-signaling defects (Campbell et al., 2006) and alters the production of spinal cord interneurons in zebrafish (Nornes, Newman, Wells, Verdile,

Martins & Lardelli, 2009), paralleling phenotypes observed in *psen1*^{-/-} and *psen2*^{-/-} mice (Shen, Bronson, Chen, Xia, Selkoe & Tonegawa, 1997).

Zebrafish are also valuable for studying the etiology of AD, especially the role of hypoxia as a putative risk factor (Newman, Verdile, Martins & Lardelli, 2011). Under low oxygen conditions, mitochondria may release free radicals that increase oxidative stress (Bell et al., 2007; Moussavi Nik, Croft, Mori & Lardelli, 2014). Hypoxic conditions are easily reproduced in the zebrafish by reducing water oxygen levels or via chemical mimicry of hypoxia by sodium azide (Moussavi Nik, Newman & Lardelli, 2011). Similar to humans, hypoxic conditions in the larval and adult zebrafish upregulate several AD-related genes, including *sen1*, *psen2*, *appa*, *appb* and *bace1* (Moussavi Nik, Newman & Lardelli, 2011).

Pharmacological intervention may also help model the cognitive deficits associated with AD. For example, the cholinergic system (mediating learning and memory) is affected by AD (Fibiger, 1991), as AD patients show reduced nicotinic (nAChR) and muscarinic (mAChR) binding sites, as well as reduced acetylcholinesterase (AChE) activity (Lombardo & Maskos, 2015; Perry, Tomlinson, Blessed, Bergmann, Gibson & Perry, 1978). The muscarinic antagonist scopolamine impairs zebrafish memory without causing locomotor deficits or anxiety-like behavior (Cognato et al., 2012; Gupta, 2014; Richetti et al., 2011). Pretreatment with quercetin and rutin, two flavonoids, protects against scopolamine-induced memory impairment (Richetti et al., 2011). Flavonoids act as AChE inhibitors and can enhance learning/memory and synaptic plasticity (Ahmed & Gilani, 2009; Havsteen, 2002; Spencer, 2008). Scopolamine-induced memory impairment in zebrafish is also ameliorated by pretreatment with physostigmine, an AChE inhibitor (Kim, Lee, Kim, Jung & Lee, 2010). The ability of scopolamine to produce amnesic effects while preserving normal locomotor activity provides evidence contributing to the involvement of the cholinergic system in fish

learning and memory, and lends credence to the use of the zebrafish as a tool for drug discovery and medicines that can treat neurodegenerative diseases, including AD.

3.4. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a debilitating progressive neurodegenerative disorder affecting motor neurons in the brain and spinal cord (Rowland & Shneider, 2001). Zebrafish are a particularly attractive model for studying the function and dysfunction of spinal cord circuitry due to visual transparency at early stages of life, and because there is a high degree of functional and anatomical similarity between the zebrafish spinal cord and humans (Fetcho & O'Malley, 1995; Friedrich, Jacobson & Zhu, 2010; McGown et al., 2013). Similar to AD, there are two broad types of ALS: familial and sporadic ALS (Kiernan et al., 2011). Roughly 10% of ALS cases are inherited. The etiology of ALS is poorly understood, with a high degree of variability in genetic mutations that contribute to ALS. Nevertheless, SOD1 is the most well-understood gene to be associated with ALS (Rosen et al., 1993), and mutations in the SOD1 gene account for 20% of familial ALS cases (Valdmanis & Rouleau, 2008).

Larval zebrafish over-expressing mutant *Sod1* have abnormal neuromuscular junctions (NMJ) which worsen as the fish matures (Ramesh et al., 2010). Larval mutant fish present progressive decrease in NMJ volume (Ramesh et al., 2010), poorer performance in the forced swim test (Plaut, 2000; Ramesh et al., 2010) and reduced responsivity to repeated stimulation (Ramesh et al., 2010). Together, this indicates a defect in the neural input to the muscle, rather than defects in the intrinsic properties of the muscle (Ramesh et al., 2010). Early identification of pathogenic processes is also possible in the zebrafish through the heat-shock stress response (HSR). The HSR mechanism refolds damaged proteins in stressed cells, and is a useful tool for monitoring cellular perturbations (McGown et al., 2013). In *sod1* mutant zebrafish harboring the HSR reporter gene (*hsp70-DsRed*), fluorescence facilitates

disease mapping and spread throughout the brain (McGown et al., 2013). This method has also been used to identify neuroprotective compounds and biological targets with the potential to ameliorate early disease processes that are not yet fully understood (McGown et al., 2013).

In addition to the utility of the zebrafish in monitoring the progress of ALS symptoms, genetic mutants and pharmacological models also help identify the molecular mechanisms of this disease. For instance, the loss of function of the zebrafish orthologue *C9orf72* leads to axonal degeneration of motor neurons, and is accompanied by decreased swim speed and motility of larval zebrafish (Ciura et al., 2013). The motor deficits caused by knockdown of *C9orf72* implicate it in ALS and related neurodegenerative disorders (Ciura et al., 2013). Gene editing techniques, such as TALEN- or CRISPR, may also be used to insert point mutations in the zebrafish genome (Armstrong, Liao, You, Lissouba, Chen & Drapeau, 2016), resulting in mutant zebrafish lines recapitulating ALS. This novel methodology shows promise in the development of mutant models for other neuropsychiatric diseases as well (Armstrong, Liao, You, Lissouba, Chen & Drapeau, 2016). Pharmacological intervention with neurotoxins like beta-methylamino-alanine (BMAA) can also be relevant to modeling ALS. Pericardiac injection of BMAA during embryonic development alters protein homeostasis and glutamate signaling, whereas fish exposed to a sublethal dose of BMAA display reduced heart rate and abnormal spinal axis formation, but can be rescued pharmacologically (e.g., by inhibiting the endocannabinoid enzyme fatty acid amide hydrolase) (Froyset, Khan & Fladmark, 2016; Purdie, Samsudin, Eddy & Codd, 2009).

4. Zebrafish sensitivity to CNS drug classes

The well-documented similarity of zebrafish and mammalian neurotransmitter systems (Panula et al., 2010; Panula et al., 2006) contributes to the fact that zebrafish models display similar pharmacology and sensitivity to various CNS drugs. Using selected classes of

neuroactive drugs as examples, we will further illustrate this aspect of zebrafish models, and its relevance to the search for novel therapeutic approaches.

4.1. Antiepileptic drugs

PTZ is one of the most widely used convulsant agents in rodents and zebrafish, and produces robust seizure phenotypes suppressed to varying degrees by a wide range of known anti-epileptic drugs (Cunliffe, 2016). PTZ induction of seizures is also an effective way of medium-throughput testing for the discovery of new anti-epileptic treatments (Baxendale et al., 2012; Cunliffe, 2016). As small molecule screens may be conducted in zebrafish as early as 2 dpf, the efficacy of potential treatments is evaluated not only through behavioral testing, but through the monitoring of neural responses (e.g., *c-fos*) (Baxendale et al., 2012). Exposure to PTZ increases *C-fos* expression which is attenuated by classic anti-convulsant agents, as well as anti-inflammatory agents, natural and synthetic steroids, antioxidants, vasodilatory agents, pesticides and herbicides (Baxendale et al., 2012). However, while these drugs attenuate PTZ-induced seizures, the mechanism of their action remains unclear. In addition to PTZ, other drugs evoke seizure-like states in zebrafish (Winter, Redfern, Hayfield, Owen, Valentin & Hutchinson, 2008). Kainic acid (KA) is a common convulsant agent in rodents, and is able to produce similar effects in zebrafish (Alfaro, Ripoll- Gómez & Burgos, 2011). Glutamate receptor antagonists diminish KA-induced seizures, underscoring the utility of the zebrafish model to study glutamatergic excitatory neurotransmission. Also pertinent to the study of anti-epileptic treatment is the combination of genetic manipulation with pharmacological interventions (Cunliffe, 2015). For instance, clemizole (a histaminergic antagonist) is efficient in treating genetically evoked seizures on *scn1lab* zebrafish (Grone & Baraban, 2015), a model of Dravet syndrome (Baraban, Dinday & Hortopan, 2013) caused by SCN1A mutations with spontaneous seizures insensitive to major anti-epileptic drugs.

4.2. Antipsychotics

First generation (typical) antipsychotics are high-affinity antagonists of dopamine D2 receptors, and are the most effective treatment of psychoses (Lieberman et al., 2005). However, they produce severe side effects, including tremors, paranoia and anxiety (Miyamoto, Duncan, Marx & Lieberman, 2005). The second-generation ‘atypical’ antipsychotics demonstrate a lower affinity for D2 receptors and fewer side effects, relative to typical antipsychotics (Kane, Honigfeld, Singer & Meltzer, 1988). However, there remains a great need for the identification of novel treatments for psychoses, and zebrafish models can be highly useful in this endeavor. For example, administration of MK-801 induces hyperlocomotion (similar to psychomotor agitation, a characteristic symptom of schizophrenia (Seibt et al., 2010)), as well as social and cognitive deficits (Seibt, Piato, da Luz Oliveira, Capiotti, Vianna & Bonan, 2011). MK-801-induced locomotor effects are reversed by typical (haloperidol) and atypical (olanzapine and sulpiride) antipsychotics (Seibt et al., 2010). However, fish exposed to MK-801 perform poorly in an inhibitory avoidance task, and their social and cognitive deficits are restored by atypical, but not typical, antipsychotics (Seibt, Piato, da Luz Oliveira, Capiotti, Vianna & Bonan, 2011). Importantly, atypical antipsychotics have affinities for dopaminergic as well as serotonergic, glutamatergic and other neurosignaling pathways. For example, resperidone acts via D2 and serotonin 5-HT₂ receptors, and shows promise as an anxiolytic substance (Idalencio et al., 2015). Stressed fish exposed to resperidone spend more time in top of the novel tank test, have fewer transitions to the dark in the light-dark test (Magno, Fontes, Gonçalves & Gouveia, 2015) and show lower cortisol levels (Idalencio et al., 2015). The purinergic system has been recently implicated in schizophrenia (Lara & Souza, 2000), especially since adenosine, the final product in the ectonucleotidase cascade, modulates dopamine and glutamate (Lara & Souza, 2000). In zebrafish, haloperidol reduces ATP hydrolysis and adenosine deamination, thereby

reducing synaptic adenosine levels (Seibt, da Luz Oliveira, Bogo, Senger & Bonan, 2015). The sensitivity of zebrafish ATP hydrolysis to haloperidol suggests an extracellular mechanism of action, potentially relevant to pharmacological targets (Seibt, da Luz Oliveira, Bogo, Senger & Bonan, 2015).

4.3. Drugs of abuse

Substance abuse and addiction are easily modeled in larval and adult zebrafish (Stewart et al., 2011). For example, addiction, tolerance and withdrawal can be studied using aquatic conditioned place preference (CPP) paradigms (Collier & Echevarria, 2013; Collier, Khan, Caramillo, Mohn & Echevarria, 2014; Mathur & Guo, 2010). A typical CPP setup consists of two distinct environments, which differ in their colors, visual patterns or environmental cues (Darland & Dowling, 2001). The protocol consists of three steps: initial determination of environment preference, conditioning session, and testing of final place preference. From the conditioning session, three outcomes are possible: preference for the non-preferred side, aversion of the preferred side, or no change. In zebrafish, CPP protocols generally take ~3 days (Collier, Khan, Caramillo, Mohn & Echevarria, 2014), but may also run for several weeks (Kily et al., 2008). This protocol is widely used in zebrafish, rodents and other model organisms to investigate the behavioral effects of psychoactive compounds and associative learning (Lucke-Wold, 2011) but, despite the ability to elucidate reward-seeking behavior, does not measure the drug's abuse potential (see further).

