Journal of Neuroscience Methods 234 (2014) 54–58

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic Neuroscience

Fish in behavior research: Unique tools with a great promise!

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HIGHLIGHTS

- Comparative behavioral neuroscience answers phenogenetic and phylogenetic questions.
- Study of new species increases translational relevance in biomedical research.
- Zebrafish strike a balance between practical simplicity and system complexity.
- Analysis of evolutionarily older species helps us find the core mechanisms.

ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form 15 April 2014
Accepted 15 April 2014
Available online 22 April 2014

Keywords:
Comparative behavioral brain research
Zebrafish
Phenomics
Phenotyping

ABSTRACT

Fish represent the most diverse class of vertebrates on Earth and also an unprecedented, but as of yet still largely untapped, resource for comparative analyses that can illuminate answers to questions about both how organisms work and how they evolved. The current review is a general discussion of some of the basic principles of why adding new species such as fish to the short list of biomedical model organisms (mainly the house mouse and the rat) has merit. In addition to the general points, it also reviews some questions about a newcomer, the zebrafish, which is rapidly gaining popularity in brain and behavior research. It discusses some examples demonstrating the advantages and disadvantages of the zebrafish mainly in the context of biomedical research. It is followed by other articles that further elaborate on these questions.

Contents

1. Introduction

1.1. The most diverse group of vertebrates: the teleosts

Teleost (ray finned bony) fishes arose during the Triassic period about 250 million years ago and represent the largest group among vertebrate classes with over 30,000 species living today (Nelson, 2006; Lévêque et al., 2008). Teleost fish inhabit perhaps the most diverse habitats and geographical regions on Earth.

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http://dx.doi.org/10.1016/j.jneumeth.2014.04.015

Ranging from open waters of the Pacific Ocean to the caves of Mexico (e.g. the blind cave tetra, *Astyanax mexicanus*), from the abyss of water depths below 4000 m (bathypelagic fish, e.g. the humpback anglerfish, *Melanocetus johnsonii*) to the streams and lakes of the Himalayan Mountain range (e.g. Tibetan Stone Loach, *Triplophysa stoliczkai*) fish can be found in practically any body of water. Fish are perhaps the most successful vertebrates of our geological time. With their enormous diversity (Lévéque et al., 2008) comes an amazing array of anatomical, physiological and behavioral adaptations. If one just looks at reproductive behavior one finds all the types of reproductive strategies and mating systems ever described in the animal kingdom represented among fish species, including parthenogenesis as well as various forms of sexual reproduction such as polyandry (one female breeding with multiple males), polygyny (one male breeding with multiple females), polygynandry (multiple males breeding with multiple females), and also monogamy and biparental care for the young (one male breeding with one female and rearing their offspring together). The diversity of teleosts is an enormous, and as of today largely untapped, resource. It offers unprecedented possibilities for comparative analyses that can illuminate answers to both proximate questions about how the body and the brain of vertebrates function as well as ultimate questions about how vertebrates evolved.

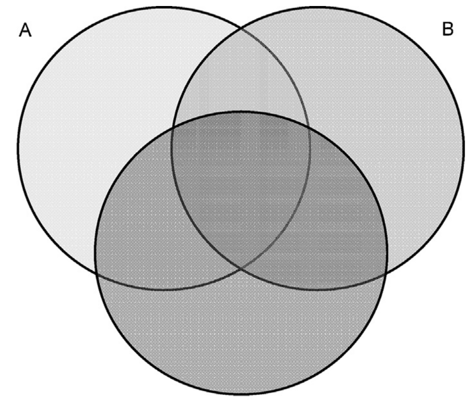

Fig. 1. The power of comparative analysis. Each of the three circles shown on this Venn diagram represents the collection of biological mechanisms present in a given species (A, B or C) associated with a particular behavior of interest (for example social behavior). Note that the behavior of interest is user defined and may appear somewhat similar at the phenotypical level across the studied species but one does not a priori know if the apparent similarities at the level of behavior represent construct (mechanistic) similarities. The goal of the comparison of species is exactly that: identification of mechanistic similarities. The overlapping areas between the circles represent mechanisms common across at least two of the species. The area in the middle that overlaps with all three circles represents mechanisms that are common to all three species and thus is expected to involve the evolutionarily conserved features most fundamental to the behavior of interest. By analyzing multiple species (three or more), such conserved mechanisms are more easily found and thus the core aspects of biology underlying the behavior of interest may be better identified and understood.

