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REVIEW ARTICLE

Zebrafish models in neuropsychopharmacology and CNS drug discovery

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Despite the high prevalence of neuropsychiatric disorders, their aetiology 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. As well as 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 modelling and the discovery of novel drug targets.

Abbreviations

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid β A4 precursor protein; BMAA, β-mythylamino-alanine; CPP, conditioned place preference; dpf, days post fertilization; GR, glucocorticoid receptors; HPA, hypothalamus-pituitary-adrenal; HPI, hypothalamus-pituitary-interrenal; HSR, heat-shock stress response; MO, morpholino-modified antisense oligonucleotide; NAC, N-acetylcysteine; NMJ, neuromuscular junction; PPI, pre-pulse inhibition; PSEN1, presenilin1; PSEN2, presenlinin2; PTZ, pentylenetetrazole; SNRI, selective noradrenaline reuptake inhibitors; SSRI, selective 5-HT (serotonin) reuptake inhibitor; UCMS, unpredictable chronic mild stressors; WGD, whole-genome duplication

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Tables of Links

TARGETS	
Other protein targets[a]	**Enzymes**[d]
TNF-α	AChE
Nuclear hormone receptors[b]	COX-2
GR	PSEN1
Transporters[c]	PSEN2
VMAT2	

LIGANDS	
ACTH	Kainic acid (KA)
Amyloid β (APP)	MK-801
CRH	Methylene blue (MB)
GABA	NMDA
IL-6	

These Tables list key protein targets and ligands in this article which 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 ([a,b,c,d]Alexander *et al.*, 2015a,b,c,d).

Introduction: zebrafish as an emerging animal model

Widespread and debilitating, neuropsychiatric disorders have poorly understood mechanisms and often lack effective therapies (Garakani *et al.,* 2006; Griebel and Holmes, 2013). The identification of clinically relevant biomarkers, the underlying neurobiological mechanisms and genetic and environmental factors of psychopathology, are critical steps in discovering efficacious treatments (Caspi and Moffitt, 2006; Nestler, 2013). While rodent models of human brain disorders have long been employed in this effort, they are often impeded by high-costs and experimental inefficiency (Cryan and Holmes, 2005).

The zebrafish (*Danio rerio*) has recently received attention as a powerful animal model for a wide range of human brain disorders (Kalueff *et al.,* 2014a,b; Stewart *et al.,* 2015c). 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 *et al.,* 2014a,b). 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 (Ullmann *et al.,* 2010; Wulliman *et al.,* 2012; Mueller and Wullimann, 2015).

Modelling human conditions in zebrafish enables the discovery of potential therapeutic targets and their underlying molecular interactions (Table 1). For example, in a recent study the therapeutic potential of methylene blue (MB) was revealed in a mutant mTDP-43 zebrafish; this was found by analysing the efficacy of various compounds to ameliorate the amyotrophic lateral sclerosis (ALS)-like phenotype (Vaccaro *et al.,* 2012). Likewise, the mTDP-43 mutant zebrafish presents with short, abnormally branched motor axons, increased oxidative stress and an aberrant escape response (Vaccaro *et al.,* 2012). The administration of MB, a neuroprotective agent, corrects the 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.,* 2012, 2013). The identification of ER stress as a potential target for ALS drug treatment prompted further testing of 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 haematopoietic stem cells (HSC) recently discovered in zebrafish (Zon, 2014), which have now become therapies in patients (North *et al.,* 2007). The original screening of nearly 2500 small molecules in zebrafish identified 35 'leads' that up-regulate vital HSC genes, *runx1* and *c-myb,* 10 of which modulate the prostaglandin pathway, indicating that it is involved in HSC regulation. One of these potent candidates, 16,16-dimethyl PGE_2 ($dmPGE_2$), was next tested in a mouse model where it was shown to increase the number of HSC grafted (North *et al.,* 2007; Zon, 2014). Subsequent preclinical testing using a primate blood model yielded successful results, allowing the drug to move to an approved Phase I clinical trial (Goessling *et al.,* 2011). These studies have recently yielded positive results in leukaemia 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 preclinical *in vivo* models highly amenable to experimental, pharmacological and genetic manipulations (Barros *et al.,* 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; Wyart and Del Bene, 2011; Stewart *et al.,* 2015a). Together with recent developments in genome editing techniques (e.g. CRISPR/Cas) and automated 3D behavioural phenotyping, this makes zebrafish an ideal model to study genotype–phenotype and genotype-drug-phenotype

Table 1

Particular examples of translational successes using the zebrafish model for drug discovery

Human disease	Zebrafish model	Outcome	References
Pontocerebellar hyperplasia	*Tsen54* antisense morpholino	Linking a loss-of-function mutation in the *tsen54* gene to brain hypoplasia	(Kasher *et al.*, 2011)
	R44X-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 conditions	(Schaffer *et al.*, 2014)
Spinal cord injury	*Heat shock transgenic lines*	Zebrafish show high capacity for axonal regeneration following spinal cord injury, especially through the activation of Fgf signalling. Increasing Fgf signalling in mammalian spinal injury sites may encourage glial cell differentiation and lead to favourable conditions for axonal regeneration	(Goldshmit *et al.*, 2012)
Schizophrenia	Tg(*huC*:eGFP)	The *Rgs4* gene is associated with the onset and development of schizophrenia. Using the transgenic zebrafish line, *rgs4* was found to be essential for axon formation, providing the first *in vivo* evidence supporting the role of *rgs4* in schizophrenia	(Cheng *et al.*, 2013)

