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Preface

Gaining translational momentum: More zebrafish models for neuroscience research

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ABSTRACT

Zebrafish (*Danio rerio*) are rapidly becoming a popular model organism in translational neuroscience and biological psychiatry research. Here we discuss conceptual, practical and other related aspects of using zebrafish in this field ("from tank to bedside"), and critically evaluate both advantages and limitations of zebrafish models of human brain disorders. We emphasize the need to more actively develop zebrafish models for neuroscience research focusing on complex traits.

1. Introduction

In classical physics, "translational momentum" is a vector with direction and magnitude, a function of mass and velocity of an object. In neuroscience, this term has a different meaning. "Translational" is becoming a critical concept in biomedicine, linking human disorders to animal models and biomarkers using the "bench to bedside" approach (Garner et al., 2009; Weinshilboum and Wang, 2004). As the number of valid experimental models of brain disorders continues to grow, the focus of translational neuroscience moves to recently emerging species, such as zebrafish (*Danio rerio*) (Stewart and Kalueff, 2014). Multiple reasons, summarized in Table 1, outline why over the last decade, the zebrafish has become a new "rising star" in biomedicine (Fig. 1), as part of the "tank to bedside" approach. With the growing number of zebrafish centers and laboratories worldwide (Kalueff et al., 2014), this species is especially gaining momentum in neuroscience.

Importantly, zebrafish are a relatively complex *vertebrate* species, physiologically homologous to mammals and possessing all major neurotransmitters, hormones and receptors (Alsop and Vijayan, 2009; Mueller et al., 2004; Panula et al., 2006). Zebrafish are currently used to study a wide range of neurobehavioral domains, including anxiety (see further), sociality (Engeszer et al., 2004; Gerlai et al., 2009a; Wright et al., 2006), sleep (Cirelli and Tononi, 2000; Zhdanova et al., 2008), reward (Bretaud et al., 2007; Kily et al., 2008; Ninkovic and Bally-Cuif, 2006) and cognition (Colwill et al., 2005; Williams et al., 2002). In addition to well-established *larval* zebrafish models (Borla et al., 2002; Saint-Amant and Drapeau, 1998, 2001), recent evidence strongly supports the importance of studying *adult* zebrafish phenotypes (Bencan et al., 2009; Blaser et al., 2010; Gerlai et al., 2009b; Maximino et al., 2010; Sackerman et al., 2010; Stewart et al., 2010). Given the impetus zebrafish have gained in neuroscience (Kalueff et al., 2014; Kari et al., 2007; Stewart et al., 2014), the Special Issue of this journal dedicated to novel zebrafish models of brain disorders is therefore very timely.

2. Why zebrafish?

While early research described fish behavior as simple and stereotyped (Rose, 2002, 2007), recent studies demonstrate complex, context-dependent behavioral responses in zebrafish (Agetsuma et al., 2010; Ahmed et al., 2011; Blaser and Gerlai, 2006; Gerlai, 2010; Jesuthasan and Mathuru, 2008; Levin et al., 2007; Speedie and Gerlai, 2008). Consider, for example, affective disorders, such as anxiety — currently one of the most common human brain disorders, affecting millions worldwide. Exposed to stimuli that evoke fear or anxiety, zebrafish display a range of clear-cut quantifiable behaviors, including markedly reduced exploration, increased scototaxis (dark preference), geotaxis (diving/bottom dwelling), thigmotaxis (preference of peripheral areas), freezing (immobility) and erratic movements (sudden bouts of high-velocity darting with

Abbreviations: CNS, Central Nervous System; BBB, blood-brain barrier; HTS, high-throughput screens; DSM, The Diagnostic and Statistical Manual of Mental Disorders; 3D, three-dimensional; IACUC, Institutional Animal Care and Use Committee; DMSO, Dimethyl sulfoxide; NIH, The National Institutes of Health; ZNRC, Zebrafish Neuroscience Research Consortium.

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Table 1
Selected reasons for the growing role of zebrafish in biomedical research and translational neuroscience.