4.3.1. Alcohol and nicotine

Ethanol produces a characteristic dose-dependent effect in zebrafish. At low doses (<0.5%) ethanol increases locomotion, swim speed, and shoaling behaviors (Gerlai, Lahav, Guo & Rosenthal, 2000). A 20-min exposure to 1.00% ethanol is anxiolytic in zebrafish, whereas longer exposure to the same dose (or higher doses) impairs their locomotion and induces sedation (Gerlai, Lahav, Guo & Rosenthal, 2000; Pannia, Tran, Rampersad & Gerlai,

2014; Rosemberg et al., 2012; Tran & Gerlai, 2013). The rewarding effect of ethanol in zebrafish is seen after a single exposure to 0.25-1% (Collier, Khan, Caramillo, Mohn & Echevarria, 2014) or 1.5% (Mathur, Berberoglu & Guo, 2011), reliably changing fish CPP. A prolonged CPP paradigm (e.g., daily conditioning for 4 weeks) produces robust behavioral responses which persist following abstinence, indicating the establishment of dependence-related behavior (Kily et al., 2008). Some reports evaluating the chronic ethanol CPP treatment note the development of tolerance, as indicated by lower drug-induced hyperactivity, and decreased anxiolytic effects (Gerlai, Lee & Blaser, 2006). Drug abstinence following chronic (1 week) exposure produces robust withdrawal symptoms in adult zebrafish, including anxiety-like behavior and elevated cortisol (Cachat et al., 2011a).

Zebrafish also produce a wide range of dose-dependent responses to nicotine (Kily et al., 2008; Levin, Bencan & Cerutti, 2007). At low to moderate doses (e.g., 3 – 300 μ M), nicotine evokes anxiolytic responses in the novel tank test (Levin, Bencan & Cerutti, 2007) and robust CPP that persist following a period of abstinence (Kily et al., 2008). The behavioral effects of nicotine are also susceptible to genetic variation, allowing the researchers to identify genetic candidates for human nicotine addiction (Petzold et al., 2009). Furthermore, microarray analyses of whole brain samples from nicotine-treated fish reveal an upregulation of several genes implicated in the development of drug dependence, including genes for calcineurin B and hypocretin receptor, which have both been previously linked to synaptic plasticity and neurotransmission in drug dependence (Kily et al., 2008).

4.3.2. Cocaine and amphetamines

Administration of cocaine (5, 10, and 15mg/L) to adult zebrafish produces robust arousal states, as indicated by an extension of the fins, slow circling and remaining low in the water column (Darland & Dowling, 2001). Surrounded by conspecifics, cocaine-treated zebrafish engage in aggressive behavior through dominance displays and chasing (Darland &

Dowling, 2001). Abstinence from the drug results in withdrawal symptoms within 72 h, wherein animals experience anxiety-like behavior and basal hyperlocomotion (López-Patiño, Yu, Cabral & Zhdanova, 2008). Withdrawal symptoms are counteracted by the administration of non-sedative dose of diazepam (5 μ M) or cocaine (1.5 μ M) (López-Patiño, Yu, Cabral & Zhdanova, 2008). Cocaine also produces dose-dependent CPP responses, with 10 mg/L causing the most robust response (Darland & Dowling, 2001). Cross-breeding wild-type females with males mutagenized through repeated exposure to N-ethyl-nitrosourea (ENA) yielded an F₁ generation, outcrossing of which results in F₂ generation tested for cocaine sensitivity in the CPP task. Low responding F₂ siblings were crossbred to yield F₃ generation which display low sensitivity to cocaine in the CPP task, demonstrating a genetic basis for the altered behavior profile (Darland & Dowling, 2001).

Methamphetamine is a potent psychostimulant with high addiction potential, and its abuse is comorbid with psychiatric disorders, including anxiety and depression (Akindipe, Wilson & Stein, 2014). Currently, there are no effective medications for the treatments of methamphetamine abuse. The zebrafish demonstrates sensitivity to methamphetamine and is a useful model to study effective medications, and methamphetamine-related comorbidities (Mi et al., 2016). For instance, the acute administration of methamphetamine induces avoidant behavior and increases swim speed in the open field and mirror stimulation task (Mi et al., 2016), attenuated by *I*-Scoulerine, an agent acting on dopaminergic and serotonergic systems (Mi et al., 2016). The cholinergic system may also play a role in modulating the rewarding effects of various psychoactive drugs, and genetic impairment of AChE does reduce amphetamine-induced CPP in adult zebrafish (Ninkovic et al., 2006). Finally, the pharmacological inhibition of AChE reduces addictive potential of cocaine and morphine in mice (Hikida, Kitabatake, Pastan & Nakanishi, 2003), suggesting that targeting the acetylcholine system may lead to reducing the addictive properties of drugs.

4.3.3. Hallucinogens

Hallucinogenic agents can be classified under three broad categories: (1) classic serotonergic psychedelics, (2) dissociatives, which primarily act as NMDA antagonists, and (3) deliriants, which act as anticholinergic agents (Kyzar & Kalueff, 2016). Classic serotonergic psychedelics (e.g., lysergic acid diethylamide (LSD), mescaline and psilocybin) alter zebrafish locomotion, shoaling and anxiety-like behaviors and whole body cortisol levels (Kyzar & Kalueff, 2016). Ketamine, a dissociative psychedelics, produces a dose-dependent anxiolytic effect in the zebrafish, and decreases whole body cortisol levels (De Campos, Bruni & De Martinis, 2015). The deliriant psychedelic atropine affects cholinergic neural activity in the zebrafish (Park, Lee, Kim & Lee, 2008). Although hallucinogens remain understudied in zebrafish, the available data demonstrate their sensitivity to various known drugs, and may allow for the discovery of therapeutic targets, especially given the growing recent interest in hallucinogenic agents (Kyzar & Kalueff, 2016).

4.3.4. Sedatives

Sedatives are generally prescribed for the treatment of anxiety disorders, and produce anxiety reduction, disinhibition and sedation, mainly modulating the histaminergic, GABA-ergic and adrenergic systems (Koob, 1992). Zebrafish share similarity to the mammalian GABA-A and B receptor subunits, and histamine H1 receptor (Renier et al., 2007), and are highly sensitive to a wide range of sedatives. For example, high doses of chlordiazepoxide significantly reduce swim speed (Bencan, Sledge & Levin, 2009), whereas diazepam has a biphasic effect on anxiety, with low-to-moderate doses reducing bottom dwelling, and higher doses causing sedation (Bencan, Sledge & Levin, 2009). Chronic 2-week exposure to diazepam followed by abstinence produces withdrawal-like symptoms in zebrafish, including anxiety in the light dark preference task (Cachat et al., 2011a). While this highlights the utility of zebrafish as a model for sedative-related withdrawal, there is a clear

lack of studies that evaluate the rewarding or aversive effects of sedatives in zebrafish (which can easily utilize the CPP protocol to generate invaluable information on behavioral effects of these drugs).

5. Perspectives on small molecule and genetic screening in zebrafish

5.1. Understanding genetic and anatomical differences from mammalian models

A member of the teleost group, the zebrafish has arisen from a common ancestor ~340 million years ago (Amores, Catchen, Ferrara, Fontenot & Postlethwait, 2011). The ancestor had undergone an additional round of whole-genome duplication (WGD), an event that is responsible for the diversification of gene function and phenotype in zebrafish (Meyer & Schartl, 1999). Of the homologous genes, 71.4% of human genes have at least one zebrafish orthologue and, 47% of human genes have a one-to-one zebrafish orthologue (Amores, Catchen, Ferrara, Fontenot & Postlethwait, 2011). Of the genes for which zebrafish have more than one orthologue, only few have been studied and functionally characterized. Thus, the current lack of understanding of many zebrafish orthologues of human genes is a potential problem with this model. For instance, humans possess three *Period (Per)* genes: *Per1*, *Per2*, and *Per3* (Wang, 2008) encoding regulatory elements in the circadian clock, which are also responsible for growth, rest, and hormone production (Danilova, Krupnik, Sugden & Zhdanova, 2004; Pando & Sassone-Corsi, 2002; Vatine, Vallone, Gothilf & Foulkes, 2011). Zebrafish have two *per1* genes (*per1a* and *per1b*), but only one *per2* and one *per3* (Wang, 2008). The *per1a* and *per1b* genes show distinct temporal and spatial expression, and their roles in the circadian clock is poorly understood (Wang, 2008). Transgenic models may help to elucidate the functions of the zebrafish *per1* genes, and provide insight to their role in maintaining circadian rhythms. The serotonin transporter (*sert*) genes have also been duplicated during WGD in zebrafish, which possess two *sert* genes: *serta* and *sertb* (Wang, Takai, Yoshioka & Shirabe, 2006). These genes have high homology

to vertebrate serotonin transporter genes, suggesting a conservation of function (Wang, Takai, Yoshioka & Shirabe, 2006). Thus, despite an additional WGD, it does not render the zebrafish model unusable. Rather, the study and functional identification of genes may help better understand molecular interactions, which will further clarify the efficacy of drugs and their therapeutic targets.

Furthermore, despite significant neuroanatomical similarity discussed above, some differences between zebrafish and mammals must be critically considered. For example, while several regions in the mammalian brain do not have clear structural homologous counterparts in zebrafish, including the substantia nigra and hippocampus (Mueller, Dong, Berberoglu & Guo, 2011; Panula et al., 2010), they share functional homology with selected groups of zebrafish neurons. Thus, a small population of dopaminergic cells in the posterior tuberculum is a strong candidate for the zebrafish homologue of the substantia nigra (Kaslin & Panula, 2001), as shown by neurotoxin lesion studies (Sallinen et al., 2009). Likewise, the lateral part of the zebrafish pallium contains homologous structures to the mammalian hippocampus (von Trotha, Vernier & Bally-Cuif, 2014), thereby fostering further cognitive studies in zebrafish models. One stark difference from humans is the fact that zebrafish lack a cortex, its homologue, or even molecular markers that may be used to identify a cortex region (Mueller, Dong, Berberoglu & Guo, 2011; Northcutt, 2008). This aspect may limit translation of findings between zebrafish and humans, especially on aberrant executive functioning commonly observed in psychiatric diseases (Parker, Brock, Walton & Brennan, 2013). However, given potential limitations of the model, it is necessary to evaluate its face and construct validity. Face validity determines whether the model resembles the disease in question, while construct validity determines whether the model measures what it has set out to measure. Because the rodent models often fulfill these validity criteria, many zebrafish behavioral tasks have been modified from rodent paradigms (Levin, Bencan & Cerutti, 2007).

For instance, for modeling anxiety disorders, the zebrafish has become an adept model in the identification of stress-inducing stimuli and psychoactive agents in various novelty-based paradigms (Abreu, Giacomini, Kalueff & Barcellos, 2016; Bencan, Sledge & Levin, 2009; Levin, Bencan & Cerutti, 2007). Deficits in cognitive and behavioral flexibility, commonly reported in patients with psychiatric diagnoses, may also be modeled in zebrafish. Behavioral flexibility - the ability to adapt responses to changing environmental conditions - is often studied in rodents using a reversal of contingencies in choice-discrimination tasks (Ragozzino, Detrick & Kesner, 1999; Saus et al., 2010). These tasks rely on the ability of the animal to demonstrate a reversal of learning. Zebrafish have demonstrated the ability to adapt to changing environmental contingencies, and their capacity for reversal learning shows similar patterns to that observed in rodents (Colwill, Raymond, Ferreira & Escudero, 2005). Thus, although zebrafish may lack a proper cortex, they retain the ability to perform executive functions, such as maintaining attention and behavioral flexibility (Parker, Brock, Walton & Brennan, 2013). However, we still know relatively little about the neural circuits and how different neurotransmitter systems may functionally interact in zebrafish (Parker, Brock, Walton & Brennan, 2013). Identifying the function of neural circuits in this fish, and their reciprocity with other systems, become critical to understanding the molecular basis of behavior, and in identifying therapeutic targets for diseases.