relationships (Kokel *et al.*, 2010; Cachat *et al.*, 2011b; Hwang *et al.*, 2013; Stewart *et al.*, 2015b). 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 (Kalueff *et al.*, 2014b; Fonseka *et al.*, 2016). Complementing larval models, adult zebrafish exhibit complex behaviours (Kalueff *et al.*, 2013) relevant to cognition (Blaser and Vira, 2014; Gerlai, 2016), reward (Collier *et al.*, 2014; von Trotha *et al.*, 2014), social behaviour (Gerlai, 2014; Qin *et al.*, 2014) and effects (Jesuthasan, 2012; Gerlai, 2013; Wang *et al.*, 2016a). Numerous experimental paradigms have been converted for aquatic models to investigate major behavioural phenotypes, which are well-conserved in zebrafish and mammals (Renier *et al.*, 2007; Stewart *et al.*, 2014a).

Rats and mice are currently the most commonly employed animals to study normal and abnormal brain functioning; nearly 1/3 of all published neuroscience papers in 2015 utilized rodent models, and <11% used other animal models, including zebrafish (Keifer and 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 *et al.*, 2014a; Wyatt *et al.*, 2015). As 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.

Zebrafish CNS

The overall architecture, neuroanatomical features and cellular morphology of the zebrafish CNS are generally similar to those of mammals (Kalueff *et al.*, 2014b). For example, the medial teleost pallium contains homologous structures to the mammalian amygdala (Martín *et al.*, 2011; Mueller *et al.*, 2011; Portavella *et al.*, 2004; von Trotha *et al.*, 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 *et al.*, 2006), social anxiety disorders (Stein *et al.*, 2002; Furmark *et al.*, 2004) and drug abuse (Mead *et al.*, 1999; Buffalari and See, 2010). 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 behaviour in a conditioned place preference (CPP) assay (von Trotha *et al.*, 2014), collectively supporting the role of zebrafish medial pallium as a homologous structure to the mammalian amygdala, with evolutionarily conserved functions in modulating key behaviours.

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

(Ahrens *et al.,* 2013). Optogenetic neuromodulation of the transparent and genetically accessible larval zebrafish is particularly useful for investigating the neural circuitry underlying behaviours relevant to brain disorders (Knafo and 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 (Douglass *et al.,* 2008; Arrenberg *et al.,* 2009). To date, optogenetic studies in zebrafish have largely focused on several simpler behaviours, such as escape (Douglass *et al.,* 2008), locomotion (Arrenberg *et al.,* 2009; Ljunggren *et al.,* 2014) and sensory processing (Kubo *et al.,* 2014). However, optogenetic neuromodulation in zebrafish helps future research to create robust models of complex human neuropsychiatric disorders (Tye and Deisseroth, 2012; Stewart *et al.,* 2015c). Furthermore, the imaging of neural activity in adult zebrafish is more challenging due to their larger and opaque brains. Contrast-enhanced X-ray micro-computer tomography with iodine as a contrasting agent has been recently applied in adult zebrafish, and this provided 3D visualization of zebrafish brain anatomy in intact animals (Babaei *et al.,* 2016). Optical coherence tomography has also recently been used *in vivo* in adult zebrafish to non-invasively generate real-time cross-sectional images at high resolution, that are then reconstructed in 3D (Rao *et al.,* 2009; Zhang and Yuan, 2015).

Neurochemistry is generally conserved across vertebrate species, as they share major neurotransmitters, receptors and transporters (Panula *et al.,* 2006, 2010; Alsop and Vijayan, 2008). Thus, zebrafish are sensitive to major classes of pharmacological agents, such as psychostimulants (Ninkovic and Bally-Cuif, 2006), opiates (Lau *et al.,* 2006), ethanol (Tran *et al.,* 2015), hallucinogens (Stewart *et al.,* 2013), anxiolytics (Bencan *et al.,* 2009), antidepressants (Stewart *et al.,* 2014b) and antiopsychotics (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, GABA, acetylcholine, dopamine, 5-HT, noradrenaline and histamine (Stewart *et al.,* 2015c). For instance, the major pathways and receptor subtypes of the dopamine system are all present in zebrafish, with the exception of the D_5 receptor (Panula *et al.,* 2006, 2010; Maximino and Herculano, 2010). The amino acid sequence recently compared between zebrafish and humans for D_1–D_4 receptors shows 100% amino acid homology in the binding site for D_1 and D_3, and 85–95% for D_2 and D_4 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 *et al.,* 2006; Kyzar *et al.,* 2014) and agonists predictably increase zebrafish locomotion (Irons *et al.,* 2013), paralleling similar effects in rodents (Mobini *et al.,* 2000; Akhisaroglu *et al.,* 2005). The dopamine agonist apomorphine produces a U-shaped dose–response relationship for distance travelled in larval zebrafish, with low-doses increasing time spent in the centre (anxiolytic effect) and high-doses increasing thigmotaxis (anxiogenic effect) (Ek *et al.,* 2016). Strikingly, by paralleling similar drug actions in rats (Ek *et al.,* 2016), these findings further support the translational value of neuropharmacological studies in zebrafish.

