Current Zoology, 2017, 63(1), 35–44
doi: 10.1093/cz/zow077
Advance Access Publication Date: 11 July 2016
Article

OXFORD

Article

Animated images in the analysis of zebrafish behavior

Robert Gerlai*

Department of Psychology, University of Toronto Mississauga, 3359 Mississauga Road North, Rm CCT4004, Mississauga, Ontario, Canada L5L 1C6

*Address correspondence to Robert Gerlai. E-mail: robert_gerlai@yahoo.com.

Received on 24 March 2016; accepted on 22 June 2016

Abstract

This invited review is based upon a recent oral paper I presented at the Virtual Reality Symposium of the 34th International Ethological Conference (2015, Cairns, Australia), and as such it describes studies conducted mainly in my own laboratory. It reviews how we utilized visual stimuli for inducing behavioral responses in the zebrafish with a focus on shoaling, group forming behavior. The zebrafish is gaining increasing popularity in neuroscience. With this interest, its behavior is also more frequently studied. One of the many advantages of the zebrafish over traditional laboratory rodents is that this species is diurnal, and it relies heavily upon its visual system. Thus, similarly to our own species, zebrafish respond to visual stimuli in a robust and easily quantifiable manner. For the past decade, we have been exploring how to use such visual stimuli, and have developed numerous paradigms with which we can induce and quantify a variety of behavioral responses, including shoaling. This review summarizes some of these studies, and discusses questions including whether one should use live fish as stimulus, whether and how one could present animated (moving images) of fish, and how one could optimize a range of stimulus presentation parameters to elicit the most robust responses in zebrafish. Although the zebrafish is a relative newcomer in ethology and behavioral neuroscience, and although many of our findings only represent the first steps in this research, our results suggest that the behavioral analysis of the zebrafish will have an important place in biomedical research.

Key words: alcohol, animated images, high-throughput screening, learning and memory, schooling fish, shoaling, social behavior, zebrafish.

Introduction: Animal Psychology is not a Soft Science

Behavioral analysis, and perhaps psychology in general, has sometimes been looked down on as a softer science, especially when compared with other subdisciplines of biology such as genetics or neuroscience. This view is often echoed implicitly or explicitly based on a key and recurrent issue: replicability. Behavior often exhibits apparently higher variation (within experiment, across experiment, and/or across laboratories) compared with other biological features the scientist may chose to measure, and thus the findings may appear to be difficult to replicate (Crabbe et al. 1999). Having been worked in the fields of genetics and neuroscience, I debate the general validity of this argument. Nevertheless, I also acknowledge that indeed behavior can be frustratingly variable. However, this variability may be viewed not as a disadvantage, but rather as an opportunity. One may argue that the variability comes from behavior being more flexible, resulting from plasticity and the complexity of the brain. As such, behavior may be viewed as a phenotypical measure that is more responsive to environmental (extrinsic) as well as internal (intrinsic) factors than most other phenotypes, and this is where the opportunity lies. Behavioral analysis may be viewed as a tool with which one can efficiently probe the functioning of the central nervous system (CNS), a tool that allows one to detect even small and otherwise obscured functional changes in the brain

(Gerlai 2002, 2014a). These changes may be induced by environmental factors, but may also be the result of other experimental manipulations, for example, genetic (Godinho and Nolan 2006; Takao and Miyakawa 2006; Haesemeyer and Schier 2015) or pharmacological manipulations (Kokel et al. 2012; Alexandrov et al. 2015; Haesemeyer and Schier 2015). Thus, behavioral analysis may be utilized for testing how the animal responds to the ever-changing environment, and it may also be used to screen drugs or mutations thus allowing one to answer fundamental mechanistic questions about the brain.

Another reason why animal psychology is occasionally considered a soft science is that behavioral analysis appears to be very easy to conduct. For example, one of the most frequently utilized learning paradigms employed in neurobehavioral genetic research of relational memory is the Morris Water Maze (Schenk and Morris 1985). This maze is not employed with fish, instead mice or rats are required to swim and find a hidden target, a platform placed just below the water level in the maze. This task gained much attention among neuroscientists as it allowed the investigator to analyze the functioning of a crucial part of the brain, the hippocampus (Bures et al. 1997; Gerlai 2001). But all this task requires is a large pool of water made murky with white water-color, a pedestal (platform) that is placed underneath the water surface, and a stop watch with which the experimenter measures how quickly the rat or mouse manages to get onto the platform and thus out of the water. It appears easy and simple. The simplicity of the task, however, is misleading (Gerlai 2001). Decades of research showed that depending on how one runs the task, depending on numerous procedural, physical, and timing parameters of the task, and also depending on the genotype (strain origin) of the rodent tested in it, widely different results may be obtained (Lipp and Wolfer 1998; Wahlsten et al. 2005).

Why is this discussion relevant to the focus of this review? The talks presented at the virtual reality symposium of the 34th International Ethological Conference in Cairns Australia nicely demonstrated that animal psychology methods are not simple, nor they are easy or imprecise. They are sophisticated, and can be conducted in a manner that allows precision, that is, increased replicability and consistency across experiments. One source of both these features (sophistication and consistency) comes from the increasingly powerful computer hardware and ingenious software applications employed in animal behavior research. This review will discuss neither of these features, however. It will not focus on the actual computer solutions. Instead, it will discuss HOW these methods may be utilized in animal research whose aim is to understand how the brain of the simplest vertebrate, fish, works.

Why Study Fish, and Why Zebrafish among all the Species?

Fish are the simplest vertebrate species. Analysis of fish represents a reductionist approach, one which allows the investigator to study fundamental and evolutionarily more ancient aspects of our biology (Gerlai 2014b). The questions answered by fish research are important not only because of the evolutionarily ancient aspect of this taxon, but also because fish, and zebrafish in particular, have been shown to provide translationally relevant answers (Kalueff et al. 2014). Translational relevance is, of course, the result of the continuity of the evolutionary process. It manifests, for example, as conserved nucleotide sequence among zebrafish and human (or other mammalian) genes, as similarity of neurotransmitter systems of the zebrafish and human brains, and as efficacy of drugs in zebrafish originally developed for mammals (Kalueff et al. 2014; Stewart et al. 2014a, 2014b), to mention but a few features.

In addition to the relative simplicity of the zebrafish, this species has many other advantages that are now being recognized, and which make this little fish compete well with the traditional laboratory rodent species in biomedical research. Zebrafish are small (4 cm long), very prolific (a single female can produce 200 eggs at each spawning and may spawn multiple times a week), and easy to maintain (the zebrafish is a group forming fish and can be crowded in small tanks). It also happens to develop very fast (completes its organogenesis within 5 days), and remains practically transparent during this process. Due to these features, about 4 decades ago developmental biologists discovered the zebrafish (Streisinger et al. 1981; Granato and Nüsslein-Volhard 1996), and geneticists started to assemble a molecular biology tool set specifically designed for this species (Kimmel 1989; Granato and Nüsslein-Volhard 1996; Patton and Zon 2001). By now, the zebrafish has become one of the most preferred species of geneticists with powerful genome engineering tools and forward and reverse genetic techniques readily available for it (Clark et al. 2011; Blackburn et al. 2013).

The main bottleneck of zebrafish research concerned with understanding how the brain functions is the analysis of its behavior (Sison et al. 2006). However, even this area of research has seen a rapid growth with number of publications on zebrafish behavior showing a quasi-exponential increase over the past decade (Kalueff et al. 2014). The current review will focus on one aspect of this new development, the use of visual stimuli in the behavioral analysis of shoaling (group forming) responses in zebrafish. This focus is admittedly biased, as it is based on the studies conducted in my own laboratory. Clearly, other stimuli have also been successfully employed and behavioral tests other than those focused on shoaling are also important. For example, robotic fish in the context of antipredatory behavior have started to be employed in zebrafish (Ladu et al. 2015).