5.2. Perspectives on automated and high-throughput screening

Zebrafish models are highly amenable to behavioral, genomic and proteomic testing (Jones & Norton, 2015; Purushothaman et al., 2015), as they combine a relative neural simplicity with behavioral complexity sufficient for studying multiple behavioral processes from sleep (Purushothaman et al., 2015; Rihel et al., 2010; Zhdanova, 2006) to anxiety (Richendrfer, Pelkowski, Colwill & Creton, 2012; Stewart, Gaikwad, Kyzar, Green, Roth & Kalueff, 2012). Custom-made and commercial video-tracking software can record a wide

range of zebrafish behavioral measures, including velocity, distance traveled, place preference (e.g., top vs. bottom, light vs. dark, center vs. periphery), and specific patterns (e.g., erratic swimming, stereotypic circling) (Conklin, Lee, Schlabach & Woods, 2015; Pérez-Escudero, Vicente-Page, Hinz, Arganda & de Polavieja, 2014). The automation of zebrafish video-tracking enables several behavioral outcomes to be recorded simultaneously, removing the need to repeat the experiment and/or watch and re-watch videos manually each time a new outcome is measured (Conklin, Lee, Schlabach & Woods, 2015; Pérez-Escudero, Vicente-Page, Hinz, Arganda & de Polavieja). It is also possible to record zebrafish social groups, for example, assessing fish shoaling behaviors, presently capable of tracking multiple (e.g., 8-16) animals per arena (Noldus, 2016b) to extract rich behavioral data from average inter-fish distance to shoal polarization and cohesion (Stewart, Braubach, Spitsbergen, Gerlai & Kalueff, 2014b). Larval zebrafish allow for recording of even more (e.g., 96) animals, tracking their swim patterns simultaneously (Noldus, 2016a). An added advantage of technological advancements in this rapidly growing field of zebrafish phenomics is the automatization of drug administration, and the computerization of stimulus exposure - e.g., in drug addiction or fear conditioning paradigms (Saverino & Gerlai, 2008), which collectively improves the standardization of testing procedures, efficient data collection, as well as increased throughput and data reproducibility (Love, Pichler, Dodd, Copp & Greenwood, 2004; Stewart, Gerlai & Kalueff, 2015).

Zebrafish are further amenable to high-throughput in-vivo screening as their multiple behavioral parameters can be monitored in 3D (Stewart et al., 2015a). For example, the X, Y, and Z swim trajectories can be traced by two cameras, generating two 2D-trajectory files integrated to produce a 3D trace of the swim pattern which can help identify unique drug-induced phenotypic profiles (Stewart et al., 2015a). Advances in behavioral recognitions allow for a more detailed in vivo analysis of behavioral phenotype (Stewart, Gerlai &

Kalueff, 2015). For instance, software that can discriminate between the tail, mid-body and nose of the zebrafish are well capable of quantifying locomotion, and interpreting complex behaviors such as chasing or nipping, chasing (Kalueff et al., 2013b; Stewart, Gerlai & Kalueff, 2015). These methods are especially useful in polypharmacology studies using pharmacological agents that act on multiple targets (McCarroll, Gendele, Keiser & Kokel, 2016). As many psychiatric disorders are linked to deficits in several neurotransmitter systems and have multigenic etiologies (Kendler, Aggen & Neale, 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), this possibility in zebrafish screens becomes particularly important. Computational techniques, such as hierarchical clustering or Similarity Ensemble Approach (SEA), also help identify target hits and prediction of target interactions with psychoactive compounds. The combination of behavioral phenotyping and computation techniques are useful in the development and discovery of new medical targets, including *in-vivo* behavioral phenotyping of single target compounds in 2-D or 3-D tracking, producing their unique swim traces (thigmotaxis, scototaxis, average swim speed, etc.), identifying the compounds which produce the desired behavioral phenotype (hit compounds), and their subsequent in-depth analyses with algorithms that predict their biological target(s) to generate hypotheses of target combinations (McCarroll, Gendele, Keiser & Kokel, 2016). Once identified, multiple hit compounds can then be tested in combination *in-vivo* (McCarroll, Gendele, Keiser & Kokel, 2016), probing their ability to work in concert to achieve a desired therapeutic outcome.

5.3. Perspectives on genetic zebrafish models

As already noted, genetic manipulations are critical on animal studies to identify candidate genes associated in the etiology of a disease. Short-term genetic manipulation is achieved through injection of morpholino-modified antisense oligonucleotides (MOs) (Nasevicius & Ekker, 2000), or small interfering RNA (siRNA) (de Rienzo, Gutzman & Sive,

2012) to engage in loss-of-function studies (Kalueff, Stewart & Gerlai, 2014). MOs target specific translational inhibitors and effectively reduce gene expression (Nasevicius & Ekker, 2000). RNA interference (RNAi) is a process in which RNA molecules inhibit the translation of targeted mRNA molecules (de Rienzo, Gutzman & Sive, 2012). These methods demonstrate efficacy in targeting specific genes and in producing altered phenotypes, although the efficacy of MOs has been questioned recently (Kok et al., 2015; McCammon & Sive, 2015).

The development of mutant zebrafish provides a more stable behavioral phenotype, because rather than produce a knockdown of a given gene, it completely eliminates the target gene product (Amsterdam & Hopkins, 2006; Stewart et al., 2014). Mutants are created through retroviral insertional mutagenesis, wherein a DNA basepairs are integrated into the organism's preexisting DNA (Amsterdam & Hopkins, 2006), or through chemical mutagenesis. Chemical mutagenesis involves exposing the male zebrafish to the methylating agent ethylnitrosourea weeks before mating in order to allow the mutation to fix in the spermatogonia just before they mature to sperm (Amsterdam & Hopkins, 2006; Wienholds, van Eeden, Kusters, Mude, Plasterk & Cuppin, 2003). Mutant and morphant zebrafish are used in a wide range of studies, and provide a deeper understanding on the roles and importance of specific receptors and biological targets (Griffiths, Schoonheim, Ziv, Voelker, Baier & Gahtan, 2012a; Haesemeyer & Schier, 2015). For instance, in developing a mutant model of autism, a highly active set of genes was discovered with a large genetic target, providing a deeper look in to the functional changes associated with gene deletion and duplication (Blaker-Lee, Gupta, McCammon, De Rienzo & Sive, 2012). Furthermore, the size of the genetic target, which had previously been unknown, was elucidated allowing for targeted assays in higher vertebrates and mammals (Blaker-Lee, Gupta, McCammon, De Rienzo & Sive, 2012). Similarly, loss of function mutations for the synaptic machinery genes

stxbp1a and *stxbp1b* produce robust phenotypes (Grone et al., 2016). In humans, these genes are linked to various neurodevelopmental disorders and epilepsy (Carvill et al., 2014; Saitsu et al., 2008). Homozygous *stxbp1a* knockdown results in immobility, reduced heart rate, reduced metabolism, and early death (Grone et al., 2016). Heterozygous *stxbp1a* knockdown produces markedly fewer deleterious effects; aside from a slight reduction in behavioral response to a startle stimulus, larval zebrafish produce normal behavior (Grone et al., 2016). Homozygous *stxbp1b* mutations yield zebrafish that present with epileptic seizures, along with normal mobility, metabolism and heart rate (Grone et al., 2016). The wide range of behavioral and physiological effects of the loss of function mutations for *stxbp1a* and *stxbp1b*, coupled with the functional similarity to the mammalian genes (Saitsu et al., 2008), highlight the potential for the zebrafish model to be used in the mechanistic and epigenetic study of neurodevelopmental and neuropsychiatric diseases.

6. Conclusion

Neuropsychiatric conditions afflict human population globally, and have tremendous personal and societal costs (Garakani, Mathew & Charney, 2006; Griebel & Holmes, 2013a). Animal models have long been used in neuropsychiatric studies to better understand human disease states, and play a key role in the identification of biological and molecular targets, aiming at developing safer and more effective treatments (Keifer & Summers, 2016; Krishnan & Nestler, 2011). Zebrafish are a promising new animal model which continues to provide important insights into the etiology of CNS diseases (Kalueff, Echevarria & Stewart, 2014b; Kalueff, Stewart & Gerlai, 2014). The homology of key brain regions between zebrafish and mammals underscores the utility of zebrafish models in neurobehavioral and neuropsychiatric studies. Furthermore, the conservation of neural pathways between zebrafish and mammals allows for the bi-directional translation of findings (Renier et al., 2007; Stewart, Braubach, Spitsbergen, Gerlai & Kalueff, 2014b). Current genetic tools,

tracking techniques and statistical algorithms foster gaining a deeper understanding of molecular pathways, developing new compounds or repurposing established drugs (Stewart et al., 2015a). Together with high sensitivity of zebrafish to known anxiolytic, antipsychotic and other CNS drugs, this provides researchers with a well-rounded model organism capable of identifying molecular targets for drug treatment and empirical testing of these hypotheses (Hwang et al., 2013; Kokel et al., 2010; Stewart et al., 2015a).

Conflict of interest:

The authors declare that there are no conflicts of interest.

Acknowledgements:

The study was coordinated through the International Zebrafish Neuroscience Research Consortium (ZNRC), and this collaboration was funded by St. Petersburg State University, Ural Federal University and Guangdong Ocean University. AVK is the Chair of ZNRC, and his research is supported by the Russian Foundation for Basic Research (RFBR) grant 16-04-00851.

Figure 1. The use of automated video-tracking to simultaneously assess multiple phenotypes in larval zebrafish. Panel (a) shows a 96-well holding plate to administer several compounds to larval zebrafish. Fish behaviors are recorded by an overhead camera, and images are processed through tracking software. Swim traces garnered from the tracking software allow the researcher to assess the effects of the compounds administered. Panel (b) shows an example of a swim trace in which the larval zebrafish remains towards the walls (wall-hugging behavior). Panel (c) shows an example of the opposite swim pattern in which the larval zebrafish actively explores its environment, including the center of the tank.