There is mounting evidence implicating alterations in the neuroendocrine system in various brain disorders, including depression (Herbert, 2013; Holsboer, 2001), anxiety (Hek *et al.,* 2013; Korte, 2001), addiction (Keedwell *et al.,* 2001; Lovallo, 2006) and Alzheimer's disease (AD) (Belanoff *et al.,* 2001; Wahbeh *et al.,* 2008). Activation of the neuroendocrine hypothalamus-pituitary-interrenal (HPI) axis of zebrafish releases cortisol that acts on glucocorticoid receptors (GR), similar to the hypothalamus-pituitary-adrenal (HPA) axis in humans (Alsop and Vijayan, 2009; Griffiths *et al.,* 2012b; Pavlidis *et al.,* 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; Pavlidis *et al.,* 2011; Félix *et al.,* 2013). For example, genetic mutation of the GR gene in adult gr^{s357} mutant zebrafish disrupts negative feedback and cortisol signalling 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 5-HT (serotonin) reuptake inhibitor (SSRI) fluoxetine. This also emphasizes the high degree of evolutionarily conservation between the neuroendocrine system and its modulation between zebrafish and humans (Griffiths *et al.,* 2012b; Ziv *et al.,* 2013).

Zebrafish models of major CNS disorders

A clear advantage of non-human animals (like zebrafish) for modelling brain disorders is, as already mentioned, their amenability to experimental, genetic and pharmacological manipulations. Furthermore, the behavioural 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).

Depression and anxiety

Stress is a common risk factor for developing affective disorders, including major depressive disorder (Strüber *et al.,* 2014; Lucassen *et al.,* 2016) and anxiety (Bystritsky, 2006). In mammals, the stress response is mainly mediated by the interplay between the hypothalamus, the pituitary and adrenal glands, which, collectively, form the HPA axis (Smith and 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 *et al.,* 2011) and thereby triggering depression (Zhou *et al.,* 2011).

Depression has been extensively modelled in rodents (Deussing, 2006; Krishnan and Nestler, 2011) utilizing early life (Fumagalli *et al.,* 2007) and adulthood stresses (Seligman *et al.,* 1975) as well as pharmacological interventions (Barr

and 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 modelled in animals (Seligman and Beagley, 1975; Porsolt, 2000). 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 *et al.,* 2017; Marcon *et al.,* 2016; Piato *et al.,* 2011). Adult fish exposed to 7–14 days of UCMS exhibit reduced locomotion, altered shoaling behaviour and body colour (Gerlai *et al.,* 2000). When applied to zebrafish raised in social isolation for 5 months, UCMS increases anxiety-like behaviours in the novel tank test and reduces body weight and whole-brain dopamine and 5-HT metabolite, 5-HIAA, levels, compared with zebrafish raised in groups (Fulcher *et al.,* 2017). The effects of UCMS on exploratory and group/shoaling behaviours are reversed by fluoxetine (an SSRI) and bromazepam, a benzodiazepine anxiolytic (Marcon *et al.,* 2016). In addition, several key pro-inflammatory molecules, such as TNF-α, IL-6 and 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 *et al.,* 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 *et al.,* 2014). Finally, several genetic models have been used to study depression in the zebrafish. For instance, larval zebrafish with mutant GR (gr/s357) display heightened physiological responses (e.g. higher whole body cortisol levels) and dysfunctional HPI axis (Griffiths *et al.,* 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 *et al.,* 2005; Kessler, 2007). There are several types of anxiety disorder, including panic disorder, post-traumatic stress disorder, 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 (Anxiety Disorders Association of America, 2016). The first line of treatment for anxiety disorders is typically a regimen of SSRIs or cognitive behavioural therapy (Bystritsky, 2006). Patients who do not respond to these treatments are then given selective noradrenaline reuptake inhibitors (SNRIs) or tricyclic antidepressants (Bystritsky, 2006). However, SNRIs or tricyclics increase the risk for tolerance and dependence (Otto *et al.,* 1993), thereby limiting their use. Furthermore, although many treatments for anxiety disorders exist, approximately 30% of patients show no improvement (Brown *et al.,* 1996). This necessitates the identification and development of treatments that are devoid of these limitations in efficacy and tolerance (Griebel and Holmes, 2013).

One of the problems with developing new treatments has been the identification of biochemical targets, genetic variants or mechanisms of action for the onset of the disorder (Bystritsky, 2006; Insel *et al.,* 2011; Griebel and Holmes, 2013), indicating the need for animal models. The zebrafish model is particularly amenable to high-throughput anxiolytic drug screens (Lundegaard *et al.,* 2015). The larval zebrafish hatch 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 behaviours (Richendrfer *et al.,* 2012); see Figure 1. For instance, staying near the periphery of the arena (thigmotaxis) reflects anxiety-like behaviour and is heightened following exposure to anxiogenic stimuli or drugs (Stewart *et al.,* 2012). In adult fish, measures of anxiety include a latency to explore the top or higher tendency to remain in the bottom (Stewart *et al.,* 2012) in the novel tank test (Figure 2). In the light–dark test, the fish are allowed to freely explore brightly light and dark arenas, but when zebrafish spend more time in the dark (scototaxis) this is indicative of an anxiety-like response, which can be bidirectionally influenced by anxiolytic or anxiogenic treatments (Kalueff *et al.,* 2013). 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 endeavour 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 and 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 *et al.,* 2013). NAC plays a role in maintaining oxidative balance germane to anxiety and has been shown to modulate central glutamatergic pathways (Dean *et al.,* 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 and 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 *et al.,* 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). This heightened anxiety-like response is attenuated by exposure to MEK inhibitors, anti-cancer treatment (Lundegaard *et al.,* 2015), suggesting the MEK crosstalk as a potential alternative target for treatments of anxiety.