Visual Stimuli: Ethological Relevance, Sophistication, and Control

Given the focus of the Virtual Reality Symposium of the International Ethological Congress held in 2015 in Cairns, Australia, all speakers, including myself focused on visual stimuli. Nevertheless, fish utilize stimuli of multiple modalities. They can hear (Ladich 2014), smell (Hamdaniel and Døving 2007), and perceive low frequency vibration via their lateral line (Bleckmann and Zelick 2009) (somewhat equivalent to tactile stimulus perception in terrestrial species). Is the focus of research on visual stimuli and is the use of these stimuli as the method of experimental manipulation well justified? At this point, the answer to these questions is not well known. Nevertheless, it is clear that most fish species, with the zebrafish included, are diurnal, that is, active during the day. Being diurnal means that the visual system of the zebrafish is well developed, and visual cues represent salient features of the environment for this species (Neuhauss 2003). This is an important advantage of the zebrafish over the nocturnal laboratory rodents. Visual cues are much easier to manipulate than auditory or olfactory or lateral lines cues. Their on-set, off-set, location, and many other particular features of the stimulus can be precisely controlled. The equipment (e.g., monitors, cameras, computers) one employs to present and control visual stimuli is readily available from electronics stores for a relatively low cost, because our own species is also

diurnal and uses visual stimuli in our daily life. Last, for the latter reason, the face validity, and often also the construct validity, of zebrafish paradigms and biological models of human brain function utilizing visual stimuli is high (Neuhauss 2003).

Nevertheless, vision is only one of several modalities fish use, and the question whether employing stimuli of other modalities is required or necessary in behavioral brain research remains to be answered. Some studies, including those with robotic fish have shown promise, for example (Ladu et al. 2015). In these studies, the actual physical presence of the robotic fish provides not only visual but also auditory and lateral line stimuli to the experimental fish. The complication with robotic fish, however, is that controlling the movement and sound of the robot in a manner that makes its behavior ethologically relevant is rather complicated not only because of technical limitations, but also because of our limited understanding of the role of such stimuli in fish behavior. In this review, thus I focus on the modality that is best studied and most easily controlled: visual stimuli. But what visual stimuli should we use and how should we present them?

The answer to this question has now been found in a comprehensive review published in this special issue (Chouinard-Thuly et al. 2017). In my laboratory, we have been studying the effects of visual stimuli in two main contexts, a form of social behavior, shoaling (group forming) (Miller and Gerlai 2012), and fear or anxiety (or anti-predatory behaviors) (Gerlai 2010a). Each of these different contexts represents idiosyncratic challenges, but has a common guiding principle: ethological relevance (Gerlai and Clayton 1999). In other words, we attempt to use stimuli that make sense from the perspectives of the evolutionary history and ecology of the zebrafish. In the current review, I only discuss our studies on shoaling behavior in the context of the methodological questions surrounding the use of visual stimuli.

Shoaling Behavior: How can we Induce and Measure It?

Shoaling (group forming) is a fundamental and robust feature of the zebrafish (Gerlai 2014c). Zebrafish shoal in nature and in the laboratory (Miller and Gerlai 2007; 2011 and references therein, also see Parker et al. 2014). In nature, zebrafish have been observed to aggregate in groups with the size of these groups varying from only a few individuals to several hundred individuals (Engeszer et al. 2007). In the laboratory, shoaling has been induced and quantified in two fundamentally different ways. One of them is to employ freely moving fish and to record numerous shoaling behavior-related parameters, including inter-individual distance among shoal members (the average of all distances between a given focal fish and every other shoal member), nearest neighbor distance (the smallest distance between the focal fish and its neighbor), frequency of excursions (departures from the shoal), and polarization (the degree of synchronization of movement) of the shoal (Miller and Gerlai 2008, 2012). The second one, the focus of this section, is to use visual stimuli and measure the response of a single experimental subject to these stimuli (Saverino and Gerlai 2008). What visual stimuli should we use and how should we deliver them?

Pioneering studies have shown that zebrafish are not indifferent to the features of fish with which they form groups. Zebrafish do not shoal with everyone. For example, zebrafish have been found to shoal well with conspecifics of the same color or different color, but they have been found not to shoal with a hetero-specific shoaling species (white cloud *Tanichthys albonubes*) or a hetero-specific non-shoaling species of fish (platy *Xiphophorus maculatus*)

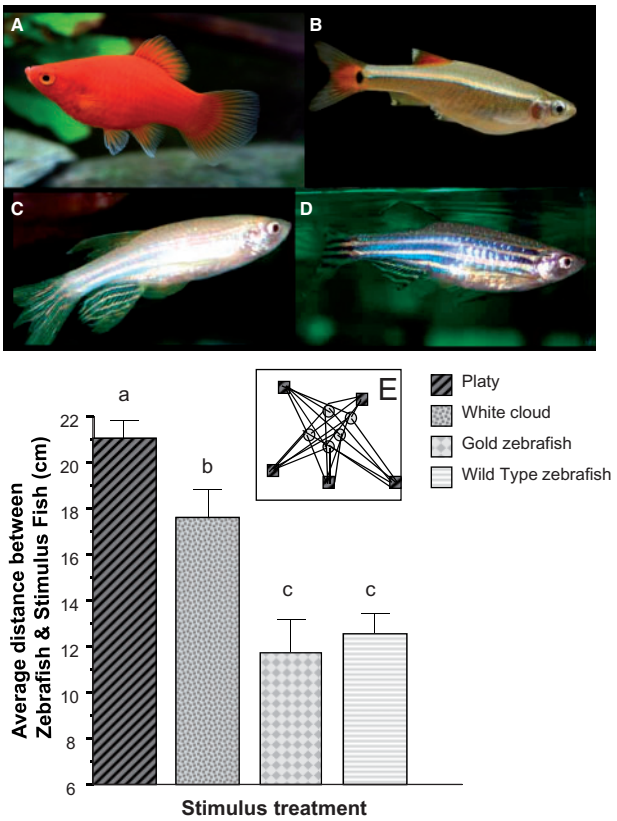

Figure 1. Zebrafish do not shoal with everyone. For each recording session, 5 wild type experimental fish were mixed with 5 stimulus fish. All fish were allowed to swim freely and inter-individual distances between experimental zebrafish (the focal fish) and all stimulus fish were measured (as illustrated in panel E). Four different type of stimulus fish were used: (A) Platy *Xiphophorus maculatus*, a hetero-specific non-shoaling fish; (B) white cloud *Tanichthys albonubes*, a hetero-specific shoaling fish; (C) a gold color variant of zebrafish *Danio rerio*, a conspecific shoaling fish; and wild type zebrafish *Danio rerio*, a conspecific shoaling fish marked with a small tail clip to distinguish them from the experimental fish, which otherwise looked identical to the stimulus fish. The graph shows that experimental zebrafish swam significantly closer to conspecifics irrespective of their color when compared with hetero-specific shoaling or non-shoaling fish. Mean ± SEM are shown. The small letters above the bars represent the results of a Tukey Honestly Significant Difference multiple comparison post hoc analysis. Bars with different letter designations significantly ($P < 0.05$) differ. Note that the results could not distinguish what features of the stimulus fish (e.g., their shape, color, pattern, movement, olfactory cues, etc.) drove the shoaling decision of the experimental zebrafish, or whether it was the experimental zebrafish's behavior or the stimulus fish's behavior or both, that led to differential shoal cohesion. For further details, full description of results and statistical analyses, see Saverino and Gerlai (2008). Modified from Saverino and Gerlai (2008).

