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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.

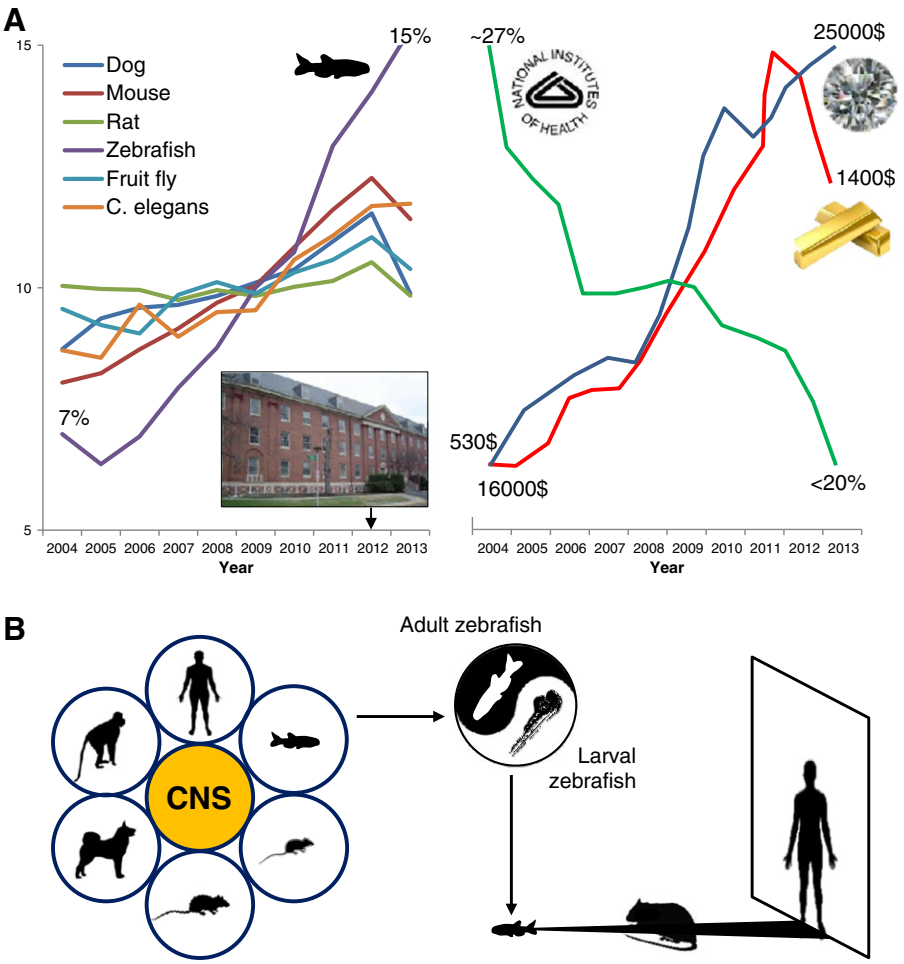

Fig. 1. The growing utility of zebrafish models in biomedicine. A: The number of publications using zebrafish and other common model organisms in the last 10 years (based on PubMed analyses, January 2014, data expressed as % publication per year, of the total for the respective species taken as 100%). For comparison of trends, the right diagram shows historical prices ($) of gold (per ounce, www.goldprice.org) and diamonds (www.wealthymatters.com), as well as the NIH funding rate for investigators with a PhD degree (%, www.nexus.od.nih.gov) for 2004–2013. Note that zebrafish literature, gold and diamonds show a similar rise in the last 10 years. Inset photo: The NIH Zebrafish Research Facility (opened in Building 6 at Bethesda campus) is the world's largest zebrafish research facility, able to house up to 20,000 tanks and 100,000 fish. B: Various vertebrate model organisms used in CNS research. Note the "dual" nature of zebrafish models (with larval and adult zebrafish available complementarily for brain research, both being equally important models for different research purposes). Right image: translational cross-species approaches to human brain disorders, focusing on conserved, evolutionarily ancient and "shared" pathways (implicated in brain pathogenesis) between various vertebrate species ("from tank to bedside"); see (Stewart et al., 2014) for details.

neurophenotyping technologies further foster innovative research using zebrafish models (Cachat et al., 2011; Kalueff et al., 2014; Maaswinkel et al., 2013).