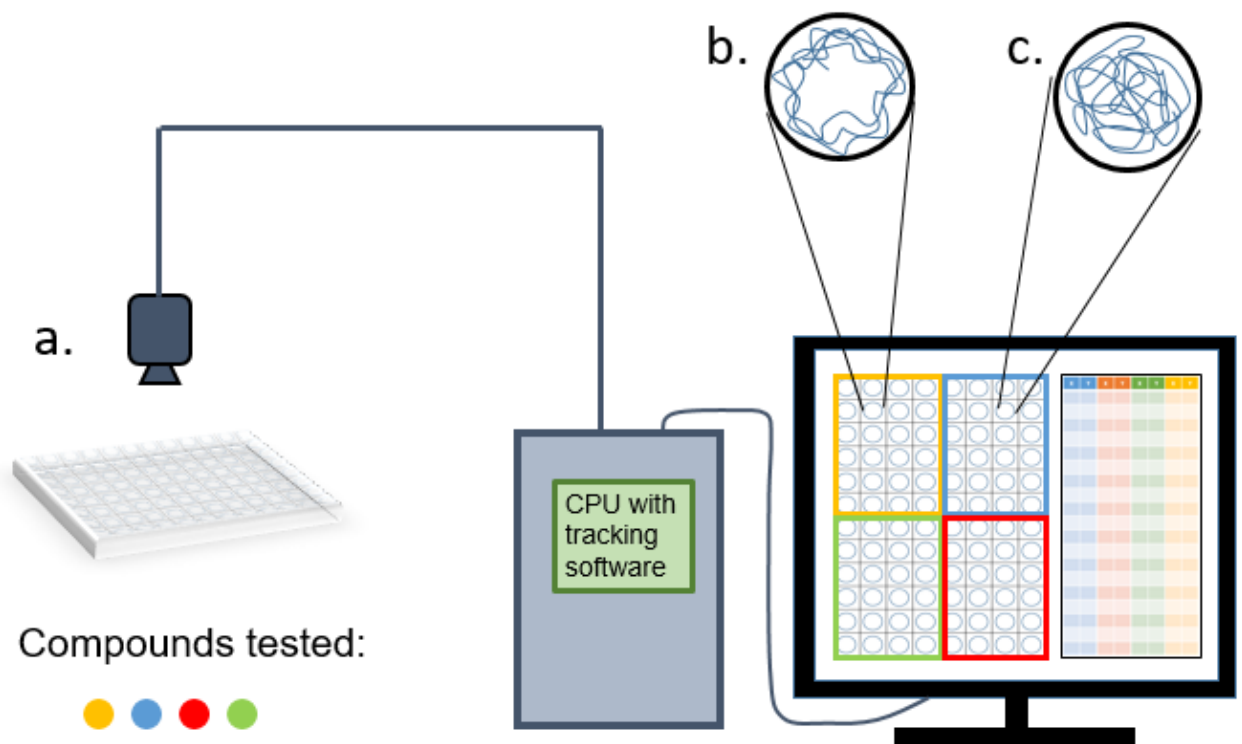
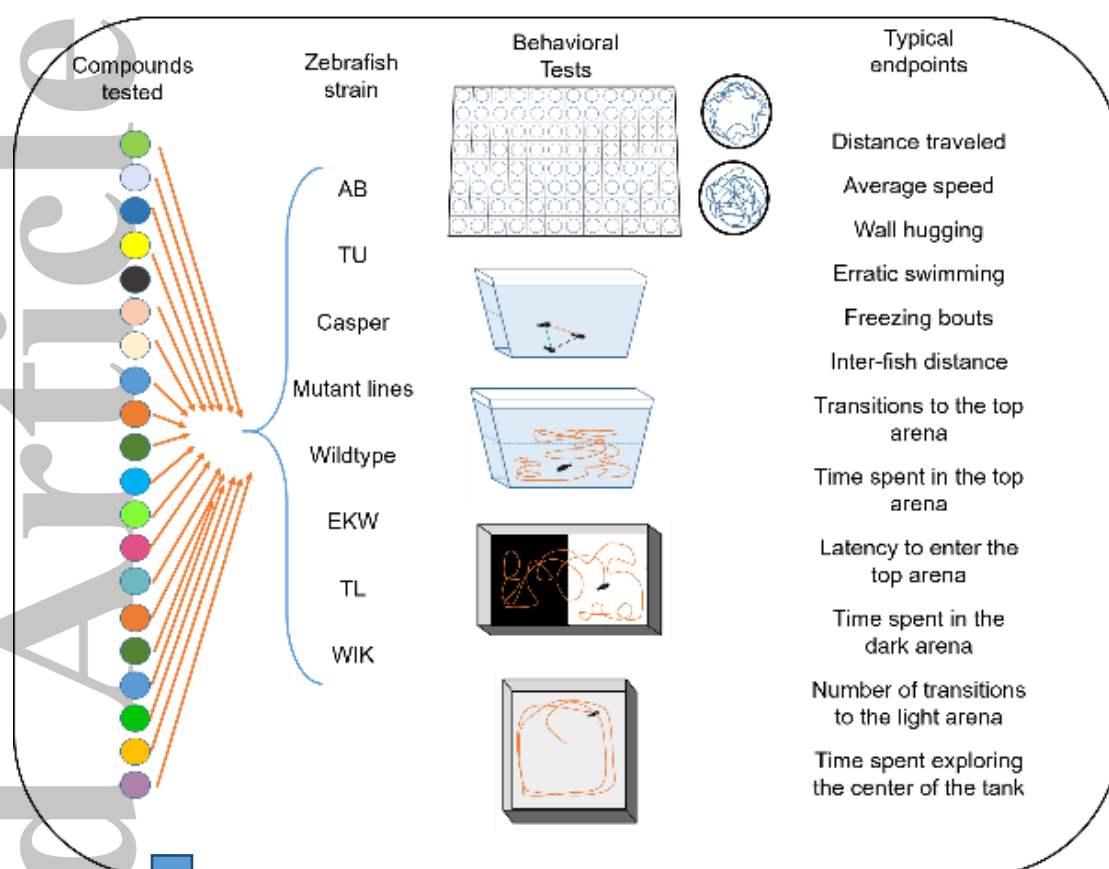


Figure 2. The use of computational techniques to identify novel therapeutic targets in adult zebrafish. Testing psychoactive compounds across various strains and transgenic lines of zebrafish in a wide range of behavioral and cognitive tasks can be used to generate a data library. Computational tools, such as hierarchical modeling and similarity ensemble approach (SEA), can help identify target hits, enabling the predictions about the effects of various drug combinations. The generated hypotheses may then be tested *in vivo* using larval or adult animals.

Generating data library



Identifying molecular targets, generating hypotheses for target combinations

Testing drug combinations in vivo

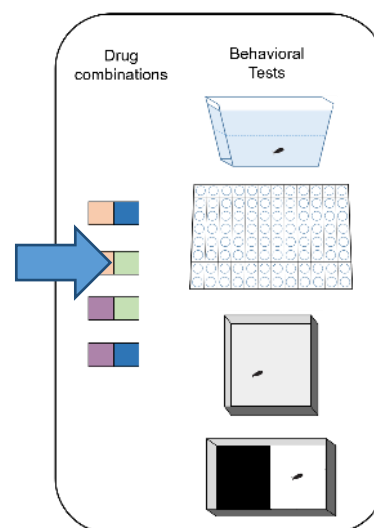
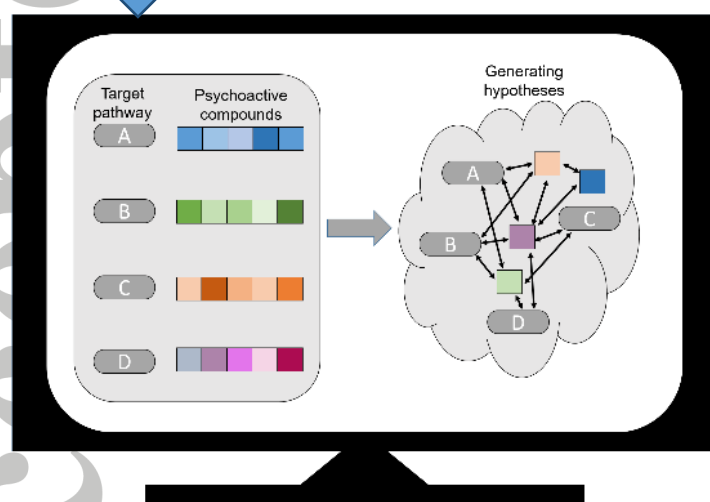


Table 1: Selected examples of translational successes using the zebrafish model for drug discovery

Human disease	Zebrafish model	Outcome	References
Pontocerebellar hyperplasia	<i>Tsen54</i> antisense morpholino	Linking a loss-of-function mutation in the <i>tsen54</i> gene to brain hypoplasia	(Kasher et al., 2011)
	<i>R44X</i> -loss-of-function mutant	Linking homozygous mutation of CLP1 (a member of the tRNA splicing endonuclease complex, TSEN) to abnormal spinal neurons, curved body, small head and eyes, and an early death in fish, helped identify this mutation as a risk factor for human condition	(Schaffer et al., 2014)
Spinal cord injury	<i>Heat shock transgenic lines</i>	Zebrafish show high capacity for axonal regeneration following spinal cord injury, especially through the activation of Fgf signaling. Increasing Fgf signaling in mammalian spinal injury sites may encourage glial cell differentiation, and lead to favorable conditions for axonal regeneration	(Goldshmit, Sztal, Jusuf, Hall, Nguyen-Chi & Currie, 2012)
Schizophrenia	Tg(<i>huC</i> :eGFP)	The <i>Rgs4</i> gene is associated with the onset and development of schizophrenia. Using the transgenic zebrafish line, <i>rgs4</i> was found to be essential for axon formation, providing the first in vivo evidence supporting the role of <i>rgs4</i> in schizophrenia	(Cheng et al., 2013)

References

Abreu MS, Giacomini AC, Kalueff AV, & Barcellos LJ (2016). The smell of “anxiety”: Behavioral modulation by experimental anosmia in zebrafish. *Physiology & behavior* 157: 67-71.

Anxiety Disorders Association of America Website. [Online] Available from ADAA.org. [Accessed].

Ahmed T, & Gilani A-H (2009). Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. *Pharmacology Biochemistry and Behavior* 91: 554-559.

Ahrens MB, Orger MB, Robson DN, Li JM, & Keller PJ (2013). Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nature methods* 10: 413-420.

Akhisaroglu M, Kurtuncu M, Manev H, & Uz T (2005). Diurnal rhythms in quinpirole-induced locomotor behaviors and striatal D2/D3 receptor levels in mice. *Pharmacology Biochemistry and Behavior* 80: 371-377.

Akindipe T, Wilson D, & Stein DJ (2014). Psychiatric disorders in individuals with methamphetamine dependence: prevalence and risk factors. *Metabolic Brain Disease* 29: 351-357.

Alfaro JM, Ripoll-Gómez J, & Burgos JS (2011). Kainate administered to adult zebrafish causes seizures similar to those in rodent models. *European Journal of Neuroscience* 33: 1252-1255.

Alsop D, & Vijayan M (2009). The zebrafish stress axis: molecular fallout from the teleost-specific genome duplication event. *General and comparative endocrinology* 161: 62-66.

Alsop D, & Vijayan MM (2008). Development of the corticosteroid stress axis and receptor expression in zebrafish. *American journal of physiology Regulatory, integrative and comparative physiology* 294: R711-719.

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders: DSM-5.ed.* Association A.P.: Washington, D.C.

Amores A, Catchen J, Ferrara A, Fontenot Q, & Postlethwait JH (2011). Genome Evolution and Meiotic Maps by Massively Parallel DNA Sequencing: Spotted Gar, an Outgroup for the Teleost Genome Duplication. *Genetics* 188: 799-808.

Amsterdam A, & Hopkins S (2006). Mutagenesis strategies in zebrafish for identifying genes involved in development and disease. *Trends in genetics* 22: 473-478.

Andrea Galimberti C, Magri F, Copello F, Arbasino C, Cravello L, Casu M, *et al.* (2005). Seizure frequency and cortisol and dehydroepiandrosterone sulfate (DHEAS) levels in women with epilepsy receiving antiepileptic drug treatment. *Epilepsia* 46: 517-523.

Armstrong GAB, Liao M, You Z, Lissouba A, Chen BE, & Drapeau P (2016). Homology Directed Knockin of Point Mutations in the Zebrafish *tardbp* and *fus* Genes in ALS Using the CRISPR/Cas9 System. *PloS one* 11: e0150188.

Arrenberg AB, Del Bene F, & Baier H (2009). Optical control of zebrafish behavior with halorhodopsin. *Proceedings of the National Academy of Sciences* 106: 17968-17973.

Ashburn TT, & Thor KB (2004). Drug repositioning: identifying and developing new uses for existing drugs. *Nature Reviews Drug Discovery* 3: 673-683.

Babaei F, Hong TLC, Yeung K, Cheng SH, & Lam YW (2016). Contrast-Enhanced X-ray Micro-Computed Tomography as a Versatile Method for Anatomical Studies of Adult Zebrafish. *Zebrafish*.

Babin PJ, Thisse C, Durliat M, Andre M, Akimenko M-A, & Thisse B (1997). Both apolipoprotein E and A-I genes are present in a nonmammalian vertebrate and are highly expressed during embryonic development. *Proceedings of the National Academy of Sciences* 94: 8622-8627.

Baraban SC, Dinday MT, & Hortopan GA (2013). Drug screening in *Scn1a* mutant zebrafish identifies clemzole as a potential Dravet syndrome treatment. *Nature Communications* 4.

Baraban SC, Taylor MR, Castro PA, & Baier H (2005). Pentylentetrazole induced changes in zebrafish behavior, neural activity and *c-fos* expression. *Neuroscience* 131: 759-768.

Barr AM, & Markou A (2005). Psychostimulant withdrawal as an inducing condition in animal models of depression. *Neuroscience biobehavior reviews* 29: 675-706.