(Saverino and Gerlai 2008) (Figure 1). What stimuli the experimental zebrafish based their decision on whether to shoal or not to shoal are not known at this point. Nevertheless, subsequent analysis of visual stimuli using computer animation demonstrated that both the color and the shape of moving images of fish make a difference. In this study, experimental zebrafish were presented with a choice (Saverino and Gerlai 2008). On one side of the tank, the single experimental subject was shown animated (moving) images of a wild type zebrafish, and on the other side a similarly moving but altered images. The alterations ranged from color changes (yellow or red tinted images), modification of stripe pattern (wild type zebrafish are horizontally striped, and the modified images had no stripe or

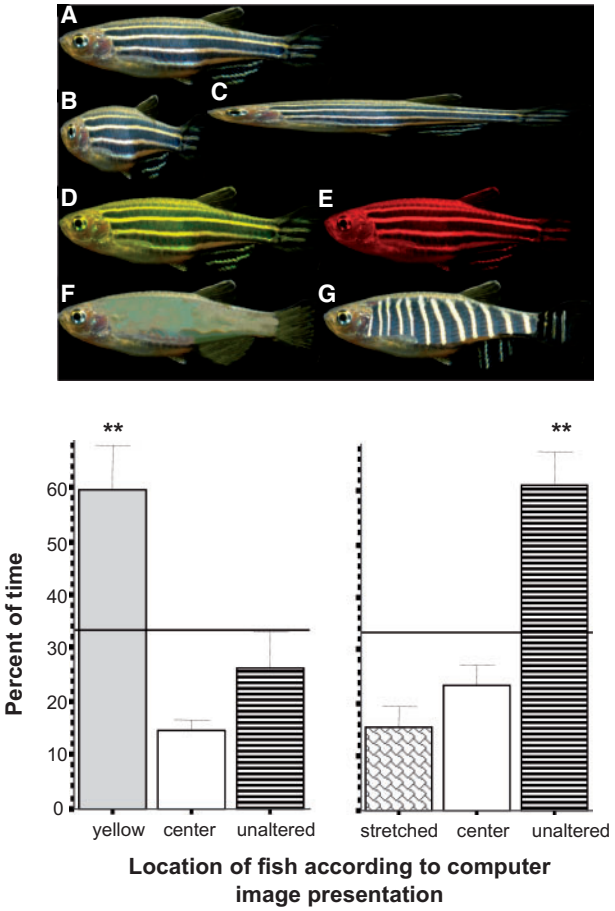

Figure 2. Examples of visual stimulus feature preference/avoidance in zebrafish. The upper panel shows the different images shown to zebrafish. On one side of the experimental tank, a group of animated (moving) images of unaltered, wild type zebrafish (A) were presented, while on the opposite side, a group of animated images of one of the other six modified photos (B, C, D, E, F, or G) were shown (image D shows a yellow and E a red coloured zebrafish). The bar graphs show the percent of time a single experimental fish spent in the third of the tank near the wild type images, the center of the tank, and in the third of the tank near the modified images. Chance level performance (33%) is indicated by the solid horizontal line. Mean ± SEM are shown. Asterisks denote significant difference compared with chance ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Two experimental condition examples are shown: The left bar graph shows that experimental zebrafish exhibited a significant preference toward the yellow colored images. The bar graph on the right shows that zebrafish exhibited a significant avoidance of the elongated images. For further details, full description of results and statistical analyses, see Saverino and Gerlai (2008). Modified from Saverino and Gerlai (2008).

had horizontal stripes), or the shape of the images (elongated or more round body). Preference was evaluated by measuring how much time the experimental fish spent in the proximity of one versus the other image side. The results were somewhat surprising. Zebrafish were found to exhibit equal preference to images with wild type stripe pattern versus no stripe or vertical stripe pattern (Saverino and Gerlai 2008). They also did not show any change in the strength of preference toward the more rounded body shape, but showed a robust avoidance reaction toward the side where the elongated images were shown (Figure 2) (Saverino and Gerlai 2008). A possible explanation for the latter is that the elongated images resembled a natural predator of the zebrafish, the needle fish (Saverino and Gerlai 2008). Also interesting was the finding that showed that zebrafish exhibited a differential preference toward yellow colored images over the wild type colored images (Figure 2) (Saverino and Gerlai 2008). The potential explanation for this finding may be that during spawning, and also during aggressive encounters, zebrafish appear more vividly colored associated with enhanced golden, or yellow hue. Last, zebrafish are also sensitive to several other aspects of the visual stimuli intended to induce shoaling responses. For example, we have started to investigate the effects of the size and the number of conspecific images (Fernandes et al. 2015a) as well as the speed, location, and numerous other features of these images on shoaling responses in zebrafish (Mahabir S, Gerlai R, unpublished data). Although these results are preliminary, they signify the need to conduct systematic and parametric analysis of numerous visual features as to how important or unimportant they may be for shoaling decisions in the zebrafish. Briefly, we do not yet know what a Platonian essence of zebrafishness is for the zebrafish, but we already do know that not every feature matters with the same weight and some features do not matter at all.

These findings raise an important practical question too: how sophisticated must the image-presentation method be to induce maximal behavioral responses in the experimental zebrafish? Are visual cues alone sufficient to induce maximal shoaling responses, or do zebrafish also need olfactory, auditory, and/or lateral line cues when making shoaling decisions? Would realistic 3D image movement be required to induce maximal shoaling responses, or could simpler, more rudimentary image movement patterns be sufficient? Could only a subset of visual stimuli characteristic of a real zebrafish be sufficient? The above questions have started to be addressed by a recent study (Qin et al. 2014). In this study, we compared the strength of shoaling of experimental fish induced by different stimuli, including live stimulus fish inside the tank of the experimental zebrafish, live stimulus fish outside the tank of the experimental fish, presentation of a pre-recorded video showing live stimulus fish on a computer monitor, or presentation of animated (moving) images of zebrafish, respectively. Shoaling strength was quantified as the distance between the stimulus and the experimental fish during stimulus presentation (Figure 3). The shorter the distance was, the stronger the shoaling response was considered. Live stimulus fish inside the tank of the experimental fish (separated from the experimental fish by a perforated and transparent acrylic sheet) allowed the experimental fish to perceive all stimuli irrespective of their modality. Presenting live stimulus fish outside of the experimental fish allowed the stimulus fish to perceive only visual cues of the stimulus fish. Presentation of video-recordings of live stimulus fish also provided visual cues only, but in this test the stimulus fish could not interact with or respond to the behavior of the experimental fish. Last, presentation of computer animated 2D images of zebrafish provided stimuli that neither interacted, nor moved realistically in 3D. Interestingly, comparison of shoaling strength across these different conditions found no differences the effect of the employed stimuli. All of these stimuli were effective and induced a robust shoaling response (Qin et al. 2014). Thus, we concluded that zebrafish did not require realistic 3D image software presentation tools, and did not require interactive image responses, nor did they need perception of stimuli of modalities other than visual in order to exhibit a maximal shoaling response. A simple 2D animated image presentation suffices. Although this finding may seem surprising at first sight, ethologists have long known that even complex vertebrates may use only a limited number of cues in a range of behavioral contexts. The concept of key stimulus triggering fixed action patterns is well appreciated in animal behavioral studies