As with other models, zebrafish have their limitations (Table 2). Should this preclude us from using zebrafish to complement more traditional (*e.g.*, rodent) models of brain disorders? Absolutely not — first, considering the innovative nature of neuroscience *per se* (Kalueff et al., 2007), and second, because the advantages of using zebrafish in neuroscience (Table 1) outweigh the risks and limitations. In fact, it has been argued recently that major brain disorders can be modeled in zebrafish (Table 3), perhaps, in a much cheaper and more straightforward way, as compared to other vertebrate models (Gerlai, 2012; Kabashi et al., 2011; Kalueff et al., 2014).

Is it easy to develop and introduce new models of brain disorders? Clearly not, as it is often difficult to identify phenotypes and syndromes to model them across species. In addition, new ideas and paradigms in science are often met with reservations (Kalueff et al., 2007). For example, Table 4 summarizes common reservations and concerns about zebrafish models, which we have encountered ourselves or often hear from dedicated colleagues who work with other organisms. Importantly, while most of these concerns are reasonable, they are relatively easy to address. Furthermore, although some concerns are zebrafish-specific, others are more general, and can be raised for any model organism, including rodents (Table 4). Thus, the goals of this preface (and the Special Issue itself) are to acknowledge the potential and limitations of zebrafish models of complex brain disorders, and – more importantly – to encourage the field to move full speed ahead toward applying this knowledge to urgent needs of translational neuroscience.

3. Concluding remarks

One interesting aspect is worth mentioning here, putting current zebrafish research in a historical perspective. The history of Science can be both encouraging and ironic. For example, 110 years ago, Ivan Pavlov (Fig. 3) won the Nobel Prize for his groundbreaking study of the physiology of digestion. This line of research has later contributed to his theory of conditioned reflexes (Pavlovian conditioning), for which Pavlov remains one of the world's most renowned and influential physiologists. Back then, all "serious" science was performed in dogs, prompting Pavlov to acknowledge "man's best friend" in his 1904 Nobel lecture. He further expressed

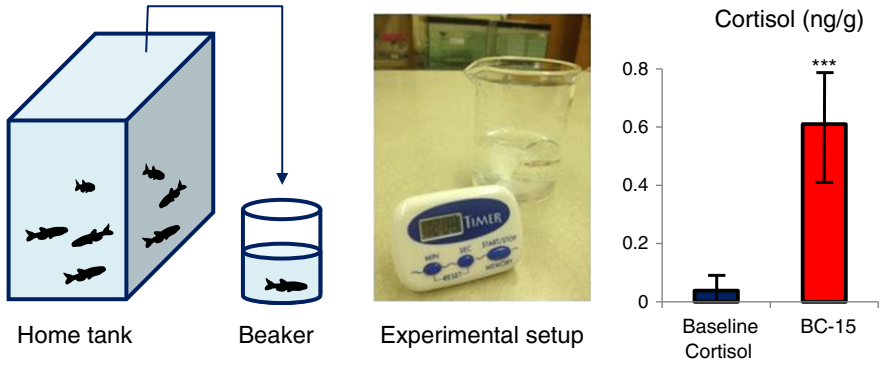

Fig. 2. A diagram illustrating the beaker stressor test and elevated whole-body cortisol levels in adult zebrafish following an acute exposure. The graph shows the average whole-body cortisol level of 10 subjects for each condition (baseline cortisol from instant anesthetization *vs.* a 15-min exposure to the beaker stressor (BC-15); *** < 0.0001, *U*-test).

his gratitude by commissioning the world's first Monument to the Dog in 1935. One can only imagine what would happen if someone told the fiery Pavlov that "primitive" rats and mice will replace his beloved dogs, becoming the neuroscience's most popular model organisms for decades. Perhaps, Pavlov would have been even more surprised to learn that zebrafish are widely used today to study conditioning (Agetsuma et al., 2010; Okamoto and Aizawa, 2013; Okamoto et al., 2012) and other related complex CNS phenomena (Collier and Echevarria, 2013; Kalueff et al., 2013; Miller et al., 2013; Nguyen et al., 2013; Parker et al., 2013).

Thus, although the landscape of animal model organisms we use today has changed markedly since Pavlov (Fig. 1), this history lesson is interesting. For example, it shows that the subject of "mainstream neuroscience" today may not be in the spotlight 50 years later. Therefore, we shall remain open-minded when it comes to accepting new model organisms: perhaps, 10–15 years from now (or even sooner), "zebrafish psychiatry" will be taught in colleges in the Introductory Neuroscience courses. Also, the Monument to the Zebrafish is still yet to be built — something that our International Zebrafish Neuroscience Research Consortium (ZNRC) is discussing, and welcomes feedback from colleagues on whether this initiative is timely.

In summary, the zebrafish is rapidly gaining its "translational momentum" in biological psychiatry, becoming a valuable tool to study the normal and pathological brains. And while this little Asian fish prefers shallow waters to big fast rivers in the wild, the laboratory zebrafish is boldly entering mainstream neuroscience.