Barros TP, Alderton WK, Reynolds HM, Roach aG, & Berghmans S (2008). Zebrafish: an emerging technology for in vivo pharmacological assessment to identify potential safety liabilities in early drug discovery. *British journal of pharmacology* 154: 1400-1413.

Baxendale S, Holdsworth CJ, Santoscoy PLM, Harrison MRM, Fox J, Parkin A, *et al.* (2012). Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures. *Disease Models & Mechanisms* 5: 773-784.

Belanoff JK, Gross K, Yager A, & Schatzberg AF (2001). Corticosteroids and cognition. *Journal of psychiatric research* 35: 127-145.

Bell E, Klimova TA, Eisenbart J, Moraes CT, Murphy MP, Buinger GRS, *et al.* (2007). The Qo site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production. *Journal of Cell Biology* 177: 1029-1036.

Bencan Z, Sledge D, & Levin ED (2009). Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *Pharmacology Biochemistry and Behavior* 94: 75-80.

Berk M, Malhi GS, Gray LJ, & Dean OM (2013). The promise of N-acetylcysteine in neuropsychiatry. *Trends in Pharmacological Sciences* 34: 167-177.

Blaker-Lee A, Gupta S, McCammon JM, De Rienzo G, & Sive HL (2012). Zebrafish homologs of genes within 16p11.2, a genomic region associated with brain disorders, are active during brain development, and include two deletion dosage sensor genes. *Disease Models & Mechanisms* 5: 834-851.

Blaser R, & Vira D (2014). Experiments on learning in zebrafish (*Danio rerio*): A promising model of neurocognitive function. *Neuroscience & Biobehavioral Reviews* 42: 224-231.

Braff DL, Geyer MA, & Swerdlow NR (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology* 156: 234-258.

Brennan CH (2011). Zebrafish behavioural assays of translational relevance for the study of psychiatric disease. *Reviews in the neurosciences* 22: 37-48.

Brown C, Schulberg HC, Madonia MJ, Shear MK, & Houck PR (1996). Treatment outcomes for primary care patients with major depression and lifetime anxiety disorders. *American Journal of Psychiatry* 153: 1293-1300.

Bruni G, Rennekamp AJ, Velenich A, McCarroll M, Gendele L, Fertsch E, *et al.* (2016). Zebrafish behavioral profiling identifies multitarget antipsychotic-like compounds. *Nature chemical biology*.

Buffalari DM, & See RE (2010). Amygdala mechanisms of Pavlovian psychostimulant conditioning and relapse. In *Behavioral Neuroscience of Drug Addiction*. Springer, pp 73-99.

Burgess HA, & Granato M (2007). Sensorimotor gating in larval zebrafish. *The Journal of neuroscience* 27: 4984-4994.

Bystritsky A (2006). Treatment-resistant anxiety disorders. *Molecular Psychiatry* 11: 805-814.

Cachat JM, Canavello PR, Elegante MF, Bartels BK, Hart PC, Bergner CL, *et al.* (2011a). Modeling withdrawal syndrome in zebrafish. *Behavioural Brain Research* 208: 371-376.

Cachat JM, Stewart A, Utterback E, Hart P, Gaikwad S, Wong K, *et al.* (2011b). Three-dimensional neurophenotyping of adult zebrafish behavior. *PloS one* 6: e17597.

Campbell WA, Yang H, Zetterberg H, Baulac S, Sears JA, Liu T, *et al.* (2006). Zebrafish lacking Alzheimer presenilin enhancer 2 (Pen-2) demonstrate excessive p53-dependent apoptosis and neuronal loss. *Journal of Neurochemistry* 96: 1423-1440.

Canavello PR, Cachat JM, Beeson EC, Laffoon AL, Grimes C, Haymore WA, *et al.* (2011). Measuring endocrine (cortisol) responses of zebrafish to stress. In Zebrafish neurobehavioral protocols. Springer, pp 135-142.

Carvill GL, Weckhuysen S, McMahon JM, Hartmann C, Moller RS, Hjalgrim H, *et al.* (2014). GABRA1 and STXBP1: Novel genetic causes of Dravet syndrome. *Neurology* 82: 1245-1253.

Caspi A, & Moffitt TE (2006). Gene–environment interactions in psychiatry: joining forces with neuroscience. *Nature Reviews Neuroscience* 7: 583-590.

Cheng Y-C, Scotting PJ, Hsu L-S, Lin S-J, Shih H-Y, Hsieh F-Y, *et al.* (2013). Zebrafish *rgs4* is essential for motility and axonogenesis mediated by Akt signaling. *Cellular and Molecular Life Sciences* 70: 935-950.

Ciura S, Lattante S, Le Ber I, Latouche M, Tostivint H, Brice A, *et al.* (2013). Loss of function of C9orf72 causes motor deficits in a zebrafish model of amyotrophic lateral sclerosis. *Annals of neurology* 74: 180-187.

Cognato GdP, Bortolotto JW, Blazina AR, Christoff RR, Lara DR, Vianna MR, *et al.* (2012). Y-Maze memory task in zebrafish (*Danio rerio*): the role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. *Neurobiology of learning and memory* 98: 321-328.

Collier AD, & Echevarria DJ (2013). The utility of the zebrafish model in conditioned place preference to assess the rewarding effects of drugs. *Behavioural Pharmacology* 24: 375-383.

Collier AD, Khan KM, Caramillo EM, Mohn RS, & Echevarria DJ (2014). Zebrafish and conditioned place preference: a translational model of drug reward. *Progress in neuro-psychopharmacology & biological psychiatry* 55: 16-25.

Colwill RM, Raymond J, Ferreira L, & Escudero H (2005). Visual discrimination learning in zebrafish (*Danio rerio*). *Behavior Processes* 70: 19-31.

Conklin EE, Lee KL, Schlabach SA, & Woods IG (2015). VideoHacking: Automated Tracking and Quantification of Locomotor Behavior with Open Source Software and Off-the-Shelf Video Equipment. *The Journal of Undergraduate Neuroscience Education (JUNE)* 13: A120-A125.

Cortese BM, & Phan KL (2005). The role of glutamate in anxiety and related disorders. *CNS Spectrum* 10: 820-830.

Cryan JF, & Holmes A (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nature reviews Drug discovery* 4: 775-790.

Cunliffe VT (2015). Building a zebrafish toolkit for investigating the pathobiology of epilepsy and identifying new treatments for epileptic seizures. *Journal of neuroscience methods*.

Cunliffe VT (2016). Building a zebrafish toolkit for investigating the pathobiology of epilepsy and identifying new treatments for epileptic seizures. *Journal of neuroscience methods* 260: 91-95.

Cunliffe VT, Baines RA, Giachello CNG, Lin W-H, Morgan A, Reuber M, *et al.* (2015). Epilepsy research methods update: Understanding the causes of epileptic seizures and identifying new treatments using non-mammalian model organisms. *Seizure* 24: 44-51.

Cutler C, Multani P, Robbins D, Kim HT, Le T, Hoggatt J, *et al.* (2013). Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. *Blood* 122: 3074-3081.

Danilova NP, Krupnik VE, Sugden D, & Zhdanova I (2004). Melatonin stimulates cell proliferation in zebrafish embryo and accelerates its development. *The FASEB Journal* 18: 751-753.

Darland T, & Dowling JE (2001). Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proceedings of the National Academy of Sciences* 98: 11691-11696.

De Campos EG, Bruni AT, & De Martinis BS (2015). Ketamine induces anxiolytic effects in adult zebrafish: A multivariate statistics approach. *Behavioural brain research*.

de Rienzo G, Gutzman JH, & Sive HL (2012). Efficient shRNA-Mediated Inhibition of Gene Expression in Zebrafish. *Zebrafish* 9: 97-107.

Dean OM, Giorlando F, & Berk M (2011). N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. *Journal of Psychiatry Neuroscience* 36: 78-86.

Desmond D, Kyzar E, Gaikwad S, Green J, Riehl R, Roth A, *et al.* (2012). Assessing epilepsy-related behavioral phenotypes in adult zebrafish. *Zebrafish protocols for neurobehavioral research*: 313-322.

Deussing JM (2006). Animal models of depression. *Drug Discovery Today: Disease Models* 3: 375-383.

Douglass AD, Kraves S, Deisseroth K, Schier AF, & Engert F (2008). Escape behavior elicited by single, channelrhodopsin-2-evoked spikes in zebrafish somatosensory neurons. *Current Biology* 18: 1133-1137.

Ek F, Malo M, Åberg Andersson M, Wedding C, Kronborg J, Svensson P, *et al.* (2016). Behavioral analysis of dopaminergic activation in zebrafish and rats reveals similar phenotypes. *ACS chemical neuroscience*.

Engel J (2013) *Seizures and epilepsy*. vol. 83. Oxford University Press.

Félix AS, Faustino AI, Cabral EM, & Oliveira RF (2013). Noninvasive measurement of steroid hormones in zebrafish holding-water. *Zebrafish* 10: 110-115.

Fetcho J, & O'Malley DM (1995). Visualization of active neural circuitry in the spinal cord of intact zebrafish. *Journal of neurophysiology* 73: 399-406.

Fibiger HC (1991). Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. *Trends in neurosciences* 14: 220-223.

Fonseka TM, Wen XY, Foster JA, & Kennedy SH (2016). Zebrafish models of major depressive disorders. *Journal of neuroscience research* 94: 3-14.

Friedrich RW, Jacobson GA, & Zhu P (2010). Circuit neuroscience in zebrafish. *Current Biology* 20: R371-R381.

Froyset AK, Khan EA, & Fladmark KE (2016). Quantitative proteomics analysis of zebrafish exposed to sub-lethal dosages of β -methyl-amino-L-alanine (BMAA). *Scientific Reports* 6.

Fulcher N, Tran S, Shams S, Chatterjee D, & Gerlai R (2016). Neurochemical and Behavioral Responses to Unpredictable Chronic Mild Stress Following Developmental Isolation: The Zebrafish as a Model for Major Depression. *Zebrafish*.

Fumagalli F, Molteni R, Racagni G, & Riva MA (2007). Stress during development: Impact on neuroplasticity and relevance to psychopathology. *Progress in neurobiology* 81: 197-217.

Furmark T, Tillfors M, Garpenstrand H, Marteinsdottir I, Långström B, Oreland L, *et al.* (2004). Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neuroscience letters* 362: 189-192.

Galecki P, Talarowska M, Bobińska K, & Szemraj J (2014). COX-2 gene expression is correlated with cognitive function in recurrent depressive disorder. *Psychiatry Research* 215: 488-490.

Garakani A, Mathew S, & Charney DS (2006). Neurobiology of anxiety disorders and implications for treatment. *Mount Sinai Journal of Medicine* 73: 941-949.

Gerlai R (2013). Antipredatory behavior of zebrafish: adaptive function and a tool for translational research. *Evolutionary psychology : an international journal of evolutionary approaches to psychology and behavior* 11: 591-605.

Gerlai R (2014). Social behavior of zebrafish: From synthetic images to biological mechanisms of shoaling. *Journal of neuroscience methods*.

Gerlai R (2016). Learning and memory in zebrafish (*Danio rerio*). *Methods in Cell Biology*.

Gerlai R, Lahav M, Guo S, & Rosenthal A (2000). Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacology, Biochemistry and Behavior* 67: 773-782.

Gerlai R, Lee V, & Blaser RE (2006). Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*). *Pharmacology, Biochemistry and Behavior* 85: 752-761.

Geyer MA, Krebs-Thomson K, Braff DL, & Swerdlow NR (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* 156: 117-154.

Giacomini NJ, Rose B, Kobayashi K, & Guo S (2006). Antipsychotics produce locomotor impairment in larval zebrafish. *Neurotoxicology and teratology* 28: 245-250.

Goessling W, Allen RS, Guan X, Jin P, Uchida N, Dovey N, *et al.* (2011). Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. *Cell stem cell* 8: 445-458.

Goldshmit Y, Sztal TE, Jusuf PR, Hall TE, Nguyen-Chi M, & Currie PD (2012). Fgf-Dependent Glial Cell Bridges Facilitate Spinal Cord Regeneration in Zebrafish. *The Journal of neuroscience* 32: 7477-7492.