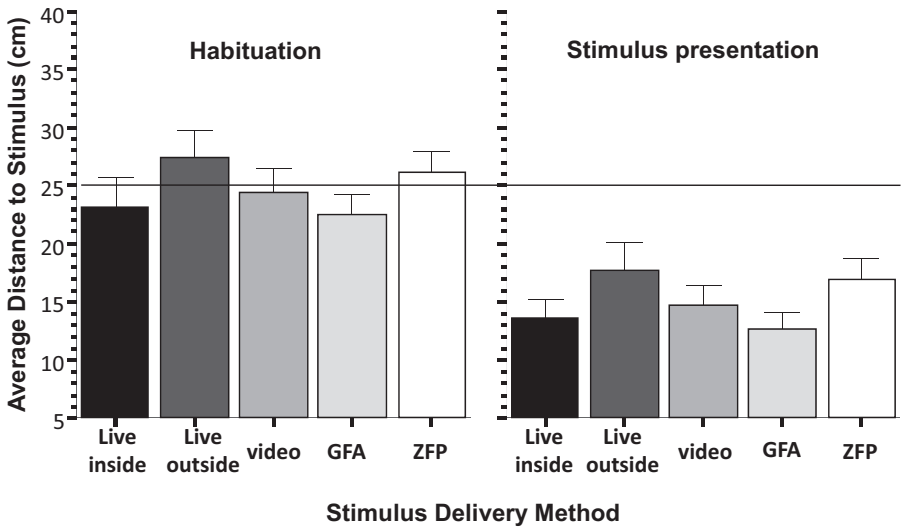

Figure 3. Comparison of different stimuli according to their effect on the shoaling response of experimental zebrafish. A single experimental fish was allowed to view one of the following different stimuli: live conspecifics inside the experimental tank, live conspecifics outside the experimental tank, video-recording of live conspecifics played back on a computer screen adjacent to the side wall of the experimental tank, animated images of conspecifics presented by the GFA software (developed inhouse) on a computer screen adjacent to one of the side walls of the experimental tank, and animated images of conspecifics presented by the ZFP software (developed inhouse) on a computer screen adjacent to one of the side walls of the experimental tank. The distance between the experimental fish from the stimulus is measured (shoaling response). The bar graph on the left shows the performance of experimental fish during the habituation session, when no stimuli are available. The bar graph on the right shows the performance of the experimental fish during stimulus presentation. Chance performance is indicated by the solid horizontal line (25 cm, the midpoint of the experimental tank). Mean ± SEM are shown. The results show that all stimulus conditions induced a robust shoaling response that was statistically indistinguishable across the different conditions. For further details and statistical analyses see Qin et al. (2014). Modified from Qin et al. (2014).

(Huntingford 1984; Takeuchi et al. 1987). For example, stickleback males respond equally vigorously with aggressive display to an approaching dominant male opponent or to a non-fish shaped object whose bottom part is colored red (Tinbergen 1951). Similarly, sea gull chicks will peck equally enthusiastically at the orange tipped beak of their mothers and also at a short stick presented to them with a similar color pattern (for review see Cate 2009). Zebrafish may also use such key stimuli when deciding whether to shoal or not to shoal and may not need fully realistic rendering of the color, pattern, shape, and movement of live zebrafish.

However, some cautionary notes must be made about this conclusion. Conceptually, it is easy to see why zebrafish would require only a simplified virtual reality presentation. Instead of perceiving all stimuli that characterize shoal mates, they may only pay attention and/or respond to a very small subset of key features of their shoal mates. Thus, one may conclude that realistic 3D-like animation of zebrafish-like images is not necessary. Nevertheless, the above-discussed findings cannot be considered as final proof for this argument. The reason is that our assumption that there is a linear negative correlation between shoaling strength and the distance to the shoal stimulus may not be entirely correct. It is possible that this measure is not sensitive enough to, and thus may not properly quantify, true shoal preference. An alternative method, often employed in fish research, is the choice task, in which the contrasted stimuli are presented on two opposite ends or two different parts of the test apparatus (e.g., Saverino and Gerlai 2008; Gómez-Laplaza and Gerlai 2011). The second point to which I draw the reader's attention here is the potential context-specific nature of key stimuli. For example, under highly aversive conditions zebrafish may not exhibit selective preference for particular stripe patterns, and thus may shoal well with all fish as long as these fish are about the same size, shape, and general color as the experimental subject. This may be because areal predators (fishing birds) do not see the stripe pattern differences that the fish exhibit on their sides, and thus the oddity effect may not work for this type of stimulus (Landeau and Terborgh 1986). However, in the context of reproductive behavior, preference for the horizontal stripe pattern in the zebrafish is likely to be crucial. This is because there are several *danio* and *devario* species sympatric in nature with the zebrafish that, although exhibiting the same olive brown color on their back (when viewed from above), show highly different and species-specific color and stripe patterns on their side.

In summary, the investigation of what stimuli to use for the induction of shoaling is only beginning. Nevertheless, it is already clear that computer-aided animations can be employed effectively. Presentation of animated images allows consistent stimulus delivery, which is expected to reduce error variation within and across experiments. The on-set and offset of stimulus delivery can be precisely controlled, and numerous aspects of the presented visual stimuli may be systematically dissected and investigated. It is thus likely that soon we will have a detailed catalog of what visual stimuli we need to present and in what contexts to induce the desired behavioral responses in zebrafish. It is also likely that during the establishment of these methodological details, numerous findings related to ethological, ecological, and evolutionary fitness questions will be discovered. Last, the developed methods will allow us to use zebrafish in biomedical research, that is, in the analysis of brain function of the zebrafish and in the modeling of brain dysfunction associated human disorders (Gerlai 2010b; Kalueff et al. 2014), a topic we discuss in the last section of this review.

Shoaling, Biological Mechanisms, and Disease Models

The number of genes that express mRNA in the brain of a vertebrate, including that of the zebrafish, is currently estimated to be around 10–15 thousand, well over 50% of the entire genome of the