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Table 3
Zebrafish models relevant to human neuropsychiatric disorders (based on "The Diagnostic and Statistical Manual of Mental Disorders" (DSM-5) (American Psychiatric Association, 2013). + Pertinent zebrafish models available (including models targeting selected, but not all symptoms); ? unclear, or no models available yet; n/a the specific disorder is not applicable to zebrafish.

Major groups of human neuropsychiatric disorders	Availability of relevant models in:	
	rodents	zebrafish
Neurodevelopmental disorders	+	+
Schizophrenia and other psychotic disorders	+	+
Bipolar and related disorders	+	+
Depressive disorders	+	+
Anxiety disorders	+	+
Obsessive–compulsive and related disorders	+	?
Trauma- and stressor-related disorders	+	+
Dissociative disorders	n/a	n/a
Somatic symptom and related disorders	n/a	n/a
Feeding and eating disorders	+	+
Elimination disorders	?	?
Sleep–wake disorders	+	+
Sexual/gender dysfunctions	+	?
Disruptive, impulse-control, and conduct disorders	n/a	n/a
Substance-related and addictive disorders	+	+
Neurocognitive disorders	+	+
Personality disorders	n/a	n/a
Paraphilic disorders	n/a	n/a

Table 4
Common reasons why neuroscientists can be cautious with applying zebrafish models.

Considerations	Comments and recommendations
General concerns	
Why use zebrafish?	Table 1 summarizes multiple advantages of applying zebrafish models to translational neuroscience research
Conservative nature of Science: Is it "safe" to try a new model organism, such as zebrafish?	Science constantly evolves. As scientists, we shall be creative, innovative and thinking outside the box (Kalueff et al., 2007). Necessity is the mother of invention.
Lack of understanding of physiological complexity: Do zebrafish have brains?	Zebrafish show a remarkable genetic and physiological homology to humans. Many systems are highly conserved, and many genes show >95% homology.
Lack of information and knowledgebase: Can zebrafish feel pain? Do they have affective behaviors? Can you model anxiety or depression in fish?	Yes, see Kalueff et al. (2013) for details of major behavioral domains, phenotypes and syndromes in zebrafish
Specific practical/methodological concerns	
Genome duplication concerns: Zebrafish have a duplicated genome. The system is just too complicated	Zebrafish genome duplication affects some, but not all, genes. In most cases, the "duplicated" copies encode very similar proteins (*e.g.*, receptors or transporters) with similar/overlapping pharmacology and biochemistry (Kalueff et al., 2014)
Fiscal concerns: Zebrafish may be too expensive for my lab	Many labs we know are switching to zebrafish to *save* money (*e.g.*, zebrafish models can be 500–1000 times cheaper than similar mammalian studies).
How can I give drugs to fish?	Use the same delivery methods as with other model organisms: *e.g.*, systemic (*via* immersion), intraperitoneally, subcutaneously, with food, or using intracerebroventricular injections.
I cannot figure out zebrafish doses. It may be very difficult for various drugs to relate human or rodent doses to zebrafish doses	Species difference in physiology and pharmacology are common in biomedicine. However, comparative analyses of relative potencies of effective doses for various psychotropic drugs in zebrafish revealed similar ranking of drugs' activity across various species, illustrating translational value of zebrafish models for CNS drug screening (Kalueff et al., 2014). Together with the growing body of zebrafish pharmacological/toxicological literature and the availability of efficient low-cost HTS, this contributes to establishing a library of zebrafish doses for major pharmacological agents
I do not understand fish behavior! Can I decode fish behavior well enough to model complex brain disorders?	Like with any other area of science, it takes time, as more models become developed, and zebrafish responses become clearer now than a decade ago (*e.g.*, see Kalueff et al., 2013). Numerous behavioral tests have also been developed, which are both simple to perform and accepted by the field as valid assays (*e.g.*, see Kalueff and Cachat, 2010; Kalueff and Stewart, 2012 for detailed protocol reviews).
"Cultural" concerns	
Habits: My mentor worked with rodents, and the mentor of my mentor worked with rodents	Many discoveries in biomedicine were made using novel model organisms. Try a new field (plus this will reduce competition with your mentors, so they get their grants funded too).
My peers think zebrafish research is not very "mainstream" in neuroscience	Generating more new knowledge changes the field, and zebrafish research is rapidly becoming *the* mainstream neuroscience (also see Fig. 3 for a historical perspective)
The zebrafish field is too small, and may not be worth investing in it. Will I have job in this field?	The zebrafish field is rapidly growing (Fig. 1), and may become a perfect academic investment (Stewart et al., 2014). Also, in addition to academic and government research, there is a growing number of companies developing and using zebrafish *in-vivo* HTS to discover new therapies
Resource- and use-related concerns	
Lack of husbandry skills: I do not know how to raise the fish. We do not have the fish facility.	It is generally quite easy; but always ask your vivarium staff, if their help is needed. To start new zebrafish projects, the laboratory only needs a small room (if you start a new model organism, your university may be very supportive space-wise; more space will become available if you start bringing grants)
Concerns about animal care and use committees (IACUCs): They will raise too many questions if I work with fish	IACUCs are becoming well-aware of the rapidly growing utility of zebrafish in biomedicine. While they sometimes may have "interesting" views on zebrafish research, try to educate and work with IACUCs. Using a lower vertebrate adheres to the 3R principles
Funding concerns: The NIH does not like zebrafish. My study section will reject our grant if I propose to use fish	The NIH is very interested in zebrafish research. Recently, they built the worlds' biggest zebrafish facility in Bethesda, MD (Fig. 1), and also have the trans-NIH zebrafish initiative. The NIH is also getting more and more zebrafish grant applications each cycle, and now tries to include zebrafish experts in their panels. The NIH study sections are also "warming up" to zebrafish projects
Are there enough zebrafish data resources?	There are multiple outstanding public-access biomedical databases available to zebrafish investigators, including the Zebrafish Information Network (the zebrafish model organism database, www.ZFIN.org), the Zebrafish Genome (ENSEMBL) database, the Zebrafish Genome (UCSC) database, the Zebrafish International Resource Center (ZIRC, www.zebrafish.org), the Zebrafish Neurophenome Project (ZNP, www.tulane.edu/~znpindex) and Zebrafish Brain Atlas (www.zebrafishbrain.org)