Green J, Collins C, Kyzar EJ, Pham M, Roth A, Gaikwad S, *et al.* (2012). Automated high-throughput neurophenotyping of zebrafish social behavior. *Journal of neuroscience methods* 210: 266-271.

Griebel G, & Holmes A (2013a). 50 years of hurdles and hope in anxiolytic drug discovery. *Nature Reviews Drug Discovery* 12: 667-687.

Griebel G, & Holmes A (2013b). 50 years of hurdles and hope in anxiolytic drug discovery. *Nature reviews Drug discovery* 12: 667-687.

Griffiths BB, Schoonheim PJ, Ziv L, Voelker L, Baier H, & Gahtan E (2012a). A zebrafish model of glucocorticoid resistance shows serotonergic modulation of the stress response. *Frontiers in Behavioral Neuroscience* 6: 1-10.

Griffiths BB, Schoonheim PJ, Ziv L, Voelker L, Baier H, & Gahtan E (2012b). A zebrafish model of glucocorticoid resistance shows serotonergic modulation of the stress response. *Front Behav Neurosci* 6: 68.

Grone BP, & Baraban SC (2015). Animal models in epilepsy research: legacies and new directions. *Nature neuroscience* 18: 339-343.

Grone BP, Marchese M, Hamling KR, Kumar MG, Krasniak CS, Sicca F, *et al.* (2016). Epilepsy, Behavioral Abnormalities, and Physiological Comorbidities in Syntaxin-Binding Protein 1 (STXBP1) Mutant Zebrafish. *PloS one* 11: e0151148.

Gupta (2014). Assessment of locomotion behavior in adult Zebrafish after acute exposure to different pharmacological reference compounds. In Drug Development and Therapeutics. Medknow Publications and Media Pvt. Ltd., p 127.

Haesemeyer M, & Schier AF (2015). The study of psychiatric disease genes and drugs in zebrafish. *Current opinion in neurobiology* 30: 122-130.

Havsteen BH (2002). The biochemistry and medical significance of the flavonoids. *Pharmacology & therapeutics* 96: 67-202.

Hek K, Direk N, Newson RS, Hofman A, Hoogendijk WJ, Mulder CL, *et al.* (2013). Anxiety disorders and salivary cortisol levels in older adults: a population-based study. *Psychoneuroendocrinology* 38: 300-305.

Herbert J (2013). Cortisol and depression: three questions for psychiatry. *Psychological medicine* 43: 449-469.

Hikida T, Kitabatake Y, Pastan I, & Nakanishi S (2003). Acetylcholine enhancement in the nucleus accumbens prevents addictive behaviors of cocaine and morphine. *Proceedings of the National Academy of Sciences* 100: 6169-6173.

Holsboer F (2001). Stress, hypercortisolism and corticosteroid receptors in depression: implicatons for therapy. *Journal of affective disorders* 62: 77-91.

Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, *et al.* (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 496: 498-503.

Howell KR, Kutiyawalla A, & Pillai A (2011). Long-Term Continuous Corticosterone Treatment Decreases VEGF Receptor-2 Expression in Frontal Cortex. *PLoS One* 6: e20198.

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, *et al.* (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature biotechnology* 31: 227-229.

Idalencio R, Kalichak F, Rosa JGS, de Oliveira TA, Koakoski G, Gusso D, *et al.* (2015). Waterborne risperidone decreases stress response in zebrafish. *PloS one* 10: e0140800.

Insel TR, Voon V, Nye JS, Brown VJ, Altevogt BM, Bullmore ET, *et al.* (2011). Innovative solutions to novel drug development in mental health. *Neuroscience & Biobehavioral Reviews* 37.

Irons T, Kelly P, Hunter D, Macphail R, & Padilla S (2013). Acute administration of dopaminergic drugs has differential effects on locomotion in larval zebrafish. *Pharmacology Biochemistry and Behavior* 103: 792-813.

Jesuthasan S (2012). Fear, anxiety, and control in the zebrafish. *Developmental neurobiology* 72: 395-403.

Jones LJ, & Norton W (2015). Using zebrafish to uncover the genetic and neural basis of aggression, a frequent comorbid symptom of psychiatric disorders. *Behavioural Brain Research* 276: 171-180.

Kalueff AV, Echevarria D, & Stewart AM (2014a). Gaining translational momentum: More zebrafish models for neuroscience research. *Progress in neuro-psychopharmacology & biological psychiatry*.

Kalueff AV, Echevarria DJ, & Stewart AM (2014b). Gaining translational momentum: More zebrafish models for neuroscience research. *Progress in neuro-psychopharmacology & biological psychiatry*.

Kalueff AV, Gebhardt M, Stewart AM, Cachat JM, Brimmer M, Chawla JS, *et al.* (2013a). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish* 10: 70-86.

Kalueff AV, Gebhardt M, Stewart AM, Cachat JM, Brimmer M, Chawla JS, *et al.* (2013b). Towards a Comprehensive Catalog of Zebrafish Behavior 1.0 and Beyond. *Zebrafish* 10: 70-86.

Kalueff AV, Stewart AM, & Gerlai R (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in pharmacological sciences* 35: 63-75.

Kane J, Honigfeld G, Singer J, & Meltzer H (1988). Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Archives of General Psychiatry* 45: 789-796.

Kasher PR, Namavar Y, van Tjin P, Fluiter K, Sizarov A, Kamermans M, *et al.* (2011). Impairment of the tRNA-splicing endonuclease subunit 54 (*tsen54*) gene causes neurological abnormalities and larval death in zebrafish models of pontocerebellar hypoplasia. *Human molecular genetics* 20: 1574-1580.

Kaslin J, & Panula P (2001). Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). *Journal of Comparative Neurology* 440: 342-377.

Keedwell PA, Poon L, Papadopoulos AS, Marshall E, & Checkley SA (2001). Salivary cortisol measurements during a medically assisted alcohol withdrawal. *Addiction biology* 6: 247-257.

Keifer J, & Summers CH (2016). Putting the “Biology” Back into “Neurobiology”: The Strength of Diversity in Animal Model Systems for Neuroscience Research. *Frontiers in Systems Neuroscience* 10: 69.

Kendler KS, Aggen SH, & Neale MC (2013). Evidence for multiple genetic factors underlying dsm-iv criteria for major depression. *JAMA Psychiatry* 70: 599-607.

Kessler RC (2007). The global burden of anxiety and mood disorders: Putting ESEMeD findings into perspective. *Journal of Clinical Psychiatry* 68: 10-19.

Kessler RC, Chiu WT, Demler O, Merikangas KR, & Walters EE (2005). Prevalence severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. . *Archives of General Psychiatry* 62: 617-627.

Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardimn O, *et al.* (2011). Amyotrophic lateral sclerosis. *The Lancet* 377: 942-955.

Kily LJ, Cowe YCM, Hussain O, Patel S, McElwaine S, Cotter FE, *et al.* (2008). Gene expression changes in a zebrafish model of drug dependency suggest conservation of neuro-adaptation pathways. *Journal of Experimental Biology* 211: 1623-1634.

Kim Y-H, Lee Y, Kim D, Jung MW, & Lee C-J (2010). Scopolamine-induced learning impairment reversed by physostigmine in zebrafish. *Neuroscience research* 67: 156-161.

Knafo S, & Wyart C (2015). Optogenetic neuromodulation: New tools for monitoring and breaking neural circuits. *Annals of Physical and Rehabilitation Medicine* 58: 259-264.

Kok FO, Shin M, Ni C-W, Gupta A, Grosse AS, van Impel A, *et al.* (2015). Reverse Genetic Screening Reveals Poor Correlation between Morpholino-Induced and Mutant Phenotypes in Zebrafish. *Developmental Cell* 31: 97-108.

Kokel D, Bryan J, Laggner C, White R, Cheung CYJ, Mateus R, *et al.* (2010). Rapid behavior-based identification of neuroactive small molecules in the zebrafish. *Nature chemical biology* 6: 231-237.

Koob GF (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *TIPS* 13: 177-184.

Korte S (2001). Corticosteroids in relation to fear, anxiety and psychopathology. *Neuroscience & Biobehavioral Reviews* 25: 117-142.

Krishnan V, & Nestler EJ (2011). Animal Models of Depression: Molecular Perspectives. *Current Topics in Behavioral Neuroscience* 7: 121-147.

Kubo F, Hablitzel B, Dal Maschio M, Driever W, Baier H, & Arrenberg AB (2014). Functional architecture of an optic flow-responsive area that drives horizontal eye movements in zebrafish. *Neuron* 81: 1344-1359.

Kumari V, Soni W, & Sharma T (1999). Normalization of Information Processing Deficits in Schizophrenia With Clozapine. *The American Journal of Psychiatry* 156: 1046-1051.

- Kyzar E, Stewart AM, Landsman S, Collins C, Gebhardt M, Robinson K, *et al.* (2014). Behavioral effects of bidirectional modulation of brain monoamines by reserpine and d-amphetamine in zebrafish. *Brain research* 1527: 108-116.
- Kyzar EJ, & Kalueff AV (2016). Exploring Hallucinogen Pharmacology and Psychedelic Medicine with Zebrafish Models. *Zebrafish*.
- Lara DR, & Souza DO (2000). Schizophrenia: a purinergic hypothesis. *Medical Hypotheses* 54: 157-166.
- Lau B, Bretaud S, Huang Y, Lin E, & Guo S (2006). Dissociation of food and opiate preference by a genetic mutation in zebrafish. *Genes, brain, and behavior* 5: 497-505.
- Levin ED, Bencan Z, & Cerutti DT (2007). Anxiolytic effects of nicotine in zebrafish. *Physiology & behavior* 90: 54-58.
- Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, *et al.* (2005). Effectiveness of Antipsychotic Drugs in Patients with Chronic Schizophrenia. *The New England Journal of Medicine* 353: 1209-1223.
- Ljunggren EE, Haupt S, Ausborn J, Ampatzis K, & El Manira A (2014). Optogenetic activation of excitatory premotor interneurons is sufficient to generate coordinated locomotor activity in larval zebrafish. *The Journal of Neuroscience* 34: 134-139.
- Loeblich S, & Nedivi E (2009). The function of activity-regulated genes in the nervous system. *Physiological reviews* 89: 1079-1103.
- Lombardo S, & Maskos U (2015). Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. *Neuropharmacology* 96: 255-262.
- López-Patiño M, Yu L, Cabral EM, & Zhdanova I (2008). Anxiogenic effects of cocaine withdrawal in zebrafish. *Physiology & behavior* 93: 160-171.
- Lovallo WR (2006). Cortisol secretion patterns in addiction and addiction risk. *International Journal of Psychophysiology* 59: 195-202.
- Love DR, Pichler FB, Dodd A, Copp BR, & Greenwood DR (2004). Technology for high-throughput screens: the present and future using zebrafish. *Current opinion in biotechnology* 15: 564-571.
- Lucassen P, Oomen C, Schouten M, Encinas J, & Fitzsimons C (2016). Adult Neurogenesis, Chronic Stress and Depression. *Adult Neurogenesis in the Hippocampus: Health, Psychopathology, and Brain Disease*: 177.
- Lucke-Wold B (2011). The Varied Uses of Conditioned Place Preference in Behavioral Neuroscience Research: An Investigation of Alcohol Administration in Model Organisms. *Impulse* (Columbia, SC) 2011.

Lundegaard PR, Anastasaki C, Grant NJ, Sillito RR, Zich J, Zeng Z, *et al.* (2015). MEK Inhibitors Reverse cAMP-Mediated Anxiety in Zebrafish. *Chemistry & Biology* 22: 1335-1346.

Magno LDP, Fontes A, Gonçalves BMN, & Gouveia A (2015). Pharmacological study of the light/dark preference test in zebrafish (*Danio rerio*): Waterborne administration. *Pharmacology Biochemistry and Behavior* 135: 169-176.

