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Zebrafish Neurobehavioral Protocols

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Preface to the Series

Under the guidance of its founders Alan Boulton and Glen Baker, the Neuromethods series by Humana Press has been very successful since the first volume appeared in 1985. In about 17 years, 37 volumes have been published. In 2006, Springer Science + Business Media made a renewed commitment to this series. The new program will focus on methods that are either unique to the nervous system and excitable cells or which need special consideration to be applied to the neurosciences. The program will strike a balance between recent and exciting developments like those concerning new animal models of disease, imaging, in vivo methods, and more established techniques. These include immunocytochemistry and electrophysiological technologies. New trainees in neurosciences still need a sound footing in these older methods in order to apply a critical approach to their results. The careful application of methods is probably the most important step in the process of scientific inquiry. In the past, new methodologies led the way in developing new disciplines in the biological and medical sciences. For example, Physiology emerged out of Anatomy in the nineteenth century by harnessing new methods based on the newly discovered phenomenon of electricity. Nowadays, the relationships between disciplines and methods are more complex. Methods are now widely shared between disciplines and research areas. New developments in electronic publishing also make it possible for scientists to download chapters or protocols selectively within a very short time of encountering them. This new approach has been taken into account in the design of individual volumes and chapters in this series.

Wolfgang Walz

Preface

The use of zebrafish (*Danio rerio*) in neurobehavioral research has dramatically increased over the past decades. This has led to the development of novel behavioral assays to quantify a variety of behaviors seen in larval and adult zebrafish. There has also been an increasing trend toward the use of automated video-tracking software to analyze the behaviors observed in these assays. The ability to correlate behavioral patterns with physiological endpoints on an individual is another advantage of using zebrafish in neurobehavioral research. As such, zebrafish are rapidly emerging as a promising, high-throughput animal model for biomedical research.

The present book is written by the leading experts in zebrafish research, many of which are active members of the Zebrafish Neuroscience Research Consortium (ZNRC). This volume is composed of protocols detailing three major research areas, including (1) the use and interpretation of video-aided quantification of zebrafish behaviors, (2) descriptions of novel assays commonly used to quantify emotionality, as well as learning, memory, and social behaviors in zebrafish, and (3) the quantification of circulating cortisol levels and the subsequent correlation to anxiety-like behaviors in zebrafish. This book will serve as a useful *practical* complement to another book of this series, *Zebrafish Models in Neurobehavioral Research (Vol. 52)*, which covers scientific/theoretical problems and neurobiological principles of zebrafish behavior.

The first chapter of the present book describes the principles of video-tracking in zebrafish research, making note of the advantages of video analysis. These include the ability to obtain an increased number of behavioral endpoints, many of which are not quantifiable using traditional observation techniques, as well as improved precision when quantifying certain zebrafish behaviors that are otherwise highly subjective. In line with this, Chapter 2 presents a novel approach to analyze data produced with automated behavioral recording. Termed the "videogram", this single image forms a density map of zebrafish activity in a video sequence, serving as a direct, repeatable, and unbiased measure of animal activity.

Chapter 3 explains how automated video-tracking technologies can be connected with a behavioral assay in larval zebrafish. Focusing on the quantification of avoidance behaviors in larva, this protocol describes two assays, the "bouncing-ball assay" and the "two-fish assay", which measure responses to a threatening stimulus as well as avoidance behavior, respectively.

The following chapters of this book describe more specific behavioral paradigms to examine the wider behavioral repertoire of zebrafish. This includes Chapter 4's quantification of zebrafish responses to chemical alarm cues or substances that indicate the presence of predation risk. Several assays of zebrafish learning and memory are summarized in the subsequent chapters, including a modified T-maze test of the acquisition and extinction of reward-visual stimulus association, simple conditioned place preference assays for assessing the reinforcing properties of drugs of abuse, as well as a method for studying conditioning olfactory behaviors in adult zebrafish. Additionally, Chapter 8 provides a detailed proto-

col for a light/dark plus maze novel environment test, which measures thigmotaxis and scototaxis in order to assess anxiety-like behaviors in zebrafish.

Chapters 9 and 10 are logically interconnected and describe assays of zebrafish social behavior. The first contribution describes methods for simple, fast, and accurate assessment of drug-induced effects on social and motor behaviors in zebrafish. Such behavioral paradigms may be particularly useful in conjunction with high-throughput drug screening. The second protocol outlines an assay for identification, characterization, and quantification of agonistic behaviors in zebrafish, which can be used to assess the effects of pharmacological and genetic manipulations in this species.

Chapter 11 provides a protocol for determining circulating cortisol levels in zebrafish. Such physiological quantification is highly applicable to behavioral studies of fear and anxiety-like responses in zebrafish, as the zebrafish acute stress response is analogous to that of humans, resulting in increased production and secretion of cortisol into the blood.

Chapters 12 and 13 provide some further protocols that can be used to phenotype zebrafish behavior in novelty tests. The first protocol explains how to analyze an interesting behavioral pattern recently observed in zebrafish – their natural tendency to form preferred safe zones, or "homebases". The second assay parallels Chapter 8 and is based on the fish's inherent tendency of scototaxis (dark preference). This chapter illustrates, using two variations of the light/dark box test, how this simple paradigm can be used to assess zebrafish behavior evoked by anxiogenic or anxiolytic drug administration.

While most drugs are administered to zebrafish by immersion of a fish into a drug solution, Chapter 14 discusses an alternative method of drug administration, which utilizes intraperitoneal injection to treat zebrafish with a pharmacological agent. This protocol is especially useful for those working with drugs that may not be conducive for immersion, such as insoluble or highly toxic compounds.

The previous chapters are further complemented by Chapter 15, which instructs the reader on how to employ Maximum Predictive Value (MPV) to determine how sensitive a particular model is to various pharmacological manipulations. As a particular example, this chapter outlines how to utilize this measure to validate behavioral endpoints in the novel tank test when assessing anxiety-like behavior.

The final Chapter 16 presents a highly innovative approach to zebrafish behavior based on three-dimensional reconstructions of zebrafish swim traces to better understand their behavior. This protocol logically summarizes other chapters in this volume, providing a methodology for using video-tracking technology to more comprehensively characterize zebrafish behavior. This contribution will be especially useful for analyzing automated endpoints for drug- and class-specific zebrafish phenotypes in parallel.

Overall, as the reader will learn from this book, zebrafish offer an excellent opportunity to perform steadfast scientific investigations in a robust and high-throughput manner. All this remarkably separates the zebrafish from other classical laboratory animals. Moreover, the relative ease at which zebrafish can be housed, reproduced, and handled has prompted their introduction into teaching laboratories. Given the value of zebrafish in the research and teaching laboratories, we hope that this book will be accessible to a wide range of expertise. The Editors acknowledge the important role of ZNRC in promoting zebrafish research, including many protocols described here by active participating laboratories. The present compilation of neurobehavioral protocols is particularly timely, as it provides the first practical introduction to the exciting field of zebrafish behavioral research. Perhaps

even more importantly, all assays described herein can be performed, creatively modified, further improved, and combined in almost limitless ways, again epitomizing the growing potential of zebrafish in modern scientific inquiry.

Allan V. Kalueff
Jonathan M. Cachat

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