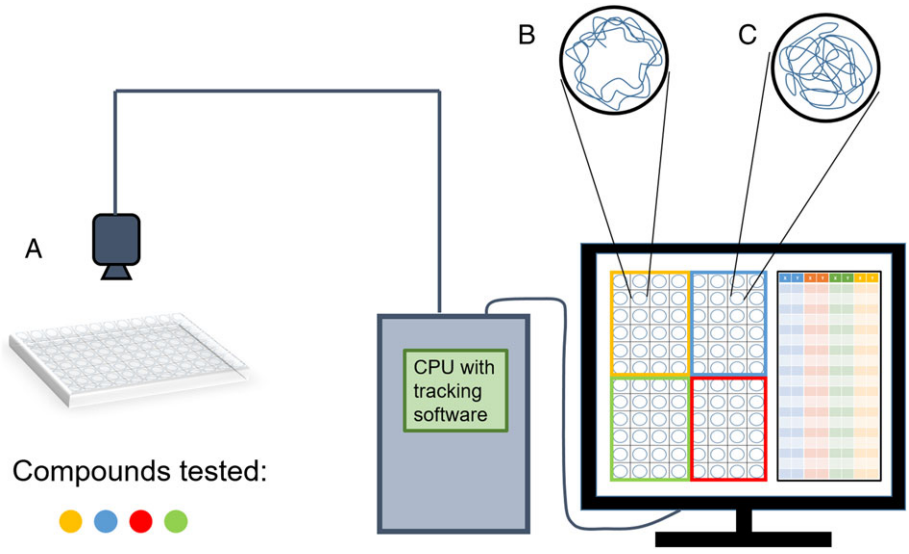

Figure 1

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 behaviours 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 stay close to the walls (wall-hugging behaviour). Panel (C) shows an example of the opposite swim pattern in which the larval zebrafish actively explore their environment, including the centre of the tank.

Epilepsy

Epilepsy, which affects approximately 50 million people worldwide (WHO, 2016), is characterized by recurrent convulsions/seizures, behavioural impairments, pathological neural activity and endocrine dysfunction (Andrea Galimberti *et al.,* 2005; Zhang and Liu, 2008; Green *et al.,* 2012; Engel, 2013). Epilepsy can be modelled in larval and adult zebrafish (primarily by administration of convulsant drugs and genetic modifications) and evaluated by various behavioural and physiological endpoints (Wong *et al.,* 2010; Desmond *et al.,* 2012; Cunliffe, 2015). Characteristic behaviours 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 *et al.,* 2005; Zdebik *et al.,* 2013). Experimental seizures in zebrafish can be induced by acute caffeine (250 mg·L^{-1}), pentylenetetrazole (PTZ, 2.5 g·L^{-1}) and picrotoxin (100 mg·L^{-1}), 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 *et al.,* 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 (Loebrich and Nedivi, 2009; Cunliffe *et al.,* 2015). Finally, various genetic techniques enable the greater exploration of function for specific candidate genes (Teng *et al.,* 2010; Mahmood *et al.,* 2013; Cunliffe, 2015) or anti-epileptic treatments using high-throughput and rapid screening in zebrafish (Baxendale *et al.,* 2012; Baraban *et al.,* 2013; Cunliffe, 2015).

Psychosis

Psychosis manifests as disturbances in cognition, affect, motor activity and social behaviour (American Psychiatric Association, 2013) and is often accompanied by aberrant glutamatergic signalling (Merritt *et al.,* 2013; Schobel *et al.,* 2013). The glutamate 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 *et al.,* 2013). MK-801 is a potent NMDA antagonist used to model schizophrenia in rodents, zebrafish and other animal models (Moghaddam and Jackson, 2003; Swain *et al.,* 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 *et al.,* 2001). Schizophrenia patients show impaired PPI (Braff *et al.,* 2001), which can be rescued by antipsychotic therapy (Kumari *et al.,* 1999; Geyer *et al.,* 2001). PPI is reliably reproduced in larval zebrafish, including genetic mutants with reduced PPI currently available (Burgess and 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.

Alzheimer's disease

AD is a progressive neurodegenerative disease resulting in cognitive deficits, delusions, hallucinations and changes in mood and behaviour (Voisin and Vellas, 2009). One of the hallmark symptoms of AD is the development of neurofibrillary tangles and amyloid β plaques (Newman *et al.,* 2011). There are two broad classes of AD: sporadic AD (developing at age > 65), and familial AD (fAD), developing much earlier (Rossor *et al.,* 1984). Sporadic AD accounts for