Mahmood F, Fu S, Cooke J, Wilson SW, Cooper JD, & Russell C (2013). A zebrafish model of CLN2 disease is deficient in tripeptidyl peptidase 1 and displays progressive neurodegeneration accompanied by a reduction in proliferation *Brain* 136: 1488-1507.

Marcon M, Herrmann AP, Mocelin R, Rambo CL, Koakoski G, Abreu MS, *et al.* (2016). Prevention of unpredictable chronic stress-related phenomena in zebrafish exposed to bromazepam, fluoxetine and nortriptyline. *Psychopharmacology* 233: 3815-3824.

Martín I, Gómez A, Salas C, Puerto A, & Rodríguez F (2011). Dorsomedial pallium lesions impair taste aversion learning in goldfish. *Neurobiology of Learning and Memory* 96: 297-305.

Mathur P, Berberoglu MA, & Guo S (2011). Preference for ethanol in zebrafish following a single exposure. *Behavioral Brain Research* 217: 128-133.

Mathur P, & Guo S (2010). Use of zebrafish as a model to understand mechanisms of addiction and complex neurobehavioral phenotypes. *Neurobiology of disease* 40: 66-72.

Maximino C, & Herculano AM (2010). A review of monoaminergic neuropsychopharmacology in zebrafish. *Zebrafish* 7: 359-378.

McCammon JM, & Sive H (2015). Challenges in understanding psychiatric disorders and developing therapeutics: a role for zebrafish. *Disease Models and Mechanisms* 8: 647-656.

McCarroll M, Gendele L, Keiser M, & Kokel D (2016). Leveraging large-scale behavioral profiling in zebrafish to explore neuroactive polypharmacology. *ASC Chemical Biology* 11: 842-849.

McGown A, McDermid JR, Panagiotaki N, Tong H, Al Mashhadi S, Redhead N, *et al.* (2013). Early interneuron dysfunction in ALS: insights from a mutant *sod1* zebrafish model. *Annals of neurology* 73: 246-258.

Mead AN, Vasilaki A, Spyraiki C, Duka T, & Stephens DN (1999). AMPA-receptor involvement in c-fos expression in the medial prefrontal cortex and amygdala dissociates neural substrates of conditioned activity and conditioned reward. *European Journal of Neuroscience* 11: 4089-4098.

Merritt K, McGuire P, & Egerton A (2013). Relationship between glutamate dysfunction and symptoms and cognitive function in psychosis. *Frontiers in Psychiatry*: 86.

Meyer A, & Schartl M (1999). Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions. *Current opinion in cell biology* 11: 699-704.

Mi G, Gao Y, Yan H, Jin X, Ye E, Liu S, *et al.* (2016). l-Scoulerine attenuates behavioural changes induced by methamphetamine in zebrafish and mice. *Behavioral Brain Research* 298: 97-104.

Miyamoto S, Duncan GE, Marx CE, & Lieberman JA (2005). Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Molecular Psychiatry* 10: 79-104.

Mobini S, Chiang T-J, Ho M-Y, Bradshaw C, & Szabadi E (2000). Comparison of the effects of clozapine, haloperidol, chlorpromazine and d-amphetamine on performance on a time-constrained progressive ratio schedule and on locomotor behaviour in the rat. *Psychopharmacology* 152: 47-54.

Mocelin R, Herrmann AP, Marcon M, Rambo CL, Rohden A, Bevilaqua F, *et al.* (2015). N-acetylcysteine prevents stress-induced anxiety behavior in zebrafish. *Pharmacology, Biochemistry and Behavior* 139: 121-126.

Moghaddam B, & Jackson ME (2003). Glutamatergic animal models of schizophrenia. *Annals of the New York Academy of Sciences* 1003: 131-137.

Moussavi Nik SH, Croft K, Mori TA, & Lardelli M (2014). The Comparison of Methods for Measuring Oxidative Stress in Zebrafish Brains. *Zebrafish* 11: 248-254.

Moussavi Nik SH, Newman M, & Lardelli M (2011). The response of HMGA1 to changes in oxygen availability is evolutionarily conserved. *Experimental Cell Research* 317: 1503-1512.

Mueller T, Dong Z, Berberoglu MA, & Guo S (2011). The dorsal pallium in zebrafish, *Danio rerio* (Cyprinidae, Teleostei). *Brain research* 1381: 95-105.

Mueller T, & Wullimann M (2015) *Atlas of early zebrafish brain development: a tool for molecular neurogenetics*. Academic Press.

Nasevicius A, & Ekker SC (2000). Effective targeted gene 'knockdown' in zebrafish. *Nature Genetics* 26: 216-220.

Nestler EJ (2013). The origins of molecular psychiatry. *Journal of molecular psychiatry* 1: 1-2.

Newman M, Verdile G, Martins RN, & Lardelli M (2011). Zebrafish as a tool in Alzheimer's disease research. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1812: 346-352.

Nguyen M, Stewart AM, & Kalueff AV (2014). Aquatic blues: Modeling depression and antidepressant action in zebrafish. *Progress in neuro-psychopharmacology & biological psychiatry*.

Ninkovic J, & Bally-Cuif L (2006). The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. *Methods* 39: 262-274.

Ninkovic J, Folchert A, Makhankov YV, Neuhauss SCF, Sillaber I, Straehle U, *et al.* (2006). Genetic identification of AChE as a positive modulator of addiction to the psychostimulant D-amphetamine in zebrafish. *Journal of Neurobiology* 66: 463-475.

Daniovision. [Online] Available from <http://www.noldus.com/animal-behavior-research/products/daniovision>. [Accessed].

Shoaling behavior in zebrafish. [Online] Available from <http://www.noldus.com/animal-behavior-research/solutions/research-fish/shoaling-behavior>. [Accessed].

Nornes S, Casper G, Esther C, Ey P, & Lardelli M (2003). Developmental control of Presenilin1 expression, endoproteolysis, and interaction in zebrafish embryos. *Experimental Cell Research* 289: 124-132.

Nornes S, Newman M, Wells S, Verdile G, Martins RN, & Lardelli M (2009). Independent and cooperative action of Psen2 with Psen1 in zebrafish embryos. *Experimental Cell Research* 315: 2791-2801.

North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, *et al.* (2007). Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* 447: 1007-1011.

Northcutt RG (2008). Forebrain evolution in bony fishes. *Brain Research Bulletin* 18: 191-205.

Orger MB, & Portugues R (2016). Correlating Whole Brain Neural Activity with Behavior in Head-Fixed Larval Zebrafish. *Methods in molecular biology* (Clifton, NJ) 1451: 307.

Otto MW, Pollack MH, Sachs GS, Reiter SR, Meltzer-Brody S, & Rosenbaum JF (1993). Discontinuation of benzodiazepine treatment: efficacy of cognitive-behavioral therapy for patients with panic disorder. *American Journal of Psychiatry* 150: 1485-1490.

Pando M, & Sassone-Corsi P (2002). Unraveling the mechanisms of the vertebrate circadian clock: zebrafish may light the way. *BioEssays* 24: 419-426.

Pannia E, Tran S, Rampersad M, & Gerlai R (2014). Acute ethanol exposure induces behavioural differences in two zebrafish (*Danio rerio*) strains: a time course analysis. *Behavioral Brain Research* 259: 174-185.

Panula P, Chen Y-C, Priyadarshini M, Kudo H, Semenova S, Sundvik M, *et al.* (2010). The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. *Neurobiology of disease* 40: 46-57.

Panula P, Sallinen V, Sundvik M, Kolehmainen J, Torkko V, Tiittula A, *et al.* (2006). Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases. *Zebrafish* 3: 235-247.

Park E, Lee Y, Kim YK, & Lee CJ (2008). Cholinergic modulation of neural activity in the telencephalon of the zebrafish. *Neuroscience letters* 439: 79-83.

Parker MO, Brock AJ, Walton RT, & Brennan CH (2013). The role of zebrafish (*Danio rerio*) in dissecting the genetics and neural circuits of executive function. *Frontiers in Neural Circuits* 7: 1-13.

Pavlidis M, Sundvik M, Chen Y-C, & Panula P (2011). Adaptive changes in zebrafish brain in dominant–subordinate behavioral context. *Behavioural brain research* 225: 529-537.

Pavlidis M, Theodoridi A, & Tsalafouta A (2015). Neuroendocrine regulation of the stress response in adult zebrafish, *Danio rerio*. *Progress in neuro-psychopharmacology & biological psychiatry*.

Pérez-Escudero A, Vicente-Page J, Hinz RC, Arganda S, & de Polavieja GG (2014). idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nature Methods* 11: 743-748.

Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH, & Perry RH (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br Med J* 2: 1457-1459.

Petzold AM, Balciunas D, Sivasubbu S, Clark K, J., Bedell VM, Westcot SE, *et al.* (2009). Nicotine response genetics in the zebrafish. *Proceedings of the National Academy of Sciences* 106: 18662-18667.

Piato AL, Capiotti KM, Tamborski AR, Oses JP, Barcellos LJG, Bogo MR, *et al.* (2011). Unpredictable chronic stress model in zebrafish (*Danio rerio*): Behavioral and physiological responses. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 35: 561-567.

Plaut I (2000). Effects of fin size on swimming performance, swimming behaviour and routine activity of zebrafish *danio rerio*. *The Journal of Experimental Biology* 203: 813-820.

Porsolt RD (2000). Animal models of depression: utility for transgenic research. *Reviews in the neurosciences* 11: 53-58.

Portavella M, Torres B, & Salas C (2004). Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *The Journal of Neuroscience* 24: 2335-2342.

Purdie EL, Samsudin S, Eddy FB, & Codd GA (2009). Effects of the cyanobacterial neurotoxin β -N-methylamino-L-alanine on the early-life stage development of zebrafish (*Danio rerio*). *Aquatic toxicology* 95: 279-284.

Purushothaman S, Saxena S, Meghah V, Lakshmi M, Singh SK, C. S, *et al.* (2015). Proteomic and gene expression analysis of zebrafish brain undergoing continuous light/dark stress. *Journal of sleep research*.

Qin M, Wong A, Seguin D, & Gerlai R (2014). Induction of social behavior in zebrafish: live versus computer animated fish as stimuli. *Zebrafish* 11: 185-197.

Ragozzino ME, Detrick S, & Kesner RP (1999). Involvement of the prelimbic–infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. *Journal of Neuroscience* 19: 4585-4594.

Ramesh T, Lyon AN, Pineda RH, Wang C, Janssen PM, Canan BD, *et al.* (2010). A genetic model of amyotrophic lateral sclerosis in zebrafish displays phenotypic hallmarks of motoneuron disease. *Disease Models and Mechanisms* 3: 652-662.

Rao KD, Alex A, Verma Y, Thampi S, & Gupta PK (2009). Real-time in vivo imaging of adult Zebrafish brain using optical coherence tomography. *Journal of biophotonics* 2: 288-291.

Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, *et al.* (2000). Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biological psychiatry* 47: 769-776.

Renier C, Faraco JH, Bourgin P, Motley T, Bonaventure P, Rosa F, *et al.* (2007). Genomic and functional conservation of sedative-hypnotic targets in the zebrafish. *Pharmacogenetics and genomics* 17: 237-253.

Richendrfer H, Pelkowski SD, Colwill RM, & Creton R (2012). On the edge: pharmacological evidence for anxiety-related behavior in zebrafish larvae. *Behavioural Brain Research* 228: 99-106.

Richetti S, Blank M, Capiotti K, Piatto A, Bogo M, Vianna M, *et al.* (2011). Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. *Behavioural Brain Research* 217: 10-15.

Rihel J, Prober D, Arvanites A, Lam K, Zimmerman S, Jang S, *et al.* (2010). Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science* 327: 348-351.

Rosemberg DB, Braga MM, Rico EP, Loss CM, Córdova SD, Mussulini BHM, *et al.* (2012). Behavioral effects of taurine pretreatment in zebrafish acutely exposed to ethanol. *Neuropharmacology* 63: 613-623.