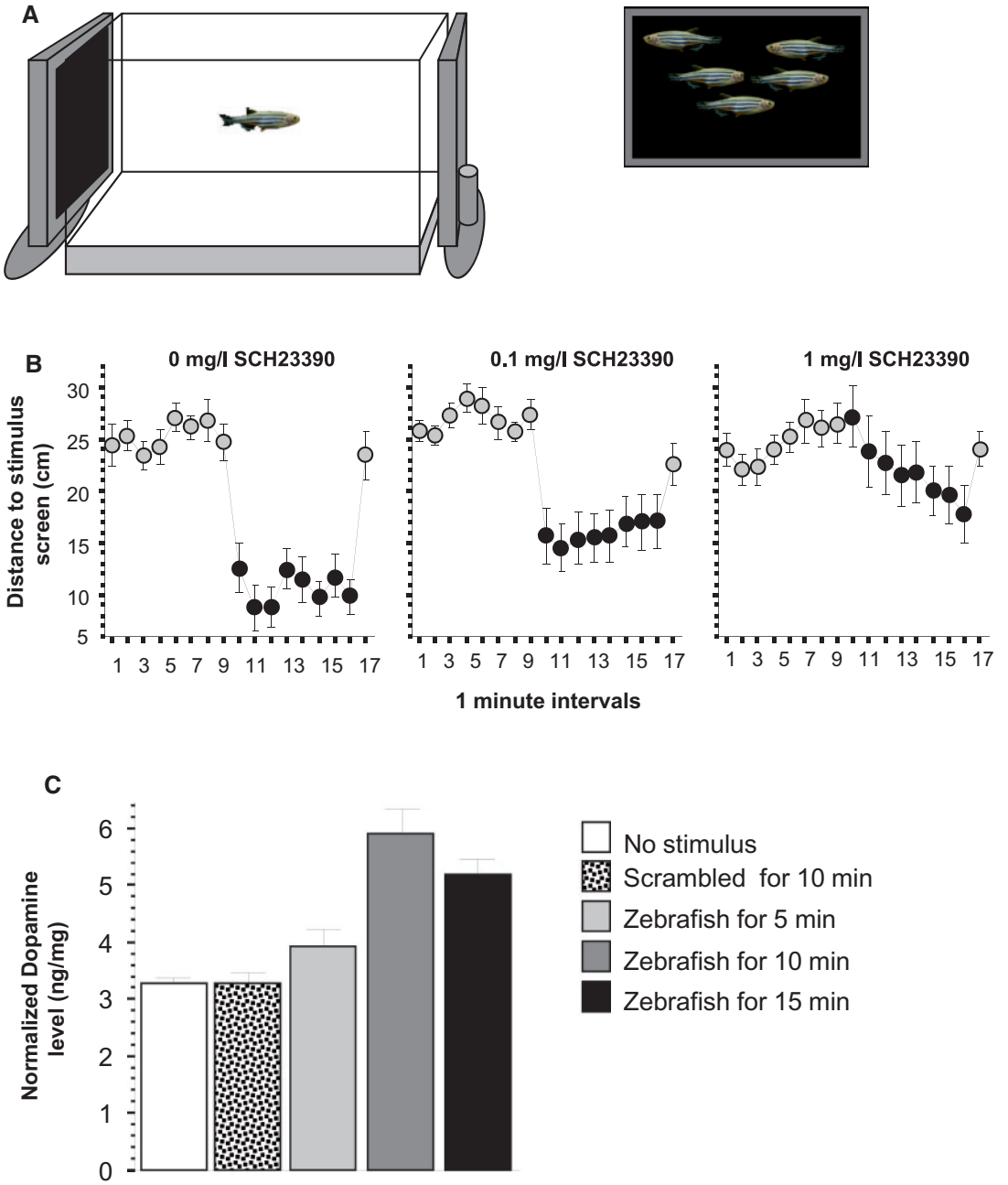

Figure 4. The dopaminergic system is involved in shoaling. Panel A shows the apparatus used to induce shoaling and the image presentation screen with 5 moving zebrafish images. Note the two computer monitors placed adjacent to opposite sides of the tank. Also note that during a 10 min long habituation period neither of these screens shows any images. Subsequently, one of the two monitors presents animated images of zebrafish. Image presentation lasts for 8 min after which again both monitors show a blank (black) screen. Note that during the habituation period experimental zebrafish (tested singly) swim, on average, about 25 cm away from the stimulus screen (gray filled circles), the midpoint of the tank considered chance level performance. However, also note that in response to the animated images, the distance robustly decreases to about 10 cm (black filled circles), a response we regard as measure of the strength of shoaling (Panel B line graph on the left). Last, note that this shoaling response significantly and dose dependently diminishes when the experimental fish have been exposed to 0.1 mg/l or 1 mg/l bath concentration of SCH23390, a dopamine D1-receptor antagonist (panel B, line graphs in the middle and on the right, observe the reduced difference between the habituation period, gray filled circles and the stimulus period, black filled circles, performance). Mean ± SEM are shown. Panel C shows the amount of dopamine measured using HPLC from whole brain of zebrafish exposed to different stimuli (see legend). Note that a moving image in which the pixels of a prior zebrafish image were scrambled into a rectangular shaped object does not increase dopamine levels compared with control fish that were shown no images at all. However, presentation of animated images of conspecifics for does increase dopamine levels in the brain of zebrafish in an image presentation length dependent manner. Mean ± SEM are shown. For further methodological details, statistical analysis results and interpretation, see Scerbina et al. (2012); Saif et al. (2013). Modified from Scerbina et al. (2012) and Saif et al. (2013).

given species (Lein et al. 2007; Pan et al. 2011). Imaging neuronal activity in response to a simple visual stimulus in live immobilized zebrafish shows waves of a dazzling array of neuronal activity changes (Ahrens et al. 2013; Perez et al. 2015). Perception, processing, and responding to social stimuli may engage many parts of the zebrafish brain. Social behaviors, including shoaling, are arguably some of the most complex functions the zebrafish brain may perform. How can we understand all this complexity? Clearly, we are quite far from having a complete account of neurobiological mechanisms underlying vertebrate social behavior, but over the past several years a lot of information has already been accumulated on this subject.

Instead of reviewing this already vast literature, here I present a proof of principle example from my own laboratory, a narrowly focused hypothesis driven research on dopamine and the role of the dopaminergic system in zebrafish shoaling behavior. This focus has been warranted by the observation that the sight of conspecifics is rewarding in zebrafish (Al-Imari and Gerlai 2008) and that the dopaminergic system is involved in reward in a variety of vertebrate species (Hoebel 1985; Fibiger and Phillips 1988; Wise and Rompre 1989). Zebrafish have been found to perform well in an associative learning task in which the reinforcement applied was visual access to live conspecific stimulus fish (Al-Imari and Gerlai 2008). When presentation of an otherwise neutral color cue card (the conditioned stimulus, CS) was paired with the presentation of live conspecifics (the unconditioned stimulus, or US), zebrafish quickly learned the association between these two sets of stimuli. After 20 pairing trials, when the color cue was presented alone, the trained experimental zebrafish preferred to stay in close proximity to the color cue (Al-Imari and Gerlai 2008). However, zebrafish that received the CS and US presented randomly during training, that is, not in a paired fashion, showed no preference for the CS (the cue card). In addition to proving the ability of zebrafish to acquire associative memory, this learning task also demonstrated that the sight of conspecifics is rewarding.

The dopaminergic system plays a fundamentally important role in reward and motivation in vertebrates. Thus, we wanted to investigate whether shoaling in zebrafish is mediated by this neurotransmitter system. We employed a dopamine D1-receptor antagonist SCH23390, which has known selectivity for D1-Receptors, at least in mammals (Caine et al. 1995). D1-R is one of the most abundantly expressed dopamine receptors in the zebrafish brain (Boehmler et al. 2004; Li et al. 2007). Most proteins compared between zebrafish and mammals have been found to possess high amino-acid sequence similarity especially at functionally important parts of the proteins. Based on such evolutionary conservation across these distant species one may expect drugs developed for mammalian species (including humans) to show similar efficacy in the zebrafish. Thus, using the SCH23390 D1-R antagonist, we expected to significantly impair the functioning of the dopaminergic system. Interestingly, zebrafish immersed in the D1-R antagonist drug solution 30 min prior to a shoaling paradigm exhibited significant and dose-dependent disruption of shoaling without any obvious motor side effects (Figure 4, panel B) (Scerbina et al. 2012). Although promising, such disruption may still be a result of altered performance features unrelated to shoaling itself. For example, unknown off-target drug effects may have impaired motor function (albeit not detected) or visual perception, or may have affected motivational features unrelated to shoaling, for example, fear and anxiety. Many of these putative changes may have modified shoaling responses. To examine this possibility we used an alternative approach. We induced shoaling using visual stimuli, and measured the response of the dopaminergic system to this stimulation (Saif et al. 2013). We removed the brains of zebrafish immediately after the stimulation and quantified the amount of dopamine and DOPAC (3,4-Dihydroxyphenylacetic acid, dopamine's metabolite) from the brain homogenates. Our results confirmed that the dopaminergic system is involved in shoaling (Figure 4, panel C). Presentation of the shoaling images significantly increased the amount of dopamine and DOPAC in the brains of zebrafish (Saif et al. 2013). Importantly, presentation of animated images not resembling zebrafish did not induce the shoaling response, and these images also did not increase dopamine and DOPAC levels in the brain of the tested zebrafish (Saif et al. 2013). Last, the activation of the dopaminergic system by the presentation of animated conspecifics appeared to be specific to this neurotransmitter system, as changes in other neurochemicals including in the amount of serotonin and 5HIAA (serotonin's metabolite) were not induced by the presentation of animated conspecifics (Saif et al. 2013). The above results strongly suggest that the sight of conspecifics is a rewarding stimulus, and that shoaling is mediated by the dopaminergic system, which both may explain the strong shoal-forming tendency of the zebrafish. How can these results be utilized in modeling human disorders?

We have been studying the effect of alcohol in zebrafish (Gerlai et al. 2000; Gerlai 2015). Alcohol is a complex drug that engages multiple neurotransmitter systems, one of which is the dopaminergic system. Dopamine has been shown to play fundamental roles in drug, including alcohol, abuse (Samson et al. 1992). However, also importantly, alcohol has been long known to significantly affect several domains of social behavior in humans (Boyatzis 1977; Wilson 1977). In addition to its acute and chronic effects, alcohol is also known to alter embryonic development (Sampson et al. 1997). When pregnant women drink, their children often end up suffering from a lifelong disease, more or less severe forms of fetal alcohol spectrum disorders, FASD (Sampson et al. 1997). Fetal alcohol spectrum disorders represent a huge societal burden and suffering with an estimated frequency of occurrence in children that can exceed 10% depending on country or region examined (Roozen et al. 2016). Particularly prevalent are the milder forms of the disease (May et al. 2009). A behavioral feature common to a range of FASD cases, including the milder forms, has been increasingly recognized: abnormal social behavior (Thomas et al. 1998; O'Connor et al. 2006; Kully-Martens et al. 2011; Rasmussen et al. 2011).