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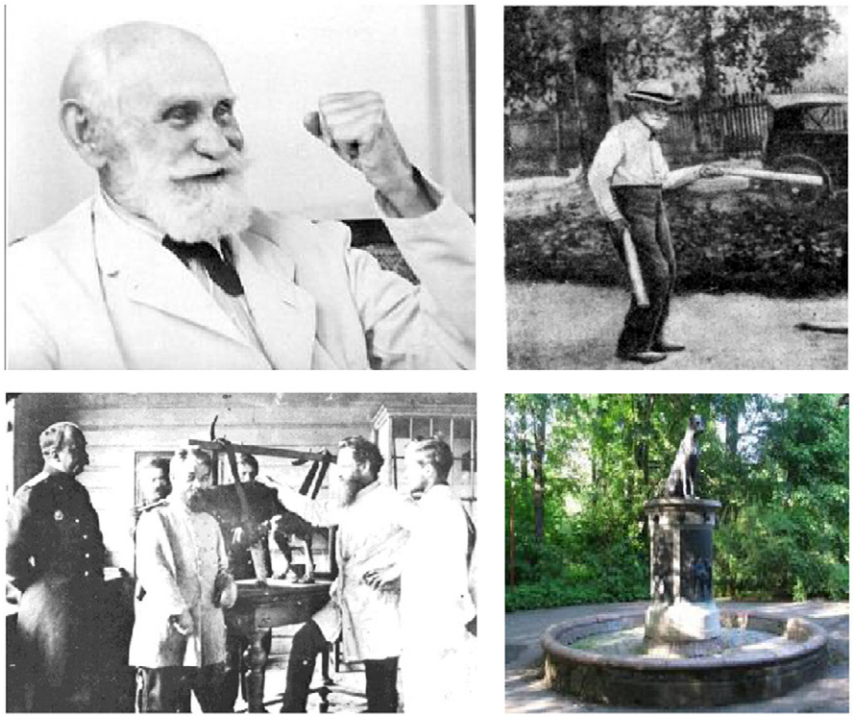

Fig. 3. History lessons: Pavlov and 110 years later. Academy Professor Ivan Pavlov (1849–1936) won the 1904 Nobel Prize in Physiology or Medicine for his groundbreaking work on the physiology of digestion, the research he had performed in dogs. Bottom right panel — Monument to the Dog at the Institute of Experimental Biology, commissioned by Pavlov in 1935 to honor the dog's service to biomedical science (photos courtesy of Pavlov Museum and the Institute of Experimental Medicine, St. Petersburg, Russia). Pavlovian conditioning is currently commonly studied in fish species, including zebrafish. Monument to the Zebrafish is still to be built.

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