2. The advantage of adding a new species to the mix for biomedical research: finding common features across species increases translational relevance

In the current review, however, I will not focus on multiple species and the comparative approach. Instead I will briefly introduce a single teleost species, the zebrafish (*Danio rerio*) that is rapidly gaining acceptance in biomedical research. Some important points about comparative approaches, however, first have to be made even with this one-species-mind. All too often, investigators including myself argue why our chosen species is better than other ones, and indeed each of these favorites perhaps possesses some features that may be advantageous from the perspectives of biomedical research, the context of this paper. Nevertheless, the power of comparative studies is sometimes overlooked. The Holy Grail of biomedical research is translational relevance, i.e. the promise that research done with a laboratory species will provide useful information applicable to the human clinic. Most biomedical research has been conducted using a single "model" organism, the house mouse or the rat. The problem with this, however, is that two species (mice or rat and men in this case) may differ in many ways. Consider the example of identifying a particular behavioral feature in the mouse and finding associated genetic effects, say a set of over or under-expressed genes. The correlation between the behavioral feature and gene expression characteristics may be strong but it may not necessarily mean causal relationship. We do not know how many or which genes really underlie or explain the behavioral phenotype of interest. In order to answer this question one would need to independently and systematically manipulate every possible gene and every possible combination of genes. And what if the relationship among the behavioral and gene expression profiles is species-specific, i.e. different between the mouse and human? Finding phenotypical correlations among species-specific features in one species does not guarantee that such correlations will be present in the other, and perhaps even more importantly, even if they are present in both species, causal relationships among these correlations is not at all certain. Consider the example of autism. Numerous mouse models have been suggested for the analysis of human autism. One of these is the BTBR mouse strain that shows some features that resemble the behavioral abnormalities seen in human autistic patients. For example, these mice exhibit reduced social interactions, impaired play, low exploratory behavior, unusual vocalizations and heightened anxiety (e.g. McFarlane et al., 2008). This strain is also characterized by many other strain specific features including the absence of the corpus callosum, and a, very attractive, black-tanned fur color. It is unlikely, however, that these latter strain-specific features have anything to do with the idiosyncratic behavioral characteristics of this mouse strain. Thus, the question of what mechanisms underlie the abnormal behavioral characteristics is not easy to answer. One way to provide answers to this question is to compare the features of these mice to human, but humans are not easy to experiment with for ethical and practical reasons. This is where zebrafish come in.

Adding a new species to the mix of comparisons can robustly enhance our ability to identify biological features and mechanisms that are relevant to the studied behavioral phenomena, abnormal social behavior in this particular example. Why is this so? It is a matter of Boolean algebra. Consider a Venn diagram (Fig. 1) in which each circle represents the collection of biological features or mechanisms of a given species that are apparently (and without the assumption or knowledge of causality) are associated with a behavior of interest, abnormal social behavior in our example. Two species may give some overlap between biological features of each of these species but in the overlapping area perhaps a large number of irrelevant features still may dilute the relevant ones. Using multiple species will allow one to identify a much smaller area of overlap, mechanisms that are common across all the studied species and ones that all are associated with the behavior of interest. In summary, using multiple species will allow one to identify common features across species and these commonalities will likely be shared not just among the studied laboratory organisms but also with humans. That is, the comparative approach increases translational relevance. Another point to consider before I move to the specifics of why zebrafish are becoming increasingly popular in