We decided to attempt to model this disease with zebrafish. Such a model is not unrealistic. Unlike in many other human CNS disorders, FASD has a clearly indentified cause: embryonic alcohol exposure. We decided to model this disease by administering alcohol at doses that would represent the milder, and more prevalent forms of FASD, ones in which children do not suffer from major physical abnormalities but rather only exhibit behavioral changes including abnormal social behavior (May et al. 2009). We exposed zebrafish 24 h after fertilization for 2 h to alcohol ranging between 0.25 and 1.00 vol/vol% (Fernandes and Gerlai 2009). This developmental time point represents the end of the segmentation and the beginning of the pharyngula stage of zebrafish development, which corresponds approximately to the late first or early second trimester of human fetal development (Kimmel et al. 1995; also see http://zfin.org/zf_info/zfbook/stages/and http://www.ehd.org/virtual-human-embryo/). At this developmental stage, the major structural components of the brain have started to form, but neuronal connections have not been finalized and neuronal migration is still ongoing.

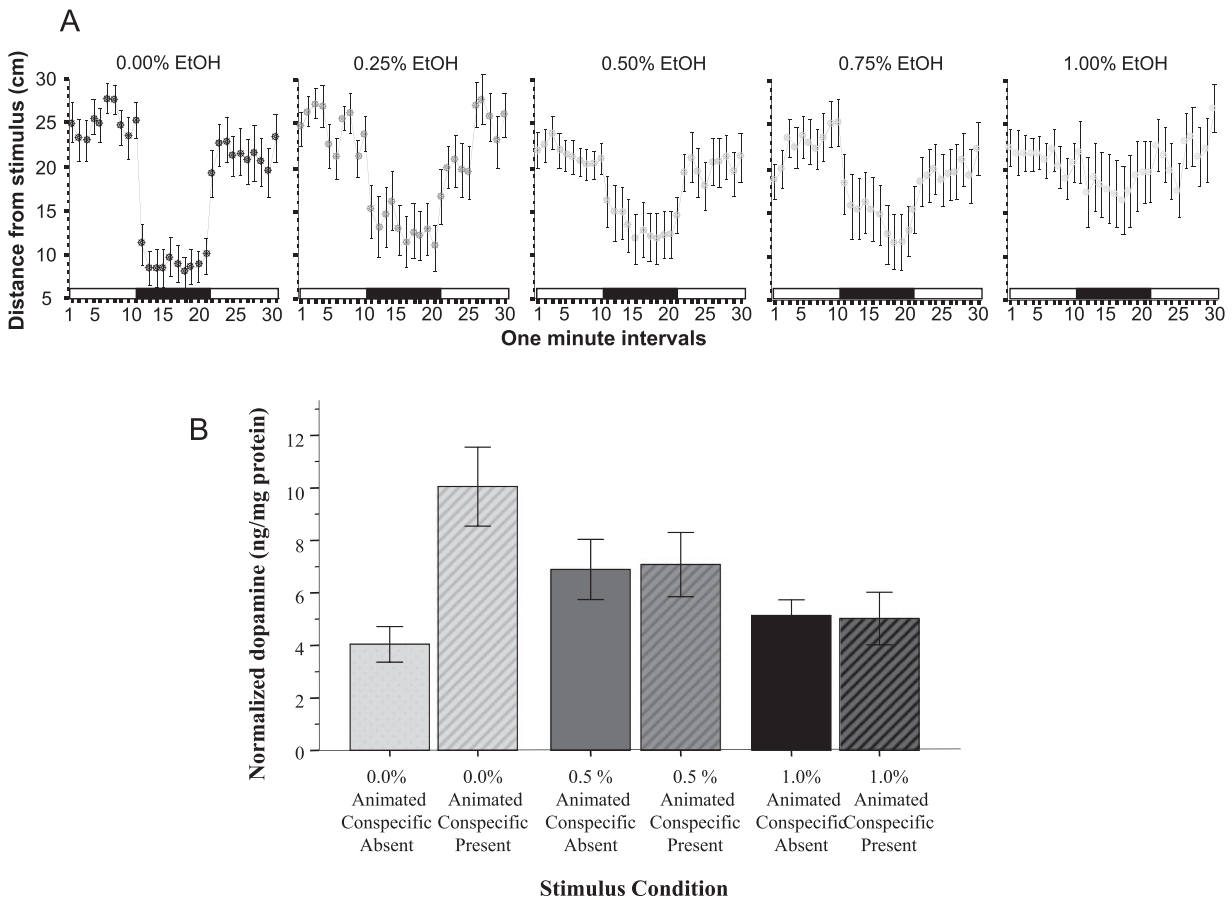

Figure 5. Exposure to alcohol during embryonic development (2 h long immersion at 24th h post-fertilization) significantly diminishes responding to animated images of conspecifics at the behavioral (A) and neurochemistry (B) level. The behavioral test paradigm and apparatus is similar to that indicated in Figure 4. (A) Distance to the stimulus screen significantly diminishes when animated images of conspecifics are being shown, a response that is significantly and dose dependently diminishes in adult fish that were exposed to alcohol during their embryonic development. Mean ± SEM are shown. The stimulus period is indicated by the black solid horizontal bar underneath the line graphs. The unfilled horizontal bar underneath the line graphs represent the periods when the stimulus was off. The concentration of alcohol to which experimental fish were exposed during their embryonic development is indicated above the corresponding line graph. (B) The amount of dopamine relative to total brain protein weight is significantly increased in response to sight of animated conspecifics in control alcohol unexposed zebrafish. This dopamine response is, however, absent in adult fish that were exposed to alcohol during their embryonic development. Mean ± SEM are shown. The concentration of alcohol administered during embryonic development as well as stimulus condition (animated conspecifics absent versus present) is indicated underneath the bar graph. Modified from Fernandes and Gerlai (2009) and from Fernandes et al. (2015b).

Interestingly, unlike in prior studies where zebrafish embryos were exposed to higher doses of alcohol and for longer periods of alcohol administration (e.g., Bilotta et al. 2004; Arenzana et al. 2006), our zebrafish showed no changes in their anatomy or growth rate, and they experienced no increases in mortality (Fernandes and Gerlai 2009). In fact, the exposed fish seemed completely unaltered. However, when we quantified their behavior during their adult stage, we found a significant and dose-dependent impairment in their response to animated images of conspecifics (Figure 5, panel A) (Fernandes and Gerlai 2009). Such impairment could be due to many factors, including impaired vision, impaired motor function, altered fear, etc. However, by now we have excluded all these possibilities (Fernandes and Gerlai 2009; Buske and Gerlai 2011; Seguin D, Gerlai R, unpublished data). What we have been left with is social behavior itself. It appears that embryonic alcohol exposure disrupted the development of the zebrafish brain leading to abnormal responding to conspecific images. Also importantly, we have shown that the impairment is replicable in a real shoaling situation: embryonic alcohol exposed fish form significantly less tight shoals than control alcohol unexposed fish do (Buske and Gerlai 2011).