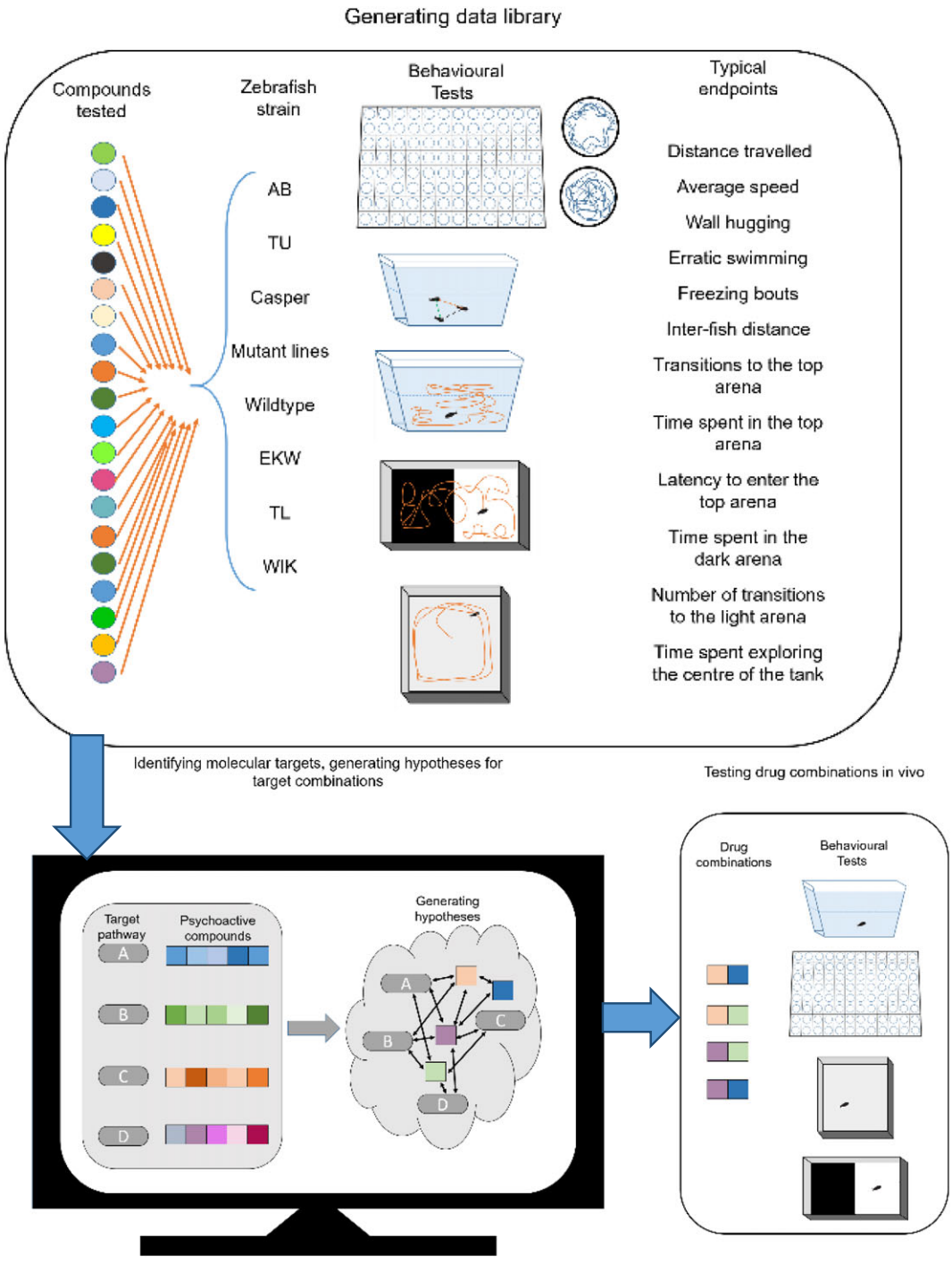

Figure 2

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 behavioural and cognitive tasks can be used to generate a data library. Computational tools, such as hierarchical modelling and similarity ensemble approach (SEA), can help identify target hits, enabling the predictions about the effects of various drug combinations. The hypotheses generated may then be tested *in vivo* using larval or adult animals.

more than 95% of all AD cases (Newman *et al.*, 2011) and is linked to the apolipoprotein E ε4 allele (Selkoe, 2001). The zebrafish orthologue of this gene is *apoE* (Babin *et al.*, 1997). Early onset fAD is hereditary and has been linked to mutations in the *presenilin1* (*PSEN1*), *presenlinin2* (*PSEN2*) and amyloid β a4 precursor protein (APP) genes, orthologous to the zebrafish *psen1*, *psen2*, *appa* and *appb* genes (Newman *et al.*, 2011). The injection of transcription-blocking morpholinos for *psen1* disrupts notch signalling and results in aberrant somite formation (Nornes *et al.*, 2003, 2009;

Campbell *et al.,* 2006). *Psen2* blocking produces notch-signalling defects (Campbell *et al.,* 2006) and alters the production of spinal cord interneurons in zebrafish (Nornes *et al.,* 2009), paralleling phenotypes observed in *psen1*$^{-/-}$ and *psen2*$^{-/-}$ mice (Shen *et al.,* 1997).

Zebrafish are also valuable for studying the aetiology of AD, especially the role of hypoxia as a putative risk factor (Newman *et al.,* 2011). Under low-oxygen conditions, mitochondria may release free radicals that increase oxidative stress (Bell *et al.,* 2007; Moussavi Nik *et al.,* 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 *et al.,* 2011). Similar to humans, hypoxic conditions in the larval and adult zebrafish up-regulate several AD-related genes, including *sen1*, *psen2*, *appa*, *appb* and *bace1* (Moussavi Nik *et al.,* 2011).

Pharmacological interventions may also help model the cognitive deficits associated with AD. For example, the cholinergic system (which mediates 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 AChE activity (Perry *et al.,* 1978; Lombardo and Maskos, 2015). The muscarinic antagonist scopolamine impairs zebrafish memory without causing locomotor deficits or anxiety-like behaviour (Richetti *et al.,* 2011; Cognato *et al.,* 2012; Gupta, 2014). 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 (Havsteen, 2002; Spencer, 2008; Ahmed and Gilani, 2009). Scopolamine-induced memory impairment in zebrafish is also ameliorated by pretreatment with physostigmine, an AChE inhibitor (Kim *et al.,* 2010). The ability of scopolamine to produce amnesia 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.