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, *et al.* (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362: 59-62.

Rossor MN, Iversen LL, Reynolds GP, Mountjoy CQ, & Roth M (1984). Neurochemical characteristics of early and late onset types of Alzheimer's disease. *British medical journal* 288: 961-964.

Rowland LP, & Shneider NA (2001). Amyotrophic Lateral Sclerosis. *The New England Journal of Medicine* 344: 1688-1700.

Saito H, Kato M, Mizuguchi T, Hamada K, Osaka H, Tohyama J, *et al.* (2008). De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nature Genetics* 40: 782-788.

Sallinen J, Torkko V, Sundvik M, Reenila I, Khrustalyov D, Kaslin J, *et al.* (2009). MPTP and MPP⁺ target specific aminergic cell populations in larval zebrafish. *Journal of Neurochemistry* 108: 719-731.

Saus E, Brunet A, Armengol I, Alonso P, Crespo JM, Fernandez-Aranda F, *et al.* (2010). Comprehensive copy number variant (CNV) analysis of neuronal pathways genes in psychiatric disorders identifies rare variants within patients. *Journal of psychiatric research* 44: 971-978.

Saverino C, & Gerlai R (2008). The social zebrafish: Behavioral responses to conspecific, heterospecific, and computer animated fish. *Behavioral Brain Research* 191: 77-87.

Schaffer AE, Eggens VRC, Caglayan AO, Reuter MS, Scott E, Coufal NG, *et al.* (2014). CLP1 Founder Mutation Links tRNA Splicing and Maturation to Cerebellar Development and Neurodegeneration. *Cell* 157: 651-663.

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511: 421-427.

Schobel SA, Chaudhury NH, Khan UA, Paniagua B, Styner MA, Asllani I, *et al.* (2013). Imaging patients with psychosis and a mouse model establishes a spreading pattern of hippocampal dysfunction and implicates glutamate as a driver. *Neuron* 78: 81-93.

Seibt KJ, da Luz Oliveira R, Bogo MR, Senger MR, & Bonan CD (2015). Investigation into effects of antipsychotics on ectonucleotidase and adenosine deaminase in zebrafish brain. *Fish physiology and biochemistry* 41: 1383-1392.

Seibt KJ, da Luz Oliveira R, Zimmermann FF, Capiotti KM, Bogo MR, Ghisleni G, *et al.* (2010). Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*). *Behavioural brain research* 214: 417-422.

- Seibt KJ, Piatto AL, da Luz Oliveira R, Capiotti KM, Vianna MRM, & Bonan CD (2011). Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (*Danio rerio*). *Behavioural Brain Research* 224: 135-139.
- Seligman ME, & Beagley G (1975). Learned helplessness in the rat. *Journal of Comparative and Physiological Psychology* 88: 534-541.
- Seligman ME, Rosellini RA, & Kozak MJ (1975). Learned helplessness in the rat: time course, immunization, and reversibility. *Journal of Comparative and Physiological Psychology* 88: 542-547.
- Selkoe DJ (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiological reviews* 81: 741-766.
- Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, & Tonegawa S (1997). Skeletal and CNS Defects in Presenilin-1-Deficient Mice. *Cell* 89: 629-639.
- Shin LM, Rauch SL, & Pitman RK (2006). Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Annals of the New York Academy of Sciences* 1071: 67-79.
- Smith SM, & Vale WW (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience* 8: 383-395.
- Spencer JP (2008). Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. *Proceedings of the Nutrition Society* 67: 238-252.
- Stein MB, Goldin PR, Sareen J, Zorrilla LT, & Brown GG (2002). Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch Gen Psychiatry* 59: 1027-1034.
- Stewart AM, Braubach O, Spitsbergen J, Gerlai R, & Kalueff AV (2014a). Zebrafish models for translational neuroscience research: from tank to bedside. *Trends Neurosci* 37: 264-278.
- Stewart AM, Braubach O, Spitsbergen J, Gerlai R, & Kalueff AV (2014b). Zebrafish models for translational neuroscience research: from tank to bedside. . *Trends in neurosciences* 37: 264-278.
- Stewart AM, Cachat J, Gaikwad S, Robinson KS, Gebhardt M, & Kalueff AV (2013). Perspectives on experimental models of serotonin syndrome in zebrafish. *Neurochemistry international* 62: 893-902.
- Stewart AM, Gaikwad S, Kyzar E, Green J, Roth A, & Kalueff AV (2012). Modeling anxiety using adult zebrafish: a conceptual review. *Neuropharmacology* 62: 135-143.
- Stewart AM, Gerlai R, & Kalueff AV (2015). Developing highER-throughput zebrafish screens for in-vivo CNS drug discovery. *Frontiers in behavioral neuroscience* 9.

Stewart AM, Grieco F, Tegelenbosch RA, Kyzar EJ, Nguyen M, Kaluyeva A, *et al.* (2015a). A novel 3D method of locomotor analysis in adult zebrafish: Implications for automated detection of CNS drug-evoked phenotypes. *Journal of neuroscience methods* 255: 66-74.

Stewart AM, Grossman L, Nguyen M, Maximino C, Rosemberg DB, Echevarria DJ, *et al.* (2014). Aquatic toxicology of fluoxetine: Understanding the knowns and the unknowns. *Aquatic toxicology* (Amsterdam, Netherlands).

Stewart AM, Ullmann JFP, Norton WHJ, Parker MO, Brennan CH, Gerlai R, *et al.* (2015b). Molecular psychiatry of zebrafish. *Molecular psychiatry* 20: 2-17.

Stewart AM, Wong K, Cachat JM, Gaikwad S, Kyzar E, Wu N, *et al.* (2011). Zebrafish models to study drug abuse-related phenotypes. *Reviews in the neurosciences* 22: 95-105.

Strüber N, Strüber D, & Roth G (2014). Impact of early adversity on glucocorticoid regulation and later mental disorders. *Neuroscience & Biobehavioral Reviews* 38: 17-37.

Swain H, Sigstad C, & Scalzo (2004). Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). *Neurotoxicology and teratology* 26: 725-729.

Swerdlow NR, Geyer MA, & Braff DL (2001). Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology* 156: 194-215.

Teng Y, Xie X, Walker S, Rempala G, Kozlowski D, Mumm J, *et al.* (2010). Knockdown of zebrafish *Lgi1a* results in abnormal development, brain defects and a seizure-like behavioral phenotype *Human molecular genetics* 19: 4409-4420.

Tran S, Chatterjee D, & Gerlai R (2015). An integrative analysis of ethanol tolerance and withdrawal in zebrafish (*Danio rerio*). *Behavioural brain research* 276: 161-170.

Tran S, & Gerlai R (2013). Time-course of behavioural changes induced by ethanol in zebrafish (*Danio rerio*). *Behavioral Brain Research* 252: 204-213.

Tye KM, & Deisseroth K (2012). Optogenetic investigation of neural circuits underlying brain disease in animal models. *Nature Reviews Neuroscience* 13: 251-266.

Ullmann JF, Cowin G, Kurniawan ND, & Collin SP (2010). A three-dimensional digital atlas of the zebrafish brain. *Neuroimage* 51: 76-82.

Vaccaro A, Patten SA, Aggad D, Julien C, Maios C, Kabashi E, *et al.* (2013). Pharmacological reduction of ER stress protects against TDP-43 neuronal toxicity in vivo. *Neurobiology of disease* 55: 64-75.

Vaccaro A, Patten SA, Ciura S, Maios C, Therrien M, Drapeau P, *et al.* (2012). Methylene blue protects against TDP-43 and FUS neuronal toxicity in *C. elegans* and *D. rerio*. . PLoS One 7: e42117.

Valdmanis PN, & Rouleau GA (2008). Genetics of familial amyotrophic lateral sclerosis. *Neurology* 70: 144-152.

Vatine G, Vallone D, Gothilf Y, & Foulkes N (2011). It's time to swim! Zebrafish and the circadian clock. *FEBS Letters* 4: 19.

Voisin T, & Vellas B (2009). Diagnosis and treatment of patients with severe Alzheimer's disease. *Drugs Aging* 26: 135-144.

von Trotha JW, Vernier P, & Bally-Cuif L (2014). Emotions and motivated behavior converge on an amygdala-like structure in the zebrafish. *The European journal of neuroscience*.

Wahbeh H, Kishiyama SS, Zajdel D, & Oken BS (2008). Salivary cortisol awakening response in mild Alzheimer disease, caregivers, and noncaregivers. *Alzheimer Disease & Associated Disorders* 22: 181-183.

Wang H (2008). Comparative analysis of *Period* genes in teleost fish genomes. *Journal of molecular evolution* 67: 29-40.

Wang Y, Li S, Liu W, Wang F, Hu L-F, Zhong Z-m, *et al.* (2016a). Vesicular Monoamine Transporter 2 (Vmat2) knockdown elicits anxiety-like behavior in zebrafish. *Biochemical and Biophysical Research Communications*.

Wang Y, Li S, Liu W, Wang F, Hu L-F, Zhong Z, *et al.* (2016b). Vesicular monoamine transporter 2 (Vmat2) knockdown elicits anxiety-like behavior in zebrafish. *Biochemical and Biophysical Research Communications* 470: 792-797.

Wang Y, Takai R, Yoshioka H, & Shirabe K (2006). Characterization and Expression of Serotonin Transporter Genes in Zebrafish. *Journal of Experimental Medicine* 208: 267-274.

WHO (2016). Epilepsy (Fact sheet No. 999). World Health Organization.

Wienholds E, van Eeden F, Kusters M, Mude J, Plasterk RHA, & Cuppin E (2003). Efficient Target-Selected Mutagenesis in Zebrafish. *Genome Research* 13: 2700-2707.

Winter MJ, Redfern WS, Hayfield AJ, Owen SF, Valentin J-P, & Hutchinson TH (2008). Validation of a larval zebrafish locomotor assay for assessing the seizure liability of early-stage development drugs. *Journal of Pharmacological and Toxicological Methods* 57: 176-187.

Wong K, Stewart AM, Gilder T, Wu N, Frank K, Gaikwad S, *et al.* (2010). Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish. *Brain research* 1348: 209-215.

Wulliman MF, Rupp B, & Reichert H (2012) *Neuroanatomy of the zebrafish brain: a topological atlas*. Birkhäuser.

Wyart C, & Del Bene F (2011). Let there be light: zebrafish neurobiology and the optogenetic revolution. *Reviews in the neurosciences* 22: 121-130.

Wyatt C, Bartoszek EM, & Yaksi E (2015). Methods for studying the zebrafish brain: past, present and future. *European Journal of Neuroscience* 42: 1746-1763.

Zdebik AA, Mahmood F, Stanescu HC, Kleta R, Bockenhauer D, & Russell C (2013). Epilepsy in *kcnj10* morphant zebrafish assessed with a novel method for long-term EEG recordings. *PloS one* 8: e79765.

Zhang J, & Yuan Z In vivo non-invasive imaging of the adult zebrafish brain with a 1325 nm long range spectral-domain optical coherence tomography system.1-3.

Zhang S-W, & Liu Y-X (2008). Changes of serum adrenocorticotrophic hormone and cortisol levels during sleep seizures. *Neuroscience bulletin* 24: 84-88.

Zhdanova I (2006). Sleep in Zebrafish. *Zebrafish* 3: 215-226.

Zhou QG, Zhu LJ, Wu HY, Luo CX, Chang L, & Zhu DY (2011). Hippocampal neuronal nitric oxide synthase mediates the stress-related depressive behaviors of glucocorticoids by downregulating glucocorticoid receptor. *Journal of Neuroscience* 31: 7579-7590.

Ziv L, Muto A, Schoonheim PJ, Meijsing SH, Strasser D, Ingraham HA, *et al.* (2013). An affective disorder in zebrafish with mutation of the glucocorticoid receptor. *Molecular psychiatry* 18: 681-691.

Zon LI (2014). Translational Research: The Path for Bringing Discovery to Patients. *Cell Stem Cell* 14: 146-148.