What mechanism could explain the impaired shoaling response? It is likely that a teratogen-like alcohol induces a complex array of developmental changes that may manifest as complicated network of biochemical and structural abnormalities in the adult brain. We have started to map these abnormalities, but here I only focus on our proof of concept, that is, hypothesis driven approach. Given what we learned about the involvement of the dopaminergic system in shoaling and given the known interaction between alcohol and dopamine, we decided to check the dopaminergic system as a potential mediator of the embryonic alcohol exposure induced impairment in shoaling. We measured how the dopaminergic system responds to the presentation of conspecific images in fish that were never exposed to alcohol and in fish that were exposed to alcohol during their embryonic development (Fernandes et al. 2015b). Interestingly, we found that although the baseline levels of dopamine and DOPAC were unaltered in alcohol-exposed fish, when these fish were shown the conspecific images, they did not increase dopamine and DOPAC levels in their brain, whereas fish that were never exposed to alcohol showed a robust dopamine and DOPAC response (Fernandes et al. 2015b). In summary, we discovered that

embryonic alcohol treatment disrupts shoaling and impairs dopaminergic responses to social stimuli when tested in adult fish.

The above example demonstrates how one can utilize presentation of visual stimuli in the analysis of zebrafish behavior and how such behavioral analysis may guide or complement modeling human CNS disorders or other neurobiological mechanisms-related studies. Clearly, the presentation of computer-animated visual stimuli adds sophistication, experimental control, and precision to animal behavioral studies. When paired with automated recording and quantification of behavioral responses, for example, with the use of video-tracking systems, such virtual-reality systems can be utilized for the analysis of a large number of subjects, because the experimenter does not need to be present and because a large number of apparati may be run in parallel. The ability to run multiple tests at the same time significantly increases throughput, which may allow one to conduct large-scale mutagenesis and/or drug screening studies (e.g., Rihel et al. 2010; also see review by Gerlai 2014a). Such screening studies in turn may allow one to systematically analyze complex neurobiological phenomena, including the mechanisms of social behavior of vertebrates, and/or the mechanisms underlying fetal alcohol exposure induced changes in the brain. Once these mechanisms are discovered and better understood, this knowledge may be utilized in the development of therapeutic applications, and also in the identification of biomarkers that would aid diagnosis. Clearly, these are long-term goals, but I suggest the increasingly sophisticated virtual-reality methods coupled with the fast-paced development of molecular tool custom designed for the zebrafish, will make this little vertebrate an excellent tool for the neuroscientist.

Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council (311637-06) and Brain Canada Foundation grants.

References

Ahrens MB, Orger MB, Robson DN, Li JM, Keller PJ, 2013. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nat Methods* **10**:413–420.

Alexandrov V, Brunner D, Hanania T, Leahy E, 2015. High-throughput analysis of behavior for drug discovery. *Eur J Pharmacol* **750**:82–89.

Al-Imari L, Gerlai R, 2008. Sight of conspecifics as reward in associative learning tasks for zebrafish *Danio rerio*. *Behav Brain Res* **189**:216–219.

Arenzana FJ, Carvan MJ III, Aijón J, Sánchez-González R, Arévalo R et al., 2006. Teratogenic effects of ethanol exposure on zebrafish visual system development. *Neurotoxicol Teratol* **28**:342–348.

Bilotta J, Barnett JA, Hancock L, Saszik S, 2004. Ethanol exposure alters zebrafish development: a novel model of fetal alcohol syndrome. *Neurotoxicol Teratol* **26**:737–743.

Blackburn PR, Campbell JM, Clark KJ, Ekker SC, 2013. The CRISPR system—keeping zebrafish gene targeting fresh. *Zebrafish* **10**:116–118.

Bleckmann H, Zelick R, 2009. Lateral line system of fish. *Integr Zool* **4**:13–25.

Boehmler W, Obrecht-Pflumio S, Canfield V, Thisse C, Thisse B et al., 2004. Evolution and expression of D2 and D3 dopamine receptor genes in zebrafish. *Dev Dyn* **230**:481–493.

Boyatzis RE, 1977. Alcohol and interpersonal aggression. *Adv Exp Med Biol* **85B**:345–375.

Bures J, Fenton AA, Kaminsky Y, Zinyuk L, 1997. Place cells and place navigation. *Proc Natl Acad Sci USA* **94**:343–350.

Buske C, Gerlai R, 2011. Early embryonic ethanol exposure impairs shoaling and the dopaminergic and serotoninergic systems in adult zebrafish. *Neurotoxicol Teratol* **33**:698–707.

Caine SB, Heinrichs SC, Coffin VL, Koob GF, 1995. Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Res* **692**:47–56.

Cate C, 2009. Niko Tinbergen and the red patch on the herring gull's beak. *Anim Behav* **77**:785–794.

Chouinard-Thuly L, Gierszewski S, Rosenthal GG, Reader SM, Rieucau G et al., 2017. Technical and conceptual considerations for using animated stimuli in studies of animal behaviour. *Curr Zool* **63**:5–19.

Clark KJ, Voytas DF, Ekker SC, 2011. A TALE of two nucleases: gene targeting for the masses? *Zebrafish* **8**:147–149.

Crabbe JC, Wahlsten D, Dudek BC, 1999. Genetics of mouse behavior: interactions with laboratory environment. *Science* **284**:1670–1672.

Engeszer RE, Patterson LB, Rao AA, Parichy DM, 2007. Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish* **4**:21–40.

Fernandes Y, Gerlai R, 2009. Long-term behavioral changes in response to early developmental exposure to ethanol in zebrafish. *Alcohol Clin Exp Res* **33**:601–609.

Fernandes Y, Rampersad M, Jia J, Gerlai R, 2015a. The effect of the number and size of animated conspecific images on shoaling responses of zebrafish. *Pharmacol Biochem Behav* **139**:94–102.

Fernandes Y, Rampersad M, Gerlai R, 2015b. Embryonic alcohol exposure impairs the dopaminergic system and social behavioural responses in adult zebrafish. *Int J Neuropsychopharmacol* **18**:1–8.

Fibiger HC, Phillips AG, 1988. Mesocorticolimbic dopamine systems and reward. *Ann NY Acad Sci* **537**:206–215.

Gerlai R, 2001. Behavioral tests of hippocampal function: simple paradigms, complex problems. *Behav Brain Res* **125**:269–277.

Gerlai R, 2002. Phenomics: fiction or the future? *Trends Neurosci* **25**:506–509.

Gerlai R, 2014a. Zebrafish phenomics: behavioral screens and phenotyping of mutagenized fish. *Curr Opin Behav Sci* **2**:21–27.

Gerlai R, 2014b. Fish in behavior research: unique tools with a great promise!. *J Neurosci Methods* **234**:54–58.

Gerlai R, 2014c. Social behavior of zebrafish: from synthetic images to biological mechanisms of shoaling. *J Neurosci Methods* **234**:59–65.

Gerlai R, 2015. Embryonic alcohol exposure: towards the development of a zebrafish model of fetal alcohol spectrum disorders. *Dev Psychobiol* **57**:787–798.

Gerlai R, 2010a. Zebrafish antipredatory responses: a future for translational research? *Behav Brain Res* **207**:223–231.

Gerlai R, 2010b. High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. *Molecules* **15**:2609–2622.

Gerlai R, Clayton NS, 1999. Analysing hippocampal function in transgenic mice: an ethological perspective. *Trends Neurosci* **22**:47–51.

Gerlai R, Lahav M, Guo S, Rosenthal A, 2000. Drinks like a fish: zebra fish *Danio rerio* as a behavior genetic model to study alcohol effects. *Pharmacol Biochem Behav* **67**:773–782.

Granato M, Nüsslein-Volhard C, 1996. Fishing for genes controlling development. *Curr Opin Genet Dev* **6**:461–468.

Godinho SI, Nolan PM, 2006. The role of mutagenesis in defining genes in behaviour. *Eur J Hum Genet* **14**:651–659.

Gómez-Laplaza LM, Gerlai R, 2011. Can Angelfish (Pterophyllum scalare. count? Discrimination between different shoal sizes follows Weber's law. *Anim Cogn* **14**:1–9.