Amyotrophic lateral sclerosis

ALS is a debilitating progressive neurodegenerative disorder affecting motor neurons in the brain and spinal cord (Rowland and 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 and O'Malley, 1995; Friedrich *et al.,* 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 aetiology 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 and Rouleau, 2008).

Larval zebrafish over-expressing mutant *Sod1* have abnormal neuromuscular junctions (NMJ) that worsen as the fish matures (Ramesh *et al.,* 2010). Larval mutant fish present a progressive decrease in NMJ volume (Ramesh *et al.,* 2010), poorer performance in the forced swim test (Plaut, 2000; Ramesh *et al.,* 2010) and reduced responses 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 the 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 harbouring 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 *et al.,* 2016), resulting in mutant zebrafish lines replicating ALS. This novel methodology also shows promise in the development of mutant models for other neuropsychiatric diseases (Armstrong *et al.,* 2016). Pharmacological intervention with neurotoxins like β-mythylamino-alanin (BMAA) can also be relevant to modelling ALS. Pericardiac injection of BMAA during embryonic development alters protein homeostasis and glutamate signalling, 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) (Purdie *et al.,* 2009; Froyset *et al.,* 2016).

Zebrafish sensitivity to CNS drug classes

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

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 behavioural testing but also 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, vasodilators, 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 *et al.,* 2008). Kainic acid (KA) is a common convulsant agent in rodents and is able to produce similar effects in zebrafish (Alfaro *et al.,* 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 histamine receptor antagonist) is effective at treating genetically-evoked seizures in *scn1lab* zebrafish (Grone and Baraban, 2015), a model of Dravet syndrome (Baraban *et al.,* 2013) caused by SCN1A mutations with spontaneous seizures insensitive to major anti-epileptic drugs.

Antipsychotics

First-generation (typical) antipsychotics are high-affinity antagonists of dopamine D_2 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 *et al.,* 2005). The second-generation 'atypical' antipsychotics demonstrate a lower affinity for D_2 receptors and fewer side effects, relative to typical antipsychotics (Kane *et al.,* 1988). However, there remains a great need for the identification of novel treatments for psychoses, and zebrafish models can be highly useful in this endeavour. For example, the administration of MK-801 induces hyper-locomotion [similar to psychomotor agitation, a characteristic symptom of schizophrenia (Seibt *et al.,* 2010)] and social and cognitive deficits (Seibt *et al.,* 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 *et al.,* 2011). Importantly, atypical antipsychotics have affinities for dopaminergic as well as serotonergic, glutamatergic and other neurosignalling pathways. For example, resperidone acts via D_2 and 5-HT_2 receptors and shows promise as an anxiolytic substance (Idalencio *et al.,* 2015). Stressed fish exposed to resperidone spend more time in the top of the novel tank test, have fewer transitions to the dark in the light–dark test (Magno *et al.,* 2015) and show lower cortisol levels (Idalencio *et al.,* 2015). The purinergic system has been recently implicated in schizophrenia (Lara and Souza, 2000), especially since adenosine, the final product in the ectonucleotidase cascade, modulates dopamine and glutamate (Lara and Souza, 2000). In zebrafish, haloperidol reduces ATP hydrolysis and adenosine deamination, thereby reducing synaptic adenosine levels (Seibt *et al.,* 2015). The sensitivity of zebrafish ATP hydrolysis to haloperidol suggests an extracellular mechanism of action, potentially relevant to pharmacological targets (Seibt *et al.,* 2015).

Drugs of abuse

Substance abuse and addiction are easily modelled in larval and adult zebrafish (Stewart *et al.,* 2011). For example, addiction, tolerance and withdrawal can be studied using aquatic CPP paradigms (Mathur and Guo, 2010; Collier and Echevarria, 2013; Collier *et al.,* 2014). A typical CPP set-up consists of two distinct environments, which differ in their colours, visual patterns or environmental cues (Darland and 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 *et al.,* 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 behavioural effects of psychoactive compounds and associative learning (Lucke-Wold, 2011) but, despite the ability to elucidate reward-seeking behaviour, does not measure the drug's abuse potential (see further).

Alcohol and nicotine. Ethanol produces a characteristic dose-dependent effect on zebrafish. At low doses (<0.5%), ethanol increases locomotion, swim speed and shoaling behaviours (Gerlai *et al.,* 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 *et al.,* 2000; Rosemberg *et al.,* 2012; Tran and Gerlai, 2013; Pannia *et al.,* 2014). The rewarding effect of ethanol in zebrafish is seen after a single exposure to 0.25–1% (Collier *et al.,* 2014) or 1.5% (Mathur *et al.,* 2011), reliably changing fish CPP. A prolonged CPP paradigm (e.g. daily conditioning for 4 weeks) produces robust behavioural responses, which persist following abstinence, indicating the establishment of dependence-related behaviour (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 *et al.,* 2006). Drug abstinence following chronic (1 week) exposure produces robust withdrawal symptoms in adult zebrafish, including anxiety-like behaviour and elevated cortisol (Cachat *et al.,* 2011a).