Model benefits	Comments
General	An *in-vivo* model (has more translational validity than *in-vitro* models)
	Vertebrate species with common organ systems and cell types
	High (80–85%) genetic homology to humans (Barbazuk et al., 2000; Howe et al., 2013)
	Sufficient physiological complexity combined with high physiological homology to humans, and conserved physiological systems
	Genetically tractable organism with fully sequenced genome (Howe et al., 2013)
	Reproduce quickly and abundantly (*e.g.*, a single female lays several hundred eggs each week)
	Development from eggs with a transparent chorion; this enables monitoring the developing embryos and their organs, as well as manipulating these processes *in-vivo* (*e.g.*, by injecting drugs or genes). Transparent embryos and larvae are also good for genetic and pharmacological manipulations
	External development; Zebrafish can be exposed to various environmental factors (drugs, toxins) neonatally outside of maternal organism, in more experimentally controllable environment
	Rapid development (hatch in <3 days and become mature by day 90; good to study neurodevelopmental disorders). All major organs form within 1 dpf, and the fish start feeding and swim freely within 3 dpf
	Extended lifespan (zebrafish live longer than mice, and may be a good model to study aging) (Kalueff et al., 2014)
	High potential for medium- and high-throughput screens (HTS) (Laggner et al., 2012; Stewart and Kalueff, 2014)
	Availability of "two models in one": larval and adult zebrafish (Kalueff et al., 2014)
	Adherence, as a lower vertebrate, to the 3R principles of ethical research (Replacement, Refinement, Reduction)
Practical	Space/cost-efficient, low-cost model (1 × 500–1000 *vs.* rodents) (Kalueff et al., 2014)
	Availability of various zebrafish strains, including over 1000 transgenic and mutant zebrafish strains (Kalueff et al., 2014)
	Ease of genetic manipulation, availability of a wide range of genetic tools to study zebrafish biology
	Ease of experimental (*e.g.*, pharmacological) manipulation (hundreds of zebrafish can be simultaneously drug-treated acutely or chronically in their home tanks) (Kalueff et al., 2014)
	Smaller, simpler brains which can be better assessed using newest imaging techniques, including 3D microscopy, optical studies of neuronal activity and noninvasive photoablations of individual neurons (Braubach et al., 2012)
	Highly social animals (can be used to study autism and other aberrant social behaviors (Maaswinkel et al., 2013; Mahabir et al., 2013; Miller et al., 2013; Saif et al., 2013)
Additional considerations	"Robustness of phenotypes" (as a simpler organism, zebrafish responses to experimental manipulations can be dissected better, in a more clear-cut "black-or-white" fashion (Kalueff et al., 2013))
	Possibility to assess zebrafish skin coloration as a marker of sensitivity to selected neuroactive drugs (Kalueff et al., 2014)
	Evolutionarily distant immune system from humans (good for CNS cancer research, as human cancer cells can be injected into zebrafish, and can survive there)
	Possibility of studying zebrafish swimming in 3D space (Cachat et al., 2011)
	Excellent *in-vivo* models for student learning and training in behavioral and experimental neuroscience (Bilotta et al., 1999; Fields et al., 2009; Shuda and Kearns-Sixsmith, 2009).

rapid successive turns) (Cachat et al., 2010; Egan et al., 2009; Wong et al., 2010).

These behavioral phenotypes are strikingly analogous to those of both rodents and humans. Additionally, several physiological biomarkers traditionally explored in stress research (*e.g.*, brain *c-fos* expression and systemic cortisol levels) are strongly correlated with stress behaviors, and function in similar or identical roles across these species (Egan et al., 2009; Lau et al., 2011). For example, a recently developed "beaker stress" model is both succinct and parsimonious while retaining the ability to yield dynamic and robust data in zebrafish (Fig. 2). The beaker stressor capitalizes on the sociability of the zebrafish, a defining element of this species. In a typical beaker stress protocol, an individual fish is removed from its shoal, separated and confined in a 250-ml beaker filled with 100 ml of tank water. Acute 15-min exposure to this stressor can produce a 15-fold increase in baseline cortisol levels (Fig. 2), as well as robust anxiety-like behavior.

In addition, mounting evidence suggests that neurochemical alterations can serve as reliable biomarkers of zebrafish states (*e.g.*, changes in brain monoamine levels, mediated by various stress-related stimuli or social interactions; Teles et al., 2013). Moreover, a comprehensive glossary of larval and adult zebrafish behavior has been completed, to help investigators correctly recognize and interpret zebrafish phenotypes (Kalueff et al., 2013). Finally, recent advances in automated

Table 2
Selected limitations of zebrafish in biomedical research and translational neuroscience.

Model limitations	Comments
General limitations[a]	No animal model can fully recapitulate complex human brain disorder
	CNS and some complex behaviors develop over time (*e.g.*, social behaviors are not overt in larval fish; Buske and Gerlai, 2011, 2012)
Zebrafish-specific limitations	Duplication of genome (some fish genes have two copies instead of one, as in mammals) (Kalueff et al., 2014)
	Not as many well-characterized inbred strains as mice have (note that zebrafish, and fish in general, unlike rodents, do not tolerate inbreeding, and rapidly lose fertility with inbreeding) (Kalueff et al., 2014)
	Drugs which are not water-soluble can be problematic to administer by water immersion (but use other routes, see Table 4). Also, note that one can use a vehicle or other method to solubilize the drugs. For example, we typically use 0.5–1% DMSO as a vehicle for most of our drug studies.
	Limited applications of 3D analyses to larval zebrafish, especially HTS (Cachat et al., 2011)
	Some species differences in the blood–brain barrier (BBB). While zebrafish develop a BBB similar to that of humans, species differences exist, and may affect the permeability of certain drugs.
	Unclear "test battery" effects (the role of various tests within a phenotyping battery, well-reported in rodents, needs further studies in zebrafish)
	Parental care is not known (but is key for modeling some developmental disorders, such as autism, as well as influencing behavioral characteristics, and may require alternative species to be used; Kalueff et al., 2013)
	Certain brain areas are not as developed as in mammals (*e.g.*, cortex), and some CNS structures in zebrafish are still difficult to map to their mammalian counterparts (this knowledge gap may complicate the interpretation of circuitry–behavior interplay)
	Dosage: due to species differences in physiology, it may be difficult to directly translate human or rodent doses into zebrafish doses

[a] Common to all animal experimental models.