Haesemeyer M, Schier AF, 2015. The study of psychiatric disease genes and drugs in zebrafish. *Curr Opin Neurobiol* **30**:122–130.

Hamdaniel H, Døving KB, 2007. The functional organization of the fish olfactory system. *Prog Neurobiol* **82**:80–86.

Hoebel BG, 1985. Brain neurotransmitters in food and drug reward. *Am J Clin Nutr* **42**:1133–1150.

Huntingford F, 1984. *The Study of Animal Behaviour*. London: Chapman and Hall, 411.

Kalueff AV, Stewart AM, Gerlai R, 2014. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol Sci* **35**:63–75.

Kimmel CB, 1989. Genetics and early development of zebrafish. *Trends Genet* **5**:283–288.

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF, 1995. Stages of embryonic development of the zebrafish. *Dev Dyn* **203**:253–310.

Kokel D, Rennekamp AJ, Shah AH, Liebel U, Peterson RT, 2012. Behavioral barcoding in the cloud: embracing data-intensive digital phenotyping in neuropharmacology. *Trends Biotechnol* **30**:421–425.

Kully-Martens K, Denys K, Treit S, Tamana S, Rasmussen C, 2011. A review of social skills deficits in individuals with fetal alcohol spectrum disorders and prenatal alcohol exposure: profiles, mechanisms, and interventions. *Alcohol Clin Exp Res* **36**:568–576.

Ladich F, 2014. Fish bioacoustics. *Curr Opin Neurobiol* **28**:121–127.

Ladu F, Bartolini T, Panitz SG, Chiarotti F, Butail S et al., 2015. Live predators, robots, and computer-animated images elicit differential avoidance responses in zebrafish. *Zebrafish* **12**:205–214.

Landeau L, Terborgh J, 1986. Oddity and the 'confusion effect' in predation. *Anim Behav* **34**:1372–1380.

Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A et al., 2007. Genome-wide atlas of gene expression in the adult mouse brain. *Nature* **445**:168–176.

Li P, Shah S, Huang L, Carr AL, Gao Y et al., 2007. Cloning and spatial and temporal expression of the zebrafish dopamine D1 receptor. *Dev Dyn* **236**:1339–1346.

Lipp HP, Wolfer DP, 1998. Genetically modified mice and cognition. *Curr Opin Neurobiol* **8**:272–280.

May PA, Gossage JP, Kalberg WO, Robinson LK, Buckley D et al., 2009. Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Dev Disabil Res Revs* **15**:176–192.

Miller N, Gerlai R, 2007. Quantification of shoaling behaviour in zebrafish *Danio rerio*. *Behav Brain Res* **184**:157–166.

Miller N, Gerlai R, 2008. Oscillations in shoal cohesion in zebrafish *Danio rerio*. *Behav Brain Res* **193**:148–151.

Miller N, Gerlai R, 2011. Shoaling in zebrafish: what we don't know. *Rev Neurosci* **22**:17–25.

Miller N, Gerlai R, 2012. From schooling to shoaling: patterns of collective motion in zebrafish *Danio rerio*. *PLoS ONE* **7**:e48865.

Neuhauss SC, 2003. Behavioral genetic approaches to visual system development and function in zebrafish. *J Neurobiol* **54**:148–160.

O'Connor MJ, Frankel F, Paley B, Schonfeld AM, Carpenter E et al., 2006. A controlled social skills training for children with fetal alcohol spectrum disorders. *J Consult Clin Psychol* **74**:639–648.

Pan Y, Mo K, Razak Z, Westwood JT, Gerlai R, 2011. Chronic alcohol exposure induced gene expression changes in the zebrafish brain. *Behav Brain Res* **216**:66–76.

Parker MO, Annan LV, Kanellopoulos AH, Brock AJ, Combe FJ et al., 2014. The utility of zebrafish to study the mechanisms by which ethanol affects social behavior and anxiety during early brain development. *Prog Neuropsychopharmacol Biol Psychiatry* **55**:94–100.

Patton EE, Zon LI, 2001. The art and design of genetic screens: zebrafish. *Nat Rev Genet* **2**:956–966.

Perez CC, Lauri A, Symvoulidis P, Cappetta M, Erdmann A et al., 2015. Calcium neuroimaging in behaving zebrafish larvae using a turn-key light field camera. *J Biomed Opt* **20**:096009.

Qin M, Wong A, Seguin D, Gerlai R, 2014. Induction of social behaviour in zebrafish: live versus computer animated fish as stimuli. *Zebrafish* **11**:185–197.

Rasmussen C, Becker M, McLennan J, Urichuk L, Andrew G, 2011. An evaluation of social skills in children with and without prenatal alcohol exposure. *Child Care Health Dev* **37**:711–718.

Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S et al., 2010. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science* **327**:348–351.

Roozen S, Peters GJ, Kok G, Townend D, Nijhuis J et al., 2016. Worldwide prevalence of fetal alcohol spectrum disorders: a systematic literature review including meta-analysis. *Alcohol Clin Exp Res* **40**:18–32.

Saif M, Chatterjee D, Buske C, Gerlai R, 2013. Sight of conspecific images induces changes in neurochemistry in zebrafish. *Behav Brain Res* **243**:294–299.

Sampson PD, Streissguth AP, Bookstein FL, Little RE, Clarren SK et al., 1997. Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. *Teratology* **56**:317–326.

Samson HH, Tolliver GA, Haraguchi M, Hodge CW, 1992. Alcohol self-administration: role of mesolimbic dopamine. *Ann N Y Acad Sci* **654**:242–253.

Saverino C, Gerlai R, 2008. The social zebrafish: behavioral responses to conspecific, heterospecific, and computer animated fish. *Behav Brain Res* **191**:77–87.

Schenk F, Morris RG, 1985. Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Exp Brain Res* **58**:11–28.

Scerbina T, Chatterjee D, Gerlai R, 2012. Dopamine receptor antagonism disrupts social preference in zebrafish, a strain comparison study. *Amino Acids* **43**:2059–2072.

Sison M, Cawker J, Buske C, Gerlai R, 2006. Fishing for genes influencing vertebrate behavior: zebrafish making headway. *Lab Anim* **35**:33–39.

Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff A, 2014a. Zebrafish models for translational neuroscience research: from tank to bedside. *Trends Neurosci* **37**:264–278.

Stewart AM, Ullmann JFP, Norton WH, Brennan CH, Parker MO et al., 2014b. Molecular psychiatry of zebrafish. *Mol Psychiatry* **20**:2–17.

Streisinger G, Walker C, Dower N, Knauber D, Singer F, 1981. Production of clones of homozygous diploid zebra fish *Brachydanio rerio*. *Nature* **291**:293–296.

Takao K, Miyakawa T, 2006. Investigating gene-to-behavior pathways in psychiatric disorders: the use of a comprehensive behavioral test battery on genetically engineered mice. *Ann N Y Acad Sci* **1086**:144–159.

Takeuchi H, Takei K, Satou M, Matsushima T, Okumoto N et al., 1987. Visual cues as key stimuli for courtship behaviour in male himé salmon (landlocked red salmon *Oncorhynchus nerka*). *Anim Behav* **35**:936–939.

Tinbergen N, 1951. *The Study of Instinct*. New York: Oxford University Press, 237.

Thomas SE, Kelly SJ, Mattson SN, Riley EP, 1998. Comparison of social abilities of children with fetal alcohol syndrome to those of children with similar IQ scores and normal controls. *Alcohol Clin Exp Res* **22**:528–533.

Wahlsten D, Cooper SF, Crabbe JC, 2005. Different rankings of inbred mouse strains on the Morris maze and a refined 4-arm water escape task. *Behav Brain Res* **165**:36–51.

Wilson GT, 1977. Alcohol and human sexual behavior. *Behav Res Ther* **15**:239–352.

Wise RA, Rompre PP, 1989. Brain dopamine and reward. *Annu Rev Psychol* **40**:191–225.