Zebrafish also produce a wide range of dose-dependent responses to nicotine (Levin *et al.,* 2007; Kily *et al.,* 2008). At low to moderate doses (e.g. 3–300 μM), nicotine evokes anxiolytic responses in the novel tank test (Levin *et al.,* 2007) and robust CPP that persist following a period of abstinence (Kily *et al.,* 2008). The behavioural 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 up-regulation of several genes

implicated in the development of drug dependence, including genes for calcineurin B and the hypocretin receptor, which have both been previously linked to synaptic plasticity and neurotransmission in drug dependence (Kily *et al.,* 2008).

Cocaine and amphetamines. Administration of cocaine (5, 10 and 15 mg·L^{-1}) 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 and Dowling, 2001). When surrounded by conspecifics, cocaine-treated zebrafish engage in aggressive behaviour through dominance displays and chasing (Darland and Dowling, 2001). Abstinence from the drug results in withdrawal symptoms within 72 h, wherein animals experience anxiety-like behaviour and basal hyperlocomotion (López-Patiño *et al.,* 2008). Withdrawal symptoms are counteracted by the administration of a non-sedative dose of diazepam (5 μM) or cocaine (1.5 μM) (López-Patiño *et al.,* 2008). Cocaine also produces dose-dependent CPP responses, with 10 mg·L^{-1} causing the most robust response (Darland and Dowling, 2001). The cross-breeding of wild-type females with males mutagenized through repeated exposure to N-ethyl-nitrosourea (ENA) yielded an F_1 generation, outcrossing of which results in F_2 generation tested for cocaine sensitivity in the CPP task. Low-responding F_2 siblings were crossbred to yield F_3 generation, which display low sensitivity to cocaine in the CPP task, demonstrating a genetic basis for the altered behaviour profile (Darland and Dowling, 2001).

Methamphetamine is a potent psychostimulant with high addiction potential, and its abuse is comorbid with psychiatric disorders, including anxiety and depression (Akindipe *et al.,* 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 behaviour and increases swim speed in the open field and mirror stimulation task (Mi *et al.,* 2016), and this can effect can be 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 the addictive potential of cocaine and morphine in mice (Hikida *et al.,* 2003), suggesting that targeting the acetylcholine system may lead to a reduction in the addictive properties of drugs.

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 and Kalueff, 2016). Classic serotonergic psychedelics [e.g. lysergic acid diethylamide (LSD), mescaline and psilocybin] alter zebrafish locomotion, shoaling and anxiety-like behaviours and whole body cortisol levels (Kyzar and Kalueff, 2016). Ketamine, a dissociative psychedelic, produces a dose-dependent anxiolytic effect in the zebrafish and decreases whole body cortisol levels (De Campos *et al.,* 2015). The deliriant psychedelic atropine affects cholinergic neural activity in the zebrafish (Park *et al.,* 2008). Although hallucinogens remain understudied in zebrafish, the data available 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 and Kalueff, 2016).

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 similarities with the mammalian $GABA_A$ and $GABA_B$ receptor subunits and histamine H_1 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 *et al.,* 2009), whereas diazepam has a biphasic effect on anxiety, with low-to-moderate doses reducing bottom dwelling, and higher doses causing sedation (Bencan *et al.,* 2009). Chronic 2 week exposure to diazepam following 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 behavioural effects of these drugs).

Perspectives on small molecule and genetic screening in zebrafish

Understanding genetic and anatomical differences from mammalian models

As a member of the teleost group, the zebrafish originated from a common ancestor ~340 million years ago (Amores *et al.,* 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 and 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 *et al.,* 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 (Pando and Sassone-Corsi, 2002; Danilova *et al.,* 2004; Vatine *et al.,* 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 are poorly understood (Wang, 2008). Transgenic models may help to elucidate the functions of the zebrafish per1 genes and provide insights into their role in maintaining circadian rhythms. The 5-HT transporter (*sert*) genes have also been duplicated during WGD in zebrafish, which possess two *sert* genes: *serta* and *sertb* (Wang *et al.,* 2006). These genes have high homology to vertebrate 5-HT transporter genes, suggesting a conservation of function (Wang *et al.,* 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 the 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 *et al.,* 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 and 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 *et al.,* 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 (Northcutt, 2008; Mueller *et al.,* 2011). This aspect may limit the translation of findings between zebrafish and humans, especially on aberrant executive functioning commonly observed in psychiatric diseases (Parker *et al.,* 2013). However, given the 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 fulfil these validity criteria, many zebrafish behavioural tasks have been modified from rodent paradigms (Levin *et al.,* 2007). For instance, for modelling anxiety disorders, the zebrafish has become an adept model in the identification of stress-inducing stimuli and psychoactive agents in various novelty-based paradigms (Levin *et al.,* 2007; Bencan *et al.,* 2009; Abreu *et al.,* 2016). Deficits in cognitive and behavioural flexibility, commonly reported in patients with psychiatric diagnoses, may also be modelled in zebrafish. Behavioural 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 *et al.,* 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 *et al.,* 2005). Thus, although zebrafish may lack a proper cortex, they retain the ability to perform executive functions, such as maintaining attention and behavioural flexibility (Parker *et al.,* 2013). However, we still know relatively little about the neural circuits and how different neurotransmitter systems may functionally interact in zebrafish (Parker *et al.,* 2013). Identifying the function of neural circuits in this fish and their reciprocity with other systems becomes critical to understanding the molecular basis of behaviour, and in identifying therapeutic targets for diseases.

Perspectives on automated and high-throughput screening

Zebrafish models are highly amenable to behavioural, genomic and proteomic testing (Jones and Norton, 2015; Purushothaman *et al.,* 2015), as they combine a relative neural simplicity with behavioural complexity sufficient for studying multiple behavioural processes from sleep (Zhdanova, 2006; Rihel *et al.,* 2010; Purushothaman *et al.,* 2015) to anxiety (Richendrfer *et al.,* 2012; Stewart *et al.,* 2012). Custom-made and commercial video-tracking software can record a wide range of zebrafish behavioural measures, including velocity, distance travelled, place preference (e.g. top vs. bottom, light vs. dark, centre vs. periphery) and specific patterns (e.g. erratic swimming, stereotypic circling) (Pérez-Escudero *et al.,* 2014; Conklin *et al.,* 2015). The automation of zebrafish video-tracking enables several behavioural 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 (Pérez-Escudero *et al.,* 2014; Conklin *et al.,* 2015). It is also possible to record zebrafish social groups, for example, assessing fish shoaling behaviours, presently capable of tracking multiple (e.g. 8–16) animals per arena (Noldus, 2016b) to extract rich behavioural data from average inter-fish distance to shoal polarization and cohesion (Stewart *et al.*, 2014a). 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 and Gerlai, 2008), which collectively improves the standardization of testing procedures, and provides efficient data collection, increased throughput and data reproducibility (Love *et al.*, 2004; Stewart *et al.*, 2015a).

Zebrafish are further amenable to high-throughput *in vivo* screening as their multiple behavioural parameters can be monitored in 3D (Stewart *et al.*, 2015b). 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.*, 2015b). Advances in behavioural recognitions allow for a more detailed *in vivo* analysis of behavioural phenotype (Stewart *et al.,* 2015a). 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

behaviours such as chasing or nipping, chasing (Kalueff *et al.*, 2013; Stewart *et al.*, 2015a). These methods are especially useful in polypharmacology studies using pharmacological agents that act on multiple targets (McCarroll *et al.*, 2016). As many psychiatric disorders are linked to deficits in several neurotransmitter systems and have multigenic aetiologies (Kendler *et al.*, 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, also help identify target hits and prediction of target interactions with psychoactive compounds. The combination of behavioural phenotyping and computation techniques is useful in the development and discovery of new medical targets, including *in vivo* behavioural phenotyping of single target compounds in 2D or 3D tracking, producing their unique swim traces (thigmotaxis, scototaxis, average swim speed, etc.), identifying the compounds that produce the desired behavioural phenotype (hit compounds) and their subsequent in-depth analyses with algorithms that predict their biological target(s) to generate hypotheses of target combinations (McCarroll *et al.*, 2016). Once identified, multiple hit compounds can then be tested in combination *in vivo* (McCarroll *et al.*, 2016), probing their ability to work in concert to achieve a desired therapeutic outcome.

Perspectives on genetic zebrafish models

As already noted, genetic manipulations are critical on animal studies to identify candidate genes associated in the aetiology of a disease. Short-term genetic manipulation is achieved through injection of morpholino-modified antisense oligonucleotides (MOs) (Nasevicius and Ekker, 2000) or small interfering RNA (siRNA; de Rienzo *et al.*, 2012) to engage in loss-of-function studies (Kalueff *et al.*, 2014b). MOs target specific translational inhibitors and effectively reduce gene expression (Nasevicius and Ekker, 2000). RNA interference (RNAi) is a process in which RNA molecules inhibit the translation of targeted mRNA molecules (de Rienzo *et al.*, 2012). These methods demonstrate efficacy in targeting specific genes and in producing altered phenotypes, although, recently, the efficacy of MOs has been questioned (Kok *et al.*, 2015; McCammon and Sive, 2015).

The development of mutant zebrafish provides a more stable behavioural phenotype, because rather than produce a knockdown of a given gene, it completely eliminates the target gene product (Amsterdam and Hopkins, 2006; Stewart *et al.*, 2014b). Mutants are created through retroviral insertional mutagenesis, wherein DNA basepairs are integrated into the organism's pre-existing DNA (Amsterdam and 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 and Hopkins, 2006; Wienholds *et al.*, 2003). Mutant and morphant zebrafish are used in a wide range of studies and provide a deeper understanding of the roles and importance of specific receptors and biological targets (Griffiths *et al.*, 2012a; Haesemeyer and 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 *et al.*, 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 *et al.*, 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 (Saitsu *et al.*, 2008; Carvill *et al.*, 2014). 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 behavioural response to a startle stimulus, larval zebrafish demonstrate normal behaviour (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 behavioural 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.

Conclusion

Neuropsychiatric conditions afflict the human population globally and have tremendous personal and societal costs (Garakani *et al.*, 2006; Griebel and Holmes, 2013). 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, with the aim of developing safer and more effective treatments (Krishnan and Nestler, 2011; Keifer and Summers, 2016). Zebrafish are a promising new animal model, which continues to provide important insights into the aetiology of CNS diseases (Kalueff *et al.*, 2014a,b). 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 *et al.*, 2014a). The current genetic tools, tracking techniques and statistical algorithms foster the gaining of a deeper understanding of molecular pathways, the development of new compounds or repurposing of established drugs (Stewart *et al.*, 2015b). Taken together with the 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 their hypotheses (Kokel *et al.*, 2010; Hwang *et al.*, 2013; Stewart *et al.*, 2015b).

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Conflict of interest

The authors declare no conflicts of interest.

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